Longitudinal Field Studies Will Guide a Paradigm Shift in Dengue Prevention

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Abstract The transition from prescribed to adapted dengue prevention will need to be guided by meaningful goals and accomplished with effective tools. Goals will be reached if enhanced vector control is framed by an improved understanding of vector ecology in pathogen transmission. Longitudinal field studies that capture entomologic, virologic, and epidemiologic information are the most effective was to assess fundamental assumptions and refine new techniques. The following are key tasks that need to be addressed to meet these objectives. Design operationally and epidemiologically effective ways to assess risk of DV transmission and set goals for disease prevention. Create an inexpensive and effective tool for monitoring adult *Ae. aegypti* population density. Develop a rapid, sensitive, specific, and inexpensive way to estimate serotype specific herd immunity that can be used to predict risk of epidemic DV transmission. Encourage the use of dengue vaccines as public health tools to artificially elevate immunity in an integrated disease prevention program with vector control. Evaluate more effective and operationally feasible means of reducing adult *Ae. aegypti* density reduction that can be readily adapted to situation specific circumstances. Promote field-based prospective longitudinal cohort research in disease endemic locations that assesses adaptive intervention strategies based on relationships among measures of entomologic and epidemiologic risk, dengue incidence, and severity of disease. Accomplishing these tasks will translate into the most important attributable benefit from vector control for dengue, reduction of disease burden and death.

Keywords Dengue · Vector control · Ecology · Epidemiology · Pathogen transmission

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P.W. Atkinson (ed.), *Vector Biology, Ecology and Control*,
DOI 10.1007/978-90-481-2458-9_10, © Springer Science+Business Media B.V. 2010

Introduction

When done properly, vector control is a well-documented and effective strategy for prevention of mosquito-borne disease. Familiar examples of successful mosquito vector interventions include the worldwide reduction of malaria in temperate regions and parts of Asia during the 1950s and 1960s (Curtis 2000; Rugemalila et al. 2006), yellow fever during construction of the Panama Canal, yellow fever throughout most of the Americas during the 1950s and 1960s (Soper 1967), dengue in Cuba and Singapore (Ooi et al. 2006), and more recently dengue in parts of Vietnam (Kay and Nam 2005). That these programs significantly improved public health is indisputable. Why then is disease burden from vector-borne diseases like malaria (Sachs and Malaney 2002) and dengue increasing (WHO 2006a)? Why has vector control not been effectively applied more often so that it reduces or appreciably minimizes disease? Unsuccessful programs are often attributed to a lack of resources, lack of political will or ineffective implementation (Attaran 2004; Gubler 1989; Halstead 1993; Killeen et al. 2002). Just as responsible for control failures are deficiencies in understanding relationships between vector ecology and pathogen transmission dynamics, the most appropriate methods for assessing and responding to appreciable risk, and the failure to use existing knowledge or surveillance information to make informed control decisions. It is reasonable to conclude that despite more than a century of vector-borne disease investigation, fundamental concepts in disease prevention remain incompletely defined and underutilized.

The goal of this chapter is to illustrate the power of improved ecologic and epidemiologic understanding for increased effectiveness of vector control for dengue. The concepts and processes we discuss are not limited to dengue and, therefore, consideration should be given for their application to other vector-borne diseases. We assert that a better understanding of virus transmission dynamics, concepts, and tools and strategies for disease prevention will fundamentally change and significantly improve public health programs for dengue prevention. Current programs, which emphasize universally prescribed surveillance and control, have hindered development of an appropriate conceptual and factual foundation for adaptive disease prevention programs and help to explain why contemporary vector control programs too often fall short of public health expectations.

Our principal recommendation is that enhancing dengue prevention will require locally adaptable tools and strategies. To accomplish this there is an urgent need for more comprehensive, longitudinal field studies of vector-borne diseases that (1) quantitatively define relationships between the most meaningful measures of risk and human infection and (2) use that information to direct public health measures that prevent or minimize disease. Information necessary to fill this knowledge gap should be obtained in the framework of interrelated longitudinal cohort studies that progressively build on one another, providing an increasingly detailed understanding of fundamental processes in pathogen transmission, epidemiology, and disease control. Based on our experience, critical missing knowledge of risk assessment and disease prevention can only be gained by carrying out integrative research that embraces the vector, pathogen, and human host. Too often vector-borne disease

specialists study the arthropod vector, disease, or pathogen separately. Only by studying the system in total over a considerable period of time will we gain the greater insight into the complexity of interactions between components of transmission and disease that are essential for design, implementation, and evaluation of increasingly more successful disease prevention programs. In the case of dengue, until a vaccine or chemotherapy become available, control programs will continue to be limited to vector control, which in most cases means reducing mosquito vector populations. But do we understand *Ae. aegypti* and dengue virus (DV) transmission well enough to make specific recommendations for modifications in vector populations, short of vector eradication, that will result in a predictable public health outcome? Review of relevant literature clearly indicates that the answer to this critical question is no.

Dengue Epidemiology and Ecology

Worldwide, DV infections cause more human morbidity and mortality than any other arthropod-borne virus disease (Farrar et al. 2007; Gubler 2002; 2004; Gubler and Kuno 1997; Kuno 1995; MacKenzie et al. 2006; Monath 1994). It is estimated that 2.5–3 billion people are at risk of infection in tropical parts of the world each year. In urban centers of Southeast Asia, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are among the leading causes of pediatric hospitalization. During the last 30 years dengue has emerged as a major international public health threat in the Americas (Rigau-Perez et al. 1998; WHO 2006b).

