Risk Factors for Dengue in Peru: 2000 - 2019

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Abstract

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Background

Worldwide, dengue fever is the most common arboviral disease in humans with an estimated 50–100 million dengue infections annually. In the last four decades, there has been a substantial increase in dengue fever cases, which is thought to be driven by human population growth and movement, urbanization, climate change, and socioeconomic factors. Public health practitioners have long been interested in the relationship between dengue fever and local weather because of the well-documented role that weather plays in vector species proliferation. This relationship is particularly salient in Peru, because more than half of the population is at risk for dengue infection and regions of the country are particularly sensitive to El Niño Southern Oscillation and its widespread impact on local weather through atmospheric teleconnections.

Methods

We aimed to explore the association between reported dengue cases in Peru and demographic and environmental factors, over the time period 2000 to 2019. Our unit of analysis was administrative districts. In an exploratory, descriptive analysis, we examined the bivariate relationship between reported confirmed dengue cases and demographic and environmental variables. Based on these findings and

biologic rationale, we used a multivariate analysis to examine the association between dengue cases and temperature, using the monthly number of confirmed dengue cases, basic demographic variables associated with districts and monthly average temperature and monthly average accumulated precipitation. In a secondary analysis, we examined the association between reported dengue fever cases and El Niño events accounting for district population, natural region designation, and number of healthcare facilities for the time period 2000 to 2019.

Results

We found that both temperature and precipitation were positively associated with higher dengue incidence. However, the effect of precipitation was not as great as the effect for temperature. Natural region classification was also found to play a role, but this could be a result of differences in temperature and precipitation. We also found that El Niño time periods, as defined by the Oceanic Niño Index were not significantly associated with increased dengue incidence across Peru. However, when the El Niño Coastal Index was used, there was an overall positive significant association between El Niño time periods and dengue incidence.

Conclusion

Findings from this study complement other studies on dengue and weather and increase our understanding of how changing environmental and human factors impact dengue fever cases in Peru. Additionally, our findings could potentially provide information useful to Peruvian health authorities for the prevention and control of dengue fever or future modeling studies.

Background

Overview of Dengue Globally

Dengue is a mosquito-borne viral disease that has significant socioeconomic and health impacts in the tropics and subtropics (1). More than one-third of the world's population lives in areas that place them at risk for dengue infection (2). The disease is caused by the dengue virus, an RNA virus in the *Flaviviridae* family, which is transmitted to humans via the bite of an infected mosquito. Four closely related serotypes (DENV 1, 2, 3, and 4) are currently circulating (3). In the Western Hemisphere, the mosquito *Aedes aegypti (Ae. aegypti)* is the predominant vector species of the dengue viruses (4). As opposed to some other arboviruses, including malaria, dengue is often concentrated in urban areas due to the characteristics of *Ae. aegypti*, a domesticated and urban vector (5,6).

Dengue Disease

While the full global burden of dengue remains unknown, there has been a rapid increase in cases worldwide with a greater than 30-times increase in recent decades, thought to be due to factors including population growth and increased density, urbanization, and human migration (3,5). The global burden of dengue is estimated to be around 390 million infections per year, with 96 million clinically apparent cases (7). However, these estimates are believed to be an underestimate of the true burden of dengue due to cases with mild clinical symptoms, poor surveillance, and misdiagnosis of infection (5). Only around 20 percent of people infected with dengue have clinical symptoms and those with mild symptoms may not seek medical care. Additionally, the general symptomatology of dengue may lead to medical professionals misdiagnosing the disease (8). Though cases are often asymptomatic or mild, five percent of those infected will develop a severe manifestation (dengue hemorrhagic fever) that can be life-threatening (9).

Clinical Disease

People infected with a single serotype of dengue gain a long-lived immunity to that serotype and short-lived cross-immunity to other serotypes (10). Dengue can lead to high fever, chills, headaches, rash and extreme muscle, joint, and/or bone pain. It can also manifest as dengue hemorrhagic fever, which generally occurs in people who have had prior infection with dengue virus and in children under the age of ten (11). Dengue infection and dengue hemorrhagic fever are treated through supportive therapy, though dengue hemorrhagic fever often requires hospitalization (12). Globally, dengue causes 500,000 hospitalizations annually and dengue hemorrhagic fever has an estimated case fatality of two-and-a-half percent, but when untreated the fatality rate can be greater than 20 percent (12–14).

Aedes aegypti and Dengue

In addition to human demographic changes, the worldwide spread of *Ae. aegypti* has contributed to the recent increase in dengue incidence. *Ae. aegypti* is thought to have originated in Africa and spread in the seventeenth and eighteenth centuries to countries with year-round tropical climates (4). Currently, the vector is found throughout tropical and subtropical regions (generally between the latitudes 35° north and 35° south), including Africa, the Middle East, Southeast Asia, the Pacific and Indian Islands, Northern Australia, and the Americas (15–17). Though *Ae. aegypti* can be found throughout these regions, the distribution of the vector is also constrained by elevation; the vector is uncommon above 1000 meters due to the effect of low temperatures on the mosquito life cycle (17). Researchers believe that the range of *Ae*. *aegypti* could continue to expand in upcoming decades due to global climate temperature increase,

global travel, and human activities that create additional habitat and larger vector reservoirs (5,15,18). In a 2019 study, Messina et al. predicted that population growth in endemic dengue regions coupled with some virus range expansion will lead an additional 2.25 billion people will be at risk for dengue in 2080 compared to 2015, bringing the total population at risk to 60 percent of the world's population (18).

Ae. aegypti are closely associated with humans, human dwellings, and urban areas (8,19). Adult female *Ae. aegypti* are highly anthropophilic daytime biters and have been found to spend their adult lifetime in close proximity to the location where they emerged from the larval stage (17,20). The adult females lay their eggs in natural pools and artificial water-holding containers near human dwellings to hatch into larvae and transform into adults, a development process can take a little over a week when environmental conditions are favorable (8,20). Newly hatched female mosquitoes become infected with the dengue virus when they ingest infected human blood (17). On average, within eight to twelve days, depending on temperature and environmental conditions, the virus spreads systemically from the vector's midgut and can be transmitted to new human hosts through future bloodmeals. Once a mosquito is infected, it remains infective throughout its entire lifecycle and can spread the virus to the multiple people it will feed on before producing eggs (17).

Dengue in Peru

Dengue is endemic in some regions of Peru and according to the World Health Organization's "Global Strategy For Dengue Prevention and Control, 2012-2012," is among the 30 most highly endemic countries for dengue cases, as reported to WHO, 2004 to 2010 (21). The Peru Ministerio de Salud considers dengue to be an important re-emerging disease and it is estimated that the accumulated national incidence of the disease is 239.1 cases per 100,000 inhabitants (22,23). Currently, more than half of the population of Peru lives in areas that places them at risk for dengue infection, and 17 of the 25 departments, including 256 districts, are believed to be inhabited by *Ae. aegypti* mosquitoes (24,25).

The first suspected dengue outbreaks in the Americas were in 1635, and the first dengue-like disease was reported in Lima, Peru in 1851 (26). In the 1940s, the Pan American Sanitary Bureau worked to eradicate *Ae. aegypti* in South America in the fight against yellow fever, and by the mid-20th century many countries in the Americas, including Peru, successfully reduced the *Ae. aegypti* population (26,27). However, after the eradication efforts declined in the 1960s due to many factors, including a lack of political will and political importance, inadequate community and health sector support, insufficient funding for equipment and staff, surveillance system decline and a slow-moving response to mosquito reinfestation. In addition, vector resistance to the organochlorine insecticides used, including dichlorodiphenyltrichloroethane (DDT), contributed to the decline of the eradication effort (26,28). The *Ae. aegypti* population quickly rebounded and spread passively, bolstered by increased urbanization, overcrowding, poor sanitation, the growth in international and domestic travel (26,28). The rebound of *Ae. aegypti* in the Americas, led to an increase in reported dengue cases in the 1980s and an unmatched rise in the during the 2000 to 2010 time period, with large Pan American outbreaks in 2002 and 2010 (26). In the last three decades, there has been a 4.6-fold increase in reported dengue cases in the Americas (26).

In Peru, *Ae. aegypti* reintroduction in 1984 led to the subsequent reestablishment of dengue serotype DENV-1 in 1990 (26,27,29). The country had a major outbreak of dengue in 2001 with 23,329 reported cases and it is believed that there are recurring dengue outbreaks every three to five years, with increasing cases over time (26). During the 2001 outbreak, serotypes DENV-2, DENV-3, and DENV-4 were all found circulating in Peru and the first hemorrhagic dengue was recorded (26,29). Peru began monitoring dengue cases in 1990. The Ministerio de Salud's Dirección General de Epidemiología is currently in charge of Peru's national passive dengue surveillance system. This system is used to track the distribution of dengue cases, detect outbreaks, identify circulating serotypes, and monitor vector species' distribution and behavior (23). Dengue case data are collected from a network of over 7,000 geographic reporting units, encompassing 95% of Peru's health centers (30). Probable cases of classic dengue are generally collected and reported weekly, but reporting will increase to daily during an outbreak. Reporting units must immediately report probable cases of hemorrhagic dengue (23).

Dengue and Its Prevention and Control in Peru

In response to the rise in dengue incidence in the Americas, the Pan American Health Organization (PAHO) developed the Integrated Management Strategy for Dengue Prevention and Control in 2003. As of 2010, 19 countries and territories, including Peru, have enacted this strategy which is based on six components: epidemiology, entomology, healthcare, laboratory, social communication and environment (26). Efforts to control the spread and impact of dengue in Peru have focused on the use of vaccines and vector control. In December 2015, the first dengue vaccine, Dengvaxia®, was licensed and approved for use in countries with endemic dengue, including Peru. By November 2016, the vaccine was available to people between the ages of 9 and 45 in private clinics in Peru (31). Though the vaccine benefits the overall population at risk, there have been some concerns about its efficacy in some individuals (32). Most notably, in November 2017, Sanofi Pasteur, the producers of Dengvaxia®, warned that the vaccine could increase the risk of severe dengue in certain circumstances, particularly in individuals who have not had dengue infection previously (33). Based on these findings, the WHO concluded that pre-vaccination screening (e.g., serological testing for previous infection) would be the preferred strategy. However, the WHO also acknowledges that this strategy must be evaluated as the country level for factors including affordability, dengue hospitalization rates, and test specificity and sensitivity (34). Additionally, because there is no rapid test that has been validated for screening for past dengue infection, determining an individual's serostatus can be difficult (33,35). Due to the limitations of the dengue vaccine, vector control activities remain important in areas with endemic dengue.

In Peru, the Ministero de Salud's Dirección General de Salud Ambiental is responsible for developing environmental health standards, supervising and evaluating compliance, and providing technical assistance, while regional and local governments and organizations are responsible for coordinating with the national office to conduct surveillance and control vectors and pest species of public health significance (25). The Dirección General de Salud Ambiental has classified locations by scenarios for dengue risks, including localities without the presence of the vector or cases of dengue, locations with the presence of the vector but without the presence of dengue cases, and locations with the presence of the vector and dengue and severe dengue cases. These classifications are then used to guide surveillance and intervention activities, which range from quarterly entomological surveillance at ten percent (more than

500 dwellings) to monthly entomological surveys and biological or chemical mosquito larva control activities (25). Spatial application or nebulization of insecticides are used to control the adult mosquito population when specific conditions are met (25). Generally, this strategy is employed in well-defined areas to both optimize resources and prevent or control epidemic outbreaks (25,29). In addition to mosquito control actions, community education and house inspections anchor the dengue prevention strategy. For example, in Iquitos, a city where dengue is endemic, health promotion messaging, centering symptoms recognition and breeding site eradication, is being used for dengue control (24).

Weather and Dengue

All insects are sensitive to environmental conditions, including factors like temperature, humidity, and rainfall. The relationship between environmental conditions and vector species populations, including *Ae. aegypti* mosquitoes, has been well studied due to their role in disease transmission. Like all insects, mosquitoes are poikilotherms and are greatly affected by thermal stress (36). Temperature has a significant impact on both the survival of *Ae. aegypti* mosquitoes and their ability to act as an efficient vector for pathogens. *Ae. aegypti* mosquitoes can survive at temperatures above 10 °C and below 40 °C, but female flight performance is optimized at 21 °C (36,37). In addition to the relationship between temperature and survival, temperature also has impacts on *Ae. aegypti* mosquitoes' feeding behavior and reproduction. Female *Ae. aegypti* will feed between 15 °C and 36 °C, with more frequent feeding in warmer environments compared to cooler environments (36). Generally at increasing temperatures, the length of the female's gonotrophic cycle is reduced and the developmental time between hatching and adult emergence is shorter, with optimal temperatures of 26 °C to 30 °C for the shortest gonotrophic cycle and 32 °C for fastest larval development (36). Finally, temperature can have a direct impact on the infection rate of dengue, with a shorter extrinsic incubation period in mosquitoes incubated at higher temperatures (38).

