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Aedes aegypti density and the risk of dengue-virus transmission

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Abstract

Using genetically modified mosquitoes to control vector-borne diseases will require specific, quantitative targets for the extent to which populations of competent mosquito vectors need to be reduced in order to produce predictable public-health outcomes. Unfortunately, dengue researchers do not have an entomological measure for predicting the risk of human dengue infection and disease that is as effective as they would like. The situation is further complicated by the fact that contemporary dengue control is based on the assumption, which has not been thoroughly tested, that a reduction in adult *Aedes aegypti* population densities will decrease risk of virus transmission. *Ae. aegypti* eradication is not considered feasible and there are no commercially available dengue vaccines or clinical cures. Herein we discuss four interrelated questions that need to be addressed for the proper evaluation and implementation of genetically modified mosquitoes for dengue control. In specific terms, what is an acceptable level of dengue risk? What are the mosquito densities necessary to achieve that goal? What is the best way to measure entomological risk? Because most dengue risk factors are likely to exhibit spatial dependence, at what geographic scale are the components of dengue transmission important? We conclude with two recommendations for improving dengue surveillance and control. First, there is an urgent need for field-based prospective longitudinal cohort studies on the relationships among measures of *Ae. aegypti* density, dengue incidence, and severity of disease. Second, new rapid, inexpensive, and operationally amenable methodologies are needed to evaluate and monitor the impact of vector-control strategies on disease reduction. Unless competent mosquito vectors are eliminated entirely, predicting and evaluating success following release of genetically modified *Ae. aegypti* will require a more thorough understanding of the relationship between vector density and the risk of human disease.

Keywords: *Aedes aegypti*; dengue; arbovirus; transmission; risk; surveillance; control; transgenic mosquito

Introduction

The conceptual basis for the population-replacement paradigm of mosquito-borne pathogen control is that human disease will be decreased following the introduction of

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a gene into a mosquito population that confers resistance to the pathogen (James 2000). The goal is to replace an existing susceptible mosquito population with one that is composed of refractory conspecifics by altering vector competence, or the capacity for mosquitoes to become infected with and transmit a pathogen. Modification of the structure of an existing population is a strategic departure from earlier genetic tactics, which aimed to reduce mosquito density by interfering with their reproduction (see chapters by Reisen and Lounibos elsewhere in this volume). Although details of the two approaches are different, the desired outcomes are equivalent;i.e., to reduce the number of competent mosquito vectors and, as a result, disease in humans will decrease.

Successful application of population replacement will rely heavily on knowledge of relationships among mosquito density, human infection with the mosquito-borne pathogen, and severity of human disease. Those associations become unimportant only if population replacement is perfect; that is, all competent mosquitoes are eliminated and immigration of competent mosquitoes is permanently prevented. Short of competent vector eradication, understanding the relationship between mosquito density and human infection is critical for a realistic probability of disease prevention. Successful application of population replacement will require specific, quantitative targets for the extent to which populations of competent vectors need to be reduced in order to produce predictable public-health outcomes (Scott et al. 2002).

In this chapter we discuss the relationship between density of *Aedes aegypti* and human dengue virus infections. Although critical for successful application of population replacement, the material covered has broad application that transcends genetically modified mosquitoes (GMM). It is relevant to any method that is intended to reduce disease by reducing, but not eliminating, *Ae. aegypti* populations. When appropriate we raise important unanswered questions, make recommendations for how knowledge gaps could be filled, and suggest which new methodologies would be most helpful. To place this discussion in its proper perspective, it must be noted that vector density is one of many topics related to a thorough evaluation and application of population-replacement technology. Other issues – for example, spread and stability of introduced genes, the evolutionary consequences of mosquito transformation, quantitative assessments of mosquito biology and disease control, and components of vector capacity other than vector density – are discussed elsewhere in this volume and in recent publications (Scott et al. 2002).

Relationships between mosquito density and pathogen transmission

Malariologists (Charlwood et al. 1998) have been more successful than dengue researchers in relating vector density to infection and disease (Scott et al. 2002). The entomological inoculation rate (EIR), which is defined as the number of sporozoite-infected mosquitoes biting a person per unit of time, is a robust measure of entomological risk for transmission of malaria parasites. Recent prospective epidemiological studies produced valuable insights into the relationships between EIR and malaria morbidity and mortality (Smith, Leuenberger and Lengeler 2001). Results were not always intuitively obvious, fortifying the need for additional field-based studies that directly link entomology with epidemiology. For example, in southern Tanzania the risk of human infection increased with the EIR when the prevalence of human infection was low (Boëte and Koella 2003). But when parasite prevalence was high, transmission became saturated and an increase in EIR did not result in a rise in detection of parasites in infants. Another concern is that long-term vector-density

reduction will drive mosquito abundance toward the minimal entomological threshold and result in unstable malaria transmission. When this happens, a higher proportion of people will survive to adulthood without being infected with malaria parasites. Primary infections in adults will result in more severe disease than if the initial infections were predominately in children who developed immunity and consequently experienced less severe disease as adults. This phenomenon is known as the "rebound effect" and is a possible adverse outcome that can occur when pathogen transmission is reduced but not eliminated. The goal of a GMM release, similarly, would most likely be to reduce the number of new human infections rather than to eliminate them. Recent results from bed-net studies indicate, however, that reduction in contact between humans and infected mosquitoes does not result in a detectable increase in severity of disease, even after years of bed-net usage (Maxwell et al. 2002). A complicating factor for malaria, that is less of a problem for dengue, is that because parasites in a given location are often transmitted by more than one mosquito species, rendering one species or chromosomal form resistant will not prevent transmission by other, non-transformed mosquitoes. The relative contribution of different mosquito populations to the local burden of malaria will need to be understood to predict the impact of a GMM release. Overall, an improved understanding of the relationships among various measures of entomological risk, human infection, and disease prevalence and incidence would be important contributions for strengthening predictions for the effect of population replacement on malaria transmission.

