Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world-wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Lucas Trower Date

Examining the impact of heterogeneity in timing of health seeking behavior on the power to detect seasonal effects of disease, using Buruli ulcer as the example

By

Lucas Trower

Master of Public Health

Epidemiology

_____ _____

Michael Kramer & Lancer Waller

Committee Co-Chairs

Examining the impact of heterogeneity in timing of health seeking behavior on the power to detect seasonal effects of disease, using Buruli ulcer as the example

By

Lucas Trower

B.S. Biology

University of Illinois Champaign-Urbana

2014

Faculty Thesis Advisors: Michael Kramer, Lance A. Waller

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2018

Abstract

Examining the impact of heterogeneity in timing of health seeking behavior on the power to detect seasonal effects of disease, using Buruli ulcer as the example

By Lucas Trower

The ability to detect seasonality of disease incidence is an important factor in mitigating the spread of the disease, because it helps public health officials prepare for potential outbreaks. Some diseases, such as Buruli ulcer, are rare, and seasonality may be hard to detect due to the low number of cases. On top of having a low number of cases, there is a long incubation period, and people who are infected may delay seeking treatment, which can potentially lead to a lower probability of detecting true seasonality in disease transmission or incidence. We conducted simulations to see if delay in seeking treatment among infected individuals reduces our ability to accurately capture the underlying seasonality of the causative disease. We used Buruli ulcer as our disease of interest, because there has yet to be confirmation of seasonality, although it is highly suspected it occurs around the rainy seasons in endemic countries. We created a simulated seasonality for Buruli ulcer with high probability of detection of seasonality in the absence of incubation and treatment delays. We next introduced delays such as incubation period and time-to-seek treatment to quantify the resulting reduction in detecting seasonality. Our results indicate a delay in seeking treatment can have a measureable effect on our ability to detect seasonality for a disease such as Buruli ulcer.

Examining the impact of heterogeneity in timing of health seeking behavior on the power to detect seasonal effects of disease, using Buruli ulcer as the example

By

Lucas Trower

B.S. Biology

University of Illinois Champaign-Urbana

2014

Faculty Thesis Advisor: Michael Kramer, Lance Waller

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2018

Chapter I

Background

I. BURULI ULCER AND PREVALENCE

Buruli ulcer (BU) is one of the 20 neglected tropical diseases defined by the WHO (20) and is caused by infection with *Mycobacterium ulcerans* yielding a necrotizing bacterial skin infection via a toxin called mycolactone. Mycolactone affects scaffolding proteins such as actin (6 Guienen-Mace) and disrupts cells' ability to adhere to one another and causes cell death (7 Sarfo). Mycolactone also causes neuronal process degradation often resulting in painless necrotizing of the skin.

Mycobacterium ulcerans belongs to the same family of bacteria causing tuberculosis and leprosy and is the third most prevalent human mycobacteria infection following these (1 WHO). Buruli ulcer has currently been reported in at least 33 countries with a majority of these countries being located in tropical, subtropical, and temperate climates within South America, Africa, and Pacific regions including Japan and Australia (1 WHO). A majority of the cases reported to WHO annually come from the African countries of the Democratic Republic of Congo, Benin, Côte d'Ivoire, Cameroon, and Ghana. There were 2,037 new cases of Buruli ulcer in 2015 coming from 13 of the 15 countries that regularly report BU to WHO. New cases of Buruli ulcer typically affect children under the age of

15, however, new cases of Buruli ulcer in places like Japan and Australia have a greater incidence of cases in adults older than 50 (2 O'Brien).

Clinical Signs and Categorization of Buruli Ulcer

Buruli ulcer is characterized in two stages: active and inactive. The active stage is further broken down into two phases: non-ulcerative and ulcerative.

In the non-ulcerative phase, Buruli ulcer can typically present itself in 4 different forms. The *papule* form which is most commonly seen in Australia is a very small, red, raised section of the skin that is painless. The *nodule* form is most commonly found in Africa and is a lesion that extends to the subcutaneous tissue. This form is also painless, but there have been accounts of the nodule being itchy. The *plaque* form is a painless lesion that is elevated and is greater than 2 cm in diameter with ill-defined edges (3). The *edematous* form is a less common but more severe non-ulcerative forms. There is a very extensive non-pitting swelling that is firm and painless and may involve all or part of the affected limb. This form may also be accompanied by fever.

The distinctive features of the ulcerative phase of Buruli ulcer include open skin ulcers with undermining edges, a white cotton wool-like appearance, and thickening and darkening of the skin surrounding the lesion. Similar to the non-ulcerative phase, these are typically presented as painless, but they are

progressive. Roughly 85% of the infections are found on the limbs with the lower limbs being twice as likely to be infected compared to the upper extremities.

The inactive form is described as a "previous infection with characteristic depressed stellate scars with or without other sequelae" (5).

The severity of Buruli ulcer is classified into 3 categories. *Category I* includes small lesions including the papule which accounts for about 32% of the diagnoses. *Category II* includes non-ulcerative and ulcerative plaque and edematous forms which accounts for 35% of the diagnoses. Finally, *category III* includes disseminated and mixed forms such as osteitis, osteomyelitis, joint involvement which account for 33% of diagnoses. Greater than 90% of the cases in Australia and Japan are identified as Category I (5).

II. GAPS IN EPIDEMIOLOGIC KNOWLEDGE SURROUNDING BURULI ULCER

WHO has categorized Buruli ulcer as a neglected tropical disease, and some aspects are not well known. In particular, Buruli ulcer transmission is not well understood, and there are no current clinical preventative measures, such as a vaccine. *M. ulcerans* DNA has been detected in "environmental samples including detritus, soil, biofilms, water filtrates, fish, frogs, snails, insects and other invertebrates" (23 Merritt). *M. ulcerans* is also very closely related to *M.*

marinum that is a disease of fish but can be an opportunistic infection in humans when injured skin is exposed to aquatic environments (24 Akram). Only one case of human-to-human transmission has been reported, but it was suspected BU was on the skin of the patient when bitten by another person, forcing the bacteria into the puncture wound (23 Merritt).

Previous studies have only made associations between the disease and certain risk factors such as water and insect bites, discussed below. One feature that is not known but is suspected relates to the aquatic associations and strong local seasonality in rainfall, i.e., does Buruli ulcer incidence exhibits seasonal patterns? Multiple analytic challenges inhibit the detection of seasonal patterns within Buruli ulcer incidence data. This simulation study explores two of these: our ability to detect seasonality against the strength of the signal and differences in health seeking behavior, in order to better understand the statistical power of detecting potential seasonal trends within Buruli ulcer surveillance data.

Risk Factors for Buruli Ulcer

Risk factors such as recent insect bites and interaction with water are among the most commonly found for Buruli ulcer. Mosquitoes are a species of interest for consideration as a potential vector of mechanical transmission. A recent experimental study demonstrated that a puncture to the epidermis by

either a needle or an inoculated mosquito, both with low doses of *M. ulcerans*, was sufficient enough for mice to develop Buruli ulcer. However, mice that had their tails dipped into a solution of the pathogen did not develop the disease (9). Buruli ulcer has been shown to be associated with human contact with either slow moving or stagnant waters (10, 11, 12). Stagnant waters are a breeding ground for mosquitoes, so if mosquitoes are in fact the vectors for BU, then it would stand to reason an increase in stagnant pools of water during the wet season would increase the prevalence of cases due to an increased mosquito population. However, stagnant waters may also encourage the development of *M. ulcerans* in the aquatic environment itself including in plant biofilms and on aquatic insects (23).