The impact of humidity on *Ae. aegypti* is less well studied than temperature and rainfall. Studies have shown that humidity appears to interact with temperature and can have an impact on female fecundity. A laboratory study found that as temperature increased from 25 °C to 35 °C, there was a reduction in oviposition rate, but that the intensity of the decrease was tempered by higher relative humidity (80% vs 60%) (39). A 2012 study of the effects of humidity on feeding behavior have found that when hosts are readily available there is not a change in the rate in host biting, which contradicted previous studies (40).

Rainfall can also have a large impact on *Ae. aegypti* populations. As rainfall increases, it can generate new water filled containers for breeding, including natural and artificial containers and human-made garbage (41). However, the timing of the rainfall is also important because female *Ae. aegypti* lay their eggs above the water level and the eggs hatch only when they become wet (42). Research has shown that this allows the species to be competitive during periods of drought, due to their desiccation resistant eggs, but places them at risk for a winter rainfall to cause the eggs to hatch too early and the larvae to die from cold exposure (42). Additionally, copious rainfall could potentially sweep eggs or larvae out of the container they are developing in, though a study conducted by Koenraadt and Harrington found that *Ae. aegypti* are resistant to such flushing (43). While weather has been found to impact *Ae. aegypti* reproduction,

behavior, and survival these effects may vary by geographical location, partially because of interactions between social factors, climate, and the vector species (44).

El Niño Southern Oscillation (ENSO) and Dengue

The El Niño Southern Oscillation (ENSO) is considered to be one of Earth's most significant weather-producing phenomena, in which fluctuating ocean temperatures, trade wind strength, and atmospheric pressure lead to far reaching climate condition changes (45–47). El Niño and La Niña events generally occur every two to seven years in an irregular cycle with neutral periods interspaced (47,48). El Niño warming events occur when atmospheric pressure is altered, rising in the western Pacific Ocean and falling along the eastern region. This shift causes the trade winds to weaken, which in turn allows for warm water to migrate eastward and the upwelling of cool water near the coast of Peru to become reduced. As El Niño progresses, the warming sea surface temperature (SST) and trade winds reinforce each other building the event, eventually leading to widespread impacts on local weather through teleconnections (48). The El Niño event usually ends when the boreal winter starts and lessens the feedback loop between the warming SST and trade winds (49). La Niña occurs when the trade winds grow stronger increasing upwelling and causing a cooling of SST in the eastern Pacific Ocean (47).

Researchers use several different indices, each utilizing a different area, time scale, or atmospheric and oceanic indicators, to monitor the ENSO (50,51). the National Oceanic and Atmospheric Administration (NOAA) officially uses two of these indices to classify ENSO events, the Niño 3.4 index and the Oceanic Niño Index (ONI) (51). The Niño 3.4 and ONI indices are both oceanic indices and rely on sea surface temperature anomalies in the 3.4 region, between 5° N and 5° S and 170° W to 120° W, to track and classify El Niño (51). While both indices use the same region, they classify El Niño events using different criteria. The Niño 3.4 index uses a 5-month running mean of Niño 3.4 SST anomalies and classifies El Niño and La Niña events when SST anomaly exceeds either a positive or negative 0.4 °C for six months or longer. Conversely, the ONI index uses a 3-month running mean and declares the onset of an El Niño and La Niña events when the 3.4 region SST anomaly exceeds either a positive or negative 0.5 °C for a three month period (51).

Peru is in a unique position and uses two indices to measure both remote and local ENSO impacts. Remote impacts, which can cause weather change through atmospheric teleconnections, can be monitored using Niño 3.4 or ONI indices, while local impacts, which can lead to coastal rain and marine ecosystem disturbances, are currently monitored using a different index developed by Peru's Multisectorial Committee for the Study of the El Niño Phenomenon (ENFEN) (52). In 2011, the ENFEN established an index, called the El Niño Coastal Index (ICEN), to track local El Niño and La Niña events in Peru. This index is calculated using a three-month moving average of SST anomalies in the Niño 1 + 2 region, between 0 °S-10 °S, 90 °W-80 °W, with respect to the period 1981-2010. The ICEN index classifies El Niño events when the SST anomaly exceeds 0.4 °C and La Niña events when the SST anomaly is less than -1.0 °C (52,53).

El Niño events can have worldwide impacts through teleconnections, altering regional rainfall, temperature, and sunlight availability (48). However, these impacts vary between El Niño events based on factors including El Niño event intensity and form (Eastern Pacific and Central Pacific) (48,54). Some El

Niño events strongly impact South America's coasts and Peru can be particularly sensitive because of its long coastline and position on the Pacific Ocean (55). The coast of Peru is generally dry, but during strong El Niño events there can be heavy rainfall in coastal region north of Lima (55). However, the local impact of El Niño events varies across the country's different regions. A study of the impact of El Niño and La Niña found that strong El Niño events lead to heavy rainfall north of the Pacific hydrographic drainage, but drought in the Lake Titicaca and Amazon drainages (56). It has also been found that La Niña events may also bring rainfall the Peruvian central Andes (57). In addition to rainfall, El Niño events can cause increased temperatures and heat waves in some regions, particularly along the coast (58). In theory, the changes in temperature and precipitation changes that appear with El Niño can have a large impact on dengue case counts through the changes to the vector population.

Studies of Weather and Dengue Risk in Latin America

Due to the of the relationships between local weather and mosquitoes, public health professionals have long been interested in how weather affects dengue risk. In addition, there is a lot of interest in the relationship between El Niño and vectorborne diseases, as it has become more feasible to reliably forecast El Niño and La Niña events. In the future, these forecasts could serve as an early warning system for diseases like dengue (59). Researches have taken many different approaches to examine the relationship between climate variables and dengue, including multiple linear regression models and wavelet analyses.

While there have been many studies examining the relationship between climate variables, including local weather and ENSO, and dengue in Latin America, the results have not been consistent. Research in Mexico, Puerto Rico, Thailand, Ecuador, and Venezuela consistently found that local weather variables (rainfall, temperature, humidity) were significantly associated with dengue, but that the association between ENSO and dengue differed by study (44,60–64). Studies in Mexico, southern coastal Ecuador, and Venezuela found significant positive associations between El Niño events and dengue (60,62,63). Additionally, a study of the association between SST and dengue in Yucatan, Mexico found that SST can explain up to 26% of dengue fever variability (65). Conversely, studies in Costa Rica and Honduras found a positive association between La Niña and dengue (66,67). Fuller et al. believes that the association in Costa Rica may be driven by increases in humidity during La Niña events (66).

In contrast to the studies that have found a significant relationship between the ENSO and dengue cases, other studies in Latin America, including studies in Puerto Rico and Mexico, have only found weak associations between the two variables (61,64). The inconsistent relationship between ENSO and dengue was explored in a paper that examined 4,863,598 cases of dengue fever in the Americas (Mexico and countries in Central America, Northern Caribbean islands, and South America) between 1995 and 2004. Ferreira found that four countries, Cuba, Belize, Guyana, and Costa Rica, had higher dengue incidence rates with higher El Niño activity, but that the relationship varied by country and year (68). Overall, the relationship between ENSO and dengue is not clear and consistent across Latin America, which in part may be due to the fact that all of these studies used different modeling methodology,
time scales, and spatial scales.

Studies of Dengue Risk in Peru

There have been two published studies focusing on the relationship between weather and dengue in Peru. Both of these studies found that weather was associated with dengue, but neither study included data on vector populations (30,69). The first of these studies examined the association between demographic and climate factors and dengue epidemics, using weekly dengue surveillance data, at the province and region level between 1994 and 2008 (30). Chowell et al. used a wavelet time series analysis to study the average timing of dengue epidemics in three natural regions (jungle, coastal, and mountain) and the timing of the epidemic peak at the province level. In addition, they examined the association between mean temperature and dengue cases in geographic regions. The study found that the majority of dengue cases were reported in the jungle and coastal regions and that dengue was most persistent in the jungle region, reporting more weeks with dengue cases. The study also found that jungle and mountain regions have an epidemic peak of dengue about six weeks prior to the peak in the coastal region, peaking in March in the jungle regions and April-May in the coastal regions. The results of the wavelet coherence analysis for mean temperature and precipitation between the jungle and coastal region show that there was significant coherence for the seasonal component for mean temperature, but that the pattern for precipitation was not clear. This study indicates that dengue epidemic timing is not the same in the different natural regions and that these differences may stem from the relationship between seasonal temperature cycles and mosquito vector density. In addition, the study suggests that dengue moves from the jungle region or bordering countries to the mountains and coastal region (30).

The second study examined the association between local weather variables, including temperature and relative humidity, and local potential for dengue transmission, local potential for epidemic magnitude, and seasonal timing of local dengue epidemics using weekly dengue surveillance data at the district level during the period 1994 to 2012 (69). Campbell et al. used binary classification-trees to examine the relationship between local temperature and humidity and dengue location, timing, and intensity. In addition, they mapped weekly district-level transmission potential, using a 2-dimensional weather-space pairing of temperature versus humidity. The study found that a combined weather criteria of mean temperature >22 °C for 7 or more weeks per year and a minimum temperature >14 °C for 33 weeks or more per year was able to correctly classify districts as dengue-positive or dengue-negative with 95 percent sensitivity and specificity, indicating that these conditions need to be met for dengue transmission to occur. Annual temperature range at the district-level was found to predict cumulative incidence magnitude, with the annual duration of time spent above 22 °C being the best predictor of magnitude for lower incidence districts and the annual duration of time spent above 25 °C being the best predictor higher incidence districts. Humidity worked in conjunction with temperature, producing the largest epidemics when humidity was greater than 80 percent for more than 6 weeks per year or 75% for more than 19 weeks per year and the temperature was above 25 °C. The study also found that local temperature-humidity weather-space was important factor in the magnitude of dengue epidemics, revealing that the longer amount of time that an area spends at an optimal temperature-precipitation, the higher the probability for dengue transmission. Elevated-dengue risk areas in Peru were found to have a greater amount of time in these ideal conditions for transmission. Overall, the researchers found that local weather variables were key in determining the location, timing, and magnitude of dengue transmission-potential (69).

While these two studies have well characterized the relationship between weather and dengue risk, we felt that an updated study of the surveillance data worthwhile and could help assess whether new patterns of transmission are developing due to the spread of virus. In addition, this study builds on the previous studies in two important ways. First, our study used a robust and high-quality weather dataset from the National Aeronautics and Space Administration's (NASA) Global Land Data Assimilation System (GLDAS) instead of relying on meteorological stations located throughout the country. Second, our study adds an analysis of the association between El Niño and reported dengue. Given the potential value of being able to predict increases in dengue incidence from changes in local weather and El Niño events for dengue prevention and control efforts, the inconsistent results of the relationship between ENSO and dengue in previous studies, and the availability of 19 years of dengue surveillance data, we undertook an analysis to explore whether there is a significant association between temperature and dengue incidence and El Niño events and dengue incidence in Peru from 2000 to 2019.

Study Aims

The purpose of this study is to explore associations between environmental and demographic variables and confirmed dengue cases reported to the national passive surveillance system in Peru from 2000 to 2019.

Primary Aim: To determine which environmental and demographic risk factors were significantly associated with risk of reported dengue cases at the district level in Peru between 2000 and 2019.

Secondary Aim: To assess the levels of association between temperature, precipitation, El Niño, and dengue cases in Peru districts between 2000 and 2019.

Methods

Study Area

Peru is a country located in the western region of South America, bordered by the Pacific Ocean and five other countries: Ecuador, Columbia, Brazil, Bolivia, and Chile (70). The country has a total surface area of 1,285,216 km^2 and is comprised of 24 departments, one constitutional province, 196 provinces, and 1,874 administrative districts (71). An estimated 31,331,228 people live in Peru, with the majority of population, 58.0 percent, residing in the coastal region (i.e. costa region), 28.1 percent of the population living in the Andean highlands (i.e. sierra region), and a much smaller population, 13.9 percent, inhabiting the jungle (i.e. selva alta and selva baja regions) (72).

Mainly due to the presence of the Andes mountain range running north to south across the country, Peru has a wide variety of natural regions. Generally, these regions can be divided into three classifications called costa, sierra, and selva (71). The costa region is a narrow strip of land that runs between the Pacific Ocean and the western Andes and is comprised of dunes, marine terraces, small hills, and alluvial fans. This region makes up about 11 percent of the total surface of Peru (71). The Andes mountain range

encompasses the sierra region giving this region a heterogeneous landscape filled with mountain peaks, gorges, valleys, and plateaus. This region covers about 26 percent of the land area of Peru (71). The selva region covers the majority, 63 percent, of Peru's surface and is further divided into high jungle (selva alta) and low jungle (selva baja). The high jungle is located in the eastern foothills of the Andes mountains at elevations higher than 400 m above sea level, while the low jungle is part of the Amazon River basin and characterized by lush tropical flora and flooding (71).

While Peru is located in the tropics, the climate is tempered by the county's elevation, which ranges from 0 to 6,746 m above sea level (73). There are generally two seasons in Peru, but the weather in each season varies depending on the natural region. In the central costa region, there is a cloudy and cool winter season from April to November and a hot and dry season summer from December to March, while in the northern costa region, summer is hot but with rainfall (30). In the sierra region, it is dry from May to October with a rainy season from November to April. And in the selva region, it is dry and hot from May to October and rainy and hot from November to April (30).

