Outbreak Investigation of *Plasmodium vivax* Malaria in a Region of Guatemala Targeted for Malaria Elimination

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Abstract. The Department of Santa Rosa, Guatemala, is targeted for malaria elimination. However, compared with 2011, a 13-fold increase in cases was reported in 2012. To describe the epidemiology of malaria in Santa Rosa in the setting of the apparent outbreak, demographic and microscopic data from 2008 to 2013 were analyzed. In April 2012, a new surveillance strategy, funded by the Global Fund to Fight AIDS, Tuberculosis and Malaria, was introduced involving more active case detection, centralized microscopy, increased community engagement, and expanded vector control. Interviews with vector control personnel and site visits were conducted in June 2013. From 2008 to 2013, 337 cases of malaria were reported. The increase in cases occurred largely after the new surveillance strategy was implemented. Most (137/165; 83%) 2012 cases came from one town near a lake. *Plasmodium vivax* was the malaria species detected in all cases. Cases were detected where malaria was not previously reported. Monthly rainfall or/and temperature did not correlate with cases. Interviews with public health personnel suggested that the new funding, staffing, and strategy were responsible for improved quality of malaria detection and control and thus the increase in reported cases. Improvements in surveillance, case detection, and funding appear responsible for the temporary increase in cases, which thus may paradoxically indicate progress toward elimination.

INTRODUCTION

As of 2015, 95 countries were considered malaria endemic, defined as having ongoing local transmission. Of these 95, the World Health Organization (WHO) estimates that 21 have the potential to achieve elimination by 2020 and 28 have declared a national policy of malaria elimination or are pursuing spatially progressive elimination within their borders. Recently, the governments of the countries of the Mesoamerican region (all of Central America plus the Caribbean islands) pledged to eliminate malaria in the region by 2020. Elimination in this region appears to be the most feasible of all the remaining endemic regions, with only 500 deaths from malaria reported in all of the Americas in 2015, most south of Panama. Mexico, El Salvador, Costa Rica, and Panama are in the elimination phase in 2016, defined as fewer than one case per 1,000 persons at risk per year, with Costa Rica reporting zero cases in 2014 while the four other Central American countries (Belize, Guatemala, Honduras, and Nicaragua) are considered among the more favorable targets for elimination. Guatemala, for example, has made significant progress in the past two decades. The estimated number of cases in Guatemala has decreased from over 50,000 in 2000 to fewer than 9,000 in 2012. The last documented death in Guatemala from malaria occurred in 2006. In the sparsely populated jungle region of Petén Norte, 5,512 cases of malaria were reported in 2006, including 1,000 cases of *Plasmodium falciparum* malaria. Following control measures, in 2012 in Petén Norte, there were only 38 cases of *Plasmodium vivax* and zero cases of *P. falciparum* (Global Fund for AIDS, TB and Malaria, personal communication, 2013).

Guatemala is divided into 27 health jurisdictions called “Departamento Áreas de Salud” (DAS); in 2012, over 70% of malaria cases occurred in two DAS: Escuintla, which borders the Department of Santa Rosa, and Alta Verapaz (Global Fund for AIDS, TB and Malaria, personal communication, 2013). Santa Rosa Department is in the southeast part of Guatemala with a population of approximately 340,000. Santa Rosa reported fewer than 75 cases of malaria per year from 2006 to 2011 with Annual Parasite Index of 0.22 (75 positive slides per year x 1,000/population of 340,000), and is therefore a candidate department for malaria elimination (Global Fund for AIDS, TB and Malaria, personal communication, 2013). Santa Rosa Department has had other recent health successes, including eliminating onchocerciasis in 2007 and eliminating *Rhodnius prolixus* and reducing prevalence of Chagas disease. From 2011 to 2012, however, the Santa Rosa DAS reported a sharp increase in the number of malaria cases from 12 to 165. The objective of this study is to describe the epidemiology of malaria in Santa Rosa from 2008 to 2013, to understand the origins of this increase in reported malaria cases and aid potential elimination efforts.

METHODS

Twenty DAS workers were interviewed by various study authors regarding the history of malaria control efforts. The members of the vector control team were interviewed as a focus group, whereas the DAS epidemiologist, DAS microscopist, and DAS director were interviewed individually as key informants. There was no compensation for participation. Key points of discussion were the history of malaria control efforts, as well as the impression of the workers and their reasoning behind their impression. Data were recorded and thematically analyzed, with analysis focused on information relevant to the large increase in cases in 2012.
The Department of Santa Rosa has three regions, each making up approximately one-third of the land area: a relatively high-altitude region of 1,300 m in the north; an intermediate region, containing the town of Barberena, a lake long suspected to harbor malaria-infected mosquitoes (the Laguna de Pino), and the department capital of Cuilapa at approximately 1,000 m; and a Pacific coastal plain (Figure 1).

