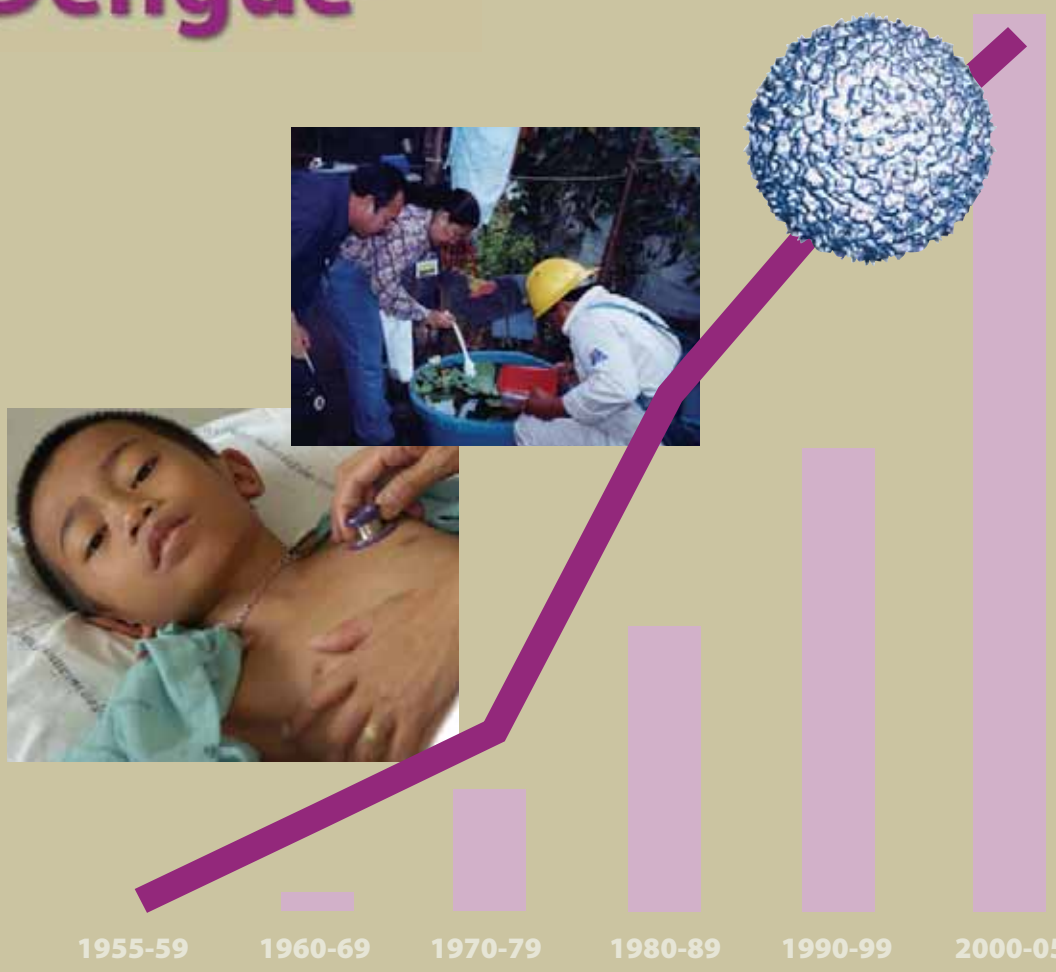
Scientific Working Group

World Health Organization

Report on

Dengue



1–5 October 2006
Geneva, Switzerland

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by
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TDR/SWG/08

Report of the Scientific Working Group meeting on Dengue

Geneva, 1–5 October 2006

TDR/SWG/08

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Executive summary

Dengue is the most rapidly spreading vector borne disease. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. Because of the rapidly increasing public health importance of this disease, in 1999 dengue was incorporated in the portfolio of the UNICEF, UNDP, World Bank, WHO Special Programme for Research and Training in Tropical Diseases (TDR). The 2002 World Health Assembly Resolution WHA55.17 urged greater commitment to dengue among Member States and WHO; of particular significance is the 2005 Revision of the International Health Regulations (WHA58.3), which includes dengue as an example of a disease that may constitute a public health emergency of international concern.