Unfortunately, dengue researchers do not have an entomological measure for predicting the risk of human infection and disease that is as effective as the EIR is for malaria risk predictions. Virus infection rates in *Ae. aegypti* are typically too low (Kuno 1997) to base a surveillance program on an EIR or its equivalent. This is especially true when one considers the currently available technology for collecting adult female *Ae. aegypti* and detecting virus in them. In addition, sterilizing immunity that follows a dengue-virus infection, which is different from the non-sterilizing response associated with malaria, can lead to spatially and temporally explicit patterns of virus transmission. For example, the probability of transmission will be low in an area regardless of the magnitude of measures of entomological risk, if human herd immunity is high. Conversely, if herd immunity is low, relatively low population densities of *Ae. aegypti* could precipitate an epidemic.

In a large, cohort-based prospective study Morrison and Scott (unpublished data) determined that traditional indices for *Ae. aegypti* density are correlated with prevalence of human dengue infections, but are at best weakly correlated with the incidence. The relationship of *Ae. aegypti* indices to the diversity of dengue-related disease is essentially unknown. Inconsistent associations from research prior to that of Morrison and Scott may in part be attributable to relatively small sample sizes and short durations of study. Moreover, *Ae. aegypti* survive and efficiently transmit dengue virus even when their population densities are remarkably low (Kuno 1997). Efficient virus transmission at low vector densities has been attributed to this mosquito's propensity to imbibe blood meals almost exclusively from humans and to do so frequently (0.6 – 0.8 meals per day), something that increases their contact with human hosts and as a result enhances their opportunities for contracting or transmitting a virus infection (Scott et al. 2000a).

Reduction of vector populations is currently the only way to prevent dengue because there is no commercially available vaccine or clinical cure. A successful population-replacement strategy that reduces mosquito density below threshold levels but falls short of *Ae. aegypti* eradication will reduce human herd immunity and

accordingly increase the risk of virus transmission. Minimal entomological thresholds for dengue, which are low even without vector control (Kuno 1997) because of *Ae. aegypti*'s unusually efficient ability to transmit virus, will need to be reduced to even lower levels. A public-health program that requires decreasingly smaller numbers of competent mosquito vectors will be difficult to manage operationally and, more importantly, to sustain over any considerable period of time. Nevertheless, without information on the relationship between vector density and disease risk dengue prevention programmes will lack specific targets for vector densities. Accordingly, predicting and validating entomological thresholds is one of the most important contributions that could be made to our understanding of dengue epidemiology and the application of population-replacement strategies for disease prevention.

Entomological assumptions of dengue control

Methodologies for the surveillance and control of *Ae. aegypti* are rooted in techniques that were developed for mosquito eradication in order to prevent yellow fever. In the 1950s and 1960s a hemisphere-wide campaign to eradicate *Ae. aegypti* was initiated in the New World (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994). The programme was successful, eliminating the mosquito from most of Latin America. It relied heavily on vector surveillance to detect the presence or absence of *Ae. aegypti*. Although programmes to eliminate *Ae. aegypti* helped define the value of vector eradication for disease prevention, eradication efforts provided little insight into the quantitative relationships between mosquito abundance and dynamics of virus transmission (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994; Gubler and Kuno 1997; Reiter and Gubler 1997). For a variety of reasons – including changes in political and public-health priorities, changes in human demographics, increases in human travel, mosquito resistance to insecticides, and, perhaps most importantly, the inability to sustain the funding and infrastructure requirements of eradication – reinfestation of cleared areas began in the 1970s. Subsequently, in 1994 the Pan-American Health Organization (PAHO) declared eradication of *Ae. aegypti* an unattainable goal (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994).

The new goal of dengue prevention and control programmes became the "cost-effective utilization of limited resources to reduce vector populations to levels at which they are no longer of significant public-health importance" (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994). The implicit assumption of this approach is that a reduction in the adult *Ae. aegypti* populations will decrease risk of virus transmission. In fact, it could be interpreted that any reduction, no matter how small, will reduce disease (Reiter and Gubler 1997). Although this recommendation makes intuitive sense, it is not precise enough to be applied in an operational context. How should public-health entomologists identify and then reduce mosquito populations to the level at which they are no longer significant? Vector-control programmes require a different kind of surveillance from the presence or absence criteria used in the eradication paradigm. For control the objective is to maintain *Ae. aegypti* populations below or close to minimal transmission thresholds, slow the force of dengue-virus transmission, and reduce sequential infections with heterologous serotypes, which are positively associated with increased risk of severe disease (Vaughn et al. 2000). The fundamental premise is that disease can be managed by reducing *Ae. aegypti*

population densities. However, no well-controlled field studies have been carried out to define the dynamic relationships between *Ae. aegypti* density and human virus infections. Assumptions about the relationship between mosquito density and dengue risk were not necessary for eradication programmes. They are, however, essential for a vector-control strategy, including those based on the release to GMM, and as such they must be defined and validated.

The shift in focus from mosquito eradication to control prompted a re-evaluation of *Ae. aegypti* surveillance techniques. Unfortunately, associations between existing indices and dengue transmission have not proven to be satisfactorily predictive. This may be because the most commonly applied indices are based on easy to sample immature *Ae. aegypti* that do not transmit virus. Only adult females transmit virus, and because they do not enter standard mosquito traps they are difficult to collect in the context of a geographically diverse surveillance programme. Development of new methodologies to collect adult *Ae. aegypti*, especially females, for surveillance purposes would be a most valuable contribution to dengue prevention including the evaluation of the population replacement strategies.