Dengue fever (DF), DHF, and DSS are caused by four closely related, but antigenically distinct, single-stranded RNA viruses (DV-1, DV-2, DV-3, and DV-4) in the genus *Flavivirus*, family Flaviridae. All four serotypes cause a range of human disease, including asymptomatic infections, undifferentiated fever, and classic DF (Gubler 2002c, 2004; Gubler and Kuno 1997; Rothman and Ennis 1999). Sequential infections with different serotypes are possible because infection with one serotype provides lifelong protection from a homologous infection, but is only briefly cross-protective against heterologous serotypes. The etiology of serious illness is not completely understood but is suspected to be due to immune enhancement and/or variation in virus virulence (Gubler 2002c, 2004; Kochel et al. 2002; MacKenzie et al. 2006; Monath 1994; Rothman and Ennis 1999; Watts et al. 1999). It is estimated that annually there are between 50 and 100 million DF cases and 250,000–500,000 DHF/DSS cases worldwide. If untreated, the case fatality rate for DHF/DSS can approach 30–40%; with supportive therapy, less than 1% of severely ill patients die (Halstead 1993).

DVs generally persist in endemic foci by a horizontal *Ae. aegypti*-human transmission cycle (Gubler 1989a; Rodhain and Rosen 1997). After an incubation period of 3–15 days (typically 4–7 days) in the human, disease symptoms are first observed (Focks et al. 1995; Waterman and Gubler 1989). Viremia often precedes fever, typically lasts ~5 days, and usually subsides in concert with the inability to detect virus in the blood (Vaughn et al. 2000). Mosquito vectors become infective after

biting a viremic individual and surviving an extrinsic incubation period of 7–14 days (Watts et al. 1987). Although other mosquitoes in the subgenus *Stegomyia* have been incriminated as vectors, *Ae. aegypti* is the most important dengue vector worldwide (Gubler and Kuno 1997). Once infective, *Ae. aegypti* can transmit virus each time they probe their mouthparts into a human or imbibe a blood meal (Putnam and Scott 1995a, b).

Aedes aegypti is uniquely adapted to a close association with humans and efficient transmission of DV. Immature forms develop primarily in artificial, man-made containers (Gubler 1989a). Highly anthropophilic, females rest inside houses where they feed frequently and preferentially on human blood (Scott et al. 1993b, 2000b), which confers a fitness advantage (Scott et al. 1997; Morrison et al. 1999; Harrington et al. 2001a). Because food, mates, and substrates for laying eggs are readily available within the human habitations where female *Ae. aegypti* reside, dispersal beyond 100m is not necessary and is detected in only a very small proportion of the adult population (Morland and Hayes 1958; McDonald 1977; Trpis and Hausermann 1986; WHO 1997, 1999; Edman et al. 1998; Harrington et al. 2001a, b, 2005). This indicates that most dispersal of DV occurs via movement of viremic human hosts. These features make *Ae. aegypti* an efficient vector and DV transmission can occur even when *Ae. aegypti* population densities are very low (Kuno 1995).

Dengue Control

Presently, dengue control is dependent on the reduction or elimination of *Ae. aegypti*. Although dengue vaccines are a focus of attention (Pediatric Dengue Vaccine Initiative funded by the Bill and Melinda Gates Foundation[1]), currently there is no licensed vaccine. Developing a dengue vaccine is a challenge because it will need to be tetravalent to avoid the risk of immune enhancement. Even after a vaccine or drug is available, we expect that vector control will remain important. The benefits of a vaccine will be limited by its safety profile, efficacy, cost, and capacity for delivery (DeRoeck et al. 2003; Shepard et al. 2004). Although a variety of dengue vaccines are being developed and there are promising leads for antidengue drugs at the time of this writing (Farrar et al. 2007), none of the vaccine candidates have been evaluated in Phase III trials and licensing is not imminent for clinical use of prospective drugs. Critical information on efficacy and cost was, therefore, not available. Even with superior efficiency, which considering the complexity of dengue disease we can not assume without rigorous evaluation, a dengue vaccine will clearly not protect against infection with other mosquito-borne viruses. Furthermore, in order for there to be widespread application of a dengue vaccine in endemic countries the cost would need to be low (no more than $0.50 per dose) and preferably applied in a single dose (DeRoeck et al. 2003). In a best-case scenario there will be perfect protection against all DVs and perhaps some

[1] See http://www.pdvi.org.

cross-protection for other *Ae. aegypti*-borne viruses in the genus *Flavivirus* (i.e., yellow fever). A dengue vaccine will not protect against infection with nonflaviviruses and, realistically, complete vaccine coverage seems unlikely. Conversely, effective vector control reduces risk of infection for all *Ae. aegypti*-borne arboviruses (e.g., dengue, yellow fever, and chikungunya) across the human population. This alone is a compelling reason for continuing *Ae. aegypti* control after an effective DV vaccine becomes available.

Current vector control methodologies for *Ae. aegypti* surveillance and control emphasize techniques that were developed for mosquito eradication to prevent yellow fever (see "Measuring Mosquito Density," below). Although those programs were initially successful in helping to define the role of vector eradication in disease prevention, the approach taken provided little insight into quantitative relationships between mosquito abundance and DV transmission (PAHO 1994; Gubler and Kuno 1997; Reiter and Gubler 1997; Scott and Morrison 2003). For a variety of reasons, mostly changing urban environments and limited economic resources, in 1994 the Pan American Health Organization (PAHO) departed from the eradication paradigm and declared eradication of *Ae. aegypti* an unattainable goal (PAHO 1994). The new goal of dengue control programs is cost-effective utilization of limited resources to reduce vector populations to levels at which they are no longer of significant public health importance (Gubler 1989b, PAHO 1994).