It was against this background that the Dengue Scientific Working Group of 60 experts from 20 countries including WHO staff from four Regions and Headquarters met in Geneva in October 2006 to review existing knowledge on dengue and establish priorities for future dengue research aimed at improving dengue treatment, prevention and control. The goal of the Scientific Working Group was to outline a research agenda by identifying bottlenecks and making detailed and specific research recommendations. The SWG wanted to identify areas of research that could lead to tangible benefits for people in disease endemic countries within the coming years as well as outline a strategic vision for applied and basic research from which benefits would be felt in the medium to long term.

As a result of major demographic changes, rapid urbanization on a massive scale, global travel and environmental change, the world, particularly the tropical world, faces enormous future challenges from emerging infectious diseases. Dengue epitomizes these challenges. In the early years of the 21st Century we are collectively failing to meet the challenge posed by dengue as the disease spreads unabated and almost 40% of the world's population now lives at risk of contracting the disease. There is currently no specific clinically useful diagnostic test, no drugs, and no vaccine, and we have failed to widely or effectively implement existing vector control and clinical management measures that we know would help to reduce the vector population and reduce case fatality rates. Yet there has never been a more optimistic time to be involved in dengue and dengue research, and interest in the disease has attracted a new generation of talented and committed clinicians and scientists. Modern science, from clinical medicine to basic research on pathophysiology, drug and vaccine discovery, through to the social and behavioural sciences and vector biology and control, offers a unique opportunity to make a tangible and substantial impact on dengue over the next decade. But in order to achieve what

is possible, a paradigm shift is required in our current approach. The dengue research community needs to: push for much greater implementation of existing knowledge to reduce case fatality rates, extend basic and clinical research to understand the underlying pathophysiology, aid diagnostics and drug discovery and further improve clinical outcome, speed up the development of vaccine candidates including moving as quickly as possible to efficacy trials, and gather evidence for implementing best practices for control of the vector.

All of this is possible in the next ten years. But to achieve this, dengue needs a much stronger voice within dengue endemic countries and within the global public health community to persuade society, funding agencies and policy-makers of the importance of the disease. We are at a critical epidemiological juncture in infectious, particularly viral, emerging diseases at the start of the 21st Century, and in many ways dengue serves as a model for how we might meet that challenge. The lessons learned from dengue will have implications for a number of other diseases and our approach to their control. The implementation of the best of existing knowledge and practice supplemented by future research applied in an integrated, holistic fashion can be expected to significantly change the lives of individuals living in dengue-endemic countries in the coming years. The Scientific Working Group hopes this research agenda will help provide a strategic plan for how we might collectively achieve the aims of reducing morbidity and mortality based on better understanding of the pathophysiology associated with dengue, on implementation strategy, and on reduction of virus transmission.

GLOBAL DENGUE RESEARCH AGENDA

The priority dengue research areas are organized around four major research streams, which will provide evidence and information for policy-makers and control programmes and lead to more cost-effective strategies that will reverse the epidemiological trend.

Stream 1: Research related to reducing disease severity and case fatality

OPTIMIZATION OF CLINICAL MANAGEMENT

An efficient out-patient system and clinical and laboratory indicators of early dengue virus infection, plasma leakage and shock, as well as an effective and safe method of managing severe haemorrhage, dengue in pregnancy, and patients with co-morbidity, need to be validated in order to scale up the use of improved treatment guidelines.

It is recommended to analyse:
- New methods and guidelines for triage and out-patient care.
- The validity, role and accessibility of available and new diagnostics.
- The predictive value of prognostic markers (host and viral, non-invasive measurement of vascular leakage).
- Standardized approaches to determining and documenting severe disease and response to treatment.
- Best practices for the treatment of dengue including early treatment to reduce severity, treatment of established shock, and effective and safe management of haemorrhage.
- The impact of co-morbidities on disease severity, and the effect of pregnancy.
- The causes of dengue deaths (including treatment failures).