Prior to 2012, data on patients in Santa Rosa Department who presented with symptoms to area hospitals or health centers and were subsequently smear-positive for malaria were reported to the DAS based in Cuilapa. Microscopy was performed at the health posts or centers, and paper forms were used to record demographic and illness data such as age, sex, town, date of symptom onset, and species of *Plasmodium*. The data from these paper forms were entered into a computerized database and stored at Cuilapa.

In July 2012, a more active surveillance program was initiated with support from the Global Fund to fight AIDS, TB and Malaria.9 The enhanced surveillance system included 1) the scale-up of the passive surveillance system through a network of volunteer collaborators by recruiting additional health “voluntarios” or community health workers (CHWs); 2) training CHWs to take blood smears from any patient with a fever; and 3) treating smear-positive patients for malaria with 3 days of chloroquine and 14 days of primaquine to obtain radical cure. Primaquine was added to the standard Guatemala regimen in 2011, although not with directly observed therapy.10 The strategy also included more active outreach to suspected high-risk communities for engagement, education, and examination of symptomatic individuals. The DAS hired 25 additional workers, increasing the team size from 25 to 50, 10 of whom were exclusively dedicated to malaria work, including data collection, data entry, and data management. Prior to this, the team of 25 vector control workers split its time between malaria, Chagas' disease, dengue fever, and other vector-borne illnesses, with no workers dedicated exclusively to malaria. To handle the increased number of blood smears, a reference microscopist certified by the Laboratorio Nacional de Salud was hired and provided with a new microscope. Although previously smears were read at decentralized health posts and centers, all slides beginning in April 2012 were sent to this microscopist in Cuilapa, and a subset of slides was sent to the national laboratory in Guatemala City for confirmation and quality control. Data continued to be registered on paper forms and transferred to the central database.

The mosquito control strategy changed as well in June 2012. Prior to 2012, it largely involved spraying insecticides and larvicides at high-risk breeding sites. The new strategy in July 2012 required the team to begin cleaning up breeding sites by removing vegetation in shallow water where *Anopheles pseudopunctipennis*, *Anopheles darlingi*, and *Anopheles albimanus* mosquitoes, the three predominant species in Guatemala, preferentially lay their eggs.11,12 *Anopheles pseudopunctipennis* is the predominant species in the inland region where most cases occur. The malaria-exclusive workers intentionally involved community members in this time-consuming work to transfer both skills and ownership to the local community. Use of larvicides at mosquito breeding sites and insecticides ceased on grounds that the harm to the environment and cost did not justify further benefit in mosquito control. Indoor residual spraying of houses continued, especially in outbreaks.

A detailed census of the entire Santa Rosa Department was also undertaken by the DAS in 2012–2013 to determine the number of people at risk for malaria. “At risk” was defined as living in a town with a malaria case in the preceding 12 months. These data and the data collected on slide confirmed cases were used for descriptive epidemiology from 2008 to 2013.

All analyses were carried out in Stata version 12.13 Relationships between rainfall and malaria cases were tested using various models for count data, with the number of cases per month as the dependent variable assuming near constant population month to month. Environmental
variables (rainfall in centimeters per month, daily average temperature, and interaction term of rainfall × temperature) were lagged by 0, 1, 2, or 3 months to reflect the approximate time necessary for mosquitoes to breed and infect people, and for disease to develop. Data on rainfall and temperature were obtained from the Guatemala National Institute of Meteorology. The models tested were random effects negative binomial using the Stata code xtnbreg (which controls for serial autocorrelation), autoregressive Poisson using one or two autoregressive terms (using the Stata code arpois because xtpoisson did not converge), ordinary least squares (OLS) with first differences (as the outcome variable was approximately normally distributed around zero), or OLS with first differences and clustering by month. The preferred model was selected by Bayesian information criterion and was the random effects negative binomial with lagged independent variables. First differences and the number of autoregressive terms were selected to control for serial autocorrelation based on examining partial autocorrelation plots, which indicated that nearly all autocorrelation could be accounted for by one autoregressive term or first differences. Maps were constructed using ArcGIS 10.1.