Seasonality

Detecting seasonality for a disease is beneficial, because it can help improve the accuracy of public health surveillance and our ability to predict when we can expect a sudden surge of cases of a particular disease. A straightforward example is the seasonal flu. Each year the WHO relies on surveillance data to predict the most common strain of influenza that will be circulating within the northern and southern hemispheres. This allows countries such as the United States to prepare for flu season in order to mitigate the severity and number of cases.

When it comes to determining the seasonality of diseases, the most common statistical tests typically focus on 3 different general temporal patterns: sawtooth, spiked, and sinusoidal (19). A sawtooth pattern will have a steady rise over time with a sudden fall in the number of cases. Spiked patterns will exhibit both a sudden increase and decrease in cases, while sinusoidal patterns will take on relatively smooth sine/cosine function shapes (19).

Being able to understand the seasonality of a disease has benefits when it comes to public health. If we know the mechanisms for what causes a disease to be seasonal, we might be able to prevent cases. If the cases are linked to an environmental factor, such as water and mosquitoes as in the case of Buruli ulcer, understanding seasonal associations can help predict future changes in anticipated incidence patterns based on the climate change in an endemic area, e.g., climate change may lead to an increased interaction between people and high-risk combinations of environmental risk factors.

Buruli Ulcer in Ghana

Ghana is one of several countries that routinely reports the number of new cases of Buruli ulcer each year to the WHO. In 2016, Ghana reported 371 new cases of Buruli ulcer giving Ghana an incidence density rate (IDR) of about 1.32 cases per 100,000 person-years. Like many other resource poor countries, most

of these cases come from younger people in the population (18) which makes sense given the age distribution is skewed towards a younger demographic (17).

Seasonality of Buruli Ulcer in Ghana

As previously mentioned, seasonal patterns for Buruli ulcer have not been clearly observed, and Ghana is no exception. Ghana has a tropical climate which lends itself to wet and dry seasons. Since Buruli ulcer has been correlated with water/rainfall, it is not hard to imagine that if BU does have seasonality it would follow the pattern of the wet and dry seasons. Not only would there be an seasonal variation in exposure to water (especially stagnant water), there would also be variation in breeding habitats for mosquitoes and aquatic biting insects. If mosquitoes and aquatic insects are vectors of BU, then it would make sense that there would be an increase in transmission and incidence of BU around the wet season. With this in mind, we might expect Buruli ulcer to have a sinusoidal shape to its seasonality since it would be following the rather smooth increase and decrease in rainfall within the region.

Health Seeking Habits of People Infected with Buruli Ulcer

Most of the 33 countries with confirmed cases of BU are resource poor, and lack proper healthcare throughout the country (21). In these low resource areas, delay in seeking treatment at a health facility is a very common and potentially serious problem for BU patients. The median delay for seeking healthcare at a facility in Benin was 34 days (IQR 15—90, mean 146 days) (13), and there were many reasons why patients delayed seeking treatment from proper healthcare facilities. The main external influences that delayed seeking treatment included advice from others, the perceived cost of treatment, duration of admission, and distance. The main internal influences that caused delays in seeking treatment included confidence in the hospitals themselves, self-diagnosis of the cause of the disease, perceived severity of the disease, and fear of treatment (13).

Problems with Detection

On top of the lack of knowledge that surrounds Buruli ulcer, it is a fairly rare disease which can make attempts at accurate and reliable detection difficult. Since Buruli ulcer has been shown to be linked to water, we might suspect seasonality of BU to be around the wet season in West Africa. Since the disease is so rare, the signal of any potential seasonality might be so weak that it is difficult to detect it. Another thing to consider when surveilling for this rare disease is the incubation period between acquiring the infection to the emergence of symptoms. Buruli ulcer has been shown to incubate anywhere from 1 to 9 months

with a mean incubation period of 135 days (IQR 109–160 days) (14). Adding that on top of the median delay of approximately 34 days for a patient to seek treatment can make the ability to track the seasonality of the disease troublesome since a diagnosis may occur many months after the initial infection. With such high variations in incubation periods and delays in seeking treatment, any signal might be shifted to a different time in the year, or even lost in the variability due to an even further reduction in signal. If the phase of the seasonality is shifted away from the truth, surveillance efforts will be biased and potential prevention and control efforts could be implemented at the wrong time resulting in a waste of resources. Since Buruli ulcer is a neglected tropical disease, it received limited attention until the WHO classified it as such, and even then, the challenges above make it difficult to study seasonal patterns in this disease.

In the sections below, we review statistical methods for detecting seasonality and use simulation studies to explore the impact of the potential reporting features above (potential variation in incubation period, and potential delays in health seeking behavior) on the power of traditional tests to detect seasonal signals of varying strength.

Analyzing Seasonal Health Data

Adrian G. Barnett and Annette J. Dobson (19) authored *Analysing Seasonal Health Data,* defining and evaluating statistical methods to analyze seasonal data, with public health practitioners in mind. As noted above, because the prevalence of Buruli ulcer is low, the incubation period is long, and there are typically additional delays in seeking proper treatment when symptoms do arise, it will be challenging to detect seasonality for this disease. If there is a seasonality in BU incidence, the goal of our simulation study to quantify effects on the detection of seasonality influenced by differences of incidence, incubation periods, and/or timely health seeking behaviors.

Chapter 2

Manuscript

Examining the impact of heterogeneity in timing of health seeking behavior on the power to detect seasonal effects of disease, using Buruli ulcer as the example

Lucas Trower[1], Michael Kramer, PhD[1], Lance A. Waller, PhD[2]

[1]*Department of Epidemiology*

[2]*Department of Biostatistics and Bioinformatics*

ABSTRACT

The ability to detect seasonality of diseases is an important factor in mitigating the spread of the disease, because it helps public health officials prepare for potential outbreaks. Some diseases, such as Buruli ulcer, are rare, and it might be hard to detect if there is seasonality due to the low number of cases. On top of having a low number of cases, there is a long incubation period, and people who are infected may delay seeking treatment, which can potentially lead to a lower probability of detecting true seasonality in disease transmission or

incidence. We conducted simulations to illustrate how delay in seeking treatment among infected individuals reduces our ability to accurately capture the underlying seasonality of the causative disease. We used Buruli ulcer as our disease of interest because there has yet to be confirmation of seasonality, although it is highly suspected it occurs around the rainy seasons in endemic countries. We created a simulated seasonality for Buruli ulcer with excellent probability of detection of seasonality in unperturbed data and introduced delays such as incubation period and time-to-seek treatment to quantify any resulting reduction in our ability to statistically detect seasonality. Our results indicate a delay in seeking treatment can have a measureable effect on our ability to detect seasonality for a disease such as Buruli ulcer.

INTRODUCTION

Buruli ulcer (BU) is one of the 20 neglected tropical diseases defined by the WHO (20) and is caused by infection with *Mycobacterium ulcerans* yielding a necrotizing bacterial skin infection via a toxin called mycolactone. Mycolactone affects scaffolding proteins such as actin (6 Guienen-Mace) and disrupts cells' ability to adhere to one another and causes cell death (7 Sarfo). Mycolactone also causes neuronal process degradation often resulting in painless necrotizing of the skin.

Mycobacterium ulcerans belongs to the same family of bacteria causing tuberculosis and leprosy and is the third most prevalent human mycobacteria

infection following these (1 WHO). Buruli ulcer has currently been reported in at least 33 countries with a majority of these countries being located in tropical, subtropical, and temperate climates within South America, Africa, and Pacific regions including Japan and Australia (1 WHO). A majority of the cases reported to WHO annually come from the African countries of the Democratic Republic of Congo, Benin, Côte d'Ivoire, Cameroon, and Ghana. There were 2,037 new cases of Buruli ulcer in 2015 coming from 13 of the 15 countries that regularly report BU to WHO. New cases of Buruli ulcer typically affect children under the age of 15, however, new cases of Buruli ulcer in places like Japan and Australia have a greater incidence of cases in adults older than 50 (2 O'Brien).