PROCESS AND IMPACT EVALUATION OF STAFF TRAINING

Training programmes in case management can help to rapidly reduce case fatality. However, training has to be standardized and adapted to the prevailing local health care system and best practice has to be identified and implemented in dengue-endemic countries.

It is recommended to analyse:
- The process and impact of existing/future training programmes.

CRITICAL ISSUES IN DENGUE PATHOGENESIS

A better understanding of dengue pathogenesis will provide a foundation for future rational clinical interventions. In particular we need to understand the changes underlying: endothelial permeability in plasma leakage, dengue virus diversity, and immune response to dengue viruses.

It is recommended to analyse:
- The physiological and molecular mechanisms leading to vascular leak and haemorrhage.
- The host genetic factors associated with dengue severity.
- The dengue viral factors and antigenic subtypes related to tropism, epidemic potential, and virulence.
- The mechanisms of antibody-mediated enhancement and protection.
- The mechanisms of virus entry and cellular/tissue tropism.
- T and B cell responses and their relation to immunopathology and protection.
- Genetic predisposition to dengue.

Stream 2: Research related to transmission control through improved vector management

DEVELOPMENT AND EVALUATION OF VECTOR CONTROL TOOLS AND STRATEGIES

The effectiveness of powerful vector control tools has been compromised by issues of delivery, coverage and acceptability. Promising new tools and approaches need to be evaluated.

It is recommended to analyse:
- The efficacy of new vector control tools and strategies in different contexts.
- Combinations of new and/or existing tools in different contexts.
- The scaling up of successful pilot projects to state or national level.

SURVEILLANCE AND RESPONSE

Strengthening of surveillance systems through development and validation of reliable risk indicators and the application of information technology is needed for improved decision-making.

It is recommended to analyse:
- Improved methods and their application/standardization in operational contexts.
- The development and utilization of early warning and response systems.
- The triggers that will allow effective response to incipient epidemics.
- The contribution of information technology to decision-making.

Stream 3: Research related to primary and secondary prevention

VACCINES

Vaccines offer the greatest hope for dengue prevention and there are several candidates in clinical development. The identification of vaccine components that are suitably safe and immunogenic, and of immune correlates of protection, should accelerate successful vaccine development and regulatory approval.

It is recommended to analyse:
- New vaccine candidates, adjuvants, and vaccination strategies.
- Correlates of protective immunity for use as an endpoint in vaccine trials.
- Immune responses in vaccine trials and natural infections.
- Prospects for phase 3 and 4 vaccine evaluation trials in multiple field sites.

- Issues associated with future vaccine usage and coverage, including cost-effectiveness studies of implementation.

DRUGS

Anti-dengue drugs may have prophylactic (e.g. outbreak prevention) and therapeutic (e.g. prevention of severe disease) uses, including potential for impact on incidence and severity of ensuing disease. Anti-viral drug discovery for dengue has accelerated in recent years along with our knowledge of 'drugable' targets in the virus.

It is recommended to analyse:
- Viral-encoded proteins for drug, diagnostics and vaccine design.
- New (including natural) products or existing licensed drugs.

Stream 4: Health policy research contributing to adequate public health response

There is a contradiction between the high priority afforded to dengue at political level and the low level of resources allocated to dengue prevention and control program activities. Health policy research will facilitate a redress of this imbalance.

It is recommended to analyse:
- Tools for rational decision-making and adequate prioritization of dengue, such as studies of dengue burden and costs of illness.
- Factors leading to success or failure of national programmes.
- Decision-making that results in declaration of state of emergency.
- The importance and burden of dengue in less studied regions (e.g. Africa).

CONCLUSION

The Scientific Working Group hopes this research agenda will help provide a strategic plan for how we might collectively achieve the aims of reducing dengue morbidity and mortality and its negative socioeconomic impact. Donors and the research community are encouraged to take part in this major programme and to contribute through timely information to the database TDR is establishing for keeping track of research activities and relevant findings.

