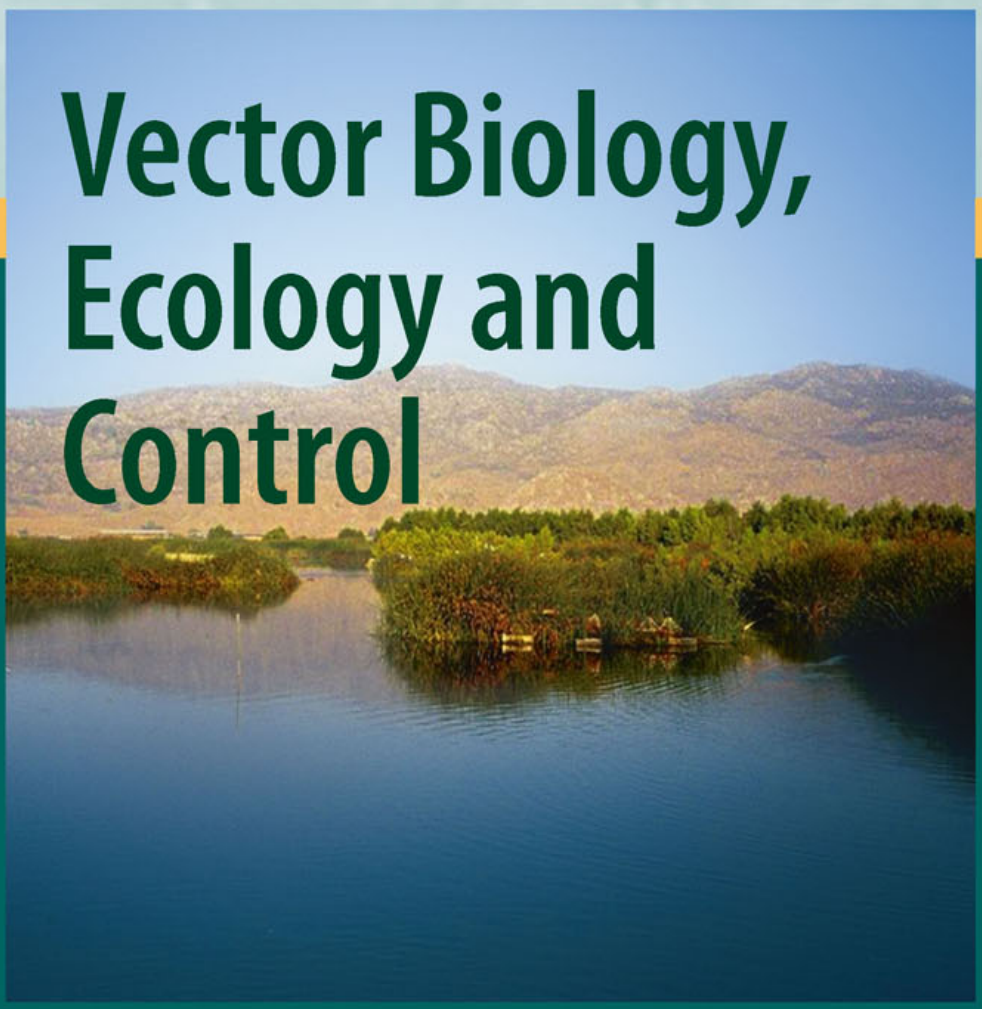
Peter W. Atkinson
Editor

Vector Biology, Ecology and Control



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Editor
Prof. Peter W. Atkinson
University of California, Riverside
Inst. Integrative Genome
Biology & Center for Disease Vector Control
Dept. Entomology
Riverside CA 92521
USA
peter.atkinson@ucr.edu

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Contributors

Assaf Anyamba Goddard Space Flight Center, Biospheric Sciences Branch, Greenbelt, MD 20771, USA, Assaf@ltpmail.gsfc.nasa.gov

Peter W. Atkinson Department of Entomology, Center for Disease Vectors Research, University of California, Riverside, CA 92521, USA, peter.atkinson@ucr.edu

Barry J. Beaty Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, bbeaty@colostate.edu

Norbert Becker University of Heidelberg, German Mosquito Control Association (KABS/GFS), 67165 Waldsee, Germany, norbertfbecker@web.de

Scott Bernhardt Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA

William Black Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, William.Black@colostate.edu

Carol Blair Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, Carol.Blair@colostate.edu

Seth C. Britch USDA-Center for Medical Agricultural and Veterinary Entomology, Gainesville, FL 32608, USA, Seth.Britch@ars.usda.gov

Ian Brooks National Center for Supercomputer Application, University of Illinois, Urbana-Champaign, IL 61801, USA, ian@ncsa.illinois.edu

David Brown Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624, USA, dabrown@fightthebite.net

Peter Burgess The Transparency and Accountability Network, Tr-Ac-Net, NY 10021, USA, peterbnyc@gmail.com

Jean-Paul Chretien Division of Preventive Medicine, Department of Defense Global Emerging Infections Surveillance and Response System, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA, Jean-Paul.Chretien@na.amedd.army.mil

Christopher F. Curtis† London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK

Lars Eisen Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, Lars.Eisen@colostate.edu

Darwin Elizondo-Quiroga Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA

Jose Farfan-Ale Universidad Autónoma de Yucatán, Mérida, Yucatán 97000, México

Brian A. Federici Department of Entomology and Interdepartmental Graduate Program in Cell, Molecular and Developmental Biology, University of California, Riverside, Riverside, CA 92521, USA, brian.federici@ucr.edu

Alexander Franz Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, Alexander.Franz@colostate.edu

Duane J. Gubler Department of Tropical Medicine, Medical Microbiology and Pharmacology, Asia-Pacific Institute Tropical Medicine and Infectious Diseases, John A. Burns School of Medicine, University of Hawai‘i, Honolulu, HI 96813, USA, dgubler@hawaii.edu

Anthony A. James Departments of Microbiology & Molecular Genetics and Molecular Biology & Biochemistry, University of California, Irvine, CA 92697, USA, aajames@uci.edu

A. Marm Kilpatrick Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95604, USA, marm@biology.ucsc.edu

Bart G.J. Knols Laboratory of Entomology, Wageningen University & Research Centre, 6700 EH, Wageningen; MalariaWorld, K&S Consulting, 6669 CP, Dodewaard, The Netherlands, bart.knols@wur.nl

Laura D. Kramer Department of Health, Arbovirus Laboratories, Wadsworth Center, Albany, NY 12201, USA, kramer@wadsworth.org

Kenneth J. Linthicum USDA-Center for Medical Agricultural & Veterinary Entomology, Gainesville, FL 32608, USA, Kenneth.Linthicum@ars.usda.gov

Saul Lozano-Fuentes Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA

Amy C. Morrison Department of Entomology, University of California, Davis, CA 95616, USA, aegypti@terra.com.pe

Robert J. Novak Division of Infectious Diseases, William C. Gorgas Center for Geographic Medicine, University of Alabama-Birmingham, Birmingham, AL 35294, USA, rjnovak@uab.edu

† The author is deceased

Ken E. Olson Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, Kenneth.Olson@colostate.edu

William K. Reisen Center for Vector borne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA, arbo123@pacbell.net

Donald R. Roberts Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA, droberts@usuhs.mil

Richard C. Russell Department of Medical Entomology, University of Sydney, Westmead, NSW 2145, Australia, rrussell@mail.usyd.edu.au

Irma Sanchez-Vargas Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA, irma.sanchez-vargas@colostate.edu

Thomas W. Scott Department of Entomology, University of California, Davis, CA 95616, USA, twscott@ucdavis.edu

Jennifer Small Goddard Space Flight Center, Biospheric Sciences Branch, Greenbelt, MD 20771, USA, jsmall@pop900.gsfc.nasa.gov

Apiwat Tawatsin Department of Medical Sciences, National Institute of Health, Ministry of Public Health, Nonthaburi 11000, Thailand, apiwat@dmsc.moph.go.th

UsavadeeThavara Department of Medical Sciences, National Institute of Health, Ministry of Public Health, Nonthaburi 11000, Thailand

Compton J. Tucker Goddard Space Flight Center, Biospheric Sciences Branch, Greenbelt, MD 20771, USA, compton@ltpmailx.gsfc.nasa.gov

Ingeborg van Schayk MalariaWorld, K&S Consulting, 6669 CP Dodewaard, The Netherlands, inga@malaria-world.com

Introduction

Mir S. Mulla joined the faculty of the Entomology Department at the University of California, Riverside in 1956, only two years after the Riverside campus was established as an independent campus within the University of California system. Prior to his appointment, Mir received his B.S. from Cornell University and then moved to the University of California, Berkeley to pursue his graduate studies. His Ph.D. from Berkeley, awarded in 1955, completed his formal American education which was the purpose of his immigration from his native Kandahar in Afghanistan.

In his over 50 years at Riverside, Mir has made an incalculable impact on vector biology both within the United States and in developing countries throughout the world. Within Southern California, Mir's basic and applied research led to the rapid and sustainable control of mosquitoes and eye gnats in the Coachella Valley and so directly enabled this region to grow to the thriving, large community it is today. In 2006 his efforts in facilitating the development of the low desert of southern California were recognized through the dedication of the Mir S. Mulla Biological Control Facility by the Coachella Valley Mosquito and Vector Control District. His success has been so profound that it remains somewhat cryptic to the many who now reside in, visit, and enjoy, this region of California, oblivious to the insect problems that severely restrained development until Mir and his students first applied their expertise many decades ago.

Mir has taken his expertise in biological control to many developing countries throughout the world leading to the successful control of mosquitoes in regions in which vector-borne disease is endemic. His research on developing new microbial control agents and his systematic testing of formulations in the laboratory, followed by trials in simulated field conditions and then in the field continues though his "retirement", further establishing his legacy of achieving sustainable, environmentally friendly and effective control of disease vectors throughout the world. Throughout his long career Mir has provided selfless service to the World Health Organization and to the many mosquito and vector control districts throughout California.

To celebrate Mir's 50 years of service to the University of California, Riverside, and to the state of California, his colleagues from California, the United States and the rest of the world gathered in Riverside for a symposium in vector biology in his honor. His long list of graduate students now run research programs in their own

countries while his many collaborators continue to employ the strategies developed by Mir at Riverside. Mir's tireless work ethic and attention to experimental detail are well known and were celebrated at this symposium as were the significant and lasting contributions he has made to global health, decades before this term enjoyed the common usage it does today.

Part I
Global Perspectives on Vector-Borne Disease

The Role of Global Climate Patterns in the Spatial and Temporal Distribution of Vector-Borne Disease

Kenneth J. Linthicum, Assaf Anyamba, Jean-Paul Chretien, Jennifer Small, Compton J. Tucker, and Seth C. Britch

Abstract Global climate variability patterns, such as those associated with the El Niño/Southern Oscillation (ENSO) phenomena, have been shown to have an impact on vector-borne infectious disease outbreaks. Evidence of the links between ENSO driven climate anomalies and infectious diseases, particularly those transmitted by insects, can allow us to provide improved long range forecasts of an epidemic or epizootic. Using satellite generated data developing climate anomalies suggested potential disease risks for 2006 and 2007. Sea surface temperatures in the equatorial east Pacific Ocean anomalously increased significantly during July–October 2006 indicating the typical development of El Niño conditions. The persistence of these conditions led to extremes in global-scale climate anomalies comparable to what has been observed during similar conditions in the past. The 2006 development of El Niño conditions had significant implications for global public health. Extremes in climate events with above normal rainfall and flooding in some regions and extended drought periods in other regions occurred. Forecasting disease is critical for timely and effective planning of operational control programs. Here we describe global climate anomalies that led to forecasts of elevated disease risks that gave decision makers additional tools to make rational judgments concerning implementation of disease prevention and mitigation strategies.

Keywords Climate change · ENSO · Disease · Vectors

Introduction

The earth's climate and ecosystems, comprising all living organisms including the disease agents of plants, animals, and humans, are intertwined in a complex association that we are attempting to better understand. Variability in global climate

K.J. Linthicum (✉)
USDA-Center for Medical, Agricultural & Veterinary Entomology, Gainesville, FL 32608, USA
e-mail: Kenneth.Linthicum@ars.usda.gov

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patterns has been shown to have an impact on the temporal and spatial distribution of infectious diseases (Nicholls 1993; Epstein 2002). The most well-known phenomenon influencing the global climate variability at interannual time scales is the El Niño/Southern Oscillation (ENSO). El Niño is the name given to a large-scale ocean-atmosphere climate phenomenon that is linked to periodic warming in sea surface temperature in the central equatorial Pacific. The opposite phenomenon is La Niña, a cold phase of ENSO. Given the large size of the Pacific Ocean, the variability in sea surface temperatures in this basin greatly influence global atmospheric circulation with pronounced impact on global-scale tropical precipitation extending into the northern hemisphere, particularly North America.

ENSO driven climate anomalies have been linked to infectious diseases, including diarrheal diseases in Peru (Checkley et al. 1997) and cholera (Pascual et al. 2000). Outbreaks of insect transmitted diseases such as Murray Valley encephalitis (Nicholls 1986), bluetongue (Baylis et al. 1999), Rift Valley fever (RVF) (Linthicum et al. 1999), African Horse sickness (Baylis et al. 1999), Ross River virus disease (Woodruff et al. 2002), dengue (Linthicum et al. 2007), chikungunya (Chretien et al. 2006), and malaria (Bouma et al. 1996; Bouma and Dye 1997) have been associated with El Niño or other climate anomalies. To properly time and efficiently plan effective operational disease and vector control programs it is important to forecast the risk, timing, and spatial extent of ENSO related human and animal disease outbreaks when such climate anomalies emerge. It is important to understand that forecasts must be precise, accurate, and timely for decision makers to respond effectively (Kovats et al. 2003). Here we describe how global climate anomalies that developed in mid 2006 through early 2007 indicated that potential disease risks were elevated in various parts of the world, and how decision makers were able to make rational judgments concerning a large RVF outbreak in the Horn of Africa and attempt to implement a wide-range of disease mitigation strategies.

Methods

We used current and forecasted ENSO anomalies (deviations from the long term mean) as based on model forecast and observed measurements of Sea Surface Temperatures (SSTs) and compared to the 1997–1998 ENSO event to infer potential disease risks for the end of 2006 and beginning of 2007. The 1997–1998 El Niño was the largest and warmest to develop in the Pacific Ocean in the past 100 years, and serves as a milestone for seasonal forecasting. Warm ENSO events are exemplified by above normal SSTs in the eastern Pacific (EP) and sometimes above normal SSTs in the western Indian Ocean (WIO). Warm ENSO events are known to increase precipitation over most eastern Pacific Ocean islands and the Peruvian coast, the US Southwest, and equatorial East Africa, and result in severe droughts in the western Pacific region (Indonesian archipelago, Philippines), Australia, northeast Brazil, and southern Africa (Glantz 1991; Chagas and Puppi 1986; Cane 1986; Rasmusson 1991; Ropelewski and Halpert 1987).

Variability is SSTs has an impact on atmospheric circulation patterns, especially influence precipitation producing mechanisms. Changes in atmospheric circulation can be inferred from Outgoing Longwave Radiation (OLR) measurements. In simple terms OLR is an indicator of both how warm the earth's surface is and how clear the atmosphere is overhead. Warm surfaces radiate more in the longwave range, while low values of OLR are typically with cool clouds in the atmosphere. Therefore high OLR values are indicative of dry land surfaces and the atmosphere above while the lowest values can be used to infer deep convective clouds which produce rainfall. The global scale changes in precipitation described above can be indirectly derived from OLR data and displayed as anomalies. The OLR data used in this paper are generated by the Advanced Very High Resolution Radiometer sensors on board a series of National Oceanic and Atmospheric Administration (NOAA) satellites.

An unscheduled El Niño conditions advisory issued in the fall of 2006 by NOAA's Climate Prediction Center (CPC) indicated El Niño conditions would peak during the Northern Hemisphere winter of 2006–2007, followed by weakening during March–May 2007 (NOAA 2006). This advisory was used to form both the genesis of an earlier paper forecasting global and local conditions conducive for elevated disease risk (Anyamba et al. 2006) and this paper which describes some of the subsequent observed disease activities.

Results and Discussion

Development of Climatic Conditions in Fall 2006

Following the fall 2006 NOAA CPC advisory (NOAA 2006), which indicated El Niño conditions had developed in the tropical Pacific and were forecast to likely continue into early 2007, Anyamba et al. (2006) started examining global SST and OLR data sets and found that SSTs increased significantly in the EP during September (Fig. 1) and October 2006. In September (Fig. 1) and October 2006, positive OLR anomaly conditions, indicating suppressed convection and precipitation, were observed across all of Indonesia, Malaysia, and most of the Philippines, which are usually the first areas to experience ENSO-related impacts. This dryness continued for the remainder of 2006 into the early part of 2007. Negative OLR anomalies, indicating enhanced convection and precipitation, were observed eastwards between the date line and Papua New Guinea, and to the west in the equatorial WIO region extending into equatorial East Africa.

Using the SST anomalies for the 1997/98 period as a reference for the most significant ENSO event (Fig. 2) and the climate forecast for the next 3–9 months, Anyamba et al. (2006) concluded that there would be a high likelihood for drought conditions to prevail over southeast Asia, Mexico, northeast Brazil, and southern Africa, and above normal rainfall and flood conditions to occur over coastal Peru, southern California, the U.S. Gulf Coast and Florida, and eastern Africa.

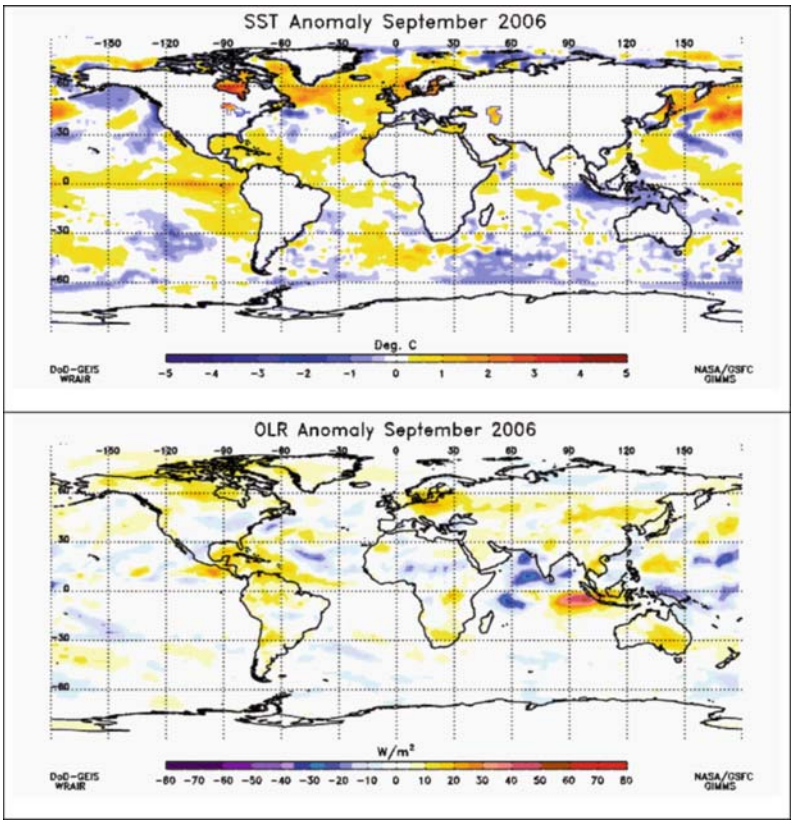

Fig. 1 Sea surface temperature (SST) anomalies for September 2006 (*top*) and outgoing longwave radiation (OLR) anomalies for September 2006 (*bottom*). SSTs are shown in degrees Celsius and OLR is shown as watts per square meter. Positive (negative) SST anomalies in the western equatorial Indian Ocean are associated with negative (positive) OLR anomalies in East Africa. The opposite patterns occur over southeast Asia where the SSTs along the Indonesian Archipelago are cold (*blue in color*) and the OLR data depict very dry conditions (*red in color*)

Forecasted and Reported Increased Disease Outbreaks

Extremes in climatic conditions may affect vector abundance and biology in different ways, either elevating or decreasing the risk of outbreaks of various vector-borne infectious diseases (Epstein 2001). Drought conditions can suppress predators of *Anopheles* malaria vectors, enhancing populations (Gabaldon 1949; Bouma and Dye 1997), reduce populations of *Aedes* and *Culex* mosquitoes that transmit RVF in sub-Saharan Africa (Fig. 3), or elevate dengue transmission due to increased water storage habitat for *Aedes aegypti* and increased temperatures enhancing vectorial capacity (Watts et al. 1987). On the other hand, heavy rains can boost food supplies and lead to elevated rodent populations (Engelthaler et al. 1999), and create ideal

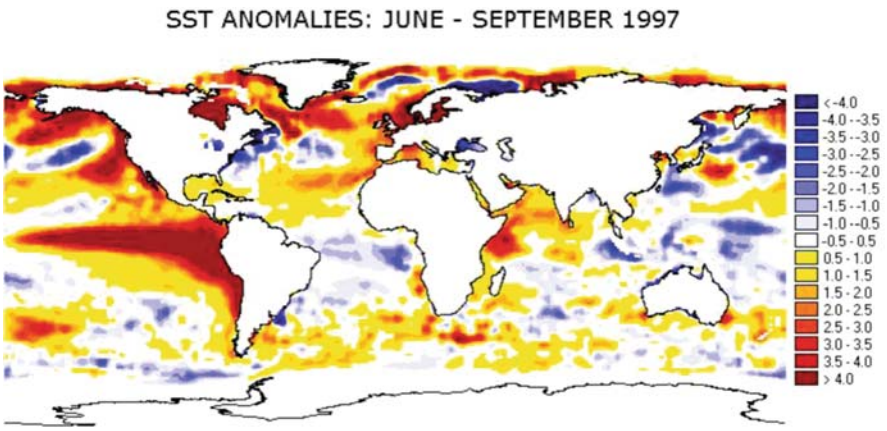

Fig. 2 Sea Surface Temperature (SST) anomalies for June through September 1997. Above normal SSTs have developed in the equatorial eastern Pacific Ocean (>3°C) and also in the equatorial Indian Ocean (~2°C) typical of warm ENSO events. The SST anomalies are computed with respect to 1982–1999 base period means

Fig. 3 Savanna grasslands in East Africa where *Aedes mcintoshi* (*left*) mosquito eggs harbor Rift Valley fever (RVF) virus during drought conditions (*center*) and adult *Aedes* and *Culex* mosquitoes transmit RVF after flooding of habitats and enhanced vegetation production during heavy rainfall periods (*right*). Note absence of clouds during drought conditions when OLR anomalies will likely be positive (*center*), and presence of clouds during especially wet conditions when OLR anomalies will be negative (*right*)

conditions for mosquito breeding and propagation of RVF virus (Fig. 3) (Davies et al. 1985; Linthicum et al. 1985).

Previous ENSO events have been strongly associated with disease outbreaks over time and space, with clusters of mosquito and rodent-borne illnesses. Based upon observations in 2006 and forecast information Anyamba et al. (2006) identified regions of North and South America, Africa, and Asia as being at increased risk for disease outbreaks (Fig. 4). A preliminary analysis of the quality of predictions of the warm ENSO phenomenon impacting various diseases throughout the world is discussed below following disease forecast information.

In Indonesia, Malaysia, Thailand, and most of the southeast Asia islands it was forecast that there would be increased dengue fever (DHF) transmission caused by

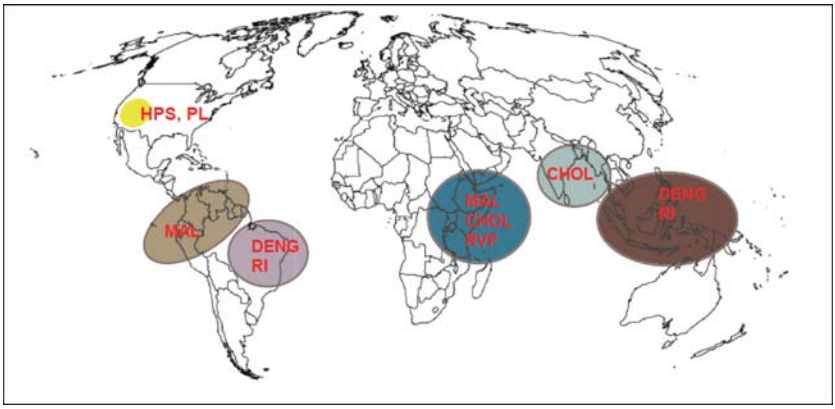

Fig. 4 Hotspots of potential elevated risk for disease outbreaks under El Niño conditions from 2006 to 2007. Diseases abbreviations as follows: DENG Dengue fever; RI Repiratory Illiness, CHOL Cholera, MAL Malaria, RVF Rift Valley fever, HPS Hanta Virus Pulmonary Syndrome, PL Plague. Adapted from Anyamba et al. 2006

drought conditions which (1) increase water storage around houses leading to elevated *Aedes aegypti* populations and (2) elevate ambient air temperatures which will reduce the extrinsic incubation period for the virus in vector mosquitoes, thus increasing vectorial capacity (Watts et al. 1987; Linthicum et al. 2007; Linthicum et al. unpublished observations). The Government of Indonesia reported in April 2007 that it considered the current DHF outbreak in early 2007 to be "an extraordinary situation." Uncontrolled burning of tropical forests and consequent smoke haze may also result from extreme drought. Thailand reported an increase in respiratory illness and issued alerts due to such haze during extreme drought that occurred in early 2007.

In Coastal Peru, Ecuador, Venezuela, and Colombia it was forecast that there would be an increased risk of malaria due to elevated *Anopheles* vector populations which will develop when various types of immature habitats are flooded after heavy rainfall follows a period of drought (Gabaldon 1949; Bouma and Dye 1997). There had been no reports of increased malaria transmission as of mid-2007.

In Bangladesh and coastal India it was forecast that elevated risk of cholera due to elevated SSTs and of inland incursion of plankton-laden water rich in *Vibrio cholerae*, the bacterium that causes cholera (Pascual et al. 2000). In addition to elevated SSTs, heavy rains wash nutrients into waterways and may trigger plankton blooms. Cholera cases are reported to be occurring in and around Delhi at this time but there are no reports from Bangladesh or coastal India in 2007.

In the U.S. Southwest (New Mexico, Arizona) it was forecast thought that there would be increased risk for hantavirus pulmonary syndrome and plague due to elevated rodent populations caused by heavy rainfall (Engelthaler et al. 1999; Parmenter et al. 1999). There has been a report of two hantavirus pulmonary syndrome human cases in Colorado by April 2007, more than would normally be

expected at this point in a normal year. New Mexico has reported a hantavirus case and deer mice rodent numbers are increasing.

In northeast Brazil it was forecast that drought conditions would lead to increased DHF and respiratory illness. Dengue transmission has been reported to be elevated in the period January through April 2007 by 20% over the same period in 2006.

In East Africa (Ethiopia, Kenya, Somalia, and Uganda) heavy rainfall in dry land areas was predicted to increase risk for RVF and malaria resulting from elevated mosquito vector populations, and increase risk for flooding-induced cholera (Loevinsohn 1994; Lindblade et al. 1999; Linthicum et al. 1999; Pascual et al. 2000;

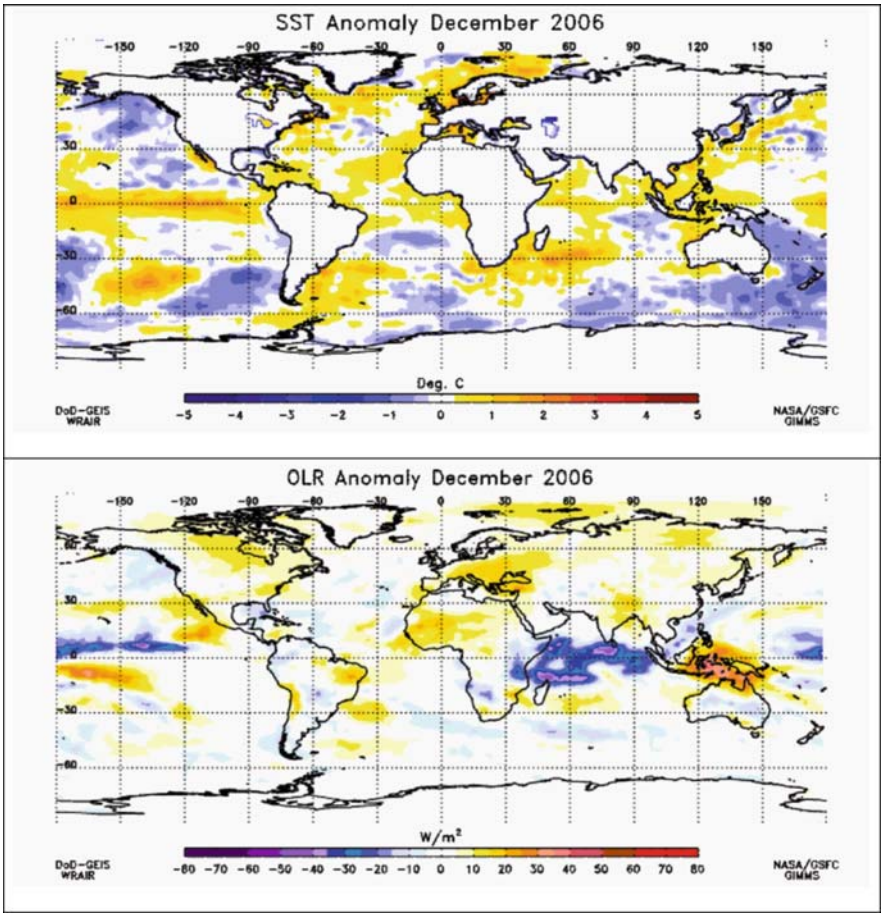

Fig. 5 Sea surface temperature (SST) anomalies for December 2006 (*top*) and outgoing longwave radiation (OLR) anomalies for December 2006 (*bottom*). SSTs are shown in degrees Celsius and OLR is shown as watts per square meter. Positive SST anomalies (depicted by *yellow-orange-red colors*) in the western equatorial Indian Ocean are associated with negative OLR anomalies (depicted by *blue colors*) in East Africa

Anyamba et al. 2002). Positive SST anomalies in the western equatorial Indian Ocean in September–October 2006 ranged from 0.3 to 0.4°C above normal and in December 2006 were almost 2°C above normal, and the warm ocean temperatures continued to produce heavy rainfall over much of the Indian Ocean and the Horn of Africa as depicted by negative OLR anomalies (Fig. 5). Anomalously high rainfall in October–December 2006 in most of Kenya and Tanzania ranged from 50 to 200 mm per month above normal. Increased levels of cholera cases were reported in Somalia, Djibouti, Kenya, and Tanzania in the first half of 2007. RVF was confirmed in patients from Garissa District in the North Eastern Province of Kenya in late December (MMWR 2007). Significant disease activity was also reported from Somalia in early 2007 and Tanzania thorough at least May 2007 (WHO Pandemic Alert and Response 2007). By February 2007 RVF cases in humans had been reported in Somalia, Kenya, and Tanzania (Fig. 6). The February 2007 RVF risk map based upon ecological studies (Anyamba et al. 2002) and published at the URL: http://www.geis.ha.osd.mil/RVFWeb/index.htm also depicted risk in Ethiopia, Sudan and Uganda but no human or animal cases were reported from these countries (Fig. 6). We observed as late as February 2007 that sheep that were allowed to graze in and around flooded mosquito habitats in savanna areas (Linthicum et al. 1984) became infected with RVF; however, cattle that were kept in feed lots several kilometers from flooded mosquito habitats remained uninfected (Fig. 7). A complete time series for the evolution of Nino 3.4 and WIO SSTs anomalies, and OLR anomalies over the Horn of Africa for the period August 2006 to May

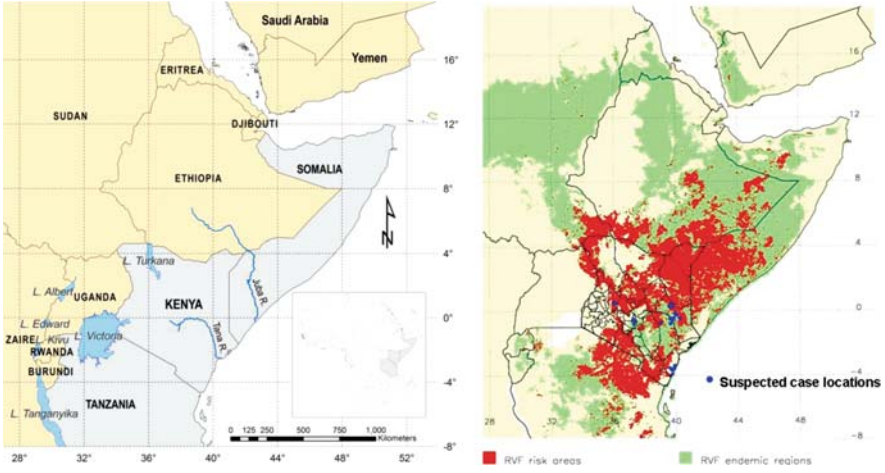

Fig. 6 Map of the Horn of Africa with countries affected by the 2006–2007 Rift Valley fever RVF outbreak depicted in gray and those countries not reporting cases depicted in beige color (*left*) and corresponding RVF risk map for February 2007. RVF endemic areas are depicted in green, and represent areas with climatological mean values that range between 0.15 and 0.4 Normalized Difference Vegetation Index (NDVI) units and receive between 200 and 800 mm/year of rainfall. Areas determined to be at risk from the RVF risk model are depicted in *red* and locations of suspected case locations in Kenya depicted by *blue dots*

Fig. 7 Sheep grazing in savanna grasslands in February 2007 in Kenya in and around flooded mosquito habitats which historically are known to be the source RVF virus (*left*), RVF infected sheep that had previously been grazing in mosquito habitats (*center*), and healthy cattle that were kept in feed lots several kilometers away from floodwater mosquito habitats (*right*)

2007 is shown in Fig. 8. It is clear that warming of SSTs in the Pacific and Indian Oceans as early as August 2006 and continuing through January 2007 quickly led to the development and maintenance of convective clouds over the Horn of Africa and the development of rainy conditions which lead to the RVF outbreak in December 2006 as described by Anyamba et al. (2009). Conversely, the cooling of the Pacific in February 2007 quickly reduced convective cloud conditions even though the Indian Ocean remained warm, resulting in the cessation in RVF transmission by mosquitoes.

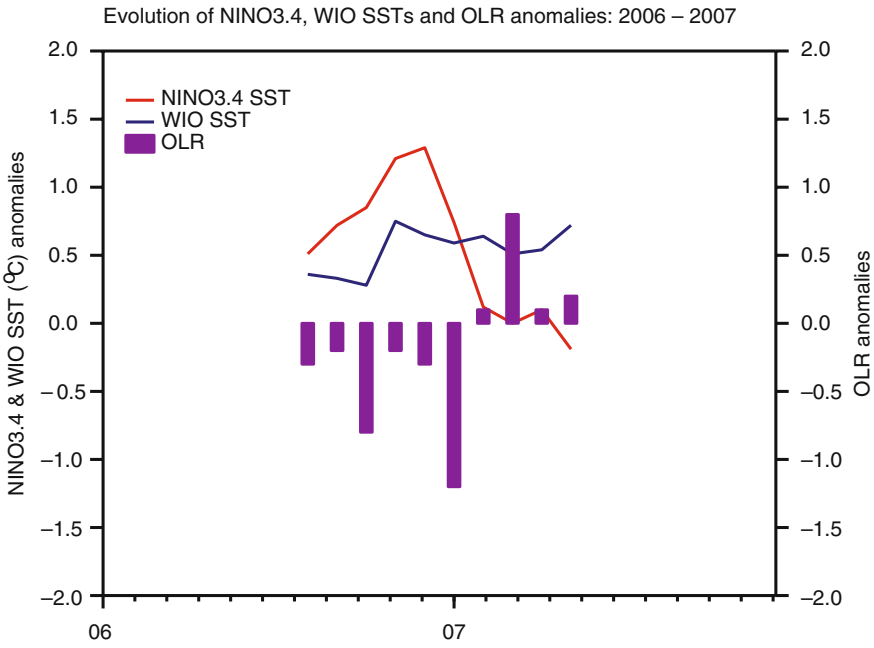

Fig. 8 Evolution of Nino 3.4 and WIO SSTs anomalies, and OLR anomalies over the Horn of Africa and the Indian Ocean for the period August 2006 to May 2007

Conclusions

Global climate analysis products including SSTs and OLR, which can serve as an indicator of rainfall, are useful in illustrating the situation of global climate anomalies with implications for public health. The fall-winter development of El Niño conditions first observed in September 2006 had important implications in early 2007 for global public health, and were similar to those observed during the large 1997/98 El Niño event and other such past events. These events have been demonstrated to have had a significant impact on vector borne diseases and their vectors. We recognize that all ENSOs do not behave alike, and climate change and warmer SSTs overall may be altering ENSOs and thus exaggerating the droughts and floods where they occur. It will be important to continue to monitor ENSO teleconnections and disease relationships in the future as climate changes. The development of El Niño conditions will continue to have important implications for global public health. The forecasting of epidemics or epizootics, like the RVF outbreak in the Horn of Africa in December 2006 to May 2007, is critical for timely and efficient planning of operational control programs if the forecast is able to precisely and accurately define the spatial and temporal range of disease outbreaks.

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The DDT Story: Environmentalism Over Rights to Health and Life

Donald R. Roberts

Abstract The insecticide DDT proved to be a potent tool in the control of insect vectors of human pathogens and directly led to the saving of many lives throughout the world during the middle of the 20th century. Reaction to environmental concerns over the use of DDT and other insecticides led to ban on the use of DDT. The cost-benefit outcome of this decision is discussed in light of the re-emergence of many vector-borne diseases over the last several decades.

Keywords DDT · Mosquito control · Toxicology · Environment

Introduction

The modern era of insecticide toxicology was launched by discovery of the insecticidal properties of DDT. The global significance of DDT for control of major human diseases was succinctly reviewed in 1957 by de Bustamante, Brazil's national malaria control program coordinator. He stated

> Until 1945–1946, preventive methods employed against malaria in Brasil, as in the rest of the world, were generally directed against the aquatic phases of the vectors (draining, larvicides, destruction of bromeliads, etc. . ..). These methods, however, were only applied in the principal cities of each state and the only measure available for rural populations exposed to malaria was free distribution of specific drugs (de Bustamante 1957).

As described by de Bustamante, advent of DDT brought dramatic changes in how governments could control malaria and other important human diseases. Standard methods of DDT use evolved quickly. For malaria, it was applied indoors at concentrations of 2 g/m^2 of wall surface. DDT spraying, referred to as indoor residual spraying (IRS), was deployed within highly disciplined and centralized programs that also included malaria case detection and treatment.

D.R. Roberts (✉)
Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

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The First Uses of IRS

Scientists in the United States began experimenting with DDT for control of malaria in 1943, and shortly thereafter, began using it operationally for malaria control in the War Areas within the U.S. However broad use of DDT did not get fully underway in developing countries until 1946. One such country was Guyana. Dr. George Giglioli supervised the trials and operational use of DDT in Guyana. The early DDT work in Guyana is elegantly described in Giglioli's autobiography, "Demerara Doctor." (Curtis 2006).

Guyana was intensely malarious. As shown in Table 1, malaria rates dropped precipitously once spray programs were initiated.

The remarkable results of the house-spraying campaign also brought dramatic reductions in infant and maternal mortalities (Table 2).

Giglioli reviewed the improvements in health and human welfare that accompanied the public health use of DDT. Particularly enlightening are the vital statistics that Giglioli compiled for Guyana Sugar Estates (Curtis 2006). He reported a 32% mortality, a 78% decrease in malaria deaths, a 50% decrease in deaths from anaemia, as well as many other improvements in health statistics.

The benefits of spraying houses were so universally high that programs were quickly initiated in many countries around the world. Those programs brought spectacular reductions in malaria and equally spectacular improvements in health. Successes of those programs eventually led to creation of the World Health

Table 1 Impact of DDT sprayed walls on malaria in Guyana (Giglioli 1951). DDT spraying began in mid-1946. Data are average values per year

	1943–45		1949–50		
Population surveyed	Spleen rate	Parasite rate	Spleen rate	Parasite rate	(%) Reduction
Rural					
279,475	33.3	37.7	7.1	0.22	99
Urban					
84,962	6.0	26.8	0.75	1.1	96

Table 2 Reductions in infant and maternal mortality in Guyana with use of DDT. DDT spraying began in mid-1946. Data are average values per year

1943–45		1949–50		
Population surveyed	Mortality per 1,000 live births	Population surveyed	Mortality per 1,000 live births	(%) Reduction
Infants				
368,498	126	408,331	77.5	39
Maternal				
368,498	12.67	408,331	5.5	56

Organization's global malaria eradication program as adopted by the 8th World Health Assembly in 1955.

Judging from official reports, the startup of organized global eradication did not get fully underway until 1959, which was the year when the first statistical reports on eradication programs began in the Americas. An estimated 300 million people had already been freed of endemic malaria even before the global program began. By end of eradication in 1969, another 600,000 million, for a total of almost one billion people, in originally malarious areas were largely freed of endemic malaria. The program brought enormous benefits to a huge proportion of the world's population. Unfortunately, neither many of the successes nor the programs themselves would survive the destructive forces that evolved during the 1960s.

Impact of the Anti-insecticide Movement

The anti-insecticide movement was one destructive force that evolved in the 1960s. The movement got its start through Rachel Carson's book, *Silent Spring*. Carson's book was published in 1962. In that book Carson described her imaginings of insidious DDT harm to wildlife. She claimed DDT was bringing the robin to the brink of extinction. This claim was false. Even in 1962 the robin was increasing in abundance, not declining at all.

Rachel Carson's book was a treatise on fear. She used such phrases as "evil spell"; "mysterious maladies"; "the cattle and sheep sickened and died"; "Everywhere was a shadow of death" to terrify and mobilize the public. Her book was devoid of scientific merit but it was, nevertheless, a publishing triumph. It changed the public's perception of DDT and other insecticides.

A fundamental premise of "Silent Spring" was the natural world had no experience or defense of DDT-like chemicals. At the time her book was written, information on natural chemicals, especially of organohalogens, was limited. Today, almost fifty years later, through the research of Gordon Gribble, Walter Vetter, and others, we know there is an abundance of natural organohalogens that are lipophilic, persistent, and accumulate in the food chain. One such chemical is Q1. It is a natural product with 7 chlorine atoms (DDT has only five). Q1 is abundant, widely distributed, accumulates in the food chain, and is even found in human breast milk.

Another example is a group of chemicals known as BC. The BCs are natual products. They contain 4 bromine atoms and are abundant, lipophilic, widely distributed, and accumulate in the food chain. Sponges produce BC compounds.

The wide distribution of natural products that are DDT-like is revealed by analysis of fat from common dolphins. One such analysis revealed the most abundant compound as BC-1, the second is Q1, the third is BC-2, the fourth is BC-3, and the fifth compound is p, p'-DDE. In this example DDE is much less abundant than the natural products, as are the PCBs.

Through decades of discovery that brought knowledge of a large world of natural and persistent chemicals, focus of the environmental movement remained largely on DDT. Ignored, but present all along, were the PCBs, dioxins, furans, a great diversity

of natural chemical insecticides, repellent, irritants, anti-feedants, etc. Some of these chemicals are more toxic, persistent, accumulative and abundant than DDT.

Male euglossine bees, *Eufreisa purpurata*, harvest large quantities of DDT from sprayed house walls in the Amazon Basin. This behavior is another example of how the natural world uses and interacts with DDT and other DDT-like compounds. Remarkably, the bees are not harmed by high DDT concentrations. Specimens stored in the museum since 1980 were analyzed for presence of DDT and other chlorinated chemicals. In addition to DDT, the bees contained a group of three highly chlorinated compounds, among others. It is hypothetically possible that male bees harvest DDT in order to strip chlorines from DDT as building blocks for other compounds. How the male bees use these natural chemicals is unknown. The three new compounds found in bees contain 8 chlorine atoms and are the most highly chlorinated natural products yet discovered.

As stated above, a major premise of Carson's work is the natural world has no experience with manmade chemicals like DDT. As shown in preceding paragraphs, that fundamental premise is wrong. It is now obvious that life evolved by making, using and coping with DDT-like chemicals. Carson's attack on DDT and other insecticides was instrumental in eliminating disease control programs around the world. However, Rachel Carson's book is not singly responsible for growth in anti-insecticide activism or the demise of effective malaria control programs. Another 1960s book was written on an entirely different ideological basis and it too preached against the use of DDT in malaria control programs. The book was *The Population Bomb* by Paul Ehrlich.

In his book Ehrlich proposed that growth of human populations was endangering life on earth. Such thinking was not new as illustrated by the following quote from Garrett Hardin:

"Every life saved this year in a poor country diminishes the quality of life for subsequent generations."

It was just a small step from believing that population growth was endangering life on earth to believing that public health use of DDT was harmful because it improved conditions for growth of human populations. This belief came to maturity in Paul Ehrlich's *The Population Bomb*. His book sold almost two million copies. It, like *Silent Spring*, was a treatise on fear. He used scary predictions to mobilize public opinion against national programs to prevent diseases and save lives.

Ehrlich predicted in the prologue of his book that "In the 1970s and 1980s hundred of millions of people will starve to death in spite of any crash programs embarked upon today. At this late date nothing can prevent a substantial increase in world death rate,. . ." He said there were only two solutions to our problems, either a death rate solution or a birth rate solution. Ehrlich blamed population growth on medical science, stating that ". . .medical science was the straw that broke the camel's back. While lowering death rates in the ODCs [overly developed countries] was due in part to other factors, there is no question that 'instant death control,' exported by the ODCs, has been responsible for the drastic lowering of death rates in the UDCs [under developed countries]." In these comments, Ehrlich was mostly referring to use of DDT in national malaria control programs. He referred to use

of DDT as "exported death control." He illustrated this with many descriptions of how spraying houses with DDT reduced malaria infections and malarial deaths. He stated "The power of exported death control can best be seen by an examination of the classic case of Ceylon's assault on malaria after World War II." In his descriptions of population density in India, he stated that the ". . .problems of Delhi and Calcutta are our problems too. Americans have helped to create them; we help to prevent their solution." Taken in context, the solution would be to stop spraying houses and to allow increasing malaria and, as a consequence, increasing malarial deaths. In his descriptions of population problems in Colombia, he states that Colombia is an extremely poor country ". . .with a doubling time of 21 years. Death control did not reach Colombia until after World War II. Before it arrived, a woman could expect to have two or three children survive to reproductive age if she went through ten pregnancies. Now, in spite of malnutrition, medical technology keeps seven or eight alive." The primary technology that arrived after WWII was DDT. Ehrlich attacked WHO for its support of national malaria control programs, stating "The World Health Organization. . . refuses to give up DDT for malaria control, claiming that hundreds of millions are doomed without it." It would seem that for those who subscribe to his ideology, the doom of hundreds of millions was precisely the outcome they wanted.

Converging Ideologies

All told, rich funding and powerful advocacy of the anti-insecticide movement and the political pressure and generous funding for population control combined to stall and eventually end many organized malaria control programs. Momentum for ideologies driving opposition to national malaria control programs evolved with the help of major science journals. Science magazine was a major outlet for anti-DDT literature. A review of papers published in Science magazine will reveal a pattern of publications similar to what is described in Table 3. In this table, papers dealing with the specific topic of DDT peaked during the time 1968–72. Of 70 papers published during the five years, only 3–4 letters were favorable to DDT and these were written mostly to contest outrageous claims by anti-DDT advocates. Charles

Table 3 Papers/letters on DDT published in Science magazine from 1968 to 1992

Year of publication	Number of papers/letters in Science magazine
1968–72	70
1973–77	10
1978–82	6
1983–87	1
1988–92	0

Statistics extracted from a PubMed search on keywords: journal "Science" and "DDT"

Wurster, chief science officer of the Environmental Defense Fund, authored seven articles in Science magazine on DDT. Wurster was an anti-DDT activist and not, in any meaningful way, a DDT expert. Yet he authored about 10% of all papers published on the topic of DDT in Science from 1968 to 1972. Science magazine also published a seriously flawed mathematical model of DDT harm.

The mathematical model was published in Science magazine in 1970. The author's calculated that DDT in long-lived species in a higher trophic level would continue to increase long after addition of DDT ended. They predicted that the accumulation would result in all members of trophic level being killed, reproductive failure if not killed, and of DDT passing to next higher trophic level if there was no effect. This fear-invoking model speculated that DDT, once present in obliterated population, would then be concentrated into fewer remaining species. Authors also proposed that whether this could be repeated in a series of systematic obliterations of species in upper trophic levels was unknown. The paper was written to invoke fear. The lack of a scientific basis for the model was a particularly egregious aspect of this particular paper. The model was based on an assumption of no DDT degradation or DDT metabolism. Earlier papers in Science magazine had already established that DDT was broken down in living organisms. Thus, reviewers and editors alike had to know that the fundamental assumption of the model was wrong. We can now look back and examine the model with full knowledge of what actually happened after DDT was banned in the United States.

It is now a matter of historical record that once DDT was banned in the United States, DDT declined dramatically in all higher trophic levels, it did not increase as predicted by the model in Science magazine. There were no obliterations in higher trophic levels. Not one species became extinct as a result of DDT use. DDT persisted at continually declining levels in some soils where it had been heavily used in agriculture, as well as in soils and sediments in and around formulation and production facilities. However its bioavailability was greatly reduced and, even in highly contaminated sites, there was almost no definable harm to soil fauna, soil flora, or wildlife. The model was just one example of highly biased DDT papers published in Science magazine. Another example was a paper about the Bermuda Petrel.

The Bermuda petrel paper was written by Charles Wurster and published in *Science* magazine. Wurster claimed DDT was threatening extinction of the Bermuda petrel. It was an amazing claim considering the petrel had been thought extinct for 300 years. Not until 1951 were a few nesting birds discovered. In reality, the petrel was endangered from loss of nesting habitats and from competition for nesting sites. So Wurster's prediction that DDT might cause extinction of the Bermuda petrel by the mid-1970s was a clever deception. The Bermuda petrel paper was given credibility because it was published in a highly prestigious magazine.

The obvious goal of outrageous claims by those within the environmental movement was to mobilize public and scientific opinion against DDT and other insecticides. All told, the environmental movement's use of fear was very successful. In a span of just a few years new and heavily funded organizations and bureaucracies came into existence. Additionally there was enormous growth in organizations and funding for population control. The anti-insecticide and population

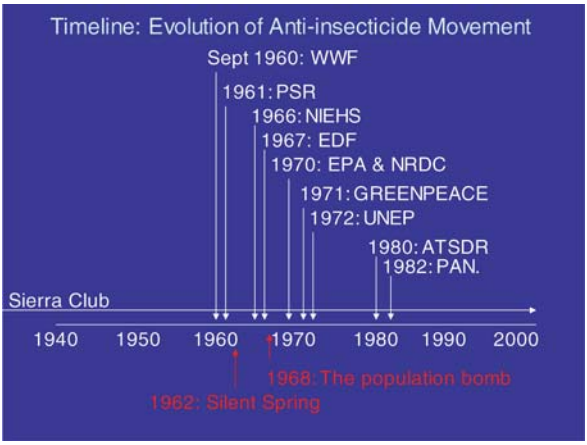

Fig. 1 Timeline for selected organizations of the anti-insecticide movement (WWF: World Wildlife Fund; PSR: Physicians for Social Responsibility; NIEHS: National Institute of Environmental Health Sciences; EDF: Environmental Defense Fund; EPA: Environmental Protection Agency; NRDC: Natural Resources Defense Concil; UNEP: United Nations Environment Programme; ATSDR: Agency for Toxic Substances and Disease Registry; PAN: Pesticide Action Network)

control movements were allied and their ascendancy over public health followed similar timelines. The timeline for appearance of a few environmental organizations and activist groups is presented in Fig. 1.

The first and ultimately influential of these groups were not benevolent or objective, but intimately tied to pernicious ideologies of the anti-insecticide and population control movements. This includes two of the most prominent environmentalist organizations today. The Sierra Club was an old organization that actively opposed DDT and other insecticides. David Brower, executive director of the Sierra Club, wrote the forward to Paul Ehrlich's "The Population Bomb." (New York Times). Brower was an outspoken advocate for controlling growth of human populations.

Sir Julian Huxley created the World Wildlife Fund (WWF) in England in 1960. Huxley too was a life-long advocate for controlling growth of human populations and growth of populations in Africa in particular. So, it was this constellation of ideologies that went after DDT in the 1960s. In later years, with DDT continually restricted from use in agriculture, the activists increasingly focused on stopping use of DDT in malaria control programs.

Failure of Public Health Advocacy

Individuals within and outside of the public health profession responded to attacks on DDT and other insecticides. However there was no coordinated response

and there were no well-funded advocacy groups to defend use of public health insecticides.

Despite the myths circulated by anti-insecticide activists, DDT was not a lucrative industry. DDT had no patent protections and was only a $20 million business, spread over four companies, in the U.S. It was not big business by any standard. Environmentalists claimed those who defended DDT were in the pockets of the insecticide industry. The fictitious money of the industry was just not there and most who defended DDT did so for public health and humanitarian reasons, not for commercial interests. The chemical industry itself was prepared to reap greater profits once DDT was gone. Replacement insecticides were patented, and more profitable and expensive than DDT.

In contrast to lack of organized advocacy on the public health side of the DDT struggle, the anti-insecticide movement mounted a coordinated and well-funded attack. They exaggerated threats and motivated opposition to insecticides through fear. They employed the press, the courts and prominent politicians. Editors of prestigious science journals were sympathetic to their cause. Those working to control malaria and other diseases in developing countries were caught like deer in the headlights of an oncoming car. Public health advocates for DDT were essentially defenseless against well-publicized fear tactics of the anti-insecticide movement. A colossal public health disaster has resulted from anti-insecticide and population control ideologies gaining control over what is done to control malaria and other insect-borne diseases.

Failures of Malaria Control Policies

The footprints of the public health disaster can be seen in the timeline for changes in global policies and strategies for control of malaria. The scale of the disaster is described in the patterns of re-emerging malaria and declining numbers of sprayed houses for four countries of the Americas (Fig. 2).

The anti-DDT campaign reached full maturity in the United States in 1969. The same year the WHA adopted resolution 22.39 and ended the time-limited approach to malaria eradication. Additionally, in 1969 the US ended the *Aedes aegypti* eradication program (program that relied on use of DDT) and the UN created the United Nations Population Fund (for controlling growth of human populations). Up to that time, UNICEF had been a major funding source for malaria eradication. By 1969 UNICEF had already become a major participant in the population control movement it quickly changed its funding priorities. By 1972 UNICEF had largely stopped funding house spray programs.

The US de-listed DDT for use in agriculture. The de-listing of DDT in the US and DDT bans in other developed countries caused a tremendous drop in supply of the chemical, a huge increase in price and a decline in its use for public health.

The next major policy change against house spray programs occurred in 1979 when the WHO changed its global malaria control strategy to de-emphasize spraying houses and emphasized instead, case detection and treatment. In 1985 the World

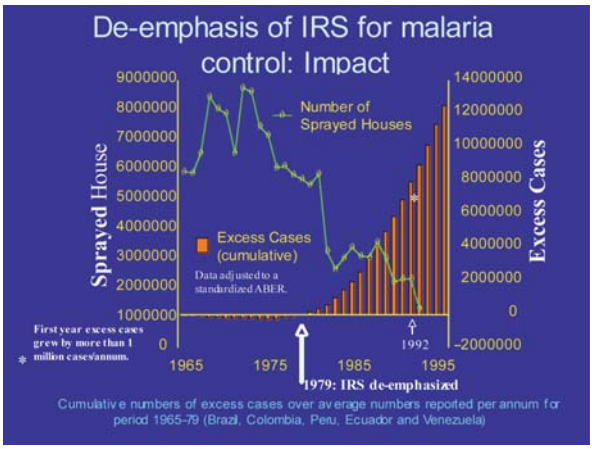

Fig. 2 Malaria increases and declining numbers of sprayed houses

Health Assembly adopted a resolution to decentralize malaria control programs by moving them into primary health care (PHC) systems. Organizationally, PHC systems are not compatible with organizational requirements of a house spray program, so the 1985 resolution was another nail in the coffin of IRS programs. The result of those changes is visible in large malaria increases and declining numbers of sprayed houses, as revealed in Fig. 2.

By the mid-1990s activists were still not satisfied with the pace of declining DDT use. In 1997, with input from environmental activists and no definable consultation from the malaria control community, the WHA adopted resolution 50.13 calling on member countries to reduce reliance on use of insecticides for disease control. This resolution was adopted even as malaria and other insect-borne diseases were continuing a long upward spiral, leaving more and more of the world's most vulnerable people either sick or dead.

With successful adoption of WHA resolution 50.13, anti-DDT advocates began almost immediately negotiating the Persistent Organics Pollutants treat for a global ban on DDT and other chemicals. In almost the last hour of negotiations, the global public health community finally came together to effectively lobby against a global ban on public health use of DDT. As a result of concerned people working together, DDT was exempted from elimination and today it can still be used in disease control programs.

DDT, A Unique Public Health Tool and Still Needed

DDT is a unique chemical compound and it is still needed for control of malaria and other diseases. DDT is unique because it functions mostly as a spatial repellent that stops mosquitoes from entering houses and biting people and transmitting

malaria while they sleep. Of all chemicals presently recommended for IRS, only DDT functions as a spatial repellent. Furthermore, its chemical actions on mosquito behavior probably account for most malaria mosquitoes still being susceptible to its toxic actions. DDT is also a contact irritant that will drive mosquitoes from treated surfaces. The contact irritant action will reduce biting rates and reduce opportunities for disease transmission. With more prolonged contact with treated surfaces, DDT will also kill mosquitoes that enter houses to feed. Altogether, no other chemical recommended for IRS has this unique profile of actions.

Failure to Find a DDT Substitute

Failure to find an adequate DDT replacement is due, in part, to the anti-DDT campaign itself. As stated above, what can be done to control malaria has, for many years, been largely under the control of the anti-insecticide movement. As a consequence, research funding generally reflect the priorities of that movement. Thus, globally, over time, hundreds of millions of dollars have been spent in research and advocacy for DDT elimination. In contrast, almost zero dollars have been spent to find a legitimate DDT replacement and almost zero dollars have been spent to improve the way we use DDT in malaria control programs. The whole subject matter of public health insecticides became an orphaned and moribund science. WHO compounded the lack of funding for chemical discovery by adhering to an entrenched view that toxicity is the only desirable chemical action of a public health insecticide. This view, of course, ignores how DDT actually functions. Today, DDT continues to be the cheapest and most long-acting chemical in the malaria control arsenal and it is still needed for control of important diseases.

Need for Advocacy for Public Health Use of Insecticides

The propaganda war against use of public health insecticides continues unabated. The global public health community must invest more in advocacy for public health insecticides, and for use of DDT in particular. The non-profit advocacy group, Africa Fighting Malaria, has become the leading proponent for IRS, for use of DDT, and renewed funding for research and development of new insecticides.

There should be no doubt that we need a replacement for DDT. However, a substitute for DDT will not become available unless there is new research and development funding. Additionally, a DDT replacement will not be discovered unless research is broadened to include spatial repellent and contact irritant actions.