A successful dengue-control programme based on vector control will require that four interrelated questions be answered (see Figure 1). First, in specific terms, what is an acceptable level of dengue risk? Second, what are the mosquito densities necessary to achieve that goal? Answering this question requires a detailed understanding of the
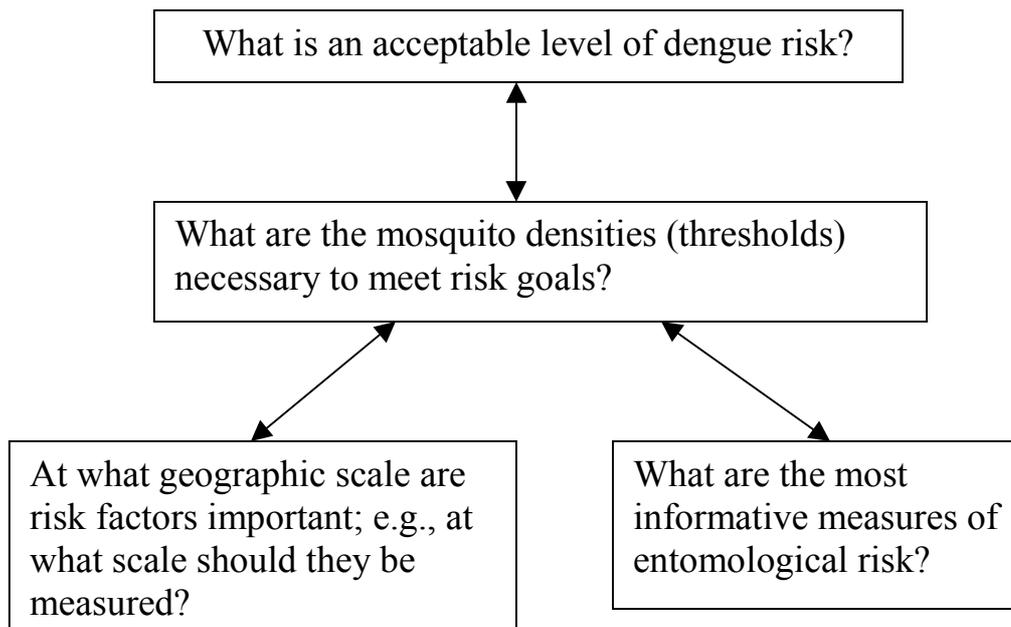

Figure 1. Four interrelated questions for determining entomological thresholds for prevention of dengue virus-related illness.

relationship between mosquito density and dengue transmission, so that one can predict the public-health impact of specified reductions in the abundance of competent mosquito vectors. Third, what is the best way to measure entomological risk? By this we mean identification of methods and survey designs. Fourth – because most dengue risk factors are likely to exhibit spatial dependence – at what geographic scale are the components of dengue transmission important? Because parameters and processes important at one scale are frequently not important or not predictive at another scale

(Liebhold, Rossi and Kemp 1993), the appropriate geographic scale must be identified for important dengue risk factors.

The remainder of this chapter is a review of key elements of these issues with our suggestions for how they can be clarified. A fundamental component that is woven throughout our discussion is that the answers to these questions can and should be derived from field-based research.

What is an acceptable level of dengue risk?

Defining an acceptable level of dengue risk will be a complex and dynamic process that will depend on the resources, public-health priorities, and history of dengue in the country or region affected. A likely acceptable and overreaching goal will be the desire to prevent large, explosive epidemics. The objective will be to reduce the force of virus transmission. In order to understand transmission well enough to predict outcomes of interventions with reasonable certainty, considerably more needs to be learned about the relationship between transmission dynamics and severe disease. This should be a priority for study. In practice, public-health officials will most often set goals based on the individual needs of their country or region. Goals will need to be dynamic; that is, they will need to fluctuate as virus transmission and successes in disease prevention rise and fall. So that, for example, goals could range from no deaths in a community to no hospitalizations to no children missing school with a dengue illness to specified reductions in any of these outcomes. It cannot be overemphasized that the goal will be to prevent disease, which varies in severity and is not always a consequence of infection; some dengue infections are asymptomatic. Characterizing the relationship of mosquito density to human infection will be easier than with disease. Infection is a binary yes or no response. Disease is a continuous variable with multiple different outcomes ranging from the absence of disease to death. Defining these relationships will constitute the informational basis for the development of effective public-health policy and surveillance.

What are the mosquito densities (thresholds) necessary to meet risk goals?

A conceptual representation of the relationship between mosquito vector density and the risk of a person being infected with an arbovirus is illustrated graphically in Figure 2. In this scenario there are two thresholds. The maximum threshold is a density above which additional mosquitoes will not increase the risk of human infection because the system is saturated. Conversely, at densities below the minimum threshold the risk of infection does not decrease because there are too few mosquitoes to sustain transmission. Transmission has ceased or if virus is introduced its basic reproductive rate is always less than 1 and it fails to persist. Between those two densities it is predicted that there is a functional relationship linking density and risk, such that reduction in mosquito density results in a corresponding decrease in infection risk. Figure 2 represents one possible example of what is a dynamic and complex association. That is to say, the relationship is not a single curve. Instead it is a theoretically infinite series of different-shaped curves representing different circumstances and conditions. We expect that the shape of the curve, or the nature of the relationship between density and risk, will vary temporally and spatially depending on factors like human herd immunity, density of human hosts, characteristics of mosquito-human interaction, virus introductions into the system, virulence of virus strains, and weather – for example, temperature and relative humidity – that affect mosquito biology and mosquito-virus interactions.