Aedes aegypti control programs worldwide vary widely, in many cases driven by country-specific economic constraints on local health agencies. Most countries use a combination of vector surveillance, chemical treatment of *Ae. aegypti* larval habitats, and either regular or emergency applications of ultra low volume (ULV) space sprays. Aerosol insecticides are effective if they reach female *Ae. aegypti* resting indoors, where they otherwise avoid insecticide contact (Reiter and Gubler 1997). This means that space sprays need to be applied inside houses using backpack applicators rather than from high-profile trucks moving down city streets or from airplanes flying over houses. Farther up the product development pipeline, disease control based on genetic manipulation of mosquito vectors is being investigated in the laboratory (Beaty 2000; James 2005) and will require extensive field evaluation before it can be deployed (Scott et al. 2002; Louis and Knols 2006). Successful dengue vector control programs in Singapore and Cuba (Ooi et al. 2006), promising results from trials with insecticide-treated materials in Latin America (Kroeger et al. 2006), and cost-effective larval control in Cambodia (Suaya et al. 2007) fortify the notion that properly done vector control effectively prevents dengue disease. Enhancing tools and strategies for vector surveillance and control should be a priority in the fight against dengue.

The PAHO strategy emphasizes vector surveillance, with the objectives of maintaining *Ae. aegypti* populations below or close to transmission thresholds, slowing DV transmission, and accordingly, reducing sequential infections with heterologous serotypes that can increase the incidence of serious disease (Vaughn et al. 2000). Although intuitively reasonable, this approach has not been systematically validated and the implication is that controlling serious disease rather than all disease is a viable public health goal. No well-controlled field studies have been published that clearly define the key relationships between vector density and human

infection. There is an urgent need for entomological and epidemiological data that refine understanding of relationships among entomological risk factors, incidence of human infection, and clinical disease manifestations. This has rarely been done for any vector-borne disease, exceptions being arbovirus studies of western and St. Louis encephalitis viruses in southern California by Reeves and his colleagues (Reeves 1971; Olson et al. 1979). Yet reduction of vector populations remains a prominent, underlying premise of many current public health recommendations for control of a long list of vector-borne diseases, including dengue. Prospective studies are urgently needed to test and refine fundamental assumptions of this strategy for dengue control.

Establishing Goals for Dengue Prevention Programs

A fundamental observation in dengue prevention is that there is no single method or approach that works in all situations (Scott and Morrison 2003). Ecology and epidemiology of virus transmission vary from one place and/or time to another. To help establish dynamic goals for disease prevention programs that can be adapted across the diversity of situations in which dengue exists, we developed four interrelated questions that assist in goal setting. The concepts discussed are not limited to dengue, and therefore, can be applied to other vector-borne diseases.[2] Location-specific answers to these questions are important steps in the development of adaptive dengue control programs.

What is an acceptable level of dengue risk? This is a complex question. The answer will be situation and location-specific depending on historical patterns of local DV transmission, available resources, and competing public health priorities. In order to reach properly informed decisions, entomologic and epidemiologic data will need to be considered. That will require appropriate coordination, sharing of relevant information, and teamwork among different public health entities (e.g., vector control and epidemiology departments) (Ooi et al. 2006). Goals will likely change as epidemiologic conditions and public health expectations change. This implies that the definition of what constitutes acceptable risk will vary from eradication of all clinically apparent dengue cases to "living with dengue but not DHF." Consideration of this issue is an important part of the paradigm shift away from universally prescribed control actions and toward local experts developing a dynamic system for repeatedly reevaluating what are the most effective control tools, strategies, and application protocols for their particular situation.

What are the mosquito densities (thresholds) necessary to meet agreed upon risk goals? The new policy for dengue control implies that although there may be some DV transmission, properly applied vector control will reduce or eliminate severe disease (Gubler 1989b; PAHO 1994). The objective, therefore, is to lower the force of infection and thus minimize severe disease by managing the density of

[2] See Scott and Morrison (2003) for additional discussion on each topic.

mosquito vector populations. This is a tricky proposition. How does one know when vector populations have been reduced to levels at which they are no longer significant? What constitutes no longer significant? What exactly are the epidemiological objectives that guide this approach?

Control strategies that do not aim for vector eradication, like this one, require surveillance (entomological and epidemiological) that informs disease prevention responses. In this case, the objective is to identify an entomological threshold below which there will be no epidemic transmission. Values above the thresholds will trigger control actions. Although the concept is straightforward, implementation is challenging. Without the appropriate knowledge and analytical tools, it can be difficult to distinguish between the mere presence of a vector species and situations when vector control is required to prevent an epidemic (Peterson and Higley 2002). Operationally friendly systems for estimating action thresholds from locally available surveillance, weather, and human population data would be a significant addition to the armature against dengue.

Thresholds for DV transmission can fluctuate depending on mosquito density, overall immunity of the local human population (i.e., herd immunity), introduction of novel virus serotypes or genotypes, the nature of contact between mosquito vectors and human hosts, human density, and weather (Scott and Morrison 2003). Temperature is particularly important because of its inverse relationship with extrinsic incubation. Even after key parameters have been identified, estimation can require acquisition of data that are hard to obtain (e.g., site-specific herd immunity) or can be encumbered by complicated assumptions (e.g., spatially and temporally explicit knowledge of mosquito density, survival, and human biting behavior).