Over the last 30–40 years anti-DDT propaganda has allowed billions of new malaria infections and millions of preventable deaths. Without advocacy to counter a continuing barrage of anti-insecticide propaganda, millions more will die. For this reason alone, our public health community should throw its support behind AFM's advocacy work.

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"Dave Brower [then executive director of the Sierra Club] expressed the consensus of the environmental movement on the subject in 1966 when he said, 'We feel you don't have a conservation policy unless you have a population policy.'" Taken from: Role of U.S. population stabilization at the beginning of the modern environmental movement (Population Issues and the 1970-Era Environmental Movement: http://www.numbersusa.com/interests/env_modernmove.html)

Vector-Borne Diseases in the 21st Century: Counting Up or Counting Down?

Anthony A. James

Abstract Medical entomology has undergone significant change during the past fifty years of Mir Mulla's service and leadership in the field. Significant advances have been made on a number of fronts, both in the field and in the lab leading to ongoing efforts to rein in vector-borne disease. These are discussed along with the identification of areas in which new research directions and synergies are needed.

Keywords Mosquito · Vector control · Insecticide · Biological control

Mir Mulla's half-century career as a medical entomologist overlaps a period of biological discovery during which some of the most profound advances have been made in understanding the physical basis of life. These include discoveries and achievements of such fundamental significance as the solving of the structure of DNA and its mechanisms for replication, and more recently, deriving the complete sequences of the genomes of humans and other organisms. Technological developments made possible many of these advances and they now permit detailed and complex analyses of biological systems on scales from the micro to the macro. Each advance challenges scientists to consider how the new knowledge may factor into some of the solutions they seek and to revise their efforts accordingly. Medical entomology as a discipline is not immune to the influences of these advances, and it is worthwhile to think about what has happened in the last fifty years and ask whether we can predict based on past events what we will need to meet the demands of the next fifty years. This exercise is limited to (and by) the perspective of the author, and will

A.A. James (✉)
Departments of Microbiology & Molecular Genetics and Molecular Biology & Biochemistry, University of California, Irvine, CA 92697, USA
e-mail: aajames@uci.edu

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focus on a few aspects of medical entomology that address arthropod vector-borne disease pathogens.

One thing we can be certain of as scientists is that there always will be significant changes in the way we interpret and conduct our work. Looking back, we see a progression of advances in medical entomology originating in basic and applied sciences. Fusions of insight and technology from chemistry, ecology and behavioral sciences resulted in a synergy that produced a number of significant successes in preventing and treating vector-borne diseases. Chemistry provided a powerful armamentarium of insecticides, repellants and drugs that were used successfully against the vectors and the pathogens they transmit. Although insecticides (for example, Paris green) were used for over a century, the implementation prior to and following the Second World War of DDT-based control practices led to many of the major achievements made by medical entomologists. Gladwell (2001), writing for a general audience, describes in great detail the impact that DDT had on morbidity and mortality from malaria, and estimates that tens of millions of lives were saved as a consequence of its widespread use. Ecological research provided valuable information about the biology of vectors that influenced when, where and how chemicals could be used to attack specific species. Indoor residual spraying for mosquitoes that transmit malaria remains to this day one of the most effective methods for halting epidemics (Curtis 2002). Development of insecticide-treated bednets benefited from an understanding of the host-seeking and post-feeding resting behavior of mosquitoes. Properly used, bednets can achieve significant reductions in severe disease (Lengeler 2004). Studies of ecology also supported environmental management to eliminate breeding habitats for the immature stages of vectors. Removing or modifying water sources can reduce significantly the population sizes of a number of vector species (for example, mosquitoes and black flies), and result in less transmission. Behavioral sciences, drawing on knowledge of ecology, increased popular awareness of the sources of disease and their modes of transmission, and produced recommendations for practices that prevent individuals from putting themselves at risk for exposure. Proper clothing, screening of windows and use of bednets are all effective by reducing contact of infected vectors with individuals. Basic research was spurred with the idea of developing supplementary approaches to vector control. We learned much about vector physiology as a result of efforts to try and define special properties of their biology that differ from other organisms. The strategy is to find biological processes unique to vectors and develop chemicals that target them specifically while having no effect on other species. Juvenile hormones are a favored target and methoprene is a product of these efforts (Wright 1976), although it is not used widely in vector control. Permethrin is a versatile compound that acts both as a repellent and insecticide. Biological control agents, including viruses (Becnel and White 2007), fish (Walton 2007), pathogenic fungi (Scholte et al. 2004; Kanzok and Jacobs-Lorena 2006), and predatory arthropods (Marten and Reid 2007; Schreiber 2007), have a high degree of safety and have been demonstrated to be effective in some circumstances. All of these practices combined with good and inexpensive drugs (malaria) and vaccines (some virus-caused diseases) reduced greatly disease burdens in some areas of the world.

With all of these great tools, where do we stand? While some good things have been done, the overall circumstances remain daunting. Sustained efforts have halted the increase in prevalence of Chagas disease (American trypanosomiasis) (Moncayo and Ortiz-Yanine 2006). We also see that progress with lymphatic filariasis is encouraging, with significant reductions in prevalence in parts of Africa and Asia (Mohammed et al. 2006; Stolk et al. 2003). The use of a good prophylactic and therapeutic drug, mectizan, has had a major positive effect on reducing the incidence and prevalence of onchocerciasis (river blindness) (Thylefors and Alleman 2006). However, vector-borne diseases as a group are increasing in incidence and prevalence (Remme et al. 2002). Malaria, dengue and leishmaniasis lead this surge with some contribution from African trypanosomiasis. The statistics are well-known to all who work in the field, well in excess of one million deaths per year, and an estimated loss of $4 - 5 \times 10^7$ DALYs (disability-adjusted life years) due to premature death, disability and daily work productivity. The complexity of the problem is illustrated further by the number and diversity of pathogens that are resurgent or emerging. Gratz (1999) recognized 15 diseases as resurgent and another dozen as emerging. This list is modified with the emergence of West Nile fever in the United States (Kramer et al. 2007) and the recent outbreak of chikungunya in Italy (Rezza et al. 2007; Chretien and Linthicum 2007) (Table 1). The latter outbreak is particularly instructive because the observed epidemiology results most likely from a single mutation in the virus that permits the mosquito, *Aedes albopictus*, to be a more competent vector (Tsetsarkin et al. 2007), combined with the recent (since 1990) invasion of that mosquito into a region where it was not previously found (Rezza et al. 2007). Yellow fever is among the resurgent. After great reductions in incidence and prevalence, some countries in South America and Africa appear to have stabilized its prevalence, while others experience sporadic epidemics (Barrett and Higgs 2007).

Table 1 Resurgent and emerging vector-borne diseases[a]

Resurgent	Emerging
Chikungunya	Barmah forest virus
Crimean-Congo hemorrhagic fever	Cat flea typhus
Dengue	Cat-scratch disease
Japanese encephalitis	Chikungunya
Leishmaniasis	Dengue hemorraghic fever
Lyme disease	Erlichiosis
Malaria	Kyasanur forest disease
Plague	O'nyong-nyong fever
Rift valley fever	Oriental spotted fever
Ross river virus	Oropouche virus
Trench fever	Posasi virus
Venezuelan equine encephalitis	Rocio virus
Yellow fever	West Nile virus

[a] Adapted from Gratz (1999).

Why do we still have problems with vector-borne diseases? As can be expected, the answer is complex. In some cases, it is a result of loss of effectiveness of our tools. Chemical resistance, be it to insecticides or drugs, eventually makes obsolete some of our best treatments. Long periods of perceived success are halted abruptly by selection of resistant vectors and pathogens. Deployment of new insecticides is hampered by their development costs and awareness by concerned public health workers of their effects on non-target organisms. Scientists now have more stringent criteria for new chemicals that emphasize safety as well as efficacy, and this has slowed the discovery and adoption of novel insecticides.

Sustainability of efforts also jeopardizes our ability to maintain control (Spielman 2006). A signature feature of good public health practices is that when they are working well, nothing appears to be happening. There are no epidemics and there is reduced morbidity and mortality. The will and ability to continue to carry out public health practices in the absence of a perceived threat are diminished. Why continue to pay for something that obviously is not a problem? Sustainability also is difficult when resources are limited and there are other public health demands for the funds that are used for controlling vector-borne diseases.

Changes in the characteristics of growing human populations such as immune status and international migration also lead to increased incidence and prevalence of specific diseases. New and complex human-made or human-induced ecologies contribute to the shift in the spectrum of vector-borne pathogens, with some organisms presenting novel threats and others re-emerging. At least twelve vector-borne diseases, including malaria and dengue, are predicted to be sensitive to climate change (Haines et al. 2006). Global warming and changes in rainfall patterns and abundance are expected to increase the sizes and distribution of vector populations, leading to upsurges in incidence and prevalence of these diseases.

Funding for research in medical entomology continues to be a challenge. Considerable excitement accompanied the decision by the Bill and Melinda Gates Foundation to include vector biology in the scope of its activities (http://www.gatesfoundation.org/default.htm) while the research portfolios in other charitable institutions were re-focused on other priorities. Remarkably, there has been a ~3–4 fold increase from 1975 to 2005 in grants awarded to vector biologists by the National Institutes of Health (USA), and the number of publications in leading journals increased >10-fold from the 1980s to now (Beaty et al. 2009). While these increases in research productivity are welcome, it is a challenge to keep them sustained. Furthermore, these increases are distributed unevenly among the global research community. While authorship on manuscripts from scientists from disease-endemic countries is becoming more representative, research funds for these same scientists are becoming more difficult to get. The recent shift in emphasis at the WHO/TDR in molecular entomology from individual to group grants is an index of these difficulties (http://www.who.int/tdr/grants/strategic-emphases/default.htm), and reflects also the difficult decisions that must be made by some funding agencies trying to optimize the impact of their limited resources.

The increases in funding and productivity in the developed world result in part from significant changes in research approaches and technologies, and the

recruitment of new investigators to the field. The new technologies include those derived from molecular biology and have been applied to differentiating members of species complexes (Favia and Louis 1999), identifying genes involved in parasite-refractory and insecticide-resistance phenotypes (Vernick et al. 2005; Hemingway et al. 2004), and assaying type and prevalence of pathogens in vector insects (e.g. de Paiva et al. 2007; Barker 1989). The genomes of three major mosquito vectors have been sequenced (Holt et al. 2002; Nene et al. 2007; http://www.vectorbase.org/index.php). These efforts have contributed to comprehensive studies of genes involved in refractory mechanisms (Riehle et al. 2006), the immune system (Dimopoulos 2003), olfaction (Fox et al. 2001; Bohbot et al. 2007) and insecticide resistance (David et al. 2005). It is now possible to use transient and stable methods for expressing exogenous genes in mosquitoes to make strains that are resistant to viruses and malaria parasites (Franz et al. 2006; Ito et al. 2002). These strains are the basis for population replacement strategies for controlling pathogen transmission (James et al. 1999). Similar technologies are being used to produce lethal strains that can be used in population reduction (Alphey 2002).

This review of medical entomology is admittedly cursory, however, there are few conclusions we can make: (1) The challenges of vector-borne diseases will always be with us. While we may have technologies that work now for specific vectors and pathogens, past experience supports the conclusion that we have no permanent solutions. From a biological perspective, we anticipate resistance to current chemicals to put more pressure on developing sustainable approaches for insecticide use. Even more confounding, we can expect the emergence of new pathogens that will require novel solutions. Therefore, we need to adopt an intellectual posture that accommodates the long-term commitment that working in medical entomology requires. We must support the use of the best tools currently available to alleviate the immediate disease burdens while at the same time develop novel control approaches for resurgent and new threats. This perspective also is important for sustaining funding for medical entomology. If governmental and private agencies recognize the dynamic nature of this field, hopefully they will understand that long-term commitments are needed to meet the continued threats of the disease pathogens; (2) We must encourage new thinking and the development of new tools. The past fifty years have shown a rapid evolution in our understanding of vectors and the pathogens they transmit. There is no reason to expect that the pace of new knowledge acquisition will slow. We must make efforts to support development and testing of the new ideas for control that will emerge from this increased knowledge; (3) New approaches are likely to be multidisciplinary. Just as knowledge of vector behavior provides information on the best methods for applying insecticides, novel approaches are going to draw on a number of disciplines to maximize their efficacy. Multidisciplinary efforts work best when driven by the needs of a practical goal. Here medical entomology has been at the forefront in examples of these types of applications. The use of any "integrated" approach requires a multidisciplinary perspective; (4) There is a need for more people to work in this field. The intellectual resources (genomes, complex modeling, epidemiology) are vast, and will require many new people to get involved.

Meeting the challenges raised in the conclusions requires significant progress in several research and applied areas, and a few of these are highlighted here. First, and perhaps foremost, there has to be a better representation and involvement of the end-users and intended beneficiaries of these efforts. With proper training, stake-holders and scientists from the disease-endemic countries can contribute to the formulation of source-originated solutions. It is reasonable to expect that a person who lives in a disease-endemic country will understand better the problems and provide novel insight for solutions. This requires building the capability and empowering of local scientists and public health workers. This is a goal that although recognized by both funding agencies (WHO/TDR 2002) and individuals, has seen few successes. The reasons for failure are many, and include few resources available in home countries. However, just because this task is difficult, does not mean it should be abandoned. Indeed, the failure to make significant progress is an argument that efforts to come up with creative solutions need to be intensified.

The quest for new ideas should encourage the exploration of other scientific disciplines for solutions to vector-borne diseases. A sustained interest in genetics and the opportunities it may provide has been evident for the last twenty years. We add to that a new knowledge of genomics and the tools of genetic manipulation and expectations are high to see soon a practical application of this work. A basic change in the way insecticides are being used is in progress. Although novel chemicals are needed, a more immediate impact can be made by rethinking the way the ones in use now are deployed. Managing insecticide resistance could sustain the utility of the currently-available chemicals (Hemingway et al. 2006).

Laboratory scientists need better tools. For example, many important vector species cannot be established easily as laboratory cultures (for example, the malaria vector mosquito, *Anopheles darlingi*). Other species, although adapted in laboratories, are difficult to maintain in large numbers, or as distinct genetic stocks. The ability to maintain stocks and lines for long-term analysis requires development of cryopreservation or other such techniques. Challenge assays for testing vector competence for specific pathogens are not available widely, and thus prevent segments of the research community from working in this area. Bioinformatics and genomic resources are in their early days of practical use, and while a vast amount of information is accessible through excellent internet sites (for example: http://www.vectorbase.org/index.php and http://www.angagepuci.bio.uci.edu), work is on-going to make them more useful to a wider spectrum of scientists. In addition, there is a need for the genome sequences of more vector species for comparative studies.

Field studies also could benefit from better tools. Methods for real-time tracking of individual vectors would revolutionize our understanding of pathogen transmission. A high-precision method for determining the age of an individual vector also would be helpful. Well-designed behavioral studies could lead to the colonization of different species as well as influence our analyses of transmission dynamics. Population studies are needed for understanding the range of the vectors and the pathogens they transmit. Modeling represents an interface between laboratory and field work, and needs to be expanded to a larger arena. Fusing models of

different vector control practices with out-come predictions on epidemiology and cost-effectiveness could play a major role in stake-holder decisions on the use of alternative control options.

Comprehensive training for future medical entomologist is urgent, and not just for those in disease-endemic countries. Students should know the basics of entomology, parasitology, molecular biology, transmission and population genetics, field biology, ecology, epidemiology and public health. Few, if any, existing curricula teach elements of all of these disciplines. Thus, there is a continual need for specialized courses and workshops to fill in gaps in these knowledge requirements.

Counting up or counting down? Well, we see elements of both. Our challenges are many and the list is longer than the few items addressed here. However, fifty years is a long time. We should take inspiration from Dr. Mulla, back it up with our optimism and intellect, roll up our sleeves and get to work!

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Part II
Emerging and Invasive Vector-Borne Diseases

The Global Threat of Emergent/Re-emergent Vector-Borne Diseases

Duane J. Gubler

Abstract The past 30 years has witnessed a dramatic re-emergence of epidemic vector-borne diseases throughout much of the world. Factors contributing to this are many, but the principal drivers have been complacency and de-emphasis of infectious diseases in pubic health policy, increased population growth, uncontrolled urbanization without concomitant attention to water and waste management, increased globalization and the ease with which modern air transport can quickly spread pathogens and their vectors. The re-emergence of parasitic, bacterial and viral vector-borne pathogens is described. This re-emergence increases the current and future need for preventative measures to contain disease outbreaks and for international cooperation and collaboration to constantly monitor the outbreak of these debilitating and deadly diseases.

Keywords Dengue · West Nile · Yellow fever · Plague · Lyme disease · Vector-borne diseases · Mosquitoes · Ticks · Urbanization · Globalization

Introduction

At the beginning of the 20th century, epidemic vector-borne diseases were among the most important global public health problems (Gubler 1998, 2002a). Diseases such as yellow fever (YF), dengue fever (DF), plague, louse-borne Typhus, malaria,

D.J. Gubler (✉)
Program in Emerging Infections Diseases, Duke-Nus Graduate Medical School, 8 College Road, Singapore 169857
Department of Tropical Medicine, Medical Microbiology and Pharmacology, Asia-Pacific Institute Tropical Medicine and Infectious Diseases, John A. Burns School of Medicine, University of Hawai'i, Honolulu, HI 96813, USA
e-mail: dgubler@hawaii.edu

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etc., caused explosive epidemics affecting thousands of people. Subsequently, other vector-borne diseases were identified as major causes of disease in both humans and domestic animals. As the natural history of these diseases became better understood, prevention and control measures, primarily directed at the arthropod vectors, were highly successful in controlling disease transmission. Effective prevention and control accelerated in the post-World War II years with the advent of new insecticides, drugs, and vaccines. By the 1960s, the majority of important vector-borne diseases had been effectively controlled in most parts of the world, and those that were not yet controlled were targeted for more intensive programs using new vaccines, drugs, and insecticides.

Unfortunately, "success led to failure"; some of the successful programs, such as the *Aedes aegypti* eradication program that effectively controlled epidemic YF and DF throughout the American tropics for over 40 years, and the global malaria eradication program that effectively controlled malaria in Asian and American countries, were disbanded in the 1970s because the diseases were no longer major public health problems (Gubler 1989, 2004; Gubler and Wilson 2005; IOM 1992). Additionally, residual insecticides were replaced with less effective chemicals used as space sprays to control adult mosquitoes. The 1970s ushered in a 25-year period characterized by decreasing resources for infectious diseases, decay of the public health infrastructure to control vector-borne diseases, and a general perception that vector-borne diseases were no longer important public health problems. Coincident with this period of complacency, however, was the development of global trends that have contributed to the reemergence of epidemic infectious diseases in general, and vector-borne diseases in particular, in the past 25 years. In addition to the emergence of newly recognized diseases, there was increased incidence and geographic expansion of well-known diseases that were once effectively controlled (Gubler 1989, 1998; IOM 1992, 2003; Mahy and Murphy 2005). This paper will briefly review the changing epidemiology of several of the most important vector-borne diseases and discuss the lessons learned from this global reemergence.

The Reemergence of Epidemic Vector-Borne Diseases As Public Health Problems

The earliest indications that epidemic vector-borne diseases might reemerge came in the early 1970s. Subsequent warnings were ignored by public health officials and policy makers because of competing priorities for limited resources (Gubler 1980, 1987, 1989; IOM 1992). The 1980s ushered in a period with increased epidemic vector-borne disease activity associated with expanding geographic distribution of both the vectors and the pathogens via modern transportation and globalization. It was not until the Institute of Medicine (IOM) report on emerging infectious diseases that policy makers took notice (IOM 1992), and not until after the 1994 plague epidemic in India that new resources were allocated to emerging infectious diseases (Fritz et al. 1996; WHO 1994).

Parasitic, bacterial, and viral pathogens may be transmitted by blood-sucking arthropods. Mosquitoes, which primarily transmit parasitic and viral diseases, are the most important arthropod vectors; ticks, which primarily transmit bacteria and viruses, are next in importance.

Parasitic Diseases

Of the parasitic infections transmitted by arthropods, malaria is by far the most important, although there has also been a reemergence of leishmaniasis and African trypanosomiasis. The principal problem area for malaria is Africa, where 95% of all global cases occur, most of them in children under five years of age (Gubler and Wilson 2005). This disease is dealt with elsewhere and will not be considered further here.

Bacterial Diseases

Two newly recognized vector-borne bacterial diseases, Lyme disease, caused by *Borrelia burgdorferi,* and ehrlichiosis, caused by *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum,* and *Ehrlichia ewingui,* have emerged as important public health problems in the past three decades (Dumler et al. 2007; Steere et al. 2004). Both have small rodents as their natural vertebrate reservoir host, with hard ticks as their principal vectors. Both diseases are found primarily in temperate regions of the world, where emergence has been associated with environmental change. Figure 1 shows the dramatic increase in reported cases of Lyme disease in the United States since the Centers for Disease Control and Prevention (CDC) began surveillance in 1982. The increased transmission in the United States is directly related to reforestation of the northeastern United States, allowing the mouse and deer populations to increase unchecked, which in turn has allowed the tick population to increase. A final factor has been the trend in recent decades to build houses in woodlots where humans share the ecology with deer, mice, and ticks; thus most transmission to humans in the northeastern United States where the majority of cases of Lyme disease occur, is residential (Steere et al. 2004).

Plague, caused by *Yersinia pestis*, is the most important reemergent bacterial vector-borne disease. The current global increase in case reports of plague is primarily due to outbreaks in Africa. However, it is the potential of plague to cause explosive epidemics of pneumonic disease, transmitted person-to-person and with high mortality that makes it important as a reemergent infectious disease and as a potential bioterrorist threat. This was illustrated in 1994 when an outbreak of plague occurred in Surat, Gujarat, India (WHO 1994). Although this was a small outbreak (most likely less than 50 cases) that should have been a relatively unimportant local public health event, it became a global public health emergency. The reasons for this are complicated and beyond the scope of this article, but it is a

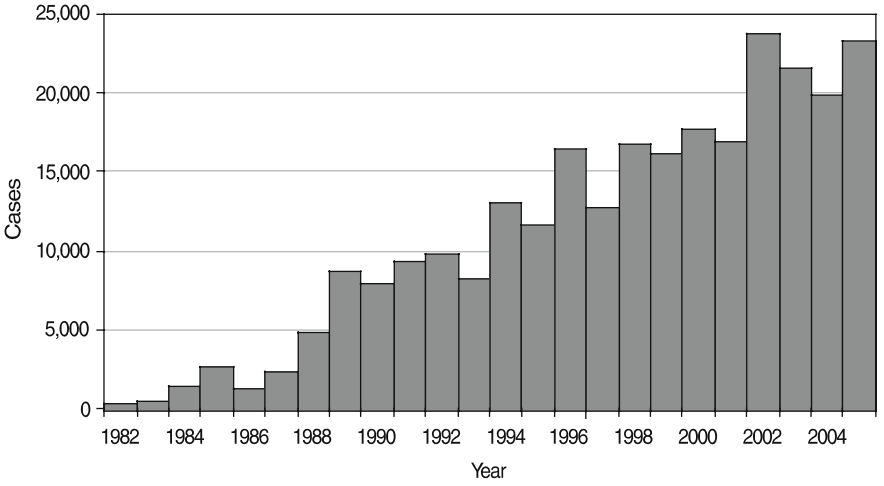

Fig. 1 Reported Lyme disease cases by year, United States, 1982–2005. Source: Adapted from Gubler (1998) and CDC (2006), courtesy, Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO

classic case of "success breeding failure." Briefly, because the Indian Health Service had successfully controlled epidemic plague in India for over 30 years (the last confirmed human plague case prior to 1994 was in 1966), laboratory, clinical, and epidemiologic capacity to diagnose and control plague had deteriorated. Thus, when the Surat outbreak occurred, the clinical and laboratory diagnosis was confused, creating lack of confidence in public health agencies and ultimately panic when it was finally announced that the disease was pneumonic plague. Within a few weeks in early October 1994, an estimated 500,000 people fled Surat, a city of about 2 million people at that time. Many of these people traveled to other urban areas in India, and within days, newspapers were reporting plague cases in other cities. The World Health Organization implemented Article 11 of the International Health Regulations (WHO 1983) for the first time in 33 years because it was thought that people with pneumonic plague might board airplanes in India and transport the disease to other urban centers around the world (Fig. 2). Many countries stopped air travel and trade with India and most implemented enhanced surveillance for imported plague cases via airplane travel. This was the first global emerging infectious disease epidemic that impacted the global economy since infectious diseases were controlled in the 1950s. It is estimated that this small outbreak cost India US$3 billion (John 1999) and the global economy US$5–$6 billion. Fortunately, there were no cases of plague exported from India (Fritz et al. 1996), but this epidemic was the "wake-up call" that modern transportation and globalization were major drivers of pandemic infectious diseases. It was this epidemic that helped stimulate in the first funding of CDC's Emerging Infectious Disease Program.

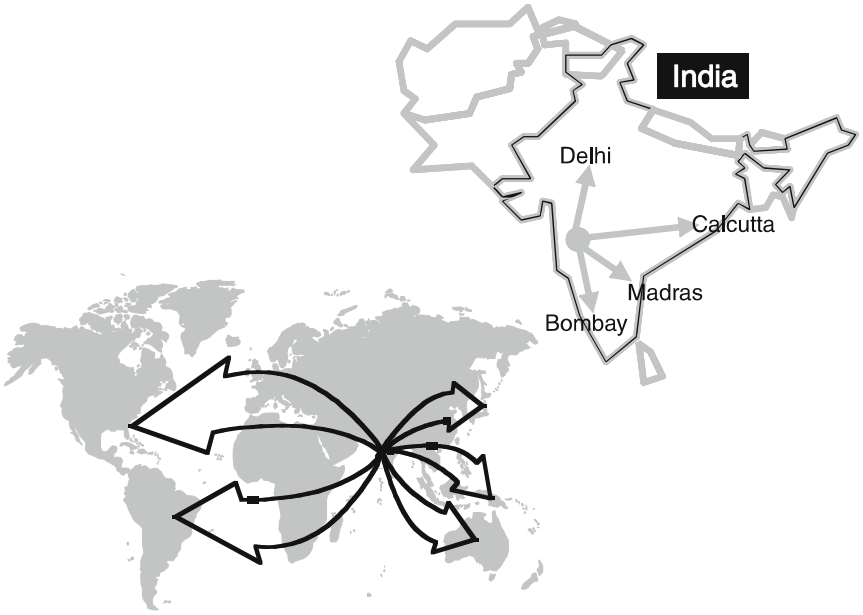

Fig. 2 Suspected spread of pneumonic plague from India, 1994. Source: Courtesy, Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO

Arboviral Diseases

Of the vector-borne diseases, it is the arboviruses that have become the most important causes of reemergent epidemic disease (Gubler 1996, 2002a). In 2007, there are few places on Earth where there is no risk of infection with one or more of these viral diseases, most of which are transmitted by mosquitoes. The more important reemergent epidemic arboviral diseases are presented in Table 1. They include members

Table 1 Emergent/Re-emergent arboviral diseases of humans

- Dengue hemorrhagic fever
- Yellow fever
- West Nile fever
- Japanese encephalitis
- Chikungunya
- Rift valley fever
- Alkumra fever (Kyasanui forest disease)
- Venezuelan equine encephalitis
- Epidemic polyarthritis
- Barmah forest
- Oropouche
- California encephalitis
- Crimean-Congo hemorrhagic fever

of three families (*Togaviridae*, *Flaviviridae*, and *Bunyaviridae*). Three diseases – dengue fever, West Nile, and yellow fever – will be discussed as case studies to illustrate the changing epidemiology of arboviral diseases.

West Nile Virus

West Nile virus (WNV) (*Flaviviridae,* genus *Flavivirus*), an African virus, belongs to the[1] Japanese encephalitis virus (JEV) sero-group, which includes a number of closely related viruses, including JEV in Asia, St. Louis encephalitis virus in the Americas, and Murray Valley encephalitis virus in Australia. All have a similar transmission cycle involving birds as the natural vertebrate hosts and *Culex* species mosquitoes as the enzootic/epizootic vectors, and all cause severe and fatal neurologic disease in humans and domestic animals, which are generally thought to be incidental hosts, as well as in birds.

The clinical illness associated with WNV in humans ranges from asymptomatic infection to viral syndrome to neurologic disease (Hayes and Gubler 2006), but historically it has been considered among the least virulent of the Japanese encephalitis sero-group viruses (Hayes 1988); recent epidemics, however, have changed that perception.

From the time WNV was first isolated from the blood of a febrile patient in the West Nile province of Uganda in 1937 (Smithburn et al. 1940) until the fall of 1999, it was considered relatively unimportant as a human and animal pathogen. The virus was enzootic throughout Africa, West and Central Asia, the Middle East, and the Mediterranean, with occasional extension into Europe (Hayes 1988). A subtype of WNV (Kunjin) is also found in Australia (Hall et al. 2002). A characteristic of WNV epidemiology during this 62-year history (1937–1999) was that it caused epidemics only occasionally, and the illness in humans, horses, and birds was generally either asymptomatic or mild; neurologic disease and death were rare (Marfin and Gubler 2001; Murgue et al. 2001, 2002).

In late August 1999, an astute physician in Queens, New York, identified a cluster of elderly patients with viral encephalitis (Asnis et al. 2000). Because of the age group involved and the clinical presentation, these cases were initially thought to be St. Louis encephalitis, but subsequent serologic and virologic investigation showed them to be caused by WNV (Lanciotti et al. 1999). The epidemic investigation, which focused only on neurologic disease, identified 62 cases with 7 (11%) deaths, all of them in New York City (Nash et al. 2001). Epidemiologic studies, however, showed widespread transmission throughout New York City, with thousands of infections (Montashari et al. 2001; Nash et al. 2001). The virus caused a high fatality rate in birds, especially those in the family *Corvidae* (Komar 2003). Genetic sequence of the infecting virus suggested it was imported from the Middle East, most likely from Israel (Lanciotti et al. 1999). Although it will never be known for sure, epidemiologic and virologic evidence suggests the virus was introduced in

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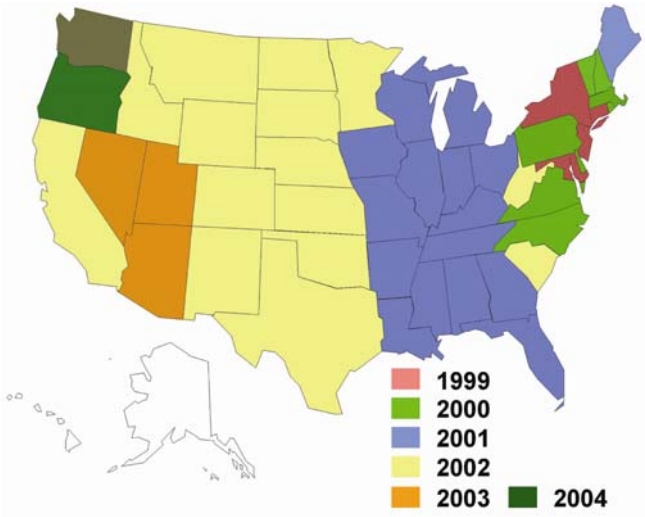

Fig. 3 The spread of West Nile virus through the United States, 1999–2004

the spring or early summer of 1999, most likely via infected humans arriving from Israel, which was experiencing an epidemic of WNV in Tel Aviv at the time (Giladi et al. 2001; Marfin and Gubler 2001).

Over the next five years, WNV rapidly moved westward across the United States to the west coast (Fig. 3), north into Canada, and south into Mexico, the Caribbean, and Central America. In 2002, it caused the largest epidemic of meningoencephalitis in U.S. history with nearly 3,000 cases of neurologic disease and 284 deaths. That same year, there was a large epizootic in equines with over 14,500 cases of neurologic disease and a case fatality rate of nearly 30% (Campbell et al. 2002). The epidemic curve for human cases in the United States is shown in Fig. 4. In 2003, another large epidemic occurred, but the epicenter of transmission was in the plains states and the majority of the reported cases were not neurologic disease (Hayes and Gubler 2006). Since 2003, the virus has persisted with seasonal transmission during the summer months, but at a lower level; the majority of cases have been in the plains and western states.

WNV was first detected south of the U.S. border in 2001 when a human case of neuro-invasive disease was reported in the Cayman Islands (Campbell et al. 2002), and birds collected in Jamaica in early 2002 were positive for WNV-neutralizing antibodies (Komar and Clark 2006). In 2002, WNV activity was reported in birds and/or equines in Mexico (in six states) and on the Caribbean islands of Hispaniola (Greater Antilles) and Guadeloupe (Lesser Antilles). Most likely, the virus was also present in Mexico in 2001, since a cow with WNV-neutralizing antibody was detected in the southern state of Chiapas in July of 2001 (Ulloa et al. 2003). In 2003, the virus was detected in 22 states of Mexico; in Belize, Guatemala, and El Salvador in Central America; and in Cuba, Puerto Rico, and the Bahamas in the Caribbean. In 2004, WNV activity was reported from northern Colombia, Trinidad,

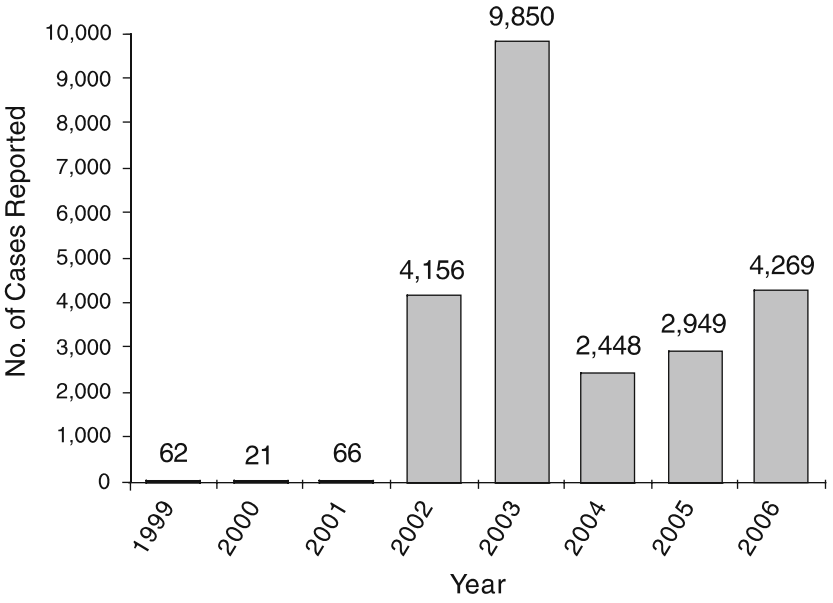

Fig. 4 Epidemic West Nile virus in the United States, 1999–2006, reported to CDC as of May 2, 2007. Source: CDC (2007)

and Venezuela, the first reported activity in South America; in 2006, Argentina reported WNV transmission (Komar and Clark 2006; Morales et al. 2006).

Migratory birds have likely played an important role in the spread of WNV in the western hemisphere (Owen et al. 2006; Rappole et al. 2000). This conclusion is supported by data on the movement of WNV in migratory birds in the Old World (Malkinson et al. 2002). Moreover, the westward movement of WNV across the United States and Canada can best be explained by introduction via migratory birds that fly south to Central and South America in the fall and north from those areas in the spring. Thus, the yearly movement westward in 2000, 2001, 2002, 2003, and 2004 shows very good correlation with the Atlantic, Mississippi, Central, and Pacific flyways of migratory birds (Figs. 3 and 5). After introduction to an area, local dispersion of WNV likely occurred via movement of resident birds, which often fly significant distances. Interestingly, the major epidemic in each region of the country occurred the following year after introduction, with the exception of the 1999 New York outbreak.

The emergence of a WNV strain with greater epidemic potential and virulence was likely a major factor in the spread of WNV in both the Old and the New Worlds (Marfin and Gubler 2001). The first evidence of this new strain of WNV was in North Africa in 1994, when an epidemic/epizootic of serologically confirmed WNV occurred in Algeria; of 50 cases with neurologic disease 20 (40%) were diagnosed as encephalitis and 8 (16%) died (Murgue et al. 2002). Over the next 5 years, epidemics/epizootics occurred in Morocco, Romania, Tunisia, Israel, Italy, and Russia, as well as jumping the Atlantic and causing the epidemic in Queens, New York (Fig. 6).

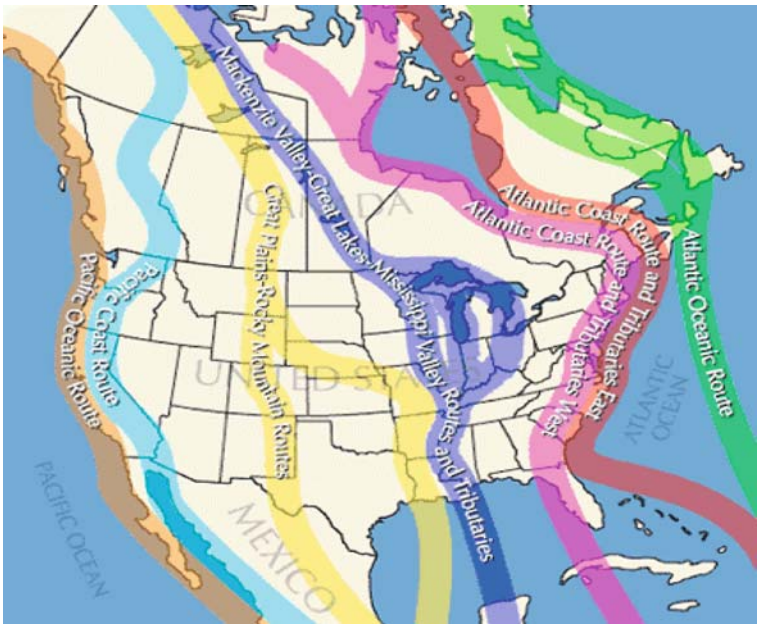

Fig. 5 Migratory bird flyways in the western hemisphere. In the fall birds fly south to areas in tropical America where they spend the winter. In the spring, they fly north again, potentially carrying the virus with them each way. Source: Reprinted from Gubler (2007a)

Fig. 6 Epidemics caused by West Nile virus, 1937–2007. The red stars indicate epidemics that have occurred since 1994 and have been associated with severe and fatal neurologic disease in humans, birds, and/or equines. Source: Reprinted from Gubler (2007a)

All of these epidemics/epizootics were unique from earlier epidemics in that they were associated with a much higher rate of severe and fatal neurologic disease in humans, equines, and/or birds. This virus most likely had better fitness and caused higher viremias in susceptible hosts, allowing it to take advantage of modern transportation and globalization to spread, first in the Mediterranean region and Europe, and then to the western hemisphere. This speculation is supported by sequence data documenting that the viruses isolated from these recent epidemics/epizootics are closely related genetically, most likely having a common origin; all belonged to the same clade (Lanciotti et al. 1999, 2002) (Fig. 7). Moreover, experimental infection of birds has documented that viruses in this clade, represented by the New York 1999 isolate, have greater virulence than virus strains isolated earlier (Brault et al. 2004; Langevin et al. 2005).

The broad vertebrate host and vector range of WNV was another important factor in the successful spread of epidemic/epizootic WNV transmission. The virus has been isolated from 62 species of mosquitoes, 317 species of birds, and more than

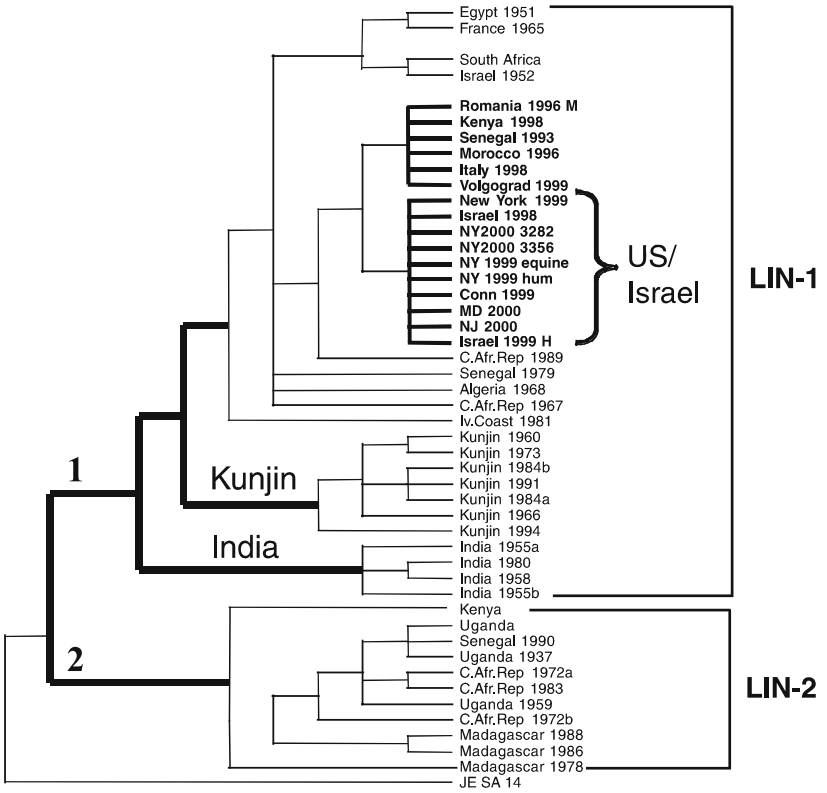

Fig. 7 Phylogenetic tree of West Nile viruses based on sequence of the envelope gene. Viruses isolated during recent epidemics all belong to the same clade, suggesting a common origin. Source: Reprinted from Gubler (2007a)

30 species of non-avian hosts since it entered the U.S. in 1999 (CDC 2007, unpublished data). The non-avian vertebrate hosts include rodents, bats, canines, felines, ungulates, and reptiles, in addition to equines and humans. It is unknown what role any of these non-avian species play in the transmission cycle of WNV, but the fact that so many mammal and opportunistic blood-feeding mosquitoes have been found infected suggests that there may be secondary transmission cycles involving mammals and mammal-feeding mosquitoes, putting humans and domestic animals at higher risk for infection.

Dengue/Dengue Hemorrhagic Fever

The dengue viruses (DENVs) are also members of the family *Flaviviridae*; there are four[2] dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4), which make up the dengue complex within the genus *Flavivirus*. While the DENVs have a primitive sylvatic maintenance cycle involving lower primates and canopy-dwelling mosquitoes in Asia and Africa, they have also established an endemic/epidemic cycle involving the highly domesticated *Ae. aegypti* mosquito and humans in the large urban centers of the tropics. They have become completely adapted to humans and current evidence suggests that the sylvatic cycle is not a major factor in the current emergence of epidemic disease (Gubler 1997; Rico-Hesse 1990).

The DENVs cause a spectrum of illness in humans ranging from inapparent infection and mild febrile illness to classic DF to severe and fatal hemorrhagic disease (WHO 1997). All age groups are affected, but in endemic areas, most illness is seen in children, who tend to have either a mild viral syndrome or the more severe dengue hemorrhagic fever (DHF), a vascular leak syndrome. DENV infection has also been associated with severe and fatal neurologic disease and massive hemorrhaging with organ failure (Sumarmo et al. 1983).

Dengue is an old disease; the principal urban vector, *Aedes aegypti*, and the viruses were spread around the world as commerce and the shipping industry expanded in the 17th, 18th, and 19th centuries. Major epidemics of DF occurred as port cities were urbanized and became infested with *Ae. aegypti*. Because the viruses depended on the shipping industry for spread, however, epidemics in different geographic regions were sporadic, occurring at 10–40-year intervals. The disease pattern changed with the ecological disruption in Southeast Asia during and after World War II. The economic development, population growth and uncontrolled urbanization in the post-war years created ideal conditions for increased transmission and spread of urban mosquito-borne diseases, initiating a global pandemic of dengue. With increased epidemic transmission, and the movement of people within and between countries, hyperendemicity (the co-circulation of multiple DENV serotypes) developed in Southeast Asian cities, and epidemic DHF, a newly described disease, emerged (Gubler 1997; Halstead 1980; WHO 1997).

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By the mid-1970s, DHF had become a leading cause of hospitalization and death among children in the region (WHO 1997, 2000). In the 1980s and 1990s, dengue transmission in Asia further intensified; epidemic DHF increased in frequency and expanded geographically west into India, Pakistan, Sri Lanka, and the Maldives, and east into China (Gubler 1997). At the same time, the geographic distribution of epidemic DHF was expanding into the Pacific islands in the 1970s and 1980s and to the American tropics in the 1980s and 1990s (Gubler 1989, 1993, 1997; Gubler and Trent 1994; Halstead 1992).

Epidemiologic changes in the Americas have been the most dramatic. In the 1950s, 1960s, and most of the 1970s, epidemic dengue was rare in the American region because the principal mosquito vector, *Aedes aegypti*, had been eradicated from most of Central and South America (Gubler 1989; Gubler and Trent 1994; Schliessman and Calheiros 1974). The eradication program was discontinued in the early 1970s, and the mosquito then began to reinvade those countries from which it had been eliminated. By the 1990s, *Aedes aegypti* had regained the geographic distribution it had before eradication was initiated (Fig. 8). This was another classic case of "success breeding failure."

Epidemic dengue invariably followed after reinfestation of a country by *Aedes aegypti*. By the 1980s, the American region was experiencing major epidemics of DF in countries that had been free of the disease for more than 35 years (Gubler 1989,1993; Gubler and Trent 1994; Pinheiro 1989). With the introduction of new viruses and increased epidemic activity came the development of hyperendemicity in American countries and the emergence of epidemic DHF, much as had occurred in Southeast Asia 25 years earlier (Gubler 1989). From 1981 to 2006, 28 American countries reported laboratory-confirmed DHF (Gubler 2002b) (Fig. 9).

While Africa has not yet had a major epidemic of DHF, sporadic cases of severe disease have occurred as epidemic DF has increased in the past 25 years. Before the

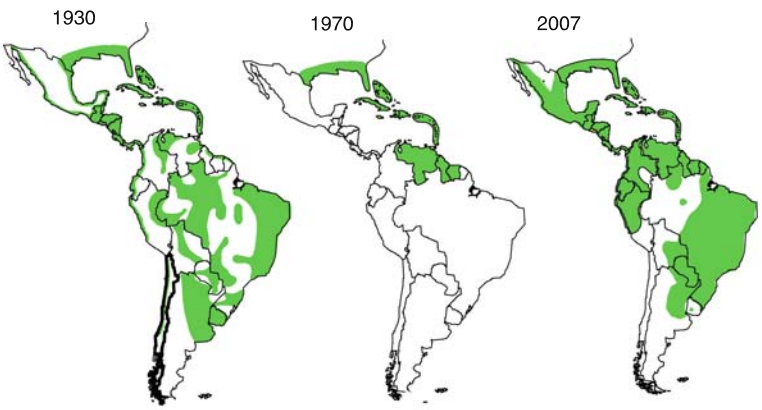

Fig. 8 Distribution of *Aedes aegypti* in American countries in 1930, 1970, and 2007. Source: Courtesy, Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO; adapted from Gubler (1998)

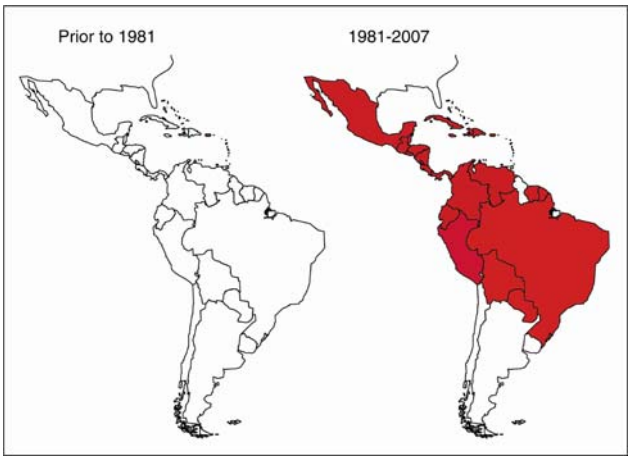

Fig. 9 Countries reporting confirmed DHF prior to 1981 and 1981–2007. Source: Courtesy, Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, CO; adapted from Gubler (1998)

1980s, little was known of the distribution of DENVs in Africa (Carey et al. 1971). Since then, however, major epidemics caused by all four serotypes have occurred in both East and West Africa (Gubler 1997; Monath 1994).

In 2007, dengue viruses and *Ae. aegypti* mosquitoes have a worldwide distribution in the tropics with 2.5–3.0 billion people living in dengue-endemic areas. Currently, DF causes more illness and death than any other arboviral disease of humans. The number of cases of DF/DHF reported to the World Health Organization (WHO) has increased dramatically in the past 3 decades (Fig. 10). Each year, an estimated 100 million dengue infections and several hundred thousand cases of DHF occur, depending on epidemic activity (Gubler 1997, 2002b, 2004; WHO 2000).

Yellow Fever

Yellow fever virus (YFV) was the first arbovirus to be isolated and the first shown to be[3] transmitted by an arthropod. It is the type species of the family (*Flaviviridae*: genus *Flavivirus*) (Gubler et al. 2007b). Its natural home is the rainforests of sub-Saharan Africa where it is maintained in a cycle involving lower primates and canopy-dwelling mosquitoes (Monath 1988). It was transported to the western hemisphere with the slave trade in the 1600s and became adapted to an urban cycle involving humans and *Aedes aegypti* mosquitoes, similar to dengue. It also established a sylvatic monkey cycle in the rain forests of the Amazon basin similar to the one in Africa.

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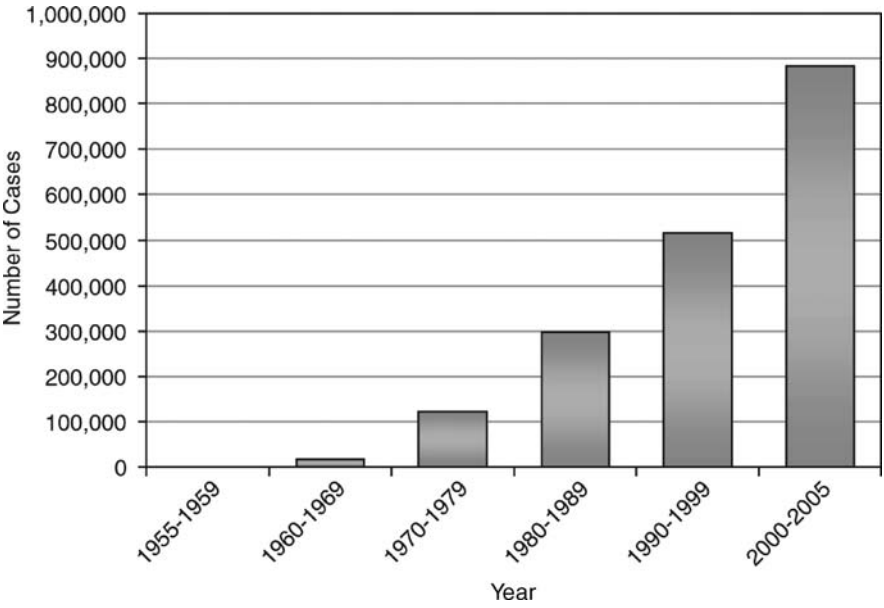

Fig. 10 Mean annual global reported cases of DEN/DHF to the World Health Organization, by decade, 1955–2005. Source: Adapted from MacKenzie et al. (2004)

The first recorded epidemic of YF occurred in 1648 and was followed by numerous epidemics in port cities of the New World, as far north as Boston (Monath 1988). Urban epidemic transmission was effectively controlled in the Americas in the 1950s, 1960s, and 1970s by the *Aedes aegypti* eradication program (see earlier discussion) (Gubler 1989; Schliessman and Calheiros 1974) (Fig. 8). The last known urban epidemic occurred in Brazil in 1942 (Monath 1988). In Francophone countries of West Africa, YF was controlled by mass vaccination programs. The result was the disappearance of major urban epidemics of YF in both Africa and the Americas. In the mid-1980s, however, the urban disease reemerged in West Africa, with major epidemics in Nigeria and increased transmission in other countries (Gubler 2004; Monath 1988; Nasidi et al. 1989; Robertson et al. 1996). Kenya experienced its first epidemic in history in 1993 (Sanders et al. 1998). In the Americas, the reinfestion of most Central and South American countries by *Aedes aegypti* has put the urban centers of the American tropics at the highest risk for epidemic urban YF in more than 60 years (Gubler 2008b). Thus, the disease continues to be an important public health problem in both Africa and the Americas.

The reemergence of epidemic YF in the past 30 years has not been as dramatic as that of DF/DHF. While there has been increased epidemic activity in both Africa and the Americas, the outbreaks have been limited and mostly associated with sylvatic cycles. Of concern is that several of the outbreaks in the Americas have occurred in or in close proximity to urban areas where *Aedes aegypti* occurs, greatly increasing the risk of urban transmission (Gubler 2004; Van der Stuyft et al. 1999). Additionally, the recent increase in ecotourism without proper immunization

has increased the risk of YF being introduced to urban areas where *Aedes aegypti* occurs (CDC 2000).

Currently, the threat is that YFV will become urbanized in the American tropics and spread geographically much as DENVs have done over the past 25 years. The biggest concern is that it will be introduced to the Asia-Pacific region, where there are approximately 1.8 billion people living in large urban centers under crowded conditions in intimate association with large populations of *Aedes aegypti* mosquitoes, thus creating ideal conditions for increased urban transmission. While there is an effective, safe, and economical vaccine for YF, its supply is limited and it would take months to increase production to the point where adequate doses could be produced. By then YFV would likely be widely distributed in the region.

If YF was introduced to the Asia-Pacific, the initial cases would most likely be misdiagnosed as DHF, leptospirosis, rickettsiosis, hantavirus disease, or malaria, thus potentially allowing it to spread and become established in widespread areas before it was identified. Thus, YF virus could be introduced and become established in Asia-Pacific countries weeks to months before it was recognized. Even after it is diagnosed, it is not likely that an effective control program could be mounted because most countries in the region do not have effective *Aedes aegypti* control programs. Once recognized as YF, it would likely cause overreaction and panic on the part of the press, the public, and health officials. Regardless of whether YF virus caused a major epidemic in this region, there would be a major public health emergency, creating social disruption and great economic loss to all countries of the region, making the Indian plague epidemic of 1994 (Fritz et al. 1996; John 1999; WHO 1994) and the 2003 SARS epidemic (Drosten et al. 2003) pale by comparison.

It is not known whether YFV would become established in Asia (Downs and Shope 1974; Gubler 2004; Monath 1989). YFV was most likely introduced sporadically to the Pacific and Asia in the past (Usinger 1944), but secondary transmission has never been documented. There are several possible explanations why there have not been YF epidemics in the Asia-Pacific region (Monath 1989). First, logistics: during the time when major YF epidemics were occurring in the Americas, the virus and the mosquitoes depended on ocean vessels to be transported to new geographical locations. The probabilities of the virus being introduced to Asia were low because the Panama Canal had not been built, and there was not as much commerce between Caribbean, Central and South American counties and Asia, as there was with the United States and Europe. Moreover, YF epidemics were not common in East Africa, thus decreasing the probability of introduction to India. Second, the high heterotypic flavivirus antibody (DENV-1, DENV-2, DENV-3, DENV-4, JEV, and many other flaviviruses of lesser importance) rates in Asian populations, while not protecting against YF infection, could possibly modulate the infection and down-regulate viremia and clinical expression, as has been shown in monkeys (Theiler and Anderson 1975), thus decreasing the likelihood of secondary transmission by mosquitoes. Third, there has been some suggestion that Asian strains of *Aedes aegypti* mosquitoes are less susceptible to YFV than American strains (Gubler et al. 1982). Finally, it is possible that evolutionary exclusion may prevent YFV from becoming established in areas where closely related flaviviruses are endemic.

Most likely, a combination of these factors has contributed to preventing YF from becoming established in Asia in the past.

The reason why urban YF has not occurred in the American tropics, despite the high risk in recent years, is not known. As noted for Asia, the high seroprevalance rates for the DENVs and other flaviviruses in most Central and South American countries could down-regulate viremia and illness, thus decreasing the risk of secondary transmission and clinical diagnosis. Additionally, the enzootic YFV may require adaptation to *Aedes aegypti* and humans, before becoming highly transmissible in the urban environment. If it does become adapted, however, it is important to remember that the logistic and demographic factors that influence arbovirus spread at the beginning of the 21st century are very different from past centuries. First, tens of millions of people travel by jet airplane between the cities of tropical America and the Asia-Pacific region every year; this provides the ideal mechanism for people incubating YFV to transport it to new geographic locations. There has been an increase in ecotourism in recent years, and since 1996, at least six tourists have died in the United States and Europe as a result of infection with YFV acquired during travel to YF endemic countries without vaccination (CDC 2000; Gubler 2004; Gubler and Wilson 2005). If urban epidemic transmission of YF begins in the Americas, there could be thousands of YFV infected people traveling to Asia-Pacific countries where *Aedes aegypti* exposure is high, thus dramatically increasing the probability that epidemic YF transmission will occur in Asia.

Why Has There Been Such a Dramatic Resurgence of Vector-Borne Diseases?

The dramatic global reemergence of epidemic vector-borne diseases in the past 25 years is[4] closely tied to global demographic, economic, and societal trends that have been evolving over the past 50 years. Complacency and deemphasis of infectious diseases as public health problems in the 1970s and 1980s resulted in a redirection of resources and ultimately to a decay of the public health infrastructure required to control these diseases. Coincident with this trend, unprecedented population growth, primarily in the cities of the developing world, facilitated transmission and geographic spread. This uncontrolled urbanization and crowding resulted in a deterioration in housing accompanied by a lack of basic services (e.g., water, sewer, and waste management). Population growth has been a major driver of environmental change in rural areas as well (e.g., deforestation, agriculture land use, and animal husbandry practice changes). All of these changes contributed to increased incidence of vector-borne infectious diseases.