Figure 2. A graphical representation of the relationship between *Aedes aegypti* density and the risk of a person being infected with dengue virus.
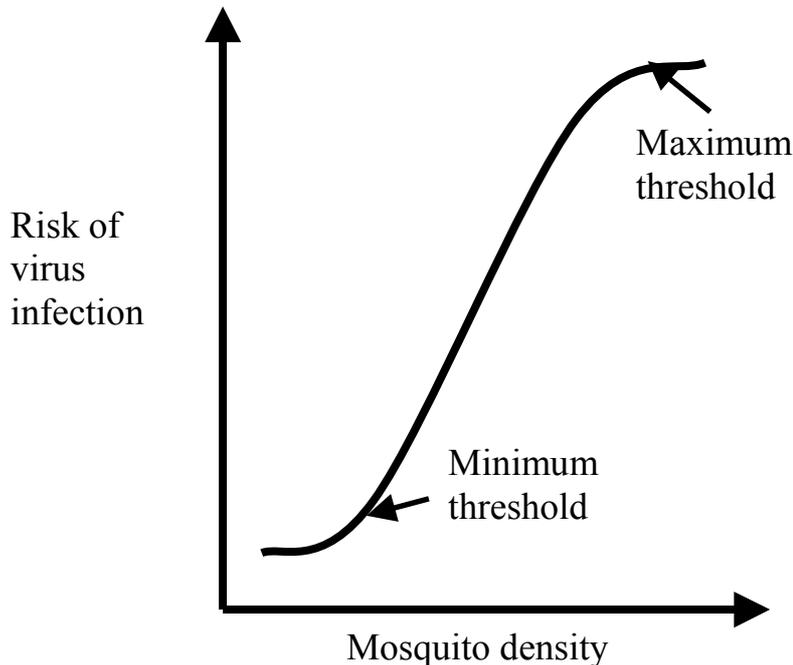
Empirical derivation of a minimal entomological threshold for arbovirus transmission, to the best of our knowledge, was done only once before and it did not include dengue. From 1965 to 1967 in southern California W.C. Reeves and his colleagues (Reeves 1971) determined the relationship between the density of *Culex tarsalis* and transmission of western equine encephalomyelitis (WEE) virus. Mosquito densities were reduced by the application of insecticide over nearly 1,500 square miles in Kern County. The number of mosquitoes collected in bird-baited traps and New Jersey light traps (NJLT) were used as relative measures of mosquito density. The magnitude of virus transmission was estimated based on infection rates of *Cx. tarsalis* and detection of WEE-specific antibodies in sentinel chickens. Reeves concluded that when less than 1 mosquito was captured per trap per night, virus transmission had been reduced to the extent that it could not be detected. When 2 - 9 mosquitoes were captured per night, low levels of transmission could be detected. When more than 10 mosquitoes were captured, transmission increased and the risk of human or equine infection rose. A similar analysis with St. Louis encephalitis (SLE) virus indicated that thresholds were higher than for WEE – transmission was undetectable when mosquito densities were above 10 per trap per night – suggesting that transmission of SLE was less efficient than WEE. Olson et al. (1979) reported similar results for their retrospective analysis of relationships between NJLT and human-incidence data collected from 1953 to 1973 throughout California. These are important studies because they demonstrate the utility of relative measures, as opposed to the prohibitive requirement of absolute counts, of mosquito vector density for predicting transmission risk. As such, mosquitoes captured per unit time can be used to develop, apply, and evaluate novel techniques, like GMM, and public-health policy for reducing arboviral disease.

Establishing epidemiologically significant levels of entomological indices for dengue has been elusive (see Table 1). In urban areas in Latin America, a container index (CI) of less than 10 (Conner and Monroe 1923) or a house index (HI) of < 5

(Soper 1967) was considered a prophylactic level for yellow-fever transmission. During a yellow-fever epidemic in Diourbel, Senegal, transmission occurred only in areas where the World Health Organization density index was > 5 (Brown 1977). PAHO (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994) recognizes three levels of infestation for dengue transmission: low (HI < 0.1%), medium (HI = 0.1-5%), and high (HI > 5%). These estimates were similarly obtained retrospectively. They require empirical verification because results from field studies indicate that there is an inconsistent relationship between larval indices and virus transmission rates (Focks and Chadee 1997).

Table 1. Estimates of dengue entomological thresholds.

Index	*Aedes aegypti* density		Author
Container index	≤ 10%	Safe zone for yellow-fever transmission	Connor and Monroe 1923
House index	< 5%	Prophylactic for yellow fever	Soper 1967
Breteau index	< 5	Absence of yellow-fever transmission	Brown 1977
House index	> 15%	Dengue hemorrhagic fever present	Brown 1977
Pupae per Person	1.05 – 0.26	Unable to sustain dengue transmission	Focks et al. 1995, 2000

In the past 30 yrs, two countries – Cuba (Armada Gessa and Figueredo Gonzalez 1986) and Singapore (Chan 1985) – have instituted successful dengue-control programmes. At both locations control was vertically oriented and incorporated source reduction, space spraying, health education, and law enforcement – there were negative consequences for non-compliance. In response to a serious dengue epidemic in 1981, Cuba reduced the national HI from 35 to 0.2. Since then, maintaining a HI of <0.01 has prevented dengue (Guzmán et al. 1999). Before control programmes were instituted in Singapore, DHF was most prevalent where HI were >15 (Brown 1977). With control, HIs were reduced from 25 to < 5 by 1973 and to < 1 by the mid-1980s. Although dengue transmission persists and has been increasing since 1986, the incidence of dengue in Singapore remains considerably lower than in neighbouring countries.

We can safely expect that transmission thresholds will vary depending on a variety of factors. The extent of dengue transmission is determined by the level of herd immunity in the human population to circulating virus serotype(s); virulence characteristics of the viral strain; survival, feeding behaviour, and abundance of *Ae. aegypti*; climate; and human density, distribution, and movement (Focks et al. 1995; Kuno 1997). An important unanswered question in this regard concerns whether all these factors need to be measured to predict risk or, if the complexity of the system can be reduced, what kind of information needs to be gathered for which components? One way to address these issues is through the application of quantitative models.