Important features of threshold values are that they are dynamic (i.e., they vary through time and space) and estimation is difficult because they are often based on data that are difficult to obtain or that require assumptions that are difficult to accept. In a practical sense development of thresholds will require the use of models (i.e., Focks et al. 1993a, b, 1995) that can be used to make relative rather than absolute comparisons (Dye 1992). An appropriate analogy is hurricane prediction, for which there are models that can be used with some degree of error to make life-saving decisions. Due to inherent variability in key dengue transmission parameters and the difficulty in some cases of obtaining accurate measurements, it would not be wise to establish a fixed threshold value for DV transmission even at the same location. We can expect, however, to be able to identify circumstances when the risk of transmission is particularly high and prioritize use of limited vector control resources to sites where they will do the most good.

Iterative modeling exercises can be used to systematically identify the most informative surveillance systems and predict intervention approaches with the highest probability of meeting local disease prevention goals. We are currently involved in a project (i.e., the Innovative Vector Control Consortium) (Hemingway et al. 2006) that includes upgrading and making more user friendly existing simulation models for *Ae. aegypti* population dynamics (Focks et al. 1993a, b) and DV transmission (Focks et al. 1995). Our goal is to make these models freely available as a component

of a web-based dengue decision support system so that at a variety of different levels (e.g., national, regional, or local) public health, vector control, or government officials can contrast and select from different surveillance and control options under a variety of site and operationally specific circumstances.

Preliminary estimations indicate that entomological thresholds for DV transmission are quite low (Focks et al. 2000). The most important reason for this is *Ae. aegypti*'s uncommon feeding behavior. Most adult female mosquitoes engage in a feeding duality. They feed on plant sugars as a substrate for the synthesis of energy reserves (i.e., glycogen and lipid) that are used for flight and maintenance activities and blood for amino acids that are used for development of eggs (Clements 1999). Female *Ae. aegypti* deviate from this pattern in ways that make them particularly dangerous vectors. In dengue endemic situations where *Ae. aegypti* live in close association with humans, females seldom feed on plant carbohydrates (Edman et al. 1992; Van Handel et al. 1994; Costero et al. 1998). They meet their energetic and reproductive needs by feeding frequently and preferentially on human blood (Scott et al. 1993a, b; Chow et al. 1993; Scott et al. 2000a, b). Patterns of multiple biting on humans are consistent with facilitation of DV transmission. Multiple meals are taken from different people, bites are heterogeneously distributed so that some people are bitten more often than others, and virus can be moved from one place to another by visitors who are bitten in homes where infected mosquitoes reside (Chow-Schaffer et al. 2000; DeBenedictis et al. 2003). Because *Ae. aegypti* tend not to disperse far (Morland and Hayes 1958; McDonald 1977; Trpis and Hausermann 1986; Edman et al. 1998; Harrington et al. 2005), energy needs for flight are reduced. Nutrients in a diet limited to human blood support mosquito maintenance activities and reproduction as long as females feed multiple times in each egg-laying cycle (Harrington et al. 2001a). The unique feature of human blood that makes this possible is believed to be the low concentration of the amino acid isoleucine compared to other vertebrate sources of blood. From an epidemiologic perspective, frequent human biting increases the opportunities for mosquito vectors to acquire DV by biting an infected person and to transmit virus after becoming infectious. From an entomological point of view, feeding frequently and preferentially on only human blood confers a fitness advantage and, therefore, females that engage in that behavior have a selective advantage (Day et al. 1994; Scott et al. 1997; Naksathit and Scott 1998; Costero et al. 1998; Morrison et al. 1999; Harrington et al. 2001a). Consequently, frequent and preferential human biting makes *Ae. aegypti* a remarkably efficient and, thus, dangerous mosquito. It does not take many *Ae. aegypti* to sustain unacceptable levels of DV transmission. The operational implications of efficient transmission are that entomological thresholds will be low and thus for vector control to be effective it will need to be thorough and sustained.

What are the most informative measures of dengue risk? To date, attempts to predict dengue epidemics have been largely unsuccessful. Public health departments worldwide remain perplexed and frustrated with their inability to assess dengue risk in a meaningful way. In places where fewer than all four serotypes are transmitted (i.e., Latin America and parts of Asia), surveillance systems have been proposed for detecting the introduction of novel DV serotypes (Gubler and Casta-Velez 1991).

In endemic regions of Southeast Asia, where there is an overall pattern of three to four year cyclical increases in disease (Hay et al. 2000; Cummings et al. 2004), viral surveillance has been more informative than current entomological techniques for managing DV transmission. Nevertheless retrospectively – and to some extent arbitrarily – prescribed entomological indices are heavily relied upon to assess dengue risk and the effectiveness of vector control programs (Focks and Chadee 1997; Focks et al. 2000; Scott and Morrison 2003). An operationally valuable early warning system for dengue, which is in great demand by public health officials (DeRoeck et al. 2003), will need to include data on human herd immunity, *Ae. aegypti* and human population densities, contact rates between vectors and humans, and ambient temperature.