1. Dengue as a public health problem and efforts to increase understanding and control

This document provides an overview of the state of the art of dengue research as well as recommendations for a global priority research agenda aiming to provide information for policy and practice and allow evidence-based decisions to be made in order to reverse the ongoing exponential increase in dengue, including in its severe forms. Throughout the document, 'dengue' is used as a generic term which includes uncomplicated and severe forms of the disease such as dengue haemorrhagic fever, dengue shock syndrome, dengue fever with haemorrhage, and, in some contexts, the silent or unapparent forms of dengue infection.

DENGUE AS AN EXPANDING PUBLIC HEALTH PROBLEM

Dengue is the most rapidly spreading arboviral disease in the tropics and subtropics. The global burden of dengue has increased at least four-fold over the last three decades and almost half the world's population is estimated to be at risk (see Nathan and Dayal Drager, working paper [WP] 3.1).

The primary vectors *Aedes aegypti* and the probably less important *Aedes albopictus* have spread throughout the tropics. An estimated 50 million dengue infections now occur annually, particularly in South-East Asia, the Americas, and the Western Pacific islands. About 500 000 severe dengue cases are reported annually and some 19 000 dengue-related deaths were registered in 2002. Despite the fact that dengue is accepted as an important public health problem in the WHO South-East Asia Region (SEAR), Western Pacific Region (WPR) and Americas Region (AMR), without reliable data about the burden of disease in the African Region (AFR) (see Sang, WP 3.3) and the Eastern Mediterranean Region (EMR), no detailed documentation of the real burden of dengue exists. Estimates starting from the 1980s (see Suaya, Shepard and Beatty, WP 3.2) show dengue as an emerging disease with disastrous consequences for peoples health and household economy, and for society in general. As the registration and reporting of dengue varies from country to country and region to region, and surveillance systems are generally weak (see Ooi, Gubler and Nam, WP 7.1), it is difficult to make comparisons between countries, monitor international trends, and measure the impact of interventions.

INTERNATIONAL EFFORTS TO CONTROL DENGUE

Because of the rapidly increasing public health importance of this disease, a 2002 World Health Assembly Resolution (WHA55.17)[1] urged greater commitment to dengue among Member States and WHO, while the 2005 Revision of the International Health Regulations (WHA 58.3)[2] included dengue as a disease that may constitute a public health emergency of international concern. In 1999 dengue was incorporated in the portfolio of

the Special Programme for Research and Training in Tropical Diseases (TDR) and the WHO Initiative for Vaccine Research (IVR). Other WHO departments are also directly or indirectly involved in dengue research and an inter-departmental working group on prevention and control of dengue was established, which is chaired by the department for control of Neglected Tropical Diseases (WHO/NTD).

Research activities supported through WHO, including through its regional offices in the Americas (PAHO), South-East Asia (SEARO) and Western Pacific (WPRO), emphasize three major research lines: improved vector control, case management, and primary prevention through vaccine discovery and development (Kroeger et al. 2006).

Other major dengue initiatives include the Pediatric Dengue Vaccine Initiative (PDVI) and the Innovative Vector Control Consortium (IVCC), both supported by the Bill and Melinda Gates Foundation and others. DENFRAME and DENCO are research consortia supported by the European Commission. Clinical dengue research in Asia is supported by the Wellcome Trust; ecosystems research for dengue control is supported by the Canadian International Development Research Centre (IDRC) and TDR; dengue drug discovery, accelerated by the Novartis Institute for Tropical Diseases, is supported by Novartis and the Singapore Economic Development Board and the academic sector (see Selisko et al, WP 4.3); dengue control in the Mekong Delta is supported by the Asian Development Bank and, in the Americas, by the Inter-American Development Bank. Major initiatives in the discovery and development of dengue vaccines and diagnostics as well as of insecticides come from the industrial sector supported and/or coordinated by WHO and other partners (see Barrett and Hombach, WP 4.2; Buchy, WP 4.4). Major publications have been produced in relation to dengue vector control and clinical management (see Nathan and Dayal Drager, WP 3.1).