The ethics committee of the Universidad del Valle reviewed the protocol and the Division of Global Health Protection of the Centers for Disease Control and Prevention determined the protocol to be research not involving humans since databases did not include patient names.

RESULTS

From 2008 through 2013, 337 cases of malaria were reported. Fifty-four percent of reported malaria cases were in men (Table 1). The median age was 20 years; the Guatemalan median age is 20.7 years. Six percent of patients were less than 6 years of age while 86% of cases were 6–55 years of age. *Plasmodium vivax* constituted 331/337 (98%) of the cases. The last case of *P. falciparum* in Santa Rosa Department was reported in December 2010 (Table 1, Figure 2).

From 2008 to 2011, a steady decline occurred in the number of reported cases of all species in Santa Rosa (Figure 2), similar to the rest of the country (Global Fund for AIDS, TB and Malaria, personal communication, 2013). However, in 2012, a 13-fold increase was observed, from 12 cases in 2011 to 165 in 2012. Eighty-three percent of all cases in 2012 (137/165) were located within 2 km of the Laguna de Pino, a marshy lake near the town of Aldea El Cerinal at an altitude of 1,080 m, a known high incidence focus based on previous reporting (Figures 1 and 3). The incidence in Aldea El Cerinal in 2012 was 12.3 cases per 1,000 person-years. From 2009 to 2011, 75% of cases (59/79) in Santa Rosa Department occurred in this town, and 99% (78/79) occurred in persons whose reported home was within 6 km of the lake.

A new surveillance strategy began in April 2012, and interventions such as removal of vegetation began from mosquito breeding sites, as identified by the community under the direction of the DAS team. While in the four...
previous years the proportion of cases in the first and second half of the year were approximately equal, in the second half of 2012, 111 cases were reported compared with 54 cases in the first half. Additionally, in 2012 cases were reported in towns where no cases had been reported through the previous passive detection system (i.e., in the towns of Oratorio, Chiquimulilla, and Aldea el Hawaii, all on the coastal plain) (Figure 3). The number of reported cases peaked in July 2012 with a steady downward trend since then (Figure 4). The slide positivity rate (SPR) decreased from 7.6% in 2012 to 4.6% in 2013 (2,171 and 913 slides, respectively). SPR was not available before 2012.

To evaluate the contribution of the intervention year and control for climatic variables such as rainfall and temperature, count-based regression models that controlled for autocorrelation were used (Supplemental Table 1). A dummy variable for the intervention year showed a consistent and significantly positive association with case reporting.
There were no statistically significant relationships between climate and case reporting, after controlling for serial autocorrelation, under a variety of model specifications.

To fully deduce the effect of the various new malaria control strategies that were implemented, qualitative data revealed important insights to complement the quantitative findings. In a focus group discussion, the DAS workers identified their new ability to devote time exclusively to the many activities necessary for detection and control of malaria as the most important change from 2011 to 2012. Funding allowed them to hire more workers, and devote 10 workers exclusively to malaria. Previously they had to divide their time between all vector-borne diseases in Santa Rosa, which made access to coastal parts of Santa Rosa Department difficult and infrequent. Unanimously, they believed that the number of cases they were finding near Aida el Cerinal, in addition to cases in other parts of the DAS, were simply not being detected. The new strategy allowed sufficient resources to do active surveillance to discover additional cases. They reported detecting malaria infection in febrile patients who previously might not have approached the local community health volunteer or the clinic. They are also optimistic about future adherence to bed net usage as a result of increased engagement, since previously bed net usage, even in Aida el Cerinal, was uneven.

Of the 165 cases in 2012, 49% were reported through the community health volunteers. This represents a slight but nonsignificant increase in reporting by these volunteers; from 2008 to 2011, their contribution was 45% of the reported cases. The other sources of reported cases in 2012 were the local hospital (8%), local health centers (28%), DAS workers (8%), and others (7%).

The microscopist indicated that some of the malaria burden results from imported labor for agriculture. Near the Laguna de Pino, labor is imported for coffee plantations that harvest from January to March, and to the coastal area for sugar cane harvesting from October to April. Many workers come from the neighboring department of Escuintla, which has the highest malaria incidence in Guatemala, as well as the departments of Petén, Quiche, and Alta Vera Paz, which have malaria as well. He expressed concern that total elimination of malaria will require malaria control among seasonal workers infected with malaria.