Many urban agglomerations (population >5 million) have emerged in the past 50 years, and most have an international airport through which millions of passengers pass every year (Wilcox et al. 2007). In addition, globalization has insured an

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equally dramatic increase in the movement of animals and commodities between population centers. The jet airplane provides the ideal mechanism by which pathogens of all kinds move around the world in infected humans, vertebrate host animals, and vectors. A classic example of how urbanization combined with globalization has influenced the geographic expansion of disease is illustrated by the DENVs (Fig. 11). In 1970, only Southeast Asian countries were hyperendemic with

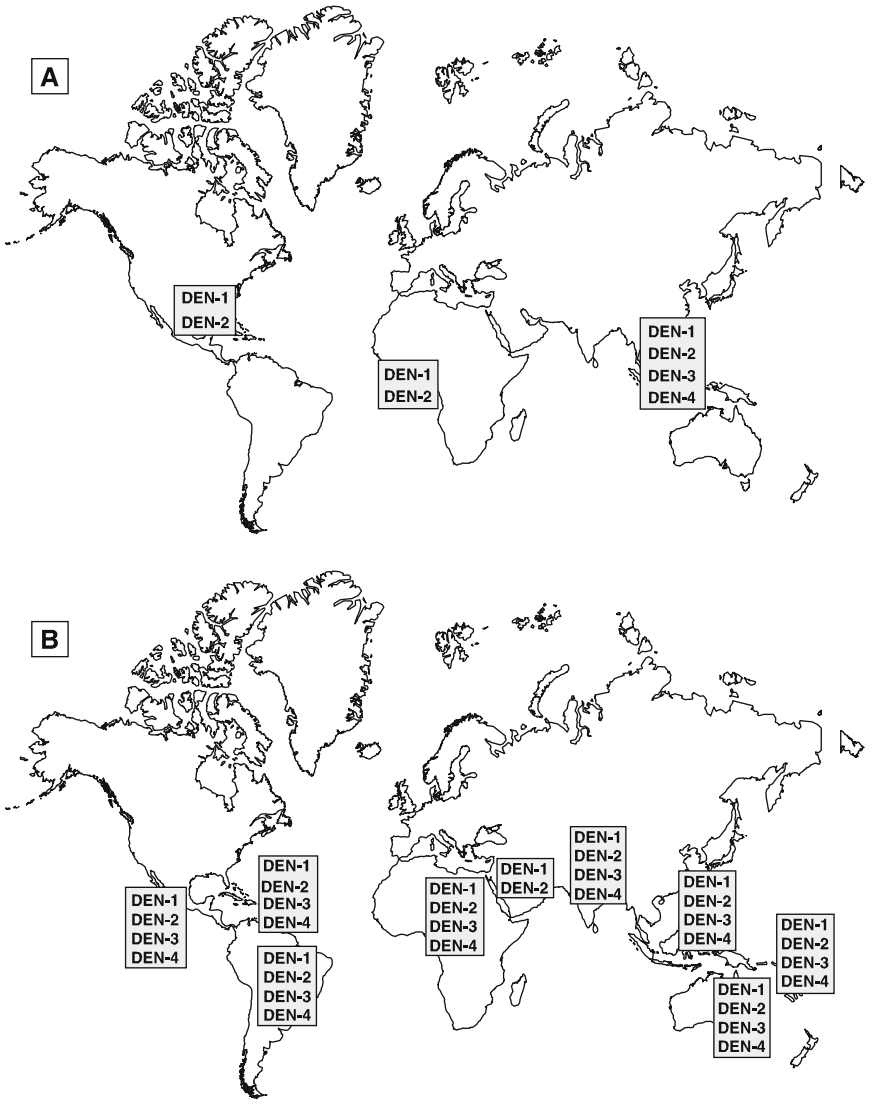

Fig. 11 The global distribution of dengue virus serotypes, (**a**) 1970 and (**b**) 2007. Source: Adapted from Mackenzie et al. (2004)

multiple virus serotypes co-circulating, as a result of World War II. The rest of the tropical world was hypoendemic with only a single DENV serotype circulating, or nonendemic (Fig. 11a). In 2007, the whole of the tropical world is hyperendemic as a direct result of urbanization, lack of mosquito control, and increased movement of viruses in people via modern transportation (Fig. 11b). The result has been increased frequency of larger epidemics, and the emergence of the severe and fatal form of disease, DHF, in most tropical areas of the world. Globalizaton and modern transportation were also responsible for the recent spread of WNV to and throughout the western hemisphere (Fig. 6). Increased transmission is a major driver of genetic change in all of these viruses, which can result in virus strains with greater virulence or epidemic potential being spread around the globe. The concern is that YF or RVF will be the next vector-borne diseases to spread because of globalization and modern transportation.

There are many other vector-borne diseases that have the potential for geographic spread. As an illustration of movement of infectious disease pathogens, Table 2 lists some of the exotic diseases introduced into the United States in recent years. It should be noted that the majority of these pathogens are vector-borne, zoonotic, and viruses. In addition, five species of exotic mosquitoes have been introduced and have become established in the country in the past 25 years. Some of the more important epidemic vector-borne diseases affecting humans at the beginning of the new millennium and which have the potential to spread via modern transportation are shown in Table 3. Again, it should be noted that most are zoonotic viral diseases. There is reason to believe that, sooner or later, one or more known or unknown pathogens will cause devastating epidemic disease.

Table 2 Exotic infectious diseases that have recently been introduced to the United States

Diseases	Autocthonous transmission
• West Nile fever	Yes
• Yellow fever	No
• Mayaro fever	No
• Dengue fever	Yes
• Chikungunya	No
• SARS	No
• Monkeypox	Yes
• CJD/BSE	No
• HIV/AIDS	Yes
• Lassa fever	No
• Malaria	Yes
• Leishmaniasis	Yes
• Chagas disease	Yes
• Cyclospora	Yes
• Cholera	No

Table 3 Principal epidemic vector-borne diseases affecting humans at the beginning of the twenty first century

• Malaria
• Plague
• Leishmaniasis
• African trypanosomiasis
• Relapsing fever
• Yellow fever
• Dengue fever and dengue hemorrhagic fever
• West Nile encephalitis
• Japanese endephalitis
• Rift valley fever
• Venezuelan equine encephalitis
• Chikungunya
• Epidemic polyarthritis
• Other arboviruses
• Zoonoses

Lessons Learned and Challenges to Reverse the Trend

At the dawn of the 21st century, epidemic infectious diseases have come "full circle" in that[5] many of the diseases that caused epidemics in the early 1900s, and which were effectively controlled in the middle part of the 20th century, have reemerged to become major public health problems. Complacency and competing priorities for limited resources have resulted in inadequate resources to continue prevention and control programs when there is no apparent disease problem. Only when an epidemic occurs do policy makers respond by implementing emergency response plans, but by then it is usually too late to have any impact on transmission.

In today's world of modern transportation and globalization, we have learned to expect the unexpected: that old diseases will reemerge and new diseases will emerge, and that modern transportation and globalization will disperse them around the world. Once introduced and established, it is unlikely that zoonotic disease agents can be eliminated from an area.

We have learned that international cooperation and collaboration are critical to developing and maintaining effective early warning disease detection and emergency response systems. Unfortunately, while elaborate epidemic preparedness and response plans are often drawn up, these plans are most often not implemented until it is too late to impact disease transmission because the decision to declare an emergency is one that often has important political and economic implications. As a result, public health problems that should remain localized have the potential to become more widespread because of modern transportation and the mobility of people.

[5]Reprinted in part with permission from Gubler (2008). Copyright 2008.

We have learned that we must emphasize prevention. Local public health infrastructure must be rebuilt and maintained in order to contain disease outbreaks as local public health events instead of letting them spread around the world via modern transportation. The public and the press require accurate and reliable information in order to prevent panic and overreaction.

We have learned that most newly emergent infectious diseases will likely be caused by zoonotic pathogens, and those that cause major regional or global epidemics that impact the global economy will likely originate in Asia. This has been the case for the past 25 years, and demographic, societal, and economic trends suggest this trend will continue for the indefinite future (Table 4). Thus, it is projected that most of the world's population growth will occur in the cities of Asia in the next 25 years, and most of the world's economic growth will occur in Asian countries. Changes in animal husbandry and agricultural practices, combined with regional human behavior and cultural practices, and increased trade, will all facilitate the emergence of exotic zoonotic pathogens in a region where people from rural areas continue to migrate to large urban centers, and from which the movement of people, animals, and commodities increase the risk of dispersal via modern transportation and globalization.

Finally, if we hope to reverse the trend of emerging and reemerging infectious diseases, the movement of pathogens and arthropod vectors via modern transportation must be addressed. This problem has important political and economic implications, but if it is not dealt with, the long-term costs will far exceed those required to proactively address the problem. Local public health infrastructure, including laboratory and epidemiologic capacity, must be developed in all countries, but especially in those tropical developing countries where new diseases may emerge. Effective laboratory-based, active disease surveillance systems are needed in every country, as are public health personnel that can respond rapidly and effectively to control epidemic transmission before it spreads. We need new tools (vaccines, drugs, insecticides, diagnostic tests, etc.), and finally, we need to better understand the ecology of newly emerging diseases in order to develop effective prevention strategies; drugs or vaccines will likely not be developed for most of these pathogens.

Table 4 Pathogens of tomorrow: From whence they will come?

From Asia and Animals
• Human population growth
• Urbanization
• Environmental change
• Animal husbandry
• Agricultural practices
• Human behaviour
• Cultural practices
• Economic growth
• Trade

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The Need for Synergy and Value Creation in Contemporary Vector Research and Control

Bart G.J. Knols and Ingeborg van Schayk

Abstract Contemporary research in the field of medical entomology is hampered by systems thinking, besides work processes and organizational structures that demand focus and inhibit creative and innovative initiatives. This leads to tunnel vision, limited lateral thinking, and forced attention to minutia with limited added value. PhD projects, in particular, are severely affected by this. Students focus on a piece of work for 3–4 years to find themselves an expert on a subject of limited importance before entering post-doc life. There are simple ways to encourage lateral thinking in science and open up strategic space for unconventional, exciting and stimulating research that matters.

Here we focus on various examples of lateral approaches to create synergy (use or application of knowledge or practices from non-related fields) and subsequent added value (appropriation of that knowledge, practice or process to deliver a real benefit to your own field of research). We describe why research on genetically-engineered mosquitoes, in spite of absence of proof-of-principle, became widely considered as a potential break-through in disease control. Discarded approaches to reduce vector-host contact, for instance the use of physical barriers in house design, experience the opposite; it is hard to generate renewed interest for methods that have proven capable of substantially reducing transmission. Even larval control, with dramatic historical successes, suffers from high-tech scientific developments that seek to achieve the same goal. We cover the search for human kairomones for trap/bait development and biological control agents to control adult mosquitoes and the subsequent discovery of a fungal entomopathogen used against grasshoppers that kills mosquitoes; other examples of synergy and value creation are presented.

Keywords Medical entomology · Synergy · Value creation · Innovation · Creativity

B.G. Knols (✉)
Laboratory of Entomology, Wageningen University & Research Centre, 6700 EH, Wageningen, The Netherlands
e-mail: bart.knols@wur.nl

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Introduction

The topic of synergy and value creation, which forms the subject of this chapter, is exemplified brilliantly by Prof. Mir Mulla's research activities over the past five decades. His contributions span many different insect pests and approaches to controlling these, either in their larval or mature stages, through biological or chemical control. His research covered behavioral and cultural control of eye gnats, development of chemical and biological control tactics for mosquitoes, control of midges and black flies, and pesticide-nontarget organism interactions. He also focused on the biology and ecology of medically important insects, and abundance and distribution of house-dust mites as related to atopic allergy. As a result of this focus on a multitude of organisms, coupled with vast acquisition of broad knowledge of pest control in general, it is not surprising to see the lasting impact and success of Prof. Mulla's contributions. Several of the other eminent scientists that contributed a chapter to this volume have acquired a similar status and it is interesting to examine the underlying processes leading to their success. Is it their accumulation of knowledge and experience over time? Is their success based primarily on creative abilities and a "feel" for innovation? And perhaps more importantly, is the new generation of young scientists that will be charged with vector research and control in future heading towards a similar status in today's science world?

We spend very little time trying to understand the factors that drive research that matters – research that really makes a difference in the way we solve pest and disease problems. Contributions that make pest control strategies more cost-effective, socio-economically more acceptable, more environmentally friendly, and indeed more sustainable. For example, of the hundreds of scientific articles that appear every year in the field of malaria vector research, how many of these really change the way we practice malaria vector control in developing countries? Do elaborate models of transmission affect the way we aim to maximise coverage of bednets at villages in remote African settings? Clearly not. Many large-scale trials with nets have established that population effects occur at high coverage rates, and models have shown how dramatic the impact on disease can be (Lengeler 2004). However, of the numerous bednet campaigns currently underway, in how many of these is this knowledge actually being appropriated? How does our knowledge of mosquito population genetics affect control programmes under the responsibility of Ministries of Health and National Malaria Control Programmes? The fact that nearly seven decades of research on malaria vectors has brought us back to the application of more than one million kilograms of DDT in thirteen African nations in 2007 (Sadasivaiah et al. 2007), with possibly more wide-scale use in 2008, begs the question if research efforts are having the right focus and are really designed to deliver contributions aimed at solving the malaria crisis.

During the symposium to commemorate Prof. Mulla's long-term contributions to vector research and control, we used two quotes to highlight this observation. The first is from Abraham Maslow, who coined the phrase "If a hammer is the only tool you have, you tend to view all problems as nails". We added a famous quote from Albert Einstein: "If you do what you did, you get what you got". These statements

underscore the danger of incremental approaches to innovation. In the field of vector control this could mean our unchanging focus on insecticides. In spite of knowing that application of insecticides will always lead to resistance, we keep on searching for new compounds – the hammer is the insecticide, all pests are nails. And yes, if you spray against a pest, you will get what you got, resistance. A good example of this approach is the Innovative Vector Control Consortium (IVCC; Hemingway et al. 2006) with its focus on the discovery and development of new public health insecticides. Apparently there is a greater interest in developing strategies to deal with the aftermath of resistance (Kelly-Hope et al. 2008) than to avoid it in the first place by leaving the hammer for what it is and try out new tools.

A further problem relates to the wide-spread availability of and easy access to information, leading to convergence of ideas which undermines creative thinking. Logical incrementalism (Quinn 1978), in which small and seemingly sensible steps are made in the developmental process is the result of knowledge convergence. Radical thinking that leads to transformational change is thus inhibited – any new student in the lab first receives a pile of articles on the subject he/she will be working on. Convergence of thought rather than exploiting the opportunity of radical thought development driven by the simple concept of "becoming smarter by knowing less" (Claxton 2001).

The Death of Creativity

Creativity is made up of three components; expertise, motivation, and creative thinking skills (Fig. 1) (Amabile 2001). Expertise reflects knowledge, which can be present in many different forms (technical, procedural, patents, etc.). Motivation also knows many different forms, but can broadly be grouped into intrinsic (e.g. drive, passion) and extrinsic (e.g. money, work environment, freedom) motivation. Intrinsic motivation is often the major contributing factor for scientists ("I'm not in it for the money") and is fortunately the component that can easily and immediately be influenced by the work environment.

Considering the more wide-spread availability of information and expertise, coupled to the fact that motivation remains central in the scientific world and is

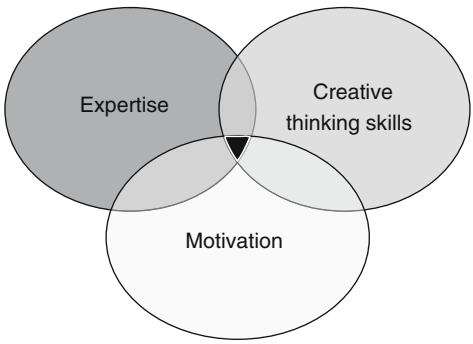

Fig. 1 The three components of creativity (after Amabile 2001). Creativity is optimal if the three components are present and integrated

considered a key driver for innovation, it is not surprising to observe that the only remaining option to differentiate is the ability to think creatively. And this is, regretfully, the one component we often ignore or worse, actively inhibit. For example, at present, funding agencies not only expect us to define our goals clearly, but also insist on seeing the path that will be taken to reach those goals. Often to the level of objectives, activities, and let's not forget the verifiable indicators. There is hardly a better way to remove the challenge for a student that joins such project than by forcing him/her to stick to predefined work plans. The freedom on how to reach goals is thus removed and creative thinking skills shut-off. The net result of this approach is often leading to tunnel vision – the student focuses on the end goals without consideration for observations and processes encountered on the way. Convergence of knowledge, logic incrementalism and tunnel vision are now influencing the innovation process to such extent that major gaps between the disciplines making up a field can no longer be bridged. Genetic control of disease vectors provides a good example of this, where the interface between the modern biotechnological approaches taking place in developed country laboratories are too distant from real-life settings in disease-endemic countries to remain meaningful (Knols and Louis 2006).

Besides challenge and freedom, there are four more inhibitors of creativity (Amabile 2001): resources, group features, and supervisory and organisational support. Many of us spend considerable time being fairly successful in channelling our creativity into resource acquisition rather than using that creativity in our research endeavours. The latter three components are heavily affected by organisational culture ("the way we do things around here"). Again, there isn't much positive to report when competitiveness in terms of where a paper is published but not the appropriation of knowledge generated is the driving factor for status and career progression within research environments and academic institutions. A further constraint is the fact that research groups normally consist of like-minded individuals. Thus we have a department of entomology and a department of chemistry. However, the assembling of homogenous groups with similar interests has frequently been shown to be the groups with the lowest levels of innovation and creativity. Similar mind-sets don't deliver grand new ideas. Finally, since work loads are linked to predefined outputs and milestones speed becomes the universal "good", and as we cannot speed up innovation this often translates in extra time at the lab. Stress becomes the norm, and is a most powerful suppressor of innovation. Then, as the stakes get higher and the need to problem solving intensifies the originality of the result lessens, often becoming stereotyped and uncreative. Given these constraints to creativity, it is hardly surprising that the overwhelming majority of research papers produced every year are incremental at best and that indeed we're back to where we were: DDT. How can this be improved?

Synergy and Value Creation

In his landmark book, *The structure of scientific revolutions*, Thomas Kuhn (1962) detailed nicely that almost any breakthrough in scientific endeavor is first a break with tradition. A classical example is penicillin: fungal contamination of petri dishes

wasn't just contamination nor was it that Fleming planned experiments that led to the discovery. But it was the discovery of antibiotics that saved millions of lives since. It is the challenge and opportunities to obtain intrinsic and extrinsic rewards that will drive young creative scientists to certain research domains. Regretfully, in the field of vector control and research, this domain is dominated by both high-tech and modern biotechnological approaches. It is apparently considered sexier to work with micro-arrays than to use a dipper to collect larvae in breeding sites in rural Africa. Youngsters may even consider that all the basic questions of field biology in malaria and other vector-borne diseases have been solved and that this domain is therefore no longer exciting and perhaps even boring. The contrary is true.

The disparity between research in the developed world and that going on in disease-endemic countries is, however, a point of concern. An army of students in Europe and the USA, for which I coined the term "Eppendorf generation", is, out of necessity, focused on the minutia, without developing the ability to see malaria as a real disease that is out there killing a child every 30s. Curtis (2002) made the case by arguing that further work in molecular entomology should be driven by the practical problems that vector control personnel has, not by what molecular biologists can and would like to do with new technological developments in the field of molecular biology.

Synergy and value creation offer good opportunities to regain focus and search for innovative solutions to old problems:

- *Synergy* is venturing beyond disciplinary boundaries to seek scope economies; it is fundamental research. Vector biologists looking at pest control in agriculture, looking at the developments in medicine, looking at developments in internet and communication technology – how can we use what is already out there creatively to advance our own discipline?
- *Value creation* deals with moving research from the bench to the field; it is operational research. How can we ensure that our research efforts actually reach the field and contribute to a reduction in disease morbidity and mortality (like in the case of malaria)?

Neither of this is easy as it requires a change in the context in which we work (Fig. 2). We need to start thinking big – do what Bill Gates did when he envisioned the world connected by computers in the 1980s. Instead of running the next gel, let's think what the actual contribution of that gel to problem solving will be. Rather than marvel at our latest paper in *Science* or *PNAS*, should we not be more concerned about the appropriation of that knowledge? What does it actually do to solve the problem? And finally, how can we release our students to become transformationalthinkers?

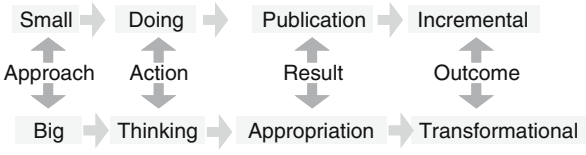

Fig. 2 Changing the conceptual framework in which we operate, to drive creativity, innovation, and transformational change

Contemporary research in medical entomology today operates like the top row in Fig. 2. There is focus on details, incremental developments, and frequently the search for publishable results, with a minimal outcome. We've become used to publishing findings and make small contributions although recently there have been moves towards working in line with the bottom row of Fig. 2. A great example is the Bill & Melinda Gates Foundation's Exploration scheme (Anonymous 2008a), which has been designed to encourage synergy in order to radically change the development process, break with traditional funding mechanisms, and involve scientists not directly working on tuberculosis, HIV, malaria etc. to apply their knowledge to these diseases and generate new ideas and approaches (Roberts and Enserink 2007). Likewise, new funding mechanisms based on submission of tenders to solve specific problems and involve anyone in society are underway (Travis 2008). Whether or not such initiatives will yield radically new ways to conduct vector control remains to be seen, but these certainly provide a platform for lateral thinking that drives synergy.

It is interesting to note that "breaking with tradition" has been successfully applied in the field of pest control. The advent of synthetic pyrethroids for the control of agricultural pests led to their initial application on bednet material (Darriet et al. 1984). With hindsight, this example of synergy is perhaps the most significant contribution to malaria vector control (certainly in sub-Saharan Africa) of the last 25 years. The seemingly simple thought of applying an insecticide to a bednet (transformational in itself) now forms the bedrock of protection from malaria with millions of nets being produced and distributed each year; true value creation (Lengeler 2004). We have discussed this example with various peers and combined with classical studies by De Meillon in South Africa in the late 1920s (De Meillon 1936), on house-entry and exiting of mosquitoes, which led to the development of indoor residual spraying, we concluded that these are the two publications that form the basis of how we control malaria in Africa today.

A second good example of breaking with tradition is pest control in greenhouses in the Netherlands. Historically, such pests would be controlled with insecticides, with resistance management tactics in place to resolve the recurring problems associated with this approach. In the early 1980s, at the time when more than 90% of all pest control was undertaken with insecticides, Prof. Joop van Lenteren pioneered research to develop powerful pest control tactics using natural enemies and predators of the major pests (e.g. mites, leaf miners). In spite of scepticism with the farmers and relentless opposition from the pesticide manufacturers, we now practice biological control in more than 90% of the greenhouses – the opposite from the situation 25 years ago. The production and sales of natural enemies and predators has become a multi-million dollar industry in Holland.

A third example, which then also demonstrates the feasibility of such paradigm shifts in developing countries, was the development of odour-baited traps and targets to control the tsetse flies in various African countries. This approach differed radically from bush and game clearing and area-wide insecticide application at the time it was initiated by pioneers like Dr. Glyn Vale and colleagues (Vale 1993). The focus here changed from targeting tsetse at resting sites, requiring area-wide

application of insecticides, to a focus on what drives tsetse host-seeking behavior. Certainly for the savannah species of tsetse, this has led to highly potent trap-bait systems.

Architecture and Vector-Borne Disease Control

Frank Snowden, in his recent book on the eradication of malaria in Italy (Snowden 2006), described how Celli, in the early 1900s, was extremely successful in controlling malaria transmission simply by adding physical protection to houses. Only 1% of humans occupying such houses contracted disease in the middle of marshes riddled by mosquitoes, whereas hundreds of people in the control group without screens fell ill. Similarly, in the southern USA, screened porches played a significant role in eradicating malaria. Yet we seem to have forgotten about the potential of this method. The synergy here lies in the use of architecture as a discipline to help us design houses in such a manner that they minimize mosquito entry. Lindsay and colleagues (2002, 2003) showed recently how house entry can be controlled by simple changes in design or use of barriers, even without the use of insecticides. Thus, although we tend to think of houses in Africa as simple mud and thatch structures, more and more advanced house designs are seen, particularly in the urban but also in rural environments, where house design and disease control has a real potential, not only for malaria, but also for the control of tuberculosis, upper respiratory infections and diarrheal diseases. The recently coined concept of the "Casa segura", by Prof. Barry Beaty and colleagues, builds on the same principles of making entire houses and their future designs more suitable to control (vector-borne) diseases. With the major African malaria vectors preferring to feed indoors, there is ample scope to not protect at the individual level (e.g. through bednet use) but at the household level (by making the entire house mosquito proof, or better still, by turning the entire house into a trap). Synergy can also be exploited by using architectural know-how to study airflows inside houses, and in particular how these can be manipulated. Value creation is the use of redirected airflow that contains human odours to a trap into which host-seeking female mosquitoes can be lured. Clearly, with African malaria vectors visiting a "point source" (i.e. the house), there will be many more things we can do to disrupt their contact with human hosts, besides using nets or indoor residual spraying. At present, we know nothing about the actual behavior of mosquitoes after they enter a house, apart from the fact that following blood feeding a considerable proportion remains indoors. Increased understanding of what drives the search for resting sites will enable more targeted application of insecticides (if these exert no excito-repellency) or other control methods. It is appreciated that such behaviors may differ for the various vectors and will depend on geographical and agro-ecological settings, which as a consequence necessitates detailed knowledge on mosquito behavior and ecology. This, in turn, hinders the development of "blanket technology" that can be applied from The Gambia to Djibouti, from Niger to South Africa (like nets or indoor residual spraying).

Genetically Modified Mosquitoes

An excellent example of synergy and a true paradigm shift is the development of genetic control strategies that essentially move away from mosquito population reduction to population replacement with insects no longer capable of transmitting pathogens. The initial synergy originates from studies that succeeded in germline transformation of *Drosophila*, in the early 1980s (Spradling and Rubin 1982). It wasn't until 1991, when a now historical meeting was held in Tucson (Anonymous 1991) that the strategy of incapacitating disease vectors through genetic transformation was coined. The thinking here was certainly transformational: rather than killing mosquitoes, let's replace them with ones that are harmless. It is not surprising, therefore, that this leap in thinking generated enormous interest from both the scientific and funding communities (Fig. 3). The strategic space that was opened up left those in search for incremental improvements of population reduction strategies far behind. Both the National Institutes of Health (NIH) and the World Health Organization (TDR) were captivated by the idea and devoted major resources (both financial and HR wise) to this new endeavor. Although genetic control strategies had been developed against mosquitoes decades before (Curtis 2006), these all focused mostly on population control. Interest in these had waned, because of technical hurdles but surely also because of the problems related to reinvasion and inability to eliminate local populations. The idea then to drive genes that confer refractoriness through populations resolved these issues, thereby increasing its appeal.

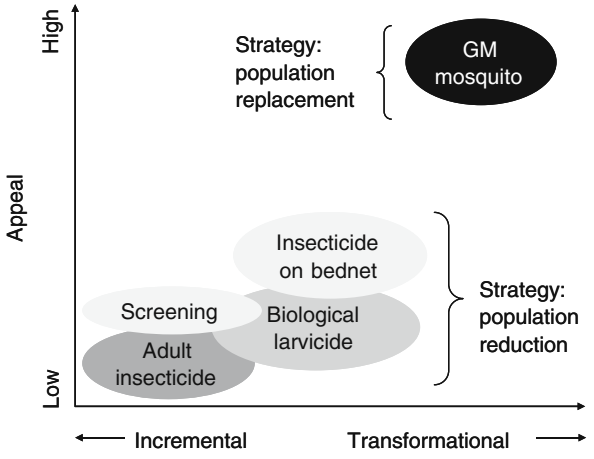

Fig. 3 The GM (genetically modified) mosquito endeavor, which opened up strategic space in vector control and resulted in high appeal to both the scientific and funding community because of its radical deviation from classical approaches

The synergy resulted in remarkable progress within a decade. Successful stable germline transformation of *An. stephensi* was accomplished in 2000 (Catteruccia et al. 2000). Just two years later the first transgenic mosquito with much reduced ability to transmit rodent malaria was created (Ito et al. 2002). The value creation of these successes has proven more difficult. To date, no efficient drive mechanism is available, no effector molecules to target human malarias (in particular

P. falciparum) have been identified, and GM mosquitoes suffer from fitness losses (Marrelli et al. 2006) although this may be compensated for when mosquitoes are actually being challenged with parasites (Marrelli et al. 2007). Much bigger hurdles are to be expected when moving towards field implementation, in the absence of proof-of-principle under near natural conditions. Although these experiments are now underway (Clayton 2006), it remains uncertain how end-users will view this approach. Not only are ethical, legal and social issues the most neglected aspects of this approach so far, also within the scientific community major scepticism abounds with nearly two-thirds of those asked "will the transgenic mosquito ever fly to control malaria" either answer "no" or "have no opinion" (Knols et al. 2007). It is imperative, therefore, to not only have the operational power to define the future of GM mosquito research, but also have criteria power. These criteria are often defined outside the scientific community yet may have major implications on the ability to further the approach towards full field evaluation. In other words, synergy is more powerful with due consideration of the potential for value creation.

It is interesting to compare this with the development of malaria vaccines. In spite of decades of research, the value creation of this approach has never been questioned because of the grand successes that have been accomplished with vaccination (e.g. smallpox eradication). Without any synergy, all vaccines are after all based on active immunization, the value of the approach remains incredibly strong, even when it is acknowledged that a commercial vaccine may be a decade away at best. What the GM approach lacks is value creation; evidence that in operational as well as biological terms there is a good chance of success.

Larval Control: Forgotten Successes

A significant change in malaria vector control followed MacDonald's book *The epidemiology and control of malaria* (1957). The now famous Ross/MacDonald equations on vectorial capacity as the driver for malaria transmission led to the abandonment of a focus on control of aquatic stages in favour of focusing on adult stages. With the adult daily survival rate of vectors as the sole factor in the equation with exponential rather than linear impact, it fitted well with the launch of the Global Eradication Campaign just two years previously that was largely based on application of residual insecticides in the intra-domiciliary domain.

What seems to have been forgotten here are the dramatic successes in controlling malaria by focusing on the control of larval stages. Long before the advent of chemical adulticides, environmental management and application of larvicides (like the arsenic compound Paris Green) was practiced around the world, sometimes at very large scales. Two examples stand out: the eradication of *Anopheles gambiae* from North-eastern Brazil (Soper and Wilson 1943; Killeen et al. 2002a), and the eradication of *An. arabiensis* from Egypt (Shousha 1948).

Considering that at present there is renewed interest in, or at least intensified debate on the potential for global malaria eradication, these historical successes should deliver elements for synergy and value creation (Killeen et al. 2002b). The

Brazil example, where following the accidental establishment of an African vector led to its spreading over 54,000 km^2 within a decade, would, in today's context, be viewed as an invasion catastrophe that cannot be averted. Actually, the global spreading of the Asian Tiger mosquito, *Aedes albopictus*, serves as a good example of our times. In spite of the knowledge that this species will invade much of Western Europe (from Italy), there is no concerted and drastic effort to avoid this, but a great effort by vector biologists to document the looming catastrophe in "The first record of Aedes albopictus in. . ." articles in the *Journal of the American Mosquito Control Association*.

In spite of the fact that excellent substitutes for Paris Green have been developed in the form of biological agents like *Bacillus thuringiensis israelensis*, with proven efficacy against African malaria vectors (Fillinger et al. 2003; Fillinger and Lindsay 2006), the uptake of this approach has been limited so far. Where it has been, results have been encouraging (Majambere et al. 2007). Given that no resistance has been found in anophelines against these biologicals, how could Soper eradicate *An. gambiae* from a huge area in Brasil, but is failure looming in its native Africa? We argue that not the tool (i.e. larval control) but the approach towards value creation can explain this. Rather than the rigorous, military-style, vertically structured campaigns that were executed in Brazil and Egypt, contemporary larval control is organised through participatory, horizontal schemes (Dongus et al. 2007; Opiyo et al. 2007). With community involvement as a (rightly so) prerequisite in any malaria control campaign it should be questioned therefore if community consent would not be adequate, following which implementation of the actual intervention remains in the hands of dedicated control teams. This than leads to the observation that not the control *method* itself but the *management* of the implementation of that method becomes crucial. This is where the difference in success between the French and Gorgas in controlling yellow fever and malaria at the Panama Canal originates from. This is why Soper was so successful in eradicating *An. gambiae* in Brazil, and why Shousha succeeded in eradicating malaria in Egypt. If value creation fails not because of the tool but because of implementation issues than this requires managers, not scientists. This is why mosquito abatement districts in the USA are highly efficient and successful in applying larval control strategies.

We conclude that, based on historical successes in Africa, larval control can be used to eliminate populations of malaria vectors, but that flaws in value creation are the underlying causes for potential failure. Management, not science, holds the key to success. We have always been struck by the success of Coca Cola, Gillette and Fa (soap). In the smallest and remotest villages in Africa these commodities are always present but bednets are nowhere to be found. It is not that bednets are not wanted (there definitely is market pull) but it is simply the distribution and supply chain management, market positioning and advertising that leads to absence in such settings. And that brings us back to synergy: what can those working in science faculties learn from those in business faculties? With perfect traps available to control tsetse and trypanosomiasis, why aren't these being applied in major parts of Africa? That's management (resourcing, production, distribution, application), not science.

Synergy and Value Creation at Wageningen University

Given the merits of synergy and value creation described above, at Wageningen University we now aim to institutionalize this approach. In spite of the constraints we face in terms of grant applications (pre-defined goals, milestones, etc.) we try to create an enabling environment that maximizes the chances of innovative discoveries. For instance, the establishment of an innovation platform, through which we invite specialists from completely unrelated disciplines (e.g. the airline and space industry) to drive synergy in the field of medical and veterinary entomology is most rewarding. Similarly, by actively undertaking exercises to unleash creative thinking in our team members, we have embarked on new topics not being researched anywhere else. Below we describe three examples of synergy and value creation that form the basis for ongoing research in our laboratory.

Identification of Attractants for Malaria Mosquitoes

The example of effective odour-baited traps for tsetse flies formed the basis for research in our group to identify human odours that can be used in a similar way to trap African malaria vectors. The immediate problem we stumbled upon in the early 1990s is the fact that humans produce hundreds of volatile organic chemicals (VOCs), any of which could play a key role in attracting host-seeking females. The classical, laborious, time-consuming and costly approach would be to sample these VOCs from humans, identify these using gas chromatography and mass spectrometry and then evaluate the influence of these on mosquito behaviour. Although not aware of it at that time, we applied synergy throughout our research to zoom in on essential compounds driving this behaviour.

First, we abandoned the above-mentioned organic chemistry approach. Instead, we used the short range behavior of mosquitoes as the starting point, by asking ourselves why mosquitoes prefer to bite certain parts of the body. Based on a study conducted by Haddow (1945) in which he observed biting patterns of the African mosquito *Aedes simpsoni* on volunteers and concluded a preference for biting the face, we set up similar experiments with four different mosquito species. By placing a motionless naked volunteer under a large bednet and introducing hungry females one-by-one into the net, we obtained biting patterns that were far from random. Of the two malaria vector species tested, we found *An. atroparvus* preferring to bite on the face, and *An. gambiae* preferring the ankles and feet (De Jong and Knols 1995). We then also determined that *An. albimanus* prefers to bite the face (Knols et al. 1994). Subsequently, we showed that this preference is odour-based and that the selection of biting sites is governed by VOCs from the preferred sites. In the case of *An. atroparvus/albimanus* we could change the biting pattern by channelling exhaled breath out of the experimental room; for *An. gambiae* we could change the pattern by washing the feet of the volunteer with a non-repellent soap

containing anti-microbial agents at 30 min intervals (De Jong and Knols 1995). With *An. gambiae* as our target species, we used this approach to focus specifically on human foot odour from then onwards.

The second, more unconventional, piece of synergy originated from the social stigma associated with foot odour. Rather than focusing on the composition of foot odour, we searched information available from the pharmaceutical industry, which has multi-million dollar efforts to develop products to quench foot odour. It was there that we found the initial links to certain aromas of cheeses closely resembling foot odour. The Dutch word "tenenkaas", used to describe strong foot odour, and literally meaning "toes-cheese" then led us to evaluate the attractiveness of pinhead quantities of various smelly cheeses in our windtunnel olfactometer. We subsequently incriminated Limburger cheese as highly attractive to the African malaria vector *An. gambiae* (Knols and De Jong 1996). At that stage we were oblivious as to why we could attract a highly anthropophilic African malaria mosquito to a Dutch dairy product.

The third piece of synergy came from the field of Food Sciences. We embarked on ploughing through literature on cheese manufacturing and came across statements like "Bacteria used in cheese production may have originated from human skin" (Sharpe et al. 1976), and "Cheese smells of feet rather than the reverse" (Jackman 1982). Rather than focusing on the cheese itself, it was apparently the microbial inoculum used to obtain a specific aroma that mattered. Eventually, we postulated that VOCs produced by the coryneform bacterium *Brevibacterium linens* on the surface-ripening Limburger cheese is closely related to *B. epidermidis*, a microbe residing in the toe clefts of human feet (Braks et al. 1999). The fourth piece of synergy thus originated from the discipline of microbiology. By the end of 1996 we had reduced the number of lead compounds from >300 to less than 15. Since that time, additional VOCs that influence the host-seeking behavior of this species have been identified, like ammonia (Braks et al. 2001), but overall most of the original lead compounds are still dominating our research efforts (including research in Tanzania and The Gambia).

All of the studies described above were conducted by one of us (BGJK) with Dr. Ruurd de Jong, an electrophysiologist that worked mostly on Colorado potato beetles. His limited knowledge of mosquitoes at the time we started working together certainly led us to become smarter by knowing less. His insightful questioning, based on knowledge about other insects, was a key driver for the four elements of synergy described above.

The process of value creation since that time has shown more difficult. With proven behavioural effects of the lead VOCs in olfactometer and electrophysiology studies, the exact composition and dose of VOCs in blends that can work under field conditions remains laborious (Qiu et al. 2007). Meanwhile, however, worn socks have been shown to effectively trap *An. gambiae* under semi-field conditions (Njiru et al. 2006; S. Moore and F. Oketch, unpublished data) and in experimental hut trials in The Gambia (M. Jawara, unpublished data) and the search for effective synthetic blends continues.

Toward Biological Control of Adult Mosquito Vectors

Given the problems associated with insecticide resistance described above, and the simple fact that at Wageningen University we do not, by default, conduct research on insecticides, we have engaged in the search for suitable alternatives to control adult mosquitoes. The synergy exploited here again originates from the field of tsetse fly control whereby scientists at the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya discovered an entomopathogen suitable for infecting and killing tsetse in the mid-1990s (e.g. Maniania 1998). This prompted us to evaluate the potential of spores of *Metarhizium anisopliae* to infect and kill *An. gambiae* and the filariasis vector *Culex quinquefasciatus* (Scholte et al. 2003). Although the use of fungi to control larval stages of mosquitoes had been the focus of research decades before (for review see Scholte et al. 2004), the use of these against adult mosquitoes was novel. Our very first pilot experiments proved successful and this has since led to broad interest in developing this technology as an alternative means to control vector-borne diseases (Thomas and Read 2007).

Like the initial use of synthetic pyrethroids, widely applied against pest in agriculture, on bednets, the synergy in this example originated from the mere observation that this fungus is known to infect and kill a broad spectrum of insects. We have since evaluated the potential impact of application of fungal spores inside houses in southern Tanzania, and have modelled the impact on malaria transmission (Scholte et al. 2005). With colleagues at the Ifakara Health Research and Development Centre (IHRDC) we are now preparing a large-scale trial encompassing several thousand households. Meanwhile we have also been able to infect the dengue and arbovirus vectors *Aedes aegypti* and *Ae. albopictus* with this fungus (Scholte et al. 2007).

The value creation of this novel method to control adult mosquitoes is dependent on a variety of technical and implementation issues (Knols and Thomas 2006). A key concern relates to the persistence of spores once applied inside local houses. However, recent identification of mosquito-killing isolates of *Beauveria bassiana* has shown spore persistence exceeding 6 months (M. Thomas, pers. comm.), bringing practical application closer. More intricate is the fact that biologicals are still viewed as inferior to chemical insecticides much in the same way natural enemies and predators were viewed in the 1980s in the greenhouse example described before. This in spite of the fact that late-killing fungi (mosquitoes succumb to infections 6–14 post exposure to fungi) have a much reduced chance of mosquitoes developing resistance against these (Thomas and Read 2007). Moreover, it has recently been shown that following exposure to fungus, insecticide-resistant strains of *An. gambiae*, *An. arabiensis*, and *An. funestus* display increased susceptiblility to the insecticides they are fully resistant to, posing fungi as an alternative insecticide-resistance management tool (Farenhorst et al., unpublished data).

At a time when the urge to develop new vector control tools is constantly on the agenda of international gatherings it is encouraging to see that research on fungi is ongoing in four African countries (Ghana, Kenya, South Africa, Tanzania), and

mass production is underway in two (Senegal, South Africa), and it is hoped that this will develop into a new strategy that can augment the limited arsenal of tools at hand today.

The Achilles Heel of Malaria

Our last example of synergy and value creation lies outside the field of medical entomology, though it was entomology that started it. In 2003, Dr. Richard Mukabana conducted experiments in Kenya on the attractiveness of humans to *An. gambiae*, when he observed marked fluctuation in attractiveness depending on the infection status with *Plasmodium falciparum* (Mukabana et al. 2004, 2007). He was subsequently involved in experiments that demonstrated that children carrying gametocytes (infectious to mosquitoes) displayed increased attractiveness to host-seeking females (Lacroix et al. 2005). The theory that *Plasmodium* parasites may alter the odour profile of hosts and thus render these more attractive to mosquitoes thus became established.

The synergy here lies in the hypothesis that changes in odour profile may be linked to VOCs present in parasite-infected blood that are different from uninfected blood and that these VOCs may be exchanged with the lung cavity at the alveolar interface. In line with the development of breathalyzers for ailments in the developed world (lung cancer, breast cancer, early detection of heart transplant rejection, tuberculosis) we quickly realized the importance of the availability of such devices for non-invasive and rapid screening of patients for malaria parasites. The advantages are numerous (Knols 2005). In particular, a biomarker for gametocyte carriage would give tremendous power of tackling the infectious reservoir with gametocidal drugs or selective protection of hosts when carrying infectious stages.

Value creation of this idea is currently underway. Given that breathalyzers for alcohol are available online for a mere 30 €, it is striking that this approach has not been tried against major diseases of the poor, including sleeping sickness, leishmaniasis, dengue, etc.

Conclusions

In this chapter we have highlighted some of the pitfalls in current research practices that lead to incrementalism and absence of highly innovative ideas. To a large extent this is determined by the gap between appropriation of knowledge and what is requested from vector biologists in academia (publications, grants, teaching and training). It surfaced that current malaria vector control (nets and indoor residual spraying) is based on just two publications in the last Century. We highlight the importance of synergy and value creation and show how these can drive transformational thinking and development of new concepts in vector control. It is concluded that approaches that worked well in the past (larval control, house improvement) have lost appeal and suffer from flaws in value creation that are largely managerial

and not science-based. New approaches, in particular genetic control of mosquitoes, will need due consideration of value creation if these are to evolve into open field implementation. Three examples of lateral thinking and application of synergy and value creation have been presented. More will be needed if we are to tackle the challenges posed by disease vectors in the years to come.

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"Dramas" Down-Under: Changes and Challenges in Australia

Richard C. Russell

Abstract In this paper I will attempt to provide an overview of some of the current issues concerning exotic pest/vector mosquitoes and invasive/emerging disease in Australia. Malaria continues to threaten Australia with its continuing high levels activity in neighboring Papua New Guinea and the Solomon Islands; there are many hundreds of imported cases and the occasional local (introduced) transmission in northern regions. Dengue viruses are the most important of the arboviruses in the Australasian region and present as episodic problems with occasional epidemics; there is virtually annual introduced activity in Queensland and control of *Aedes aegypti* continues to be problematic. Japanese encephalitis virus appears to be active annually in the Torres Strait region but not on the mainland, and the lack of human cases indicates the widespread human vaccination program has been effective. Of the alphaviruses, Ross River remains the most prevalent with many thousands of human infections annually, but Barmah Forest virus has become of particular interest for annual outbreaks affecting many hundreds in eastern coastal regions. There are relatively frequent importations of exotic mosquitoes, including *Ae. aegypti* and *Ae. albopictus*, to northern ports, but the former is still confined to Queensland and the latter established in islands of the Torres Strait but not on the mainland; however, other exotic species, including *Aedes vexans* and *Culex gelidus*, have become established on mainland Australia. There are questions of the impact of climate change on mosquito-borne disease, particularly malaria and dengue, and while it is unlikely that malaria will be reestablished, dengue may be more of an issue if there is an increase in distribution of the vector for whatever reason.

Keywords Australia · Alphaviruses · *Aedes* · *Culex* · Dengue · Malaria

R.C. Russell (✉)
Department of Medical Entomology, University of Sydney, Westmead, NSW 2145, Australia
e-mail: rrussell@mail.usyd.edu.au

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Introduction

There is an increasing concern in Australia for the introduction of exotic mosquito vectors and mosquito-borne pathogens, as well as for the emergence of indigenous pathogens. In this overview, I will attempt to cover some of the current issues.

Exotic Vectors

Overall, Australia has six exotic mosquito species (*Culex quinquefasciatus*, *Aedes aegypti*, *Ae. albopictus*, *Ae. vexans*, *Cx. gelidus*, *Cx. molestus*) established amongst its native fauna, and more than 10 exotic species have been recorded arriving at ports in Australia but have not established: *Aedes aegypti* and *Ae. albopictus* at ports other than in Queensland (QLD) where these two mosquitoes are established (albeit *Ae. albopictus* is currently not on mainland QLD), and *Ae. malayensis*/*scutellaris*, *Ae. quasirubrithorax*, *Ae. papuensis*, *Ae. dasyorrhus*, *Ae. novalbitarsis*/*subalbitarsis*, *Culex fragilis*, *Cx. spathifurca*, *Cx. sitiens* gp. species (Russell and Kay 2004). *Aedes aegypti* continues to be imported to Australian seaports from nearby infested regions, with regular cargo vessels but also illegal fishing vessels and refugee boats (Whelan et al. 2001). In the fiscal year 2005/06, there was a total of 9 interceptions in the Northern Territory (NT) (8 *Ae. aegypti* and 1 *Ae. albopictus*), in 2004/05 there were 22 interceptions comprising 22 *Ae. aegypti* and 1 *Ae. albopictus*, and in 2003/04 there were 6 *Ae. aegypti* and 1 *Ae. albopictus* interceptions (P. Whelan personal communication).

In Australia, *Ae. aegypti* remains confined generally to northern QLD, although in early 2004 an established population was detected in the NT town of Tennant Creek (seemingly introduced by road transport from QLD) and an eradication program was undertaken; the species apparently did not spread to other NT communities and the eradication efforts were proclaimed as successful in 2007. Control was based on repeated rounds of treating all potential receptacles with bifenthrin or deltamethrin residual sprays, methoprene pellets or chlorine solution; with treatments of all vacant urban and rural residential and industrial properties, waste storage and disposal areas, storm water systems, telecommunication inspection pits and manholes, and any other area found to be potentially breeding or harbouring mosquitoes. Rainwater tanks were sealed and treated with methoprene briquettes (18 g/kg S-methoprene) and pellets (40 g/kg S-methoprene) and storm water systems were cleared of underground soil obstructions. (Whelan et al. 2004, 2005; P. Whelan pers.comm.). An established infestation was also detected on Groote Eylandt in the NT in 2006 and is currently subject to a similar eradication program (Whelan 2006).

Aedes albopictus was first detected on Yorke Island in the Torres Strait between Australia and Papua New Guinea (PNG) in April 2005 (Russell et al. 2005; Ritchie et al. 2006). A delimiting survey throughout 22 communities on 17 islands in the Torres Strait and in the Northern Peninsula Area (NPA) of the mainland, with collection of larvae and pupae from containers and adults attracted to human bait, detected

Aedes albopictus in 10 island communities but not in any mainland sites. Immature forms of *Aedes albopictus* are difficult to distinguish morphologically from the local *Aedes scutellaris* but we are able to confirm identification using Polymerase Chain Reaction (PCR) methods (Beebe et al. 2007). Retrospective genetic analysis of larvae collected in April 2004 and April 2005 on Yorke Is. indicated that *Ae. albopictus* was present in low densities in 2004, and that there were three genetically distinct mitochondrial haplotypes on Yorke Is. in April 2005, suggesting one significant or multiple incursions (Ritchie et al. 2006).

An eradication program was established in early 2006 and has been progressing under supervision of an expert Technical Advisory Group (TAG).

The program has reduced *Ae. albopictus* populations on five of the ten islands, but the species has spread to an additional three islands although not to the mainland (Davis 2007).

Control was based around removing those breeding sites that could be disposed of and treating those sites that couldn't be removed, with treatments of residential properties, waste storage areas, and any other area found to be breeding or harbouring mosquitoes. Briquettes (18 g/kg S-methoprene) and pellets (40 g/kg S-methoprene) were used as insecticide, and supplemented with spray (80 g/L Bifenthrin).

Control efforts continue to target positive communities and adjacent vegetated areas. The current proposal is to fund the program until 2009. The goal is to eliminate *Ae. albopictus* from the Torres Strait, prevent future incursions and work with community councils to develop and maintain a mosquito management program (Davis 2007). The potential for the species to spread to southern states has been investigated and there is concern that it may provide a vector of dengue where none currently exists (Russell et al. 2005).

Aedes vexans (Australia) was first detected in northern Western Australia (WA) in 1996 and has been found regularly in the region since 2001 (Johansen et al. 2005a). It is thought to have been introduced by wind currents from Indonesia or possibly by the occasional light aircraft that arrives from nearby islands. This species is of interest for a number of reasons, not the least being its extraordinary global distribution through Europe, Asia and North America; it is found also in parts of Africa, Central America and the Pacific (where it has long been called *Ae. nocturnus*), indicating it has the capacity to spread widely in Australia. On the basis of northern hemisphere experience, it presents as a potential nuisance pest and possible vector of pathogens, although its competence for local arboviruses is completely unknown.

Culex gelidus, a secondary vector of Japanese encephalitis virus in Southeast Asia, was first detected in QLD in 1999. However, a check of museum specimens revealed it had been collected previously in QLD in 1994 (Ritchie et al. 2001) and also in the NT in 1996 (Whelan et al. 2001). It is thought to have arrived in the NT from QLD, and more recently moved to northern WA via cattle trucks or other vehicles from near sewage ponds, cattle yards or abattoirs.

Vector competence experiments with a NT strain of *Cx. gelidus* have shown that it was a highly efficient vector of Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV), a moderately good vector for

Ross River virus (RRV), but was refractory to Barmah Forest virus (BFV) (Andrew van den Hurk pers. comm.). In QLD, JEV (Ritchie et al. 2007) and RRV (Ritchie pers. comm.) have been detected in *Cx. gelidus* by PCR analysis, from Torres Strait islands and Cairns, respectively (Scott Ritchie pers. comm.).

It still seems to be relatively rare in WA, and in QLD and NT it appears to have not created pest problems, with high numbers of adults being found only near high organic aquatic habitats where there are high numbers of larvae, so it may be that it presents little appreciable risk as a vector to humans in most situations. Williams et al. (2005) published a CLIMEX projection of the distribution of *Cx. gelidus* in Australia, revealing a potentially wide distribution throughout coastal Australia, particularly in tropical and subtropical areas.

Exotic Pathogens

Malaria was endemic historically in northern Australia (Black 1972), but was declared eradicated by the WHO in 1981. However, there are many hundreds of imported cases annually and occasional cases of introduced transmission occur in northern regions (particularly the Torres Strait islands). There have been increasing problems with endemic malaria in PNG and the Solomon Islands in recent decades and these areas provide the greatest risk for travelers and for introduction to the northern islands and northern mainland of Australia. At least 27 species of Anopheles mosquitoes occur within Australia, and some are capable of transmitting Plasmodium species between humans.

An area of northern Australia north of 19°S has been considered to be the Australian "Malaria Receptive Zone" since the 1940s (Ford 1950). This actually represented the distribution of local malaria infections pre-1950 (Black 1972), within a zone of tropical conditions suitable for maintenance of *P. falciparum* transmission, and with the presence of a competent vector being self evident from the records of local cases.

There has been only a single outbreak of malaria in Australia where the vector species was incriminated by dissection for sporozoites. This was 1942, when *An. farauti s.l.* was found infected in Cairns (Black 1972), and the idea that this species was the principal Australian vector was supported by its reputation as a major vector in PNG. However, another species, *Anopheles hilli* was also found infected in that same investigation and, additionally, the distribution of cases in northern Australia bears no relationship to the northern/northeastern coastal distribution of *An. farauti s.l.* or of *An. hilli*. In none of the other recorded cases, from WA, NT and QLD in the "Receptive Zone", or in WA, QLD, NSW and VIC in the southern half of Australia, has a species being incriminated other than by circumstantial evidence of local distribution and abundance.

On available evidence, for the northeast of QLD, from Townsville to the Torres Strait, it could be argued that *An. farauti s.l.* is the vector "most likely", but we now know that this "species" is comprised of three discrete siblings on the Australian mainland (and more elsewhere) (Schmidt et al. 2001) and their relative vector

competence is unknown. In southern Australia, where malaria has been transmitted as far south as Melbourne in the east and south of Perth in the west, it could be concluded that *An. annulipes s.l.* is the vector "most likely", but likewise we now know that that "species" is comprised of at least 17 siblings on the Australian mainland (Foley et al. 2007), and their relative vector competence is also unknown. What we do know, however, is that mosquitoes in both the north and south of Australia have transmitted malaria, and that the "so-called" Australian malaria vector *An. farauti* has had little to do with the majority of these incidents.

The impact of climate warming on the distribution of *An. farauti* has been proposed (Bryan et al. 1996) to extend its range south by approximately 800 km (from Townsville to Gladstone) by 2030, although it was found soon after to be established at Mackay some 350 km south of Townsville (van den Hurk et al. 1998). Its distribution appears to be limited by humidity and altitude as well as temperature (Sweeney et al. 2006), but given that its distribution appears to have little association with the overall picture of malaria transmission in Australia anyway, it could be argued that whatever happens to *An. farauti* with climate change is somewhat irrelevant.

With an increase in malaria transmission patterns in neighbouring countries, whether caused by climate change or for other reasons (e.g. increasing drug or vector resistance, or control program logistics) we can expect an increase in imported malaria with travelers and refugees to Australia, so the threat of local transmission may increase concomitantly. This is more likely in northern parts because of local environmental conditions, but is possible in many parts of southern Australia in certain situations and in warm seasons.

However, for any future activity on a community scale, numbers of persons in infectious states living within susceptible communities would have to be exposed to large numbers of competent mosquitoes in favorable environmental circumstances. Without the collapse of Australia's health services, it seems highly improbable that malaria could become re-established in the "Receptive Zone", and almost impossible for southern Australian regions, even given that climate warming will somewhat lower the southern boundary of the area suitable for endemic malaria by virtue of its temperature regimes.

Dengue (DENV) continues to be the most important arboviral disease regionally; activity is episodic with occasional epidemic outbreaks. There is virtually annual introduced activity in Australia and other areas of the South Pacific (e.g. French Polynesia, the Cook Islands and Fiji in the past 12 months) and vector control continues to be problematic.

Dengue possibly occurred in Australia from the time of first European settlement, if not before, but the earliest reference is from 1873 (Lumley and Taylor 1943). Kay et al. (1984) noted that the first record of what appears to be hemorrhagic dengue (DHF) occurred in Charters Towers in 1897; there was death associated with dengue in 1885 and 1904–05, and in an earlier outbreak in 1879 an estimated 75% of the Townsville population was infected (Lumley and Taylor 1943).

In recent times, dengue transmission resumed in 1981, with cases in Thursday Island and Cairns, QLD, after an interval of 26 years (Guard et al. 1984; Kay et al. 1984), and since 1990, there has been frequent moderate activity in northern QLD

and all four serotypes of DENV have been identified. The first case of classified (WHO criteria) DHF in Australia in recent times, occurred in 1993 during an outbreak in northern QLD and was relatively mild. The 1992–93 outbreak went on to involve almost 2,000 people with half that number serologically confirmed and the rest inferred on clinical grounds. A serosurvey of 1,000 randomly recruited residents in Charters Towers (population 10,000) indicated that 20% of the population were infected (Mackenzie et al. 1996).

Following the 1993 epidemic, the Queensland Tropical Public Health Unit, based in Cairns, developed and published a Dengue Fever Management Plan for North Queensland, along with a manual on Dengue: Guidelines for Diagnosis and Management for Health Care Staff in North Queensland. The management plan aims to reduce the incidence of dengue in northern Queensland by:

(i) implementing serological and virological testing locally, and implement routine on-going surveillance for and reduction of *Ae. aegypti*
(ii) educating and motivating people to practice behaviours that reduce *Ae. aegypti* breeding
(iii) establishing more effective legislative powers to enforce prevention of breeding of *Ae. aegypti*
(iv) containing incipient dengue epidemics with emergency mosquito control procedures
(v) enabling medical and related personnel to deal with hospitalisations due to dengue
(vi) implementing prioritised applied research activities which help to reduce *Ae. aegypti*
(vii) establishing a co-ordinating structure to make the objectives of the plan achievable

At the end of 1996 an outbreak of DENV2 in the Torres Strait, apparently initiated by a traveler returning from PNG, spread southwards to Cairns in early 1997 and totaled over 200 cases (with a cumulative incidence rate of 23 per 1,000 inhabitants for the Torres Strait islands) (Hanna et al. 1998). Entomological intervention at this time included house-to-house source reduction, methoprene treatment of water tanks, and interior residual spraying (IRS) with deltamethrin inside houses on two affected islands, and dengue cases ceased within two weeks of the operations.

In the 1993 QLD outbreak mentioned above, one patient matched WHO criteria for Grade II Dengue Haemorrhagic Fever (DHF) (Row et al. 1993). Following this outbreak there was a report of 21% of 521 patients in Townsville exhibiting minor haemorrhagic manifestations (Streatfield et al. 1993). The outbreak of DENV3, with some concurrent DENV2 activity, in 1997–98, resulted in two (possibly three) mild cases of DHF and one (possibly two) cases of dengue encephalopathy (Mackenzie 1999). The first severe cases DHF since the return of dengue activity in 1981 were recorded in 2004 with two deaths (McBride 2005) – the first for a century.

It is well known that *Ae. aegypti* is widely established in Queensland, particularly in the northern coastal regions, but the vector has not been recorded from other

states for some three or more decades. Following the reappearance of dengue in Queensland in 1981, there were surveillance programs instituted in other States, with the NT, northern WA and NSW showing particular concern. Subsequently, the absence of the vector was confirmed for NSW and WA, and periodic surveys in the NT have confirmed that it remains similarly free of the vector, despite a number of importations to Darwin by sea from countries to the north, one importation by road from QLD, and at least two introductions comprising one by road from QLD and the other by sea possibly from an illegal fishing or other overseas vessel.

Dengue is considered to be not endemic in QLD, but with frequent activity of DENV in the nearby regions of PNG, the Pacific and Southeast Asia, there is a continuing (and perhaps increasing) risk for introductions to QLD. There is a strong feeling in northern Queensland that the dengue management plan has been successful in reducing the impact of epidemics since 1993; however, continuation of the joint government and community effort, in reducing vector populations and detecting imported cases of infection quickly, is required to sustain the success. In the past 5 years, new strategies have been developed for vector surveillance and monitoring, and control, wherein lethal ovitraps, both sticky (Ritchie et al. 2003; Williams et al. 2006) and pesticide treated types (Williams et al. 2007b) and BG traps (Williams et al. 2007a), are being used to reduce the extensive human effort and amount of pesticide involved with outbreak counter measures.

As mentioned above, the restricted tropical distribution of dengue activity has led to claims that climate warming will lead to a southerly and westerly extension of the disease (and presumably the vector). However, the historic distribution of *Ae. aegypti* and its transmission of dengue in Australia do not support the projections; the vector previously existed in eastern Australia at least almost to the NSW/Victorian (VIC) border, but perhaps well into VIC from where unconfirmed records are as far south as Melbourne, and it is known to have been established further south than Perth in WA (Lee et al. 1987).