2. Ongoing dengue research

TOWARDS IMPROVED VECTOR CONTROL

New methods for identifying and targeting the most productive larval habitats of the vector are needed to improve the cost-effectiveness of vector control. A multicountry project to determine the practicality and reliability of using the pupal/demographic survey of *A. aegypti* in water containers as a method for identifying breeding sites producing the majority of adult mosquitoes was based on the recommendations of a commissioned report.[3] The efficacy of targeted intervention in the most productive container habitats is now being examined in a multicentre study.[4,5] The efficacy of new vector control tools is also being analysed in multicentre studies[6,7] (also see McCall and Kittayapong, WP 6.2), while the usefulness of new information technology (IT) tools for decision-making, such as spatial analysis of vector breeding using geographical information systems (GIS) and determination of dengue vector threshold levels using mathematical modelling, will be explored by the IVCC.

Community participation is a vital component in the delivery and sustainability of effective dengue vector control, and WHO commissioned the development of a step-by-step guide on social mobilization and communication for dengue prevention and control.[8] This communication-for-behavioural impact (COMBI) approach to social mobilization has been field tested in several countries in Asia, Latin America and the Caribbean, where it contributed to the challenging task of engaging communities in more focused application of vector control measures against *Ae. aegypti* and other important vectors (see Elder and Lloyd, WP 7.3).

The TDR Steering Committee on Strategic Social, Economic and Behavioural Research provides the major coordinating input for interdisciplinary projects on vector ecology and its relation to biological and social aspects of disease transmission and control. This programme is supported by IDRC and is focused on six Asian study sites. TDR also funded several mosquito genome-based research projects that are potentially relevant to vector control, following which work to complete the *A. aegypti* genome and refine vector transformation techniques continued, leading to techniques for the stable transformation of *Aedes* into mosquitoes that are refractory to dengue infection.

The testing and evaluation of pesticides and pesticide application technologies for public health use, including for dengue prevention and control, is coordinated by the WHO Pesticide Evaluation Scheme (WHOPES, at: www.who.int/WHOPES). The Vector Ecology and Management unit of the WHO department for control of Neglected Tropical Diseases (NTD) has provided additional technical support and guidance for developing

promising applications of technologies for dengue vector control, such as those associated with long-lasting insecticidal materials (see McCall and Kittayapong, WP 6.2).

TOWARDS IMPROVED CASE MANAGEMENT

Effective management of dengue patients requires rapid diagnosis. Current diagnostic tests are however of variable quality and their performance and accuracy have not been validated; they are based on viral genome detection and are complex and expensive. Since 2004, at joint meetings between TDR and the Paediatric Dengue Vaccine Initiative (PDVI), product characteristics for dengue diagnostic tests for different indications have been defined, an inventory of existing tests drawn up, and a TDR-led strategy for dengue test development and evaluation presented.[9] Establishment of laboratory networks in South-East Asia and Latin America, and field trials of the performance of selected tests, are under way. Plans for facilitating the development of new tests with higher performance characteristics are also being developed by the European Commission supported DENFRAME consortium.

Dengue has different clinical manifestations that, according to the present classification system, fall into categories such as dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Other severe manifestations include dengue fever with unusual haemorrhage and dengue with severe organ complications. The variety of clinical pictures and complexity of the classification system are confusing for many clinicians and complicate triage and case management of patients as well as reporting[10,11,12] (also see Lum et al, WP 5.1). While the pathogenesis of dengue is gradually better understood, key areas such as the pathways of immune enhancement or the virological/immunological factors that lead to DHF and other severe forms of dengue (see Simmons et al, WP 4.1) are as yet not understood at all. Such understanding is, however, critical for vaccine development. Life-threatening complications are more likely to occur when individuals who are already immune to one of the four dengue virus serotypes become infected with another virus serotype, although the variations between different ethnic, age and nutritional groups as well as between regions are poorly understood.