**Discussion**

A region of Guatemala targeted for malaria elimination reported a 13-fold increase in cases in 2012, the same year that the local health department increased the intensity of its surveillance and control efforts with financial support from the Global Fund to Fight Malaria, TB and HIV. This analysis was undertaken to determine the cause of this increase and what it might mean for elimination efforts in Santa Rosa and in Guatemala more broadly. We did not find a specific weather event, major new focus of malaria, change of species, misdiagnosis, or occupational changes to account for this increase. Our findings suggest that this increase in cases may be the result of a strengthened health system including increased detection, improved diagnostics, and improved reporting, which may thus suggest progress toward elimination.

Differentiating between increased surveillance and a true outbreak as the cause of the large increase in reported cases required using both the qualitative and quantitative analyses. The DAS workers unanimously believed that the cases found in 2012 most likely represented a true malaria burden previously undetected or reported, based on their impressions of the baseline health status of the population near the Laguna de Pino and what their previous set of resources restricted them from reaching. The quantitative data support their impression. From 2009 to 2011, only one case was reported more than 6 km from the Laguna de Pino, similar to the results from 2012 to 2013.

The conclusion that the sharp increase in cases near the Laguna de Pino represents steps toward elimination, rather than an outbreak, is buttressed by the continued decline in cases after its initial peak through the end of 2013. SPR decreased from 2012 to 2013 despite a similar number of slides collected, suggesting no significant change in true malaria incidence (SPR was unavailable for 2011 and earlier). While during the rainy season (June–November) in 2012 a combined 125 cases were reported, in the same months of 2013 only 17 cases were reported. Another sign of malaria control progress in Santa Rosa was that, despite the increased detection, no case of *P. falciparum* was recorded. Santa Rosa has therefore not had a reported case of falciparum malaria during a period of 3 years, the necessary time to declare elimination. *Plasmodium falciparum* is still endemic in Guatemala constituting < 5% of cases, particularly in the neighboring department of Escuintla.

The factors that enabled this success merit detailed discussion as a possible set of lessons learned for other countries or regions beginning malaria elimination efforts. This study revealed five key factors as contributing to the discovery and elimination of previously undetected cases. First, according to the DAS, the most important change has been their ability to devote time exclusively to the many activities necessary for detection and control of malaria. With funding from the Global Fund the vector control team doubled their workforce, and devoted 10 workers exclusively to control of malaria. Such manpower translated into greater engagement with the community, better education of malaria workers and the general population at schools, churches, community events, military activities, and a weekly radio program. In addition, the team could also devote to time-consuming practices such as encouraging community involvement of vegetation clearance, greater recruitment and training of CHWs, more comprehensive data collection and analysis, and more prompt delivery of services. This will only increase in impact if sustained over time.

Second, surveillance intensity increased across the entire department, from the high-altitude northern region to the coastal plain. Recruitment of community health volunteers was most vigorous in areas suspected of being at high risk. These volunteers were recruited in every major town or group of villages, and cases were reported in 2012 in towns and cities where no cases had been reported for years (Figure 3). The increase in workers occurred just before the increase in case detection while there were no environmental changes clearly associated with this increase. Although it might have been expected that the increased number of
community health volunteers were those reporting the new cases, the proportion of cases reported by these volunteers remained the same. Thus, the CHWs’ most meaningful impact may have been outreach, education, and vegetation removal, rather than simply surveillance.

Third, the ability to hire a well-trained microscopist to work full time at the regional DAS allowed them to centralize and streamline the handling of blood smears, case reporting, and quality control. Increased technical capacity of human capital would be expected to increase the overall quality of a malaria control program. This input was further augmented by placing microscopists in two health clinics (Taxisco and Chiquimulilla) along a major transportation artery (Highway 2) where immigrant sugar cane workers congregate (Figure 1). An additional microscopist was strategically placed in Barberena based on epidemiological data related to Laguna de Pino.

Fourth, the Global Fund model of financial support with local leadership and implementation showed clear value, although further data collection will be necessary to continue to evaluate its effectiveness. Additionally, with the possibility of scarce funding and subsequent “donor retreat,” there is a real risk that countries could lose financial support from the Global Fund just as they approach elimination, in favor of countries with higher disease burden. Policymakers should plan to avoid either this eventuality or its consequences, and be prepared to push forward toward malaria elimination.