A differential equation model of dengue transmission was developed to estimate the basic reproductive rate of dengue and to evaluate the relative merits of different insecticide and source-reduction control strategies (Newton and Reiter 1992). Results from the model reinforce conventional thoughts about the role of herd immunity in dengue-transmission dynamics. As herd immunity increased, higher mosquito densities were needed to support dengue transmission. For example, at >80%

immunity, no transmission occurred, even when mosquito densities were high (5 mosquitoes/person). Below 80% immunity, significant transmission could occur at increasingly lower vector densities. Thus, with herd immunity at 50%, about 40% of the human population can be infected at a density of 3 mosquitoes/person, whereas with herd immunities at 10%, a similar number of cases can occur at less than 1.5 mosquitoes/person. The authors pointed out that these conclusions require validation with field data.

During the early to mid-1990s two computer simulation models were developed that can be used to estimate dengue entomological thresholds (Focks et al. 1993a; 1993b; 1995). We will refer to them as the container-inhabiting mosquito simulation model, which led to development of the dengue-transmission simulation model. The mosquito model is a habitat- and weather-driven accounting programme of the population dynamics of *Ae. aegypti*. The dengue model accounts for the dynamics of a human population driven by country- and age-specific birth and death rates.

As part of a validation of the dengue model, data from a 1978 dengue epidemic in Honduras were examined (Focks et al. 1993b). During the epidemic, a positive correlation between *Ae. aegypti* indices and seroprevalence of dengue antibody was observed, which suggests that low mosquito densities prevented dengue transmission in unaffected communities (Figueroa et al. 1982). Simulations with lower mosquito abundances indicated that this explanation was plausible. Transmission thresholds for the Honduras site were estimated to be something less than 0.5 female *Ae. aegypti*/person, which corresponded to 0.25 pupae/person. Thresholds are expected to vary in association with fluctuations in ambient environmental conditions, especially temperature, and seroprevalence of the human population to circulating or introduced viruses (Focks et al. 1995; Focks 2003). These results indicate that minimum entomological thresholds for dengue are low and dynamics in the relationship between mosquito density and human infection are complex.

There is clearly a need for *Ae. aegypti* control guidelines that include realistic, quantitative goals. It is not enough to say we must reduce the mosquito population. We need to specify how much the population must be reduced and what effect that will have on virus transmission. With limited, and sometimes dwindling, resources there are distinct advantages to being able to target locations or components of the mosquito's life cycle/behaviour where intervention can do the most good. Simulation models are a tool for examining the relationship between mosquito abundance and dengue incidence, for identifying container habitats in which mosquito larvae are most numerous, to estimate the impact of removing containers on mosquito abundance and dengue transmission, and to estimate transmission thresholds.

Simulations with data from Iquitos, Peru, illustrate how the mosquito and dengue models can be used to characterize risk of virus transmission in geographically distinct areas. Although limited by sample size and a lack of temporal concordance with weather, seroprevalence and entomological data, this example nevertheless supports the notion that there is spatial heterogeneity of dengue risk in a single city. Entomological data were collected during 1994-1995, serologic determination of prevalence and incidence rates were carried out in 1993-1995, weather data were recorded during 1987-1991, and age-specific human demographic data came from 1993. In two neighbourhoods (Maynas and Tupac Amaru), we set the following starting parameters: a dengue-1 viremic person was introduced into the community once a month. Entomological surveys indicated that the number of pupae/person/ha was slightly higher in Maynas (0.40) than in Tupac Amaru (0.32). Similarly, the average number of adult female *Ae. aegypti*/person/ha generated by the mosquito

model was 0.64 in Maynas and 0.60 in Tupac Amaru. For each neighbourhood we ran 10 simulations. Because stochastic events are sometimes important in the early phases of a mass-action phenomenon such as an epidemic, repeated simulations with the same starting conditions do not always result in similar outcomes. This is most likely to occur as conditions become intermediate between favouring the loss or the rapid expansion of transmission; that is, when the lower transmission threshold is approached.

Results from pre-intervention simulations are shown in panels A and C of Figure 3. Each point on the graph represents the dengue incidence rate (cases/1,000 population/120 days). At both sites, significant dengue-1 transmission was observed. Overall transmission rates were higher in Tupac Amaru, probably because the human population density was higher than in Maynas (353 versus 311 persons/ha). In both populations, dengue-1 epidemics occurred in most simulations.
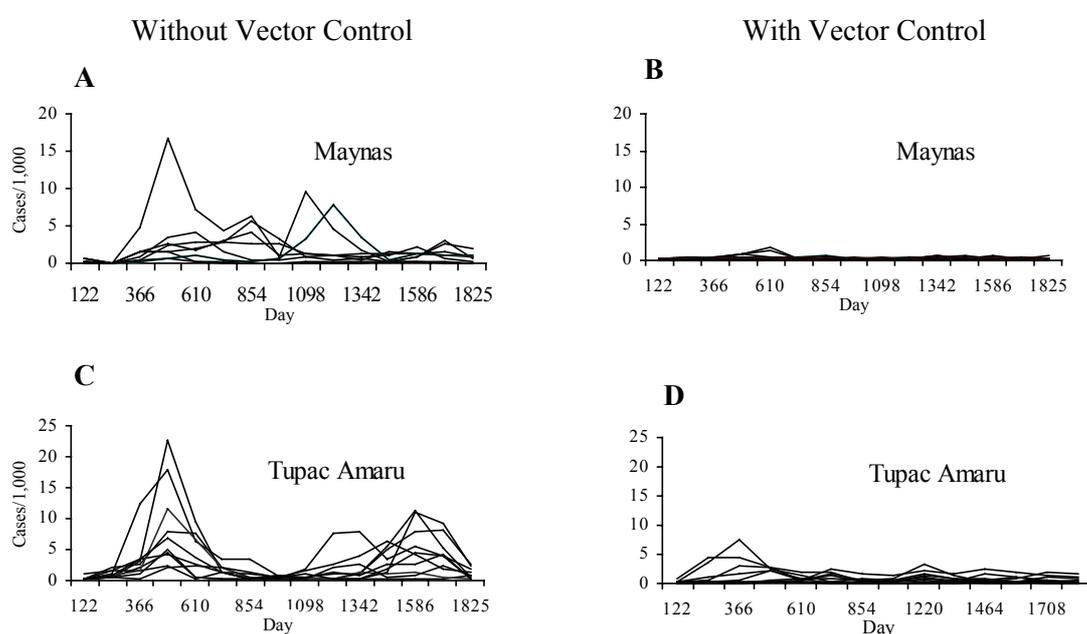