TOWARDS IMPROVED PRIMARY PREVENTION THROUGH SUPPORT OF VACCINE DEVELOPMENT AND DRUG DISCOVERY

IVR in close collaboration with PDVI provides guidance on the evaluation and testing of dengue vaccines and contributes to shaping the underpinning agenda on research issues. This facilitating role is critical since considerable challenges remain for the

successful clinical development of a dengue vaccine. Specific issues relate to the complex disease epidemiology and concerns over vaccine-induced disease enhancement through immunopathological processes (see Barrett and Hombach, WP 4.2). To increase the number of candidate dengue vaccines in the pipeline, research has been carried out to validate monkey models, characterize humoral immune responses to dengue virus and vaccine candidates, and explore novel strategies for attenuating dengue virus. To facilitate vaccine testing, international reference materials have been established and a consultation process initiated to define correlates for protection by dengue vaccines. Ongoing activities emphasize support for the clinical evaluation of dengue vaccines, in step with candidate vaccines being progressed by industry.

Dengue drug discovery has attracted the attention of industry (Novartis Institute for Tropical Diseases in Singapore) and academic institutions. Better understanding of the virus encoded proteins will lead to new opportunities for targeted drug design (see Selisko et al, WP 4.3).

TOWARDS IMPROVED EPIDEMIOLOGICAL SURVEILLANCE

DengueNet is an open-access web-based information system that allows public health officials and researchers to share a common database and foster collaboration (http://www.who.int/denguenet) (see Nathan and Dayal Drager, WP 3.1). It was created by the WHO unit of Epidemic and Pandemic Alert and Response (EPR) for collecting and analysing global standardized epidemiological data on dengue and DHF cases and deaths, and circulating virus serotypes, by geographical area and time. DengueNet is implemented by the WHO regional offices, by ministries of health of endemic countries, and by laboratories. Rapid analysis of indicators such as case fatality rates allows monitoring of the current situation during disease outbreaks and the possibility of targeting training to improve early recognition, referral and hospital case management for severe dengue.[13] Standardized global dengue surveillance data,[14] the principal result expected from full implementation of DengueNet, are critical for the public health community to define the burden of dengue and to assist governments and other stakeholders to make investment decisions about applied research and development. However, the establishment of national surveillance systems is still an enormous challenge (see Ooi, Gubler and Nam, WP 7.1).

POLICY RESEARCH, IMPLEMENTATION RESEARCH RELATED TO DELIVERY ISSUES, AND RESEARCH CAPACITY STRENGTHENING

Most of the research programmes mentioned above have inbuilt elements of policy analysis and implementation research related to service delivery issues including studies about the usefulness of modern information technology (see Martinez, WP 7.2) and the sustainability of strategies (see San Martin and Brathwaite, WP 7.4). While capacity strengthening components are an overarching theme in most research programmes, more systematic approaches to individual and institutional capacity building for dengue research is desirable.

3. Purpose and objectives of the Scientific Working Group

The main purpose of the SWG meeting was to set the global agenda for future dengue research in terms of describing what is known and which priority research areas should be covered in the future. As a starting point, a review of the existing evidence on dengue prevention and control was needed as well as an update of the ongoing dengue studies and strategies for translating research findings into policy and practice (see working papers in annexes 3–7). This, as well as interaction with dengue control and management, provides a basis for defining further priorities in dengue research.

The specific objectives of the meeting were to:

- Provide an overview of the state of the art of dengue prevention and control and research needs.
- Identify key research areas for the next few years that can inform policy and improve dengue prevention and research.

4. Global research agenda recommended by the Scientific Working Group

The priority dengue research areas are organized along four major research streams that will provide evidence and information for policy-makers and control programmes and lead to more cost-effective strategies to reverse the epidemiological trend.

Stream 1: Research related to reducing disease severity and case fatality

OPTIMIZATION OF CLINICAL MANAGEMENT

An efficient out-patient system, and clinical and laboratory indicators of early dengue, plasma leakage and shock, as well as an effective and safe method of managing severe haemorrhage, dengue in pregnancy, and patients with co-morbidity, need to be identified and validated in order to scale up improved and standardized treatment guidelines.