WHO has categorized Buruli ulcer as a neglected tropical disease, and some aspects are not well known. In particular, Buruli ulcer transmission is not well understood, and there are no current clinical preventative measures, such as a vaccine. *M. ulcerans* DNA has been detected in "environmental samples including detritus, soil, biofilms, water filtrates, fish, frogs, snails, insects and other invertebrates" (23 Merritt). *M. ulcerans* is also very closely related to *M. marinum* that is a disease of fish but can be an opportunistic infection in humans when injured skin is exposed to aquatic environments (24 Akram). Only one case of human-to-human transmission has been reported, but it was suspected BU was on the skin of the patient when bitten by another person, mechanically forcing the bacteria into the puncture wound (23 Merritt).

Previous studies have identified associations between the disease and certain risk factors such as water and insect bites, discussed below. One feature

that is not known but is suspected relates to the aquatic associations and strong local seasonality in rainfall, i.e., does Buruli ulcer incidence exhibits seasonal patterns? Multiple analytic challenges inhibit the detection of seasonal patterns within Buruli ulcer incidence data. This simulation study explores two of these: our ability to detect seasonality against the strength of the signal and differences in health seeking behavior, in order to better understand the statistical power of detecting potential seasonal trends within Buruli ulcer surveillance data.

Risk factors such as recent insect bites and interaction with water are among the most commonly found for Buruli ulcer. Mosquitoes are a species of interest for consideration as a potential vector of mechanical transmission. A recent experimental study demonstrated that a puncture to the epidermis by either a needle or an inoculated mosquito, both with low doses of *M. ulcerans*, was sufficient enough for mice to develop Buruli ulcer. However, mice that simply had their tails dipped into a solution of the pathogen did not develop the disease (9). Buruli ulcer has been shown to be associated with human contact with either slow moving or stagnant waters (10, 11, 12). Stagnant waters are a breeding ground for mosquitoes, so if mosquitoes are in fact the vectors for BU, then it would stand to reason an increase in stagnant pools of water during the wet season would increase the prevalence of cases due to an increased mosquito population. However, stagnant waters may also encourage the development of *M. ulcerans* in the aquatic environment itself including in plant biofilms and on aquatic insects (23).

Detecting seasonality for a disease is beneficial, because it can help improve the accuracy of public health surveillance and our ability to predict when we can expect a sudden surge of cases of a particular disease. A straightforward example is the seasonal flu. Each year the WHO relies on surveillance data to predict the most common strain of influenza that will be circulating within the northern and southern hemispheres. This allows countries such as the United States to prepare for flu season in order to mitigate the severity and number of cases.

When it comes to determining the seasonality of diseases, the most common statistical tests typically focus on 3 different temporal patterns: sawtooth, spiked, and sinusoidal (19). A sawtooth pattern will have a steady rise over time with a sudden fall in the number of cases. Spiked patterns will exhibit both a sudden increase and decrease in cases, while sinusoidal patterns will take on relatively smooth sine/cosine function shapes (19).

Being able to understand the seasonality of a disease has benefits when it comes to public health. If we know the mechanisms for what causes a disease to be seasonal, we might be able to prevent cases. If the cases are linked to an environmental factor, such as water and mosquitoes as in the case of Buruli ulcer, understanding seasonal associations can help predict future changes in anticipated incidence patterns based on the climate change in an endemic area, e.g., climate change may lead to an increased interaction between people and high-risk combinations of environmental risk factors.

Ghana is one of several countries that routinely reports the number of new cases of Buruli ulcer each year to the WHO. In 2016, Ghana reported 371 new cases of Buruli ulcer giving Ghana an incidence density rate (IDR) of about 1.32 cases per 100,000 person-years. Like many other resource poor countries, most of these cases come from younger people in the population (18) which makes sense given the age distribution is skewed towards a younger demographic (17).

As previously mentioned, seasonal patterns for Buruli ulcer have not been clearly observed, and Ghana is no exception. Ghana has a tropical climate which lends itself to wet and dry seasons. Since Buruli ulcer has been correlated with water/rainfall, it is not hard to imagine that if BU does have seasonality it would follow the pattern of the wet and dry seasons. Not only would there be an seasonal variation in exposure to water (especially stagnant water), there would also be variation in breeding habitats for mosquitoes and aquatic biting insects. If mosquitoes and aquatic insects are vectors of BU, then it would make sense that there would be an increase in transmission and incidence of BU around the wet season. With this in mind, we might expect Buruli ulcer to have a sinusoidal shape to its seasonality since it would be following the rather smooth increase and decrease in rainfall within the region.

Most of the 33 countries with confirmed cases of BU are resource poor, and lack proper healthcare throughout the country (21). In these low resource areas, delay in seeking treatment at a health facility is a very common and potentially serious problem for BU patients. The median delay for seeking healthcare at a facility in Benin was 34 days (IQR 15—90, mean 146 days) (13),

and there were many reasons why patients delayed seeking treatment from proper healthcare facilities. The main external influences that delayed seeking treatment included advice from others, the perceived cost of treatment, duration of admission, and distance. The main internal influences that caused delays in seeking treatment included confidence in the hospitals themselves, self-diagnosis of the cause of the disease, perceived severity of the disease, and fear of treatment (13).

On top of the lack of knowledge that surrounds Buruli ulcer, it is a fairly rare disease which can make attempts at accurate and reliable detection difficult. As noted above, since Buruli ulcer has been shown to be linked to water, we might suspect seasonality of BU to be around the wet season in West Africa. Since the disease is so rare, the signal of any potential seasonality might be so weak that it is difficult to detect. The incubation period between acquiring infection to the emergence of symptoms is another thing to consider when surveilling for this rare disease. Buruli ulcer has been shown to incubate anywhere from 1 to 9 months with a mean incubation period of 135 days (IQR 109–160 days) (14). Adding that on top of the median delay of approximately 34 days for a patient to seek treatment can make the ability to track the seasonality of the disease troublesome since a diagnosis may occur many months after the initial infection. With such high variation in incubation periods and delays in seeking treatment, any signal might be shifted to a different time in the year, or even lost in the variability due to an even further reduction in signal. If the phase of the seasonality is shifted away from the truth, surveillance efforts will be biased and

potential prevention and control efforts could be implemented at the wrong time, causing a waste of resources. Since Buruli ulcer is a neglected tropical disease, it received relatively little attention until the WHO classified it as such, and even then, the challenges above make it difficult to study this disease.

In the sections below, we review statistical methods for detecting seasonality and use simulation studies to explore the impact of the potential reporting features above (potential variation in incubation period, and potential delays in health seeking behavior) on the power of traditional tests to detect seasonal signals of varying strength.

Adrian G. Barnett and Annette J. Dobson (19) have written a book, *Analysing Seasonal Health Data,* defining and evaluating statistical methods to analyze seasonal data, and it is the first one to be done written specifically with public health practitioners in mind. Because the prevalence of Buruli ulcer is low, the incubation period is long, and there are typically additional delays in seeking proper treatment when symptoms do arise, it will be challenging to detect seasonality for this disease. If there is true seasonality in BU incidence, the goal of our simulation study is to quantify effects on the detection of seasonality influenced by differences of incidence, incubation periods, and/or timely health seeking behaviors.