Figure 3. Results of 10 computer simulations of the impact on dengue-1 virus transmission of *Ae. aegypti* control in Maynas and Tupac Amaru neighbourhoods of Iquitos, Peru. The model was set up to introduce dengue-1 virus one time per month per yr (day 120). (A) Maynas dengue-1, before insecticide or source-reduction interventions, pre-treatment. (B) Maynas dengue-1, after insecticide or source-reduction interventions, post-treatment. (C) Tupac Amaru dengue-1 pre-treatment. (D) Tupac Amaru dengue-1 post-treatment.

In the second phase of this exercise, we simulated a source-reduction intervention by removing selected containers that could serve as sites for larval development. For evaluation of a GMM release, modifications in equilibrium levels for mosquito susceptibility to virus infection or the density of competent vectors could be examined. For our study, we estimated that treating water barrels would reduce all pupal production by 69% in Maynas, based on Iquitos field data. Results from the vector-control simulations were dramatic (Figure 3, panels B and D). The estimated number of adult female *Ae. aegypti*/person/ha decreased from 0.64 to 0.27 and there was nearly complete prevention of virus transmission. Incidence rates never exceed 2 cases/1,000 population. In contrast, in Tupac Amaru simulated removal of all miscellaneous containers, which should have reduced pupal production by 43%, lowered the density of female mosquitoes/person/ha to 0.5 and reduced dengue-1

transmission 3-fold. In Maynas it appeared that targeted source reduction lowered mosquito densities below the minimum threshold. Conversely, in Tupac Amaru even though densities did not go below the minimum threshold, transmission was decreased by about half. For a complete analysis, simulations would need to be run for each endemic or introduced virus serotype. Our example was limited to dengue-1. We expect that the predicted impact on virus transmission will be virus-specific and, thus, unique for each serotype.

The next step in the application of these tools will be to carry out field-based evaluations. How well does simulation output match with actual reductions in adult female density and, more importantly, modification in virus transmission? Advantages of the simulation models are that they are flexible, allowing modification of virtually any component that an investigator might want to change, and they are stochastic, multiple simulations can be used to evaluate the probability of transmission or estimate risk. Drawbacks are that their complexity makes them difficult to follow and potential errors in component parts are amplified by the interaction of many different variables, they are not spatially explicit, they measure infections but not severity of disease, and they require considerable help to set up prior to carrying out comparative simulations.

What are the most informative measures of entomological risk?

In general, correlations among entomological indices and dengue incidence rates are inconsistent, understudied, and poorly defined. This may be because the often-used immature-mosquito indices are especially sensitive to sampling variation (Tun-Lin et al. 1996). Although more labour-intensive than the relatively simple immature-indices, monitoring adult mosquitoes, absolute pupal counts or larval productivity may be more predictive of disease risk. Two contributions are critical to breaking free from the conceptual rut in which dengue surveillance and control currently finds itself. First, there is an urgent need for rigorous field-based evaluations of the relationships among the available *Ae. aegypti* indices, virus transmission and disease. Second, new rapid and inexpensive methodologies are needed for assessing risk. As we explain below, it is becoming increasingly clear that dengue surveillance requires relatively large sampling efforts at frequent time intervals.

Larval indices: *Stegomyia* indices were developed (Conner and Monroe 1923; Breteau 1954) to monitor the progress of vector eradication efforts and to protect *Ae. aegypti*-free zones from re-infestation (Soper 1967). The house or premises index (HI: % of houses infested with larvae and/or pupae) has been used most widely, but it does not take into account the number of containers with immature mosquitoes nor the production of adults from those containers (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994). The container index (CI: % of water-holding containers infested with active immatures) only provides information on the proportion of water-holding containers that contain ≥ 1 immature mosquito; it does not account for variation in density or adult productivity. The Breteau index (BI: number of positive containers/100 houses) is considered the most informative because it establishes a relationship between positive containers and houses, but it fails to account for adults produced from containers.

Since 1971, a variety of alternative indices were proposed (Chan, Chan and Ho 1971; Bang, Bown and Onwubiko 1981; Chan 1985; Tun-Lin, Kay and Barnes 1995; Tun-Lin et al. 1996), which attempted to account better for adult productivity. In general, many of those indices were discounted because of the high degree of sample

variation and, perhaps more important, the severe logistical limitations that they posed (Tun-Lin, Kay and Barnes 1995).