Unit of Analysis

Due to the availability of disease surveillance, demographic, and environmental data by administrative district and the possible variability in value by geographic area, we elected to use administrative districts as the unit of analysis.

Variables

The outcome variable for this study is monthly reported dengue case counts at the district level for the time period 2000-2019. For predictor variables, we selected both environmental and demographic variables for this study. The environmental variables include monthly average temperature at the district level, monthly average cumulative precipitation at the district level, natural region classification of districts, and El Niño time periods. The demographic variables include annual population at the district level and number of healthcare facilities in each district. We selected local temperature and cumulative precipitation as variables because they have been found to be important predictor variables in other studies examining dengue incidence. El Niño was selected as a variable because it is a primary variable of interest for this study. Natural region classification of districts was added because of Peru's diverse geographic biomes. Each natural region has its own features and climate that may temper or exacerbate the impact of ENSO. Additionally, mosquito populations and transmission dynamics may vary across natural regions due to factors like culture, elevation, breeding habitat, etc. The number of healthcare facilities in a district was added because access to healthcare may have an impact on dengue transmission.

Epidemiological Data

Individually reported dengue cases, classified by diagnosis (dengue without alarm signs, dengue with alarm signs, and serious dengue) and type (probable, confirmed, and discarded dengue cases) are recorded in Peru's national surveillance system by district along with variables on location (district), age, sex, date of initial symptoms, date of diagnosis, and date of notification. We extracted a dataset covering all of Peru for time period January 2000 to September 2018 from the Peru Ministro de Salud (Ministry of Health). The diagnosis of a probable classic dengue case is purely clinical and includes a case with a history of recent fever (2 to 7 days) in addition to two or more symptoms, including headache, retro-orbital pain, myalgia, joint pain, rash, or hemorrhagic manifestation (23). A confirmed case definition includes a

probable classic case with any of the following tests or links: 1) isolation of dengue virus in the patient's serum, 2) a four times change in the titer of IgG or IgM antibodies in paired serum samples, 3) detection of virus genetic material through serum or other sample polymerase chain reaction (PCR), 4) detection of virus antigen via immunohistochemistry, immunofluorescence, or an enzyme-linked immunosorbent assay (ELISA) test, or 5) an epidemiological link, where the patient occupied a location with laboratory confirmed transmission in the previous 15 days and the vector species is present (23). Discarded cases are cases that were ruled as not dengue through laboratory tests or a lack of an epidemiological link. While these case definitions have changed in the study period, all of the data used has been updated to the current WHO definition (personal communication).

We utilized all dengue diagnosis types for all virus serotypes in this study. For the primary analysis, we consolidated confirmed cases into monthly counts based on the date of first symptoms. The decision to utilize monthly counts was made because the weather data was collected monthly. We also created a dataset of combined probable and confirmed dengue cases for a sensitivity analysis. The districts reporting dengue cases to the national surveillance system varied throughout the study period with districts not reporting when there were no cases seen (personal communication). Based on this understanding of the data, we substituted zeros for data that was missing from the dataset.

Population Data

Annual population estimates and census counts of each district were collected from the Peru Instituto Nacional de Estadistica e Informatica (National Institute of Statistics and Informatics) website. During the study period, Peru conducted four censuses (2005, 2007, 2012, 2017) and estimated population in between census events. Annual 2017 population estimates were also used for 2018 population estimates because 2018 estimates had not been posted online by the time the data was collected.

Natural Region Data, Healthcare Facility Data

Each district has been classified into a natural region (costa, sierra, selva alta, and selva baja) by the Peruvian government. This data was obtained from a 2015 document extracted from the University of San Martin de Porres (USMP). We were given data on the number of 2015 healthcare facilities in each district by the Ministry of Health in Peru (MINSA). For the purposes of this study we calculated the number of healthcare facilities per 1,000 persons, but did not differentiate between different types of healthcare facilities.

Local Weather Data

Monthly temperature and precipitation data at a 0.25-degree spatial resolution were obtained for the time period 2000 to 2019 from the National Aeronautics and Space Administration (NASA) Global Land Data Assimilation System (GLDAS). The GLDAS was developed by scientists at NASA and NOAA to create a global repository of high-quality, high-resolution, and near real-time land surface data (74,75). GLDAS uses ground and satellite observations and land surface models to produce field of land surface states, including precipitation and average surface temperature (74,75). Monthly GDLAS data at a 0.25-degree resolution was downloaded from the NASA EARTHDATA website for the area covering all of Peru (76). The GDLAS data was layered with a shapefile of Peru containing the district administrative boundaries in QGIS

version 3.6. The QGIS zonal statistics tool was used to determine the average monthly surface temperature and precipitation from the GDLAS pixels within the boundaries of the district. Twenty-five districts were smaller than the pixels and were not assigned an average temperature or precipitation value during the first run of zonal statistics. These districts were given a 0.125-degree buffer and the zonal statistics tool was used to determine average monthly surface temperature and precipitation for each district. For the analysis, temperature was converted from the Kelvin temperature scale to the Celsius temperature scale. Precipitation was converted from a rate (kg m^{-2} s^{-1}) to accumulated monthly precipitation using an average month length of 30.33 days.

El Niño and Sea Surface Temperature Data

Monthly ONI values and monthly sea surface temperature data for the Niño 3.4 region were downloaded from the NOAA website. The monthly ONI values were classified into El Niño time periods when the SST anomaly was greater than 0.5 °C and La Niña time periods when the SST anomaly was less than 0.5 °C. This classification was further broken down into weak La Niña (-0.5 °C to - 0.99 °C), moderate La Niña (-1.0 °C to - 1.49 °C), strong La Niña (less than or equal to -1.5 °C), neutral (between -0.5 °C and 0.5 °C), weak El Niño (0.5 °C to 0.99 °C), moderate El Niño (1.0 °C to 1.49 °C), and strong El Niño (greater than or equal to 1.5 °C). Monthly ICEN values were downloaded from the Instituto del Mar del Peru website. The monthly ICEN values were classified into El Niño time periods when the SST anomaly was greater than 0.4 °C and La Niña time periods when the SST anomaly was less than 1.0 °C. This classification was further broken down into weak La Niña (-1.0°C to - 1.2 °C), moderate La Niña (-1.2 °C to - 1.4 °C), strong La Niña (less than or equal to -1.4 °C), neutral (between -1.0 °C and 0.4 °C), weak El Niño (0.4 °C to 1.0°C), moderate El Niño (1.0 °C to 1.7°C), strong El Niño (1.7 to 3.0 °C), and very strong El Niño (greater than 3.0 °C). Additionally, we created a binary El Niño variable where weak, moderate, and strong El Niño months were grouped together as El Niño time periods and neutral and weak, moderate, and strong La Niña months were grouped together as La Niña time periods.

Analysis Overview

We conducted a descriptive exploratory analysis examining the associations and bivariate relationships between reported confirmed dengue cases and demographic and environmental variables. We followed the descriptive analysis with a multivariate analysis to examine the associations between reported confirmed dengue cases and environmental and demographic risk factors.

Descriptive Statistics

Descriptive statistics were calculated for reported confirmed dengue cases, average monthly temperature and average monthly cumulative precipitation. Histograms of reported confirmed case incidence rates were created to visually examine the distribution of dengue cases data by age and sex. Additionally, time series graphs of Niño 3.4 region SST, average monthly temperature and average monthly cumulative precipitation were created to visually examine the data for obvious patterns and trends.

Bivariate Analysis

The association between reported confirmed dengue cases and environmental and demographic variables, including temperature, precipitation, and density of healthcare facilities, were examined using

generalized simple linear regression with a Poisson distribution. Due to the large numbers of districts that did not report dengue cases for multiple months out of the year, we considered using a zero-inflated Poisson distribution, but the zeros in the data are "real zeros," indicating that the reporting unit did not see any dengue cases indicating that a zero-inflated Poisson is not necessary for these analyses. The association between reported confirmed dengue cases and natural region was examined using a Kruskal-Wallis test. We also examined the association between natural region and weather variables temperature and precipitation using Kruskal-Wallis tests and Dunn post hoc tests. We considered a variable to be significantly associated in the bivariate analysis if the p-value was less than or equal to 0.05.

Generalized Estimated Equation Analysis

After using the bivariate analyses to check for significant variables, we included these significant variables in a multivariate generalized estimating equation models (GEE). The GEE model method accounts for the correlation structure of dengue cases with the same districts and the autocorrelation that is occurring due to current cases being related to previous cases through time. In addition to the environmental variables, including monthly average temperature, monthly average cumulative precipitation, and natural region, and the demographic variables of population and number of healthcare facilities per district, we added a continuous variable for year to account for long term trends in dengue cases and dengue case reporting. After these variables were selected, we used a Pearson's correlation matrix to examine the variables for collinearity, checking for correlation values over 0.6.

The first GEE model was fit to the data with reported confirmed dengue cases as the outcome and temperature as the predictor, and the second GEE model was fit with reported confirmed dengue cases as the outcome and a binary El Niño variable, based on the ONI index, as the predictor. In both GEE models we used a Poisson distribution, an offset of district population, and specified an autocorrelation structure. Additionally, both models used districts as the unit for clustering to accommodate for the longitudinal measurements that were taken in each district. In a subanalysis, we examined the relationship between reported confirmed dengue cases and El Niño by natural region classification. Due to the heterogenous geography of Peru, we expect the effect of El Niño will vary dependent on natural region characteristics.

Sensitivity Analyses

We conducted three sensitivity analyses. In the first sensitivity analysis, we examined the association between reported confirmed and probable dengue cases combined and temperature using the same GEE model used in the primary analysis. This analysis was conducted because the number of reported confirmed dengue cases may underrepresent the true incidence of dengue in Peru, possibly due to factors such as lack of access to laboratory testing or shortages in laboratory staff and testing materials. The second sensitivity analysis examined the relationship between reported confirmed dengue cases and a binary El Niño variable in districts sensitive to El Niño, which we defined as districts in national departments that were most affected by the 1997-1998 El Niño event (77). Sensitive districts include all of the districts in the following departments: Ancash, Apurímac, Arequipa, Ayacucho, Cuzco, Ica, La Libertad, Lambayeque, Lima, Loreto, Piura, San Martín, and Tumbes. In the third sensitivity analysis, we examined the relationship between reported confirmed dengue cases and a binary El Niño variable, based on the local Peru ICEN index, using the same GEE model used in the primary analysis.

All analyses were completed using code created in R version 3.5.2 (2018-12-20)

Results

Descriptive Statistics

Dengue Incidence:

As Table 1 shows, there were a total of 331,518 confirmed dengue cases reported from 1,841 administrative districts during the study period with an annual range of 3,349 to 68,274 cases. The mean number of annual dengue cases was 17,448 cases. At the district level, there was a range of 0 to 5,944 cases per month with a mean of 0.6 cases per month over the study period.

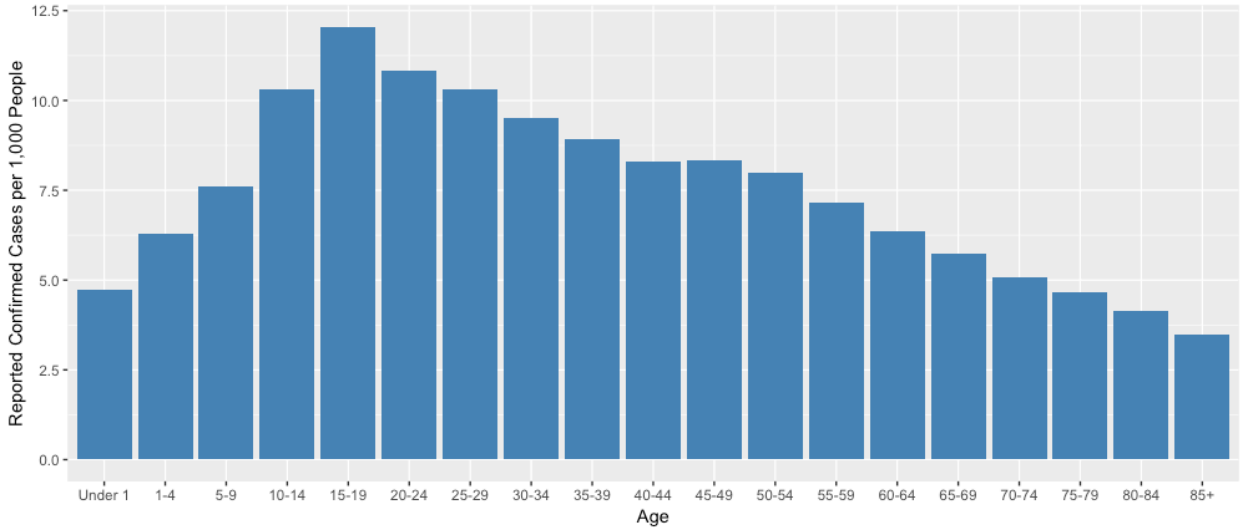
Table 1. Descriptive statistics of annual reported confirmed dengue cases and all dengue cases (confirmed and probable) in Peru, 2000 to 2019

Year	Reported All Dengue Cases: Probable Plus Confirmed	All Dengue Annual Incidence Rate (cases per 1,000 persons)	Reported Confirmed Dengue Cases	Annual Confirmed Dengue Incidence Rate (cases per 1,000 persons)
2000	5,557	0.21	893	0.03
2001	23,526	0.89	1,427	0.05
2002	8,085	0.30	2,077	0.08
2003	3,349	0.12	508	0.02
2004	9,547	0.35	1,650	0.06
2005	5,640	0.20	2,448	0.09
2006	4,022	0.14	1,219	0.04
2007	6,344	0.22	2,767	0.10
2008	12,824	0.45	6,761	0.23
2009	13,407	0.46	13,395	0.46
2010	16,842	0.57	16,842	0.57
2011	28,084	0.94	28,084	0.94
2012	28,505	0.95	28,504	0.95
2013	13,092	0.43	13,089	0.43
2014	17,234	0.56	17,222	0.56
2015	35,816	1.15	32,490	1.04
2016	25,150	0.80	22,588	0.72
2017	68,274	2.15	59,289	1.86
2018	6,190	0.19	2,381	0.07
Total	**331,488**	**0.60**	**253,634**	**0.46**

Age Distribution of Reported Confirmed Dengue Cases:

As Figure 1 Shows the age distribution of confirmed cases over the study period was unimodal, with a peak in the 15-19-year-old-age category and much lower incidence rates at the age extremes.