METHODS

For this simulation study, using BU in Ghana as an example, we used R version 3.3.3 (25) to conduct our simulation study, and utilized the package *season* version 0.3-5.

The simulation will generate incident cases with a specified population and incident density rate. Each day within a year was assigned a probability of having a case using the following formula:

$$P_i = A\{\cos(i-1)\,2\pi/365] + 1/2\}, \qquad i = 1, \ldots, 365$$

The probability was forced to sum up to 1 by taking each calendar day's probability and dividing by the total sum of the probabilities. Cases were then assigned an infection date via a random sample utilizing the adjusted probabilities. Incubation periods were generated for each case using a random variable following a normal distribution, followed by a delay in seeking treatment period using the same method. The incubation period date represents the day signs and symptoms first appear for the case, and the seek treatment date is when the case sought treatment at a proper healthcare facility. The simulation was first completed with information found within the literature for the incubation period and delay in seeking treatment for Buruli ulcer. Subsequent simulations made adjustments to either of these two parameters. Cases that progressed into the following year were wrapped around to the beginning of the simulated year with the assumption they would represent cases identified that were infected from the previous but detected in the current study year.

As the variation increased for either the incubation period or the delay in seeking treatment, cases could seek treatment before they are infected or before signs and symptoms arose at the end of the incubation period as an artifact of the random simulation. Logically this is not realistic, so the simulation was completed under two different scenarios if a case happened to fall into either of these categories. 1) If a case sought treatment for the infection before their infection date, then they were assigned a seek treatment date equal to their infection date. Under this scenario, cases had zero delay between infection and seeking treatment. 2) If a case sought treatment before the end of their incubation period, then they were assigned a seek treatment date equal to their incubation period date indicating they went in right when the symptoms started.

The total number of simulated cases each month was recorded, and data was analyzed with the *season* R package using the Cosinor function for the test for seasonality. From this analysis, we obtain estimates of the phase (where the number of cases peak in a year), amplitude (the height of the sinusoid), and whether the seasonality was statistically significant at an alpha level of 0.05 for the time of infection and the time for seeking treatment. Each simulation was conducted 100 times and we collected the averages of the outputs.

General formula used to get the outputs:

$$Y_t = c\cos(\omega_t) + s\sin(\omega_t), \qquad t = 1, \dots, n$$

where the amplitude is

$$A = \sqrt{c^2 + s^2}, \qquad (A \geq 0)$$

and the phase (in radians) is

$$P = \begin{cases} \arctan(s/c), & c \geq 0, \\ \arctan(s/c) + \pi, & c < 0, s \geq 0, \\ \arctan(s/c) - \pi, & c < 0, s > 0. \end{cases}$$

In 2016, Ghana had a total population of 28,206,728 (15). Using data from WHO (number of reported cases of BU in 2016) and worldmeter (population of Ghana), incidence of BU was 1.315289 cases/100,000 PY in Ghana (13, 14).

Based on literature suggesting BU is associated with water and rainfall, we assumed that there is an underlying seasonality corresponding to Ghana's wet and dry seasons, making the seasonality sinusoidal. The simulation will demonstrate how variations in the incubation period or the time-to-seek treatment will reduce the ability to detect the seasonality that was generated as a baseline comparison. Standard deviations held constant in the simulations were an approximation based off of the IQRs for time-to-seek treatment (11.06 days) and incubation period (46.08).

We compared the power to detect seasonality across each of the simulations to see whether there was any loss of signal strength for seasonality.

RESULTS

Scenario 1:

Cases were set to seek treatment the day infection occurred if they were simulated to seek treatment before they were infected.

Table 1 demonstrates as the incubation period variation increases there is a reduction in the detectability of seasonality from 100 percent detection when there is no variation to 10 percent detectability at 140 days standard deviation. The phase shifts from the mid-December to between the middle of March and early June. The amplitude (associated with the strength of the seasonal signal) reduced from 30.31 to 3.21. Figure 1 shows the decline in the detectability of seasonality once the variation in the incubation period is around 80 days. Figure 7 shows an example of no detection of seasonality after delays.

Table 2 demonstrates that when there is an increase in the variation of the time-to-seek treatment there is a reduction in the detectability of seasonality from 100 percent detection when there is no variation to 8 percent detectability at 120 days standard deviation. The phase shifts from the middle of December (fig. 5) to between early May and early June (fig. 6). The amplitude reduced from 22.79 to 3.60. Figure 2 shows the decline in the detectability of seasonality once the variation in the time-to-seek treatment is around 70 days.

Scenario 2:

Cases were set to seek treatment the day symptoms appeared if they were simulated to seek treatment before the end of the incubation period as opposed to

being set to seek treatment the day the infection occurred as simulated in scenario 1.

Table 3 demonstrates as the incubation period variation increases there is a reduction in the detectability of seasonality from 100 percent detection when there is no variation to 8 percent detectability at 140 days standard deviation. The phase shifts from the middle of December to between early to late June. The amplitude reduced from 30.65 to 3.25. Figure 3 shows the decline in the detectability of seasonality once the variation in the incubation period is around 80-90 days.

Table 4 demonstrates that as there is an increase in the variation of the time-to-seek treatment there is a reduction in the detectability of seasonality from 100 percent detection when there is no variation to 82 percent detectability at 260 days standard deviation. The phase shifts from the middle of December to between the middle of May and early June. The amplitude reduced from 22.79 to 3.60. Figure 4 shows the decline in the detectability of seasonality once the variation in the time-to-seek treatment is around 120 days.

DISCUSSION

These results suggest that variations in time-to-seek treatment or incubation periods for a relatively rare disease like Buruli ulcer clearly may reduce our ability to detect a seasonal pattern, even when it truly exists. In

scenario 1 where cases were set to seek treatment the day infection occurred if they were simulated to seek treatment before they were infected, the reduction in significant tests for seasonality reduced at a similar scale between variation in incubation period and time-to-seek treatment. Whereas in scenario 2 where cases were set to seek treatment the day symptoms appeared if they were simulated to seek treatment before the end of the incubation period, variation in incubation period had a similar loss in power to detect seasonality while variation in time-to-seek treatment had to have a very wide, somewhat unrealistic, variation to even make a dent in the power of seasonality detection. In this case, by the time there is any reduction in detecting seasonality there is a substantial number of cases that are seeking treatment before the end of the incubation period and are being assigned a treatment date equal to the end of their incubation period. This in turn is forcing a lot of the data to be more focused around the end of the incubation period. If people seek treatment relatively close to presenting with the first signs of symptoms, we will have a greater ability to determine a seasonal pattern of an infectious disease if there is one.

In every scenario the phase is shifted close to 4-6 months. This could have public health implications. Resources might be misused in the wrong time of the year with a relatively unknown disease if the seasonality is detected this far from when the disease peaks in infection, which may undermine the trust of public health institutions with the public.

When the detectability of seasonality went down, so did the amplitude which makes intuitive sense. A weak signal is harder to detect.

To the best of our knowledge, there has been no other study that simulated varying delays in seeking treatment and our ability to detect a seasonal pattern, or any study that used data from an actual population to investigate this question.

This study had several strengths and limitations. It is a simulation, and all simulations are technically wrong. There are just too many variables to accurately predict to have any simulation be absolutely correct. However, a simulation does allow us to utilize what is in the literature to build a baseline for what we would expect for in data relating to a disease such as Buruli ulcer. Seasonality of Buruli ulcer is highly suspected based on its association with water, aquatic biting insects, and biofilms, but has not been shown quantitatively. We assumed Buruli ulcer incidence rates have a sinusoidal shape that coincides with the wet and dry seasons. This allowed us to simulate the data as a sine function as a baseline for comparison. We added incubation and care-seeking behavior delays and wrapped cases that went into the next calendar year after the delays in seeking treatment to the beginning of our study year. We did this under the assumption these cases would represent cases that sought treatment within our study year but were infected in the previous year. Cases that sought treatment before infection were initially removed, but at a certain point in variation there were too many removed and that simulation was no longer a good representation of the number of cases per year in Ghana.