In a global context, *Ae. aegypti* has been able to establish in areas below and above the January and July isotherms of 10°C in the northern and southern latitudes, respectively, with further extensions possible during warmer months. It is clear the species historically covered most of the climatic range theoretically available to it in Australia, although for reasons not understood, it retreated dramatically following the 1940s, disappearing from NSW by 1950 and the NT between 1956 and 1969, and it was last found in WA in 1970.

With dengue activity increasing in many parts of the tropical and subtropical world, in association with rapid urbanisation in developing countries and rapid transport distributing the viruses internationally (Gubler 1997; Farrar et al. 2007), the potential for dengue viruses to be imported into Australia is likely to increase. Indeed, if there is a substantial increase in the number of imported cases of dengue into far north QLD, it could be that dengue may become endemic in this area despite a strong control program (Mackenzie et al. 1996; Hanna et al. 2006). However, unless there is a significant extension of the current distribution of *Ae. aegypti*, primarily due to factors other than climate warming, transmission will remain restricted to QLD.

Japanese encephalitis virus (JEV) was first recorded in Australia in 1995 and there have been various reviews of the subsequent activity (e.g. Russell and Kay 2004). In summary, three residents of Badu Island in the Torres Strait between Australia and PNG, developed clinical disease in 1995 and two died (Hanna et al. 1996). In late 1995, 93% of humans were vaccinated. Antibodies in humans and pigs were demonstrated on other islands, and JEV was isolated from *Cx. annulirostris* from Badu Is (Ritchie et al. 1997).

The virus was active again in the Torres Strait islands in 1998, with another human case (an unvaccinated individual) and seroconversions in pigs in the Torres Strait islands, and a human case and pig infections on mainland Australia at the Mitchell River in the southwest of Cape York, northern QLD (Hanna et al. 1999). Since 1998, there has been evidence of continuing virus activity (annually – except for 1999) in the region, although there have been no more local human cases, and van den Hurk et al. (2006) reported the first isolation of JEV from the mainland.

Nucleotide sequencing of JEV from mosquitoes and pigs from the mainland, islands and PNG, from 1998, 1997 and 1995, showed they were all closely related and suggested a common source. They were a southeast Asian topotype and most closely related to isolates from Malaysia, Thailand and Indonesia (Mackenzie 1999). Subsequent investigations of weather and wind patterns immediately prior to the outbreak indicated that the virus may have been introduced with mosquitoes blown in from PNG (Ritchie and Rochester 2001), although how the virus moved from Indonesia to PNG is uncertain.

Enzootic JEV to the north of Australia in PNG, West Papua, Timor Leste, West Timor, and other parts of eastern Indonesia, has raised concerns for mainland Australia. The likelihood that JEV could become established in Australia is difficult to estimate; there are suitable vectors (*Cx. annulirostris*) and appropriate vertebrate hosts (water birds and wild pigs) widely distributed, and these often occur together in and near wetlands.

However, more than 10 years have passed since the initial incursion to the Torres region and almost 10 years since that to the mainland, and no further southerly movement has been detected. Possible explanations relate firstly to local flavivirus antigen relationships, since viraemia for JEV was not evident in experimentally infected pigs with prior exposure to MVEV or KUNV (Williams et al. 2001). However, van den Hurk et al. (2003) found less than 5% of *Cx. annulirostris* collected away from penned pigs in Cape York has fed on pigs, with the majority of bloodmeals being from marsupials (e.g. local wallabies), indicating host feeding patterns may be important, and Hemmerter et al. (2007) have shown variation in speciation characteristics indicating vector competence may vary amongst *Culex annulirostris* populations in the Cape York region.

With respect to the virus interaction issue, it is difficult to assess whether the five flaviviruses (MVEV, KUNV, Alfuy (ALFV), Kokobera (KOKV) and Stratford (STRV)) from the Japanese encephalitis serocomplex that are already circulating in northern QLD might inhibit the establishment of JEV virus through antibody cross-protection in wildlife. However, the fact that JEV seems now to be enzootic in PNG, where a number of these flaviviruses (MVEV, KUNV, KOKV) as well as

at least one other flavivirus (Sepik virus (SEPV)) co-circulate, and the fact that JEV and the related West Nile virus (WNV) co-circulate in the Indian region, together would appear to indicate that JEV might not be excluded from Australia on simple sero-competition grounds.

Emerging Indigenous Pathogens

The Alphaviruses: Barmah Forest Virus (BFV)

The alphavirus RRV continues to be the most prevalent arbovirus infecting humans in Australia, causing many thousands of local cases annually; it also reappeared in the Pacific recently, apparently for the first time since the extraordinary 1979–80 outbreak (Marshall and Miles 1984), with serologically confirmed reports of infections occurring in travelers to Fiji during 2000, 2001, 2003 and 2004 from New Zealand (Dave Slaney pers. comm.) and Canada (Klapsing et al. 2005).

However, the emerging arbovirus of scientific interest and public health concern is Barmah Forest virus (BFV), with increasing numbers of infections of BFV being reported from across the country. Activity of BFV in Australia has been reviewed previously by Russell (1995), Russell (1998), Russell and Dwyer (2000) and Russell and Kay (2004), and more recent updates on activity in the states with most cases have been published for Queensland (Quinn et al. 2005) and NSW (Doggett and Russell 2005).

Although less prevalent nationally than RRV, BFV has been recorded in all states. Early regional outbreaks occurred in disparate parts of the country, e.g. in northern tropical regions such as Arnhem Land in the NT in 1992 (Merianos et al. 1992) and the Kimberley region of WA in 1993 (Lindsay et al. 1994), and in southern temperate regions such the south coasts of WA in 1992–94 (Lindsay et al. 1995) and NSW in 1995 (Doggett et al. 1999), and in the Gippsland region of eastern VIC in 2002 (Passmore et al. 2002).

The virus was originally recovered from *Cx. annulirostris* collected in the Barmah Forest on the Murray River in northern VIC in 1974, and mosquito species yielding BFV in Australia have been previously discussed (Russell 1995, 1998). BFV has been isolated from more than 20 species and several have been shown to be competent to transmit in the laboratory but, in general, the vectors remain relatively poorly defined. However, it appears that the saltmarsh species *Aedes camptorhynchus* and *Ae. vigilax* in coastal areas and the freshwater marsh species *Culex annulirostris* and possibly *Coquillettidia linealis* in inland rural areas are the major vectors, although some of the floodwater *Aedes* such as *Ae. normanensis* in northern Australia and *Ae. bancroftianus* and *Aedes procax* in southern Australia appear to be also involved, and *Ae. notoscriptus* may be of significance in urban/domestic situations throughout Australia.

Although BFV appears to share vectors species with RRV, outbreaks of BFV have occurred both concurrently and independently of RRV outbreaks. For instance, the first recorded outbreak in the NT in 1991–92 involved both RRV and BFV

concurrently, but when the first substantial outbreak of BFV in WA was reported from the southwest of the State during 1992–1994, it occurred in the absence of any detectable RRV activity (Lindsay et al. 1995). Recent research has further elucidated the range of mosquitoes that might be involved in regional and local transmission, particularly in Queensland and NSW, with laboratory investigations of vector competence for various species and surveillance of vector abundance in areas with records of virus transmission (Ryan and Kay 1999; Jeffery et al. 2002a, b 2006).

BFV is genotypically related to RRV and is antigenically separate from other alphaviruses. In contrast to RRV, there is high degree of homology (98–100%) between BFV sequences from isolates collected from around Australia, with no evidence of geographic or temporal divergence (Poidinger et al. 1997). Given that BFV appears to exist as one genotype, and the potential animal hosts such as for RRV are relatively sedentary, the mechanism for BFV spread from enzootic foci to elsewhere in Australia is uncertain unless birds or flying mammals (e.g. fruit bats) or humans are involved. However, while a recent study of natural arbovirus natural infections in urban vertebrates in Brisbane, QLD showed 13% of flying foxes had antibodies to RRV, none showed antibodies to BFV (Kay et al. 2007).

Overall, seroepidemiological studies in wildlife (Aldred et al. 1990; Vale et al. 1991; Johansen et al. 2005b) have shown infection in mammals, including marsupials, but antibody prevalences reported have been generally quite low compared with those for RRV virus. Laboratory studies have ruled out some domestic and peridomestic mammals, cats, dogs and possums, cats, dogs as likely reservoirs, although possums have shown relatively high prevalence of antibodies for BFV in urban environments (Boyd et al. 2001; Kay et al. 2007).

The possibility that avians are important hosts of BF virus cannot be ignored (particularly because rapidly spreading outbreaks suggest a highly mobile vertebrate host), and more data from serosurveys and viraemia studies are required before mammals and/or birds can be ascribed as hosts for the virus. Additionally, the mechanism(s) of persistence and/or reintroduction of the virus have not been defined, and although vertical transmission is a possibility (as is known for RRV) it has not yet been shown for BFV despite investigation in areas with high adult mosquito infection rates. Naish et al. (2006) developed a weather-based forecasting model for BFV disease in a region of southeastern QLD that showed associations with average minimum temperatures and high tides, but seasonal instigation of BFV activity remains to be elucidated.

Human infection with BFV was first indicated in NSW, when Vale et al. (1986) reported a widespread but lower level (2–8%) of antibodies to BFV than to RRV (5–21%) for the same study group of humans from areas on the south coast. An overall State rate of 6.5% (cf. 31.6% for RRV) was later reported for QLD (Phillips et al. 1990), and limited data for WA indicate 1–2% of the population in the southwest have BFV antibodies and approx. 0.23% of the resident population becomes infected with BFV each year.

Human disease was first demonstrated in 1988 in NSW (Boughton et al. 1988), and because of the similarity to RRV infection, it is possible that BFV infection has been underdiagnosed clinically in the past. Symptoms of BFV infection include polyarthritis, arthralgia, myalgia, fever, and rash (Boughton et al. 1988; Phillips

et al. 1990; Nash and Harrington 1991; Mackenzie and Smith 1996; Sam and Crerar 1996; Beard et al. 1997).

Although the diseases caused by RRV and BFV infection cannot be reliably distinguished by their clinical symptoms (Flexman et al. 1998), arthritis is more common and more prominent with RRV infection, and rash more common and florid with BFV infection. The arthralgia may continue for at least 6 months in up to 50% of RRV patients but in only approximately 10% of BFV infected patients, although in one study 51% of respondents noted their BFV illness lasted more than 6 months (Beard et al. 1997). Serological testing can distinguish RRV and BFV infections; however, detection of virus-specific IgM only gives a presumptive diagnosis as IgM often persists for months or years and may represent past infection (Flexman et al. 1998).

It is possible that activity of BFV may have been unrecognised in the past, because of confusion with other virus infections, but it does appear that BFV has spread and increased in incidence; cases have now been reported from all Australian states, and incidence rates prior to 2003 were approx.18% of that of RRV but post 2003 they are approx. 35% of that of RRV (Australian Government Department of Health and Ageing data http://www9.health.gov.au/cda/Source/Rpt_4.cfm).

While notifications of BFV infections have increased in recent years, the reasons for this are not understood, although certainly there may have been an increase in reporting as medical practitioners became more aware of the infection and requested serological testing after commercial test kits became widely available from 1992. There is also some recent evidence of false positives results from commercial serological test kits and at least some of the reported recent increase may be attributed to this problem.

BFV infection is a notifiable disease and national case numbers are derived from serological laboratory reports, with national serologically confirmed reports on BFV being first recorded in 1995. During the six years 1995–2000, the annual average was 782 (range 616–1038), while for the six years 2001–2005, the annual average has been 1327 (range 910–2122). While activity is usually variable between and within states, years and seasons, the most obvious increases have been in the eastern states of Queensland and NSW, (Australian Government Department of Health and Ageing data http://www9.health.gov.au/cda/Source/Rpt_4.cfm >). Indeed NSW has experienced an overall 300% increase in case numbers for the past 6 years compared with the previous six years, with the north coast regions of the state experiencing annual outbreaks.

Quinn et al. (2005) analysed records of BFV case data for 1993–2003 from Queensland to reveal incidence rates and seasonal and regional prevalence, and Doggett and Russell (2005) have done this similarly for NSW.

Conclusions

The continuing and increasing quantity and rapidity of movement of humans and cargo by sea and air will continue to provide for increasing risks of spread of vector-borne diseases. The continuing exclusion of exotic vectors from Australia depends

critically on vigilant surveillance and control activities of federal quarantine and State or Territory Health authorities. The potential for introduction of other exotic mosquito-borne pathogens, such as Chikungunya virus (CHIKV) and Rift Valley fever virus (RVFV), both of which have known vectors in Australia, is recognized as a concern by medical and veterinary authorities, with CHIKV already known to have been imported via a human infection (Druce et al. 2007) and RVFV the subject of a national Risk Assessment exercise. Concerns for the high profile WNV are complicated by the fact that, although vectors exist in Australia, the local flavivirus KUNV is now recognized as a less virulent subtype of WNV, and its close relationship with WNV may mean that cross-protection mechanisms in wildlife may lessen the likelihood of severe outcomes should WNV-NY99 be introduced. What other threats and risks, not yet evident, await us?

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Part III
Arboviruses and Their Control in the Field

Novel Strategies to Control *Aedes aegypti* and Dengue

Barry Beaty, Scott Bernhardt, William Black, Carol Blair, Lars Eisen, Darwin Elizondo-Quiroga, Jose Farfan-Ale, Saul Lozano-Fuentes, Alexander Franz, Ken E. Olson and Irma Sanchez-Vargas

Abstract Vector-borne diseases are resurgent throughout the world. There are critical needs to develop novel approaches and strategies and improved public health capacity to prevent and control these diseases. Dengue is an archetypical resurging and emerging disease. At the Arthropod-Borne and Infectious Diseases Laboratory, we are investigating novel strategies for *Aedes aegypti* and dengue control; including development of (1) transgenic *Ae. aegypti* that are innately immunologically resistant to dengue virus infection, (2) a Casa Segura (safe house) approach based upon long-lasting insecticide-treated materials to protect humans from *Ae. aegypti* in the epidemiologically most important point of contact – the home, and (3) a dengue decision support system to enhance the efficacy of *Ae. aegypti* and dengue control programs. The results provide promise for targeting the vector to prevent and control dengue.

Keywords Dengue · Casa Segura · Vector Innate Immunity · Decision Support System

Introduction

The emergence of epidemic dengue and dengue hemorrhagic fever and shock syndrome in the Americas has been a public health disaster (Gratz 1999; Gubler 2002). Because there is no vaccine, control of the principal vector, *Aedes aegypti*, is the only means for preventing dengue infections and epidemics. Physical removal of breeding containers and source reduction using chemical or biological larvicides are used to reduce immature *Ae. aegypti* populations to prevent dengue virus transmission, and larval control and space spraying targeting adult mosquitoes in and

B. Beaty (✉)
Arthropod-Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523, USA
e-mail: bbeaty@colostate.edu

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around premises with dengue infections is used to intervene in impending epidemics (PAHO 1994). Although these approaches are undoubtedly beneficial, they have not proven to be sufficient or sustainable for control of *Ae. aegypti* and dengue. Vaccines are not likely to be forthcoming in the near future. Thus, at the Arthropod-Borne and Infectious Diseases Laboratory (AIDL) we are investigating a number of novel approaches to control dengue by focusing upon the vector.

Harnessing Vector Innate Immunity to Develop Dengue Resistant *Ae. aegypti*

One novel approach being investigated for dengue prevention focuses upon the understanding of the genetic basis of vector competence of *Ae. aegypti* for dengue virus. We first demonstrated that RNA interference (RNAi) constitutes a robust innate immune response to arboviruses in cultured *Ae. albopictus* mosquito cells (Adelman et al. 2002; Olson et al. 2002; Sanchez-Vargas et al. 2004). This had provocative implications for engineering resistance to dengue virus transmission in vector populations. We then proceeded to determine if RNA could be a determinant of the vector competent phenotype for arboviruses and if this knowledge could be exploited to engineer *Ae. aegypti* that are resistant to dengue virus infection. The ultimate goal is to use this knowledge to manipulate vector populations to interrupt dengue virus transmission.

RNAi Conditions Arbovirus Infection in Mosquitoes

Studies were conducted to determine if RNAi constitutes a robust innate immune response to arboviruses in vectors in vivo (Keene, et al. 2004). RNAi is triggered in eukaryotic organisms by double-stranded RNA (dsRNA), and it results in destruction of any mRNA that has sequence identity with the dsRNA trigger. Most arboviruses are RNA viruses and form dsRNA during their replication cycles. We hypothesized that RNAi may act as an antagonist to alphavirus replication in *Anopheles gambiae*. We first demonstrated that the RNAi pathway in *An. gambiae* can be silenced by transfecting cells with dsRNA derived from exon sequence of the *An. gambiae Argonaute2* (*Agago2*) gene (Hoa et al 2003). The next step was to determine if RNA silencing of *Agago2* expression would make *An. gambiae* mosquitoes more permissive (more competent) to arbovirus infection. Studies were conducted to determine whether RNAi conditions the vector competence of *An. gambiae* for O'nyong-nyong virus (ONNV, genus Alphavirus). We genetically engineered a modified ONNV to express enhanced GFP (eGFP) as a marker. When the ONNV-eGFP was intrathoricacially injected into *An. gambiae*, the virus first replicated at the portal of entry and then spread slowly over a 9-day period to other tissues. Mosquitoes were then co-injected with ONNV-eGFP and either control dsRNA derived from the *ß-galactosidase* (*dsßgal*) gene or from ONNVnsP3 (*dsnsP3*) gene. Treatment with *E. coli dsnsP3* RNA inhibited ONNV-eGFP spread,

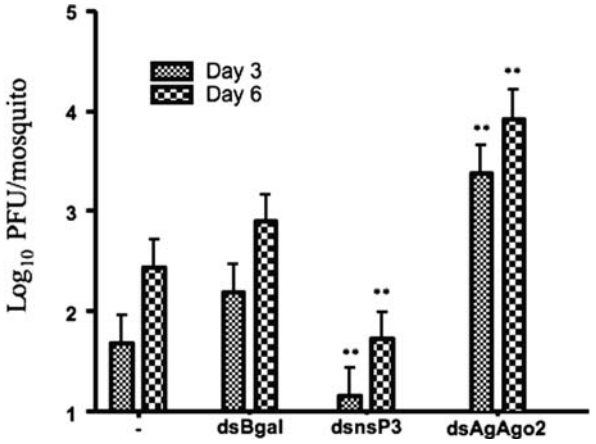

Fig. 1 Silencing the RNAi response makes *Anopheles gambiae* mosquitoes susceptible to O' nyong nynong virus (adapted from Keene et al. 2004)

as determined by eGFP expression patterns, and ONNV-eGFP titers were significantly reduced in the *dsnsP3* RNA-injected mosquitoes compared to mosquitoes co-injected with the *dsßgal* gene sequence RNA (Fig. 1). Thus *An. gambiae* did mount an RNAi response to suppress the arbovirus infection. To demonstrate unequivocally that RNAi was involved, mosquitoes were then co-injected with ONNV-eGFP and *dsAgago2* RNA, the latter of which would inhibit the vector RNAi response. Mosquitoes co-injected with virus and *Agago2* dsRNA were much more permissive to virus infection and displayed widespread eGFP expression and virus titers 16-fold higher than *dsßgal* RNA controls at three or six days after injection. Perturbation of the RNAi response by silencing Argonaute 2, a component of the RNAi-induced silencing complex, changed *An. gambiae* mosquitoes from ONNV-resistant to permissive phenotypes.

These landmark studies provided direct evidence that RNAi is an antagonist of ONNV replication in *An. gambiae*, and they suggest that the innate immune response conditions vector competence. Indeed, studies have now demonstrated that RNAi also modulates infection, replication, and transmission of dengue viruses in mosquitoes (Franz et al. 2006). Thus RNAi may be an important determinant of vector competence in all of the major arbovirus families.

Association Mapping Links Vector Competence of Ae. aegypti to the Vector Immune Response

In addition to the elegant molecular studies demonstrating a role for RNAi in arbovirus vector competence, we have also utilized field collected *Ae. aegypti* from Mexico to link RNAi genes to vector competence for dengue viruses in natural

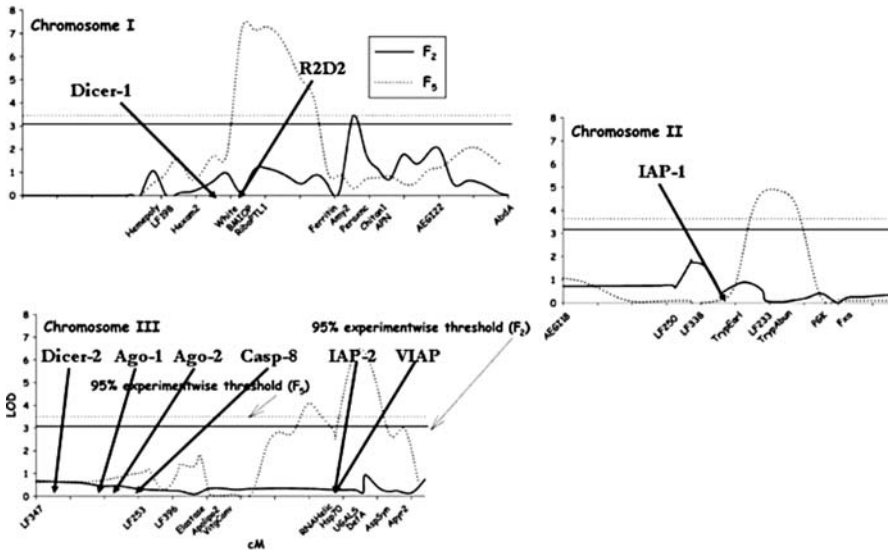

Fig. 2 QTL mapping of selected innate immunity genes of *Aedes aegypti*

populations. Previous studies of the breeding structure of *Ae. aegypti* in Mexico revealed dramatic differences in vector competence of the populations for dengue virus; following ingestion of virus-infected bloodmeals, 24–83% of the mosquitoes in populations became infected and potentially could transmit the virus (Bennett et al. 2002). *Ae. aegypti* mosquito colonies were selected for high and low vector competence relative to midgut escape and dissemination of the virus, and then used in genetic studies to identify quantitative trait loci (QTLs) associated with vector competence (Bennett et al 2005). Further genetic studies focusing on RNAi and apoptosis revealed that some genes of the mosquito RNAi response (eg. R2D2) map to major QTLs that determine vector competence. (Fig. 2). These results strongly suggest that vector competence is conditioned by the innate immune response of the vector. Additional genetic studies are underway using recent *Ae. aegypti* collections from Mexico that naturally demonstrate high and low vector competence for dengue type 2 virus (DENV-2); these studies will map additional RNAi genes on the *Ae. aegypti* linkage map and identify QTLs for vector competence in more field relevant populations.

Exploitation of the RNAi Response to Generate Transgenic Mosquitoes Immunologically Refractory to Dengue Virus Infection

Studies were then conducted to determine if the vector natural innate immune response could be exploited to generate transgenic, dengue virus-immune mosquitoes (Franz et al. 2006). In an elegant experiment, *Ae. aegypti* mosquitoes

were genetically modified to exhibit impaired vector competence for dengue type 2 viruses (DENV-2). We exploited the RNAi pathway in the mosquito midgut by constructing an effector gene that expresses an inverted-repeat (IR) RNA derived from the pre-membrane protein coding region of the DENV-2 RNA genome. The *Ae. aegypti* carboxypeptidase A promoter was used to express the IR RNA in midgut epithelial cells after ingestion of a blood meal. This inducible promoter system was chosen to minimize potential deleterious effects of constitutive expression of the construct and because it would express the effector sequence in the midgut immediately after ingestion of the blood meal, when virus would first encounter the midgut epithelial cells. This is when the virus would be most susceptible to the vector immune response. The effector gene expression cassette was inserted into the genome of an *Ae. aegypti* white-eye Puerto Rico Rexville D strain by using the non-autonomous mariner *MosI* transformation system. One transgenic family, Carb77, efficiently expressed IR RNA in the midgut after a blood meal. Carb77 mosquitoes that ingested an artificial blood meal containing DENV-2 exhibited marked reduction of viral envelope antigen expression in midguts and salivary glands after infection. DENV-2 titration of individual mosquitoes showed that most Carb77 mosquitoes were not permissive to virus replication. Mosquitoes from the Carb77 line were also significantly less capable of transmitting the virus than control mosquitoes (Fig. 3). The presence of specific siRNAs in RNA extracts from midguts of Carb77 and the loss of the resistance phenotype when the RNAi pathway was interrupted proved that DENV-2 resistance was caused by a RNAi response (Franz et al. 2006).

This landmark study is an important proof of concept for potential genetic control of dengue virus transmission. Indeed, engineering of transgenic *Ae. aegypti* that

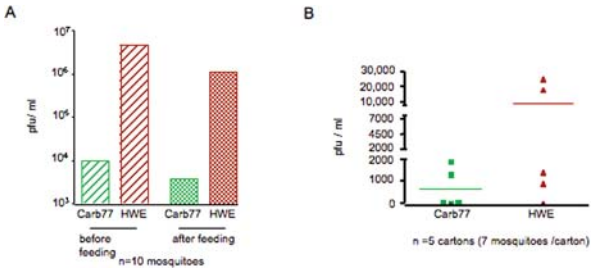

Fig. 3 Transgenic mosquitoes with an induced RNAi immune response to dengue virus are greatly restricted in their permissiveness to virus infection, replication and transmission. Characterization of the Carb77 DENV-2 resistance phenotype. One week old females received a DENV-2 (Jamaica 1409) containing, artificial bloodmeal (virus titer in the bloodmeal 10^7 pfu/ml) and were assayed 14 days later. (**a**) Virus titers of ten 14 day old individual Carb77 or HWE females were analyzed by plaque assays ("before feeding"). After feeding on a solution placed between two parafilm membranes virus titers of another 10 Carb77 or HWE females were analyzed ("after feeding"). (**b**) Five groups, of seven mosquitoes each were allowed to feed on a solution into which they could deliver infectious virus during salivation. Virus titers of these feeding solutions were analyzed by plaque assays using LLC-MK2 cells (*Bars* indicate mean virus titers.)

are immune to DENV-2 provides a powerful tool for developing population replacement strategies to control transmission of dengue viruses. This strategy is now being evaluated as part of a Gates Grand Challenge grant.

The Casa Segura ("Safe Home") – A Novel Approach to Protect the Home from *Aedes aegypti*

Another novel approach being investigated at AIDL for dengue control is based upon knowledge of the biology and behavior of *Ae. aegypti*, and the environment in which dengue virus transmission most commonly occurs. In many areas in the Americas, *Ae. aegypti* is an archetypical endophagic and endophilic vector; it feeds, rests, and if given the chance will also lay eggs inside the home. Prevention programs focus upon source reduction (larviciding) and environmental sanitation as noted above to attack the breeding sites of the vector. In addition, vector control programs respond to identification of dengue cases with space spraying for adult mosquitoes and source reduction and environmental sanitation in and around premises (PAHO 1994). Unfortunately, in today's throw-away society, breeding sites for *Ae. aegypti* are everywhere and accumulate rapidly following clean up campaigns. Since the vector is intimately associated with humans in indoor environments – (Reiter and Gubler 1997; Reiter et al. 2003; Beaty and Eisen 2008), a more efficient control strategy would be to develop and improve pesticide-based interventions that prevent the vector from entering and/or attack the vector in the epidemiologically most significant point of contact for dengue virus transmission – the home. Prevention of endophagy by *Ae. aegypti* is important not only to stop transmission of virus from infected mosquitoes to susceptible humans, but also to stop *Ae. aegypti* from feeding upon infected humans, becoming infected, and subsequently transmitting the virus to new susceptible humans. Viremia titer is directly correlated with febrile illness and disease severity (Vaughn et al. 2000). Infected humans with high virus titers are the sickest, the most infectious for vectors, and most likely to be restricted to the home environment, where the vectors feed. Preventing contact between the vectors and these important amplifying hosts will be an added benefit of the Casa Segura. Thus, interrupting endophagy by *Ae. aegypti* impacts the transmission cycle of the virus at two points and provides a unique opportunity to reduce dengue virus amplification. We are exploring a Casa Segura approach using long-lasting insecticide-treated materials (LL-ITMs) as curtains for control of adult *Ae. aegypti* in indoor environments in Mexico. The Casa Segura strategy aims to reduce transmission of virus to humans as well as infection of vectors by viremic humans, thereby dramatically reducing dengue virus transmission potential.

The Casa Segura concept is based upon the demonstrated efficacy of insecticide-treated bednets to reduce the burden of malaria by preventing pathogen transmission in the home by night time feeding vectors (e.g., N'Guessan et al. 2001; Hawley et al. 2003; Lengeler 2007). New LL-ITMs remain efficacious for >5 years. The development of these LL-ITMs for bednets is truly a landmark event in vector control.

Dengue virus transmission by *Ae. aegypti*, which is a day time feeder, also occurs most frequently in indoor environments because of the endophilic and endophagic behavior of the vector -(Reiter and Gubler 1997; Clark et al. 1994; Edman et al. 1997). Thus the use of LL- ITMs also offers great potential for prevention of dengue.

Indeed, a number of studies have demonstrated the potential for ITMs to be used as curtains to control *Ae. aegypti* in the home and to prevent dengue transmission. The studies used different insecticides and different materials as curtains, but each of the studies demonstrated the approach to be remarkably efficacious, at least in short term experiments. ITMs used as curtains dramatically reduced *Ae. aegypti* populations and also dengue virus transmission in intervention versus control homes in Viet Nam (Nam et al. 1993; Nguyen et al. 1996; Igarashi 1997; Kroeger et al. 2006),

We are currently implementing a Casa Segura approach for prevention of dengue through use of LL-ITMs (i.e., curtains covering windows in collaboration with academic partners (Universidad Autonoma de Yucatan), public health partners (Servicios de Salud de Yucatan, Mexico), and industry partners (Bayer Environmental Science, Acytex Internacional). The Casa Segura implementation in Merida, Mexico, will assess the protective efficacy of LL-ITMs (treated with deltamethrin) in a two year longitudinal study in a large city environment. The study will assess entomological outcomes (eg, abundance of adult *Ae. aegypti* in the home, pupal demographic survey, larval indices, pesticide resistance type and prevalence) and epidemiological outcomes (eg, seroconversion rates, dengue illness incidence, virus isolation) in intervention and non-intervention houses of Merida.

Potentially, LL-ITMs, such as curtains, wall-hangings etc., can protect domiciles, schools, or other structures where people are exposed to mosquito bites, for multiple years. The domicile and other indoor environments may also be protected against other pathogen vectors as well as pest insects, including nuisance *Culex* mosquitoes. Indeed, many globally important vector-borne diseases (VBDs) are transmitted principally indoors, e.g., malaria in much of sub-Saharan Africa, dengue, leishmaniasis, Chagas and filariasis. Thus the Casa Segura may provide a broad spectrum product for disease and pest management, rather than one stove piped to a particular vector or disease. Conceptually, the Casa Segura provides the protection of a western style domicile in terms of VBDs by exploiting LL-ITMs (and potentially other interventions, which will undoubtedly be forthcoming in the future) to effect a safe house.

A Dengue Decision Support System for Enhanced Vector Control and Prevention

In the "Microbial Threats to Health" report from the Institute of Medicine (Smolinski et al. 2003), among the recommendations for addressing VBDs was the following:

- Expand efforts to exploit Geographic Information System (GIS) and robust models for predicting and preventing the emergence of vector-borne and zoonotic diseases and exploit innovative systems of surveillance that capitalize on advances in information technology.

Development of the Dengue Decision Support System (DDSS)

The Innovative Vector Control Consortium (IVCC) is helping to address this challenge for dengue and malaria by funding projects to develop new tools and approaches to enhance vector and disease surveillance and control (Hemingway et al. 2006). Computer-based Decision Support Systems are widely used in many disciplines and offer great potential for improving prevention, surveillance, and control of dengue and for management of dengue control programs. Decision Support Systems provide improved logistical capacity for data management and analysis and an emphasis on evidence-based and rational decision-making leading to implementation of effective control program strategies, methodologies, and management (Eisen and Beaty 2008).

The DDSS, which is nearing completion, will take into account data related to vector, pathogen, and disease surveillance as well as vector control, pathogen control, clinical information, diagnostic testing, behavior and education of the human population, and demographic and socioeconomic conditions (Eisen and Beaty 2008). The DDSS will support local and regional vector control programs, will promote the creation of a community of vector control people, which will share best practices and knowledge, and will be rationally designed to promote information flow between local, regional, national, and even international stakeholders. The computer-based DDSS will aid and systematize the process of gathering and analyzing information, gaining new insights, generating alternatives and, ultimately, making evidence-based decisions regarding vector and disease surveillance and control (Fig. 4).

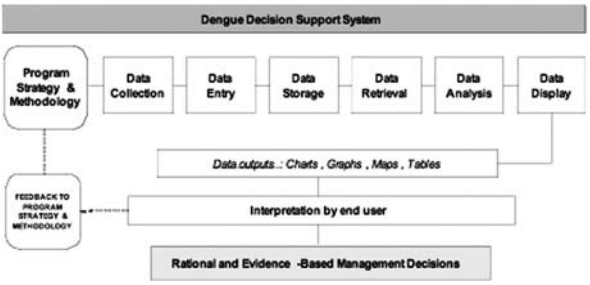

Fig. 4 Flow scheme for a dengue decision support system (adapted from a figure previously published in Eisen and Beaty 2008)

The potential for developing the DDSS has been enhanced by the emergence of GIS technology and the rapidly increasing availability of cartographic, demographic, socioeconomic, and environmental GIS-based data. GIS provides capacity for: (1) presentation of spatial and spatiotemporal patterns of risk of exposure to *Ae. aegypti* and dengue based on location-specific information (e.g., Morrison et al. 1998, 2004; Teng 2001; Rosa-Freitas et al. 2003; Getis et al. 2003; Chadee et al. 2005; Moreno-Sanchez, et al. 2006); and (2) development of predictive spatial risk models based on GIS-derived data and vector or disease measures (Rotela et al. 2007). Free mapping software (e.g., Google Earth, Google, Mountain View, California, U.S.A.; Microsoft® Virtual Earth, Microsoft Corporation, Redmond, Washington, U.S.A.) that provides access to high-quality satellite imagery and basic tools allowing the user to create and label features (place marks, lines, and polygons) are emerging as a powerful complement to GIS software for presentation of information overlaid on an image showing the physical environment. Indeed, we used freely available Google Earth imagery (see example of image quality in Fig. 5) to quickly and efficiently develop cartographic information to support DDSS implementation in Merida, Yucatan, and Chetumal, Quintana Roo, Mexico (Lozano-Fuentes et al., 2008). The developed DDSS will be used to monitor the efficacy of the Casa Segura approach to control *Ae. aegypti* in Merida.

Syndromic Surveillance for Improved Dengue Control

An important component of the DDSS is that it facilitates syndromic surveillance for dengue cases, which will potentially reduce response time for vector control by several weeks (Fig. 6). This will permit more rapid and hopefully more successful

Fig. 5 Google Earth images of Merida, Yucatan, Mexico

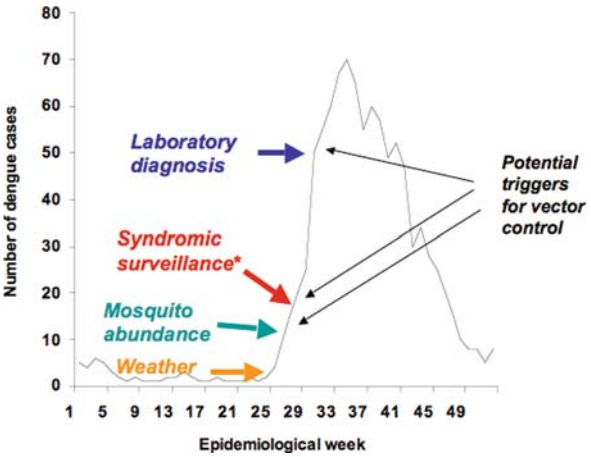

Fig. 6 Dengue warning system and syndromic surveillance provide early detection of impending epidemics. *Syndromic surveillance based on clinical diagnosis and daily electronic submission of Case Report Forms

interventions in impending epidemics. Indeed, syndromic surveillance may make the current PAHO recommendations for dengue control (ie, identifying premises, space spraying in and around premises, and source reduction around premises) much more effective (PAHO 1994). In the DDSS, clinical data from presumptive dengue cases will be entered into an electronic case report form at local health clinics and transmitted to the relevant vector and dengue control personnel daily. The clinical information will be analyzed for presumptive dengue cases using a developed algorithm to separate dengue from other commonly occurring diseases based on symptomology (Fig. 6). This offers great potential to identify impending epidemics long before laboratory diagnosed cases can be identified. The information will be provided essentially in real-time, thus minimizing vector control response time to intervene in impending epidemics (Fig. 6).

Conclusions

The needs for new tools and approaches to control dengue and other VBDs are great. The investigations at AIDL bridge the field of vector control from implementation of insecticides to exploration of innovative transgenic approaches for dengue control. We have consciously chosen to explore a wide variety of options for dengue control. VBDs are amazingly resistant to interventions, and the mosquito is a resilient and seemingly intractable foe. It is likely that successful control of many VBDs will require multiple and integrated interventions, but all will benefit from the use of a decision support system for cost-effective implementation of interventions, evidence-based information for policy decisions (Coleman et al. 2006), and effective long-term program operation and management.

Acknowledgements This paper reports results from multiple projects in the AIDL. Thus this work has been supported by a number of sources, including grants from the National Institutes

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Dengue Haemorrhagic Fever in Thailand: Current Incidence and Vector Management

Apiwat Tawatsin and Usavadee Thavara

Abstract Dengue, dengue haemorrhagic fever and dengue shock syndrome are endemic throughout South East Asia where they present a serious public health concern. The current status of these diseases in Thailand is described along with the challenges that confront those who seek to control the spread of these diseases.

Keywords Dengue · Dengue haemorrhagic fever · *Aedes aegypti* · Vector control · Thailand

Introduction

Dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) are caused by one or more of four dengue viruses (DEN-1, DEN-2, DEN-3, DEN-4) that transmit to humans through the bites of infective *Aedes* mosquitoes, *Aedes aegypti* (L.) and *Ae. albopictus* Skuse (Service 1993). The disease is now one of the major public health problems worldwide, especially in the tropical and sub-tropical regions. It is estimated that almost half of the global population are at risk of dengue infection. The disease is currently endemic in more than 100 countries in Africa, the Americas, Eastern Mediterranean, Southeast Asia and Western Pacific (WHO 2002). However, the Southeast Asia and the Western Pacific have been more seriously affected. The prevalence of DF/DHF has substantially grown during the past five decades. The annual average number of cases of DF or severe dengue (DHF/DSS) reported to the World Health Organization (WHO) have increased exponentially from 908 cases in 1950s to 925,896 cases in 2000s (Nathan and Dayal-Drager 2007). The recent global epidemic occurred in 1998, when a total of 1.2 million cases of DF/DHF, including 3,442 deaths were reported to WHO (WHO 2002).

A. Tawatsin (✉)
Department of Medical Sciences, National Institute of Health, Ministry of Public Health, Nonthaburi 11000, Thailand
e-mail: apiwat@dmsc.moph.go.th

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Incidence of DHF

DF, DHF and DSS are communicable diseases under the national surveillance system of Thailand which requires notification to the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. Among these, DHF is the majority of the reported cases (>90%) each year. DHF was first reported in Bangkok in 1949 (Wangroongsarb 1997) whereas the first epidemic occurred in 1958 (Ungchusak and Kunasol 1988). Since then, the annual incidence of DHF (morbidity per 100,000 populations) has fluctuated over time and increased from 8.9/100,000 in 1958 to 74.8/100,000 in 2006, with the highest incidence of 325/100,000 in 1987 (Fig. 1). According to classification by Nisalak et al. (2003),

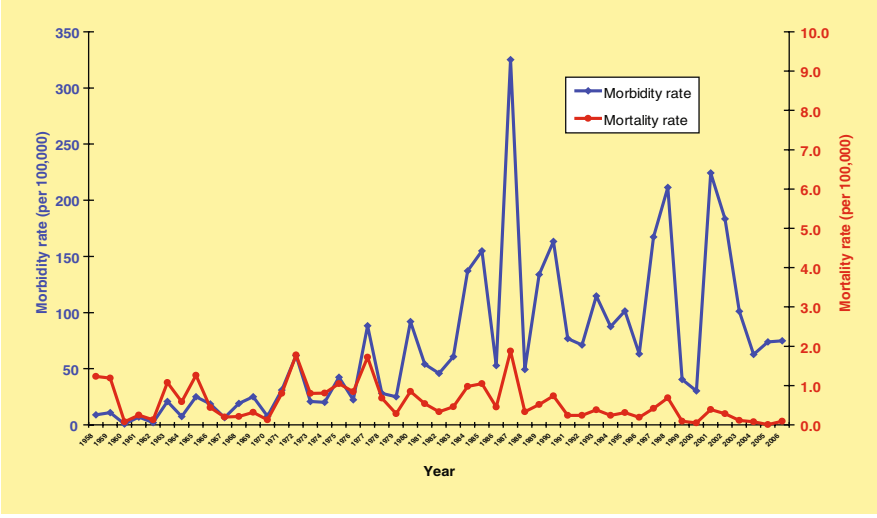

Fig. 1 Morbidity and mortality rates of DHF in Thailand, 1958–2006

the severe epidemics (annual morbidity rate >175 per 100,000) occurred in 1987, 1998, 2001 and 2002 whereas moderately severe epidemics (annual morbidity rate 134–175 per 100,000) presented in 1984, 1985, 1989, 1990 and 1997. The highest mortality rate of DHF in Thailand (1.88 per 100,000) was observed in 1987. However, it has appeared below 0.8 per 100,000 since 1988 and become less than 0.1 per 100,000 since 2004. Regarding the case fatality rate (CFR) among DHF patients, it was generally greater than 10% during 1958–1960 and dropped dramatically thereafter. CFR has been reduced to less than 1% since 1982 and remained below 0.5% during the past 18 years (Fig. 2). Recently, in 2004, 2005 and 2006, the CFR was 0.12, 0.15 and 0.13%, respectively. Based on the average number of DHF cases between 1981 and 2006, the most prevalent age group of DHF patients was 5–9, followed by 10–14, 15+ and 0–4 years, respectively (Fig. 3). DHF occurs

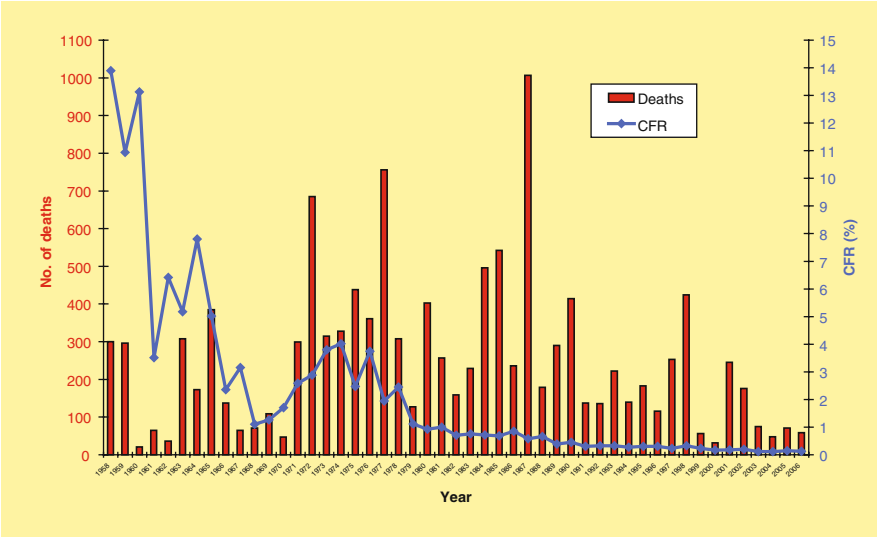

Fig. 2 Number of deaths and case fatality rate (CFR) of DHF in Thailand, 1958–2006

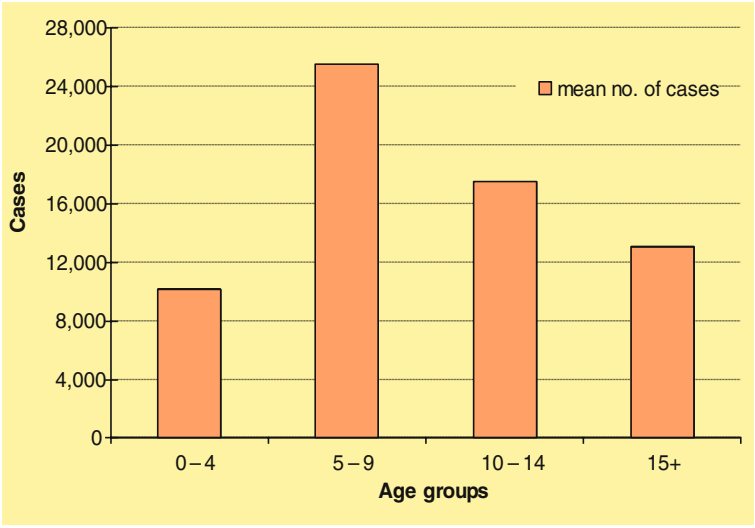

Fig. 3 Distribution of DHF in Thailand by age groups, 1981–2006

in Thailand all year round with high incidence during the rainy season lasting from May to October (Fig. 4). Until recently, DHF have been found in all provinces of Thailand; however, most cases were reported from central and southern regions in the present decade.

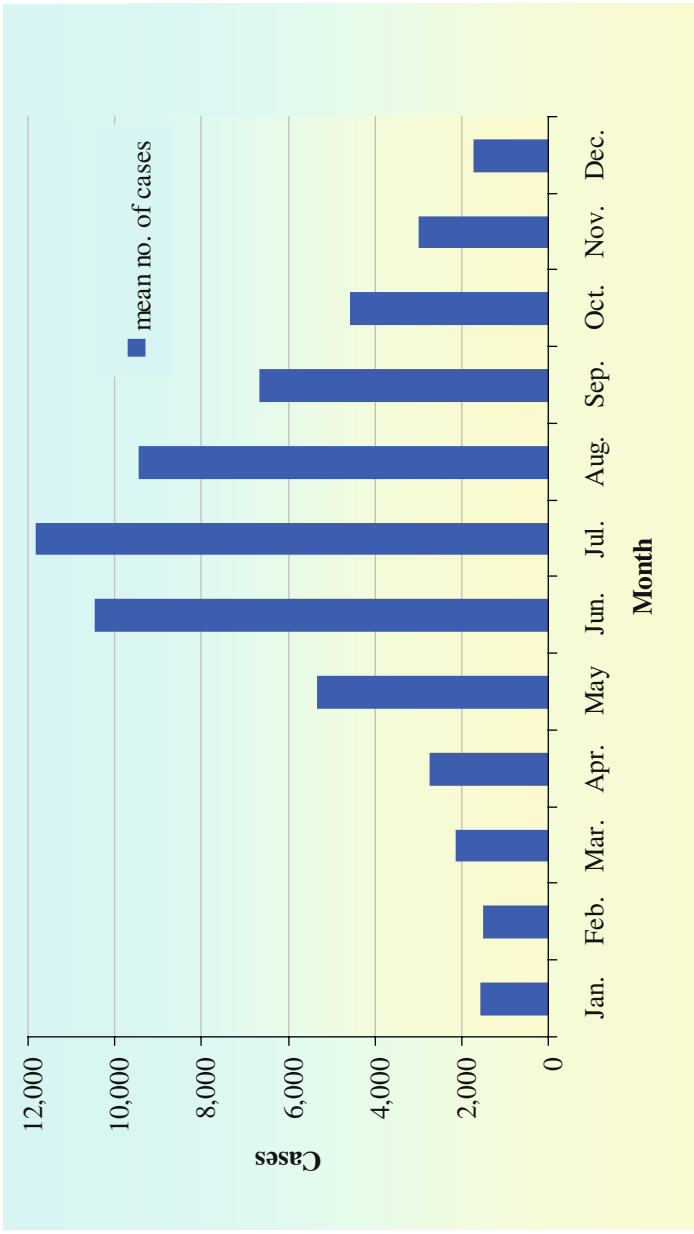

Fig. 4 Distribution of DHF in Thailand by months, 1981–2006

Distribution of Dengue Viruses

All four serotypes of dengue viruses (DEN-1, DEN-2, DEN-3, DEN-4) are endemic in Thailand (Nisalak et al. 2003). However, the predominant virus serotypes which have been frequently associated with epidemics vary from year to year. According to the data collected between 1973 and 1999 from 15,376 dengue patients admitted in the Queen Sirikit National Institute of Child Health (formerly the Bangkok Children's Hospital) in Bangkok, DEN-2 was predominant from 1973 to 1986 and 1988 to 1989; DEN-3 in 1987, and from 1995 to 1999; DEN-1 from 1990 to 1992; and DEN-4 from 1993 to 1994 (Nisalak et al. 2003). Among 50 isolations of dengue viruses obtained from DHF patients admitted at the Rayong Provincial Hospital from 1980 to 1984, DEN-2 was the predominant serotype (46%), followed by DEN-1 (32%), DEN-4 (12%) and DEN-3 (10%), respectively (Rojanasuphot et al. 1988). Recently, Anantapreecha et al. (2005) found that 45% out of 2,715 confirmed specimens collected from six hospitals scattered throughout Thailand between 1999 and 2002 were identified as DEN-1, whereas the rest were DEN-2 (32%), DEN-3 (18%) and DEN-4 (5%), respectively.

DHF Vectors

Both species of DHF vectors: *Ae. aegypti* and *Ae. albopictus* are found throughout Thailand. The main breeding places of *Ae. aegypti* in Thailand are mostly man-made water-storage containers, such as earthenware jars, water-storage drums, cement tanks, ant traps, etc (Thavara et al. 2001). *Ae. albopictus*, on the other hand, is able to breed in a wide range of natural and artificial types of breeding sources and water holding niches vary from place to place. The main breeding places of *Ae. albopictus* are mainly natural sites, such as leaf axils, tree holes, coconut husks, bamboo stumps, etc., as well as, artificial containers, for example, earthenware jars, water-storage drums, used tyres and a variety of plastic containers found in the domestic environment (Thavara et al. 2001). Although the both species normally breed in different habitats, in some certain places they may be found breeding together in the same containers (Thavara et al. 2001). The females of both species prefer to oviposit in containers with relatively clean water; however, they may also do so in waters with varying degree of contamination with organic debris.

The females of *Ae. aegypti* and *Ae. albopictus* are daytime biters. They may feed on human victims from dawn to dusk, depending on hosts available. The diel biting activity of both species is usually higher in the morning hours than in the afternoon period (Thavara et al. 2001). Very recently, we have carried out an intensive study on biting rhythm of *Ae. aegypti* during 24 h of the day, once a month for 5 months during the rainy season of 2007, and we found that the biting patterns changed from earlier reports. The average biting remained high (20.4–23.4 mosquitoes/person-h) in the morning period from 6 to 9 a.m. (Fig. 5). Then, the average biting activity declined dramatically to less than 10 mosquitoes/person-h and rose again to about 19.2 mosquitoes/person-h at dusk (6 p.m.). It is interesting to note that the biting

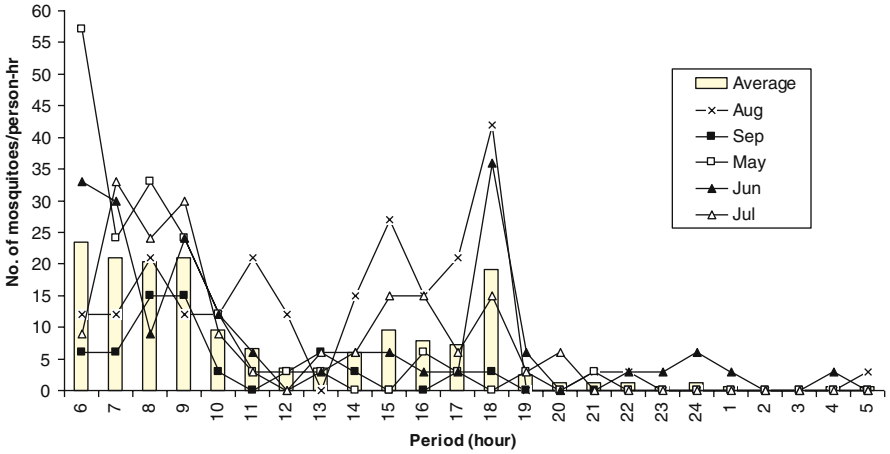

Fig. 5 Biting activity of *Ae. aegypti* during 24 h of the day conducted once a month between May and September 2007, in Nonthaburi, Thailand

activity of *Ae. aegypti* also occurred after sunset until early morning of the next day (5 a.m.), except for a couple hours between 2 and 3 a.m. (Fig. 5). *Ae. aegypti* is primarily an endophagic mosquito, rarely biting outside whereas *Ae. albopictus* is primarily an exophagic mosquito but can be found in significant numbers biting indoors (Thavara et al. 2001). Both species are persistent mosquitoes, pursuing the victim until feeding is completed. Actually, feeding continues until the mosquito is fully engorged; however, if this feeding is disturbed, it may return to feed again on the same or other victims. This thus plays an important role in dengue transmission.

In Thailand, dengue viruses have been isolated from, or detected in, wild caught *Ae. aegypti* (Watts et al. 1985; Rojanasuphot et al. 1988; Thavara et al. 1996; Tuksinvaracharn et al. 2004; Thavara et al. 2006) as well as from *Ae. albopictus* (Thavara et al. 1996; 2006). It was also found that two different serotypes of dengue viruses (DEN-2 and DEN-3) had been detected by polymerase chain reaction (PCR) technique in field-caught individual *Ae. aegypti* males and females and *Ae. albopictus* females (Thavara et al. 2006). The evidence of dengue infection in wild-caught individual *Ae. aegypti* males in the previous study obviously reveals phenomenon of transovarial transmission occurring in natural environment in Thailand. This phenomenon could maintain the viruses in the environment during dry period when populations of mosquito vectors are scarce, even though the transovarial transmission of dengue viruses by mosquito vectors could occur naturally at a relative low rate (Rosen et al. 1983). These may confirm a high incidence of DHF in Thailand.

Current DHF Vector Management

At the outset, initial vector control programs, which were implemented in late 1960s, emphasized the application of chemical sprays to control adult mosquitoes, but this intervention had little or no impact on disease transmission. As a result, the national

policy on DHF vector control was redirected to community-based strategies with emphasis on source reduction employing village health volunteers since the 1980s. The current strategies used for prevention and control of DHF vectors in Thailand are briefly summarized in Table 1. Satisfactory achievement has been achieved in some areas, depending on the strength of local health authorities and community participation.

Table 1 Current strategies used for prevention and control of DHF vectors in Thailand

Strategies used	Processes/activities
Provisional of health education	➢ School: teaching in classes ➢ Communities: public announcement, posters, brochures, leaflets ➢ Mass media: television, radio, newspapers
Massive campaigns	➢ Larval elimination on every Friday ➢ 3 regular practices for household water-storage containers: covering, changing, fish releasing
Environmental measures	➢ Source reduction of larval breeding
Larval control	➢ Physical approach: covering, draining, filling ➢ Biological agents: –: larvivorous fishes: Guppy (*Poecilia reticulata*), Siamese fighting fish (*Betta splendens*) –: Bti-based larvicides ➢ Chemical larvicides: –: temephos-based larvicides
Adult control: space spraying[a]	➢ Thermal fogs ➢ Cold aerosol sprays
Personal protection	➢ Mosquito nets ➢ Electrical mosquito-rackets ➢ Repellents

[a]Insecticides used: Deltamethrin, Cypermethrin, Tetramethrin, Bioresemethrin, Cyfluthrin, Bifenthrin, Fenitrothion, Fenthion and Malathion.

In 2007, the Department of Disease Control, Ministry of Public Health, established a new scheme for controlling of dengue diseases. This scheme includes five major key performances: rapidness of dengue reporting, complete investigation of index cases at village level, preparedness of vector control teams at the district level, rapidness of vector control at dengue-reported foci, and coverage of vector control at dengue-reported foci. The details of this new scheme are briefly described in Table 2.

Country Obstacles and Challenges

The programs for DHF vector control in Thailand have confronted some obstacles. These obstacles are listed as following.

Table 2 The key performance of a new scheme for controlling of dengue diseases established in 2007 by the Department of Disease Control, Ministry of Public Health, Thailand

Key performance	Targeted action
Rapidness of dengue reporting Objective: To strengthen the ability of dengue reporting network	➢ At least 80% of the dengue-reported cases receiving from hospitals are reported to the local health authorities of the patient dwellings within 24 h by the provincial health authorities
Complete investigation of index case at village level Objective: To find out the source of infection in order to stop epidemic	➢ At least 80% of index cases are investigated completely (index case = the first dengue case reported by hospital of each village)
Preparedness of vector control team at district level Objective: To prepare the vector control team ready for emergency control immediately after receiving case report	➢ At least one vector control team is officially appointed in each district ➢ At least one member of the vector control team is well-trained to operate the space-spraying equipment (ULV and/or thermal fog generator) ➢ At least one set of the space-spraying equipment (ULV and/or thermal fog generator) is ready to use at all time ➢ Chemical insecticides used for space-spraying and larvicides are sufficiently stocked at all time
Rapidness of vector control at dengue-reported foci Objective: To eliminate the infected mosquitoes in order to stop dengue transmission	➢ Vector control is carried out by the vector control team within 24 h after receiving report from the provincial health authorities
Coverage of vector control at dengue-reported foci Objective: To prevent the second generation of infection at the dengue-reported foci	➢ Thoroughly survey and larval control are conducted at the dengue-infected dwellings and surrounding areas ➢ Application of space spraying is carried out at the dengue-infected dwellings and 100-m surrounding areas ➢ The second application of space spraying is carried out 7 days apart from the first one at each dengue-infected dwelling ➢ Assessment of vector control is conducted at 28 days post reporting of the index case to assure the absence of the second generation of infection

Difficulty in Mobilizing Community Participation in Vector Control

The sustainability of an integrated vector control program substantially depends upon community participation and ownership (Gubler 1989). The previous community-based vector control programs in Thailand were not sustained since they were mainly operated by the public health authorities without partnership from the

targeted communities (Gubler and Clark 1996). Therefore, there is an urgent need to identify the appropriate and effective behavior for vector control to be encouraged in the communities. Recently, Kittayapong et al. (2006) demonstrated a successful community-based program employing a combination of horizontal and vertical approaches and some components of this program have been already established and routinely managed by the Local Administrative Authority with financial support from individual households. However, the sustainability of the program remains unclear and the long-term success as well as the community ownership needs to be evaluated over time.