It is recommended to analyse:

- New methods and guidelines for triage and out-patient care of dengue patients, and to validate their feasibility and results at different levels.
- The validity, role and accessibility of available and new diagnostics for dengue.
- The predictive value of prognostic markers (host/viral early warning signs) of disease severity, and to validate procedures for early recognition and treatment of plasma leakage and shock including non-invasive measurement of vascular leakage.
- Standardized approaches to determining the clinical signs of shock in children and adults, including the role and techniques of: measuring blood pressure in shock patients; diagnosing severe dengue through ultrasound, other non-invasive technology and laboratory markers (albumin, cholesterol); and response to treatment
- Best practices for effective and safe management of dengue, including early treatment to reduce severity, treatment of established shock (including using oral re-hydration therapy), and effective and safe management of haemorrhage.
- The impact of co-morbidities such as obesity, diabetes mellitus, hypertension and chronic heart diseases, and the effect of pregnancy, on severity.
- The causes of dengue deaths (including treatment failures) in order to learn lessons from negative outcomes.

PROCESS AND IMPACT EVALUATION OF STAFF TRAINING

Implementation of training programmes in case management has an immediate impact on case fatality (which is reduced), as has been shown in many countries. However, training has to be standardized and adapted to the prevailing local health care system. A review of dengue morbidity and mortality targeted at resolving the major problems in case management could include reorganization and reallocation of resources.

It is recommended to analyse the:
- Process and impact of existing/future training programmes to determine how best we can implement improved dengue case management in different health care systems and achieve greatest impact.

CRITICAL ISSUES IN DENGUE PATHOGENESIS

Better understanding of dengue pathogenesis will be the foundation for future rational clinical interventions. In particular, we need to understand the molecular and pathophysiological changes underlying: endothelial permeability in plasma leakage syndrome, dengue virus diversity (which may account for heterogeneity in virus biology including virulence and epidemic potential), and immune response to dengue viruses (which may paradoxically predispose individuals to severe disease).

It is recommended to analyse the:
- Physiological and molecular causes of vascular leak and haemorrhage.
- Host genetic factors associated with dengue severity.
- Dengue viral factors and antigenic subtypes related to tropism, epidemic potential, and virulence.
- Mechanisms of antibody-mediated enhancement and protection.
- Mechanisms of virus entry and cellular/tissue tropism.
- T and B cell responses and their relation to immunopathology and protection in primary and secondary infections.
- Genetic predisposition to dengue.

Stream 2: Research related to transmission control through improved vector management

DEVELOPMENT AND EVALUATION OF VECTOR CONTROL TOOLS AND STRATEGIES

Although powerful vector control tools are available, in practice their effectiveness has been compromised by issues of delivery, coverage and acceptability. Promising new tools and approaches need to be evaluated for: efficacy in reducing dengue transmission, cost effectiveness, community acceptance, and prospects for preventing and controlling other vector-borne diseases, while it needs to be determined how best to take promising pilot community interventions to scale in a cost-effective way.

It is recommended to analyse the:
- Efficacy of new vector control tools and strategies in different contexts.
- Effectiveness, cost, community acceptance, and feasibility of combinations of new and/or existing tools, including integrated vector management and ecosystems interventions, with a range of partners in different contexts.
- Scaling up of pilot projects to state or national level in order to identify and disseminate best practices.

SURVEILLANCE AND RESPONSE

Disease and vector surveillance are fundamental to effective programme management. Strengthening of surveillance systems through development and validation of reliable risk indicators and the application of information technology is needed for improved decision-making.

It is recommended to analyse:
- Improved methods such as the pupal demographic survey and its application in operational contexts as indicators of risk for outbreak and for informing targeted intervention.
- The development and utilization of early warning and response systems.
- The triggers (factors and information) that will allow effective response to incipient epidemics.
- The contribution of information technology (e.g. GIS, bioinformatics, DengueNet, mathematical models) to decision-making.

Stream 3: Research related to primary and secondary prevention

VACCINES

Vaccines offer the greatest hope for dengue prevention and several candidates are in clinical development. The challenge has been to identify vaccine components and immunization strategies that are suitably safe and broadly immunogenic. The identification of immune correlates of protection should accelerate successful vaccine development and regulatory approval.

It is recommended to analyse:
- Through discovery and pre-clinical research, new vaccine candidates, adjuvants, and vaccination strategies.