Human herd immunity. A key component in the transmission of an infectious disease is the proportion of people in the affected population that are susceptible to infection (Anderson and May 1991). This is especially true for a virus like dengue that causes sterilizing immunity (i.e., following exposure and an immune response a person is protected from reinfection with the same DV serotype). Results from dengue models clearly indicate that the vector densities necessary to prevent, interrupt, or decrease DV transmission are inversely proportional to seroprevalence rates of the human population (Newton and Reiter 1992; Focks et al. 1995, 2000). For example, Focks et al. (2000) predicted that when other factors remain constant entomological threshold estimates necessary for epidemic DV transmission will increase 1.5-fold when the initial seroprevalence increases from 0 to 33%, 2.1-fold when it increases from 33 to 67% and 3.2-fold when it increases from 0 to 67%. As the proportion of immune people in the population increases it is expected that it will become increasingly difficult for DV to sustain transmission.

The most specific assay for detecting serotype-specific antibody responses to a DV infection is the plaque reduction neutralization test (PRNT) (WHO 2006a). The PRNT unfortunately requires specialized laboratory facilities and equipment that are beyond the reach of most local public health units. Other serologic methods exist (e.g., enzyme-linked immunosorbent assays [ELISAs]), but they lack serotype specificity and in some cases cross-react with antibodies directed against flaviviruses that are closely related to DV. In most cases, therefore, timely and cost-effective transfer of population-based seroprevalence data is not available. There is a critical need for development of new, more cost and operationally amenable means to estimate herd immunity and, thus, susceptibility of local human populations to epidemic DV transmission.

Measuring mosquito density. Below we review the most commonly used measures of *Ae. aegypti* density that are used to assess dengue risk.

Traditional measures of *Aedes aegypti* density. The shift in focus from eradication to control programs merits a reevaluation of *Ae. aegypti* surveillance techniques. Traditional entomological surveillance techniques are based on the premise/house index (HI; percentage of houses infested with larvae and/or pupae), container index (CI; percentage of water-holding containers infested with larvae and/or pupae), and Breteau index (BI; number of positive containers per 100 houses), which were designed to detect the presence or absence of *Ae. aegypti* larvae (Conner and

Monroe 1923; Breteau 1954; Tun-Lin et al. 1995a; Focks and Chadee 1997). Several investigators discussed the limitations of traditional *Stegomyia* indices for estimating *Ae. aegypti* density and noted their poor relationship with DV transmission (Tun-Lin et al. 1995a, 1996; Focks and Chadee 1997; Reiter and Gubler 1997; Scott and Morrison 2003; Kay and Nam 2005). The major problems are that they fail to account for larval mortality, heterogeneity in container productivity, and temporal differences in *Ae. aegypti* life stages. Put simply, we cannot assume a strong positive correlation between the presence of larvae and adult female mosquitoes in a household. Moreover, factors impacting larval mortality and development such as container size, crowding, and availability of nutrients in aquatic larval habitats affect the relationship between larval and adult densities (Reiter and Gubler 1997; Arrivillaga and Barrera 2004).

Productivity analysis (Pupal and Demographic Survey). Larval productivity indices (Chan et al. 1971; Bang et al. 1981; Tun-Lin et al. 1995a, 1996) and pupal surveys, which were developed to account for heterogeneity in container productivity (Focks and Chadee 1997), are advances in entomological surveillance methods. Common to both is the quantification of either late instar larvae or pupae by container type or characteristic. Each does, however, have its limitations. The distribution of *Ae. aegypti*-infested containers and households can be highly clustered through time and space, making vector population estimates sensitive to sampling error and variation (Tun-Lin et al. 1995a, 1996; Focks and Chadee 1997; Getis et al. 2003; Morrison et al. 2004a, b). Some containers are large, inaccessible, and difficult to sample adequately. Quantitative sampling strategies for immature *Ae. aegypti* include funnel traps (Kay et al. 1992; Nam et al. 1998; Russell and Kay 1999) and standardized sweep methods using nets or dippers (Zhen and Kay 1993; Tun-Lin et al. 1995b; Knox et al. 2007). Larval productivity indices are based on quantification of third and fourth instar larvae, which are expected to be subject to less sampling variation than pupae.

In contrast, the pupal/demographic survey methodology quantifies pupae rather than larvae (Focks et al. 1993a, b; Focks and Chadee 1997) because in theory it is more practical to count the absolute number of *Ae. aegypti* pupae than other life stages (Southwood et al. 1972; Focks et al. 1981) and pupal mortality is slight and well-characterized. The number of pupae per person is correlated with the number of adults per person (Focks et al. 1981, 1995). The relative importance of a container type (i.e., production of adult mosquitoes) is defined as the product of the container abundance multiplied by the average standing crop of pupae (i.e., pupae per wet container). Theoretically, important container types, defined either phenotypically or functionally, can be identified and targeted in vector control campaigns providing a cost-efficient alternative to indiscriminate elimination of all potential habitats for immature *Ae. aegypti* development. Using pupal surveys as the basis of targeted control strategies is currently being evaluated in a multicountry study sponsored by the World Health Organization (WHO 2006a).

Mosquito collection. Adult *Ae. aegypti* are difficult to capture; they do not readily enter traps (Jones et al. 2003). Population densities are generally low, which makes it difficult to estimate population sizes and to this point has precluded routine surveillance of adults (Reiter and Gubler 1997). Adult capture techniques include

human bait (e.g., Nelson et al. 1978; Trpis and Housermann 1986), indoor sweeps with hand nets (e.g., Tidwell et al. 1990), and other manual methods. But these are labor intensive and subject to complex operator and location influences (Reiter and Gubler 1997). An attractant trap is being developed[3] but is not yet commercially available. The most effective currently available device for capturing adult *Ae. aegypti* is the battery-powered backpack aspirator (Scott et al. 1993a, b; Clark et al. 1994). Based on assessments in Thailand, backpack aspirators collect ~25% of adult *Ae. aegypti* in a house (Scott and Harrington, unpublished data). Aspirators can be used, therefore, to assess relative differences in adult population density. Entomological surveillance for dengue would be significantly advanced by the development of a simple, cost-effective trap for broad-scale sampling of adult *Ae. aegypti*.