Insufficient Supply of Materials Used for Vector Control

As the key breeding sites of *Ae. aegypti* in Thailand are water-storage jars and cement tanks in bathrooms in each house (Thavara et al. 2001; Strickman and Kittayapong 2002), the application of larvicides and releasing of larvivorous fishes in these containers are the main strategy used for larval control. However, the larvicides supplied by government agencies are insufficient to apply to all breeding sites in each village. Once a year, each family actually receives a limited amount (approximately 20–60 g) of conventional larvicides (mostly 1% temephos sand granules) that are adequate only for 1–3 containers (when applied at the dosage of 20 g/200 L) whereas there are more than 5 water-storage containers in each house. Moreover, the loose granules of these larvicides are washed out and eventually lost during the process of cleaning and washing water-storage containers. There is, therefore, an essential need for sufficient supply of larvicides or other appropriate and effective materials used for larval control. Recently, Tawatsin et al. (2007) showed long-lasting efficacy of removable and retrievable formulations of temephos-based zeolite and sand granules in sachets that lasted for at least 6 months against *Ae. aegypti* larvae in water-storage jars. These innovative formulations will minimize the waste of scanty and costly larvicides and will expand larval control capacity for treatment in large numbers of water-storage containers that are untreated owing to insufficient amounts of the larvicides currently available.

Lack of Good Management in Vector Control

There are frequently changes of the staff responsible for dengue control at various levels, ranging from the policy makers to operational staff. Staff sometimes were inexperienced to cope well with DHF epidemics. According to the decentralization of health systems in Thailand, the activities with regard to vector-borne disease control, such as pesticide procurement and application have been transferring to and eventually carried out by the Regional and Local Administrative Authorities through to year 2015. There is concern that these agencies have little or no experience in vector control and so will require training, procedures and guidelines for all aspects of vector management.

Lack of Systematic Monitoring of Larval and Adult Resistance to the Insecticides Used

Although many chemical insecticides have been used for DHF vector control in Thailand for many decades, there is no systematic monitoring of larval and adult resistance to the insecticides used in the treated areas. Recent studies on insecticide susceptibility of *Ae. aegypti* and *Ae. albopictus* disclosed the occurrence of insecticide resistance in some particular areas of Thailand (Somboon et al. 2003; Paeporn et al. 2004; Ponlawat et al. 2005; Yaicharoen et al. 2005; Jirakanjanakit et al. 2007a, b; Pethuan et al. 2007). Therefore, there is an essential need to establish the systematic monitoring of larval and adult susceptibility/resistance to insecticides in both periodicity and geographical coverage in Thailand. In 2007, the Department of Disease Control initiated a program for monitoring insecticide resistance in *Ae. aegypti* in 19 provinces having high risk of a DHF epidemic. It is expected that this program could be expanded to more provinces to cover the whole country in the near future. The information obtained from this program would be beneficial for an effective control against DHF vectors in Thailand as well as to prevent or slow the development of insecticide resistance in the vectors.

More applied research is also needed to develop and implement effective control programs against DHF vectors in Thailand. These include development of early and predictive warning systems employing epidemiological, entomological and serological data, improvement of entomological indices for vector surveillance, identification of high-risk areas to be subjected to intensive vector control and development of new and safe larvicides and their long-lasting formulations. Recently, we embarked upon the testing and evaluation of the microbial agent (*Bacillus thuringiensis israelensis* or Bti), chemicals, novel insect growth regulators (IGRs) and formulations that yielded long-lasting control of *Ae. aegypti* in water-storage containers. The test larvicides and their efficacy are briefly summarized in Table 3. These materials are expected increase our arsenal against *Ae. aegypti*, the important DHF vector.

Table 3 Newly developed formulations of chemical/microbial larvicides tested against *Ae. aegypti* larvae in 200-L water-storage jars carried out in Thailand recently

Larvicidal agents	Formulations	Dosages (a.i./L)	Effective days (>90% IE)	References
Bti	DT	19,980 ITU/L	91–112	Mulla et al. 2004
Temephos	1% SG	1 mg/L	> 180	Mulla et al. 2004
	1% ZG	1 mg/L	> 180	
Temephos	1% SG (sachet)	1 mg/L	> 180	Tawatsin et al. 2007
	1% ZG (sachet)	1 mg/L	> 180	
Novaluron	10% EC	10 μg/L	68	Mulla et al. 2003
		0.05–0.1 mg/L	175	
		0.5–1 mg/L	190	
Diflubenzuron	2% DT	0.02 mg/L	147	Thavara et al. 2007
	2% GR	0.05–1 mg/L	161	
		0.02 mg/L	154	
		0.05–1 mg/L	161	

Conclusion

Dengue diseases, including DF, DHF and DSS have remained important mosquito-borne diseases of Thailand since the late 1950s with high annual incidence but relative low mortality. All of the four dengue serotypes circulate continuously in Thailand with fluctuations in dominant serotypes from year to year and from place to place. Both species of DHF vectors: *Ae. aegypti* and *Ae. albopictus* are found throughout Thailand. The current vector control programs for DHF in Thailand consist of provision of health education to raise public awareness, massive campaigns for larval control, environmental measures for source reduction, larval control, adult control and personal protection measure. Satisfactory control has been achieved in some areas, depending on the strength of local health authorities and community participation. However, the programs have confronted some obstacles, such as difficulties to mobilize community participation in larval control measures, inadequate supply of larvicides, lack of good management in vector control programs, little use of procedures resulting from operational research on vector control and lack of systematically monitoring larval and adult susceptibility to the insecticides used. More applied research is needed to develop and implement sustainable control programs against DHF vectors. These include identification of appropriate and effective behavior for vector control to be encouraged in the communities, development of early and predictive warning systems employing epidemiological, entomological and serological data, improvement of entomological indices for vector surveillance, identification of high-risk areas to be subjected to intensive vector control, development of new and safe larvicides and their long-lasting formulations. Another important thrust toward the control of *Ae. aegypti* is the extensive field evaluations of novel IGRs, assessment of the longevity of the currently used and new temephos formulations as well as controlled released formulations of Bti. Information of these new strategies and tools will be used in developing future control programs against DHF vectors in Thailand.

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Using "Mulla's Formula" to Estimate Percent Control

William K. Reisen

Abstract In California, the endemic mosquito borne encephalitides, including West Nile virus, are contained by special districts using integrated vector management programs. These agencies combine public education, source reduction and proactive larval control to suppress mosquito abundance to the point where tangential transmission of virus to humans is rare or unlikely. However, when these methods in concert fail to prevent enzootic amplification and the risk of human infection becomes eminent or is on-going, emergency adulticide applications of pyrethrin compounds are used to interrupt transmission. The efficacy of these applications has become controversial and some cities have opted to not apply adulticides. The current paper describes how a formula developed Dr. Mir Mulla some 40 years ago is still useful in solving contemporary problems of estimating percent control, a statistic useful in evaluating intervention efficacy. This simple but effective equation accounts for changes in both control and treated populations and thereby can be applied in dynamic situations where abundance is not stable. Examples are presented from ground and aerial experimental applications in Riverside County and from emergency interventions in Sacramento County in 2005 and Yolo County in 2006.

Keywords Mulla's formula · Mosquito control · Encephalitis · California

Introduction

The on-going West Nile virus (WNV) epidemic is the largest recorded mosquito-borne encephalitis virus outbreak ever recorded in North America, the largest WNV epidemic documented globally, and has become the leading cause of infectious

W.K. Reisen (✉)
Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
e-mail: arbo123@pacbell.net

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neurological disease during the current decade, with >24,000 laboratory confirmed equine and human cases reported (http://diseasemaps.usgs.gov/). In California alone, there have been 2,045 human cases and 53 deaths reported through 2006, and the outbreak has continued into 2007. The rapid invasion of the New World by this African virus occurred in a very short time period, despite the implementation of emergency control measures augmented by federal funding provided by the Centers for Disease Control and Prevention (CDC). WNV clearly has revealed the inability of the United States public health system to contain an invasive zoonosis (Holloway 2000).

WNV is maintained and amplified within a *Culex* mosquito and avian host cycle (Komar 2003), with tangential transmission to humans and equines which frequently develop serious disease (Hayes et al. 2005). The transmission cycle showing possible options for intervention is shown in Fig. 1. The primary intervention approach has been Integrated Vector Management (IVM). The removal of avian hosts such as American crows and House sparrows does not meet with conservationist as well as general public approval. Personal protection by either staying indoors in a mosquito free environment after dusk or by applying repellents when outdoors can reduce human cases, but does not affect enzootic transmission leaving those who refuse to alter their behavior at risk (Nasci et al. 2001). Similarly, equine vaccination prevents equine cases but does not alter enzootic transmission patterns, regardless of herd immunity levels (Nielsen et al. 2007b).

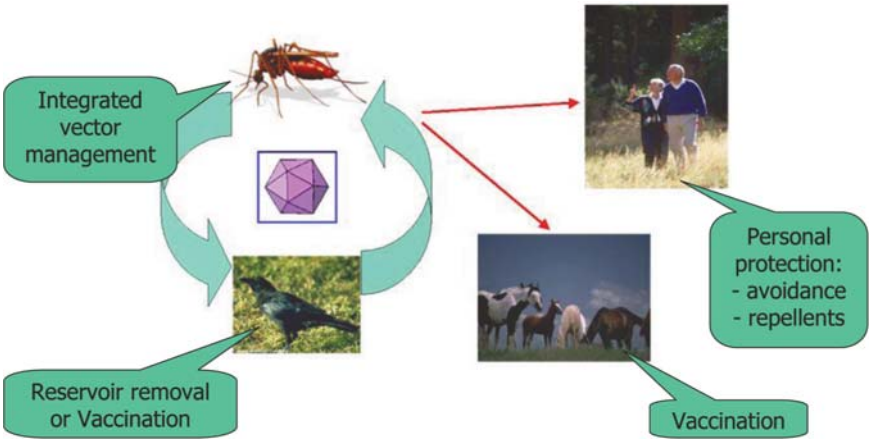

Fig. 1 Transmission cycle of West Nile virus, showing the various points for possible intervention

In response to various surveillance indicators (Kramer 2007), integrated vector management programs in California utilize an escalating cascade of suppression approaches in an attempt to maintain mosquito population size below thresholds where the risk of human infection is high (Fig. 2). Initially public education and larval control campaigns attempt to suppress populations through larval control,

an effort expected to suppress summer populations (Moon 1976). At this early time point, it is not known if viruses will be active, and surveillance is initiated to detect early season amplification. Once enzootic amplification exceeds detection thresholds, proactive control is initiated, including intensified larval control and adulticiding. If successful, virus amplification is suppressed and does not exceed epidemic thresholds (dashed line in Fig. 2). If unsuccessful, reactive epidemic management commences, usually entailing broad scale adulticiding using aircraft. If this fails and cases continue, then the only approach left is modification of human behavior or the application of effective repellents such as Deet. The time between detection of virus and the onset of human cases depends upon the rate of viral amplification and delineates the "window of opportunity" to implement control to protect the public. During hot midsummer in California when mosquito populations are large, this time "window" may be very short in duration.

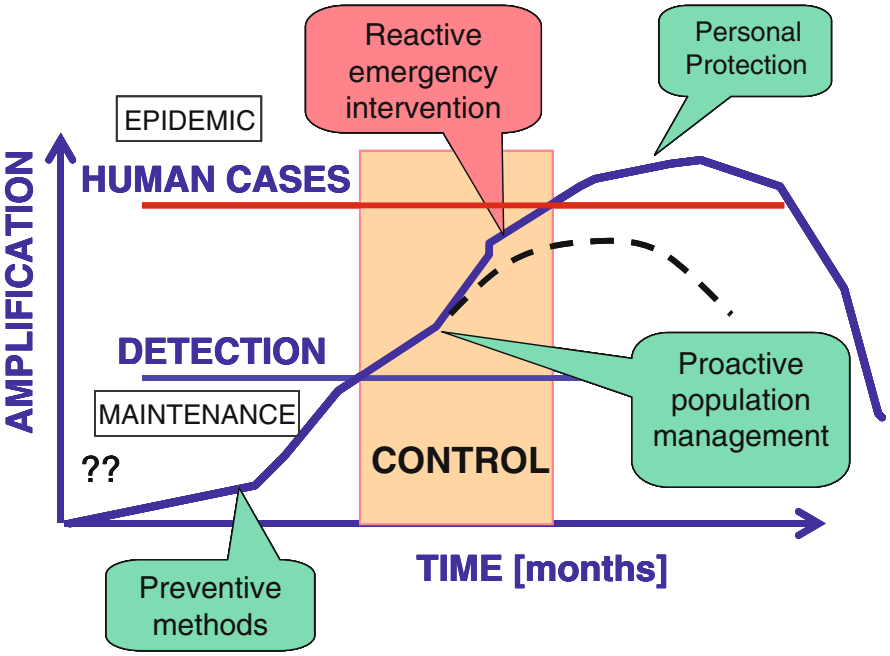

Fig. 2 Diagram depicting the surveillance and intervention paradigm during arbovirus seasonal amplification. *Dashed line* shows the attenuation of amplification following intervention, whereas the *solid line* show continued amplification above the threshold where human infection is likely

Recent outbreaks in California and the remainder of the US have triggered the widespread use of adulticides to interrupt epidemic transmission, in accordance with local (Kramer 2007) and national (Gubler et al. 2000) guidelines for arbovirus outbreak response. These applications have met with public resistance, especially in California, and concerns about pollution (Amweg et al. 2006), necessitating the

careful evaluation of application efficacy. To demonstrate the effectiveness of aerial applications on target mosquito populations, calculations must account for changes in abundance in sprayed target areas and unsprayed comparison areas during pre and post application periods. Four methods have appeared in the literature to analyze these types of data (http://www.ehabsoft.com/ldpline/onlinecontrol.htm); however, with the exception of the Henderson-Tilton formula (Henderson and Tilton 1955), these methods do not account for changes in both sprayed and unsprayed populations during pre and post spray periods. Confronted with the same difficulties in analyzing control of chironomid midges in recreational lakes (Mulla et al. 1971), Dr. Mir Mulla developed a simple, but effective, formula that accounts for changes in the abundance of the target species population in sprayed and unsprayed areas pre and post spray. The current paper given in honor of the long career of Dr. Mulla in vector control focuses on the utility of his formula in estimating percent control during experimental and epidemic adulticide applications in California, using examples taken from our recent research.

"Mulla's Formula"

In this formula, percent control or reduction (R) is calculated as:

$$\%R = 100 - \left[(C_1/T_1)\,x\,(T_2/C_2)\right] \times 100$$

where C_1 = pretreatment measure of target species abundance in unsprayed control area, C_2 = post treatment unsprayed control, T_1 = pretreatment treated or sprayed area, and T_2 = post treatment sprayed area. This formula is based on several basic assumptions:

1. Counts at individual traps are independent measures of relative abundance.
2. Ratio of abundance at traps in control and treated areas is consistent over time.
3. Changes in this ratio are due to treatment effects.

Although developed for estimates of insect larval abundance, this method of estimating percent reduction can be applied to any standardized measure in treated and control areas.

Examples

During April 2005 the Coachella Valley Mosquito and Vector Control District (CVMVCD) experimentally attempted to suppress *Culex tarsalis* abundance in a 1 m^2 area of the Coachella Valley in Riverside County, California, using three replicate ground applications of a ULV formulation of AquaReslin (Lothrop et al. 2007a). Relative abundance measured by 4–8 replicate traps within 5 strata was variable and significantly correlated over time among abundance measures within

strata in the treated area, but not with abundance measured within two unsprayed control strata (Fig. 3). When calculated using Mulla's formula, percent reduction or control was acceptable, ranging from 65 to 85%. However, even with this high level of estimated percent reduction, adult abundance rebounded rapidly and continued to increase post spray, most likely due to rapid recruitment by emergence or immigration.

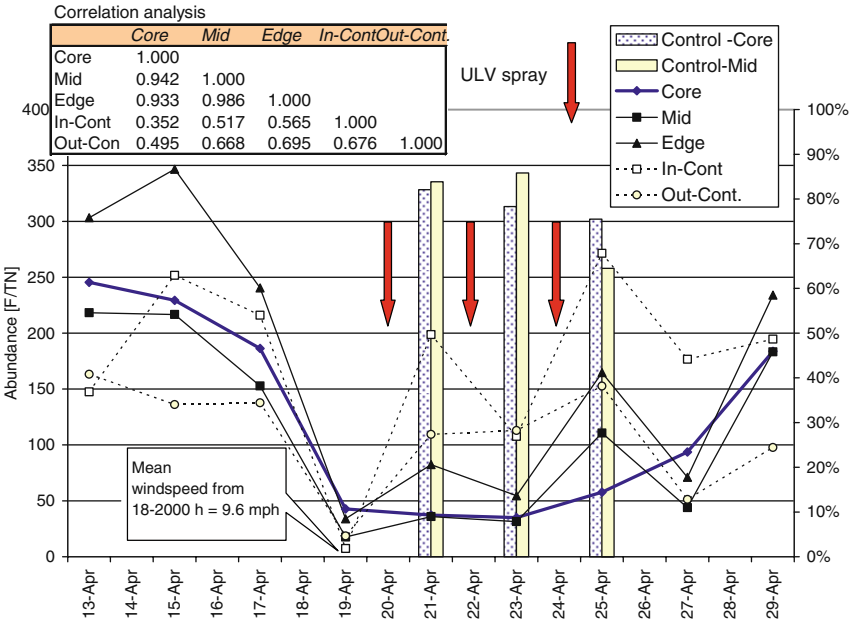

Fig. 3 Relative abundance of *Cx. tarsalis* at traps deployed within 5 strata in rural Coachella Valley during April 2005 showing the impact of replicated ground ULV application using AquaReslin. Core, mid and edge progress from the center to edge of the sprayed zone, whereas inner and outer controls were not sprayed. Shown are correlations among mean abundance per zone over time ($r > 0.67$ significant at $P = 0.05$, $df = 7$) and percent control estimated by Mulla's formula for traps in the core and mid zones compared with traps in the outer control zone. Data from Lothrop et al. (2007a)

During September 2005, a 1 mi^2 area of managed wetlands recently flooded for waterfowl was treated on three alternate nights by air using a ULV formulation of Pyrenone 25:5 mixed in a 1:2 formulation with BVA oil (Lothrop et al. 2007b). The *Cx. tarsalis* population in this area was rapidly increasing and abundance estimated within 4 of the 5 strata were significantly correlated (Fig. 4). When compared to abundance measured at outer control traps, percent reduction calculated for abundance in the core of the sprayed zone ranged from 2 to −120%, indicating that the spray did not effectively reduce abundance within the target area. This was unexpected, because kill of sentinel mosquitoes exposed in bioassay cages (Townzen and

Natvig 1973) was similar to the April 2005 ground application. These data emphasized the importance of measuring abundance in unsprayed control and sprayed areas as well as assessing spray efficacy using sentinels.

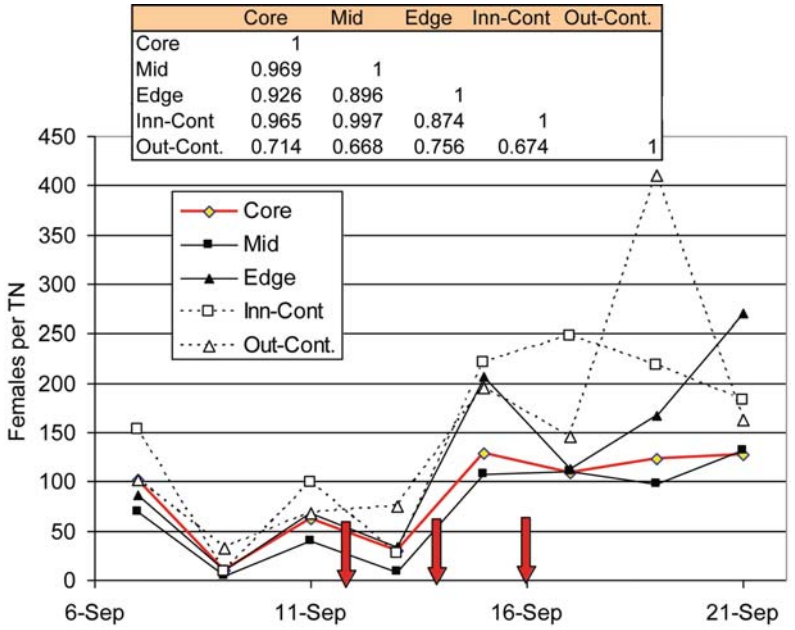

Fig. 4 Relative abundance of *Cx. tarsalis* at traps deployed within 5 strata in rural Coachella Valley during September 2005 showing the impact of replicated aerial ULV applications using Pyrenone 25:5 mixted 2:1 by volume in BVA oil. Core, mid and edge progress from the center to edge of the sprayed zone, whereas inner and outer controls were not sprayed. Shown are correlations among mean abundance per zone over time ($r > 0.71$ significant at $P = 0.05$, $df = 6$). Data from Lothrop et al. (2007b)

In July 2005, enzootic surveillance measures determined that epidemic WNV amplification was underway in Sacramento, California (Elnaimen et al. 2006). The distribution of the ensuing epiornitic was delineated by the locations of dead birds (mostly corvids, American crows, Western scrub-jays, Yellowbilled magpies) reported to the California Dead Bird Program (Fig. 5). In response to reports of the first indications of tangential transmission to humans within urban Sacramento, the Sacramento-Yolo MVCD treated 120,000 acres on 3 occasions by air using a ULV formulation of the pyrethrin compound EverGreen®. Measures of mosquito abundance, mosquito infection rates, reported dead birds and laboratory confirmed human cases during pre and post spray intervals in the northern Sacramento area were compared to comparable unsprayed areas using Mulla's formula (Fig. 6). Although the decrease in host-seeking *Culex* abundance was 76%, the associated minimum infection rate in *Culex* females decreased from 24 to 3 per 1,000 females tested. These data indicated that the adulticide application may have altered the population age structure and disproportionately eliminated the old infected females. In

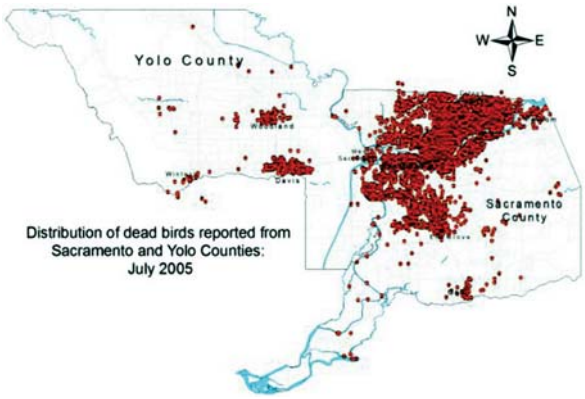

Fig. 5 Plot of reported dead birds in Sacramento and Yolo counties during July 2005. Data from Elnaimen et al. (2006)

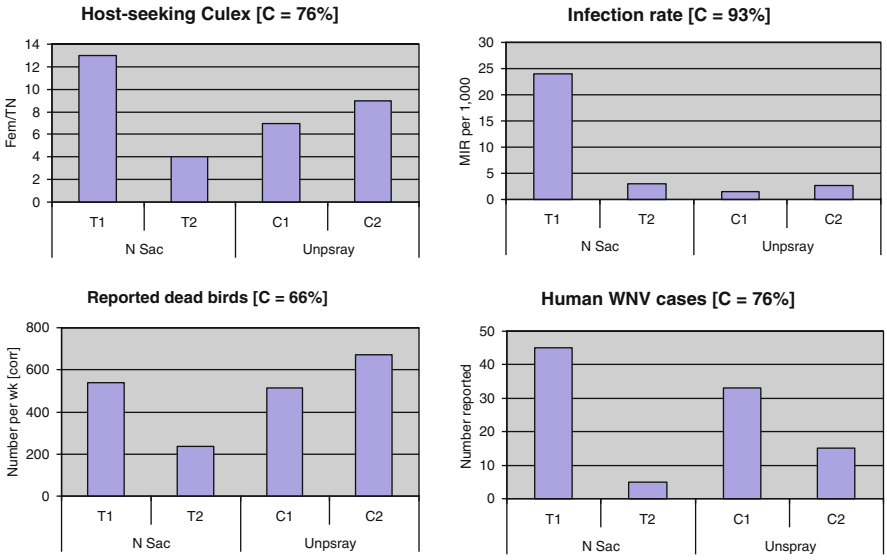

Fig. 6 Changes in host-seeking female *Culex per* trap night, female *Culex* infection rate per 1,000 tested, dead bird reports per week and human cases reported from north Sacramento sprayed and unsprayed control areas during pre and post spray period. Included are estimates of percent control using Mulla's formula. Data from unpublished reports by the Sacramento-Yolo MVCD

agreement, the numbers of dead birds reported by the public per week decreased from 537 to 235 in the sprayed zone, while the numbers remained similar in the control zone increasing slightly from 516 to 670 per week. In contrast to the dead bird reports, the numbers of human cases markedly declined in both sprayed and unsprayed areas; however, the rate of decrease in the sprayed area (45–5 cases) was disproportionately greater than in the control zone (33–15 cases), yielding a 76% reduction. Collectively, these data indicated that the marked reduction in infected *Culex* arrested both epiornitic and epidemic transmission.

During 2006 an outbreak consisting of 15 human cases occurred in Davis, California (Nielsen et al. 2007a). The rising numbers of laboratory confirmed WNV-infected dead birds and human cases during July resulted in the SYMVCD applying a ULV formulation of EverGreen by air on 8–9 Aug 06, even though the abundance of both *Cx. tarsalis* and *Cx. pipiens* were low at this time (Fig. 7). Efficacy assessed by sentinel cages was variable, ranging from 0% mortality for cages protected under canopy or wind shadows to 100% for cages positioned in the open. Percent reduction of the target population estimated by Mulla's formula was 26% for *Cx. tarsalis* questing at dry ice baited traps and 75% for *Cx. pipiens* collected by gravid female traps. Differences in percent reduction were attributed to mosquito distribution within Davis, with *Cx. tarsalis* immigrating from larval sources in the surrounding agroecosystem and *Cx. pipiens* emerging from sources in peridomestic habitats within Davis. The spray did appear to interrupt the outbreak, because there was only a single human case, one WNV positive dead bird report and only one positive *Cx. pipiens* pool documented after August 20th. Termination of the epidemic most likely also was enabled by the onset of cool weather, where the minimum temperatures remained below 14°C for the remainder of theyear.

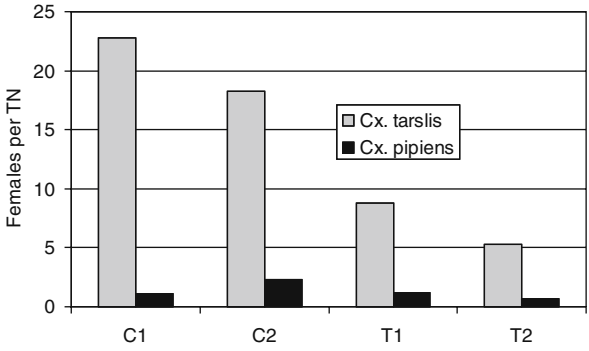

Fig. 7 Changes in mean numbers of *Cx. tarsalis* at dry ice baited traps and *Cx. pipiens* at gravid traps per night in sprayed treated zone (T) and unsprayed control zone (C) during pre (1) and post (2) spray. Data from Nielsen et al. (2007a)

Summary

Evaluation of the impact of ULV adulticiding on target mosquito populations has proven to be difficult and confounded by several factors.

- Treatment and control sites are not always independent. For example, in Coachella Valley unsprayed control traps frequently varied in a similar fashion to traps within the treated spray zones, indicating that the impact of the spray was minimal or that the spray impacted the general population in the area. The latter would seem to be the case based on the assessment of widespread control applications, such as was done in Sacramento during 2005.

- Treatment and control sites are affected differently. For example, in Davis *Cx. tarsalis* were produced in rural agricultural sources out of the spray zone, immigrated into the city, were most abundant at peripheral monitoring sites, and were minimally impacted by aerial spray over Davis. In contrast, *Cx. pipiens* were produced within the city and control of this population was similar to that observed in Sacramento during 2005.
- Weather. Variation in weather has a major impact on the application processes as well as the evaluation. In the desert habitats in Coachella Valley, hot and very dry conditions required us to formulate adulticides using BVA oil to ensure that droplets would descend from air craft and kill sentinel mosquitoes at ground level (Lothrop et al. 2007b). In addition, heat radiation off the ground can drive ground applications upwards and out of the target area. In Davis, cool weather following the aerial application most likely contributed to the termination of virus activity (Nielsen et al. 2007a).
- Avian herd immunity. Although minimally discussed in the current paper, the increase in the seroprevalence rate within the peridomestic passerine community appears to markedly dampen virus transmission. Interestingly, the 2004 WNV epidemic in Los Angeles appeared to end abruptly in September, even though no emergency adulticiding was done. Here, as soon as passerine herd immunity exceeded 25%, the epidemic ended and few further human cases or dead birds were detected (Wilson et al. 2005). Considering the elevated mortality among experimentally infected House finches and House sparrows (Komar et al. 2003; Reisen et al. 2005), 25% seroprevalence indicates that there was considerable associated depopulation and that more than 80% of the population may have been infected.
- Diapause. During the fall after the critical photoperiod for the induction of diapause, *Cx. tarsalis* (Nelson 1964; Reisen et al. 1986) and presumably *Cx. pipiens* populations, bifurcate into older parous host-seeking females not destined for diapause and non-host-seeking females destined for winter diapause. Therefore, sampling late season populations measured by dry ice baited traps will naturally decrease in abundance regardless of control efficacy.

The evaluation of intervention will remain difficult because of the volatility of mosquito population dynamics and the interplay of multiple interacting and confounding factors. Mulla's formula is useful, because it accounts for mosquito population dynamics in both sprayed and unsprayed areas, pre and post spray, is easy to use and can be applied to other quantifiable measures such as infection rates, dead bird and counts of human cases. The formula may be useful in evaluating surveillance measures providing the control sites are similar to and independent from the treated sites.

Acknowledgements I especially thank Hugh and Branka Lothrop, Donald Gomsi and the staff of the Coachella Valley MVCD who conducted the ground and aerial evaluation studies in Coachella Valley, Dave Brown and staff at the Sacramento-Yolo MVCD who applied and evaluated the emergency applications done in Sacramento and Davis. Carrie Nielsen participated in the evaluation of the Davis application. This research was funded, in part, by grants from the National Institutes of Allergy and Infectious Diseases, NIH, and the Coachella Valley and Sacramento-Yolo MVCDs.

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Longitudinal Field Studies Will Guide a Paradigm Shift in Dengue Prevention

Thomas W. Scott and Amy C. Morrison

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

T.W. Scott (✉)
Department of Entomology, University of California, Davis, CA 95616, USA
e-mail: twscott@ucdavis.edu

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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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Recombinant Bacterial Larvicides for Control of Important Mosquito Vectors of Disease

Brian A. Federici

Abstract The development of recombinant DNA techniques has made it possible to significantly improve the efficacy of *Bacillus thuringiensis* subsp. *israelensis* (Bti) currently used to control larvae of important mosquito disease vectors. As part of this symposium honoring the career of Professor Mir S. Mulla, I briefly review here advances we have made using recombinant DNA technology to combine the most potent insecticidal proteins from Bti, *B. thuringiensis* subsp. *jegathesan* (Btj), and *B. sphaericus* (Bs) into new bacterial strains that are ten-fold more toxic than wild type species of Bti and Bs used in current commercial formulations. These advances have been achieved by combining new knowledge derived from basic studies of the molecular biology and genomics of these bacteria with technical developments that enable increases in the synthesis of mosquitocidal proteins produced per unit of fermentation medium. Several bacterial strains have been constructed that have LC_{50}s in the range of 1.5–2 ng/ml of fermentation solids against fourth instars of *Culex quinquefasciatus* and *Anopheles gambiae*, compared to LC_{50}s of 13–20 ng/ml for the commercial wild type strains. These new bacterial larvicides, which are currently under commercial evaluation, offer environmentally compatible options for use as components in integrated vector control programs aimed at reducing malaria, filariasis, and many important viral diseases of humans.

Keywords Recombinant bacterial larvicides · Bacterial endotoxins · Mosquito control · Vector control · Filariasis · Malaria · Viral diseases · *Anopheles gambiae* · *Culex pipiens quinquefasciatus* · *Bacillus thuringiensis* subsp. *israelensis* · *Bacillus sphaericus*

B.A. Federici (✉)
Department of Entomology and Interdepartmental Graduate Program in Cell, Molecular and Developmental Biology, University of California, Riverside, CA 92521, USA
e-mail: brian.federici@ucr.edu

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Introduction

Mosquitoes vector the etiologic agents of many of the most important diseases of humans, including malaria, filiariasis, and Dengue Fever. From the late 1940s until the 1980s, these diseases were effectively controlled in many countries by using synthetic chemical insecticides such as DDT, parathion, and pyrethroids to suppress populations of larval and adult mosquitoes. During the late 1980s, as a result of the evolution of insecticide resistance, a reduction in vector control programs, and curtailment of chemical insecticide usage owing to their detrimental environmental effects, these diseases began to re-emerge and significantly impact human populations again, especially in sub-Saharan Africa and southeast Asia. To overcome the resurgence of these important diseases, various national and international health agencies and private foundations mounted research programs over the past two decades aimed at developing new tools to control these diseases, with a focus on vaccines, new anti-malarial drugs, new insecticides, and novel genetic approaches to disease transmission such as the development of transgenic mosquitoes refractory to the pathogens that cause these diseases. While all these strategies show promise for the development of long-term disease suppression, none will be available for use on a large scale for at least another decade. For possible use in the near term, we have focused on research efforts on using recombinant DNA techniques to develop improved bacterial insecticides for controlling the larval stages of the most important vectors of these diseases, with emphasis on controlling species of *Culex* and *Anopheles* mosquitoes.

Commercial bacterial larvicides for mosquito control have been in common use in many developed countries for more than a decade. Current products, however, are too expensive for widescale use in developing countries. In addition, until recently, bacterial larvicides were not considered viable alternatives to chemical insecticides in sub-Saharan Africa because the breeding areas are so extensive, again leading to unacceptable costs for effective vector control. However, recent studies in Kenya have shown that at least in some areas, biting rates by *Anopheles gambiae* can be reduced by more than 90% by using a combination of existing commercial formulations of *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *B. sphaericus* (Bs) (Filinger and Lindsay 2000). The relatively high cost of producing bacteria-based products in comparison to chemical insecticides is due in part to the cost of fermentation. Thus, over the past decade we focused our efforts on basic and applied research aimed at improving the potency of bacterial larvicides by increasing the insecticidal efficacy per unit of fermentation medium. Here I summarize the key results of our studies showing how we were able to use basic discoveries to recombine the most effective mosquitocidal proteins to develop bacterial strains based on several *Bacillus* species that are ten-fold more effective than existing wild type strains used in commercial formulations. These recombinant bacteria are currently under evaluation for use against a range of vector species. If proven effective under field conditions, they should be useful as components of integrated vector control programs that target reductions of various *Culex* and *Anopheles* species including those of the *Cx. pipiens* and *An. gambiae* species complexes.

Basic Biology and Mode of Action of Bt and Bti

The insecticidal bacterium, *Bacillus thuringiensis* (Bt), has been the most successful commercial microbial insecticide, and also has been the subject of the overwhelming majority of genetic engineering studies to improve efficacy. Until recently, the first subspecies discovered that was highly toxic to mosquitoes, *B. thuringiensis* subsp. *israelensis* (Bti) was the only subspecies commercialized for mosquito control. But a few years ago, Valent BioSciences (Libertyville, Illinois, USA) commercialized the 2362 strain of *B. sphaericus* (Bs), another mosquitocidal bacterium, as it was more effective against the larvae of certain *Culex* and anopheline species, especially those breeding in polluted water. Below is a brief background on insecticidal bacilli that provides information on their biology and how these bacteria kill insects.

Bt is actually a complex of bacterial subspecies that occur commonly in such habitats as soil, leaf litter, on the surfaces of leaves, in insect feces, and as a part of the flora in the midguts of many insect species (Glare and O'Callaghan 2000; Federici 1999). Bts are characterized by the production of a parasporal body during sporulation that contains one or more protein endotoxins in a crystalline form (Fig. 1). Many of these are highly insecticidal to certain insect species. Bt endotoxins are actually protoxins activated by proteolytic cleavage in the insect midgut

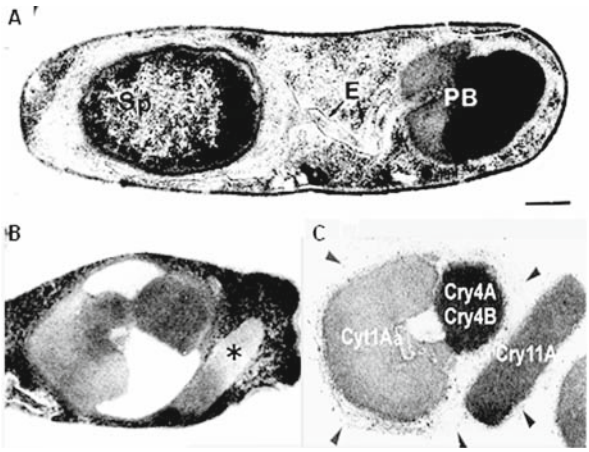

Fig. 1 Sporulating cell of *Bacillus thuringiensis* subspecies *israelensis* and parasporal bodies characteristic of this subspecies as revealed by transmission electron microscopy. (**a**) Sporulating cell illustrating the developing spore (Sp) and parasporal body. The parasporal body (PB), composed primarily of Cry4A, Cry4B, Cry11A, and Cyt1A proteins, is assembled outside the exosporium membrane (E). (**b**) Portion of sporulating cell just prior to lysis. The Cry11A crystal (*) lies adjacent to the Cyt1A and Cry4A and Cry4B inclusions. (**c**) Purified parasproal body showing the components of the parasporal body. In this subspecies, the individual protein inclusions are enveloped in a multilamellar fibrous matrix (*arrowheads*) of unknown composition, which also surrounds the crystals holding them together. A typical mature parasporal body of this subspecies measures 500–700 nm in diameter. Bar in (**a**) = 100 nm

after ingestion. The activated toxins destroy midgut epithelial cells, killing susceptible insects within a day or two of ingestion. In insect species only moderately sensitive to the toxins, such as *Spodoptera* species (caterpillars commonly known as armyworms), the spore contributes to pathogenesis by germinating and producing vegetative insecticidal proteins (Vips), proteases and phospholipases. Bt also produces other insecticidal compounds including β-exotoxin and Zwittermicin A. Some of these are synergistic, and thus their combined actions often result in death of recalcitrant lepidopteran species. The most widely used Bt is the HD1 isolate of *B. thuringiensis* subsp. *kurstaki* (Btk), an isolate that produces four major endotoxin proteins, Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa packaged into two crystalline parasporal body. The three Cry1 proteins co-crystallize forming a bipyramidal crystal, whereas Cry2Aa forms a separate cuboidal crystal. This isolate is used as the active ingredient in numerous commercially available bacterial insecticides (Dipel, Foray, Thuricide) used to control many lepidopteran pests in field and vegetable crops, and forests (Schnepf et al. 1998). Bti, on the other hand, is only highly toxic to the larvae of mosquito and blackfly species. This subspecies produces a parasporal body that contains four major endotoxins, Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa. The three Cry proteins are related to those of Btk, but have insect spectra limited to mosquitoes, black flies, and related dipterans species. Cyt proteins are unrelated to Cry protein.

As noted above, Cry and Cyt proteins are actually protoxins that must be ingested and processed by midgut enzymes to yield active toxins. Most have evolved to dissolve from the environmentally stable endotoxin crystals and be activated under the alkaline conditions, pH 8–10, that are, characteristic of the midgut lumen of caterpillars and mosquito larvae. Once activated, Cry molecules bind to glycoprotein or glycolipid receptors. In general, the former are aminopeptidases, alkaline phosphatases, or cadherins on the midgut epithelial cell microvilli. Toxin molecules oligomerize and insert into the membrane causing cell lysis. Cyt proteins are thought to have a similar mode of action, with the exception that they do not require protein receptors for binding. Instead, they bind directly to the microvillar lipid bilayer.

The underlying hypothesis for Cry and Cyt protein mode of action is known as colloid-osmotic lysis. Toxin oligomers are thought to form cation-selective pores that cause an influx of cations, especially K, into the cell. The cell then takes in water, compensating for the cation influx to maintain ionic balance, and subsequently swells and lyses. The actual cause of larval death is not known, but is thought to be nerve paralysis that results from a rise in blood pH due to the inflow of alkaline midgut juices into the hemolymph. While this is the current paradigm, there is some evidence that neither toxin type forms cationic pores. If fact, evidence is quite strong that Cyt proteins act as membrane detergents.

Of significance to understanding Bti's mode of action is that the Cyt protein synergizes the toxicity of the Bti Cry proteins, and can delay the phenotypic expression of the evolution of resistance to these. Synergistic interactions are also known to occur between the Bti Cry proteins, but the most important interactions are between the Cyt protein and Cry proteins. The Cyt protein, when combined with the Bs Bin toxin (see below), can also overcome resistance to the latter. The lipid affinity

of CytA and its ability to cause lesions in the microvillar lipid bilayer are responsible for the important properties. It is worth noting that no significant resistance under field conditions has been reported in mosquito populations despite decades of use, and this is likely due in part to the unique biochemical properties of Bti's Cyt protein.

Several commercial products based on Bti are available and are used to control both nuisance and vector mosquitoes and black flies. Formulations of Bti (Vecto-Bac, Teknar) proved particularly important in the World Health Organization's Onchocerciasis Control Program in West Africa, were they were used during the 1980s and 1990s in rotation with the chemical insecticide, temiphos, to virtually eliminate larval populations of the black fly vector, *Simulium damnosum*, of the filarial worm that causes this disease.

Basic Biology and Mode of Action of *Bacillus sphaericus* (Bs)

Since the mid-1960s it has been known that many isolates of Bs are toxic to mosquito species. Over the past three decades, three isolates have been evaluated for mosquito control, 1593 from Indonesia, 2297 from Sri Lanka, and 2362 from Nigeria (Charles et al. 1996). The 1593 and 2297 isolates were obtained from soil and water samples at mosquito breeding sites, whereas 2362 was isolated from a dead adult black fly.

The toxicity of Bs, like Bt, is the result of protein endotoxins that are produced during sporulation and assembled into a parasporal body. Bs is unusual in that the main toxin is a binary toxin, i.e., composed of two protein subunits (BinA and BinB). These are proteolytically activated in the mosquito midgut to release peptides of, respectively, 43 and 39 kDa, that associate to form the binary toxin, with the former protein constituting the binding domain, and the latter the toxin domain. The toxins bind to microvilli of the midgut epithelium, causing hypertrophy and lysis of cells, destroying the midgut and killing the mosquito larva. Exactly how the Bin toxin kills cells is not known, although researchers in France have shown that the principal receptor is an alpha-glucosidase tethered to the microvillar membrane. For many years, it has also been known that the Bin toxin must be internalized by the cell to exert toxicity, and as a result, full toxicity if typically not apparent until 48 h after treatment of larvae with Bs preparations.

A commercial product known as VectoLex (Valent BioSciences, Libertyville, Illinois) was registered recently for control of *Culex* larvae, and certain species of *Anopheles* mosquitoes. Corresponding strains have also been developed for control of *Culex* and anopheline mosquitoes in China and other countries.

Current Usage

Bti and Bs formulations are used as bacterial insecticides, i.e., applied as needed, and are available in a variety of formulations including emulsifiable concentrates, wettable powders, and granules for use against a wide range of pest and vector

mosquitoes breeding in different habitats. On a worldwide basis, millions of hectares are treated annually with products based on Bti and Bs.

Genetic Regulation of Cry and Cyt Protein Synthesis

The principal genetic factors controlling the yield of endotoxin synthesis in Bt are promoters, a 5′ mRNA stabilizing sequence and 3′ transcriptional termination sequences. The relative stability of each endotoxin is also a factor that affects yield.

With respect to promoters, Bt endotoxin synthesis is typically under the control of two strong sporulation-dependent promoters, BtI and BtII. BtI is transcribed by sigma-35 complexed with the RNA polymerase, whereas BtII transcription is regulated by sigma-28. While this is the typical state for Bt promoters, Cry3A synthesis is under the control of a weak promoter active during vegetative growth. Moderate levels of Cry3A synthesis occur in the bacterium due to the presence of a mRNA stabilizing sequence of 9 nucleotides referred to STAB-SD present in the 5′ region of the *cry3A* transcript (de Maagd et al. 2003; Agaisse and Lereclus 1996). Endotoxin synthesis can be increased by as much as 10-fold when this sequence is spliced into expression constructs for many proteins, and placed under the control of Bt sporulation-dependent promoters (Park et al. 1998; Federici et al. 2003; Park et al. 2005). The 3′ terminus non-coding terminus of most Bt genes contains a stem-loop structure that acts as a transcription terminator, but these structures also stabilize the transcript, apparently by retarding 3′ exonuclease degradation. This extends transcript half-life, leading to higher endotoxin synthesis than would occur in the absence of these terminators.

Several other factors enhance synthesis of Bt endotoxins during or after translation. For example, a 20-kDa protein encoded as the third ORF of the *cry11A* operon enhances net synthesis of many endotoxins, apparently acting as a chaperone. A 29-kDa protein encoded by the *cry2Aa* operon facilitates crystallization, and therefore yield of Cry2A. Lastly, different endotoxin proteins vary in their stability, some, such as Cry3A are much more stable than others, for example, than Cry4A (Park et al. 1998; Federici et al. 2003; Park et al. 2005). Generally, the more stable a protein, the higher the yield when these are synthesized at high levels using expression vectors.

Construction of Recombinant Bacterial Larvicides

The most common strategy for constructing recombinant Bt strains is using a shuttle expression vector that contains replication origins for both *B. thuringiensis* and *E. coli*, a multiple cloning site, and genes for antibiotic resistance, for example to ampicillin and erythromycin for easy selection of transformants. A shuttle vector such as pHT3101 containing a gene of interest is amplified in *E. coli*, isolated, and then introduced into a candidate Bt strain by electroporation.

In many cases, *cry* and *cyt* genes of *B. thuringiensis* inserted into shuttle vectors were expressed under the control of their own promoters, which generally results in a high yield of the encoded protein. In terms of promoter strength, *cyt1A* promoters are among the strongest known among *cry* and *cyt* genes. In addition, as mentioned above, the *cry3A* upstream 5′ mRNA stabilizing sequence (STAB-SD) improves stability of *cry3A* transcripts and concomitantly the yield of certain Cry proteins. To optimize Cry protein yields in Bt, a recombinant expression vector, pSTAB was developed. This vector was constructed by inserting the 660-bp DNA fragment containing *cyt1A* promoters combined with the STAB-SD sequence into the multi-cloning site of pHT3101.

Using the p*cyt1A*/STAB expression vector, which combined these different genetic elements, it was possible to significantly increase yields of several Cry proteins. For example, by expressing the *cry3A* gene using this vector, yields twelve-fold greater than those obtained with the wild type strain of *B. thuringiensis* subsp. *morrisoni* (isolate DSM2803) from which this gene was cloned were obtained. Cry3A yield obtained per unit medium using *cyt1A* promoters alone, i.e., lacking the STAB-SD sequence, was only about two-fold higher than that of the wild-type DSM280 strain. This demonstrates that most of the enhancement was due to inclusion of the STAB-SD sequence (Park et al. 1998).

The significant increase in Cry3A yield obtained using *cyt1A* promoters combined with the STAB-SD sequence led to tests of this expression vector for enhancing synthesis of other Bt endotoxins. The level of enhancement using this expression system varied depending upon the candidate protein. For example, yields of Cry11B and the Bs binary toxin were increased substantially, as much as eight-fold (Fig. 2), whereas yields of proteins such as Cry11A and Cry2A increased only 1.5–2-fold.

Perhaps the best example of the successful use of *cyt1A* promoters combined with STAB-SD comes from engineering recombinant Bti strains. This vector was used to produce several different recombinant strains that vary in complexity, ranging from a strain that produces only a single endotoxin to strains that produce as many as five endotoxins. In the simplest case, p*cyt1A*/STAB was used to synthesize the Bin toxin of Bs 2362. Using this construct, Bin synthesis was eight-fold higher than that obtained with wild type Bs 2362. The toxicity of this strain was 13-fold better than wild type Bs against *Culex* species (Federici et al. 2003; Park et al. 2005).

To improve toxicity while at the same time preventing or delaying the evolution of resistance, several strains were constructed in which toxin complexity was increased and Cyt1A was added for resistance management. One strain constructed using this strategy was a recombinant that synthesized the Bin toxin, Cyt1A and Cry11B. In this recombinant, the mosquitocidal proteins were from three different species; Bin from Bs 2362, Cry11B – a protein more toxic than Cry11A – from Bt subsp. *jegathesan*, and Cyt1A from Bti. This recombinant was constructed using a dual-plasmid expression system with two different plasmids, each with a different antibiotic resistance gene for selection. The resulting recombinant *B. thuringiensis* produced three distinct crystals and was 3–5 times as toxic to *Culex* species as either Bti IPS-82 or Bs 2362 (Fig. 3).

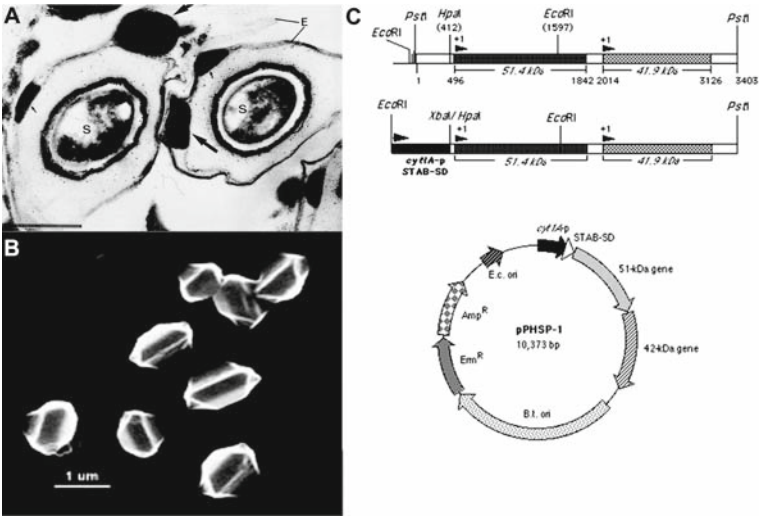

Fig. 2 Cloning of the *Bacillus sphaericus* binary toxin operon followed by engineering the operon into a shuttle expression vector designed to synthesize the binary toxin under the control of the three wild type tandem *cyt1A* promoters combined with the STAB-SD sequence. (**a**) Electron micrograph of sporulated cells showing (*arrows*) crystals of the binary toxin on the internal side of the exosporium membrane. (**b**) Scanning electron micrograph of purified binary toxin crystals synthezied in an acrystalliferous strain of *B. thuringiensis* subsp. *israelensis* using the *E. coli-B. thuringiensis* shuttle vector shown in (**c**) (*circular vector map*). The *top portion* of (**c**) shows the structure of the *B. sphaericus* operon (*top*) and the engineered operon in which expression is driven by *cyt1A* pomoters. Bars in (**a**) and (**b**) equal approximately 1 mm. Micrograph in (**a**) courtesy of Dr. Jean-Francois Charles of the Institut Pasteur, Paris, France

To construct a recombinant with an even greater range of endotoxins for both increased toxicity and resistance management, the IPS-82 strain of Bti, which produces the complement of toxins characteristic of this species, was transformed with pPHSP-1, the p*cyt1A*/STAB plasmid that produces a high level of Bs Bin toxin (Fig. 4) (Federici et al. 2003; Park et al. 2005). This recombinant was more than ten-fold more toxic than either of the parental strains to larvae of *Cx. quinquefasciatus* and *Cx. tarsalis* (Table 1). Aside from high efficacy, as noted above, this new bacterium is much less likely to select for resistance in target populations, as it combines Cyt1A from Bti, which delays the evolution of resistance to mosquitocidal Cry proteins and Bs Bin, with Bti Cry toxins and Bs Bin. The resistance management properties of this bacterium are currently under evaluation.

The markedly improved efficacy and resistance-delaying properties of this new bacterium make it an excellent candidate for development and use in vector control programs, especially to control *Culex* vectors of West Nile and other viruses as well as species of this genus that transmit filarial diseases. Moreover, larvae of *An. gambiae*, the key vector of malaria in many regions of Africa, have recently been show to be as sensitive to this recombinant as *Culex* mosquitoes (Federici et al., unpublished data). This is because of the high sensitivity of this species to the Bs

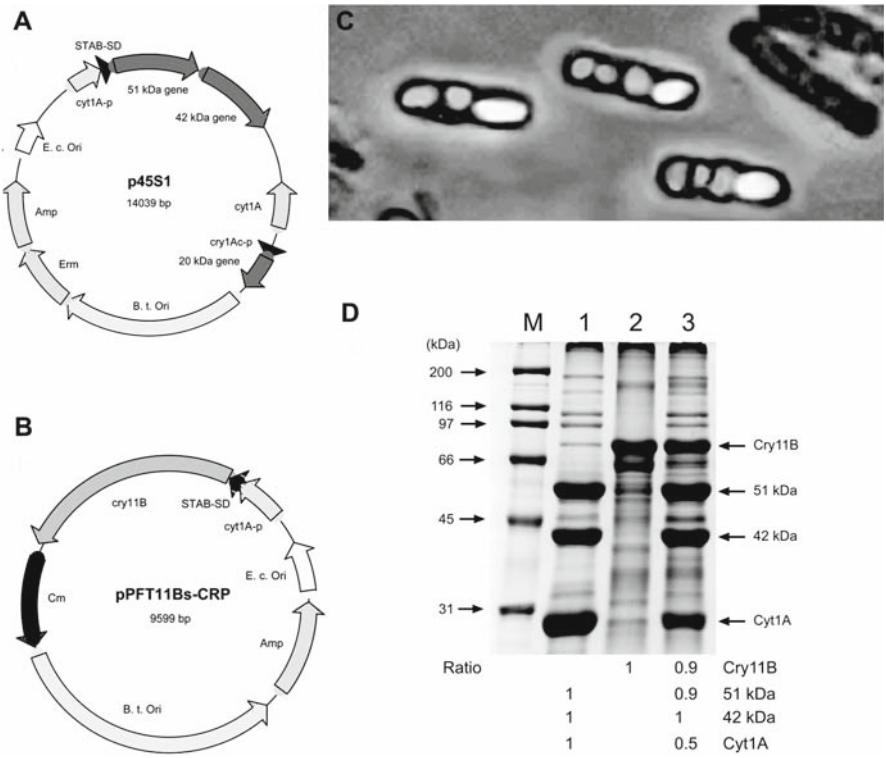

Fig. 3 Construction de novo of a unique mosquitocidal strain of *B. thuringiensis* that produces individual endotoxin crystals of the Bs Bin binary toxin, Cyt1A, and Cry11B. (**a**) Vector bearing the *cyt1A* and *B. sphaericus bin* genes. (**b**) Vector bearing the *cry11B* gene from *B. thuringiensis* subsp. *jegathesan*. These were transformed successively into the 4Q7 acrystalliferous strain of *B. thuringiensis* subsp. *israelensis* to yield the final recombinant strain that produces Bs Bin, Cyt1A, and Cry11B. (**c**) Phase-contrast micrograph of sporulated recombinant cells of *B. thuringiensis* 4Q7 containing individual crystals of Cyt1A, Cry11B, and the *B. sphaericus* binary toxin. The spore is on the right in each of these cells. (**d**) Analysis of endotoxin content in wild type and recombinant strains of *B. thuringiensis*. M, molecular size marker; lane 1, *B. thuringiensis* subsp. *israelensis* 4Q7 producing *B. sphaericus* binary toxin and Cyt1A (4Q7/p45S1); lane 2, *B. thuringiensis* subsp. *israelensis* 4Q7 producing Cry11B (4Q7/pPFT11Bs-CRP); lane 3, *B. thuringiensis* subsp. *israelensis* 4Q7 producing Cry11B, Cyt1A, and *B. sphaericus* binary toxin (4Q7/p45S1-11B). The numbers at the base of lane 3 indicate the approximate ratio of each toxin produced in the Cry11B, Cyt1A, Bin recombinant in comparison to, respectively, the Cyt1A plus Bin recombinant (lane 1) and the Cry11B recombinant (lane 2). Equal amounts of culture medium were loaded in each well. Bar in (**c**) = 1 mm

Bin toxin. This indicates this Bti/Bs recombinant should also be more cost-effective against other species of anopheline larvae sensitive to Bs Bin than the insecticides based on the wild type parental strains of these bacterial species.

The improvements in activity noted above result from the increase in toxicity per unit weight of fermentation medium. These increases significantly reduce production costs for obtaining the same level of pest or vector control. The extent to which

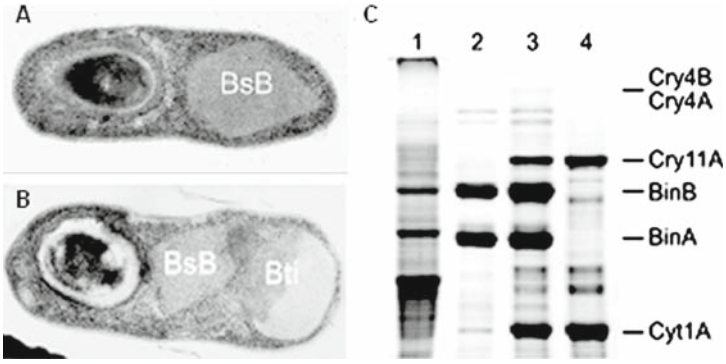

Fig. 4 Synthesis of *Bacillus sphaericus* 2362 binary toxin in *B. thuringiensis* subsp. *israelensis*. (**a**) Transmission electron micrograph of the recombinant strain Bti4Q7/BsB, engineered to synthesize only the *B. sphaericus* binary toxin. A single large crystal (BsB) is adjacent to the spore. (**b**) Transmission electron micrograph of the recombinant crystalliferous strain, BtiIPS-82/BsB, engineered to synthesize the *B. sphaericus* binary toxin in a wild type strain of *Bacillus thuringiensis* subsp. *israelensis*. This strain produces the typical IPS-82 parasporal body (Bti) and Bs 2362 binary toxin crystal (BsB). (**c**) Comparative endotoxin yields produced per unit medium by wild type *B. sphaericus* strain 2362, and wild type and engineered strains of *B. thuringiensis* subsp. *israelensis* constructed to synthesize the Bs 2362 binary toxin. Lanes: 1, wild type control *B. sphaericus* 2362 strain; 2, recombinant Bti4Q7 strain, Bti4Q7/BsB, engineered to produce Bs 2362 toxin; 3, recombinant BtiIPS-82 strain, BtiIPS-82/BsB, that produces the Bs 2362 toxin and typical Bti parasporal body; 4, wild type control Bti IPS-82 strain

Table 1 Potency of *Bacillus thuringiensis* susbsp. *israelensis* recombinants to fourth instars of *Culex quinquefasciatus*

		Improvement ratio	
Bacterial strain	LC_{50} (ng/ml)[a]	Bti IPS82 + Bs Bin versus Bti IPS82	Bt4Q7 + Bin versus Bs 2362
Bti IPS82[b] (Wild Type)	19.5	1.0	
Bs 2362[c] (Wild Type)	15.0		1.0
Bti 4Q7[d] + Bs Bin	1.4		10.0
Bti IPS82 + Bs Bin	1.5	13.0	

[a]All values have been rounded off for clarity of presentation – detailed values are presented in Park et al. (2005) and other related references.
[b]Bti IPS 82 is a wild type strain that produces all four major mosquitocidal proteins characteristic of *B. thuringiensis* subsp. *israelensis*. Commercial products contain derivatives of this strain.
[c]Bs 2362 is a wild type strain that produces the Bin toxin during sporulation as well as several Mtx during vegetative growth.
[d]Bti 4Q7 is an acrystalliferous strain derived from Bti by plasmid curing. It produces none of the major mosquitocidal proteins produced by wild type Bti.

these savings are potentially passed along to consumers, as opposed to being used to increase company profits has not been determined, as the recombinant strains discussed have not yet been commercialized.