Figure 1. Age distribution of all reported confirmed dengue (cases per 1,000 persons by age category)



*Population at risk from 2017 census data

Sex and Reported Confirmed Dengue Incidence Rates:

Figure 2. Sex distribution of all reported confirmed dengue (cases per 1,000 persons by sex)

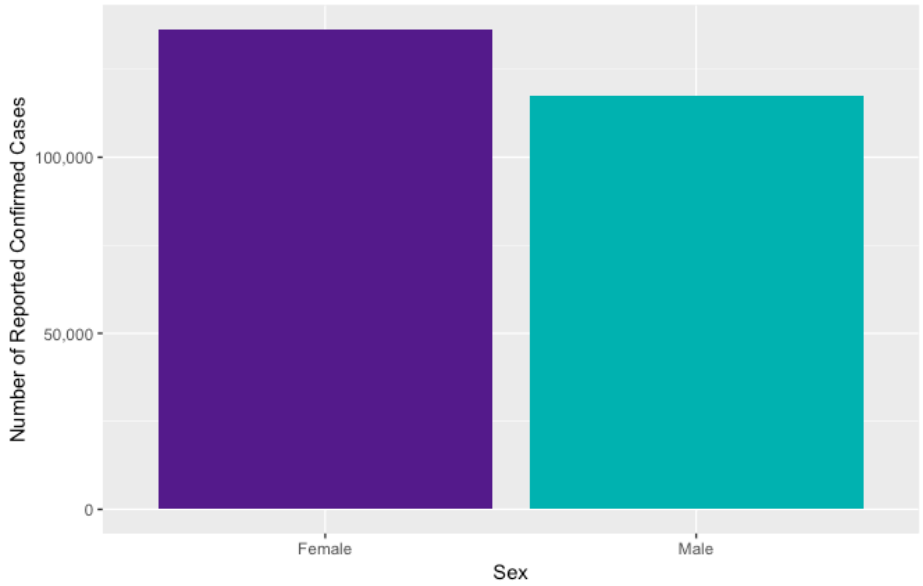
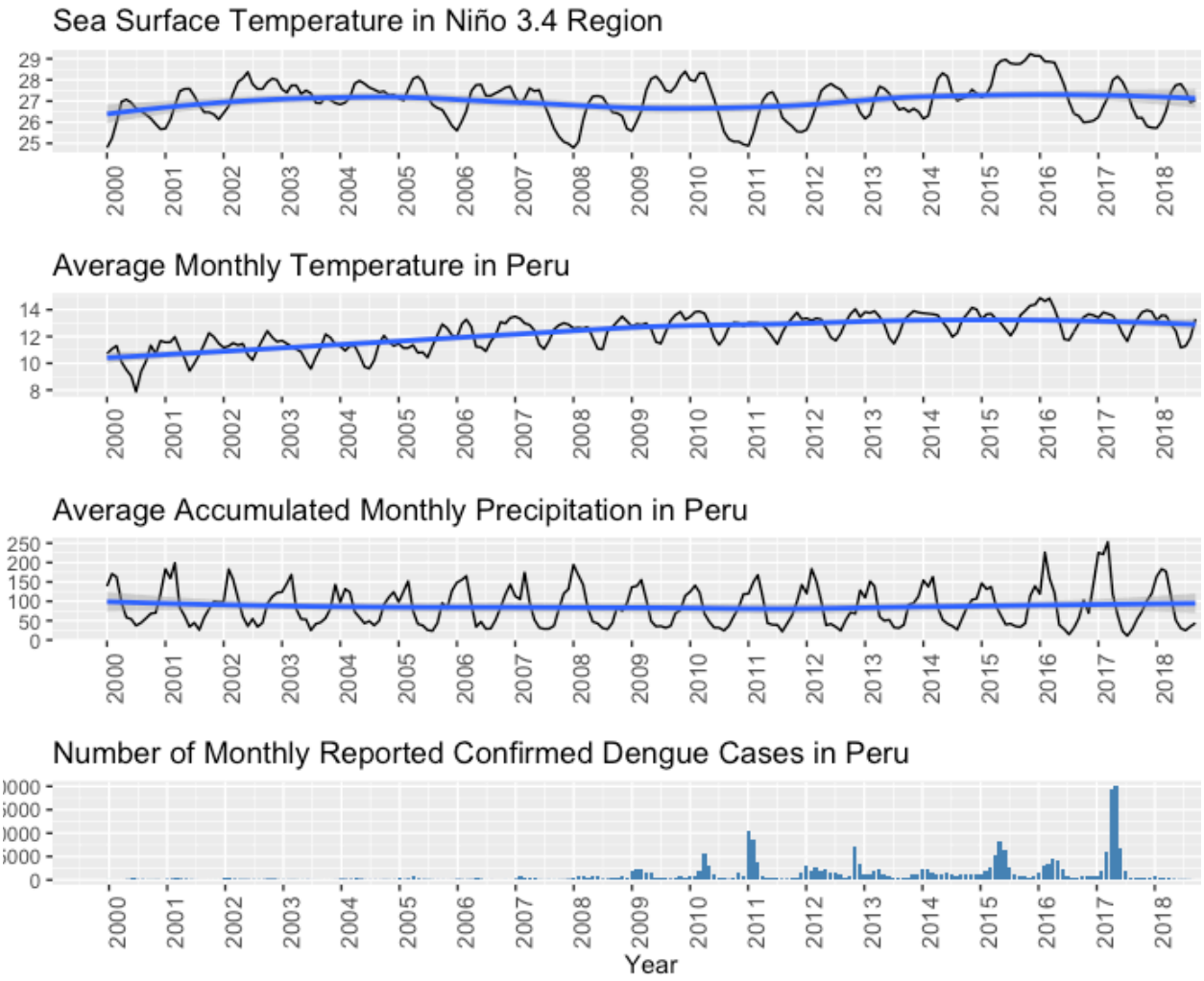


Figure 2 shows the number of reported confirmed dengue cases by sex. According to the 2017 census results, the male population represents 49.2 percent of the population and the female population is 50.8 percent of the population (71). Females have slightly more reported confirmed cases than males.

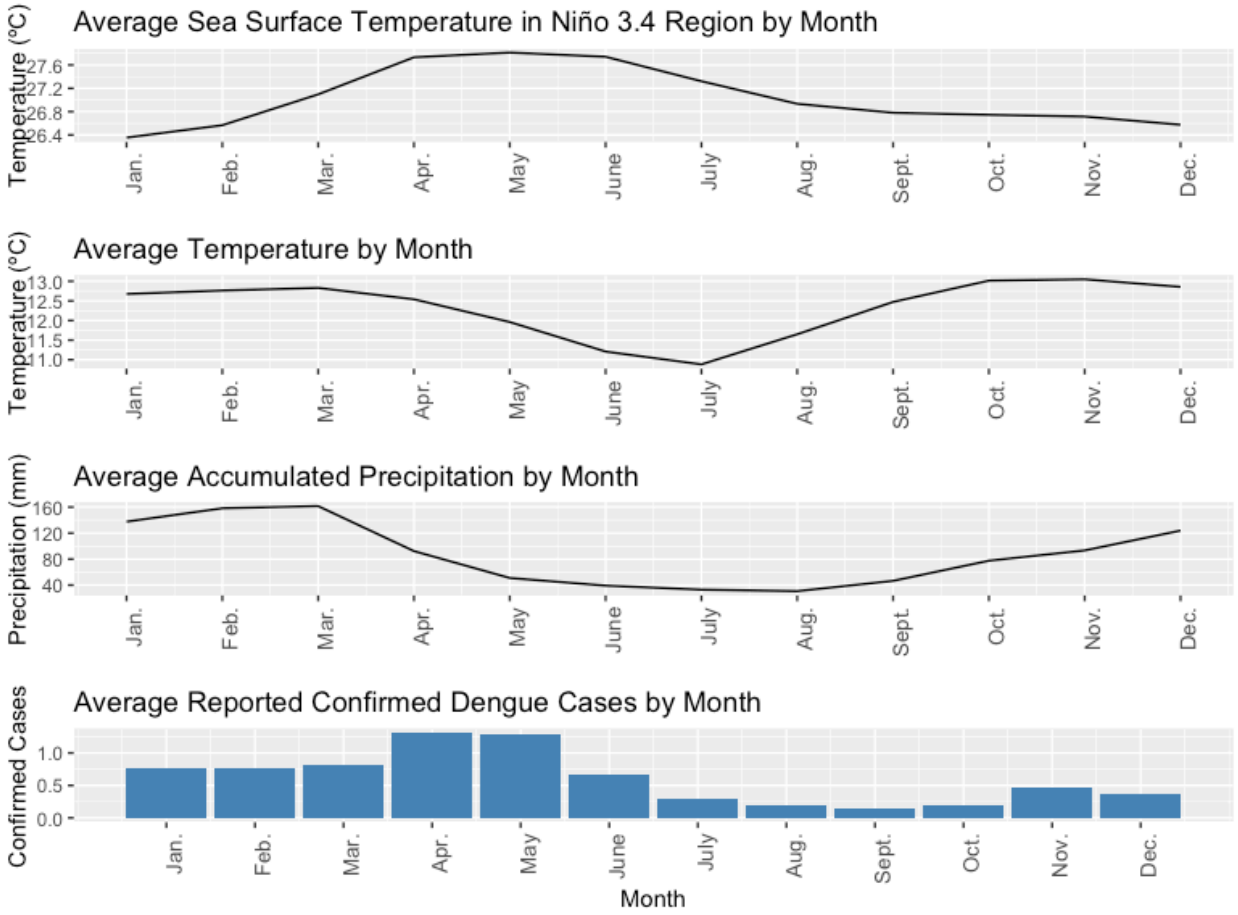
Annual Fluctuations in Sea Surface Temperature, Temperature, Precipitation, and Dengue Cases:

Figure 3. Time series plots of sea surface temperature, average monthly temperature in Peru, average monthly accumulated precipitation in Peru, and number of monthly reported confirmed dengue cases



*Blue lines are Locally Weighted Scatterplot Smoothing (LOWESS) lines

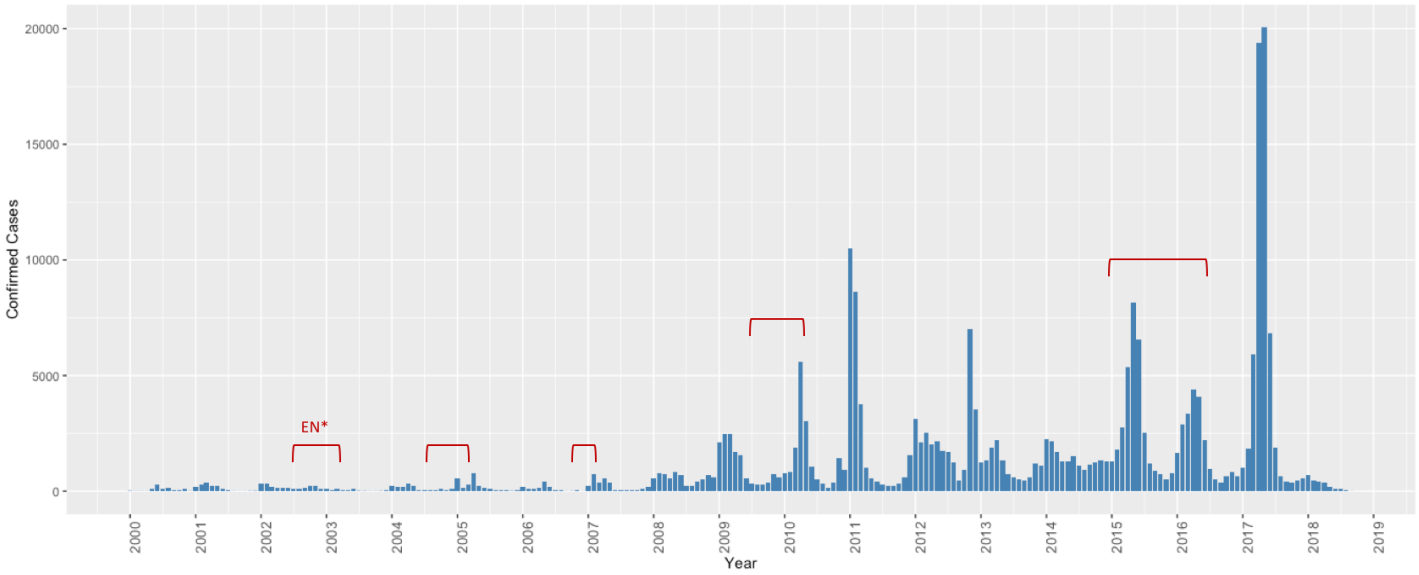
Figure 4. Annual plot of sea surface temperature, temperature in Peru, accumulated precipitation in Peru, and reported confirmed dengue cases averaged by month