Pupal methods: Advantages of using pupae as a measure of *Ae. aegypti* abundance are that (1) absolute counts of *Ae. aegypti* pupae are feasible in most domestic environments (Southwood et al. 1972; Focks et al. 1995), (2) pupal mortality is slight and well-characterized, and (3) the number of pupae/person is highly positively correlated with the number of adults mosquitoes/person (Southwood et al. 1972; Focks et al. 1981). For additional discussion on the relative merits of this approach, the reader is referred to a recent review by Focks (2003), which was intended to encourage the use of the pupal index. Disadvantages of the pupal index concern the time and manpower necessary to carry it out and sampling variation. Collecting individual pupae is time-consuming, especially from large containers. In areas where other *Aedes* species coexist in domestic habitats, pupae from each container must be held separately until they emerge as adults for proper identification. Development of *Ae. aegypti* within individual containers has an important cohort effect; that is, groups of larvae develop into pupae synchronously, so that the number of pupae observed is dependent on the day of survey. The difference of one day can result in collecting only a few pupae compared to potentially hundreds the next. Nevertheless, when it is applied to individual container types and when a sufficiently large number of houses are surveyed, the pupal index can be used to estimate adult density and the relative proportion of the adult *Ae. aegypti* population attributable to each kind of container (Focks and Chadee 1997). Large sample sizes are essential to overcome sampling problems associated with temporal and spatial variation in *Ae. aegypti* pupal production (Getis et al. (submitted)).

Adult population densities: In nature, adult *Ae. aegypti* population densities are relatively low compared to most other mosquito species and difficult to estimate, which based on current technology makes routine adult surveillance problematic (Reiter and Gubler 1997; Scott et al. 2000b). Capture techniques focus on females and include collecting mosquitoes that come to bite human bait (Nelson et al. 1978; Trpis and Häusermann 1986) or collection by indoor sweeps with hand nets (Tidwell et al. 1990) and other manual methods. A drawback to using humans to attract mosquitoes is the ethical concern of exposing collectors to virus infection. These kinds of capture techniques are labour-intensive and subject to complex operator and location influences (Reiter and Gubler 1997).

In an effort to standardize and make adult *Ae. aegypti* collection more straightforward, cardboard sticky lures are currently being evaluated (Bangs et al. 2001). Disposable cards that contain a chemical mosquito attractant and are coated with an adhesive can be placed in houses to capture mosquitoes. In a laboratory study, the infecting virus serotype was correctly detected by reverse-transcriptase polymerase chain reaction up to 30 days after experimentally infected mosquitoes were applied to the lure. Field studies will define the capture efficiency of this technique.

An indirect measure of adult female presence or absence is the oviposition trap or ovitrap. Black glass or plastic jars (ca. 500 ml) are partly filled with water. Eggs that were laid on a rough paddle or paper lining inside the trap can be collected and counted. The enhanced CDC ovitrap uses paired ovitraps with different dilutions (100% and 10%) of hay infusion and produces ca 8 times more *Ae. aegypti* eggs than regular ovitraps (Reiter, Amador and Colon 1991). Ovitraps do not provide estimates of *Ae. aegypti* population densities (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994; Reiter and Gubler 1997), but

they can give insights into relative changes in the adult female populations (*Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control* 1994). An important source of ovitrap error are biases, that have not been formally defined and likely vary from one site to another, associated with competition with other, natural oviposition sites.

The most effective adult *Ae. aegypti* collecting methodology is the backpack aspirator (Clark, Seda and Gubler 1994; Edman et al. 1992; Scott et al. 1993b; Scott et al. 1993a; Scott et al. 2000b). Mosquitoes are collected from resting sites, principally dark protected indoor sites, and densities can be estimated as the number of adults per house and as the number of houses positive for adults per number of houses sampled. Advantages of this method are that it results in collection of all physiological stages of female as well as male *Ae. aegypti*, not just females that are seeking a blood meal or laying eggs. The principal disadvantage is that it is labour-intensive and can be affected by variation in collector efficiency. Recent field studies in Thailand indicate that efficiency of skilled collectors is in the range of 20% of the mosquitoes in a house (Harrington, Edman and Scott unpublished data).

The fact that none of these methods is as informative or amenable to large-scale sampling as we would like, reinforces the statement we made earlier – a most significant contribution to dengue surveillance and control would be development of an operationally feasible technique to monitor adult female *Ae. aegypti* population densities. A similar recommendation can be made for anopheline mosquitoes and malaria surveillance.

At what geographic scale are risk factors important; e.g., at what scale should they be measured?

Without a clear understanding of the spatial dependence of risk-factor data, accurate quantification of mosquito density thresholds will not be possible. For example, if entomological risk factors, such as abundance, survival, dispersal, and feeding behaviour vary spatially we must use statistical techniques that do not assume that observations are independent. Numerous spatial statistical methods are now available that account for the spatial structure of data. At the operational level, information on the spatial characteristics of dengue risk factors will have important implications for selecting sampling strategies for surveillance, targeting control measures, and providing the framework to develop dengue risk maps.

Because they can be viewed as point processes, data on the distribution and abundance of *Ae. aegypti* and human dengue infections are well suited for spatial point pattern analysis and exploratory data analysis at different geographic scales (Gatrell et al. 1996; Getis 1999). Historically, most people studying *Ae. aegypti* have characterized temporal, rather than geographic, patterns in mosquito abundance (Gould et al. 1970; Sheppard et al. 1969; Yasuno and Pant 1970). In a few instances, spatial differences in *Ae. aegypti* population indices and rates of reported dengue cases were correlated with surveillance (Chan 1985; Morrison et al. 1998) and prospective longitudinal cohort data (Morrison and Scott unpublished data). In general, point pattern analysis allows one to test questions about clustering patterns for mosquito vectors and cases of disease among humans. For example, one can ask whether clustering patterns of dengue cases are primarily due to natural variation in *Ae. aegypti* population densities at households or whether clusters are merely the result of some *a priori* heterogeneity in the region were the study was conducted (Gatrell et al. 1996). It is also possible to determine the spatial scale over which clustering occurs and whether clusters are associated with proximity to specific

features of interest, such as village meeting places, schools or markets. Geographic scale is especially important because of the modifiable areal-unit problem (MAUP). MAUP refers to variation in results when data are combined into sets of increasingly larger areal units or alternative combinations of base units at equal or similar scales (Openshaw and Taylor 1979). Both phenomena are common problems for dengue surveillance and control programmes because data are most commonly reported for areal units defined by political rather than epidemiological boundaries.