Safety Concerns About Wild Type and Recombinant Bacterial Insecticides

In determining what types of tests should be done to evaluate the safety of bacterial insecticides, early tests were based primarily on those used to evaluate chemical insecticides. However, the tests have evolved over the decades and are now designed to evaluate the risks of Bt, specifically the infectivity of the bacteria and toxicological properties of proteins used as active ingredients. The tests are grouped into three tiers, I–III (Betz et al. 1990). Tier I consists of a series of tests aimed primarily at determining whether an isolate of a Bt subspecies, as the unformulated material, poses a risk if used at high levels, typically at least 100 times the amount recommended for field use, to different classes of non-target organisms. The principal tests include acute oral, acute pulmonary (inhalation), and acute intraperitoneal evaluations of the material against different vertebrate species, with durations from a week to more than a month, the length depending on the organism. In the most critical tests, the mammals are fed, injected with, and forced to inhale millions of Bt cells in a vegetative or sporulated form. Against invertebrates, the tests are primarily feeding and contact studies. Representative non-target vertebrates and invertebrates include mice, rats, rabbits, guinea pigs, various bird species, fish, predatory and parasitic insects, beneficial insects such as the honeybee, aquatic and marine invertebrates, and plants. If there is clear infectivity or acute toxicity in any of these tests, then the candidate bacterium would be rejected. If uncertainty exists, then Tier II tests must be conducted. These tests are similar to those of Tier I, but require multiple consecutive exposures, especially to organisms where there was evidence of toxicity or infectivity in the Tier I tests, as well as tests to determine if and when the bacterium was cleared from non-target tissues. If infectivity, toxicity, mutagenicity, or teratogenicity is detected, then Tier III tests must be undertaken. These consist of tests such as two-year feeding studies and additional testing of teratogenicity and mutagenicity. The tests can be tailored to further evaluate the hazard based on the organisms in which hazards were detected in the Tier I and II tests. It must be realized that the tests for recombinant bacteria will likely be more strict than those used to register many synthetic chemical insecticides on the market, even though many of the latter are known to be toxic to non-target invertebrates, as well as vertebrates such as fish and humans, especially if not used properly.

To date, not a single registered Bt insecticides based on Cry or Cyt proteins has had to undergo Tier II testing (Fillinger and Lindsay 2000; Betz et al. 1990; Federici 2003). In other words, no moderate or significant hazards or risks have been detected with any Bt subspecies used commercially or any Bt crop against

any of the non-target organisms studied, including mammals. As a result, all Bt insecticides (and Bt crops) registered in the US are exempted from a tolerance requirement, i.e., a specific level of insecticide residue allowed on a crop just prior to harvest. Moreover, no washing or other requirements to reduce levels consumed by humans are required. In fact, Bt insecticides can be applied to crops such as lettuce, cabbage, and tomatoes just prior to harvest, and Bt crops have no restrictions for human consumption. Moreover, if the Bts used in commercial agriculture are safe enough to be eaten, then it should be obvious that the Bti and Bs products used for mosquito control are also safe for use near human populations. It is important to realize that such a statement cannot be made for many synthetic chemical insecticides.

Aside from the various safety studies required by the US Environmental Protection Agency, programs have been mounted to monitor the health effects of spraying Bt insecticides directly on human populations. Two such recent studies, for example, were conducted during the late 1990s, one in Victoria, Canada, and another in Auckland, New Zealand (Petrie et al. 2003; Valadares et al. 2001). In both cases the Bt spray programs were undertaken to eliminate lepidopteran forest pests that had invaded these countries. To eliminate these pests, suburban residential areas inhabited by thousands of people were spayed periodically for several weeks, until the pests were eradicated. During the spray programs, and for months thereafter, the human populations were monitored for the presence of the Bt applied, and for symptoms of disease. Bacteria were easily recovered from nasal samples, for example, and from monitoring particulates in the air. In Auckland, New Zealand, some discomfort followed the sprays, but "most residents saw their health as unaffected by the spray program, and there was no significant increase in visits to general practitioners or alternative health care providers" (Valadares et al. 2001). Similar results were obtained in the populations monitored in Victoria during the Bt spray program – the "human health surveillance program failed to detect any correlation between the aerial application of *B. thuringiensis* subsp. *kurstaki* HD1-like bacteria and short-term health effects in the general adult population" (Petrie et al. 2003). This evidence of little or no significant health effects on human populations subjected to Bt sprays is in sharp contrast to the well-known toxic effects many chemical pesticides have on humans (Siegel 2001; Betz et al. 2000).

Specific safety studies on the various recombinant bacteria described above have not yet been conducted. The reasons for this include a shortage of funding for such studies, and marketing concerns by the companies that produce bacterial insecticides that the public may not accept the use of genetically engineered bacteria. There is, however, no reason to think that recombinant bacteria like those described above will have properties that would adversely affect most non-target organisms, and certainly any effects, including toxicity to these organisms, would be much less than those of synthetic chemical insecticides. Nevertheless, it is likely that it will be several before recombinant bacterial insecticides will join other recombinant organisms, such as Bt crops, as components of biological control and integrated pest management programs for agricultural pests and vectors of human and animal diseases.

Conclusions

The knowledge and variety of techniques generated by molecular biology and genomics over the past twenty years been put to use to generate recombinant bacterial larvicides for use as components in integrated vector control programs. These recombinant bacteria are much more efficacious that the wild type strains from which they were derived. The significant improvements in potency per unit of fermentation medium of recombinant bacteria should markedly reduce the costs of these new biological insecticides, bringing them within the range of costs for new chemical insecticides. The advantage of the new bacteria, aside from improved efficacy, is that they are much more environmentally compatible than most chemical insecticides. While acceptance by the public remains an issue, the extensive plantings of Bt crops in countries like the US, Australia, Argentina, and China indicates recombinant bacterial larvicides will eventually play an important role in controlling mosquito pests and vectors of disease.

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Part IV
Pest Management and Outreach in Disease Endemic Regions and in the United States: Practical, Novel and Attainable Strategies for Vector Control

Current Prospects for the Control of the Vectors of Malaria and Filariasis

Christopher F. Curtis

Abstract Mir Mulla developed the view that biological control, when reinforced with other methods, was an important component of the control of vector-borne disease. Recent developments in the use of insecticide-impregnated bed nets, the tracking of the emergence of pyrethroid resistance and the use of therapeutic drugs to combat the pathogenic agent of filariasis are discussed.

Keywords Insecticide treated nets · Malaria · Pyrethroid · Filariasis · Biological control

Introduction

Most malaria vectors bite in the middle of the night. Therefore bednets should be an effective protection but, if nets are torn, the mosquitoes find the holes unless the nets are treated with a suitable insecticide. If such treated nets are used by whole communities there is a major reduction in the population of mosquitoes old enough for the development of infective *Plasmodium* sporozoites to have gone to completion. This "bonus" of reduction of the infective biting population in a community is approximately equal to the percent of personal protection due to an individual sleeping under a net. There is a tendency to target subsidised or free nets to children and pregnant women who are the most vulnerable to malaria. However, by ensuring that a whole community is using effectively treated nets, mosquito kill would be maximised to the great benefit of those most vulnerable to malaria. Furthermore, filariasis is a disease mainly of older people and, to gain the double benefit of controlling filariasis as well as malaria, all age groups should be provided for. In tropical Africa, malaria transmission is far more intense in rural than in urban areas

C.F. Curtis (✉)
London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK

The author is deceased

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and available donor funding for malaria vector control should be concentrated on free provision of treated nets for everyone in highly malarious rural areas. Long Lasting Insecticidal Nets, which remain insecticidal despite repeated washing, are now being manufactured and distributed free of charge to tens of millions of people linked to other health protection measures, such as measles vaccination.

In tropical Africa indoor residual spraying may be most appropriate for rapid reaction to onset of occasional malaria epidemics in highland areas.

At present, only pyrethroids are used for net treatment and there is concern about a recent report of emergence of pyrethroid resistance strong enough to interfere with the practical effectiveness of treated nets and of indoor residual spraying. An attempt is advocated to eradicate this potentially disastrous gene by eradicating the local *Anopheles gambiae* population.

When *Culex* vectors of filariasis are breeding in pits and flooded cellars floating layers of expanded polystyrene can provide sustainable control. Integration of such vector control with mass administration of anti-filarial drugs to human populations gives better long term results than drug administration alone. Control of the severe biting nuisance of *Culex* populations increases the prestige of projects and there is a strong argument for such integrated control in the current effort to eliminate filariasis as a public health problem.

Recent data from Vietnam and India reinforce the view of Mir Mulla that well organized biological control, integrated with other methods, can contribute importantly to control of vectors of human disease.

The Personal Protection Provided by Insecticide Treated Nets Against Malaria Vectors

Ross (1910) pointed out that because of the tendency of many of the important malaria vector species to bite in the middle of the night (Pates and Curtis 2005), bed nets should be an effective means of malaria prevention. However, nets are easily torn, especially in mud-built rural tropical houses. A net which is even slightly torn gives no protection from biting unless it is treated with an insecticide (generally a pyrethroid) which drives away or kills mosquitoes contacting the net before they can find the holes (Curtis et al. 1996). In rooms where insecticide treated nets (ITNs) have been used collections in the morning show major reductions in numbers of blood fed mosquitoes, compared to rooms where nets have not been used (Maxwell et al. 2003). However, the reduction is not to zero and the question arose whether the few blood fed mosquitoes found in netted rooms had fed elsewhere and entered after feeding. However, Soremekun et al. (2004) tested for genetically variable microsatellites in the blood meals of mosquitoes caught in netted rooms and in the blood of the people who had slept under the nets. About 85% of the bloodmeals could be unequivocally matched (Fig. 1) to the blood of the sleepers in the same room, leaving only about 15% which had fed elsewhere (in some cases their meals could be matched with a person without a net in a neighbouring house). Thus we can

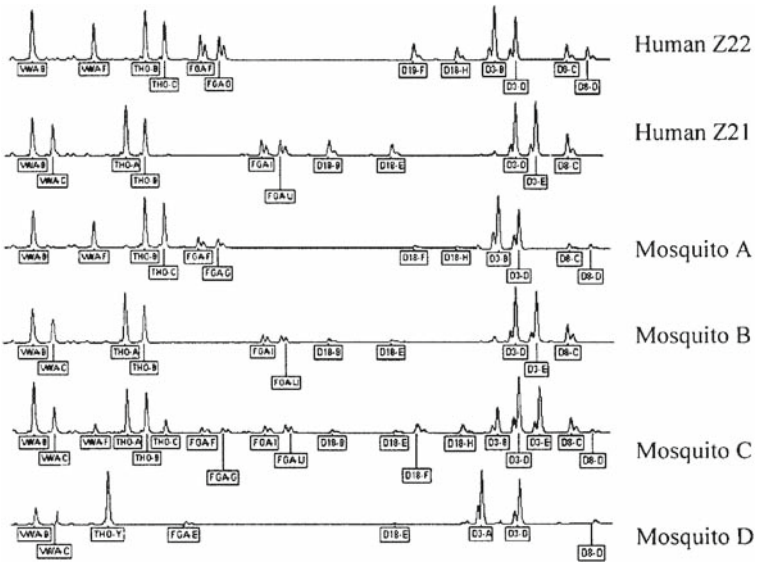

Fig. 1 "DNA fingerprinting" using microsatellites in mosquito blood meals and blood of sleepers in the rooms where the mosquitoes were caught (data of Soremekun et al. 2004). Note cases of matching with one (**a** and **b**), both (**c**) or neither (**d**) of the sleepers

conclude that ITNs give good protection to sleepers under them, but the protection is not perfect, either because some mosquitoes can bite through treated netting or they can catch a person who has got out of their net during the night.

Impact of Extensive Use ITNs on Vector Populations and the Arguments for Free Provision

Because of this proof of the imperfection of personal protection by ITNs it is particularly important that when ITNs are used by whole communities many mosquitoes are killed after being attracted to contact the nets by the odor of sleepers under the nets. By making it risky for a mosquito every time it enters a bedroom in search of a blood meal, fewer mosquitoes survive the period of approximately 12 days required for *Plasmodium* to mature to the infective sporozoite stage. Unique data were collected by Tony Wilkes (Magesa et al. 1991; Curtis et al. 2006) demonstrating the life-shortening effect of community-wide use of ITNs, or indoor residual spraying with DDT, and the consequent reduction of the number of mosquitoes with sporozoites (Fig. 2). Teklahaimanot et al. (2007) pointed out that in order to maximise the mosquito killing it is very much preferable to provide ITNs free of charge for every sleeping place in a community and not just to target free or subsidised nets to children and pregnant women who are the most vulnerable to malaria because of low immunity to this disease. Furthermore a high rate of subsidy of the retail cost

Village (type of roof on houses)	Pre-intervention		Intervention	Post-intervention	
	Mean age grade	% sporozoite +ve		Mean age grade	% sporozoite +ve
Mng'aza (thatch)	1.255	6.1%	Pyrethroid treated nets	0.792	2.3%
Mindu (thatch)	1.229	7.9%	DDT indoor Spraying	0.400	2.5%
Mlingano (iron)	0.764	4.9%	Pyrethroid treated nets	0.518	0.6%

Fig. 2 Mean age grade (method of Polovodova 1949) and sporozoite rate (by dissection and ELISA) in traditional and modern Tanzanian villages before and after DDT spraying or introduction of ITNs (Magesa et al. 1991; Curtis et al. 2006)

of nets for a whole country would probably be more expensive for donors than ex-factory purchase of large numbers of nets for free distribution targeted at lowland rural areas which have far higher malaria endemicity than urban or highland areas (Hay et al. 2005).

The data which are generally relied upon as the definitive evidence that ITNs have a major impact on morbidity and mortality (Lengeler 2005) were from five large trials in which community-wide coverage and regular re-treatment of the nets was ensured. These trials led to the conclusion that an average of 5.5 child deaths per year were prevented for every 1,000 children provided for. In operations where there has not been community-wide coverage with effectively insecticidal nets there will be sub-optimal mosquito killing and therefore sub-optimal protection of children. It would be misleading to assume that the above mentioned figure for deaths prevented would apply in such cases (Yukich et al. 2007). Another reason for preferring community-wide coverage is the accumulating evidence for interaction of HIV with malaria (Whitworth and Hewitt 2005) so that adults other than pregnant women increasingly need protection from malaria. Furthermore, it is hoped that ITNs will contribute to reduction of mosquito transmission of filariasis and this disease affects mainly adults, so targeting nets at children only would have little effect against this disease.

Until recently it has been necessary to re-treat nets with a pyrethroid once a year to compensate for the insecticide lost with repeated washing of nets. Our experience over many years in Tanzanian villages is that, with adequate advance notice to villagers, a very high percentage of the nets provided free of charge are brought on the appointed day for re-treatment until, after several years, the nets become seriously torn and tend to be discarded (Maxwell et al. 2006). The careful bringing of nets for re-treatment is contrary to the belief, held by some, that nets provided free of charge will not be looked after and conversely that very poor villagers need to be taught that they must get used to paying for nets if they want their children to be protected from malaria. Such a belief in the virtues of individuals having to pay for prevention of disease in their families has never been applied (even in the USA) to vaccination. In fact the linkage of measles vaccination campaigns with free

distribution of ITNs has provided rapid, equitable and low cost coverage in several countries (e.g. Grabowsky et al. 2005). Furthermore, "herd immunity" conferred by a high enough vaccine coverage to reduce pathogen populations is very comparable to the reduction of the infective vector population by high coverage with ITNs.

It is extremely important that provision of ITNs is not thought of as a "one-off" exercise but is sustained. In Vietnam a government program has ensured the annual re-treatment of the nets of about 10 million people since 1997 and cases and deaths from malaria have dropped dramatically during that time (see data of Tran Duc Hinh in Fig. 3 and Curtis et al. 2004). Recently long lasting insecticidal nets have been developed, whose insecticide deposit is wash-proof, thus avoiding the need to organize a re-treatment service. Over the last three years Ethiopia has obtained support from several donors which funded the free distribution of over 18 million long lasting insecticidal nets in the lowland provinces which bear the main burden of malaria (Fig. 4 based on Teklahaimanot et al. 2007). This is close

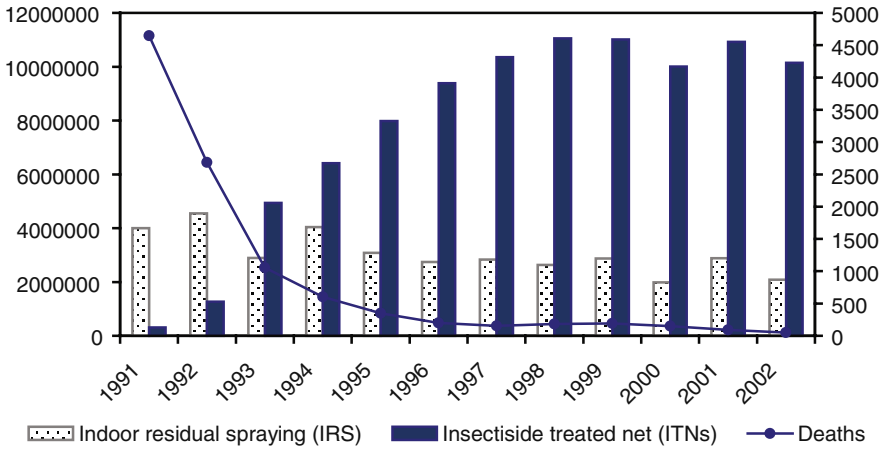

Fig. 3 Data of Tran Duc Hinh on people provided with annual net re-treatment or IRS and malaria mortality in Vietnam

Total no. Long Lasting Insecticidal Nets distributed			
Cost recovery scheme	Free distribution linked to other health protection measures and targeted to everyone in lowland malarious provinces		
1995 -2004	2005	2006	2007
1.5 million	4.7 million	7.4 million	6.7 million

Fig. 4 The target of 20 million nets for the lowland malarious provinces has almost been achieved in 3 years of free distribution with support from Global Fund, UNICEF, World Bank, DfID (UK), CIDA (Canada), JICA (Japan) and Carter Center

to the target of 20 million for full coverage of the populations of those provinces, based on an average of 1.7 people using each net. Extending this program to cover al of lowland rural tropical Africa on a prolonged basis would cost about $600 million per year, i.e. a fraction of what is spent in Europe and the USA in control of another hematophagus insect – the cat flea (Rust 2005).

Indoor Residual Spraying Versus ITNs

Malaria eradication was completed in the USA and southern Europe using Indoor Residual Spraying (IRS) in the late 1940s. Encouraging results were obtained with this method in certain parts of Africa in the past (Curtis and Mnzava 2000) and under the US President's Malaria Initiative there is a revival of interest in this method for Africa. From a multi-village trial of IRS versus with ITNs using the same pyrethroid for both purposes Curtis et al. (1998) reported very similar impact of both methods on mosquito populations and malaria, but ITNs were found to be cheaper because one-sixth as much insecticide was needed for annual treatment of a family's nets as for annual spraying of their house walls and ceilings.

It is generally considered that in highly endemic areas the logistics of sustaining a large scale IRS program are more difficult to manage than the distribution in the same area of Long Lasting Insecticidal Nets. However, an important situation where IRS may be the most appropriate solution is in highland or semi-desert areas where only exceptionally rainy years lead to a malaria problem and where people of all ages have little immunity to the disease. If a trained and equipped spray team was available to respond like a "fire brigade" to reports of such imminent epidemics, it is to be hoped that the epidemics could be suppressed. Provision of nets in such places would probably not be economic as mosquito protection would only be needed for the few months of an epidemic and, by the time of the next epidemic, the nets would probably be worn out.

The Threat of Pyrethroid Resistance

At present only pyrethroids are used for widespread net treatment. Thus there is much concern about emergence of pyrethroid resistance in vector populations. Surprisingly, it has been found that in central Côte d'Ivoire, where there is a high frequency of the *kdr* resistance gene in *Anopheles gambiae*, there is high mortality among free flying *An gambiae* entering experimental huts in which people sleep under pyrethroid treated nets (Darriet et al. 2000; Asidi et al. 2004). Furthermore Henry et al. (2005) showed an encouraging reduction of malaria associated with introduction of ITNs into villages. It is suggested that the reduced irritability of *kdr* carrying mosquitoes in contact with a pyrethroid leads to longer contacts and eventual picking up of a lethal dose. However, one should not be complacent about the situation as N'Guessan et al. (2007) have reported that in southern Benin an

apparently similar *kdr* gene is associated with failure of ITNs and IRS with a pyrethroid to achieve acceptable mosquito mortality in experimental huts.

The spread of such a gene could be disastrous to the high hopes now placed on ITNs and IRS with pyrethroids. This author therefore advocates an urgent effort to eradicate *An. gambiae,* and thus the resistance gene with the mosquitoes which carry it, in the part of southern Benin where this very alarming data have been obtained. It is suggested that a carbamate such as bendiocarb or an organophosphate such as pirimiphos methyl could be used as an adulticide and an Insect Growth Regulator with long persistence such a pyriproxifen (Yapabandara et al. 2001) should be used against the aquatic stages. It is recognized that the gene may already have spread extensively and that eradication of *An. gambiae* would be a very difficult undertaking. However, if in 10 years time the gene has spread throughout Africa and spoiled the prospects of a major impact of vector control on African malaria, those who decided in 2007 not even to try to take any practical action will be rightly criticized.

Control of Filariasis Vectors

Unlike malaria, filariasis does not kill people (except indirectly via inducing affected people to commit suicide) but it is rated as the second most important cause of disability after mental illness. In different parts of the tropical world the nematode worms which can cause elephantiasis and other filarial symptoms are transmitted by mosquitoes of four different genera. *Culex quinquefasciatus* are the vectors of *Wuchereria bancrofti* in southern and eastern India, which suffers the world's largest burden of filariasis. *Cx quinquefasciatus* are also the main vectors in urban areas in East Africa. However, in West Africa the local *Cx. quinquefasciatus* are not susceptible to *W. bancrofti* (Curtis and Graves 1983) and *Anopheles* species are the filariasis vectors. In such areas it is important to set up trials to check the supposition that in the long run ITNs, mainly introduced against malaria, will also have a beneficial effect in controlling filariasis transmission.

In India and urban East Africa much of the breeding of *Cx. quinquefasciatus* is in polluted water in pit latrines, soakage pits and buildings where the basements are flooded with sullage water from leaking pipes. In all such sites the water is retained within walls and is suitable for the application of a 1 cm thick layer of expanded polystyrene (styrofoam) beads. As first shown by Reiter (1978) this suffocates mosquito larvae (see Fig. 5) and has no adverse effects. Where there are open drains which flow at least part of the time the beads would soon be washed away. It is important to assess how much of the tropical, urban *Culex* problem is controllable with polystyrene beads. In Makunduchi, Zanzibar, there is a population of 12,000, who were badly affected by filariasis. There are 2,000 pit latrines and checking of all of them showed that at least at some seasons 550 of these pits contained water and were *Culex* breeding sites. Very few other breeding sites could be found. This mosquito population appeared to be well isolated by the ocean on two sides and several kilometres of very dry country on the others. Polystyrene was

Fig. 5 Floating layer of expanded polystyrene beads suffocating *Culex* larvae (Curtis et al. 2002)

obtained in its unexpanded form, expanded in boiling water in householders' cooking pots and applied to all the wet pit latrines. Light trapping overnight and hand collections in the mornings showed approximately a 98% reduction in number of *Culex* mosquitoes in bedrooms (Maxwell et al. 1990) which was extremely welcome in the community because of reduced mosquito nuisance. From time to time a flare-up in mosquito biting was noticed in a certain part of the community and the inhabitants informed members of the anti-mosquito team. A local search generally revealed a newly wet pit as the source of the trouble and this was quickly dealt with by a polystyrene application. On one occasion extremely heavy rain led to flooding of some pit latrines and re-application of polystyrene was needed there, but otherwise a treated pit remained mosquito-free for years.

Integration of Vector Control with Mass Anti-filarial Drug Administration

At the time of the main anti-mosquito operation a campaign was also carried out of mass administration of the anti-filarial drug di-ethyl carbamazine (DEC) to the whole human community. The drugs were delivered with the invaluable help of the local political representatives (*balozis*). A similar program was carried out in another community in Zanzibar where there was no vector control. The percentage of people positive for microfilariae, was determined by blood sampling at night, which is the only time when the microfilariae of periodic strains of *W. bancrofti* can be found in the blood. As shown in Fig. 6, in the short run both programs reduced the prevalence of microfilariae from 40–50% to about 10%, but without vector control there was re-infection leading to resurgence to the pre-intervention level over about 5 years. By contrast there was far less indication of resurgence in Makunduchi where there was sustained vector control.

The WHO policy for global elimination of filariasis as a public health problem is based on annual rounds of mass distribution of anti-filarial drugs which have been

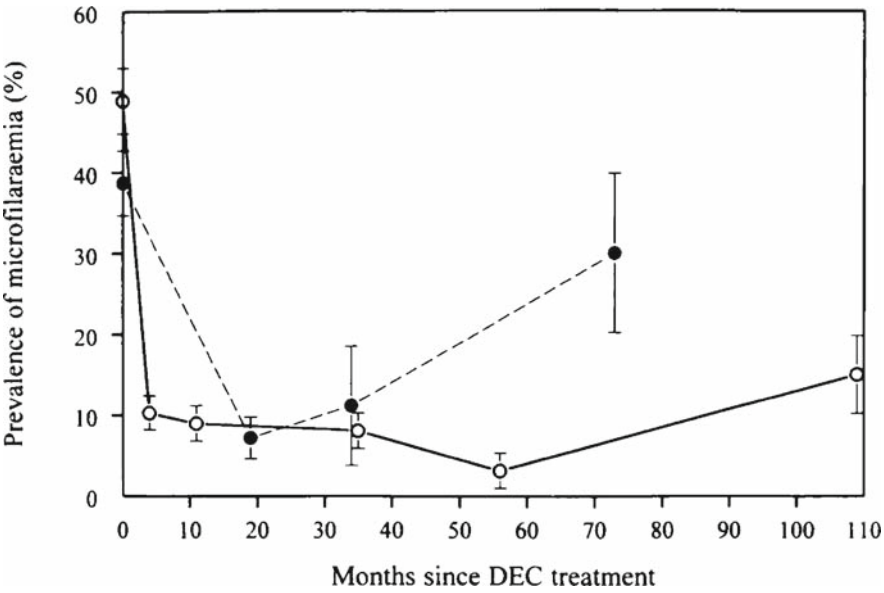

Fig. 6 Prevalence of microfilariae in two communities in Zanzibar with mass treatment in the first year with the anti-filarial drug di-ethyl carbamazine (DEC); *dashed line*: no vector control; *solid line*: *Culex* control with polystyrene beads which prevented re-infection (Maxwell et al. 1999)

provided free of charge by the manufacturers. Vector control is given little emphasis in this policy. It might be argued that equivalent results could have been achieved at Makunduchi if repeated annual rounds of mass drug administration (MDA) were used instead of vector control. However the more recent data of Sunish et al. (2007) from south India showed that with repeated rounds of MDA alone, progress in reducing prevalence of filarial antigen seemed to reach a limit. However, where this was integrated with application of polystyrene to soakage pits, adult populations of *Culex* were greatly reduced and progress continued in reducing filarial prevalence (Fig. 7). Thus there is increasing evidence in favor of integrating vector control with MDA if the ambitious target of elimination of filariasis is to be achieved.

	1999-2002	2003-2004
Annual mass drug admin.only	20.4%	19.5%
Annual mass drug admin. + vector control	17.7%	5.5%

Fig. 7 Trial in Tamil Nadu, India: filaria antigenaemia when annual mass drug administration was continued for 6 years, with or without vector control using polystyrene beads in soakage pits (Sunish et al. 2007)

Biological Control of Tropical Vector Borne Diseases

Following in the footsteps of Mir Mulla there have been some important recent examples of carefully monitored programs of biological control of tropical vectors. One has been against *Aedes aegypti* vectors of dengue using *Mesocyclops* copepods as predators of the first instar larvae of the mosquito in Vietnam (Vu Sinh Nam et al. 2000). Village teams are trained to check every water tank in their village for presence of the predator, which can be seen with the naked eye. Where they are missing from a particular tank it is generally found feasible to stock that tank from a nearby positive tank. By this method *Ae. aegypti* and dengue has been eliminated from substantial areas of Vietnam.

In Karnataka state in south India *Poecillia* and *Gambusia* larvivorous fish have been successfully used in village wells and ponds to control *Anopheles* larvae and hence to produce sustained reductions in numbers of malaria cases (Ghosh et al. 2005). After initial stocking of the sites from fish hatcheries, it has been found by half yearly checking that in most cases the fish populations are self sustaining, but when a site had lost its fish it could generally be replenished from a nearby site.

Fish may be usable to assist with control of *An. gambiae* in Africa but its breeding sites tend to be temporary and therefore less suitable for fish than the wells and ponds in India. Therefore the best hope for malaria prevention in Africa seems to be a sustained attack on the adult mosquitoes coming into houses to blood feed.

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Unraveling a Complex Transmission Cycle: Implications for Control

Laura D. Kramer and A. Marm Kilpatrick

Abstract The ability of an arbovirus such as the West Nile virus to be transmitted depends on interactions among a large number of factors including host population structure and susceptibility, mosquito population structure, feeding patterns, and vectoral capacity and the genetic makeup of the virus. The interaction of these genetic components with environmental factors at any given time plays a significant role in viral transmission, as well as viral evolution and adaptation.

Keywords West Nile virus · *Culex* · Mosquito · Arbovirus transmission

An understanding of vector – virus – vertebrate interactions will allow us to begin to make predictions of public health risk and consequently allow us to make informed decisions regarding mosquito control. The plethora of factors influencing epidemic activity of a pathogen include host factors such as population composition and density, competence and immunity; vector factors including species composition and competence, longevity; and virus factors including strain and genotype (Fig. 1). The genetics of the vector, virus, and vertebrate have a critical impact on the intensity of virus transmission. Additional layers of complexity are the influence of environmental factors such as rainfall and temperature, and the need for synchronicity leading to contact between the vector and vertebrate at a time when one of them is infectious. The focus of this paper is on the consequences of these layers of interactions on viral evolution and adaptation, and determination of the most significant drivers of virus transmission with an emphasis on the impact of heterogeneity. Recent field studies and experimental research on West Nile virus (WNV) conducted by our laboratories will be used to address these points, and in the process honor the ecological approach to control fostered by Mir Mulla.

West Nile virus (*Flavivirus; Flaviviridae*) is spherical, enveloped, and approximately 50 nm in diameter Rice 1996. It contains a host-derived lipid bilayer surrounding a nucleocapsid consisting of the viral RNA complexed with multiple

L.D. Kramer (✉)
Department of Health, Arbovirus Laboratories, Wadsworth Center, Albany, NY 12201, USA
e-mail: kramer@wadsworth.org

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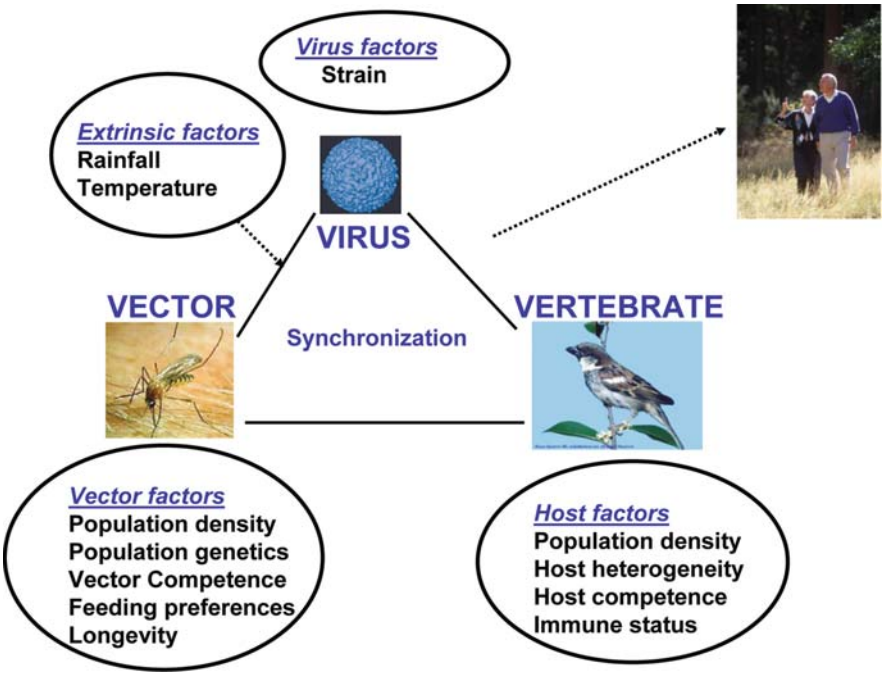

Fig. 1 Important components of the arboviral transmission cycle that impact intensity of transmission, including key aspects of virus-vector-vertebrate interaction, influence of environmental factors, and need for synchronicity (contact) among the three components

copies of the capsid protein (Fig. 2) (Mukhopadhyay et al. 2003) as depicted in Kramer et al. (2007a). The viral genome is linear, positive sense, single-stranded RNA, 11,029 kb in length. Sequence data indicate that the first WNV isolates from New York City in 1999 were 99.8% identical to an Israeli isolate from 1998 (Lanciotti et al. 1999). This introduced genotype of WNV has been termed the "Eastern" or NY99 genotype.

The primary vectors of WNV are *Culex* spp. mosquitoes, although the virus has been isolated from at least 60 additional species of ten genera (http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm). Some of the non-*Culex* species are undoubtedly incidental vectors which are not competent to transmit virus. The predominant *Culex* species in WNV transmission varies regionally: *Culex pipiens* Linnaeus is the most important vector in the northeastern, north central, and midAtlantic US (Kilpatrick et al. 2005), the sibling species *Culex quinquefasciatus* Say in the southern US, and *Culex tarsalis* Coquillet in agricultural regions in the west (Bernard and Kramer 2001). The importance of *Cx. restuans* Theobald is unknown as its distribution overlaps with *Cx. pipiens* and since their adults are difficult to distinguish, they are commonly grouped together in surveillance testing. Studies of them are uncommon, but indicate they are equally competent as *Cx. pipiens* (Ebel et al. 2005). In the southeast, *Cx. nigripalpus*

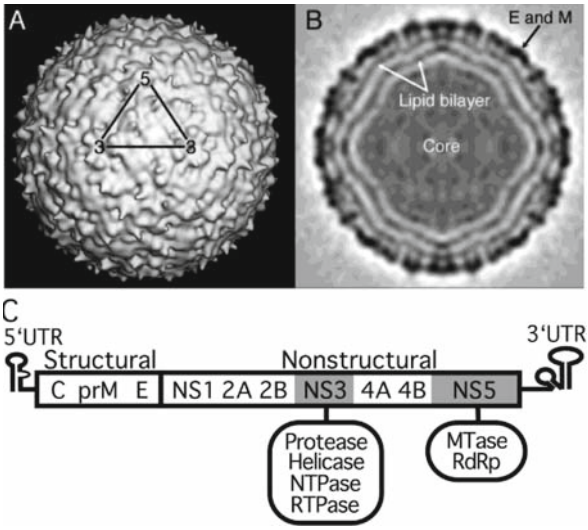

Fig. 2 WNV structure as reconstructed by cryo-EM. (**a**) A surface-shaded view with one asymmetric unit of the icosahedron indicated by the triangle. (**b**) Central section of the reconstruction, showing the concentric layers of mass density. Reproduced with permission from Mukhopadhyay et al. (2003) as modified in Kramer et al. (2007a)

Theobald is also a significant vector and *Cx. erraticus* Dyar and Knab may play a role in some areas (Cupp et al. 2007). *Culex* species, including *Cx. pipiens*, are also the most important vectors outside the US. It has been postulated that the lack of intense transmission of WNV in northern Europe is a consequence of the lack of hybridization between two biotypes of *Cx. pipiens*, form pipiens and form molestus (Fonseca et al. 2004).

Virus has been detected in over 300 native and captive avian species. Although most species appear susceptible to infection, mortality varies greatly. Mammals have been implicated as susceptible hosts, although they are thought to play a less important role, if any, in viral amplification because of their relatively lower viremia titers. Evolutionary pressures on the virus, therefore, are applied predominately by the mosquito and avian environments. WNV is one of the most widely distributed arboviruses. WNV has been isolated in Africa, the Middle East, Europe, Russia, Asia, Australia, and most recently the United States, Canada, Mexico, the Caribbean, Central and South America (Kramer et al. 2007b). The viruses are separated into two major lineages: Lineage-1 viruses include all Africa, Middle East, Europe, Russia, India, Australia, and the US. This lineage is further subdivided into Kunjin and India subtypes. Lineage-2 viruses include Sub-Saharan Africa and Madagascar (Fig. 3) (Lanciotti et al. 2002; 1999). Recently 3 additional lineages have been proposed, III [Czechoslavia; Rabensburg virus (Bakonyi et al. 2005)], IV [Russia (Lvov et al. 2004)], V [India (Bondre et al. 2007)].

The first topic my laboratory addressed was the evolution of WNV over time in New York State where the virus has been active since 1999. To study this, we sequenced isolates from American crows (*Corvus brachyrhynchos*) and *Culex pipiens* mosquitoes submitted by counties in diverse locations in NYS to the Arbovirus Lab since 1999 (Ebel et al. 2004). The entire envelope gene (1,503 nt) of more than 88 isolates of virus was sequenced. Neighbor joining analysis was conducted

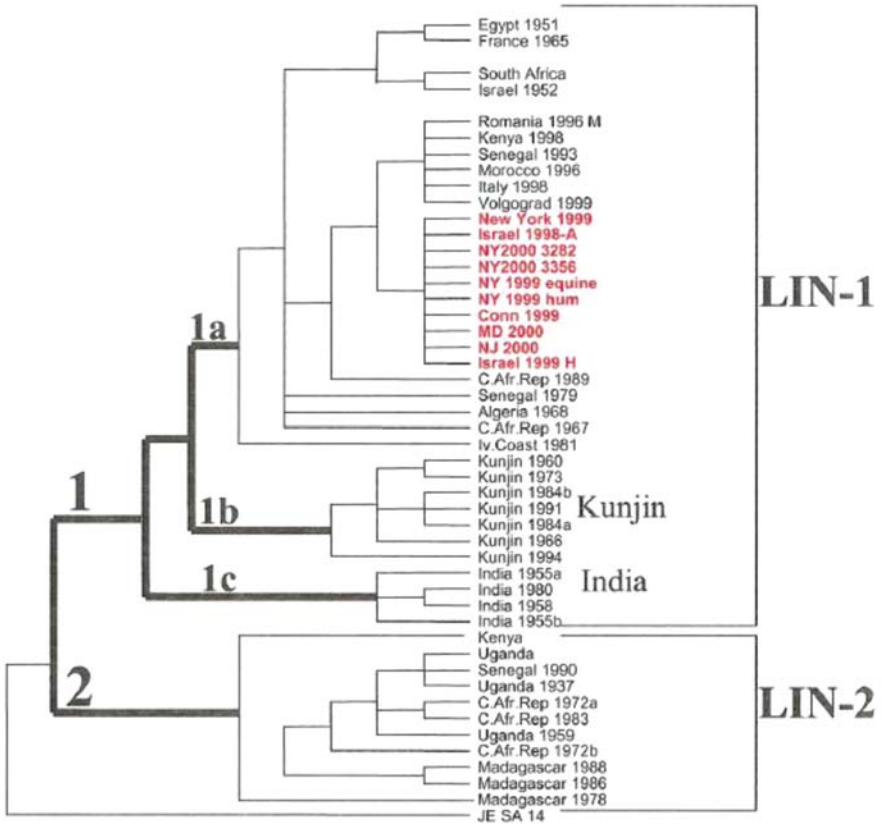

Fig. 3 Phylogenetic tree of West Nile virus isolates generated by parsimony analysis of aligned nucleotide sequences of 47 WNV strains and Japanese encephalitis virus as the outgroup, using a 255-bp region of the envelope gene (positions 1402–1656). Reproduced with permission from Lanciotti et al. (2002)

using 1,000 replicates. During the spread of WNV across North America, a new genotype ("North American dominant" or WN02) emerged and rapidly became dominant among circulating WNV strains (Davis et al. 2005; Ebel et al. 2004). The WN02 genotype consensus sequence contains three consensus nucleotide sequence changes from the NY99 genotype: a U–C change at position 1442 in the E gene, a C–U change at position 2466 in the E gene, and a C–U change at position 9352 in the NS5 gene (Davis et al. 2005; Ebel et al. 2004). The U1442C is the only non-synonymous change, resulting in a valine to alanine change at amino acid position 159 in the E protein. This conserved amino acid change is not located within the predicted receptor binding domain or any region predicted to be critical for efficient fusion of the viral envelope with the host cell membrane. The North American dominant genotype was first identified in Texas in 2001, and in New York in 2002.

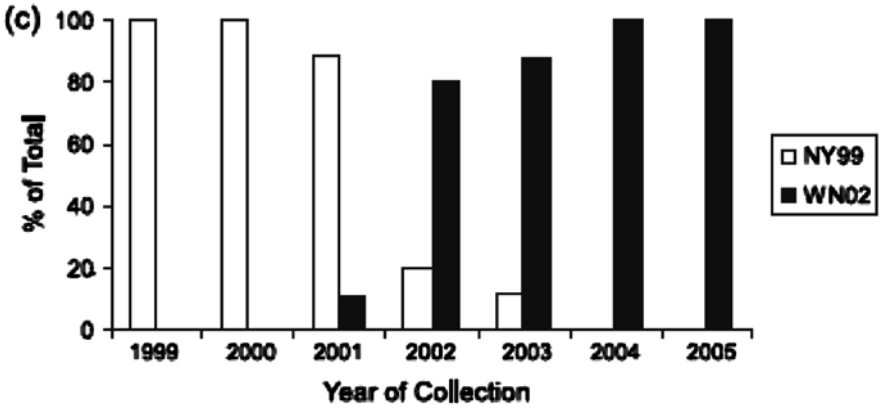

Fig. 4 Displacement of the genotype of West Nile virus introduced into the US in 1999 ("NY99") by a new genotype, "North American dominant" or "WN02". Reproduced with permission from Snappin et al. (2007). □ NY99 genotype, ■ WN02 genotype

It rapidly displaced the introduced genotype over the next 2 years so that by 2004, there was no evidence of the NY99 genotype virus in circulation (Fig. 4) (Snappin et al. 2007). In order to understand the mechanism of this rapid displacement of one genotype with another, we began with an examination of the simplest explanation, that the WN02 genotype replicates more efficiently than the NY99 genotype. We examined this first in vitro, using multi-step growth analysis, and found no differences in growth in mosquito or avian cell culture of strains of the two genotypes (Fig. 5) (Moudy et al. 2007). We then evaluated fitness of the viral strains of the two genotypes following modified protocols of Holland as described (Ciota et al. 2007) and also saw no consistent differences (Fig. 6) (Moudy et al. 2007). Studies then were conducted in vivo using *Culex pipiens* and *Culex tarsalis.* A greater proportion of both species of mosquitoes became infected with WN02 than NY99 at early time points, and this continued out to 14 days post-feeding in *Cx. tarsalis*. In early experiments, proportions of infected *Cx. pipiens* equalized by 9 days post-feeding. However later studies demonstrated the WN02 advantage continued over time and accelerated with temperature (Kilpatrick et al. 2008). Viral transmission of both genotypes begins very early at temperatures of 32°C, with a consistently greater fraction of mosquitoes transmitting WN02 than NY99. In summary, we observed a change in the basic reproductive ratio of WNV through impact on extrinsic incubation period (EIP) as well as overall vector competence when experiments were carried out beyond 14 days. These differences are most likely due to genotype-specific differences in replicative efficiency and/or cell to cell spread in the mosquito.

There are several points during the mosquito infection process that differences could occur in virus-vector interactions. When a mosquito imbibes an infectious bloodmeal, the virus enters the midgut lumen and infects and replicates in the midgut epithelial cells. It then escapes from the midgut and infects and replicates in

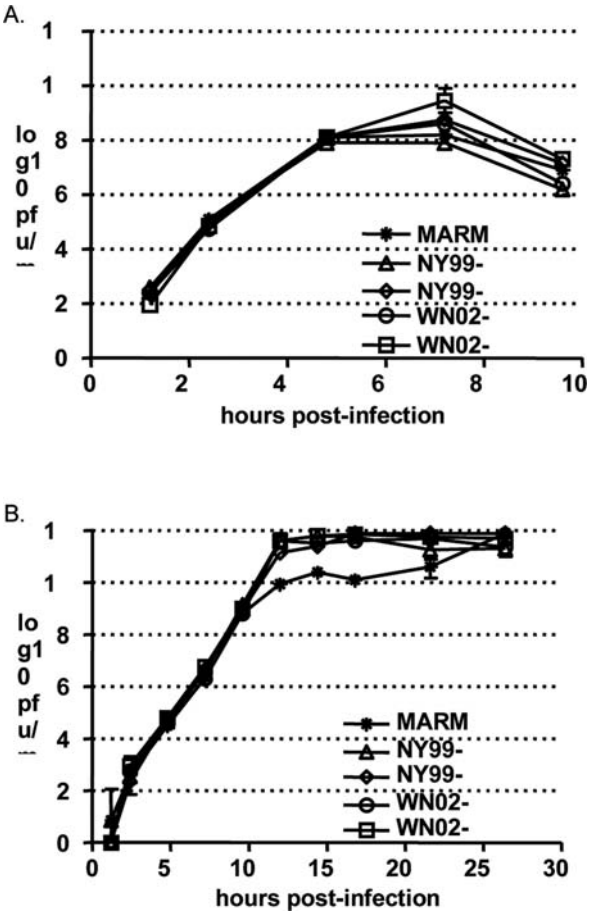

Fig. 5 Replication efficiencies of NY99 and WN02 genotypes in vitro. Confluent (**a**) DF-1 or (**b**) C6/36 cells were infected with a single strain of WNV at an MOI of 0.01, and samples of supernatant were taken at the indicated times for virus titration in Vero cells. Viral titers at each time point are shown as $\log_{10}$ pfu/mL. Reproduced with permission from Moudy et al. (2007)

the fat bodies. The virus then disseminates to numerous secondary tissues, including the salivary glands, where it infects the cells and is expelled with the mosquito's saliva during subsequent bloodmeals (Hardy 1988). To help determine at what point in the viral infection pathway the genotypes were behaving differently, we infected mosquitoes by intrathoracic (IT) inoculation. This method of infection leads directly to a disseminated infection of secondary tissues, bypassing the initial midgut infection. If we observed a difference in transmission at early times, as was seen in orally infected mosquitoes, it would suggest that WN02 genotype viruses were better able to infect and replicate in the salivary gland. However, if no difference in transmission was observed, it would suggest that the WN02 genotype viruses were better able to overcome the midgut infection barrier. Results indicated no differences in EIP following IT inoculation, although a greater proportion of mosquitoes transmitted the WN02 on days two through five. This suggests that the main difference is in the midgut, but secondary tissues also may be involved. Interestingly, WN02 viruses replicate to higher titers from two days post-inoculation until at least

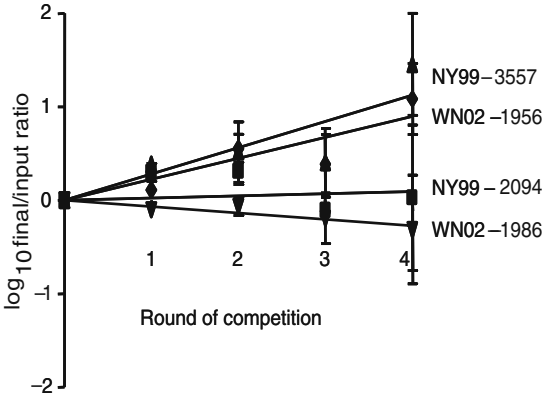

Fig. 6 Competitive fitness of NY99 and WN02 genotypes in vitro. Confluent C6/36 cells were infected with a 1:1 ratio of WNV isolate to MARM at an MOI of 0.01. At 96 h post-infection, supernatants were harvested, diluted to an MOI of 0.01, and passed onto fresh cells. A total of four rounds of competition were performed. WNV:MARM ratios were determined by plaque titration in Vero cells in the presence (to measure MARM) and absence (to measure wildtype virus) of MAb 5H10 (Bioreliance). Fitness vectors representing the relative fitness of each WNV isolate compared to MARM are shown. Reproduced with permission from Moudy et al. (2007)

9 days post-inoculation. This indicates that WN02 viruses exhibit increased replication efficiency in vivo as compared to NY99 viruses. Together with previous data, it suggests that WN02 viruses can better overcome the midgut barriers due to their increased replication efficiency. However, these studies measure overall viral replication, which can be affected by several aspects of the viral life cycle including entry, RNA replication, and exit from the host cell. Therefore, future work will focus on viral interactions with the midgut.

As with the mosquito vectors, several aspects of virus interaction with the avian amplifying hosts could play a role in strain displacement, such as a difference in levels of avian viremia or differences in virus shedding or persistence within the birds. Brault and colleagues (Brault et al. 2007) have demonstrated the importance of a single point mutation on viremia in American crows. We are in the process of comparing the two WNV genotypes in house sparrows, which were the second most important host in our study sites in the Baltimore/Washington DC area, with American robins being first (Kilpatrick et al. 2006b).

These differences between the two WNV genotypes in mosquitoes could lend a significant advantage to amplification of WN02 over NY99 strains. Since *Culex* species can take a bloodmeal every 5 days, on average, a virus that can be transmitted 5 days after infection would be able to infect susceptible avian hosts during the first bloodmeal after infection, whereas a virus that isn't transmitted until seven to nine days post-infection would not be able to infect birds until the second bloodmeal after infection. Therefore, the WN02-infected mosquito would have the potential to infect more birds than the NY99-infected mosquito, leading to greater numbers of WN02 infected birds, and subsequently increased minimal infection rates

in mosquitoes. Similarly, higher viremias in birds would lead to a larger fraction of mosquitoes biting a host becoming infected.

A second focus of our research is to determine the spatio-temporal drivers of WNV transmission in North America, focusing initially on the northeast and mid-Atlantic region of the US. It was initially demonstrated that *Cx. pipiens* is not only the predominant enzootic vector of WNV in the northeast where some of the studies took place. Minimal infection rate (MIR) and host feeding patterns of mosquitoes in New York analyzed in conjunction with vector competence data indicated *Cx. pipiens* is also an important bridge vector to humans (Kilpatrick et al. 2005). Taking into account abundance, vector competence, feeding pattern, and minimal infection rate of different species of mosquitoes, *Cx. pipiens* represents the greatest threat to humans. The species-pair *Cx. pipiens* and *Cx. restuans* accounts for >80% of the total Risk, a surrogate for human WNV infections in the New York region, over the transmission season. The combined Risk of four other important species evaluated, *Aedes japonicus*, *Aedes vexans*, *Aedes trivittatus*, and *Culex salinarius*, represented one quarter the threat posed by *Cx. pipiens and Cx. restuans.*

In the mid-Atlantic, the relative abundance of birds at two residential and three urban sites were compared to the avian hosts identified in blood meals from *Culex pipiens* and *Culex restuans* mosquitoes, the predominant enzootic vectors in the midAtlantic region of the country. It was observed that a mosquito's feeding pattern did not match the relative abundance of bird species at each of the study sites. American robins (*Turdus migratorious*), which made up only 3.7% (range among sites 1.0–7.5%) of the birds, were highly preferred at all sites, and accounted for 43.4% (range 24–71%) of *Cx. pipiens* blood meals (Kilpatrick et al. 2006b). Fish crows were also preferred at two sites, but were relatively uncommon and made up only 4 and 9% of blood meals at these sites. In contrast, house sparrows (*Passer domesticus*) were extremely abundant at all sites (56% of birds; range 42–67%) but appeared to be avoided by mosquitoes and made up 11% (range 0–21%) of the blood meals. The feeding preferences were then integrated with information from the literature on the host competence for each species, which estimates the probability of infecting a mosquito while viremic. Competence takes into account avian susceptibility, mean infectiousness, and days infectious (Komar et al. 2003). Using a conservative set of assumptions, the results indicated that American robins were likely responsible for 59.3% (range 35–88%) of the WNV-infectious mosquitoes at the five sites, whereas the much more abundant house sparrows were only responsible for 24% (range 4–40%) (Kilpatrick et al. 2006b). The impact of the focused feeding on American robins, and the heterogeneity in host competence, was to intensify transmission to this species and speed up the viral amplification. The pathogen reproductive ratio, R_0, was increased by 10.4 fold (range 4.3–15.3) compared to the situation where mosquitoes fed on each host according to their abundance, and all hosts were equally competent. A possible consequence of the increased R_0 was seen in earlier detection of WNV in mosquitoes at the sites where R_0 was increased the most by heterogeneity in mosquito feeding and host competence.

One pattern that arose in the feeding data was a seven-fold increase over the season in the fraction of *Cx. pipiens* mosquitoes that had fed on humans (Kilpatrick

et al. 2006c). Feeding shifts previously had been observed over the season in other mosquitoes, including *Cx. tarsalis* in California (Tempelis et al. 1965) and Colorado (Tempelis et al. 1967), and *Cx. nigripalpus* in Florida (Edman and Taylor 1968), but the cause for these previous feeding shifts was unknown. In the mid-Atlantic, the feeding shift coincided with the dispersal of American robins from urban and residential areas, and a decrease in feeding on robins. It was thus plausible that the feeding shift to humans occurred at least partly because of the decrease in the abundance of robins, *Cx. pipiens*' most preferred host.

The consequence of the feeding shift for WNV transmission to humans was determined using data collected at the same sites on mosquito abundance, and WNV infection prevalence. The abundance of WNV-infected mosquitoes that fed on humans was strongly correlated with the number of human WNV cases in the region two weeks later (the approximate length of the time from infection to the onset of illness), suggesting that this product was a valuable measure of the risk of human infection (Fig. 7). In fact, this was the first time a risk index had been used to successfully predict temporal variation in the number of human cases. An analysis using this risk index suggested that the number of human cases was increased 4.5 fold as a result of the feeding shift compared to the situation in which the mosquitoes fed on humans at the frequency observed in June (Fig. 7). The feeding shifts previously noted in other areas [as well as that recently observed in *Cx. erraticus* in Alabama (Hassan et al. 2003)] all likely contributed to the relatively intense epidemics of WNV observed across North America.

An alternate explanation for the shift in feeding patterns is that mosquito feeding behavior is determined by genetic ancestry. Previous work had shown that *Cx.*

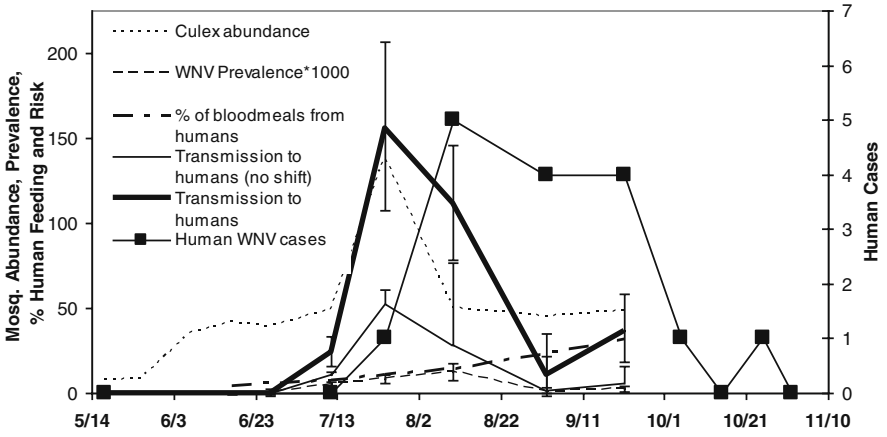

Fig. 7 Abundance of *Culex pipiens* and *Cx. restuans* mosquitoes per trap-night from CDC light traps, WNV infection rate (1,000* infection prevalence, ±1SE), percent of *Cx. pipiens* blood meals from humans, estimated human WNV infection risk (±1SE), calculated as the product of mosquito abundance, WNV infection rate, and the time-varying probability of feeding on humans (Human risk) or the June probability, 0.04, (Human risk – no shift), and the number of human WNV cases in Maryland in 2004 (adapted from {Kilpatrick et al. 2006c})

pipiens in North America were hybrids between two old world forms, *Cx. pipiens* form pipiens, which was thought to feed primarily on birds, and *Cx. pipiens* form molestus which was known to feed readily on humans (Fonseca et al. 2004) Thus, it was possible that the feeding shift in *Cx. pipiens* could have been a result of a shift in mosquito genetics over the season with form pipiens predominating early in the summer and form molestus later. In collaboration with Dina Fonseca (Rutgers University) we tested this hypothesis. Dr. Fonseca used microsatellites to determine the genetic ancestry of the same mosquitoes that were used in the feeding shift study (Kilpatrick et al. 2006c). This analysis showed that the fraction of alleles from form molestus was a significant predictor of whether the mosquito blood meal came from humans or birds (Kilpatrick et al. 2007). Thus, genetic ancestry was important in determining mosquito feeding patterns. However, the analysis also showed that there was no change in the genetic composition of the mosquito populations over time, so the feeding shift could not be explained by a shift in mosquito genetics. Taken together, these data from two studies showed that both genetic ancestry and host abundance influence mosquito feeding patterns and determine the transmission of pathogens between hosts.