Overall, we found that variation in time-to-seek treatment or incubation period for a relatively rare disease may statistically mask a seasonal pattern to the disease. If diagnoses are made very close to the onset of the infection date or the end of the incubation period, it may increase our ability to prevent, detect, and prepare for potential outbreaks of the disease. Future quantitative studies involving delays in seeking treatment and/or incubation periods impacting our ability to detect seasonality are needed to validate our findings.

References

1. World Health Organization. Buruli ulcer-Mycobacterium ulcerans infection. Geneva: World Health Organization 2000; WHO/CDS/CPE/GBUI/ 2000.1.118.

2. O'Brien DP, Friedman ND, Cowan R, et al. *Mycobacterium ulcerans* in the Elderly: More Severe Disease and Suboptimal Outcomes. Phillips RO, ed. *PLoS Neglected Tropical Diseases*. 2015;9(12):e0004253. doi:10.1371/journal.pntd.0004253.

3. Sakyi SA, Aboagye SY, Darko Otchere I, Yeboah-Manu D. Clinical and Laboratory Diagnosis of Buruli Ulcer Disease: A Systematic Review. *The*

Canadian Journal of Infectious Diseases & Medical Microbiology 2016; doi:10.1155/2016/5310718.

4. Buruli Ulcer. Centers for Disease Control and Prevention. https://www.cdc.gov/buruli-ulcer/health-care-workers.html. Published January 26, 2015. Accessed November 6, 2017.

5. Epidemiology. World Health Organization. http://www.who.int/buruli/epidemiology/en/. Accessed April 6, 2018.

6. Guenin-Macé L, Veyron-Churlet R, Thoulouze M-I, et al. Mycolactone activation of Wiskott-Aldrich syndrome proteins underpins Buruli ulcer formation. *The Journal of Clinical Investigation*. 2013;123(4):1501-1512. doi:10.1172/JCI66576.

7. Sarfo FS, Phillips R, Wansbrough-Jones M, Simmonds RE. Recent advances: role of mycolactone in the pathogenesis and monitoring of *Mycobacterium ulcerans* infection/Buruli ulcer disease. *Cellular Microbiology*. 2016;18(1):17-29. doi:10.1111/cmi.12547.

8. Anand U, Sinisi M, Fox M, et al. Mycolactone-mediated neurite degeneration and functional effects in cultured human and rat DRG neurons: Mechanisms underlying hypoalgesia in Buruli ulcer. *Molecular Pain*. 2016;12:1744806916654144. doi:10.1177/1744806916654144.

9. Wallace JR, Mangas KM, Porter JL, et al. *Mycobacterium ulcerans* low infectious dose and mechanical transmission support insect bites and puncturing injuries in the spread of Buruli ulcer. Azman AS, ed. *PLoS Neglected Tropical Diseases*. 2017;11(4):e0005553. doi:10.1371/journal.pntd.0005553.

10. 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; doi: 10.1016 /j.socscimed.2014.03.008

11. Garchitorena A, Roche B, Kamgang R, et al. *Mycobacterium ulcerans*Ecological Dynamics and Its Association with Freshwater Ecosystems and Aquatic Communities: Results from a 12-Month Environmental Survey in Cameroon. Picardeau M, ed. *PLoS Neglected Tropical Diseases*. 2014;8(5):e2879. doi:10.1371/journal.pntd.0002879.

12. Landier J, Constantin de Magny G, Garchitorena A, et al. Seasonal Patterns of Buruli Ulcer Incidence, Central Africa, 2002–2012. *Emerging Infectious Diseases*. 2015;21(8):1414-1417. doi:10.3201/eid2108.141336.

13. Mulder AA, Boerma RP, Barogui Y, Zinsou C, Johnson RC, et al. Healthcare seeking behaviour for Buruli ulcer in Benin: a model to capture therapy choice of patients and healthy community members. Trans R Soc Trop Med Hyg 2008; doi: 10.1016/j.trstmh.2008.05.026.

14. Trubiano JA, Lavender CJ, Fyfe JAM, Bittmann S, Johnson PDR. The Incubation Period of Buruli Ulcer (*Mycobacterium ulcerans* Infection). Vinetz JM, ed. *PLoS Neglected Tropical Diseases*. 2013;7(10):e2463. doi:10.1371/journal.pntd.0002463.

15. Rural population. Rural population | Data. https://data.worldbank.org/indicator/SP.RUR.TOTL?locations=GH. Accessed December 12, 2017.

16. Number of new reported casesData by country. GHO | By category | Number of new reported cases - Data by country. http://apps.who.int/gho/data/node.main.A1631. Accessed December 12, 2017.

17. The World Factbook: GHANA. Central Intelligence Agency. https://www.cia.gov/library/publications/the-world-factbook/geos/gh.html. Published April 2, 2018. Accessed April 6, 2018.

18. Ampah KA, Asare P, Binnah DD-G, et al. Burden and Historical Trend of Buruli Ulcer Prevalence in Selected Communities along the Offin River of Ghana. Small PLC, ed. *PLoS Neglected Tropical Diseases*. 2016;10(4):e0004603. doi:10.1371/journal.pntd.0004603.

19. Barnett, A. G. and Dobson, A. J. *Analysing Seasonal Health Data*. Springer Berlin Heidelberg 2010.

20. World Health Organization. http://www.who.int/neglected_diseases/diseases/en/. Accessed December 12, 2017.

21. World Health Organization. Second WHO report on neglected tropical diseases. Sustaining the drive to overcome the global impact of neglected tropical diseases. Geneva, Switzerland: WHO, 2013.

22. Aboagye SY, Ampah KA, Ross A, et al. Seasonal Pattern of *Mycobacterium ulcerans*, the Causative Agent of Buruli Ulcer, in the Environment in Ghana. *Microbial Ecology*. 2017;74(2):350-361. doi:10.1007/s00248-017-0946-6.

23. Merritt RW, Walker ED, Small PLC, et al. Ecology and Transmission of Buruli Ulcer Disease: A Systematic Review. Phillips RO, ed. *PLoS Neglected Tropical Diseases*. 2010;4(12):e911. doi:10.1371/journal.pntd.0000911.

24. Akram SM, Bhimji SS. Mycobacterium Marinum. [Updated 2017 Oct 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441883/

25. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013 Available from: http://www.R-project.org/.

Tables

Table 1. Scenario 1: Variation in the incubation period and how it impacts the amplitude, phase, and detectability of seasonality**.** Time-to-seek treatment is infection date if case sought treatment before infection.

Incubation Period SD	Average Number of Cases per Trial	Mean Amplitude	Mean Phase	Significant Seasonality (%)	Mean Amplitude after delays	Mean Phase after Delays	Significant Seasonality After Delays (%)
0	370.87	30.4546	12.545	100	30.3117	6.138	100
10	368.55	29.9435	12.533	100	29.2962	6.122	100
20	371.23	30.2486	12.526	100	28.246	6.129	100
30	371.6	30.4786	12.518	100	26.4916	6.102	100
46.08	373.11	30.5861	12.519	100	22.5277	6.128	100
60	372.03	30.7705	12.501	100	18.1522	6.116	100
70	373.4	30.7852	12.497	100	14.6288	6.079	100
80	370.88	30.4323	12.54	100	11.9716	6.08	98
90	369.38	30.0243	12.514	100	9.121	6.009	77
100	373.23	30.6377	12.512	100	6.9312	5.972	50
110	373.1	30.843	12.525	100	5.1101	5.755	27
120	371.88	30.5639	12.537	100	4.2735	5.448	20
130	366.95	30.1167	12.533	100	3.5627	5.192	11
140	368.9	30.1206	12.518	100	3.2074	4.416	10

Mean Incubation Period = 135 days; Mean Time-to-seek treatment (SD) = 34 (11.61) days; 100 trials per simulation.