As figure 3 and 4 show, mean recorded sea surface temperature, Peru land surface temperature, and Peru accumulated precipitation show clear cyclical variation over the study period. Sea surface temperature ranged from 24.79 °C to 29.23 °C, with a mean of 27.04 °C. Peru average monthly land surface temperature ranged from -6.49 °C to 29.51 °C, with a mean of 12.35 °C. The average monthly temperature follows a similar annual pattern with the lowest temperatures generally occurring in June, or July. The temperature time series plot also shows an apparent temperature increased over the study time period (linear model p-value < 0.00001). Peru's average monthly cumulative precipitation demonstrates a similar pattern with the least amount of rain occurring in June, July, or August. The mean average monthly cumulative precipitation was 86.90 mm of accumulated rainfall/month. The least amount of rainfall recorded was 0.00 mm of accumulated rainfall/month and the greatest amount was 2,950.84 mm of accumulated rainfall/month, which occurred during 2017 flooding.

Figure 4 also shows a cyclic pattern in dengue cases, with the majority of cases occurring between April and May, generally followed by a decrease in cases until October, when cases begin to rise again. Figure 3 also shows that there was an exceptionally large number of cases in 2017 and smaller peaks of cases in early 2011, late 2012, and early 2015.

Figure 5. Time series plot of reported confirmed dengue cases in Peru, 2000-2019, with El Niño periods indicated



*El Niño periods, derived from ONI index

Bivariate Results

Monthly Average Temperature and Dengue Incidence:

Figure 6. Plot of the relationship between monthly average temperature and log reported confirmed dengue cases +1

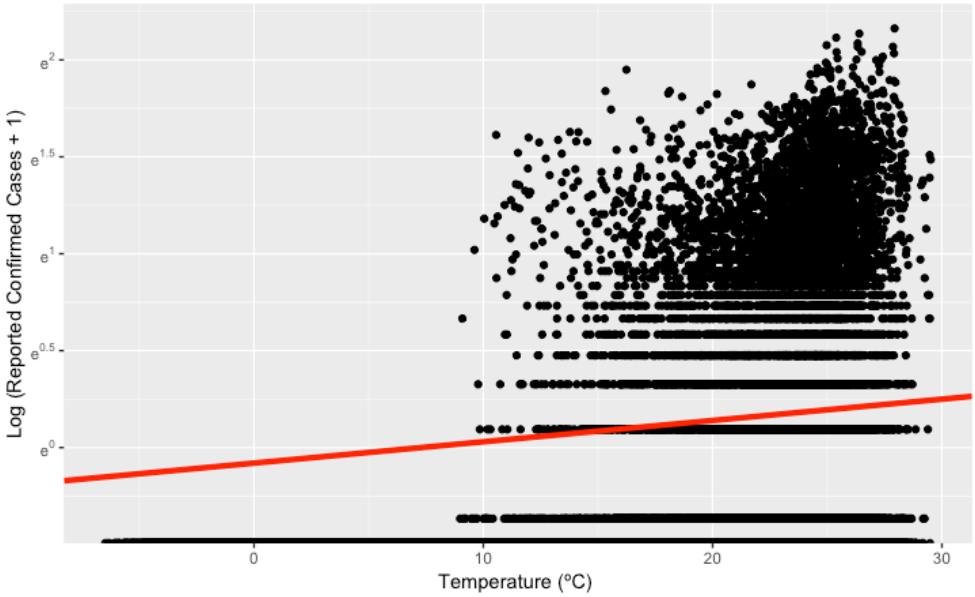


Figure 6 shows that there is a very slight positive association between temperature and dengue cases (R^2 = 0.0451, p-value < 0.0001). The red line linear regression line has the equation y = 0.011x + -0.081.

Monthly Accumulated Precipitation and Dengue Incidence:

Figure 7. Plot of the relationship between log monthly average accumulated precipitation +1 and log reported confirmed dengue cases +1

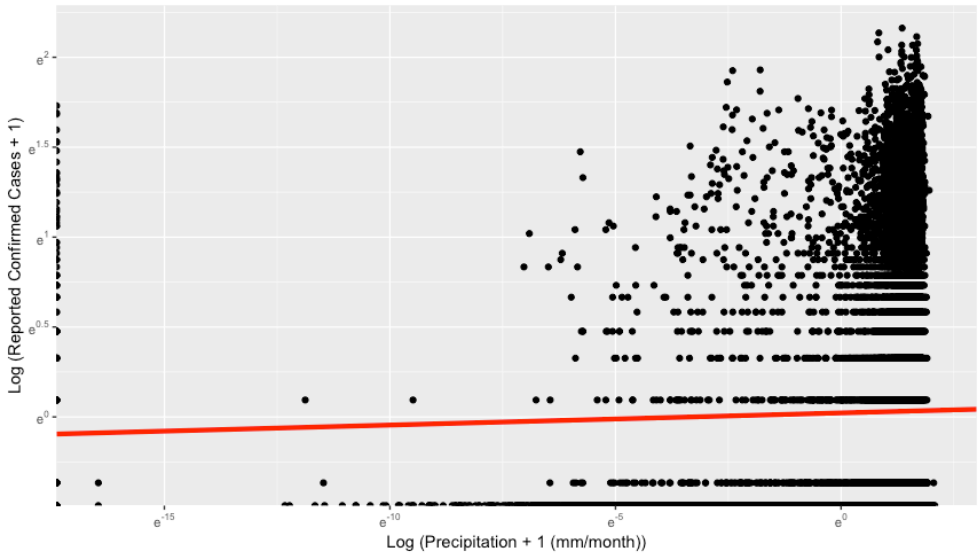
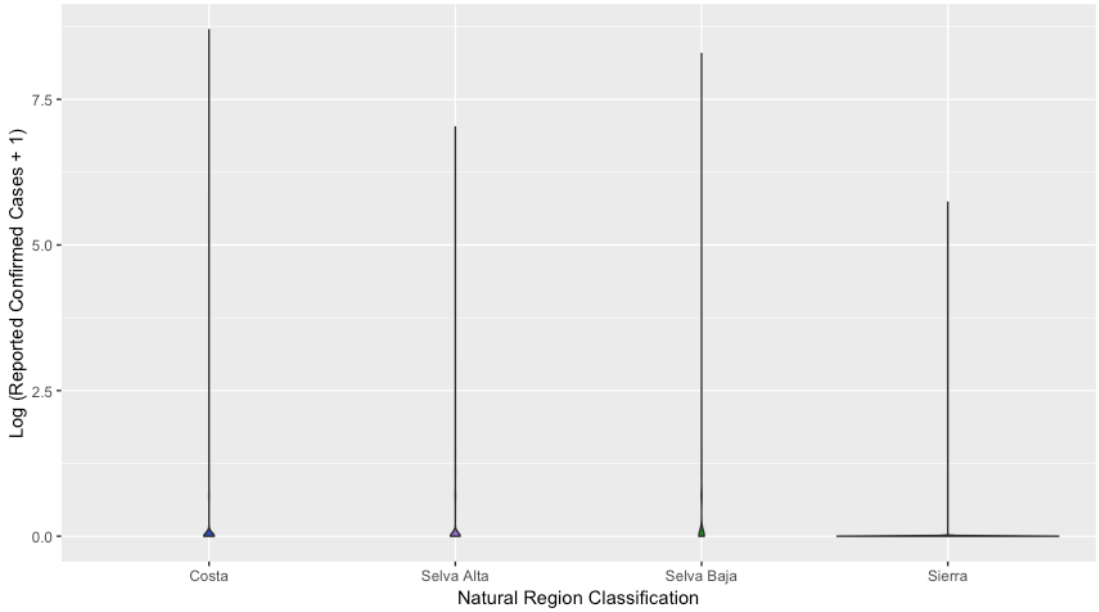


Figure 7 shows that there is an almost negligible positive association between cumulative precipitation (R^2 = 0.0005, p-value < 0.0001) and dengue cases. The red line linear regression line has the equation y = 0.007x + 0.023.

Natural Region Classification and Dengue:

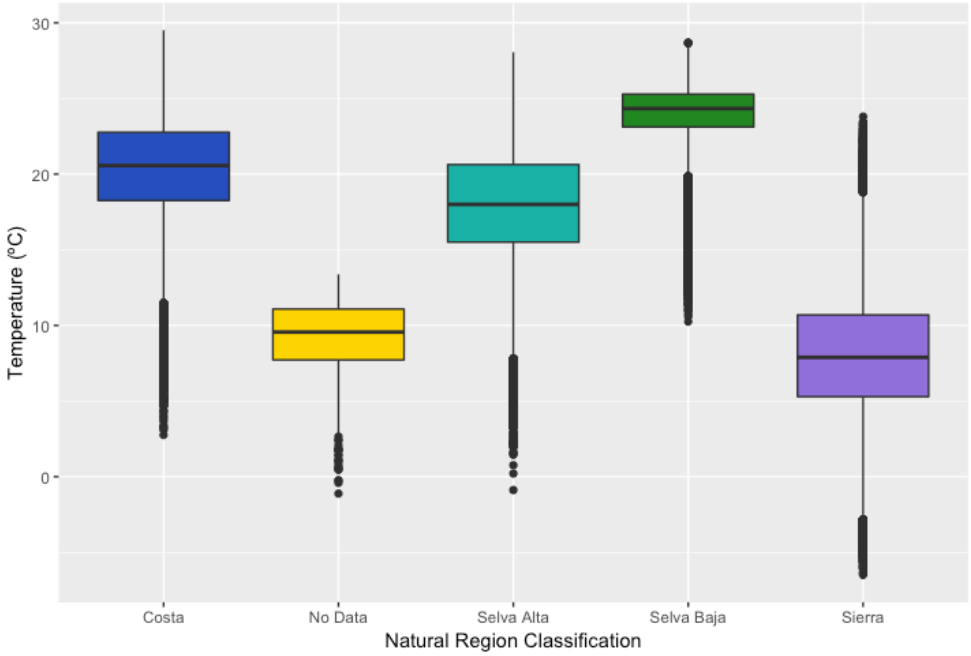
Figure 8. Violin plot of the relationship between natural region classification and log reported confirmed dengue cases +1



A Kruskal-Wallis test of log reported confirmed dengue cases +1 by natural region was significant with a p-value of <0.00001. The greatest annual incidence rate of reported confirmed dengue (11.90 cases per 1,000 person) was seen in the selva baja region in 2011, while the lowest annual incidence rate of 0 cases per 1,000 persons was in the sierra region in 2000 through 2008. At the same time, there was evidence that natural region correlated with differences in temperature and precipitation.

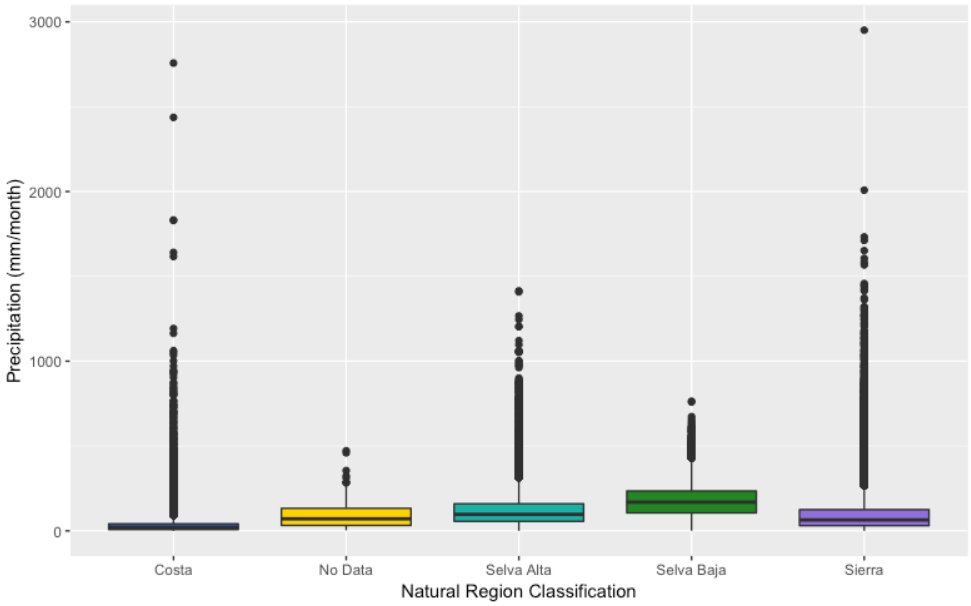
Natural Region Classification and Weather:

Figure 9. Plot of the relationship between natural region classification and average temperature in Peru, 2000-2019



As Figure 9 shows, a Kruskal-Wallis test of monthly temperature by natural region was significant with a p-value of <0.00001 and a Dunn post hoc test with a Bonferroni correction indicated that all were significant differences in temperature between all pairwise natural regions comparisons. The highest temperature region was selva baja, while the lowest temperature region was sierra. These were also the regions where the dengue incidence was also the highest and lowest respectively.