Based on our research in Iquitos, Peru, immature *Ae. aegypti* indices can be informative for characterizing spatial patterns in vector infestations (Getis et al. 2003). It has been more difficult to associate mosquito density with DV transmission. In Iquitos, only immature indices were correlated with DV seroprevalence. Conversely, only adult indices captured temporal and spatial differences in DV incidence (Morrison and Scott, unpublished data). Oviposition traps (ovitraps) can be valuable for detecting the presence or absence of *Ae. aegypti*, especially when population densities are very low. We do not, however, recommend them for assessing vector abundance because they are susceptible to significant biases from competition with natural oviposition sites.

Ambient temperature. Within a biologically amenable range (22–32°C) (Focks et al. 2000), variation in ambient temperature has well-established, important effects on *Ae. aegypti* biology and seasonal trends in dengue transmission (Watts et al. 1987; Burke et al. 1980). At less than 20°C *Ae. aegypti* eggs do not hatch. Combined mortality across all developmental stages is too high to allow populations to be sustained (i.e., $R_o < 1$) at temperatures greater than 34°C (Focks et al. 2000). Within the receptive range, temperature is negatively associated with *Ae. aegypti* development time (Gilpin and McClelland 1979), survival (Focks et al. 1993a), and extrinsic incubation of DV (Watts et al. 1987). Conversely, blood feeding frequency is positively associated with temperature (Scott et al. 2000a, b). Because increasing temperature reduces the time necessary for pupation, Focks et al. (2000) predicted that increasing temperature only 4°C, from 26 to 30°C, could increase the number of adult *Ae. aegypti* by 45%. With regard to mosquito-virus interactions, Watts et al. (1987) detected DV-2 transmission to primates only at warm temperatures (30–35°C) after 7–12 days of extrinsic incubation. Focks et al. (2000) predicted that 14 and 38% of females would survive extrinsic incubation with the potential to transmit virus to a human host when held at 22°C vs. 32°C, respectively. Because temperature has the potential to significantly affect many important aspects of *Ae. aegypti*'s role in DV transmission, it should be considered an operationally viable component of large-scale surveillance programs.

[3] See http://www.biogents.com/en/index.html and Williams et al. (2006, 2007).

At what geographic scale should dengue surveillance and control activities be carried out? Risk factors, including measures of vector densities, can predict risk differently at different geographic scales. 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 programs because data are most commonly reported for areal units defined by political rather than epidemiological boundaries. Historically, most *Ae. aegypti* ecologists have characterized temporal, rather than spatial, patterns in mosquito abundance (Sheppard et al. 1969; Gould et al. 1970; Yasuno and Pant 1970). Recent studies utilized a myriad of spatial analytical tools, including point pattern analysis (Gatrell et al. 1996; Getis 1999). The utility of these analytical tools are two-fold. First, they characterize spatial autocorrelation patterns in variables of interest. Using a practical example, we can ask if vector densities in households are more highly correlated with those in neighboring houses than houses farther away. Autocorrelation can be measured at different distances and the scale at which autocorrelation is no longer significant would represent the minimum geographic unit for which surveillance and control schemes should be applied. Recent studies demonstrate that entomological risk should be measured at a household scale (Getis et al. 2003; Morrison et al. 2004a), but the distribution of infested houses does not follow a normal distribution (Alexander et al. 2006). Consequently, sample sizes need to be high for prospective epidemiological studies and evaluation of vector interventions. Second, spatial analyses can reveal underlying patterns in different variables. 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 where the study was conducted (Gatrell et al. 1996). In this way, specific foci of transmission can potentially be identified or evaluated in relation to proximity to specific features of interest, such as village meeting places, schools, or markets. In the case of dengue, not enough is known about the role of human movement in defining the geographic scale of transmission. Although there is clear evidence of clustering of dengue cases within households (Morrison et al. 1999), how human movement patterns affect the scale of dengue transmission remains a major knowledge gap. Defining the appropriate geographic scale for measuring entomological risk and DV transmission, which will not necessarily be the same, will be an important new contribution to dengue surveillance and control (Getis et al. 2003).

Recommendations for Improved Vector Control

After the capacity to account for inherent variation in dengue risk has been improved, it will be necessary to use that information to mitigate public health threats. Just as it is for goal setting, enhancing dengue prevention requires rethinking

current control principles and, in some cases, redirecting emphasis to topics that are presently unexplored or underdeveloped. In this section we examine four conceptual shifts in vector control that will substantially improve dengue prevention.