Table 2. Scenario 1: Variation in the Time-to-seek treatment and how it impacts the amplitude, phase, and detectability of seasonality**.** Time-to-seek treatment is infection date if the case sought treatment before infection.

Time-to-Seek Treatment SD	Average Number of Cases per Trial	Mean Amplitude	Mean Phase	Significant Seasonality (%)	Mean Amplitude after delays	Mean Phase after Delays	Significant Seasonality After Delays (%)
0	373.62	30.5929	12.557	100	22.7947	6.154	100
11.06	373.11	30.5861	12.519	100	22.5277	6.128	100
20	373.35	30.6744	12.508	100	21.1346	6.089	100
30	372.53	30.5869	12.527	100	20.058	6.111	100
40	368.76	30.1296	12.53	100	17.82	6.138	99
50	367.74	30.2692	12.543	100	15.7188	6.152	100
60	372.93	30.6316	12.518	100	13.6628	6.111	100
70	366.86	29.9751	12.523	100	11.2494	6.038	95
80	367.87	30.3946	12.51	100	9.9408	5.93	83
90	372.34	30.4822	12.538	100	7.0958	6.004	57
100	368.47	30.092	12.528	100	5.4861	5.765	29
110	371.1	30.7451	12.518	100	4.0604	5.522	17
120	373.9	30.6808	12.516	100	3.6014	5.241	8

Mean Incubation Period (SD) = 135 (46.08) days; Mean Time-to-seek treatment = 34 days; 100 trials per simulation.

Table 3. Scenario 2: Variation in the incubation period and how it impacts the amplitude, phase, and detectability of seasonality**.** Time-to-seek treatment is the end of the incubation period if the case sought treatment before the end of the incubation period.

Incubation Period SD	Average Number of Cases per Trial	Mean Amplitude	Mean Phase	Significant Seasonality (%)	Mean Amplitude after delays	Mean Phase after Delays	Significant Seasonality After Delays (%)
0	371.54	30.82	12.55	100	30.65	6.14	100
10	371.32	30.71	12.55	100	30.12	6.14	100
20	367.45	30.34	12.41	100	28.54	6.11	100
30	369.21	30.05	12.53	100	26.37	6.12	100
46.08	372.04	30.55	12.53	100	22.37	6.11	100
60	370.7	30.24	12.54	100	17.57	6.13	100
70	368.93	30.55	12.53	100	14.45	6.15	100
80	371.33	30.89	12.54	100	12.20	6.20	97
90	371.8	30.47	12.52	100	9.66	6.13	87
100	370.15	30.20	12.50	100	7.47	6.16	58
110	371.6	30.58	12.50	100	5.89	6.15	39
120	372.52	30.65	12.53	100	4.57	6.24	22
130	368.14	30.30	12.42	100	3.39	6.44	9
140	372.46	30.47	12.52	100	3.25	6.66	8

Mean Incubation Period = 135 days; Mean Time-to-seek treatment (SD) = 34 (11.61) days; 100 trials per simulation.

Table 4. Scenario 2: Variation in the Time-to-seek treatment and how it impacts the amplitude, phase, and detectability of seasonality**.** Time-to-seek treatment is the end of the incubation period if the case sought treatment before the end of the incubation period.

Time-to-Seek Treatment SD	Average Number of Cases per Trial	Mean Amplitude	Mean Phase	Significant Seasonality (%)	Mean Amplitude after delays	Mean Phase after Delays	Significant Seasonality After Delays (%)
0	372.86	30.38	12.50	100	22.96	6.10	100
11.06	372.04	30.55	12.53	100	22.37	6.11	100
20	367.54	30.35	12.55	100	21.50	6.15	100
30	371.91	30.47	12.53	100	20.32	6.18	100
40	369.71	30.31	12.52	100	19.01	6.20	100
50	371.79	30.69	12.54	100	18.33	6.29	100
60	369.47	30.12	12.49	100	16.69	6.31	100
70	372.52	30.58	12.53	100	16.02	6.36	100
80	369.75	30.38	12.53	100	14.29	6.39	100
100	367.52	30.00	12.52	100	12.46	6.26	98
120	373.73	30.56	12.50	100	11.42	6.09	93
140	376.40	31.25	12.53	100	10.57	6.02	92
160	370.83	30.39	12.54	100	10.55	5.86	89
200	372.46	30.66	12.54	100	10.15	5.60	85
240	372.03	30.31	12.51	100	10.12	5.48	86
260	372.11	30.55	12.54	100	10.39	5.45	82

Mean Incubation Period (SD) = 135 (46.08) days; Mean Time-to-seek treatment = 34 days; 100 trials per simulation.

Figures

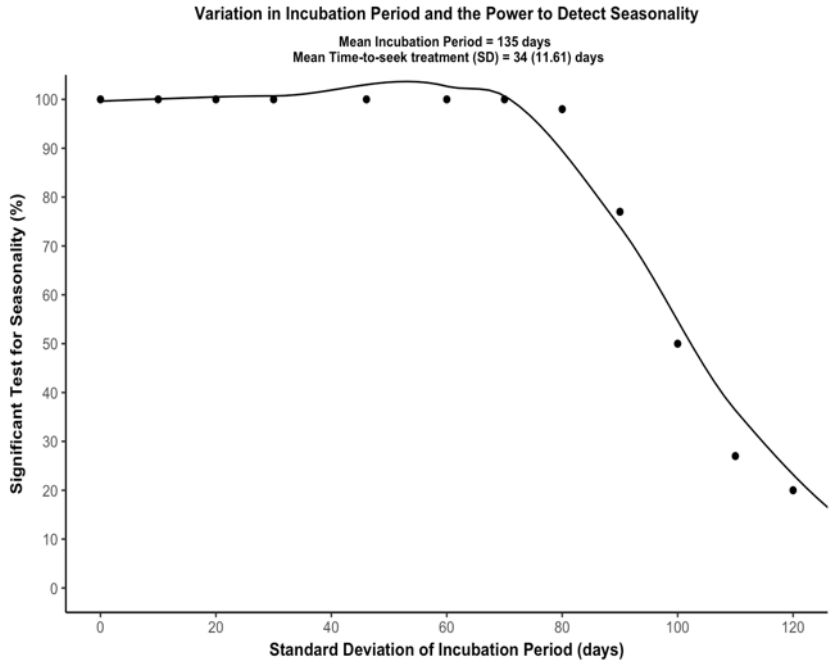

Figure 1. Variation in the incubation period from the time a case is infected to the first signs of symptoms. Cases that sought treatment before infection were set to have sought treatment on the day the infection was acquired.