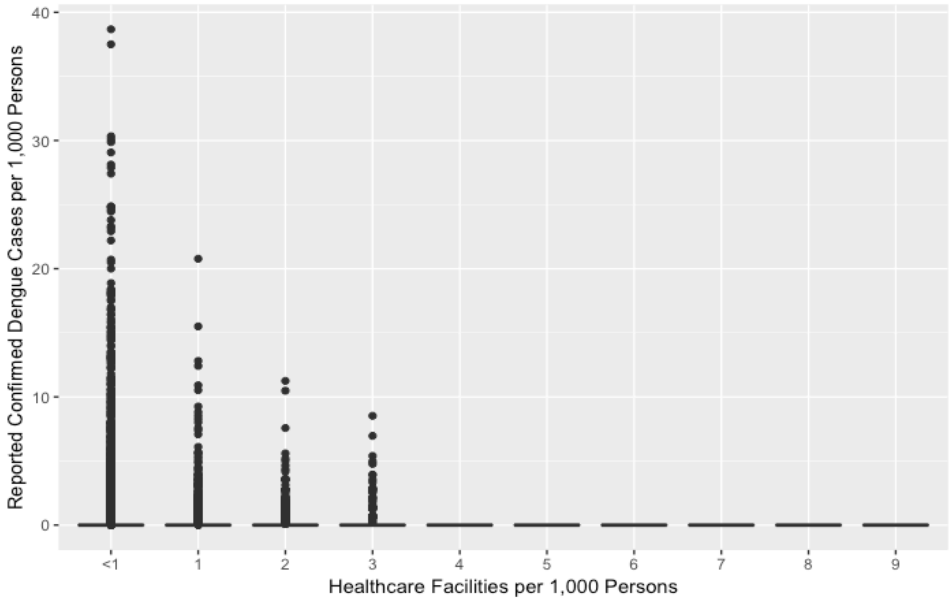
Figure 10. Plot of the relationship between natural region classification and average cumulative precipitation in Peru, 2000-2019



As with temperature, the regions demonstrated differences in monthly accumulated precipitation, as shown in Figure 10. The districts with the highest precipitation were in the selva baja region, where dengue incidence was the highest. A Kruskal-Wallis test of temperature by natural region was significant with a p-value of <0.00001 and a Dunn post hoc test with a Bonferroni correction indicated that all were significant differences between all pairwise natural regions comparisons.

Density of Healthcare Facilities and Dengue:

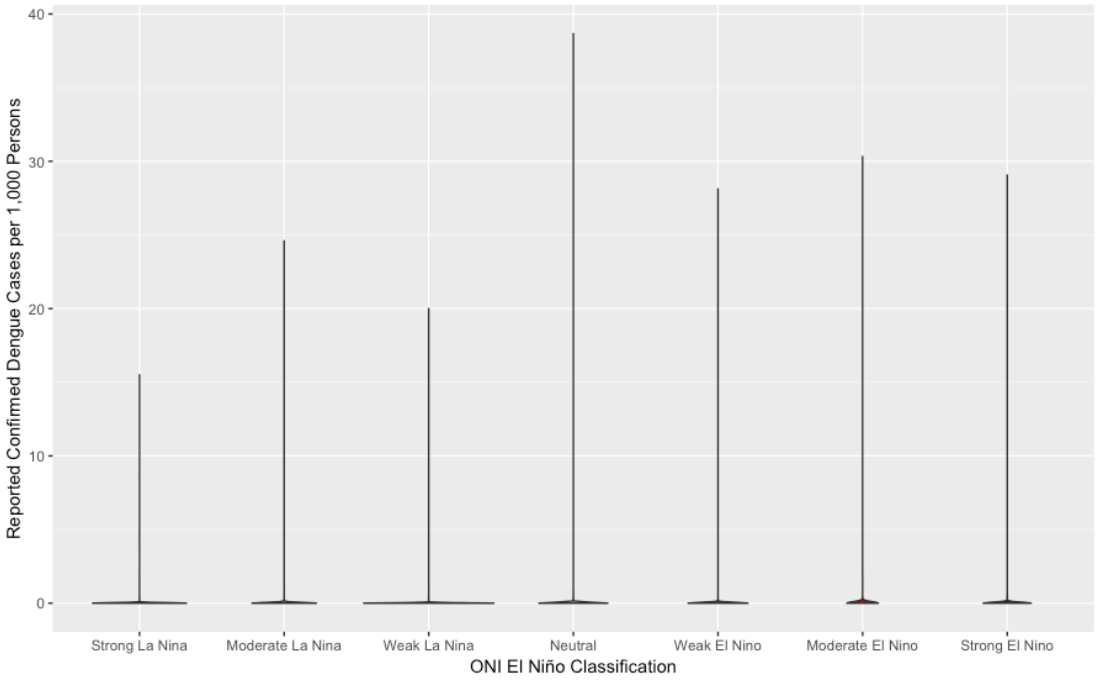
Figure 11. Plot of the relationship between density of healthcare facilities and monthly number of reported confirmed dengue cases, 2000-2019



As figure 11 shows, there was a higher reported confirmed dengue incidence in districts with fewer than one healthcare facility per 1,000 persons. A Kruskal-Wallis test of monthly confirmed dengue incidence by density of healthcare facilities was significant with a p-value of <0.00001. However, a Dunn post hoc test with a Bonferroni correction indicated that only districts with fewer than one healthcare facilities per 1,000 persons differed significantly from all other categories. No other pairwise comparisons were significant.

Bivariate Analysis of El Niño, Defined by the ONI Index, As a Risk Factor for Dengue Cases:

Figure 12. Violin plot of the relationship between ONI El Niño classification and reported confirmed dengue cases per 1,000 persons

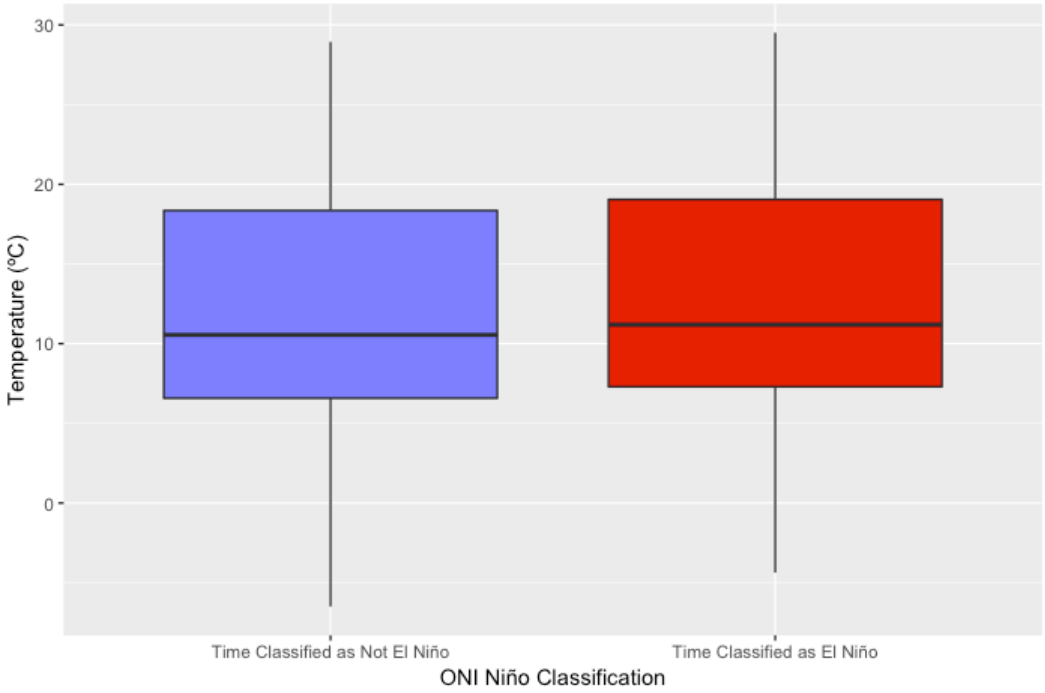


As figure 12 shows, there were significant differences between El Niño time periods and reported cases incidence. The lowest incidence was associated with strong La Niña, while the highest case incidence was associated with neutral and moderate El Niño. A Kruskal-Wallis test of cases by ONI El Niño classification was significant with a p-value of <0.00001. A Dunn post hoc test with a Bonferroni correction indicated that all were significant differences between all pairwise El Niño classification comparisons, except the comparisons between moderate El Niño and neutral classifications, the comparison between moderate La Niña and weak El Niño, the comparison between strong La Niña and weak El Niño, the comparison between moderate La Niña and weak La Niña.

El Niño, Defined by the ONI Index, and Average Weather Across Peru:

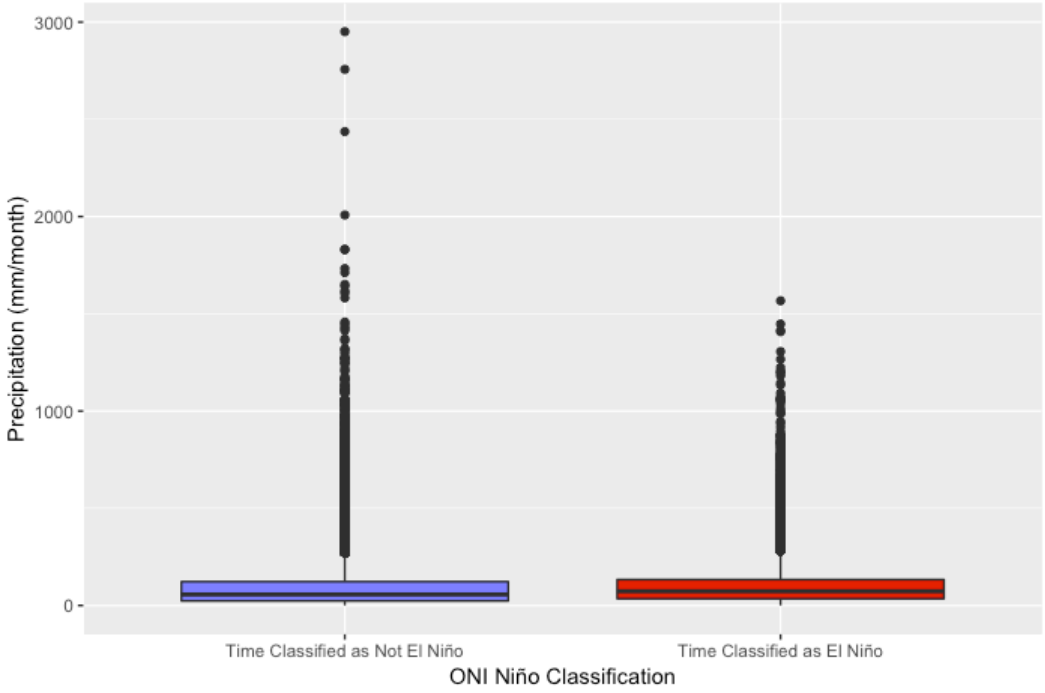
Figure 13 explores the association between El Niño events, defined by the ONI index, and average temperatures at the district level. During El Niño events, there was a trend towards slightly higher average temperatures. Using a Welch two sample t-test, we found that the average temperature during time classified as not El Niño (12.19 °C) is significantly different from the average temperature during time classified as El Niño (12.81 °C) with a p-value of <0.00001.

Figure 13. Plot of the relationship between time classified as El Niño and time classified as not El Niño and average temperature in Peru, 2000-2019



In terms of precipitation and El Niño events, figure 14 demonstrates that during time classified as not El Niño, there appeared to be more extreme precipitation events, although the mean cumulative precipitation levels for El Niño time periods and non-El Niño time periods were similar.