The Paradigm Shift from Top-Down Direction to Local Level Decision

The fundamental challenge for contemporary dengue control, regardless of the approach taken, is to develop a framework for determining in different ecologic and epidemiologic circumstances (1) what control procedures should be used; (2) how they should be applied; and (3) how they should be evaluated and/or monitored (Box 1). The underlying principle will be that there is no single approach that will work across all locations or circumstances. Although some may counter that the concept of "one size does not fit all" in vector control has been known for a long time, there is no denying that it is presently underdeveloped and underemployed. Improved dengue prevention will require a paradigm shift away from the currently common practice of universally prescribed and applied strategies to one in which local control personnel decide for themselves what is the most operationally and cost-effective strategy for their particular situation. The new approach will need to be designed to account for variation in dengue transmission at different geographic locations and at different times at the same place. Local control personnel will need to constantly evaluate their surveillance and response methods. Their goals will have to be spatially and temporally specific, accounting for local variation in ecology, epidemiology, and availability of intervention resources.

Box 1 Key Questions for Development of Innovative, Sustainable, and Cost-Effective Dengue Prevention

- What should the site and situation-specific goal(s) be for dengue prevention programs?
- How should control be monitored (i.e., what surveillance and risk assessment programs should be used)?
- What disease prevention tools are effective and currently available and which ones needed to be developed?
- What are the best integrated and adaptive control programs (e.g., dynamic application of vector control in concert with other disease prevention and management strategies)?
- What major steps need to be taken to develop, evaluate, disseminate, and ensure application of effective and sustainable dengue prevention?

Application will require:

1. Validation with longitudinal cohort studies that examine mosquito vectors and human DV infection.
2. Capacity for programmatic adaptation to site-specific circumstances.

An example of this would be use of pupal productivity analysis to target vector control at containers producing most of the adult *Ae. aegypti*. In some places most *Ae. aegypti* production is associated with water storage and those containers are easily identified and treated with larvicides. In contrast, at other locations most production comes from unmanaged containers that are transient and often missed in routine entomological inspections. Control campaigns for these two extremes would be noticeably different. In Iquitos during a severe 2002 DV-3 epidemic, local health officials deemphasized an entrenched pattern of uniform larvicide applications in preference of enhanced public awareness and container clean-up. The change was motivated by solid entomological surveillance data, which indicated that adult *Ae. aegypti* were being produced primarily from unmanaged containers rather than water storage containers.

The shift from prescribed to adaptable strategies will require application of translational research, basic and applied, to the development of novel products and strategies that reduce disease. For example, dynamic, operational tools like virus transmission models and decision support systems will be necessary to guide site- and situation-specific dengue control. For a meaningful conversion of research to improved public health, it is imperative that those responsible for preventing DV transmission use surveillance information to inform their control decisions.

Surveillance and Control of Adult Versus Immature Mosquitoes

For more than half a century dengue prevention programs focused on immature *Ae. aegypti* for surveillance and control (PAHO 1994). There are theoretical and empirical reasons for no longer strictly following that approach. With regard to surveillance, immature indices of *Ae. aegypti* density have not proven to be good predictors of DV transmission risk. Moreover, goals for immature *Ae. aegypti* surveillance are often vague and do not account for temporal and spatial variation in transmission factors. With regard to control, killing larvae is expected to have a relatively small impact on a reduction in the number of new human dengue infections, compared to killing adults.

Refocusing dengue surveillance and control on adult *Ae. aegypti* would be a significant step forward. One of the major road blocks to improved dengue surveillance is our inability to directly monitor the vector life form that transmits virus (i.e., adult females). The need for an operationally and cost-effective way to monitor adult

Ae. aegypti population fluctuations cannot be over-emphasized. And, even after we have a useful sampling technique we will need to think carefully about how best to use it. For example, unlike malariologists, dengue specialists do not have an informative measure of entomological risk like the entomological inoculation rate (EIR) (Scott and Morrison 2003). Two obstacles to a dengue EIR are (1) the difficultly in collecting adult *Ae. Aegypti* and (2) the fact that virus infection rates in *Ae. aegypti* are typically too low (Kuno 1997) to base a surveillance program on an EIR or its equivalent. An alternative approach would be to develop a dengue transmission potential (DTP) index. Leaving out mosquito virus infection status, a DTP could predict entomological risk based on the product of adult mosquito density, human-mosquito vector contact, serotype-specific susceptibility of the human population (ideally this would also include susceptibility to novel genotypes), and ambient temperature.

Dengue prevention would similarly benefit from greater attention to adult *Ae. aegypti*. Adult mosquito density has a positive nonlinear relationship with the basic reproductive number of vector-borne disease (Garett-Jones and Shidrawi 1969; Dye 1992). Control strategies directed at immature mosquitoes can only reduce the density of adult mosquitoes. Killing adults similarly reduces adult density, but more importantly it shortens vector lifespan so fewer mosquitoes survive extrinsic incubation. Because extrinsic incubation for DV is expected to be relatively long compared to an average lifespan (Styer et al. 2007), killing adults before they become infectious has a greater impact on new human DV infections than does larval control. Encouraging the development of novel strategies for killing adult *Ae. aegypti* would exploit this fundamental concept and enhance dengue prevention.

We are not recommending abandoning larval control, especially in locations and cultures with strong community participation or where conditions are particularly favorable. For instance, in Vietnam biocontrol agents were available for treating a prominent and easily recognizable container class (Kay and Nam 2005). Removal of immature *Ae. aegypti* development sites, through physical or chemical means that are targeted at containers that produce the most adults, should be considered valuable components of integrated dengue vector control programs (WHO 2006a). Our main point here is that shifting attention from immature to adult mosquitoes for surveillance and control will stimulate development of more informative and effective methods with greater impact on reducing morbidity and mortality than an immature centric approach.