A third focus of our research is to determine if and how the virus might spread to distant island groups including Hawaii, Galapagos, and Barbados (Douglas et al. 2007; Kilpatrick et al. 2006a; Kilpatrick et al. 2004). The pathways of spread that were considered included infected humans, wind transported mosquitoes, human transported mosquitoes, human transported vertebrate hosts, and migratory birds. These analyses showed that for Hawaii and Galapagos mosquitoes transported on airplanes were by far the greatest risk of introduction, whereas for Barbados migratory birds and mosquitoes on airplanes were of similar risk (Fig. 8). Other pathways were of lower risk because the concentration of virus in the blood of infected humans is too low to infect mosquitoes, very few mosquitoes have reached these islands by wind, and few vertebrate animals that could be infectious are brought to

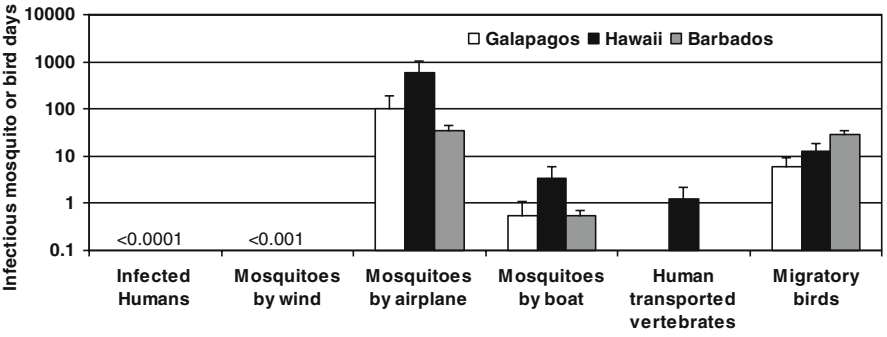

Fig. 8 Estimated risk, on a logarithmic scale, of WNV introduction to Galápagos, Hawaii, and Barbados by five pathways. Risk is quantified as the number of infectious mosquito or bird days (the number of infected animals transported, multiplied by their infectiousness, and the length of their infectious period). Data from {Kilpatrick et al. 2004}, {Kilpatrick 2006}, {Douglas et al. 2007}

these islands. Mosquitoes on boats are usually in the larval stage and the probability of vertical transmission is low. Finally, the numbers of migrating birds moving between the mainland and Hawaii and Galapagos is relatively low compared to Barbados, and both of the former island groups receive substantial flights from mainland areas where WNV is circulating. The primary management tools available to prevent the introduction of WNV and other vector borne pathogens to these island groups are (1) disinfection: killing insects on airplanes through residual chemical coatings, especially on the walls of the cargo holds where most mosquitoes are transported, or aerosols which appear to be less effective (Naumann and McLachlan 1999) and (2) control of mosquitoes around airports and areas where migratory birds arrive to decrease the chance of establishment if a mosquito or bird were to reach the island.

In summary, combined field – laboratory research on each of the three components of arbovirus transmission cycles, i.e., virus, vector, vertebrate, is critical to expanding our understanding of virus amplification leading to high risk to humans. This knowledge is essential to the development of effective control strategies.

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Sustainable Mosquito Control in California: A Template for the World

David Brown

Abstract Mosquito control in California is achieved through interactions with local mosquito abatement districts, the California State Department of Health and the University of California. This interaction has resulted in sustainable mosquito control across a large state that contains a diversity of mosquito environments. Effective mosquito control has also prevented the establishment of invasive mosquito species into California though its ports of entry and despite that large volume of freight shipped to California. The unique structure of the relationship that has effectively controlled mosquitoes in California can serve as an example to other regions of the world.

Keywords Mosquito control · California · Arbovirus · University of California

Introduction

Sustainable mosquito control has been a standard in California for over 75 years. Mosquito control has remained sustainable in California in large part through an innovative partnership involving local mosquito control districts, the State of California's Department of Public Health, and the University of California. This partnership has provided a foundation that allows the implementation of research-based control efforts at the local level that are endorsed by the State of California.

This chapter will examine the three components of this partnership and how it could be a template for other parts of the world.

D. Brown (✉)
Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624, USA
e-mail: dabrown@fightthebite.net

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Local Mosquito Control in California

Mosquito control in California officially began on May 25, 1915 through passage of the Mosquito Abatement Act. This act enabled local jurisdictions to obtain revenues and form districts for the control of mosquitoes. It is interesting to note that while mosquito control had been performed prior to this act to stem outbreaks of malaria in the northern part of the state (most notably by Professor Freeborn in Rocklin) the first districts were formed along the coastal regions of California due to the significant numbers of "pest" mosquitoes emanating from salt marsh and floodwater habitat.

Today in California there are over 60 agencies covering over 38,000 square miles and protecting over 32 million people from the threat of mosquitoes and mosquito-borne diseases (Fig. 1). The districts employ a three pronged approach of surveillance, education and control of larval and adult mosquitoes to protect the citizens within their jurisdictions. Districts in California use a combination of traps to assess adult mosquito abundance. American Light Traps, Gravid Traps and Carbon dioxide baited traps allow an assessment of adult mosquitoes at different stages of their gonotrophic cycle.

Some Districts employ a larval surveillance program as well, and initiate control based on larval abundance in known mosquito development habitats.

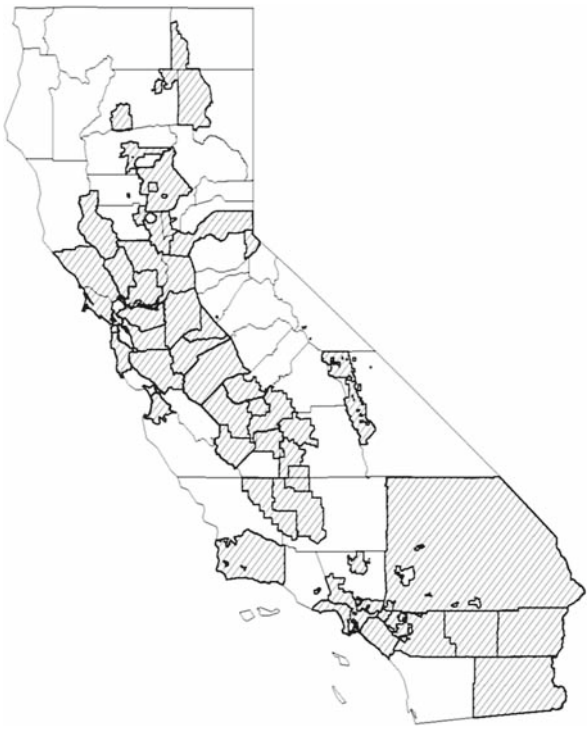

Fig. 1 Map of California mosquito control districts

Educating the citizenry on how they can reduce mosquitoes around their homes and protect themselves is a critical component of effective mosquito control. Districts in California participate in collaborative efforts to inform the public on how mosquitoes develop, how simple water management techniques can reduce mosquito development, and how repellent use can reduce exposure to mosquito bites. In addition, members of the public are encouraged to notify districts of potential larval development sites, so corrective measures can be performed.

When surveillance indicates levels of mosquitoes that require treatment, all districts use the least intrusive approach to control as is feasible for the situation. Managing the water, encouraging predation and larviciding are used to control mosquitoes in their immature state. Adult mosquito control is accomplished through ground or aerial ultra-low-volume sprays designed to reduce existing adult mosquito populations. Treatments are targeted when the mosquitoes are most active.

California Department of Public Health

The California Department of Public Health ("CDPH", formerly known as the California Department of Health Services) is the lead state agency for vector-borne disease prevention, providing a variety of services including information, technical assistance, training and program oversight to the general public and local government agencies engaged in vector control.

CDPH is responsible for administering "Cooperative Agreements" with signatory local government agencies ("Districts") that allows agencies to perform pesticide treatments for public health. The Agreement obligates signatory agencies to practice safe and effective vector control as well as meet applicable state and federal pesticide use requirements. The Agreement mandates appropriate certification and continuing education for District employees that apply pesticides. The Agreement also requires, reporting of adverse effects from pesticide applications, regular and proper calibration of all pesticide equipment, submission of monthly pesticide use reports, record-keeping and regular inspections by local agricultural commissioners. As a benefit, signatory agencies receive a variety of exemptions from various pesticide statutory codes and regulations. These include exemption from notice or posting requirements for pesticide applications. Agencies also do not need consent to make a pesticide application, and may even make pesticide applications in residential areas if there is a possibility of contamination to non-target persons or property. These and other exemptions are needed due to the unique role that public health professionals play when controlling disease vectors.

CDPH also coordinates a state-wide surveillance system for arboviruses and other vector-borne diseases. A network of sentinel chicken flocks is distributed throughout the state that provides an "early warning system" for potential arboviruses. These sentinel chicken flocks are bled at least every two weeks by local agency personnel. The blood serum collected is analyzed at a state lab for potential viruses. Results of these tests are disseminated back to the local agencies as

well as the public at large to give real-time information on potential mosquito-borne diseases in California.

It is important to note that CDPH does not perform mosquito control activities; they are, however, responsible for coordinating emergency vector control when disease outbreaks occur. Additionally, they are a strong advocate in the California Legislature for local mosquito control agencies and help educate both the public and legislators (and regulatory agencies at the state and fed level) for the need of effective and on-going mosquito control.

University of California

The University of California ("UC") has played a role in mosquito control since the early 1900s. The UC has consistently provided information on mosquito biology, examination of control measures, and evaluation of disease testing and monitoring.

One unique component of the UC system and the relationship with mosquito control agencies is the creation of the Mosquito Research Program. This program was formally established by the California legislature in 1972. It is a competitive, peer-reviewed grant program designed to develop information, materials, and techniques to assist state and local agencies in protecting the residents of California from mosquitoes and mosquito-borne diseases. The program assists UC Researchers in the preparation, submission and administration of grants both within and outside the UC system. Some examples of research that has been conducted in recent years include:

- Increased diagnostic capacity for the detection of California mosquito-borne diseases.
- An evaluation of the affects upon zooplankton or aquatic insects from pesticide applications.
- Efficacy of biological agents against vectors inhabiting constructed treatment wetlands.

A more thorough description of research projects may be found at www.ucmrp.ucdavis.edu.

In addition, the UC collaborated with the Department of Public Health and local mosquito control agencies to develop the California Mosquito-Borne Virus Surveillance and Response Plan. This plan helps guide local agencies in their control strategies, as well as to provide a document that can be shared with local municipalities to help them better understand the dynamics of mosquito populations, mosquito-borne diseases, and the control measures used to stem their spread.

This unique relationship of local agencies, state officials and academia has resulted in a comprehensive mosquito control program that is arguably second to none in the United States. It has often been referred to as the "three-legged stool" of effective mosquito control. The strong commitment of each of the legs of this stool

has enabled California to be a leader in effective, comprehensive, and sustainable mosquito control and could be used as a model anywhere else in the world. More information on these programs can be found at their websites.

www.ucmrp.ucdavis.edu
www.cdph.ca.gov
www.mvcac.org

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The Rhine Larviciding Program and Its Application to Vector Control

Norbert Becker

Abstract The use of insect-specific toxins from *Bacillus thuringiensis* is forming an increasingly important component of biological control strategies that are either being implemented or planned for use in mosquito control. The operational parameters for the most efficient use and monitoring of Bt toxins against in the field are discussed.

Keywords Mosquito control · *Bacillus thuringiensis* · Microbe · Germany

Introduction

In terms of morbidity and mortality caused by vector-borne diseases, mosquitoes are the most dangerous animals confronting mankind. They threaten more than 2 billion people and have substantially influenced the development of mankind, not only socio-economically but also politically. Undoubtedly insect-transmitted pathogens leading to epidemics and pandemics have been instrumental in the development, decline and fall of empires e.g. in Greece and Rome. Malaria was the dominant health problem in the latter days of the Roman Empire (Bruce-Chwatt and de Zulueta 1980). The Roman marshes were notorious for "mal" "aria" (bad air). This disease killed also Alexander the Great and prevented the conqueror from extending his empire, to mention but a few examples.

In Europe malaria was eradicated about 50 years ago. Current trends in re-emerging mosquito-borne infectious diseases, exemplified by increasing numbers of imported malaria cases and recent outbreaks of West Nile (WNF) and Chikungunya fever in Italy 2007, however, have given rise to growing public concern.

N. Becker (✉)
University of Heidelberg, German Mosquito Control Association (KABS/GFS),
67165 Waldsee, Germany
e-mail: norbertfbecker@web.de

P.W. Atkinson (ed.), *Vector Biology, Ecology and Control*,
DOI 10.1007/978-90-481-2458-9_15,

Mosquitoes may also cause a considerable nuisance in temperate latitudes. The mosquito species most commonly involved are the so-called floodwater mosquitoes, such as *Aedes vexans* or *Ochlerotatus sticticus* in river valleys that are regularly flooded, the snow-melt mosquitoes, e.g. *Oc. communis*, *Oc. punctor*, *Oc. hexodontus* in swampy woodlands and tundra areas, the halophilous species *Oc. caspius* and *Oc. detritus*, which breed particularly in the shallow lagoons found along the coasts of Southern Europe, Asia Minor and North Africa, or the rock-pool mosquito *Oc. mariae* found along parts of the Mediterranean rocky coasts, where mass occurrences can become a great nuisance. *Cx. pipiens molestus*, which is known as the "house mosquito" because of its presence close to human settlements can likewise make itself noticed in temperate zones as a nuisance (Becker et al. 2003).

Mosquitoes are extremely successful organisms due to their ability to adapt to a wide range of habitats. They are found throughout the world, except in deserts and permanently frozen areas. Ninety-two mosquito species of more than 3,200 recorded world-wide, traverse the European continent Mosquito larvae colonise a wide range of water-bodies, temporary and permanent, highly polluted as well as clean, large or small, stagnant or flowing, even in the smallest accumulations such as water-filled buckets, flower vases, old tyres, hoof prints or leaf axils. Adult mosquitoes vary greatly in their bionomics, e.g. concerning the host seeking, biting and migration behaviour and strategy for reproduction. It is the medical importance and the troublesome behaviour of mosquitoes that has aroused the interest of scientists.

The discovery of the life cycle of most vector-borne diseases at the end of the 19th century gave hope to being able to successfully control this scourge of humanity. The foundations for the control of the mosquitoes were established at the beginning of the last century. These included source reduction, as a means to reduce human vector contact. Vaccines, e.g. against yellow fever, and drugs, e.g. for malaria, were also developed. The development and use of DDT as a residual insecticide, achieved good results in the control of mosquitoes. In the 1950s it was believed that malaria would be exterminated by the use of DDT and chloroquine, but disillusionment quickly followed. Mosquitoes became resistant to the control measures and the weapon became blunt. Toxicological and ecotoxological problems were undesirable disadvantages with the use of unspecific and highly persistent insecticides. Despite considerable efforts of national and international organisations like the World Health Organisation (WHO), prevention of a more dramatic increase in vector-borne diseases is mainly what has been achieved up to date. Not only do the vectors and pathogens have tremendous adaptability, but also new types of diseases appear, such as dengue-haemorragic fever that was observed for the first time in South-East Asia in 1954. Altogether the greater mobility of people by modern means of transport, the intensified international trade, as well as fluctuations in climate, have resulted in a wider distribution of vector mosquitoes and disease-causing agents. The risk of becoming infected with a vector-borne disease has increased again not only in the tropics but also in Europe. The outbreak of Chikungunya fever in 2007 is an example for the steady threat of even tropical diseases in Europe and

elsewhere. The fight of mosquitoes requires not only integrated control programmes, in which all appropriate methods for control are used, but also knowledge of the biology and ecology of the target organism must be considered (Mulla 1994). Since the early 1970s the search for new environmentally compatible control tools started when the disadvantages of non-selective insecticides were evident and discribed in Rachel Carson's book "Silent Spring" (Carson 1962).

The discovery of the gram-positive, endospore-forming soil bacterium *Bacillus thuringiensis subsp. israelensis* in the Negev desert of Israel in 1976 and of the potent strains of *B. sphaericus* in recent years have inaugurated a new chapter in the control of mosquitoes and blackflies (Becker and Margalit 1993; Mulla et al. 1982, 1990; Mulla 1990; Becker et al. 2003). The new subspecies of *B. thuringiensis* is highly toxic to larvae of most mosquito species and to blackfly larvae. New strains of *B. sphaericus*, such as strain 2362 isolated from an adult blackfly in Nigeria (Weiser 1984) are much more potent than the first isolates and are particularly active against larvae of *Culex* species and *An. gambiae*.

The discovery of these microbial control agents marked the breakthrough in biological control, because of the special abilities of these microbial agents. Their protein crystals are highly toxic to target organisms and extremely environmentally safe. Mass production of the bacteria, the availability of efficient formulations and the easy handling of the formulated products make microbial control tools a successful new weapon against nuisance and vector mosquitoes.

In Germany over 1,000 km^2 of breeding areas have been treated with Bti, resulting in a reduction of the mosquito population year by year more by than 90% and without evidence of any harmful impact on the environment.

The German Mosquito Control Association (GMCA) – Kommunale Aktionsgemeinschaft Zur Bekämpfung Der Stechmückenplage (KABS)

The control of mosquitoes in Germany has a long history. In the 1920s and 1930s breeding sites were treated with petroleum oils (Becker and Ludwig 1983). During the 1950s and 1960s adulticides were used. However, in the early 1970s, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The outdoor attack rate on humans was more than 500 female mosquito bites per minute, greatly restricting the time village residents could spend outside. As a reaction to this natural disaster, 44 towns and communities on both sides of the River Rhine merged their interests in the GMCA/KABS, a united mosquito control programme founded in 1976 under the leadership of the local governor Paul Schädler. Now 98 cities and municipalities along a 310 km stretch of the Upper Rhine River, with a total population of 2.7 million people, have joined forces to control the mosquitoes, [mainly *Aedes vexans* (Meig.)] over a breeding area of some 600 km^2 of the Rhine's flood plain. The budget of the programme is

approximately 2.5 million Euro a year, which results in overall costs per person per year of approximately 1 Euro. The overall goal of the KABS is to control mosquitoes while conserving biodiversity. This goal can be reached effectively only, when biological control methods are used.

The control of *Aedes* mosquitoes by GMCA/KABS is based mainly on the use of Bti products. Domestic mosquitoes [(*Culex pipiens* (L.)] are controlled mainly by the use of Culinex®/Vectobac DT®-Bti-tablets in containers and septic tanks, as well as by the application of *B. sphaericus* to eutrophic ponds and ditches. The conservation, and promotion of predators is also an important goal of our programme. Therefore, the microbial control methods are integrated with environmental management (e.g. improving ditch systems for regulation of water levels and for provision of permanent habitats for aquatic predators such as fish).

The Prerequisites for the Development of a Microbial Mosquito-Control Strategy

For the successful use of microbial agents to control mosquitoes certain prior studies are necessary:

1. entomological studies of the biology and ecology of the native nuisance mosquito species (e.g. species composition and population dynamics related to climatical conditions);
2. Precise mapping and numbering of all major breeding sites;
3. assessment of the effective dosage in laboratory bioassays with field collected larvae (LC_{99} = minimum effective dosage) and in small field tests conducted in dominating breeding types under various abiotic and biotic conditions;
4. Adaptation of the application technique to the requirements in the field;
5. design of the control strategy based on the results obtained during the preparation phase;
6. training of field staff;
7. governmental application formalities for the use of microbial control agents.

Design of the Control Strategy. The control strategy for large-scale operation is elaborated according to several considerations.

1. The migration behaviour of the target mosquitoes. The objective of the strategy is to keep mosquitoes away from human settlements, and so the migratory behaviour of the nuisance mosquitoes needs to be considered. Species like *Ae. vexans* that readily migrate need to be controlled even in breeding sites that are far away from settlements (*Ae. vexans* can migrate more than 15 km when population pressures are high). Domestic mosquitoes (*Cx. pipiens*) that migrate no more than a few hundred metres are controlled only within the settlements and within a radius of 500 m.

2. The potential productivity of mosquitoes of a breeding site. This is a criterion for the relevance of a breeding site (assessment of the mosquito threshold for the control).
3. The climatic conditions (changes of the water level, length of rainy and dry season), which influence the occurrence of the mosquitoes.
4. The population dynamics of the target organisms. These determine the best timing of the treatment which causes the strongest negative impact on the target organisms.
5. The residual effect of the microbial control agent, which can be relevant for the sequence of re-treatments.
6. Adaptation of the control technique to the ecological conditions. According to ecological conditions, such as water level and vegetative growth, the application of Bti may be made on foot or by helicopter.
7. Development of an integrated control strategy, including predator, environmental management, and community participation.

Routine Treatments

The flood plains of the Rhine are usually inundated several times each summer. The extent of the flood water depends on the snow-melt in the Alps and on rainfall, and it is constantly necessary to monitor the water flow in the Rhine and in the flood plain. During flooding, mosquito larvae hatch within minutes or hours of the temperature exceeding 10°C. Before control measures are begun, the larval density and the larval stages are checked by means of ten sample scoops at representative breeding sites, in order to justify the action being undertaken and to establish the correct dosage and the best formulation to use. One day after application, spot sample scoops are taken at the reference breeding sites to check mosquito density and thereby establishing the efficacy of the treatment.

According to the extent of the flooding, 10–20% of the potential breeding areas of 600 km^2 has to be dealt with regularly by the 400 collaborators of the GMCA/KABS. For treating first and second larval instars, 200 g of powder formulation (Vectobac WDG, 3.000 ITU/mg) or half a liter of liquid concentrate (1.200 ITU/mg) are dissolved in 9–10 L of filtered pond water for each hectare treated and applied by a knapsack sprayer. For deeper sites or when later instars are present, the dosage is doubled. During the worst floods, a third of the area is treated with Bti granules dispensed from a helicopter (dosages: 10–20 kg/ha). From 1981 to 2007, 100 tonnes of Bti powder, more than 2,000 tonnes of Bti granules (ice or sand granules), and 50 tonnes of Bti liquid concentrates have been used, treating over 4,000 km^2 of breeding area.

Control of domestic mosquitoes is mainly carried out by householders. To assist with this, GMCA/KABS provides information on the biology of *Cx. pipiens molestus* and on appropriate control measures. Culinex®/Vectobac DT® tablets have been particularly successful. They kill *Culex* larvae in water containers over a period of several weeks. In drainage systems and large cesspools with eutrophic water

bodies, *B. sphaericus* as a liquid or powder formulation is applied against *Culex* larvae. Each year about 1 million of Culinex-Bti/B. sphaericus tablets are successfully applied against *Culex pipiens* especially in rainwater containers.

Geographic Information Systems (GIS)

Geographic Information Systems (GIS) have now become very widely used in our programme, in dealing with spatially related data. Modern information technology is used for the integration of GIS systems with database technology, and with digital mobile field data collection systems supported by a Global Positioning System (GPS).

GIS and information technology improve the survey, logistics and documentation of mosquito control operations. The possible applications range from direct digital site mapping using GPS assisted mobile devices, to timely aggregation of operational reports.

A spatially referenced database containing all features of interest is the basis for data collection and analysis. This spatial element enables thematically related features (e.g. population densities of certain species, flooding areas, plant associations and vegetation type, zones of human nuisance or disease) to be organised in separate layers of information, which can then be analysed and displayed in a user defined context.

The following applications are done:

- Analysis and query of available digital maps, aerial photos or satellite imagery, and thematic maps (e.g. hydrology, flooding zones, wetland inventories), in order to determine potential larval habitats.
- Spatial analysis to determine relationships between human nuisance or disease, and breeding sites (calculation of buffer-zones, map- and database query).
- GPS-assisted field data collection and breeding site inspection (detailed habitat mapping, larval and pupal survey). The use of handheld systems that synchronise data with the main data base, allows accurate and timely processing of results and data base updates.
- Forecasting of time and location of appropriate control activities, based on correlations between the spatial occurrence of triggering events for larval development (e.g. water levels and flooding areas, local weather data, the potential of larval development sites, and the results of current survey data).
- Preparation of operational maps to improve logistics, to calculate the quantities of control materials and manpower required, and to calculate the duration and costs of treatments.
- Storage of historical site profiles and related attribute data on the basis of operational maps enables future potential larval development, resulting from dynamic triggering events, to be predicted.
- GPS-assisted operations allow the tracking and direct digital documentation of field activities (e.g. aerial application).

– Reports and documentation of survey and control activities are assisted by user-defined database and map queries, which give immediate access to information stored in the database. The results of the queries can be visualised and printed in the form of standardised thematic maps, graphics or tables.

Monitoring the Program

Some 8% of the GMCA/KABS resources are invested in monitoring mosquito numbers, mosquito resistance and environmental impact. All the studies carried out to date show that the introduction of Bti and *B. sphaericus* has reduced the numbers of nuisance mosquitoes to a tolerable level while the ecosystem as a whole has not been damaged.

Monitoring mosquito numbers: To monitor mosquito abundance, 42 comparable sites throughout the entire inundation area are assessed. These are monitored twice a month from April to September, on each occasion for a whole night, and the mosquito density is sampled by means of carbondioxide-light traps. Catches in areas where no control measures have been undertaken serve as points of reference (100% of the mosquito population) for catches from areas being controlled, in order to determine the success of the measures (mortality rate in percent). It has been shown that since the widespread application of Bti began in 1981, over 90% of the population of *Ae. vexans* has been killed each year and, despite extreme serious flooding in the past few years, mass occurrences of mosquitoes have been successfully averted. Naturally, these control measures have had an extremely positive reception among the local people.

In September 2007 *Aedes albopictus* has been first time recorded in Germany and occurs now in 14 European countries.

Monitoring the environmental impact: It has been essential to document the environmental impact of Bti and *B. sphaericus* applications in order to provide a scientific basis for rebuting the arguments commonly brought against mosquito control by its opponents. Before large-scale application of microbial control agents was undertaken, the most important members of various aquatic groups (*Cnidaria* to *Amphibia*) were screened in the laboratory and in small-scale field trials for their susceptibility to microbial control agents. This work showed that in addition to mosquitoes and black flies, only a few species of midges were affected by Bti. For the most part these midges were much less susceptible to Bti than the target organisms. *B. sphaericus* is toxic to an even narrower range of insects: certain mosquito species, such as *Culex* species, are highly susceptible, *Aedes* species are much less susceptible, and black fly larvae as well as other insects (exception: *Psychodidae*) and nontarget organisms are not susceptible.

The development of insects in treated and untreated water is continuously monitored using emergence traps (photo collectors). The occurrence of insects in treated areas is assessed by regular light trap catches. All investigations have shown that while the numbers of *Aedes* mosquitoes are drastically reduced, all other insects

continue to develop in the water and, as winged adults, provide a food resource for birds, amphibians and bats.

Monitoring Resistance. Mosquito populations are checked at regular intervals for the development of resistance. No resistance has been detected after ten years of treatment with Bti[6,7]. To prevent resistance to Bsph developing in *Culex,* Bsph and Bti are used alternately in the control management plan for this species.

The Application of Microbial Control Agents Against Vector Mosquitoes

In cooperative programs we are aiming at reducing malaria morbidity and mortality by implementing integrated control programs including the use of microbial control agents as larvicides for the suppression of mosquito vectors to curb the potential threat of malaria, dengue and lymphatic filariasis. In addition to bed-nets, residual indoor spraying and appropriate diagnosis and treatment of malaria parasites as the major tools in the WHO Roll Back Malaria Program, microbial control agents are efficient, cost-effective and environmental safe tools to combat mosquito-born diseases such as malaria. Thus, for instance the use of DDT for indoor spraying can be reduced or avoided and the pollution of water and the accumulation of DDT in the fed body of humans can be prevented.

In our view the most effective method for controlling vector populations is to control the larvae at their breeding sources to kill the mosquitoes before they emerge as adults. When breeding sites are accessible and defined, larval control of mosquitoes has major advantages over adult control:

First, in contrast to adults, larvae are concentrated in predictable sites that can be usually easily accessed, treated or manipulated with no chance of the larvae escaping. High density of vector populations can be killed when they are condensed in a very limited area. In our studies in Cotonou, West-Africa it could be shown that usually the anophelines (*Anopheles gambiae s.l.)* bite inside the houses and lay their eggs close to the houses in defined small swamps and puddles usually in the yards of the houses. Because larvae are more concentrated than adults, it is possible to achieve successful control with less input into the environment. The principle of our work can be best described in a allegory. “If somebody wants to cut a tree, he shouldn’t start to cut branches at the top of the tree, but should cut the tree at the roots.”

Second, biological control agents are easy to handle, safe for the environment and the user. The new Water Dispersible Granules (WDGs) can be stored for years without loss of activity, easily be dissolved in water and applied.

Third, they are cost-effective. In preliminary tests it could be shown that in a district of Cotonou with 1,000 houses, less than 1 ha of breeding sites had to be controlled to eliminate the anopheline larvae (less than 500 g of the products are needed = amounts to less than 15 Dollars/district/per treatment/control phase). The costs per person per year amount to less than 0.7 Euro.

Fourth, the predators are not killed. We can utilize the power of the nature by conserving the predators which will feed upon newly hatching mosquito larvae after the treatment.

Fifth, by implementing an integrated programme which is not soley based on impregnated bednets will reduce the likelihood of resistance to pyrethroids which are used for impregnation of the nets.

Large-Scale Applications of Microbial Control Agents

Beside in Europe and North America large scale applications of Bti and Bsph are on-going in many parts of the world. Control of the main vector, *An. sinensis* in the Hubei Province (with over 20 million people on both sides of the Yangtze River) was achieved by applying microbial control agents (Bti and *B. sphaericus)* resulting in reduced malaria incidence by more than 90%, from 8.2 to 0.8 cases per 10,000 people from 1986 to 1989 respectively (Becker and Margalit 1993).

Since 2006 large-scale field applications take place in all detectable mosquito breeding sites in Cotonou, Benin, West Africa. Precise mapping and individual numbering of each significant breeding site enables rapid effective communication between field staff, and so provides a solid basis for a successful operation. All houses were numbered (right site of the streets = odd (1, 3, 5 etc.); left site of the streets even numbers (2, 4, 6 etc.). On each site of the streets two people were mapping. A group of two people needed about 4 h to map about 60 houses. Mapping forms were prepared with information about size, character of breeding sites, vegetation and larval occurrence.

Formulation selection were based upon habitat characteristics and the specific local situation. Application amounts were calculated upon surface area of the larval habitats. VectoBac®/Vectolex® WDG is used at a rate of 0.25–0.5 kg/ha, with specific rate selection depending upon water quality, species susceptibility and habitat conditions. WDGs are applied suspended in water at a total spray volume of 10 L/ha. For granular applications, 5–20 kg/ha of VectoBac/Vectolex G, with specific rate selection depending upon water quality, species susceptibility and habitat conditions density of vegetation. Vectolex WDG is also used at rates up to 10 g/m^2 (sewage tanks) in defined breeding sites to achieve a long term effect of several weeks up to several months.

Treatments are done by 12 spray teams in one district of Cotonou, each consist of two people each equipped with a Mesto knap sack sprayer (volume 5 L). Small plastic bags containing e.g. 125 or 250 g of Vectobac WDG or Vectolex WDG were be prepared for quick use in the field, each dissolved in 5 L of water and filled into the knap sack sprayer by using a net to avoid clogging of the nozzle. The size of the nozzle is 0.8 mm. According to the experience a spray team needed for about 100 houses about 5 h. For 100 houses much less than 5 L of the spray dilution were enough because of the small but very productive breeding sites. All breeding sites which were mapped are treated.

Larval densities have been assessed prior to treatment, and at 24 or 48 h post-treatment by standard dipping. The treatments are conducted twice a month. Four Treatments will be done with Vectolex WDG and the next two treatments by Vectobac WDG to avoid the risk of resistance against Bsph.

Source Reduction

Breeding places are eliminated also by physical alteration of temporary pools by drainage wherever possible and/or by filling. Environmental management approaches to vector control aim at modifying the environment to deprive the target vector population of its requirements for survival (mainly for breeding, resting and feeding). This reduces human-vector contact and renders the conditions less conducive to disease transmission.

The training component of the project is a very important issue since its output would have a strong impact on the future development and establishment of a sustainable environment-friendly control program. The activities include training in pest monitoring techniques for specific habitats, species identifications, Bti/B. sphaericus application techniques, field experimentation techniques, bioassay methodology for microbial agents statistical analysis, database and record maintenance.

The use of this innovative method of integrated biological control of *Anopheles*–vectors of malaria and other mosquito vectors of diseases, by ecologically safe methods, should result in significant improvement in public health and bring about economic benefits for the inhabitants of Benin, West-Africa.

In several East-African countries such as Kenya and Tanzania similar results have been achieved (Fillinger et al. 2003, 2004; Fillinger and Lindsay 2006). The following formulations and dosages have been used in a pilot project in Mbita, Lake Victoria, Kenya: VectoBac WDG (3,000 ITU/mg); VectoBacG (200 ITU/mg); VectoLex WDG (650 ITU/mg); VectoLex CG (50 ITU/mg). The optimum effective dosages were assessed as 0.2 kg/ha for WDGs, 4 kg/ha for Vectobac G and 2 kg/ha for Vectolex CG. The larvae of *Anopheles gambiae ss* proved to be more susceptible to B. sphaericus than to Bti. During the mapping activities 419 breeding sites have been identified/336 man made (80%). Four people have treated all breeding sites in 2–3 days. Rotation between Bti and B. sphaericus should avoid resistance against B. sphaericus. Monitoring of the adult densities in 12 houses in 14 days intervals; additionally large scale adult collections (10 houses per zone) in 2-monthly intervals revealed a reduction of the mosquito population by more than 90%. Before the treatment 51% of the breeding sites were regularly infested with *An. gambiae s.l.* and after several rounds of treatment only 7%. The occurrence of L_4 larvae reduced from 33 to 0.6%, the overall reduction of larvae by 93%. The overall reduction of adults by 92% and the costs are less than US$ 0.90/person/year.

Bti is used to control the malaria vectors *An. albimanus, An. rangeli, An. nigerrimus* and *An. sundaicus* in Peru, Ecuador, Indonesia and Malaysia, where, mosquito density was reduced by more than 85%.

Bti tablets are increasingly used against *Ae. aegypti* and *Ae. albopictus* (Mahilum et al. 2005).

Acknowledgement We are expressing our grateful thanks to Professor Dr. Mir Mulla who was the driving force for the implementation of efficient control programs world wide, so also in Germany. The German public acknowledged the contribution of Professor Mulla very much in press releases and celebrations during several stays of Professor Mulla in Germany.

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Integrated Malaria Management

Robert J. Novak, Peter Burgess, and Ian Brooks

Abstract Integrated Disease Management (IDM) as a strategy for controlling the spread of vector-borne disease utilizes the transmission cycle of the disease as the foundation for developing surveillance programs which utilize life history and environmental data. The steps and components required for the implementation of an IDM program are described with respect to controlling malaria.

Keywords Integrated disease management · Vector control · Malaria · Mosquito

Introduction

The concepts and practices of Integrated Disease Management (IDM) were largely developed following the response given to crop or agricultural pests through the use and implementation of Integrated Pest Management or IPM strategy. The initial step in IDM is to identify and define, as best as possible, the biological components of the disease/pathogen system to be managed for a specific area or landscape. The philosophy of homogeneity in landscape or ecology of the disease system to be managed is contradictory to IDM, although this working philosophy is the world standard employed by both national and international agencies. The transmission cycle of the disease is the cornerstone for developing a surveillance program based on local and factual information on the life histories of the vector(s), pathogen, host(s) including both human and animal as well as pathogen reservoirs or maintenance species. An IDM program can be initiated for all public health pathogens, since the range of critical information necessary to make evidence-based decisions

R.J. Novak (✉)
Division of Infectious Diseases, William C. Gorgas Center for Geographic Medicine, University of Alabama-Birmingham, Birmingham, AL 35294, USA
e-mail: rjnovak@uab.edu

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ranges from clinical to environmental. The surveillance Program not only monitors the multidimensional components but provides a solid basis to determine when, where and how to implement optimal management interventions for a particular area or defined human population whether these interventions are environmental, clinical or of a personal protection nature. Integrated Disease Management programs, once initiated, should always be dynamic; modifying or replacing various aspects of the program with improvements in surveillance or control technology. Management generally improves with the refinement of descriptive and predictive models for the components of the management unit. Monitoring has several important benefits. Recording trends in vector populations and disease incidence is the basis for developing and refining predictive models, as well as for determining intervention failures and the development of insecticide and/or pharmaceutical resistance.

The key components of an Integrated Malaria Management Program are illustrated in Fig. 1. An Integrated Malaria Management Program (IMM) is based upon well-established principles. Laveran's discovery of the malaria *plasmodium* in 1880 and Ross's discovery of its *Anopheline* vector in 1897 led to the development of Integrated Malaria Management (IMM) and anti-malarial drugs. By 1904 Gorgas had initiated the first anti-malarial IMM program, making possible the building of the Panama Canal. Unlike AIDS and tuberculosis, malaria cannot be transmitted directly from human to human but requires that a female *Anopheles* mosquito withdraw blood from an infected person, amplify the parasite, and inject it into a naïve person, where the parasite is again amplified. William C. Gorgas's IMM approach combined environmental modifications and mosquito vector control with human host protection using screened housing, drugs and quarantine. Although many drugs cure malaria, re-inoculation occurs and drug resistance arises as mutant parasites are selected as drug concentrations wane. Using an IMM approach where simultaneous elimination of the plasmodium parasite from humans with drugs and from mosquitoes with vector control tactics can reduce the development of both drug and insecticide resistance. Implementing environmental and clinical tactics with the help of ecology and geographic information systems (GIS), we can reduce re-infections drastically over large areas and eliminate malaria from entire countries whereas individual tactics deemed "silver bullets" are limited to small areas and historically invariably fail.

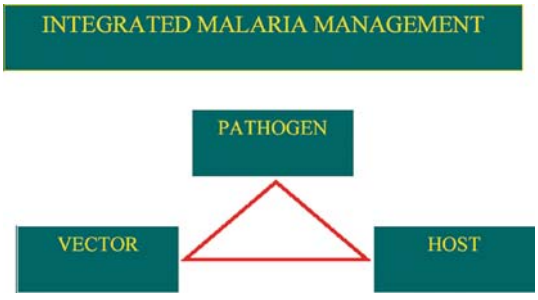

Fig. 1 The key components of an integrated malaria management program

The idea that integrated malaria management will not work in Africa is refuted by its success in tropical Panama and New Guinea. The strategy that allowed Gorgas to reduce malaria in Panama from 85 to 15% from 1906 to 1911, whereas 25% of French workers had died there earlier, was used by the US military in both World Wars I and II. High infection rates that exceeded present rates in tropical Africa were reduced successfully using the IMM (see Fig. 2) strategy of addressing the vector, parasite and human host (McCoy 1944; Russell 1968; Hays 2000). Brigadier General JS Simmons stated: "Malaria was the single most serious health hazard to allied troops in the South Pacific Area during World War II; it caused five times more casualties as did combat . . . at least on Guadalcanal (and the) Solomon Islands, this disease threatened the success of the military campaign". In 1942 the Surgeon General of the Army created an integrated system of malaria survey and control units (Hays 2000). The results from this strategy were spectacular; in New Guinea the cases of malaria decreased from >1000 per day to 1 per month in a 6 month period. In the United States and Israel, malaria was successfully managed and than eliminated using the evidence approach dictated by IMM (Kitron and Spielman 1989). Direct proof that IMM works in Africa came from the Zambian copper mines in 1920–1940 and recently from Eritrea. It is interesting to note that with the development of DDT post World War 2 to kill adult *Anopheles* species concurrently using chloroquine to eliminate the plasmodium in the human host, shifted the worlds thinking from a evidence based multi-tactic approach to a "silver bullet"

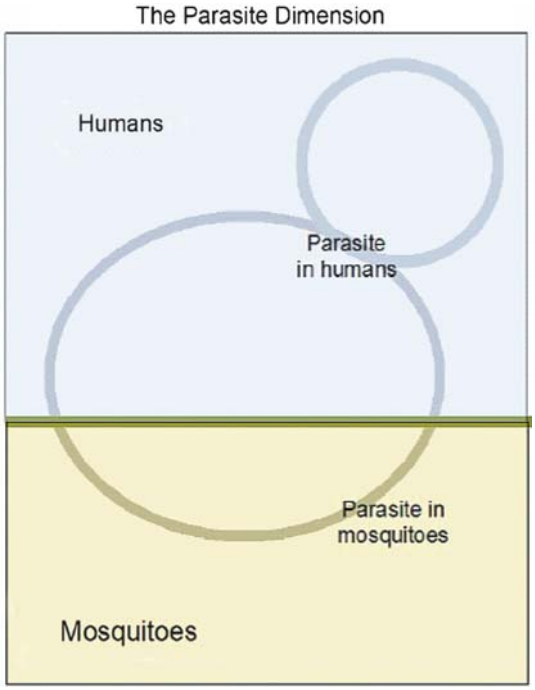

Fig. 2 The parasite moves from human to mosquito and to humans again in the course of its life cycle

approach not only to manage malaria but to eradicate both the vector and parasite from the planet. This mentality exists today with the global thought on malaria control resting on insecticide impregnated bed nets and artemesian drugs.

The overall goal of malaria control interventions is to reduce the prevalence of malaria in order to reduce both mortality and morbidity associated with mosquito-borne disease. There are two associated critical factors needed to achieve the maximum reduction in malaria prevalence, which are to use the least amount of scarce resources and the protection of the environment. The justification for these factors is that they will help insure an improved quality of life and enhance the economic performance of the area. The operational objective is to rapidly reduce the incidence making malaria a rare disease that will lead to eradication. Optimum malaria control interventions must be evidence-based focusing on malaria transmission dynamics including the human host, plasmodium parasite and vector mosquito or mosquitoes. This gathering of multidimensional information provides for the fundamental information necessary to develop an integrated plan employing a variety of operational and tactical tools to simultaneously aim to: (1) Reduce the parasite in the human population, (2) Reduce reinfection of the human population and lower the prevalence of malaria parasites in the mosquitoes, and (3) Reduce mosquito populations in and around concentrations of human (cities, towns villagers etc.). This strategy is defined as Integrated Malaria Management.

Background and History

A Integrated Malaria Management (IMM) program that is composed of a pathogen and vector surveillance component, a computer-based cyber-environment modeling system and management operational information is styled after the successful Integrated Pest Management (IPM) approach employed by agriculture. Although the term IPM was not coined until the turn of the 20th century the ability to control malaria using a combination of interventions based on data about the host, parasite and mosquito was demonstrated by William C. Gorgas, during the construction of the Panama Canal. Gorgas's basic concepts a century ago remain valid today. In fact, the key factors, vector, parasite, host protection, that Gorgas laid out were than applied by Russell, King and Soper during WW1 and 2 and by Soper with the eradication of *Anopheles gambiae* in South America. This strategy was employed by than young public health officers during WW 2, which significantly aided the Allied Armies in both the Pacific and European Theaters. After the war these same Officers employed at Universities, in government positions and private industries continued the Integrated Disease Management operating philosophy through their work and by training the next generation of public health scientists. Drs. Hess, Bradley, May and Metcalf utilized the concept of IDM by basing the control of malaria in the Tennessee River valley during the dam building era of the Tennessee Valley Authority or TVA. During post WW 2 the insecticide DDT and the antimalarial drug chloroquine prompted the Global campaign to eradicate malaria. The

campaign was a success in temperate regions of the globe but resistance to DDT and chloroquine essentially caused the eradication campaign to end in 1962. From this point in time to recent years the disease has been virtually ignored with funds being challenged to a new generation of silver bullets ranging from vaccines genetically altered vectors to bed nets. Investigations on the biology of the parasite, vector and host essential became passé. In summary the United States has a long history of integrated vector and disease management although the terms were not coined until the late 1960s early1970s. The US has for over 100 years developed the strategies, methods and tactics to control a variety of mosquitoes using integrated approaches have not had the opportunity to address the major sub Saharan African problem of malaria on a large scale.

Malaria has been eliminated in many locations that were endemic a century ago, but the scourge of malaria remains critical in Africa where WHO reports more than 3 million people die annually and 1 million are young children. A big part of this problem is that there are inadequate resources in the African health sector to control mosquitoes and malaria, and with insufficient resources, it is impossible to break the vicious cycle of malaria transmission. Instead of making the situation better, some of the limited interventions over the past years have contributed to making the situation worse by accelerating insecticide resistance in the mosquito and drug resistance in the malaria parasite.

Since 2000, African and international health leadership have recognized the challenge and have significantly committed specific resources to malaria. However, the data about program effectiveness and performance remains weak or unavailable and while there are media-like indications of success, the evidence basis for much of the reporting of success is poor.

Over the last 50 years the concept of mosquito abatement efforts to control vectors that transmit malaria, filariasis, yellow fever, dengue etc. as well as pest species have progressively shifted to adult control. Availability of equipment, the use of residual pesticides (notable DDT), strategies based on single intervention techniques (eradication of malaria/yellow fever vector and later the parasite) have shifted the focus from the optimum solutions. In time emphasis shifted from field work seeking out the sources of mosquitoes and limiting the use of pesticides to only those places where and when a routine attack with little regard for specific mosquito information or to methods that provided limited utility in providing artificial insecticide barriers. The introduction of hypertoxic residual pesticides aided in losing sight of the importance of ecological information to manage mosquito populations even when they were used in low concentrations and carelessly applied many times over broad areas. First residual insecticides were used as larvicides because they provided many answers to logistical questions, and they were effective at low concentrations. Then came the era of ground and aerial application of thermal fogs and mists. Even though it is a confirmed fact that these application techniques will eventually cause resistance if continually used or misused, the ease of application took precedence over sound empirical data. These often times indiscriminate use of pesticides violate the first principal of Integrated Pest Management (IPM) or Integrated Mosquito Management (IMM) of effective control based on the principal of attacking the

pest/vector when it is most concentrated, least mobile and most accessible or the CIA approach.

The integrated approach originally used successfully by Gorgas during the construction of the Panama Canal had a focus on the human, the habitat, the parasite and the mosquito. A similar set of interventions is the basis of IMM. There are many possible interventions and variants of these interventions. They fall into three main areas:

1. Medical
2. Personal protection
3. Mosquito (vector) control

The reason there are three areas of intervention relates to the basic biological science where there are interactions between the human, the parasite and the mosquito vector. Figures 2, 3 and 4 help to show the complex dynamic in a simple understandable form. Figure 2 is a reminder that the parasite moves from human to mosquito and to humans again in the course of its life cycle. The parasite may remain dormant in the human host for a long time principally with *Plasmodium vivax*. The parasite becomes active from time to time and causes bouts and in many cases numerous bouts of malaria. When a mosquito bites, takes a blood meal, there are several possible consequences:

1. the mosquito is malaria parasite free and the human host is not infected in which case the mosquito remains non malarial,
2. the mosquito is infected and the human host has malaria in which case the bite does not change the situation,
3. the mosquito is not infected and the human host is infected in which case the mosquito becomes infected with the malaria parasite,
4. the mosquito is infected and the human host is not in which case the host becomes malarial.

Figure 3 shows the dynamic of the mosquito/human host interaction in a simplified way. With an abundance of mosquitoes and a high prevalence of infected mosquitoes the prevalence in the human host increases. Efforts to limit contact between the mosquito and the human host helps, but is not easy to do with a high degree of effectiveness for all the times the mosquitoes may be looking for a blood meal.

With medical treatment (Fig. 4), the malaria in the human host can be reduced. This has a favorable impact on the mosquito human interaction, but it is small for the population taken as a whole. There is little impact on the mosquito population and the prevalence of malarial mosquitoes.

The mosquito population can be very favorably impacted by source control. Figure 5 shows how the population of mosquitoes can be substantially reduced by active source control. With no source control the mosquito population stabilizes at a level that is governed by general environmental considerations, of which the weather

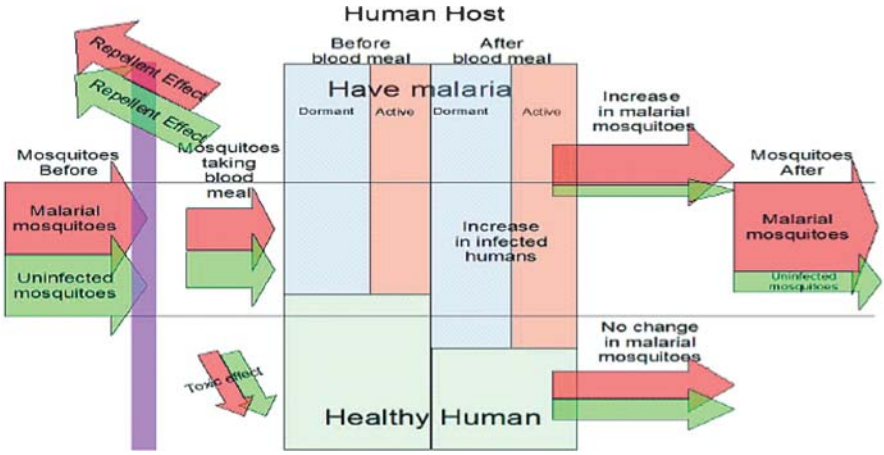

Fig. 3 The dynamic of the mosquito/human host interaction

is one of the most important. With source control the population of mosquitoes can be reduced significantly.

When there is an abundance of adult mosquitoes, the use of adulticiding (IRS, ULV etc.) will reduce the mosquito population. Figure 6 shows two situations. In the first case the adulticiding is successful and mosquitoes are killed and the population is reduced. In the second case there is resistance to the first chemical used, so the procedure is repeated using a different chemical treatment. The cost and intermediate result of adulticiding suggests that this should be a significant part of integrated malaria control interventions. On its own adulticiding is going to have little impact on the ultimate goal because there will be rapid reestablishment of the mosquito

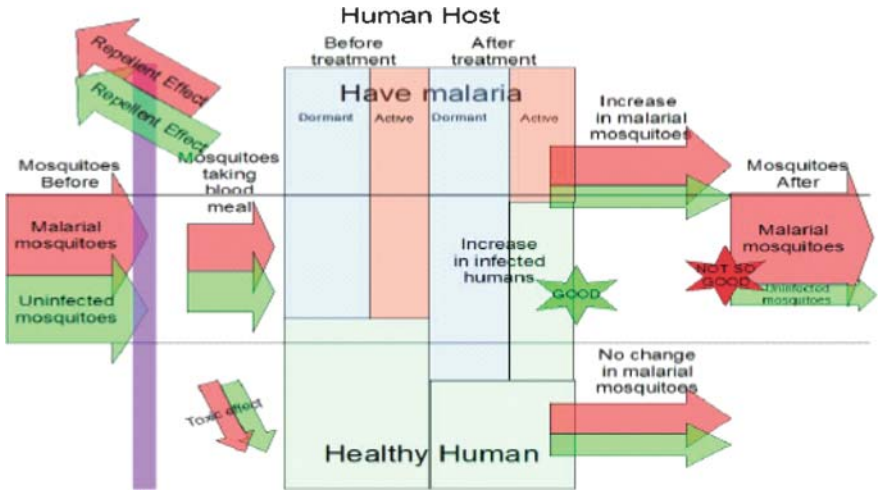

Fig. 4 The dynamic of the mosquito/human host interaction with medical treatment

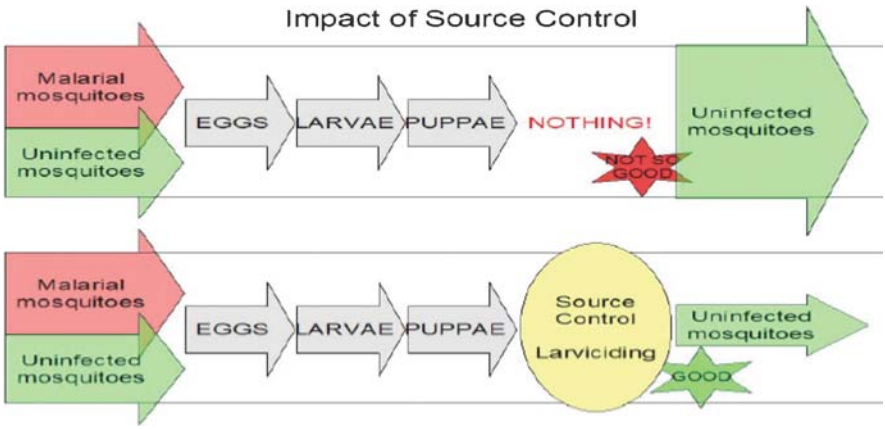

Fig. 5 Impact of the mosquito population with source control

population and because there will also be rapid reinfection of the mosquitoes with the parasite. The use of adulticiding might, however, be a very powerful factor in accelerating the impact of other interventions, specifically the medical treatment and the larviciding, and in combination give an optimized performance.

Figure 7 summarizes in a simplified way the various interventions involved in integrated mosquito and malaria management. The primary goal of reduced malaria burden in society is shown in green, in the human section before and after. The related goals, the reduction of parasite in the human host and the reduction of parasite in the mosquito population are also shown. Between before and after there are a portfolio of possible mosquito and malaria control interventions. Best performance is for the costs of the interventions to be the lowest possible for the maximum

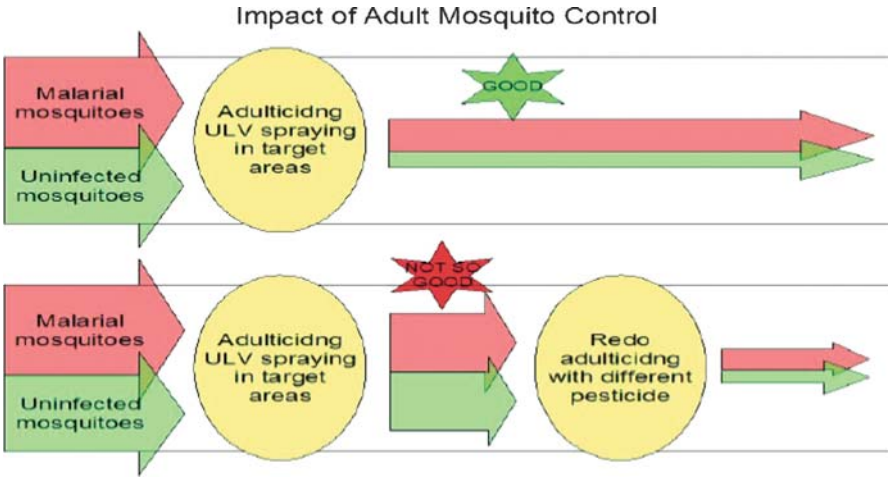

Fig. 6 Impact of the mosquito population with adult mosquito control

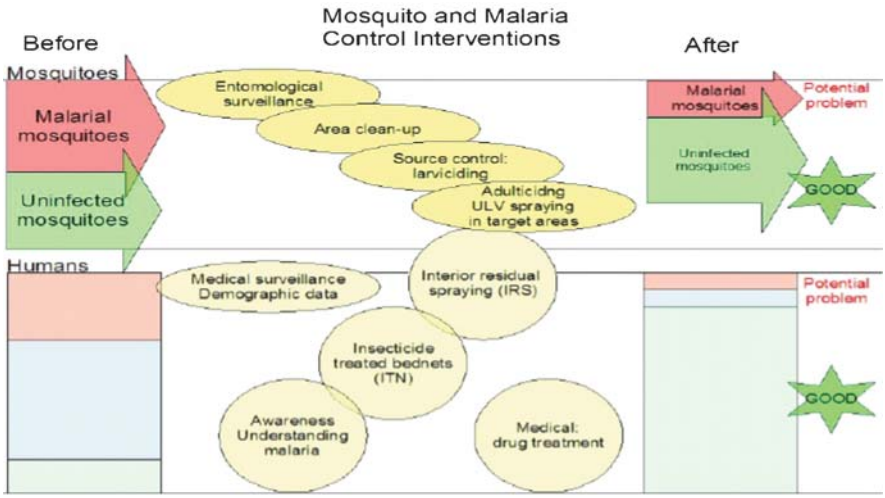

Fig. 7 The various interventions involved in integrated mosquito and malaria management

of improvement or progress towards the ultimate goal of reducing malaria burden in the community. The science of each of these interventions is quite well known. Rather little is yet known about the optimum way to combine these interventions for best performance.

The Medical Component of IMM

Malaria is a deadly disease for children and pregnant women, and some types of malaria are also deadly for everyone. Malaria in all its variations is a debilitating disease for everyone. Accordingly, the treatment of malaria is a priority for medical personnel. The popular low cost drug Chloroquine is no longer effective in most situations because of a build up of resistance to this drug. More recent drugs like Fanzidar are also showing signs of widespread resistance. A more recent therapy using Artemesinin Combination Therapy (ACT) is more powerful and there is no resistance reported as yet, and it is an effective therapy. The cost of ACT is variable and as with Chloroquine or Fansidar, out of reach economically in most communities in Africa without a subsidy. Medical treatment of active cases is a high priority in the health sector, but the issue of resistance is a major concern. Where there is perpetual reinfection, and the treatment is limited to the existing drug therapies, there is absolutely going to be resistance development, and most likely quite rapidly.

Medical treatment that addresses the active malaria bout should be supplemented by medical treatment that addresses the parasite that is simply hosted in the human subject. A bite from a non-malarial infected mosquito is a nuisance, the bite is not

dangerous. Medical treatment to reduce the prevalence of the malaria parasite in the human host is a key part of an Integrated Malaria Management regime. Medical treatment that helps to eradicate the disease is very much more cost effective than medical treatment that only addresses a presently active bout of malaria, that will reactivate in a matter of days, weeks, months and perhaps many times in a single year. Many malaria infections in humans are asymptomatic but are important epidemiologically since they provide for a source for re-infection. Therefore active surveillance of infected individuals followed by treatment are a central component of a IMM program.

Personal Protection in IMM: Community Awareness and Personal Protection

Increased individual and community awareness of mosquitoes and their role in the transmission of malaria, and the importance of treatment is very important. The community needs to know:

1. about how malaria is transmitted, and
2. about ways to control the mosquito population,
3. about how use of bednets can reduce incidence of malaria,
4. about the advantages of interior residual spraying,
5. about the ways to keep mosquitoes away from the house, and
6. about the recognition and treatment of malaria.

With better knowledge of these matters, the community becomes empowered to take control of many of the factors that have an impact on the malaria status of their community.

In order for a malaria management program to be successful and sustainable there is a critical need to get the community involved and running as much of the program as possible. In a situation where the malaria level has been reduced almost to zero, it is possible that everything can be done in the community with little external inputs with the exception of data logistics and the IMM cyber-environment modelling. If interventions are needed, there should be the implementation capacity available for the required interventions to be deployed as needed, together with the necessary funding.

Personal protection using insecticide treated bednets (ITN): For the past several years personal protection using an insecticide treated bednet has been a widely used intervention. The implementation and use of bednets have shown remarkable results in declining rates of mortality in young children and pregnant females. However, the use of bednets has not brought about similar results in a reduction of prevalence.

Fig. 8 Styles of bednet (this photograph from the CDC web page)

There are several styles of bednet (Fig. 8) and a variety of pyrethrum/pyrethroid-based chemicals are used. Not all the chemicals being used have been approved for use by the WHO and/or UNICEF.

The main goal in many of the programs has been to get young children who are at the highest risk of dying as a result of a malaria bout to sleep under a bednet and be protected. The result of these efforts seems to have been positive in that it seems that less children are dying of malaria in the critical first year, or even two or three, but it is less clear that children as a whole are growing up to adulthood. The possibility is that children survive initially, but subsequently die because malaria is so prevalent in the society at large.

Another group being targeted for bednet use are pregnant women who are also highly vulnerable to malaria. Again, the reports suggest that sleeping under a bednet reduces the incidence of malaria for the person involved, but this does not translate into less malaria in the community as a whole, and is probably unsustainable for the individual when they are no longer in the vulnerable group of pregnant women.

The cost of a bednet varies from around $2.00 to around $10.00, but it is not clear that these numbers relate to the same item, and the data are not easily to be found that show the makeup of costs. A "per year" cost of using a bednet is sometimes stated to be around $4.00 per person per year.

Personal protection using interior residual spraying (IRS): Personal protection using interior residual spraying (IRS) of the home is a proven way of reducing the impact of the mosquito vector on people in the home. There are several ways in which IRS impacts on the mosquito and malaria:

1. By the repellent effect which helps to keep mosquitoes out of the home,
2. By the toxic effect which kills the mosquito when they try to rest on the treated surfaces, as they would do after a blood meal. This operates along the following lines:

    a) In the event that the mosquito was not malarial before the blood meal the human subject will not become infected, but if the mosquito is malarial before the blood meal the human subject will be at risk of infection,
    b) If the human subject is host to the parasite before the blood meal, then the IRS toxicity will stop the mosquito transmitting the parasite to others.