Figure 14. Plot of the relationship between time classified as El Niño and time classified as not El Niño and average accumulated precipitation in Peru, 2000-2019



Correlation Matrix:

Figure 15. Correlation Matrix of all numeric variables

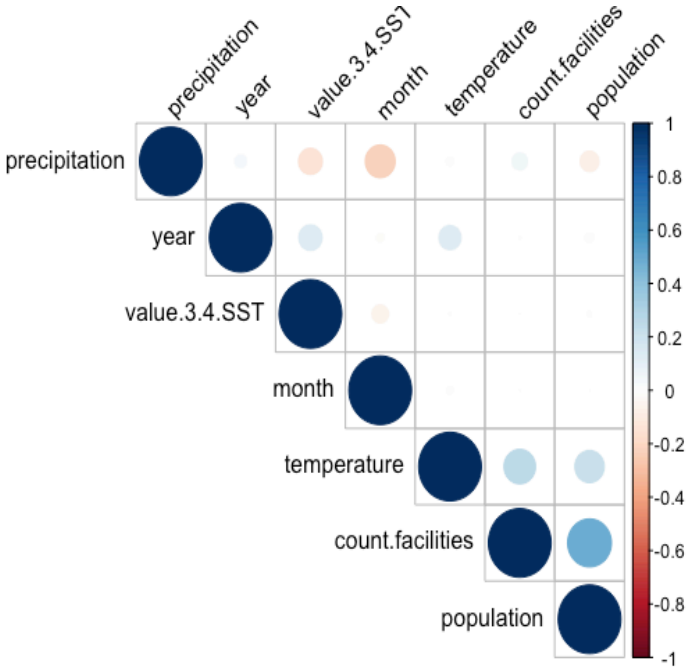


Table 2. Correlation Matrix of all numeric variables

	Year	Month	Temperature	Precipitation	No. Healthcare Facilities	Population	Niño 3.4 Sea Surface Temperature
Year	**1.0000**	**-0.0126**	**0.1224**	**0.0314**	**-0.0005**	**0.0151**	**0.1351**
Month	**-0.0126**	**1.0000**	**0.0081**	**-0.2249**	**0.0000**	**0.0000**	**-0.0667**
Temperature	**0.1224**	**0.0081**	**1.0000**	**0.0110**	**0.2513**	**0.2172**	**0.0006**
Precipitation	**0.0314**	**-0.2249**	**0.0110**	**1.0000**	**0.0578**	**-0.0862**	**-0.1420**
No. Healthcare Facilities	**-0.0005**	**0.0000**	**0.2513**	**0.0578**	**1.0000**	**0.4871**	**0.0000**
Population	**0.0151**	**0.0000**	**0.2172**	**-0.0862**	**0.4871**	**1.0000**	**0.0030**
Niño 3.4 Sea Surface Temperature	**0.1351**	**-0.0667**	**0.0006**	**-0.1420**	**0.0000**	**0.0030**	**1.0000**

The correlation matrix analyses shown here demonstrate that the different continuous risk factor variables were not highly correlated.

Primary GEE Model Results

Table 3 summarizes the results of the bivariate and multivariate GEE modeling of risk factors for districts to have reported confirmed cases of dengue, using temperature and cumulative precipitation, but not El Niño events. Both temperature and precipitation in this model, and alta selva was also positively associated with case risk.

Table 3. Coefficients of bivariate generalized linear model and multivariate generalized estimating equation model on temperature and cumulative precipitation and the monthly number of reported confirmed dengue cases in Peru, 2000-2019

	Bivariate			**Multivariate**		
Factor	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
Temperature	1.5847	1.0008	<0.00001	1.5186	1.0247	<0.00001
Precipitation	1.0023	1.0000	<0.00001	1.0021	1.0003	0.0000
Density (Count) of Healthcare Facilities	0.0139	1.0097	<0.00001	1.0040	1.0040	0.3173
Year	1.1876	1.0005	<0.00001	1.1686	1.0078	<0.00001
Dunn Post Hoc Test	**Z Score**	**Adjusted P-value**				
Natural Region: Costa	77.8265	0.0000		1.2688	1.5109	0.5640
Natural Region: Selva Alta	82.5695	0.0000		3.1205	1.5354	0.0080
Natural Region: Selva Baja	158.3884	0.0000		1.6562	91.4690	0.2639

*Values have been exponentiated

**QIC value is -741,760.5

Table 4 summarizes the results of the bivariate and multivariate GEE modeling of risk factors for districts to have reported confirmed cases of dengue using El Niño time periods, defined using the ONI index. In this model, El Niño had a slight positive association with case risk. However, this association was only significant in the bivariate analysis. In addition, all natural region classifications had positive associations with case risk.

Table 4. Coefficients of bivariate generalized linear model and multivariate generalized estimating equation model on El Niño, using the ONI index, and the monthly number of reported confirmed dengue cases in Peru, 2000-2019

	Bivariate			**Multivariate**		
Factor	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	1.0924	1.0048	<0.00001	1.0302	1.0954	0.744
Density (Count) of Healthcare Facilities	0.0139	1.0097	<0.00001	0.9915	1.0043	0.046
Year	1.1876	1.0005	<0.00001	1.1797	1.0094	<0.00001
Dunn Post Hoc Test	**Z Score**	**Adjusted P-value**				
Natural Region: Costa	77.8265	0.0000		82.9784	1.4728	<0.00001
Natural Region: Selva Alta	82.5695	0.0000		119.8954	1.4684	<0.00001
Natural Region: Selva Baja	158.3884	0.0000		464.7083	1.4665	<0.00001

*Values have been exponentiated

**QIC value is -460,222.8

Results of Subanalysis of the relationship between reported confirmed dengue cases and El Niño by natural region classification

Table 5 summarizes the results of four multivariate GEE models of confirmed cases of dengue and El Niño time periods, defined using the ONI index, in each of the four natural region classifications (costa, sierra, selva alta, and selva baja). El Niño time periods were associated with increased dengue case risk in the costa and sierra natural regions, with the sierra region having the greatest effect. El Niño also had a slight positive association with case risk in the selva alta region, though this association was not significant. In the selva baja region, El Niño was associated with a lower dengue incidence rate.

Table 5. Coefficients of multivariate generalized estimating equation model on El Niño, using the ONI index, and the monthly number of reported confirmed dengue cases by natural region, 2000-2019

	Costa			Sierra			Selva Alta			Selva Baja		
Factor	**Beta***	**San. SE***	**P-value**	**Beta***	**San. SE***	**P-value**	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	1.5383	1.1445	0.0014	9.0675	2.4584	0.0142	1.0824	1.1680	0.6100	0.4954	1.1237	<0.00001
Count of Healthcare Facilities	0.9330	1.0061	<0.00001	0.9243	1.0378	0.0340	1.0196	1.0036	<0.00001	1.0433	1.0049	<0.00001
Year	1.2837	1.0185	<0.00001	1.8463	1.2148	0.0016	1.1043	1.0089	<0.00001	1.1069	1.0042	<0.00001

*Values have been exponentiated

Table 6 summarizes the results of four multivariate GEE models of confirmed cases of dengue and El Niño time periods, defined using the ICEN index, in each of the four natural region classifications (costa, sierra, selva alta, and selva baja). The results of this analysis were similar to the results from the previous analysis. El Niño time periods were associated with increased dengue case risk in the costa, sierra, and selva alta natural regions, with the sierra region having the greatest effect. In the selva baja region, El Niño was associated with a lower dengue incidence rate.

Table 6. Coefficients of multivariate generalized estimating equation model on El Niño, using the ICEN index, and the monthly number of reported confirmed dengue cases by natural region, 2000-2019

	Costa			Sierra			Selva Alta			Selva Baja		
Factor	**Beta***	**San. SE***	**P-value**	**Beta***	**San. SE***	**P-value**	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	2.3491	1.1774	<0.00001	10.6175	1.7605	0.00003	1.5055	1.1950	0.0220	0.4906	1.1218	<0.00001
Count of Healthcare Facilities	0.9329	1.0061	<0.00001	0.9241	1.0381	0.0350	1.0196	1.0036	<0.00001	1.0433	1.0048	<0.00001
Year	1.2754	1.0201	<0.00001	1.7078	1.1004	<0.00001	1.0982	1.0085	<0.00001	1.1137	1.0041	<0.00001

*Values have been exponentiated

Sensitivity Analyses Results

Model of Temperature and Dengue, using Probable and Confirmed Reported Dengue Cases:

Table 7 summarizes the results the multivariate GEE model of risk factors for districts to have reported confirmed and probable cases of dengue, using temperature and cumulative precipitation, but not El Niño events. The results of this model are similar to the results of the model using only confirmed cases of dengue. Temperature, precipitation, and the alta selva natural region have positive associations with case risk in this model.

Table 7. Coefficients of multivariate generalized estimating equation model on temperature and cumulative precipitation and the monthly number of reported probable and confirmed dengue cases in Peru, 2000-2019

Factor	**Beta***	**San. SE***	**P-value**
Temperature	1.5354	1.0212	<0.00001
Precipitation	1.0023	1.0002	<0.00001
Density (Count) of Healthcare Facilities	1.0023	1.0033	0.4883
Year	1.0859	1.0077	<0.00001
Natural Region: Costa	1.2705	1.4368	0.5088
Natural Region: Selva Alta	3.1174	1.4525	0.0023
Natural Region: Selva Baja	1.5431	1.4820	0.2702

*Values have been exponentiated

**QIC value is -628,736.7

Modeling of El Niño, defined by the ONI index, and Dengue in Districts Sensitive to El Niño:

Table 8 summarizes the results of the bivariate and multivariate GEE modeling of risk factors for reported confirmed cases of dengue using El Niño time periods, defined using the ONI index, in districts sensitive to El Niño events. Sensitive districts include all of the districts in the following departments: Ancash, Apurímac, Arequipa, Ayacucho, Cuzco, Ica, La Libertad, Lambayeque, Lima, Loreto, Piura, San Martín, and Tumbes. In this model, El Niño had a positive association with case risk. However, this association was only significant in the bivariate analysis.

Table 8. Coefficients of bivariate generalized linear model and multivariate generalized estimating equation model of El Niño, using the ONI index, and the monthly number of reported confirmed dengue cases in districts sensitive to El Niño, 2000-2019

		Bivariate			**Multivariate**	
Factor	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	1.1921	1.0053	<0.00001	1.1322	1.1161	0.26
Density (Count) of Healthcare Facilities	0.0054	1.0137	<0.00001	0.9752	1.0058	1.60e-05
Year	1.2143	1.0006	<0.00001	1.2067	1.0124	<0.00001
Dunn Post Hoc Test	**Z Score**	**Adjusted P-value**				
Natural Region: Costa	68.21		0.0000	48.5352	1.6104	<0.00001
Natural Region: Selva Alta	32.64		<0.00001	52.7266	1.6191	<0.00001
Natural Region: Selva Baja	99.69		0.0000	218.6276	1.6127	<0.00001

*Values have been exponentiated

**QIC value is -364454.5

Modeling of El Niño, defined by the ICEN index, and Dengue:

Table 9 summarizes the results of the bivariate and multivariate GEE modeling of risk factors for districts to have reported confirmed cases of dengue using El Niño time periods, defined using the ICEN index. In this model, El Niño had a significant positive association with case risk in both the bivariate and multivariate model. All natural regions were also positively associated with case risk.

Cases and El Niño, with the ICEN index:

Table 9. Coefficients of bivariate generalized linear model and multivariate generalized estimating equation model on El Niño, using the ICEN index, and the monthly number of reported confirmed dengue cases in Peru, 2000-2019

	Bivariate			**Multivariate**		
Factor	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	1.7315	1.0041	<0.00001	1.3423	1.1131	0.006
Density (Count) of Healthcare Facilities	0.0139	1.0097	<0.00001	0.9915	1.0043	0.046
Year	1.1876	1.0005	<0.00001	1.1760	1.0092	<0.00001
Dunn Post Hoc Test	**Z Score**	**Adjusted P-value**				
Natural Region: Costa	77.8265	0.0000		83.0772	1.4729	<0.00001
Natural Region: Selva Alta	82.5695	0.0000		119.8163	1.4683	<0.00001
Natural Region: Selva Baja	158.3884	0.0000		464.8896	1.4665	<0.00001

*Values have been exponentiated

**QIC value is -465,204.2

Modeling of El Niño, defined by the ICEN index, and Dengue in Districts Sensitive to El Niño:

Table 10 summarizes the results of the bivariate and multivariate GEE modeling of results of the bivariate and multivariate GEE modeling of risk factors for reported confirmed cases of dengue using El Niño time periods, defined using the ICEN index, in districts sensitive to El Niño events. Sensitive districts include all of the districts in the following departments: Ancash, Apurímac, Arequipa, Ayacucho, Cuzco, Ica, La Libertad, Lambayeque, Lima, Loreto, Piura, San Martín, and Tumbes. In this model, El Niño had a positive association with case risk in both the bivariate and multivariate models. Additionally, the associations between El Niño and case risk were found to be greater than the associations in the model using all districts in Peru.