This technology was first applied to dengue transmission with reported cases from an epidemic in a small Puerto-Rican community – Florida, population 8,700 – during 1991 (Morrison et al. 1998). Each case was georeferenced to 10m and the data were managed in an ARC/INFO geographic information system. Twenty-six percent of the houses had more than one reported case of dengue. Using two types of pattern analysis – K-function and Knox test – significant clustering was detected in individual houses over short periods of time, 3 days or less. Beyond 3 days no clustering of cases was detected. The geographic spread of cases through the community was so rapid and extensive that their spatial distribution did not significantly change during the course of the epidemic.

The first large-scale spatially explicit study of variation in *Ae. aegypti* density is currently being carried out in the geographically isolated Amazonian city of Iquitos, Peru (Getis et al. (submitted)). Based on georeferenced larval, pupal, and adult *Ae. aegypti* samples collected in and around Iquitos households, analyses were carried out to define the (1) underlying spatial structure of *Ae. aegypti* infestations, (2) temporal stability of that structure, and (3) correlations between clusters at different *Ae. aegypti* life stages. Results from this study will have ramifications for estimating entomological risk of dengue transmission and standardizing dengue surveillance.

K-function (Ripley 1981; Getis 1984) and G_i^* (Ord and Getis 1995) analyses revealed distinct patterns of *Ae. aegypti* clustering in Iquitos. Clusters of development sites containing larvae or pupae were limited to the smallest measurement of scale; that is, 10 m or the area associated with approximately one or two houses. Adult *Ae. aegypti* were strongly clustered at 10 m and weakly out to a maximum of 30 m. Modest adult dispersal by flight (*Dengue haemorrhagic fever: diagnosis, treatment, prevention and control* 1997; *Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines* 1999; Edman et al. 1998; Harrington et al. 2001) likely accounts for similarities in spatial distribution between immature and adult *Ae. aegypti*. Limited adult distribution and the tendency for females to imbibe human blood frequently (Scott et al. 2000a) may explain the household clusters of clinically ill people over short periods of time in Florida, PR (Morrison et al. 1998). Interestingly, a cohort effect was detected in Iquitos in which stages of the mosquitoes' life cycles that are directly linked – for example, larvae to pupae – were spatially correlated to one another. If, however, a step in the development process is skipped – for example, larvae to adult – the correlation broke down. This helps explain the observation that the probability of positive containers being repeat offenders was relatively low. That is to say, why most positive containers were not infested with *Ae. aegypti* during repeated sampling. These results indicate that most containers are in flux, moving from containing immature *Ae. aegypti* and producing adults to returning to negative status. An important ecological implication of this observation is that *Ae. aegypti* populations in Iquitos appear not to be in equilibrium with the containers in which immature mosquitoes develop. Perhaps as proposed by Southwood et al. (1972) and Dye (1984) for *Ae. aegypti* in Thailand, in Iquitos availability of oviposition sites does not have a major regulating affect on adult

density. An implication of these results to *Ae. aegypti* surveillance for a GMM programme is that entomological risk must be measured at the level of the household at frequent time intervals. In Iquitos source reduction and education campaigns in conjunction with a GMM release are more likely to result in sustained reductions in *Ae. aegypti* populations and disease control than only using larviciding strategies targeted at specific container types.

Implications

To make the transition from eradication to control programmes, like population replacement, we must quantify the relationship between *Ae. aegypti* abundance and dengue virus transmission. The most effective way to characterize the density-risk association is to carry out prospective longitudinal cohort studies that measure simultaneously mosquito density, dengue incidence, and severity of disease. Study designs should not be limited to reported cases; rather they should include a variety of methods for monitoring the human population for symptomatic and asymptomatic infections. This could be done, for example, with a combination of scheduled blood draws and techniques for actively identifying disease. Prescribed serologic testing from a study cohort will capture all infections. Fever studies and monitoring attendance at some regular function, like school, will identify which of those infections resulted in disease, and from those individuals the severity of disease can be derived. Entomological and human data should be georeferenced – managed in geographic information systems – so that it can be analysed for epidemiologically relevant spatial and temporal patterns. Targeted reductions in adult female density in conjunction with incidence and disease monitoring would verify the existence of thresholds and quantify the relationship between abundance and incidence of disease. Absolute measures of disease reduction will be difficult to obtain. A more productive approach would be to note the relative affects of different control scenarios (Dye 1992). From that kind of knowledge base the likelihood increases dramatically for understanding the effects of a GMM release. Simulation models can be used for this purpose, but before that happens the predictive capabilities of models must be properly validated with prospective field data. To determine what methods will be most useful in an operational context for initially evaluating and ultimately monitoring disease reduction from a GMM release we must develop new entomological survey techniques and properly evaluate the existing techniques that are summarized above.

As defined in our introduction, we limited our discussion to the relationship between reduced vector competence and decreased disease risk. It is worth pointing out, however, that the prospects for dengue control would benefit by expanding our perspective to phenotypes other than refractoriness to pathogen infection (Rascon, Styer and Scott 2003). Examples of alternative phenotypes could include extension of the extrinsic incubation period, reduction of life span, age-dependent induction of mortality, reduction in blood-feeding frequency or the tendency to take blood meals from humans, and alterations in reproductive behaviour and biology. If these or other alternative approaches are pursued, the need for an entomological scaffolding like the one we discussed in this chapter will continue to be required for predicting outcomes and monitoring applications. An increasingly detailed understanding of the relationships between mosquito ecology and the epidemiology of the pathogens that they transmit is and will continue to be a fundamental component of improved public-health programs.

Acknowledgements

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