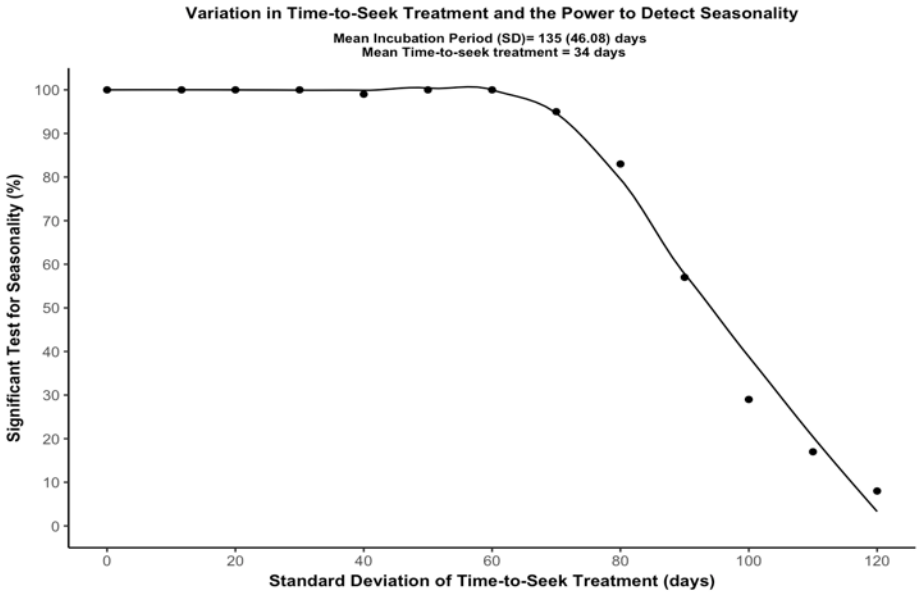

Figure 2. *Variation in the time-to-seek treatment from the time a case first shows symptoms. Cases that sought treatment before infection were set to have sought treatment on the day the infection was acquired.*

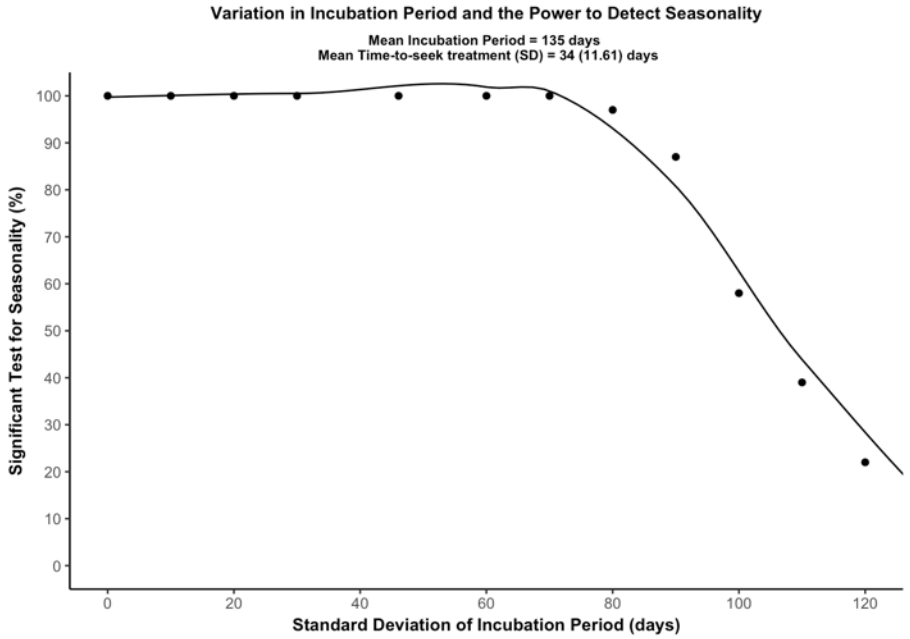

Figure 3. Variation in the incubation period from the time a case is infected to the first signs of symptoms. Cases that sought treatment before the end of the incubation period were set to have sought treatment on the day symptoms presented.

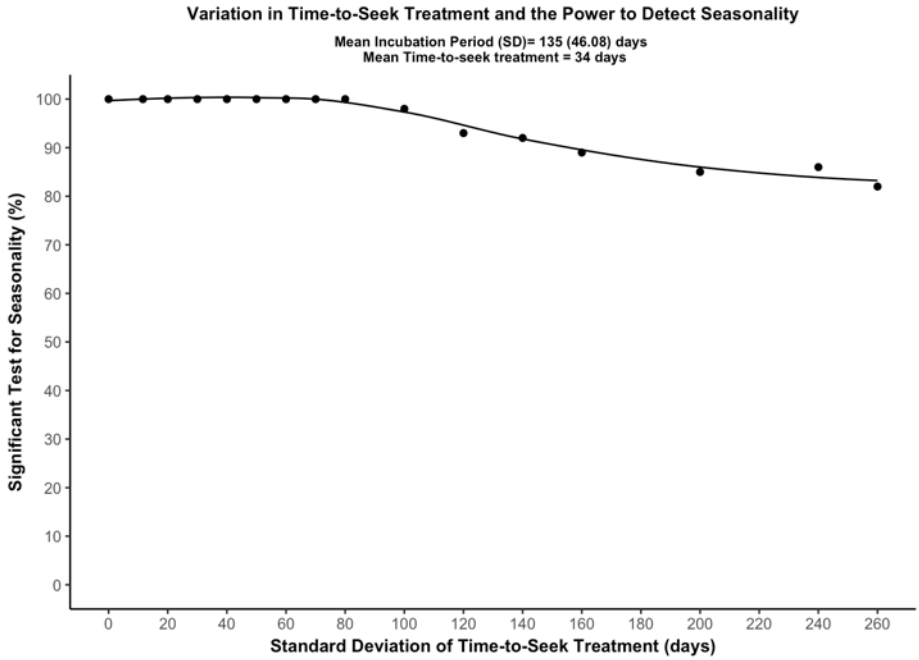

Figure 4. Variation in the time-to-seek treatment from the time a case first shows signs of symptoms. Cases that sought treatment before the end of the incubation period were set to have sought treatment on the days symptoms presented.

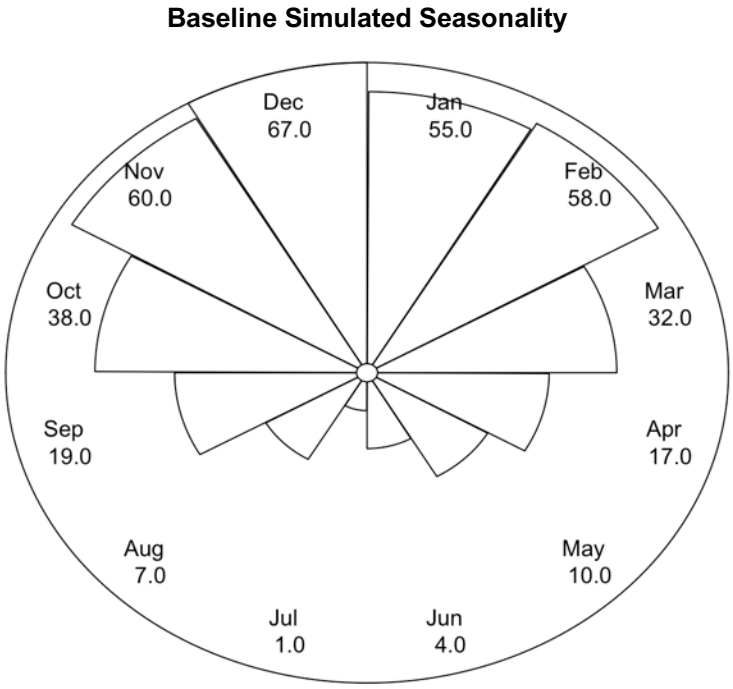

Figure 5. *Expected distribution of incident cases in a year without any delays. Total count of cases within each month are shown.*