Emphasis on Intradomicile Vector Control

Increased attention on surveillance and control of adult *Ae. aegypti* reveals the opportunity to attack them in human habitations, where they spend most of their time. Because adult *Ae. aegypti* rest, feed, mate, and reproduce in houses (Scott et al. 2000b), it is believed that this is where they make the most frequent contact with humans (DeBenedictis et al. 2003), and thus, where most people are infected. The assumption that the home is the primary point of contact for human DV infection merits rigorous validation in prospective field studies. Nevertheless,

based on existing information, attacking this species in homes is well justified. The efficacy of strategies such as indoor residual sprays (IRS) and intradomicile application of insecticide-treated materials (ITM) are strongly supported by encouraging results from a variety of *Ae. aegypti* field studies (Nam et al. 1993; Nguyen et al. 1996; Igarashi 1997; Kroeger et al. 2006). Moreover, it has been known for some time that when insecticides do not reach *Ae. aegypti* inside homes they are ineffective (Reiter and Gubler 1997). Novel products and systems for delivery of insecticidal products into homes will enhance broad-scale intradomicile dengue prevention programs. It is essential that means for detecting and managing insecticide resistance are incorporated into an overall plan for adult mosquito control programs to prevent dengue. Because intradomicile control is conceptually consistent with the current public health policy for dengue (i.e., managing disease by managing mosquito vector populations) (PAHO 1994) it should be promoted to enhance disease prevention.

Advantages from this approach transcend *Ae. aegypti* and dengue. Intradomicile insect control will decrease densities and lifespans of dengue and nondengue insect vectors and pests and, thereby, help reduce the long list of public health problems that they represent. For example, in addition to dengue, the home is a major point of infection for pathogens like malaria, lymphatic filariasis, leishmaniasis, and Chagas disease. A variety of insect vectors (e.g., *Ae. aegypti, Anopheles gambiae, An. funestus, Culex quinquefasciatus*, sandflies, and triatomids) bite and infect humans in their homes. Pest insects (e.g., bed bugs, cockroaches, filth flies, and pest mosquitoes) are similarly too often abundant in homes and can lead to the perception that control measures directed at specific vectors (i.e., *Ae. aegypti*) are not effective. Knowledge gained from an improved understanding of peridomestic insect ecology can be effectively applied in intradomicile control strategies that address a variety of disease and pest problems. In so doing, what was originally conceived as an *Ae. aegypti* control program can be leveraged into a cost- and operationally-effective public health program that reduces a variety of diseases and pest problems.

Integrated Disease Prevention: Vector Control and Vaccines

It is generally accepted that an integrated, multidimensional control strategy is superior to a single line of attack (Shea et al.2000). Thus, vector control guidelines frequently and justifiably include recommendations for disease prevention that combine different vector interventions (WHO 2006a). We propose to take the notion of integrated disease prevention a step farther, across disciplines that traditionally have not been used in combination by applying vector control and a vaccine together. The justification for our recommendation is that in concert these two methods will act sooner and be more sustainable than either method by itself. The synergetic benefit, from vector control and chemotherapy, has been documented for lymphatic filariasis (Sunish et al. 2007). Proof of principle with another vector-borne disease justifies serious consideration of a similar strategy for dengue prevention. In this approach, we view both strategies as public health tools, rather than something intended to

protect individuals. The overall goal is to sustain a lowered force of DV transmission, ideally so that the basic reproductive number (R_o) for dengue is less than one. If that is accomplished, disease would correspondingly decrease and DV transmission could conceivably be eliminated from treated areas.

The combined benefit of vector control and a vaccine comes from their complimentary impact on reducing R_o. The critical proportion of a population that must be vaccinated to eliminate transmission of a pathogen is derived by the equation $P_c = 1 - (1/R_o)$ (Anderson and May 1991). Although, R_o for any pathogen varies through time and space, if we assume that for dengue $R_o = 10$ the critical proportion to vaccinate will be 90%. If R_o can be reduced by reducing the density of vector mosquitoes a smaller proportion of susceptible people will need to be vaccinated (i.e., if $R_o = 2$ then $P_c = 50\%$). Vector control, therefore, makes it easier to meet vector-borne disease vaccine delivery goals.

The positive impact of a vaccine on vector control concerns the issue of sustainability. There are numerous examples of effective vector control over the short term (Ooi et al. 2006). The big challenge is to sustain disease suppression. This is because effective vector control lowers the incidence rate. The aim of vector control, short of vector eradication, is to lower the force of pathogen transmission. Recruitment into the population of susceptible people by birth is sufficient to gradually decrease herd immunity over time to the point where mosquito densities necessary to avoid unacceptable levels of transmission are so low that operationally they are close to vector eradication. Accordingly, over the long term, vector control becomes increasingly difficult to sustain. If, however, herd immunity can be artificially elevated by vaccination this difficult battle does not need to be fought. Vaccination can be used to sustain artificially elevated levels of herd immunity and at the same time the force of DV transmission can be diminished by vector control. The result is an operational capacity to sustain R_o below one. Vaccination as a public health tool, therefore, makes sustained vector control a realistic possibility.

Clinical cures for dengue will be important for disease management, but are not likely to have a major impact on virus transmission because DV viremia is brief (i.e., 3–7 days), many DV infections are asymptomatic (Waterman and Gubler 1989; Focks et al. 1995; Rigau-Perez et al. 1998), and most people do not seek medical attention until after they have been viremic for some time or after their viremia has subsided altogether (Vaughn et al. 2000). Drugs will be valuable in a clinical setting but are not expected to reduce DV transmission unless applied prophylactically on a broad scale.

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