Table 10. Coefficients of bivariate generalized linear model and multivariate generalized estimating equation model on El Niño using the ICEN index, and the monthly number of reported confirmed dengue cases in districts sensitive to El Niño, 2000-2019

	Bivariate			**Multivariate**		
Factor	**Beta***	**SE***	**P-value**	**Beta***	**San. SE***	**P-value**
El Niño (binary)	2.0342	1.0046	<0.00001	1.5557	1.1374	0.0006
Density (Count) of Healthcare Facilities	0.0054	1.0137	<0.00001	0.9751	1.0058	1.50e-05
Year	1.2143	1.0006	<0.00001	1.2014	1.0123	<0.00001
Dunn Post Hoc Test	**Z Score**	**Adjusted P-value**				
Natural Region: Costa	68.21		0.0000	48.5867	1.6105	<0.00001
Natural Region: Selva Alta	32.64		<0.00001	52.6223	1.6190	<0.00001
Natural Region: Selva Baja	99.69		0.0000	218.4899	1.6127	<0.00001

*Values have been exponentiated

**QIC value is -372959.2

Discussion

This study reports on an analysis of 19 years of surveillance data for dengue infection in Peru, a country experiencing severe outbreaks of the virus. The primary aim of this study was to determine which environmental and demographic risk factors had the greatest effect on dengue cases in Peru between 2000 and 2019. In our multivariate model, temperature appeared to have the largest association with dengue cases. We also found a statistically significant positive associations between case risk and cumulative precipitation. While there was a positive association between El Niño time periods and reported confirmed dengue cases, this association was only statistically significant in the multivariate model when El Niño time periods were measured using the ICEN index. Additionally, natural region classification had a significant impact on the association between El Niño time periods and reported confirmed dengue cases, with the sierra region most affected by El Niño. These results can add to existing knowledge of the dengue virus and can assist with enhanced prevention and management of new cases.

The results of the GEE model support the findings of the bivariate models and indicate that there is a positive association between dengue cases and temperature in Peru, with a 1.52 times higher dengue incidence rate for a 1 °C increase in temperature. Our finding of a positive relationship between monthly temperature and case incidence at the district level aligns closely with other studies that have found that temperature has a positive association with dengue in Latin American countries (55,56,59,67). While our model assumes a linear association, other studies have found that the relationship may be non-linear. One study in Guangzhou, China found that there was a positive linear effect between daily mean temperature and dengue at lower levels, but that the relationship became non-linear above the threshold of 28 °C (78). The non-linear effect of temperature on dengue could be further examined using a non-linear model that includes a spline function to account for this relationship.

There was also a positive association between dengue cases and precipitation, but the effect was not as high as the effect for temperature. For a one unit increase in accumulated mm of precipitation per month, there was a 1.0023 times higher dengue incidence rate. Monthly accumulated Precipitation also had a positive association with dengue incidence rate in the multivariate analysis. This result is not surprising, because many other studies have found that precipitation is an important factor in increased dengue cases in Latin America (55,56,59). Additionally, it is known that additional rainfall can create new breeding ground for *Ae. aegypti* females, who lay their eggs in man-made or natural containers of water (41). In other studies, this relationship has also been found to be non-linear and vary by region (44,78). The study in Guangzhou, China found that there was a non-linear relationship between daily rainfall and dengue below a threshold of 7 mm of rainfall (78). Another study of dengue epidemics and ENSO found epidemics followed prolonged droughts in some areas, possibly because the dry conditions forced people to stockpile water in containers creating addition breeding ground for the vector (79).

Density of healthcare facilities at the district level was negatively associated with dengue cases in the bivariate analysis, with more dengue cases reported by districts with less than one healthcare facility. However, this association became non-significant in the multivariate model. This finding could indicate that healthcare facilities are reducing dengue cases through interventions or care. However, the density

of healthcare facilities is a measure of the number of healthcare facilities per 1,000 persons, which can be inflated when a district has a small population. Therefore, this finding could simply indicate that there are fewer dengue cases in rural areas with a small population.

Year has a positive association with dengue, indicating that Peru has had an increasing number of reported dengue cases during the study period. However, it is difficult to tease out whether this increase is due to a true increase in cases or factors such as national surveillance system improvement, increasing laboratory testing capacity, or other activities in the country. For example, there was a large spike in reported confirmed dengue cases in 2017. One possible driver for this sudden increase in cases is the zika virus national emergency that was declared in July 2016 for half of the country (69). This emergency could have increased the number of people seeking care for arboviral illnesses due to their fear of zika or it could have put healthcare providers on the alert for zika cases, causing them to be more likely to order a test when a patient arrived with flu-like symptoms. Additionally, there is a cross-reaction in antibody testing between flaviviruses, including zika, and dengue which could account for the additional confirmed cases seen (70).

Natural regions were also found to play a role, but this could be a result of differences in temperature and precipitation. In addition to the bivariate generalized linear models used to examine model variables, the Kruskal-Wallis tests were used to examine the different natural regions of Peru. The results of these tests and the Dunn post hoc tests show that there are clear differences between the natural regions of Peru.

The GEE model examining the association between El Niño and dengue cases shows that there is a weakly positive, but non-significant association between time classified as El Niño, using the ONI index, and dengue incidence rate. When the GEE model was used to examine the association between El Niño and dengue incidence in El Niño sensitive districts, the association became stronger, but was still not significant. This finding is most similar to the results of a study in Puerto Rico examining the relationship between climatic variables (temperature, precipitation, and ENSO) and monthly dengue cases between 1979 and 2005. In this study, Jury also found that dengue cases were positively associated with temperature, weakly associated with local rainfall, and not significantly correlated with ENSO, as measured by the Niño 3 SST index (61).

Though the association between El Niño and dengue cases was not significant overall, this association varied widely by natural region. The sierra region had the largest positive association between El Niño and dengue cases. In the Andes, El Niño events generally lead to the equatorial Andes receiving below normal precipitation, while the coastal region and Andean foothills often experience exceptional rainfall. In addition to altered precipitation, the tropical Andes may also warm up to 1 °C during El Niño events (80). These local weather changes may allow *Ae. aegypti* to extend into previously inaccessible habitat and human populations. The costa region, which also receives additional rainfall during El Niño, had a significant and positive association between El Niño and dengue cases, though the effect was much smaller than for the sierra region. In contrast, the selva alta region did not show a significant association between El Niño events, defined by the ONI index, and dengue, and the selva baja region had a significant negative association. Usually El Niño events are associated with warming and drought in the northern,

eastern, and western Amazon rainforest, though this pattern differs depending on the El Niño type (81). Decreased rainfall could lead to fewer natural and human-made breeding sites for *Ae. aegypti*, if water is not being stockpiled in urban areas. The same analysis using the ICEN index to define El Niño events had similar results, with the exception of a significant positive association between El Niño events and dengue cases in the selva alta region. This finding indicates that the El Niño index selected can cause significant differences.

The importance of El Niño index selection is reinforced in the sensitivity analysis of El Niño and dengue cases using the ICEN index. While the primary GEE model examining the association between El Niño and dengue cases was not significant, results differed when the ICEN index was used. When the El Niño index was changed from the ONI index to the ICEN index, the GEE model shows that there is a positive and significant association between time classified as El Niño and dengue incidence rate. Additionally, this relationship become stronger and more significant when used to examine the association in sensitive districts. While the ONI index is widely used by NOAA and other agencies to predict and monitor El Niño events, the ONI is best suited to detecting basin-wide El Niño events and may not catch other types of El Niño (82). The ICEN index, based on Niño 1 + 2 region SST, is also used by Peru to monitor and detect El Niño events, and may be able to identify other varieties of El Niño (82). The discrepancy between the two indices was clearly seen in 2017, when an event called El Niño costero developed along with extreme rainfall during the 2016-2017 austral summer (83). This event caused flooding and landslides, leading to 100 deaths and 6270 reported confirmed dengue cases (83). The ONI index failed to capture this event, classifying the time as neutral, while the ICEN index classified the time as weak El Niño, correctly identifying that there was an El Niño. However, the ICEN index also did not provide early enough warning to allow Peru to prepare and inaccurately characterizing the event's true magnitude, which had extreme weather impacts despite being classified as weak El Niño (82). This finding illustrates that El Niño is a complex phenomenon that may be difficult to predict and detect. Additionally, each of the El Niño indices provide different ways to monitor the ENSO and may need to be used in conjunction to create a future model that can accurately predict dengue outbreaks in Peru. Additionally, while there were significant findings from the GEE model, it is important to remember the results of the GEE model are at a population level over all of the districts of Peru. Peru has a widely varying landscape and that these results of this model likely do not reflect what is occurring in a specific district.

Another interesting finding of this study is the age distribution of reported confirmed dengue cases. Dengue incidence appeared to have a unimodal distribution with a peak in the 15-19-year-old-age category and much lower incidence rates at the age extremes. This result generally aligns with other studies of age and dengue, which have found that in the Western Hemisphere the incidence of dengue and dengue hemorrhagic fever is higher in adults, as opposed to in Southeast Asia where children are more at risk (84). A 2007 study in Brazil found the risk of clinical dengue is lower in children than in adolescents and adults, though the authors also note that dengue virus serotype and strain are thought to play a role in the severity of the illness (85). The lower incidence of dengue at the age extremes that we found may be a result of children having a lower risk of clinical dengue disease, and thus not being captured by the surveillance system, and older adults protected by immunity gained through previous infection. Further study of the relationship between age and dengue case risk in Peru may be important

and help inform dengue intervention strategies. Additionally, northeastern Brazil has seen the age groups at risk for dengue change over time from 1998 through 2007, with the incidence rising in children, and Peru may face similar shifts in the population most at risk.

Limitations

Overall Limitations:
Peru is a very heterogeneous country with significant differences in climate and local weather by region. Thus, the effects of our predictor variables likely vary by geographic location. For example, water stressed regions, like the sierra region, are likely more sensitive to increases in precipitation than regions with consistent precipitation, including both the selva alta and selva baja regions. Additionally, monthly SST anomalies may not always accurately predict El Niño events, which develop gradually and may not continue to persist month to month. Furthermore, our study is based on based on broad geographic areas and uses a relatively long time scale of months. Data at this granularity may miss relationships that occur over short time intervals like days or weeks. Finally, due to our large sample size (1,841 administrative districts), some of the weakly statistically significant positive associations could be a result of the large sample size alone.

Data Limitations:
A major limitation to this study is the reliance on passive surveillance data. Such data is by nature influenced by a number of factors, including health-seeking behavior, healthcare staff awareness and capacity, and surveillance infrastructure and policies (86). Surveillance data can be challenging to model due to limitations in data quality and completeness. In this instance, all missing values have been treated as true zeros, based on the advice of the person who supplied the dataset. However, these zeros could be reported due to other circumstances, including some clinics not recognizing dengue cases or not having access to an adequate amount of testing materials. These factors are likely not linked to small increases in weather variables, but they could theoretically be associated with El Niño which can cause natural disasters like flooding, disrupting reporting units of the surveillance system and supply chains. For example, during the 2017 El Niño costero event, 64 health care centers were destroyed and 1,044 were damaged (87). In addition to dengue cases being missed at the clinic level, this disease is notoriously underreported, with only 20 percent of people having clinic symptoms and some of those with mild enough symptoms to not seek care at a health center (8). However, underreporting due to clinical symptoms is not likely heavily influenced by weather or El Niño events and probably has little impact on this study.

Another limitation is the lack of dengue intervention data. We did not have data on dengue prevention activities or dengue vaccination coverage in Peru during our study time period. This lack of data could have a large impact on our findings because some districts may be deploying vector control activities (e.g., insecticide or larvicide), community education programs, and other interventions (e.g., insecticide treated nets). Activities that reduce the vector population and dengue cases would attenuate the association between the environmental predictors and the outcome of dengue cases. Vaccination coverage would also generally reduce dengue cases and modify the relationship between our predictors and dengue.

Additionally, while Peru does monitor vector populations, we were not able to access this information. Therefore, our study relies on the assumed link between weather and dengue cases via the vector species, *Ae aegypti,* but cannot verify this mechanism in Peru.

Model Limitations:

While the GEE models used allowed us to account for correlation within districts and autocorrelation, they also have some limitations for modeling this type of data. The major limitation of our GEE model is that it relies on the assumption that districts are independent units. However, this assumption is not likely completely accurate because both people and vectors can easily cross district borders. However, due to the fact that our model included all of the districts in Peru and because we could not combine the districts into a fewer number of levels, we chose to not pursue a multilevel model. Future analyses could use spatial epidemiology techniques to explore the importance of proximity to other high-risk regions.

Conclusion

Using a high-quality and robust local weather dataset, demographic variables, and 19 years of dengue surveillance data we were able to examine the association between temperature, precipitation and dengue cases using a GEE model. In addition, we extended previous studies of local weather and dengue cases by adding on an analysis of the association between El Niño and dengue cases. Temperature had the highest positive association with dengue, indicating that it is an important environmental factor for dengue transmission. El Niño events were also found to have a positive association with dengue, though this relationship significantly varies depending on the El Niño index selected and natural region classification. The findings from this study increase our understanding of how changing environmental and human factors impact dengue fever cases in Peru. Additionally, our findings could potentially provide information useful to Peruvian health authorities for the prevention and control of dengue fever or future modeling studies.

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