Fifth, the shoreline of the Laguna de Pino facing the town of El Cerinal presented over 2 km of shallow water with ample vegetation ideal for breeding large numbers of mosquitoes. July 2012 was the first time that local residents recall these breeding sites had been completely removed from the lake. The cleaning was accomplished over a period of weeks and included labor from the community and the DAS workers exclusively dedicated to malaria control. This labor-intensive effort was critical in turning a lake temporarily clear of all shallow vegetation. This environmental activity will significantly impede the malaria-transmission cycle at least temporarily, and may provide evidence that such efforts could prove essential in other areas of Central America. Efforts to maintain the waters free of mosquito breeding areas in the tropics will be critical in turning a lake temporarily clear of all shallow vegetation. This environmental activity will significantly impede the malaria-transmission cycle at least temporarily, and may provide evidence that such efforts could prove essential in other areas of Central America.

The history of malaria resurgence after the Global Malaria Eradication Program (1955–1969) urges caution and continued emphasis on active surveillance for cases and interruption of transmission in Santa Rosa Department. In a tropical area that seasonally receives approximately 2 m of rainfall, has many poor households that do not reliably use bed nets despite windows that provide easy entry for mosquitoes, and has significant migratory labor from malaria-endemic regions, the natural and socioeconomic environment favors the persistence of malaria and other mosquito-borne infections unless adequate control measures are sustained. Based on this study, we have four main recommendations for Santa Rosa and similar settings.

First, the town of Aldea El Cerinal should consider consistently using bed nets and indoor residual spraying. Second, a case–control study to determine risk factors for malaria such as occupation or domestic activities, such as washing clothes in the lake, income, use of protective measures, and proximity to the Laguna de Pino would inform which control efforts may be most urgent. Third, continued surveillance and full treatment with chloroquine and primaquine (which was added to the standard Guatemala regimen in 2011), possibly with the inclusion of directly observed therapy, are important activities in ensuring that the observed gains are preserved and progress toward malaria elimination continues. Fourth, though funding from the Global Fund proved essential for resourcing the necessary technology and human capital to bring Santa Rosa close to elimination, sustainability there and across Guatemala would require considerable and regular disciplined community-based activities. Additionally, although vegetation removal for vector control is a common practice, one study indicated that it can be associated with the replacement of one mosquito species with another. Entomological evaluations of whether the current activities have reduced mosquito populations are required.

The qualitative analysis pointed out several ongoing challenges to achieving elimination in DAS, such as Santa Rosa, if pockets of malaria remain endemic in the country (such as neighboring Escuintla). Because of the movement of labor and the indolent nature of P. vivax, it is possible that a DAS close to elimination will continue to have new cases imported from other parts of Guatemala and thus maintain endemicity. The DAS workers also identified challenges in advocating consistent bed net usage as bed nets block cooling air currents, may get torn, may be difficult to install, and may be obtrusive during daylight hours. For these and many other reasons mentioned earlier, commitment to elimination will have to be sustained over many years, and draw heavily on local expertise in design, implementation, and elimination program monitoring.

Finally, while we observed no major weather event such as Tropical Storm Agatha of 2010 that created massive flooding in the area, the relationship between rainfall and temperature and malaria incidence was nonlinear and complex, and thus we cannot rule out a climatic contribution to the change in incidence. However, given all the other evidence presented here, weather was likely not the main contributor to the sharp increase in cases. Also, our regression analyses controlled for temperature and rainfall, as well as their interaction.

LIMITATIONS

Our study had several limitations. Since the method for detecting and reporting cases changed so markedly in 2012, it is difficult to know whether the increase was not at least partially due to a true outbreak of disease, rather than merely increased surveillance. Also, although there is increased outreach by community health volunteers to take blood smears from people with symptoms, it is difficult to know how many infected persons cases are still missed because of subclinical infection in semi-immunes or in persons with relapsing P. vivax malaria. Finally, whether mosquito or larval burden decreased in the Laguna de Pino and other fresh water sources after vegetation removal was not surveyed.

RECOMMENDATIONS

The history of malaria resurgence after the Global Malaria Eradication Program (1955–1969) urges caution and continued emphasis on active surveillance for cases and interruption of transmission in Santa Rosa Department. In a tropical area that seasonally receives approximately 2 m of rainfall, has many poor households that do not reliably use bed nets despite windows that provide easy entry for mosquitoes, and has significant migratory labor from malaria-endemic regions, the natural and socioeconomic environment favors the persistence of malaria and other mosquito-borne infections unless adequate control measures are sustained. Based on this study, we have four main recommendations for Santa Rosa and similar settings.

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will be required to attain the goal of making Central America the next world region free of malaria.

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