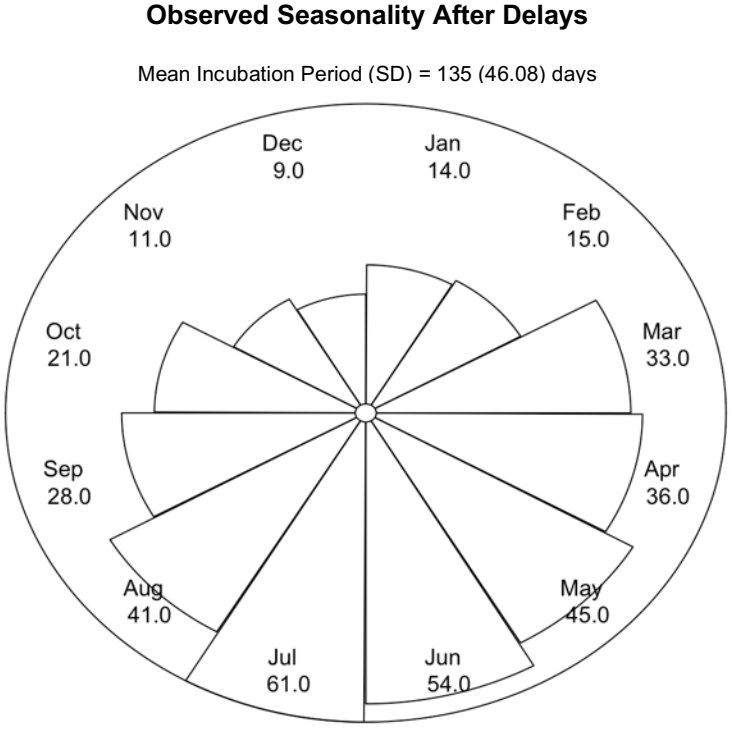

Figure 6. *Observed seasonality when incident cases are identified. Total count of cases within each month are shown.*

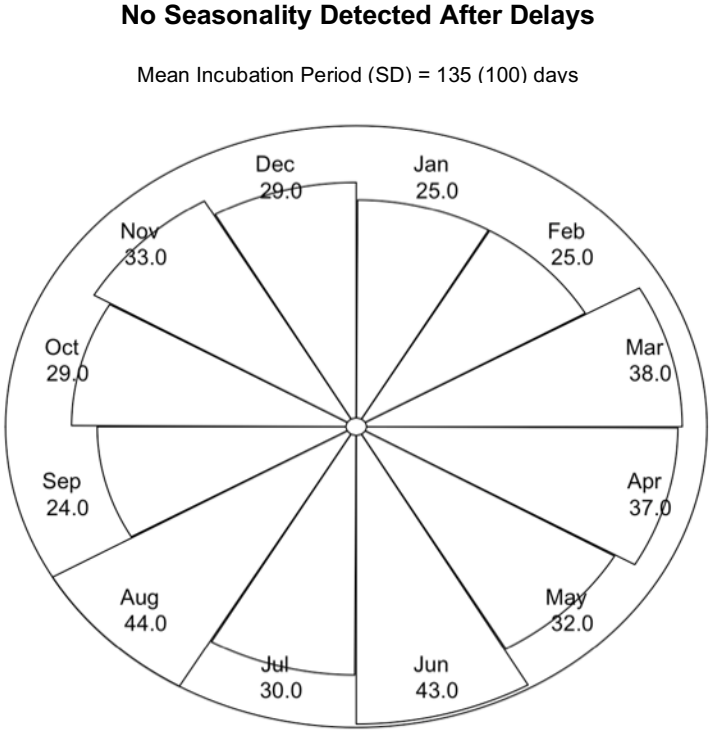

Figure 7. *No seasonality detected after delays. Total count of cases of each month are shown.*

Chapter 3

Buruli ulcer is not a well-studied disease, and many of its aspects are not well known. As one example, it is strongly suspected that Buruli ulcer has a seasonal component around the wet and dry seasons of endemic areas, however, no studies to date have found a statistically significant effect. We propose a simulation study to examine whether having variation in incubation periods or time-to-seek treatment may cause a reduction in our ability to detect seasonality (i.e., a reduction in the statistical power to detect a seasonality effect). We demonstrate that increasing variation in either incubation period or time-to-seek treatment reduces power to detect seasonality if the variation is wide enough. WE also find that shifts in temporal patterns can still yield high power but may result in detecting peak incidence in the wrong part of the year.

Our results suggest that there may be true seasonality that remains relatively undetectable, because variation in incubation or time-to-seek treatment causes such varying times that a pattern cannot be determined. Our results also suggest that a pattern can be determined, but in the wrong time of the year, which can cause public health officials to inadvertently waste resources and possibly have the public lose trust in their capabilities when the true seasonality is observed. These instances would be more crucial for diseases that are not well studied such as Buruli ulcer, or diseases that are emerging and how little to no information is known about their epidemiology.

This study had several strengths and limitations. It is a simulation, and all simulations are technically wrong. There are just too many variables to accurately predict to have any simulation be absolutely correct. However, our simulation does allow us to utilize what is in the literature to build a baseline for what we would expect for a disease such as Buruli ulcer. Seasonality of Buruli ulcer is highly suspected based on its association with water, aquatic biting insects, and biofilms, but has not been shown quantitatively. We assumed the incidence of Buruli ulcer follows a sinusoidal shape in time coinciding with the wet and dry seasons. This allowed us to simulate the data as a sine function as a baseline for comparison. We wrapped around cases that went into the next calendar year after the delays in seeking treatment to the beginning of our study year. We did this under the assumption these cases would represent cases that sought treatment within our study year but were infected in the previous year. Cases that sought treatment before infection were initially removed, but at a certain point in variation there were too many removed and not a good representation of the number of cases per year in Ghana.

Future quantitative studies involving delays in seeking treatment and/or incubation periods impacting our ability to detect seasonality are needed to validate our findings.

Also, by collecting additional data on the incubation period and/or time-to-seek treatment, we could determine where on the simulation curves we are. By having that data, we would be able to determine the expected power to detect

seasonality for that particular disease, such as BU, within a certain location. The simulation can be adapted to focus on a community, country, or global level and allow us to see if our results vary at these different scales. One would expect it would be harder to detect seasonality at the community level due to a lower number of cases and would expect an increase in power as the scale gets larger. One caution with this line of thinking is that there might be variability in the seasonality of a particular disease if it associated with local climate which varies greatly across the globe. If this is the case, it might be advantageous to analyze data by climates and not by borders.

The results from this simulation could be utilized to provide insight on current or past surveillance efforts specifically for BU. Data collected on incident cases and subsequent data analysis to detect seasonality for BU might be inaccurate for the reasons considered here. Public health officials could utilize this information to essentially reverse engineer and simulate when the infections might have actually been acquired using real data.

For each of our simulations, the power curve showed a decrease in the power to detect seasonality with increasing variation in either the incubation period or the time to seek treatment, but we did not take into account increasing variation in both parameters simultaneously which would most likely further decrease the power of detection and such studies would be of interest for future research.

Another future area of research would be to set up the simulation to allow users to plug in local values to get sample sizes required for adequate power to detect seasonality. Such an application will tell if it feasible to detect seasonality with the current data collected on the disease, or how much data would be needed for adequate detection. It could also help inform at what scale will be best suited to collect the amount of data necessary.

While the simulation was initially created using BU within Ghana as an example, it can easily be adapted for other diseases with different parameters from different countries. The simulation is flexible enough to account for these different scenarios and is useful in this sense because what is expected for one disease cannot be said to be the exactly the same for another.