3. By diverting anopheline mosquitoes from humans to animal feeding.

The use of DDT as the chemical agent for IRS is the most cost effective. DDT has a high repellent effect, is toxic to mosquitoes and remains effective for a long time. The effectiveness of DDT lasts perhaps as much as twice as long as other chemical agents. There are some mosquitoes that are resistance to DDT, but this resistance does not seem to apply to the repellent effect. In terms of cost effectiveness DDT appears to be several times better than other chemicals, being a less costly chemical, requiring less frequent application, and having a bigger impact on the malaria prevalence in the community.

IRS should, of course, be conducted by trained personnel who know and practice safety. The environment should be monitored to confirm that there is no undesirable environmental impact.

Personal protection using insecticide treated panels: One of the behaviors associated with some insecticides is a repellent effect, which keeps mosquitoes away and stops them taking blood meals. The technique has been used on an experimental basis with success.

Personal protection using other techniques: Exposure to mosquitoes can be reduced by many different techniques. Some of the approaches are expensive and therefore limited to the wealthier members of society. Living in air-conditioned space and using sprays to ensure that any mosquitoes are killed is effective and used by wealthy families and in international class hotels. Burning insecticide treated coils keeps mosquitoes away from possible blood meal targets. Wearing clothing that covers the legs and arms helps keep mosquitoes from reaching a blood meal.

Mosquito (Vector) Control or Integrated Vector Management (IVM)

The principal objective of a IVM program is to control locally produced mosquito populations in order to reduce the threat of mosquito-borne disease transmission and to achieve a low level of mosquito annoyance. To meet this objective we must employ the safest, most effective and economical procedures, methods and

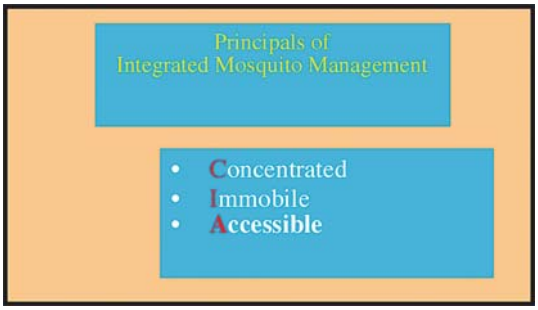

Fig. 9 The CIA approach to insect vector management

insecticides to manage mosquito populations with the least adverse impact on the environment.

The operational definition of IVM, which is based on the bionomics of the vector species can be based on three key factors based on the location that a management protocol is being developed. The key terms are Concentrated, Immobile and Accessible or the CIA approach (see Fig. 9). What this means is that in order to manage a vector population you have to establish where and when it is in its most concentrated, immobile and accessible state. This is a logical protocol since by its implementation you are going to kill the greatest number of mosquitoes with least amount of contamination to the environment. For mosquitoes the egg stage is generally considered where a species satisfies all of these 3 criteria. However, except for the elimination of oviposition or egg laying sites can be accomplished other means of reducing the population is impossible since a effective ovicidal insecticide has not been developed. Therefore for most situations the larval stage is the one that meets the criteria. This has become the tactic of choice with the advent of microbial insecticides, *Bacillus thuringiensis israelensis* the only environmental safe insecticide since it kills primarily mosquito larvae while leaving predator populations intact that may be natural regulators of mosquitoes in a aquatic habitat.

Source control or environmental management reduces the population of locally produced mosquitoes that are responsible for transmission of vector-borne pathogens and associated nuisances to human and animal populations. Killing mosquitoes at their sources, when they are in the larval stages and concentrated, immobile and accessible is the key to a cost effective program. The interventions focus on reducing the abundance of adult females, both vector and nuisance species to tolerable levels. Other measures supplement this primary intervention.

The objectives of source management in the aquatic habitat is to locate and eliminate larvae within the management area in order to reduce or eliminate adult mosquitoes in known areas of aggregation and/or concentration (Fig. 10). Larval management is the most effective and reliable way to control a mosquito population. It is considered the first line of attack see figure below. The application of insecticide when the larvae are most concentrated in the habitat also reduces the amount of insecticide, which has the dual effect of reducing potential environmental contamination as well as reducing costs.

Fig. 10 Source management in rice fields

Although controlling the adult population which is mobile is considered a second line of defense (Fig. 11) with anthropomorphic mosquito species like *Anopheles gambiae,* it can satisfy the CIA criteria and one could employ a very effect tactic, indoor Residual Spraying (IRS), to reduce the flying adult population.

Abatement plans for *Anopheles, Culex* and *Aedes* mosquito species depend on the pattern of annual and seasonal (dry and rain) rainfall and the incidence and distribution of the immature stages of the mosquitoes. In an IMM program weather data are collected and included in the IMM cyber-environment model. Climatological information especially precipitation coupled with soil moisture levels can provide

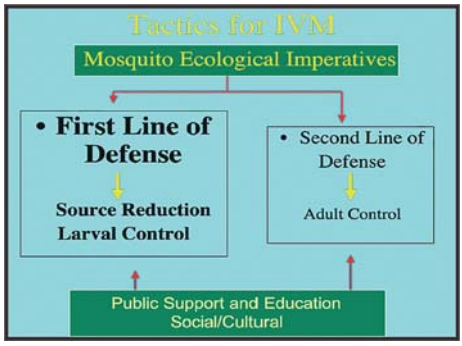

Fig. 11 Tactics for insect vector management

the kind of aquatic habitat data used to forecast or predict as well as prioritize productive sites.

Anopheles and *Culex* species have time limited estivation and/or latency capabilities in the adult, larval or egg stages and cannot remain dormant during dry periods. This is especially important in tropical and sub-tropical areas. It is imperative to locate and manage the water filled harborages that provide sustainable habitats during these times. These "seed populations" are the sources for the enormous increases in population densities that occur when the rainy season begins and aquatic habitats become numerous.

The population density of mosquitoes is directly related to weather conditions, especially precipitation (irrigation), temperature and relative humidity. Monitoring these key climatological conditions can provide the necessary information to predict which sites will be producing mosquito larvae and when. These parameters coupled with a knowledge of sub-surface water (water table) can provide additional information on where and when to begin mosquito larval applications.

A mosquito abatement area must be large enough to encompass the sources of mosquito vectors and pests it is to manage. The boundaries of mosquito control operations should be established by the affected communities taking mosquito biology into account. In most circumstances a distance of 500–1,000 m from human habitation is a default guideline for *Anopheles* species. Larval habitat surveys should be done within this area to locate any larval sites that would be a source of adult mosquitoes. Source control using larval treatments Insecticides applied for larval treatment are very effective if the insecticide application units:

1. apply insecticides to the exact sites where larvae are present as determined by the surveillance and data collection teams, and
2. everything to do with insecticides is carried out in accordance with the safety rules for humans, animals and the environment.

The personnel engaged in working with chemical and biological agents must be trained and supervised to sanitise the specific larval sites. It is optimum when the teams apply an insecticides only to that portion of the habitat occupied by the larvae while they are in their most concentrated phase (early larval instars). The insecticide prescribed must also be chosen as indicated by the environment. Protection of the environment and human health is paramount.

Surveillance data will also identify locations of breeding places that result from a variety of wastes, such as automobile tires and discarded containers of all types that collect water. All of these potential breeding places can be cleaned up and will results in a reduction in malaria producing sources.

It is often said that every "hoof mark in Africa" is a potential habitat for mosquitoes, and to the extent that this is a challenge, it may not have much impact on the success of an integrated malaria management program because the combination of interventions can be used to limit the impact of an out of control mosquito population increase on the overall health situation. The key question to determine is exactly what does a habitat produce in terms of adult mosquitoes. Several studies

have shown that hoof prints and other small habitats produce very few if any adult mosquitoes. These habitats if understood properly could be an ally to mosquito control where a female lays eggs but no adults emerge. Good filed data over time will provide this important kind of data.

While mosquito population control is best managed at the larval stage before they fly and disperse (CIA approach), modern ultra-low volume (ULV) spray technology makes it very cost effective to control flying mosquitoes. Chemicals such as Dibrom are used extensively in the United States for mosquito and vector control and the modern spray techniques available are very effective and very low cost. The cost can be as low as $2.50 per acre treated, and the per-capital cost very low depending on the population density.

Also important is that the impact of aerial ULV spraying is very fast. If an area is correctly sprayed tonight, the mosquito population will be significantly lower next morning, usually a reduction of more than 80%. If the reduction is less than this, there is a resistance problem that needs to be addressed, and changing to a different family of insecticides and re-spraying will probably deliver a reduction in the mosquito population. This is a technology that needs additional field trials in sub Saharan Africa to establish its efficacy in reducing Anopheles population, especially in large tract of irrigated lands.

In areas where mosquitoes have a very favorable habitat, a permanent reduction in the mosquito population is going to be difficult. It is possible that source control referred to above can help bring the population of mosquitoes under control.

The challenge is not to reduce the population of mosquitoes, but to reduce the population of malarial mosquitoes, and to break the currently perpetual cycle of parasite transmission from human host to human host through the mosquito vector. The role of ULV is to reduce the population of mosquitoes long enough for the other interventions to have an impact on the permanent situation.

The optimum way of doing this depends a lot on the behavior of all the interacting systems, and the data will be analyzed in order to understand these interconnections.

Community Focus: The Role of Mosquito and Vector Control Districts

Mosquito and Vector Control Districts (MVCD) or Mosquito Abatement Districts (MAD) are central to the success of vector control in the USA. They are controlled by local communities, usually paid for by local tax revenues, and are charged with mosquito and vector control as a part of public health.

Mosquito and vector control districts in the USA have a history that goes back almost 100 years. One of the first mosquito control districts was created in California in Marin County in 1915 to combat salt marsh mosquitoes. More and more mosquito abatement districts were formed through the 20s and 30s. In California and much of the USA the climate and topography supports the development of human disease vectors and nuisance pests. Because of continuing surveillance and active intervention when needed the mosquito and vector control programs have kept local malaria

cases in the USA to near zero. While malaria is under control, the risk of other vector borne diseases such as West Nile Virus makes constant vigilance important for public health where early detection and early action is a requisite to stop any impending epidemic.

Mosquito and vector control districts in the United States (as well as places like Darwin, Australia) use all the possible interventions that science shows will help to control potential vectors and be safe for human populations and the environment. Where urgent action is required the use of ultra-low-volume (ULV) spraying of pesticides like Dibrom may be used. Where surveillance shows that the vector can be controlled by larviciding, then larviciding may be used. In all control interventions great care is taken that there is no hazard to human health or to the environment. While the biological agents and chemicals used are toxic to the target vectors they are not toxic to humans and not damaging to the environment and the eco-system as a whole.

Community based MVCDs may have a role in Africa to make mosquito and vector control sustainable. Life is local, and getting malaria under control is local. The vector operates locally, and can only be controlled locally. The community has a deep knowledge of the facts about the community: the people, the geography, the entomology, the epidemiology, and the environment. The community can do surveillance on a continuing basis to provide the data needed to determine what interventions are needed to improve the health of the community and maintain an improved health status.

When MVCDs need additional support in the USA, there are easily accessible consultants, service organizations and others who can undertake the interventions and have all the equipment and experience needed. The same sort of support is needed for MVCDs in Africa, whether these are provided by government, by the private sector or by some innovative public/private partnership entity.

The IMMC has a contact network throughout the MVCD community in the United States and around the world. This community can be of long term value in maintaining success in the control of dangerous vectors like the mosquito.

Community Focus: Field Surveillance and Data Collection

Data concerning everything that has an impact on the success of mosquito and malaria control needs to be measured and the data collected and included in a process that ensures that local decisions are made correctly, as well as being used to monitor for more strategic issues in both science and operations.

Local people have to be empowered to do the surveillance and collect the data. This is not quickly done, but with awareness and training local people can be trained and will do good work at reasonable cost. Ongoing oversight and training is required as in any activity.

The data being collected will provide a range of data about the people, the geography, the entomology, the epidemiology, and the environment... in much the same way in Africa as is routine in mosquito control districts in the USA.

At the present time there are many ways of collecting data, from written papers to very sophisticated electronic devices incorporating global position information and continuously connected to a computer database. Exactly how the data are collected is less important than that the needed data are being collected.

Community Level Analysis, Feedback and Action

As much as possible the community should take responsibility for the performance of the program, and must therefore be empowered to do analysis and make local decisions to control the mosquito and malaria situation. This makes it possible to appropriate decisions to be made on a timely basis in the field where intervention is needed.

Training and Operational Support

The three most important things that a IMM program will do are: training, training and training. There is a massive amount of knowledge, but there is a challenge in getting people who need the knowledge to have it and to be in a position to use it. After training there is the need to have operational support so that a decision can be turned into practical action.

The IMM focus on data and management information helps to get decisions made that optimize the use of scarce resources, but this only will result in progress towards the ultimate goal if the ability to translate decisions into action also exists. Accordingly a IMM program must include the capacity to facilitate the interventions and undertake operational activities.

Performance Metrics and Management Information the Cyberenvironment for Integrated Malaria Management (CE for IMM)

The science involved with mosquitoes, malaria parasites and the human host is complex. While it is possible to build and operate simple models of behavior of each of the elements, and create some management information about performance, such a model would ignore many of the issues that concern scientists such as the potential for uncontrolled pandemics and the unchecked emergence of resistance and other mutations.

A cyberenvironment does better than a wall map, in essence because it can handle the same amount of data for an unlimited time series, for an unlimited number of geographic locations, and an unlimited number of different combinations of biological conditions and control interventions.

In the initial stages the cyberenvironment will not add substantial incremental value over simpler models, especially where there are effective local management information data flows. However, the cyberenvironment will have an ability to provide ongoing scientific analysis and management information very cost effectively on a long term basis. This is important because it must be anticipated that malaria will rebound to endemic levels quite quickly unless there are rapid control interventions. The best way to prevent a rebound is for there to be timely surveillance, timely analysis and timely reaction. This is exactly what a cyberenvironment for IMM will do.

A cyberenvironment (CE) for a IMM program should not be thought of as the product of one-time development projects, but as a living infrastructure that will evolve with technology and with the scientific and engineering discoveries and understanding over time. Towards this end a CE for a IMM program must be built on the principles of sustainability and adaptability using current and emerging techniques/technologies, such as web and grid services, translating/integrating middleware (e.g., MyProxy), global unique identifiers and metadata, workflow, meta-workflow and provenance, and semantic descriptions of resources and data. These types of technologies lower the architectural coupling of cyberinfrastructure and CE components while maintaining end-to-end capabilities.

The CE for an IMM Program will provide a computational framework for operational control, research and management information. All malaria-related parameters, including entomological, parasitological, socio-economic and case management data need to be tracked by household and mosquito source identifier numbers.

With these data, the cyberenvironment will be able to:

1. Simulate agent-based models of transmission and grid-based models of landscapes,
2. Tailor intervention strategies to local characteristics,
3. Integrate implementation and statistical analyses,
4. Share data and analyses for decision making,
5. Analyze progress for economic implications,
6. Serve as a data store for other future research.

A good example of a GIS CE data base is illustrated in the following satellite image (Fig. 12). The image is a 0.6 m resolution picture from Digital Globe of Kangiciri, Kenya, a village in the Mwea Rice growing Scheme. Each paddy was used to provide the grid structure and a complete data base for each of the individual grids was developed. The data-base for each grid includes biological, agricultural, treatment, irrigation and ownership which can be log in using a hand held monitor.

It is anticipated that understanding the specific interactions between the various aspects of mosquitoes, malaria and humans and the various possible interventions in specific locations will make it possible for models to optimize both the value of the interventions and the costs (Fig. 13). The synergy of integrated comprehensive actions should make very substantial improvements in cost effectiveness possible.

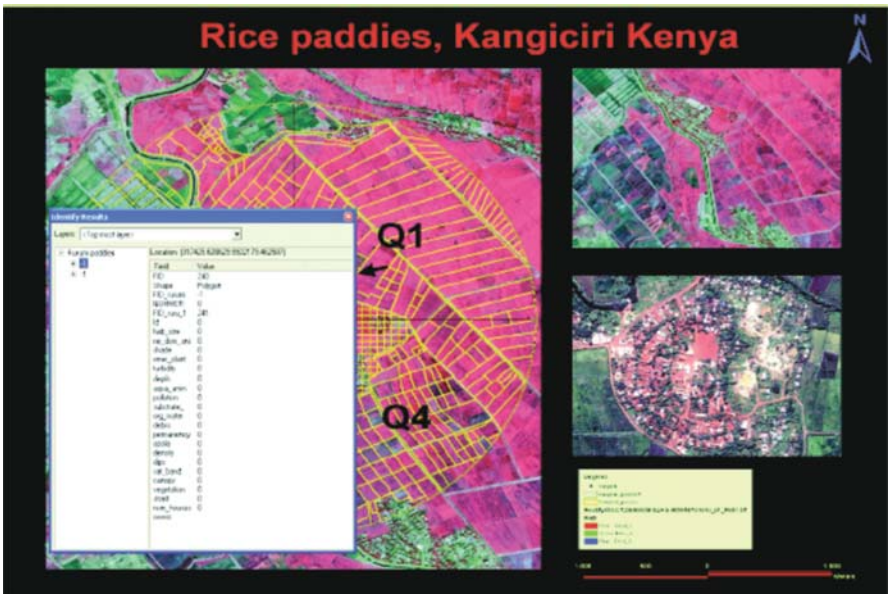

Fig. 12 Satellite image of Kangiciri, Kenya

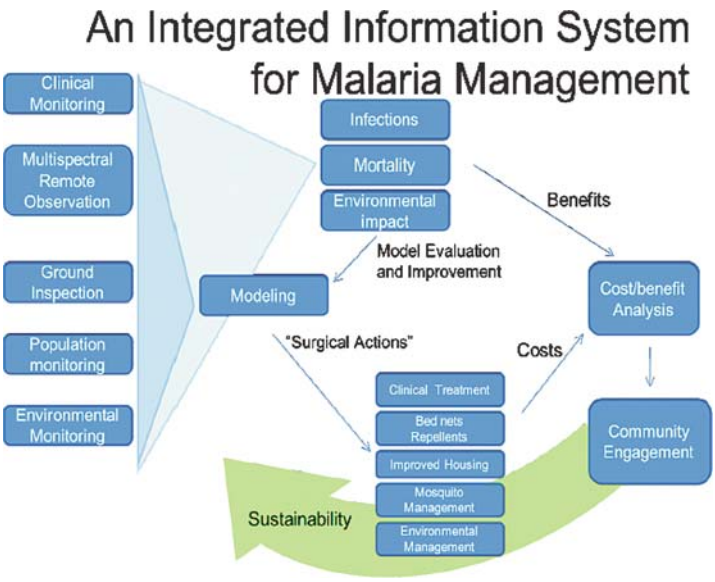

Fig. 13 Components of an integrated information system for malaria management

The proposed CE for IMM will serve not only the selected pilot locations but will also be capable for deployment into any geographic area.

The goal of the IMM CE is to provide a computational framework for operational control and research. It is also hoped that developing a framework to support all three areas of IMM as well as the economic and disease modeling will enable significant synergy and cost savings between the five groups. While an IMM CE that is currently being developed for a pilot project is in a yet to be defined region of sub Saharan Africa, is a step towards developing a CE that can be used for integrated *disease* management in any geographic area such as control of Dengue Fever in Costa Rica, HIV in Zambia and multiple diseases in Central and South America.

Collecting and handling this data in a format that will facilitate these tasks is not simple, especially considering the communication challenges associated with getting this data from remote sites in country to NCSA and returning the resulting operational support information within a workable time period. Transmission and landscape models will require ready access to very large and diverse datasets that include biological, population, behavioral, geographic, and meteorological databases. Handling and processing the datasets and multiple complex models in a heterogeneous large-scale computing environment calls for significant information technology infrastructure. Coordinating these computing and IT resources and integrating them into an effective system for decision makers, surveillance systems and responders is challenging.

To understand the feasibility of CE, consider the state of the internet in the early 1990s. At that time a significant amount of information was available, but finding it through ftp and gopher sites was unnecessarily time consuming. When NCSA developed Mosaic, these resources became easier to access. There are many resources available online today for the scientific community, including databases, sensors streaming real time data, supercomputers, visualization resources, ultra high-speed networks, and communities of researchers, but again they are difficult to use effectively. Bringing these resources together in a usable environment is the current challenge, one that NCSA is tackling with a number of CEs.

CEs incorporate collaboratory and grid technologies, web services, and other cyberinfrastructure into an overall framework that balances the need for efficient coordination with the ability to innovate. They are designed to support the full scientific lifecycle, both at the level of individual experiments, as they move from data to workflows to publication, and at the level of larger scale collaboration, where new discoveries lead to additional data, models, tools, and conceptual frameworks that augment and evolve community-scale systems. Here are examples of some of the CEs NCSA is currently developing.

The cyberenvironment for integrated malaria management is a key element for IMM management information. It facilitates rapid analysis of complex data, and makes it possible to have simple, clear, timely, material information to inform decision making. By employing the cyberenvironment surveillance system capable of developing models sing multi-stream data analysis breaking the cycle of malaria transmission and maintaining control by rapid focal identification should not only

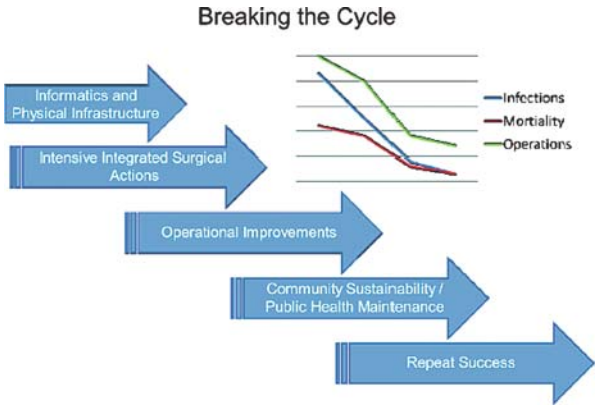

Fig. 14 Critical factors needed to break the cycle of malaria transmission

reduce but eliminate this debilitating disease. Figure 14 illustrates the critical factors needed to break the cycle.

Management Information is Important and Ubiquitous

Management information is everywhere. It is an integral part of everyone's life, but not usually recognized. In the area of sports, the measurement of performance is central to everything. In sports, in addition to the metric of winning and losing there are also the data about many other parameters of performance. Terms like "runs batted in", "throws completed", "yards run" are all part of a deeper set of metrics that help determine how to go about improving the team so that there are more wins and less losses. In the area of integrated mosquito and malaria control, the primary goal is to reduce the impact of malaria on the society ... and to do this with the minimum use of available resources. Simply put, this is getting the most VALUE for the least COST. This is the win/lose dimension of the performance metric. A secondary level of performance measurement are the operational metrics. These are measures of operational activity and their related costs. The operational activity may or may not have an immediate direct top level value, but, nevertheless do contribute to the team win ... trying winning a football game without a high performance set of line backers.

One key characteristic of management information is that it includes data that are "material" and excludes detail that has little immediate relevance. Another is that management information is timely and shows clearly what is being accomplished. Management is a pragmatic construct used almost universally in high performance corporate organizations and is simply as little data as possible at the right time that ensures that decision makers make the best possible decision. Most management information systems combine accounting information with other key metrics that reflect important operating elements. Accounting reports on their own are only a part

of management information, and operational data also is only a part of management information.

A combination of scientific data and management information helps by making it possible for non-scientific decision makers to understand the implications of their decisions and for scientists and researchers to understand the cost and value implications of their work. Though scientific data and management information are completely different in the approaches that optimize their value, they both describe the same realities, and the analysis deals with the same sets of detailed data. Moreover, scientific data tends to have the most value when the analysis can look at a very large dataset with many variables and over a significant time period. When the behaviors from the past are understood, it then becomes possible to make improved predictions about the future.

Management information, on the other hand, has the most value when it is quick, clear, easy to understand and relevant to the decisions that need to be made now. It is sometimes characterized as the least amount of information that ensures that the right decisions are being made. It is expected that IMM management information will show that there are significant opportunities to improve the cost effectiveness of the many initiatives that are presently funded and operational.

The present initiatives to combat malaria are well funded compared to the recent past. There is a commitment on the part of numerous donors to get optimum results and to be transparent and accountable. It is not yet clear whether or not the strategy is delivering optimum results, or that there is an adequate level of transparency and accountability. As fund flows have increased, there have been many reports that funds have not been used effectively to combat malaria. It remains difficult to get an accounting where there are indications that funds have not been used effectively. But even where there are clear data about activities being undertaken, there is not much associated cost accounting easily accessible for public review, and it is not easy to discern progress being made towards sustainable reductions in the burden of malaria. Accordingly an effective IMM Program will seeking to satisfy a critical need must have robust performance metrics that will make it possible for interested people and organizations to be able to see data that relates costs to results, both in terms of the intermediate activities associated with specific interventions and at the community level as a whole.

Each of the mosquito and malaria control interventions has a unique cost behavior. Because of the complex biological system and limited knowledge, the results are difficult to predict with great certainty. However, the IMM approach facilitates that data about the cost and outcome from all specific interventions becomes a part of a cumulating dataset. These interventions used in an appropriate combination provide effective, economically efficient and environmentally friendly mosquito abatement that will aid in reducing the morbidity and mortality associated with malaria and other mosquito-borne diseases. The interventions will also reduce the nuisances and pest problems associated with mosquitoes as they relate to both man and domestic animals.

The key characteristic of integrated malaria management is that ALL possible interventions are included in the analysis framework in order to be in a position

to recommend and use the interventions that are likely to give the best results at least cost.

Performance or Outcome Metrics

In the IMM approach, scientific data and management information look to the following outcome metrics:

1. Number of cases of active malaria
2. Prevalence of malaria parasite in the human population
3. Abundance of mosquitoes
4. Prevalence of malaria parasite in the mosquito population
5. Reduction in mortality associated with malaria
6. Reduction in morbidity associated with malaria

The socio-economic benefits associated with success in controlling malaria include:

1. less absence from work
2. industries like tourism less adversely affected

In the IMM approach, scientific data are compiled and analyzed to understand what it is that gets the best possible outcomes, and what is the underlying science that is driving the process. In addition, the IMM approach to scientific analysis has an element of cost analysis so that optimization does not ignore the financial and economic parameters.

In parallel, data about activities and costs are compiled so that there can be a quick, albeit crude, understanding of how much is being spent and the outcomes that are being realized. These data have the most value when the information can be used to improve immediate operations and used to improve the allocation of resources wherever resources are being used for malaria control interventions.

The three separate phases of the IMM approach are:

1. Surveillance, data collection, data analysis that determines what to do.
2. Intervention operations ... and how much did it cost.
3. Surveillance, data collection, data analysis that determines the results that were achieved in this time period and at what cost ... and what to do next.

These are reflected in Fig. 15 which shows how high performance operations integrates data collection, analysis, planning, action, more data collection, more planning, more action in a perpetual process.

The ultimate measure of success is whether the change between the initial status and the post activity status has a value that (substantially) exceeds the costs. There

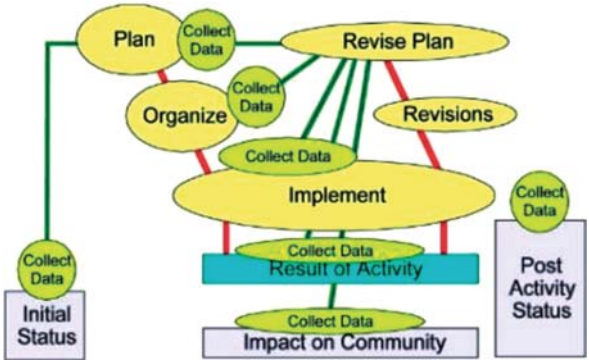

Fig. 15 Integration of data collection, analysis, planning and action in an IMM scheme

is no simple relationship between funds disbursed, interventions used, results of these activities and the ultimate impact on the community. At the present there is no understanding of how a comprehensive portfolio of control interventions can best be optimized. However, there are some things that seem to apply, and data needs to be compiled so that there is a better understanding.

There is some knowledge, and there is some experience. One reality is that the planning, implementation, review cycle is ongoing, not just a single cycle.

In Fig. 16 the activities that are implemented produce their own results or outcomes, and in turn these have an impact on the community. The metric of improvement is mainly that of impact on the community and the constituents of the community. Does the impact on the community justify the expenditure on the activities, and are the activities costing the right amount given the situation and experience elsewhere.

Over multiple cycles the aim is for the scale of the interventions to diminish and for the impact on community to get better and better, and the bad things to get smaller. This is the essence of success and sustainability. In the long run the

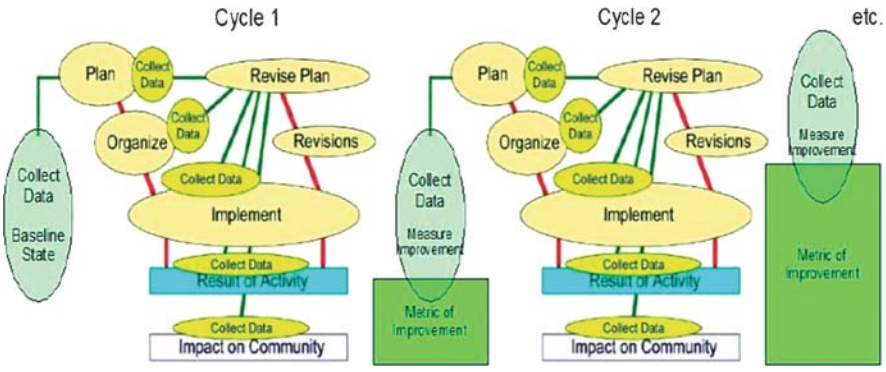

Fig. 16 Illustration of the cyclic nature of IMM

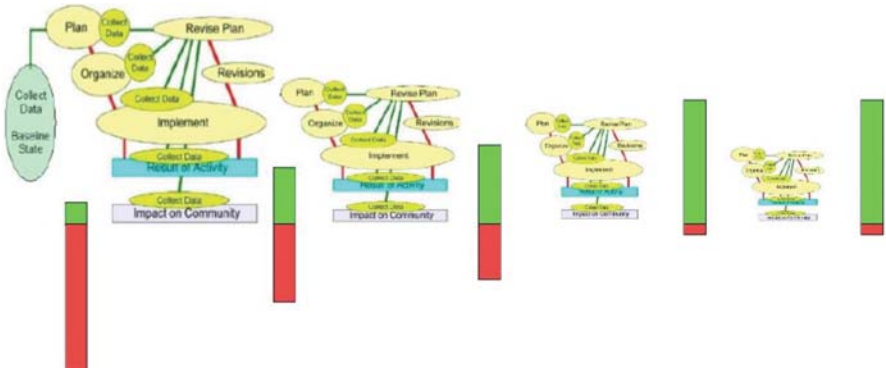

Fig. 17 Over multiple cycles of intervention the scale of the interventions diminish and the impact on the community becomes more positive

value of a good status in the community should be sufficient to pay for the cost of the essential ongoing activities that are needed to maintain the improved status (Fig. 17).

There are several different analysis cycles. At a practical level, the analysis of data needs to be done so that the results are available "in time" for the best possible decisions to be made. Thus, for example, the following needs to be done in a matter of days, or even hours:

1. A decision to do larviciding should be made in time for the larviciding intervention to be effective before the larvae become flying adult mosquitoes,
2. Identification of resistance should be made in time so that the chemical or biological agents can be changed to address the resistance issue,
3. The following interventions were done yesterday/last night ... have the expected results been achieved?

On the other hand, some scientific data analysis that is initiated now may still be ongoing in several years time. The daily, weekly or monthly accumulation of data is perpetual, and eventually the data analysis might yield real clarity about the mechanisms that drive the optimization of malaria control interventions. Periodic feedback will improve the knowledge base for decisions and improve the management models.

The purpose of surveillance and data collection is to learn what is going on in the best possible way. Surveillance data are the foundation for analysis of performance in conjunction with basic cost information derived from the financial accounting system.

Surveillance data serves not only to provide some metrics of performance, but is also vital to help understand the scientific behaviors that are going on and how best to make interventions toward the goal of reducing malaria prevalence.

Data are the most cost effective when they are used in multiple ways. In the IMM case data are used:

1. Primarily:
    1. to inform the local malaria control community using rapid local analysis and local management information techniques ... such as a wall map and colored pins, and to update a local mirror of the centralized data, and
    2. to update the IMM cyberenvironment and data analysis system for cross area comparison, alternative intervention comparison, time series analysis, etc.
2. Secondarily:
    1. to update responsible government authorities about results,
    2. to update the mosquito and malaria research community about results, and
    3. to update the local malaria control communities about results

Moving data is easy and very low cost when there is economical access to the Internet, otherwise it is more of a challenge. In general data flows need to be in electronic form so that they can be transmitted using the Internet. Figure 18 is a data flow graphic that shows the broad flows of data starting with surveillance activities:

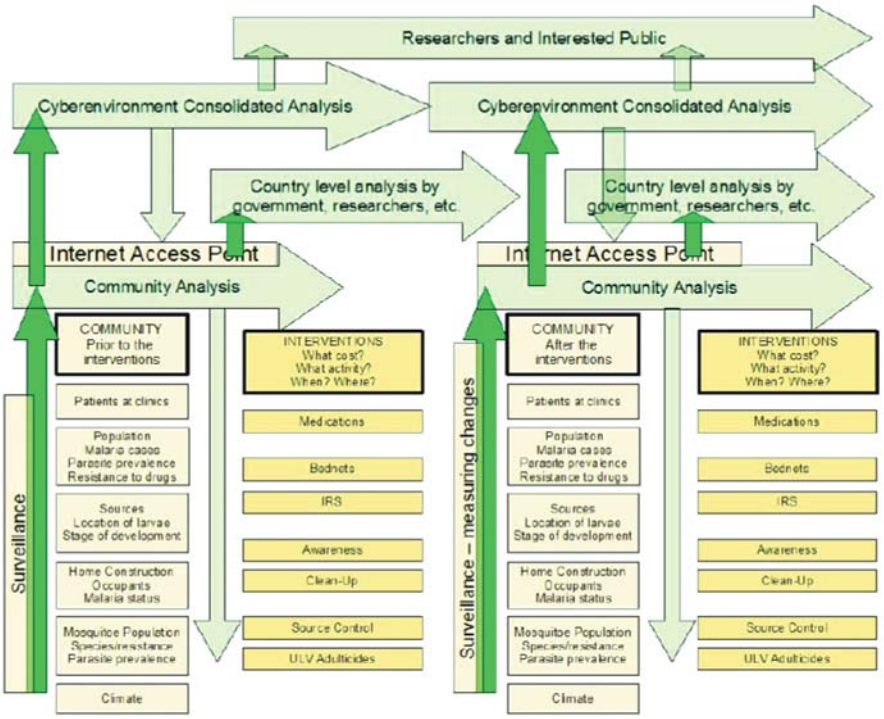

Fig. 18 Data analysis schematic illustrating the broad flows of data starting with surveillance activities

Data Flow – Data Analysis Schematic

Data flows from surveillance and data collection to a place where community activities can be coordinated and data used at that level for operational analysis and decision making . . . and the same data used in the cyber-environment in an electronic form transmitted from an Internet access point. Data flows are shown in bright green. There are also analysis actions and flows shown in light green. Data flowing into community analysis can be used almost immediately to make timely decisions about operational activities. A more in depth analysis can later confirm these decisions were the most appropriate, and give future guidance. Data and analysis from the cyber-environment are freely and easily available to the wide community of researchers and interested public. Data flows start from surveillance and field data collection. These relate to all the key performance parameters that are included in the integrated malaria management protocols. Data flows also included the operational details about the interventions including information about costs, the nature of the activities and data such as when and where the activities were carried out. The availability of an Internet access point facilitates data transfer. Cooperation with the local telecentres and other organizations with Internet access is an important way to minimize costs and to develop a local capacity for the future.

Spatial information: Mosquito and malaria control has a strong spatial characteristics that have a very large impact of control results. Accordingly spatial information and mapping are a very important part of cost effective high performance integrated malaria management.

Some of the characteristics that need to be taken into consideration include the following:

1. Where are people that are host to the malaria parasite located: where do these people live, where do they work, where do they congregate together, where do they travel to,
2. Where are the sources of mosquitoes,
3. Where do the mosquitoes travel and other details of their behaviour including when they travel and how they behave relative to homes, people and animals,
4. Where are infected mosquitoes located,
5. What mosquito and malaria control interventions have been done: when and where.

In addition to mapping that shows the simple spatial dimension of the data, there also needs to be an ability to understand the changes that occur over time about a specific place and a specific characteristic of the data.

The ultimate measure of performance is how much has been spent in order to achieve an improvement, and to a great extent money spent well today will only result in sustainable value sometime in the future. However it is also possible to relate the money spent well today with various intermediate results, that in turn will produce the sustainable long-term value.

The use of satellite images is relatively new, and the techniques can be very powerful. It is important, however, to use them in ways that are cost effective and

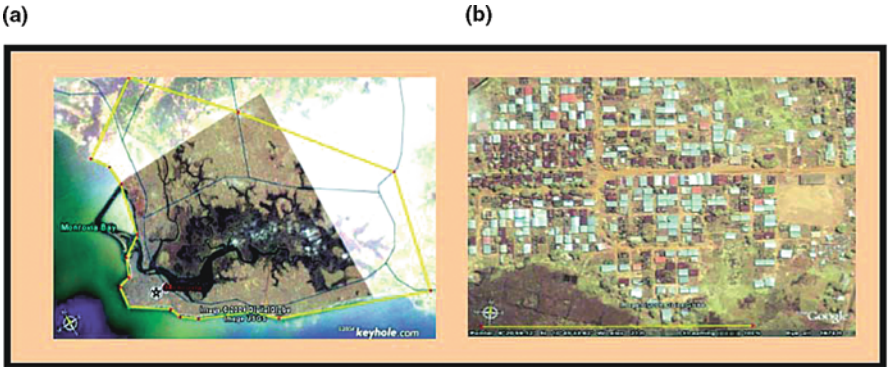

Fig. 19 (**a**) Satellite image showing the whole of Monrovia in Liberia; (**b**) Higher resolution satellite image showing individual houses in Monrovia

valuable. Satellite imagery can be used for desk review and analysis in ways that are very time saving and cost effective. The following images were accessible using Google Maps and show respectively the whole of the Monrovia are in Liberia, and a small section of the built up area of Monrovia where individual houses can be identified (Fig. 19a, b).

On the all Monrovia image, the area within the yellow line is 50,000 acres, and the dark brown area is about 15,000 acres of tidal marsh right in the middle of the city where mosquitoes breed prolifically.

Figure 20a, b are images of Stone Town and its outskirts in Zanzibar. They are images supplied by QuickBird incorporating data from the visible and near-infra-red (NIR) spectra at 0.6 m. A grid-based matrix has been overlaid.

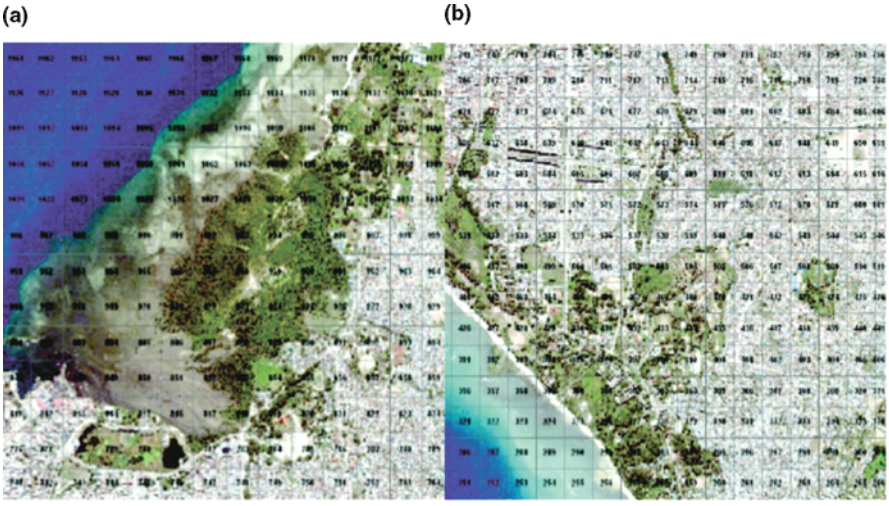

Fig. 20 (**a**) Satellite images of Stone Town, Zanzibar; (**b**) Higher resolution satellite image showing the outskirts of Stone Town, Zanzibar

These remote sensing and aerial/satellite images are the basis for a rapid build of data about the area, and a framework for rigorous spatial analysis. These images can also be used to develop an initial hypothesis about the profiles of mosquito and malaria prevalence in the area. The first phase of surveillance is to validate some of the hypothesis derived from the remote sensing. This accurate ground data is used both in the immediate process of establishing a baseline and data about the starting state, but also to initiate a process of determining what the optimum interventions strategy would be. The techniques for doing this surveillance are established, and involve building statistically valid samples relative to each square of the grid.

The early work of surveillance combines use of satellite imagery with ground verification of the conclusions derived from study of the images. This process of validating conclusions drawn from study of images should be taken very seriously because it has the potential to save both money and lives on a very large scale. There has been work done that suggests that the effectiveness of anti-malaria and anti-mosquito interventions could be improved substantially by making planning assumptions based on what can be deduced from study of satellite images. Hardly any use of this is going on at the present time on the large scale interventions that have been funded by the international community.

The cost effectiveness of data collection can be enhanced by collecting it in the best possible way. The following are some of the techniques that can be used. Some of these are incorporated in the design of the field data collection forms, whether in a paper form or electronic form.

1. Much of the data needed for efficient data processing and analysis is “permanent” and does not need to be updated.
2. Other data changes frequently and this information needs to be collected and turned into electronic form as efficiently as possible.
3. In addition their needs to be administration information to facilitate checking and holding data collection staff accountable.
4. Preprinted data collection sheets facilitate the work of the data collectors, and make it possible for the data collectors to correct erroneous permanent data.
5. Use of GPS information can be helpful in ensuring that data being collected relate to the specific location identified in the paper-work.
6. Use of emerging technologies like the SemaPedia system may be useful in helping to get accurate data entry and easy access to associated information.

Scouting or surveillance work is the foundation for meaningful data and performance metrics. Data is not easily improved after it is collected. The importance of a sound process for data collection cannot be over-emphasized. Mosquito abatement scouts or surveillance teams must be trained to sample and monitor mosquito sources, mosquito populations and weather conditions using a variety of sampling tools in order to provide both timely and accurate field data at specific locations within the geographic areas where control program is operating.

The critical goal of the program is to reduce the prevalence of the malaria parasite in the population and the incidence of malaria. Data collection about malaria prevalence and malaria incidence is needed for two purposes:

1. To facilitate the treatment of people with malaria so that their health can be improved,
2. To help reduce the pool of malaria parasite in the human host so that the level of transmission of the parasite from person to mosquito to person can be reduced,
3. To help measure the effectiveness of various medical treatments,
4. To provide a key metric of performance and a way to measure the value of the program's multiple interventions.

The local area coordinator uses the scouting surveillance data to plan for appropriate intervention activities. The plan is based on data analysis taking into consideration the mosquito species, whether a dangerous vector or a simple nuisance, the potential production by site, etc. and organizes the sites into a priority list for treatment. The idea of rapid decision-making based on local information is critical to the success of 30 community centric sustainable development and to the success of a malaria control program. A reasonable decision made at the right time is likely to be much more valuable that a perfect decision that can never be made, because the time to make it has past. In this circumstance, the detailed analysis of the situation and the recommendation concerning the optimum decision is used to help inform the local decision maker so that better decisions can be made in the future. In the case of a mosquito and vector control operation in the United States, today's surveillance information is reviewed as soon as it is possible, and by 2.30 in the afternoon a decision is made as to what interventions are going to take place overnight. The rapid identification of a problem followed by a rapid response provides the best for public health outcome as well as being the most cost effective or greatest value added interdiction.

Cyberenvironment and Data Analysis: Data Logistics

The challenges associated with data collection continue through all phases of data processing, storage and transmission. There is too little communications infrastructure, it is unreliable and it is expensive. Getting data from its original collection to the data store reliably and at low cost is a challenge. Technology is changing rapidly and it is becoming easier to communicate from remote locations.

An IMM information system provides oversight information to confirm the immediate decisions were consistent with an optimization strategy, and the give the management team information and progress and guidance about strategic direction that will optimize performance. All available paths for communications will be taken into consideration, including using local telecentres, local organizations, and others to facilitate access to computer systems and to the Internet.

Part of the data flow has to be rapidly available for decisions that need to be made from day to day. While some information can reasonably be reviewed once a month, a lot of the data are needed for decisions that are made every day. This is a challenge, but technically possible. Modern information and communications technology (ICT) is very powerful but it requires a certain level of infrastructure and a reasonable cost structure. Universal access to the Internet does not yet exist, and often access is very expensive.

In some places there are organizations that have established their own private networks. Some of these networks are operated by private corporations (such as oil industry companies) and by multilateral institutions such as the World Bank. These networks have the technical capacity to carry the data flow that could support IMM program activities. In some places there are international NGOs that have set up some form of Internet access and international data communication capability. In some places there are telecentres that have some level of data communication capacity. The telecentres have an almost universal desire to help but are challenged because of the available communications infrastructure and their precarious financial condition. The telecentre network with their community-based operations is the natural local partner for data communication. The very rapid evolution of ICT means that it is likely that cellphone data entry will be available almost universally within a short period of time. The exact manner that data will be transmitted will need to be determined for each community, and the local program.

There have been many initiatives to help organizations of many types establish telecentres in Africa to help close the "digital divide". Most of these telecentres have great difficulty surviving after the external financing ends, but they have real value both to the local community and the world community. Real value for a local community is derived from something of value happening in the community. Reducing the burden of malaria has real value in the community, and the use of the capacity of a telecentre to help with data processing and Internet access related to the data about malaria and the control interventions going on could be a very useful piece of the process, and remunerated accordingly.

Some decisions need to be taken rapidly, and simple, rapid data analysis is sufficient for good decisions to be made without a lot of sophisticated analysis. The mosquito life cycle is measured in days, and interventions are effective when they are done at the right time, and of no value when delayed. In a typical mosquito abatement district setting it is usual to find a wall map and colored pins indicating issues of concern. This works very well to pinpoint problems and plan immediate interventions.

This Analysis Complementary to the Cyberenvironment Data Analysis

The local rapid data analysis is complementary to the cyberenvironment data analysis. Exactly the same underlying data are used in the two analysis sets, but for very different purposes, albeit towards the same long term goal. What the local analysis

does is to help operational decision makers to function as effectively as possible based on the information that is available now.

The same data incorporated in the cyberenvironment (discussed next) can be used to help understand the complex behavior and help optimize future interventions.

Cost and Management Accounting for a IMM Program

Cost or analytical accounting is the activity that informs about costs and how they behave in connection with each of the IMM interventions or tactics. Cost accounting is a very simple concept, but becomes complex in most real world situations because there are a very large number of variables and "actual" cost changes all the time. One important piece of information that should come from cost or analytical accounting is information about the behavior of cost. Decision maker should have the information so that they can change costs to their advantage. This can only be done if information about cost behavior is available.

The IMM program's analytical framework must make extensive use of the standard cost technique to simplify analysis and to make comparison across different areas of the multi-disciplined-multi-tactic operation easier to understand.

A clear understanding of costs can be very helpful in making decisions about what interventions should be a priority. For example, bednets are relatively easy in that their cost does not vary much based on volume, although this is dependent on the overhead of the distribution cost which vary considerably. The unit cost of a bednet is in the range of $5.00–10.00 with a useful life of 3 years, suggesting an annual cost of between $2 and $3.50. Another example would be the costs associated with IRS which will vary depending on the chemicals used, the frequency of treatment, the staff costs and the productivity of the team. The per room cost is in the range of $2.00–12.00 per year. DDT has the advantage of having longer lasting effectiveness than most other chemicals. On the other hand medical treatment cost depends on the drug therapy being used. Chloroquine is low cost, probably under $2.00 for drugs per case, but often ineffective. ACT is effective but drug cost is probably $100.00 per case. The annual cost is high when there is rapid re-infection and a patient has 3 or more bouts of malaria in a single year. The cost of medical staff per case is quite low because staff are generally taking care of far too many patients, and the low cost of diagnosis results in a far too high level of miss-diagnosis and mistreatment of patients. The example of source management through source reduction has a relatively high cost during the phase where permanent data are being compiled, but much lower after this start up phase. Source control is cost effective when knowledge makes it possible to target the vector when it is concentrated in a small area and immobile (i.e. CIA). The two big costs are people and the chemical or biological insecticides. Aerial delivery of larvicides is cost effective in the right setting with a direct cost that depends very much on the agents being used and the area to be treated, but perhaps around $50 an acre. Similarly, adult malaria control using ULV spraying with a chemical like Dibrom has a cost that varies with the area and the method of delivery. Use of aerial spraying is very cost effective, with a direct cost

of around $2.50 an acre, and an ability to spray some 5,000 acres an hour. Note that this is very much faster and lower cost than larval control, but the areas to be treated are significantly different, especially in size.

Within the management framework these costs do not have to be minimized, they have got to be optimized so that the best possible results are achieved. The right combination of these interventions can produce very valuable results, when the same amount of money spent on any single intervention is likely to do little more than create some temporary progress, and then very little.

Cost and Management Accounting Cost and Value

Cost is how much of resources are used to make the product or deliver the service. Price is the amount that is paid in exchange for the product or service. The difference between price and cost is profit. The value can be thought of as the maximum that would be paid for the product or service by a person thinking along rational economic lines. Value adding, or value creation is the difference between value and cost. In integrated malaria management the costs and the value have something of the following profile over time.

The profiles in Fig. 21 reflect an example of costs and values over a period of about 5 years. The very different profiles for costs and values over time adds to the

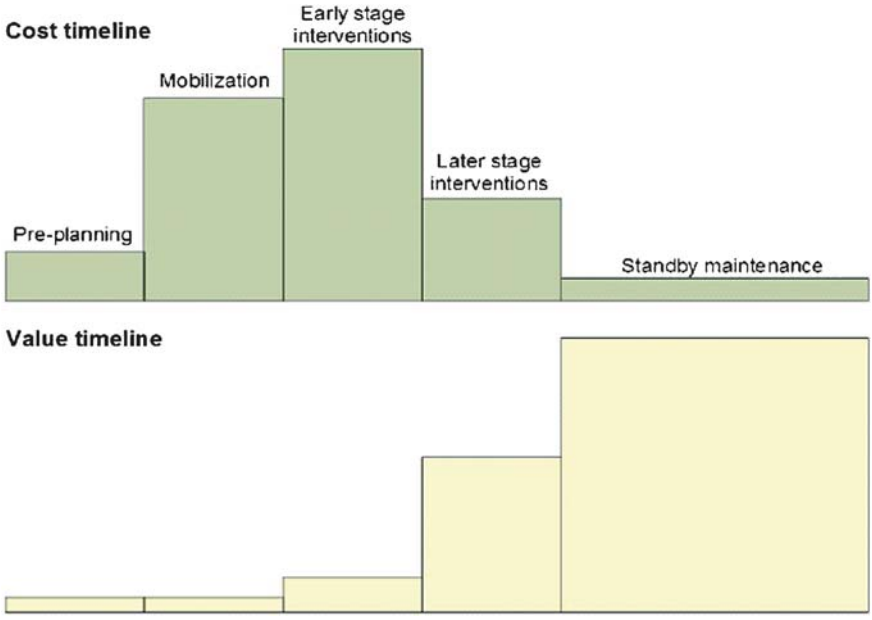

Fig. 21 Example of a profile of the costs and value of integrated malaria management over about 5 years

difficulty of interpreting performance information. Some simplifying assumptions can be made, and this is what needs to be done in the immediate future, but as fast as possible the proposed cyberenvironment for IMM should be activated to validate the assumptions and provide better decision-making rules. The CE for IMM will populate a data-store that contains prior experience incorporated in a model that makes it possible to relate current costs and current habitat data, mosquito data, and malaria data to predict possible outcomes and to manage interventions in the best possible way for maximum performance. What is more important by using the rules development by the CE for IMM program, data analysis models, rapid decisions can be made to optimize interventions. More importantly as time goes with increased empirical and operational data there will be increasing confidence that decisions will produce the intended outcomes.

In order to progress towards the ultimate goal of eradicating malaria, there are three things that need to be done:

1. Reduce incidences of malaria and the prevalence of the malaria parasite in the population through detection and drug treatment,
2. Reduce re-infection of the population with personal protection including insecticide treated bednets, screens and interior residual spraying,
3. Reduce mosquito populations near homes with integrated vector management that includes breeding area control and killing adult mosquitoes.

Achieving optimized results is not simple. Figure 22 shows the changes in various metrics over time, and how costs and value change accumulate.

The IMM cyberenvironment was developed to address this complexity and help to improve performance, but basic measures can help to keep a program going in the right direction and optimize use of resources. This graphic is grossly simplified, but even in the simple form it shows some of the challenges that have to be addressed. The bold lines for cumulative cost and value suggest that value will be growing over time, and will exceed the costs incurred, but not immediately. The indices of various intermediate results are interesting:

1. Over time it is to be expected that the population of mosquitoes will be reduced if there is effective source reduction and a reduction of adult mosquitoes.
2. Over time it is to be expected that the proportion of the human population infected with the malaria parasite will go down if there is effective anti-malaria treatment, and there are less mosquitoes and less opportunities for the mosquito to interact m with the human host and take a blood meal, especially an infected one.
3. Over time it is to be expected that the proportion of mosquito population infected with the malaria parasite will go down as the various interventions make it progressively more likely that a blood meal will not result in the mosquito being infected.

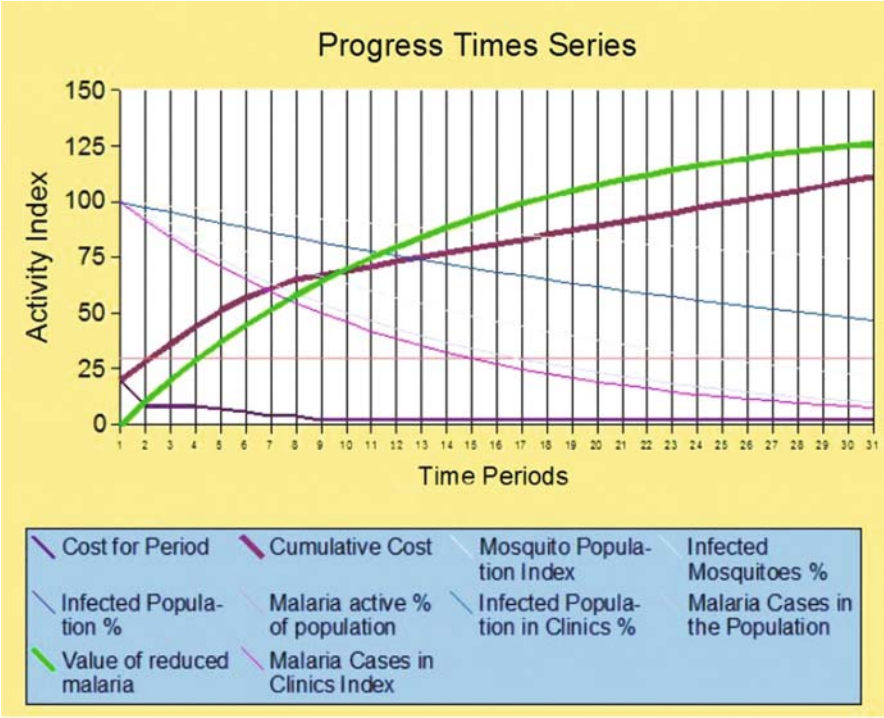

Fig. 22 This simplified graphic shows the changes in various metrics over time and how costs and value change accumulate. It suggests that value will be growing over time and will exceed the costs incurred

4. The data from the clinics may or may not be a good indicator of the status of the population as a whole. Malaria cases in the population may be a higher proportion than in the clinic, or it may be the other way round.

A successful program will get reduction in many of these key metrics, and there will be real value in the reduction in malaria in the society. Interventions that merely serve to get the intermediate results, are costly and do not serve the public interest in the long term. Worse, the process or perpetual treatment and subsequent re-infection sets the stage or resistance and a potential severe crisis over time.

The ultimate goal can be achieved using a set of mosquito and malaria control interventions. These interventions, or activities, have little value in themselves, but together can result in achievement of the ultimate goal which has enormous value. The interventions have costs. The interventions may, or may not be making a useful contribution to the ultimate goal and the ultimate value. Cost is a very simple concept, and also one that has many variants and complexities. Cost in management information must be a clear and consistent measure, supported as needed by other detail of cost to explain material issues. Performance is not based simply on cost, but on the relationship between cost and the results being achieved. Because there

are significant time lags between the time costs are incurred and time results are achieved, use of time series tables and charts is often desirable.

In summary, there are many interventions needed in order to achieve the goals of being cost effective within a IMM program including:

1. Medical case management,
2. Personal protection using insecticide treated bednets (ITN),
3. Personal protection using interior residual spraying (IRS),
4. Personal protection using screens and other repellency techniques,
5. Vector control using environmental cleanup,
6. Vector control using larviciding, and
7. Vector control using adulticidng.

In addition, their needs to be the scientific data and a management information dimension that includes:

1. Surveillance,
2. Data collection,
3. Data logistics,
4. Data analysis (cyberenvironment for IMM),
5. Management information, and
6. Feedback to operational and strategic decision makers.

The benefits of an IMM program are illustrated in Fig. 23. Today by employing the integrated philosophy of Gorgas with modern technological advances in

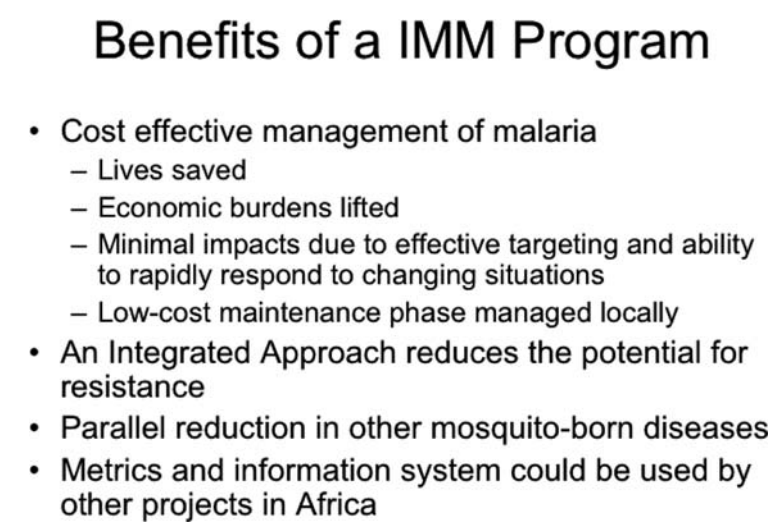

Fig. 23 A summary of the benefits derived from a Integrated Malaria Management program

malaria treatment, biology, vector control, computer science and communications the potential of managing malaria with the goal of eradication is possible throughout sub Saharan Africa. The gross economic benefits of removing this debilitating disease from prominence will enable all African nations to use their rich natural resources for the n betterment of their citizens and the world.

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