- Correlates of protective immunity for use as an endpoint in vaccine trials.
- Immune responses in vaccine trials and natural infections.
- Through the development of further field sites, including sites free of non-dengue flaviviruses, the prospects for phase 3 and 4 vaccine evaluation.
- The issues associated with future vaccine usage and coverage through vaccine implementation research.

DRUGS

Anti-dengue drugs may have prophylactic (e.g. outbreak prevention) and therapeutic (e.g. prevention of severe disease) uses, with an ensuing impact on disease incidence and severity. Anti-viral drug discovery for dengue has accelerated in recent years along with our knowledge of 'drugable' targets in the virus.

It is recommended to analyse:

- The structure of viral-encoded proteins to aid rational drug, diagnostics and vaccine design.
- New (including natural) products or existing licensed drugs with good safety profiles and to foster drug discovery efforts.

Stream 4: Health policy research contributing to adequate public health response

There is a contradiction between the high priority afforded at political level to dengue and the low level of resources actually allocated to dengue prevention and control. Health policy research will facilitate a redress of this imbalance.

It is recommended to analyse:

- The issues and events that will, through adequate dissemination of information (including of burden of disease [DALYS] and costs of illness), elevate dengue to a high priority at national and international levels.
- Case studies of national programmes to identify factors leading to success or failure of dengue prevention and control programmes in order to develop a set of best practices.
- The decision-making that results in a declaration of state of emergency to allow more timely and effective political response, and to identify the data triggers used in this process.
- The importance and burden of dengue in less studied regions, particularly Africa, and its role as contributor to 'fevers of unknown origin'.

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Dengue

Annex 1
AGENDA: Scientific Working Group on Dengue

Monday 2 October	Item	Speaker
09.00–09.30	Welcome address Overview of TDR's research strategy and vision Introduction of participants	Dr D.L. Heymann, Acting ADG/CDS Dr R. Ridley, Director TDR SWG Chair/Secretariat
09.30–09.45	Overview of activities for the SWG meeting and TDR/WHO research streams	A. Kroeger, TDR
09.45–10.10	Recent epidemiological trends, the global strategy and public health advances	M. Nathan, R. Dayal-Drager
10.10–10.30	Global dengue burden: the known and the unknown	J.A. Suaya
10.30–11.00	Coffee break	
Issues and challenges for dengue research		
11.00–11.30	Dengue research needs related to surveillance and emergency response	D. Gubler, Vu Sinh Nam, Eng Eong Ooi
11.30–12.00	Research needs related to delivery issues and behavioural change	L. Lloyd, J.L. San Martin, R. Martinez, J. Elder
12.00–12.30	Research needs related to dengue case management in the health system	L. Lum, N. Hung
12.30–13.00	Understanding pathogenesis, immune response and virus factors	M.G. Guzman, C. Simmonds
13.00–14.30	Lunch break	
14.30–15.00	Opportunities in the evaluation and development of dengue diagnostics	P. Buchy, S. Yoksan, E. Hunsperger
15.00–15.30	Opportunities in the development of dengue vaccines	A. Barrett
15.30–16.00	Coffee break	
16.00–16.30	Opportunities in the development of dengue drugs	B. Canard
16.30–17.00	Dengue vector control: tool development and strategies	P. McCall, P. Kittayapong
17.00–17.30	Dengue vector control: resistance and resistance management	J. Hemingway
17.30–18.00	Summary: dengue research needs	SWG Chair
Closure day 1 and reception		

Tuesday 3 October	Item	Name
09.00–09.30	Dengue transmission dynamics: assessment and implications for control	D. Focks, R. Barrera
Working groups		
09.30–09.45	Introduction to working groups: objectives and expected outcomes	SWG Chair/Rapporteur
09.45–10.30	Working groups I, II, III, IV (meet individually)	
10.30–11.00	Coffee break	
11.00–12.30	Working groups: continued	Working groups
12.30–14.00	Lunch break	
14.00–15.30	Working groups: continued	Working groups
15.30–1600	Coffee break	
16.00–17.30	Working groups: presentation of research priorities, rationale for selection, and recommendations	Working groups: plenary session
17.30–18.00	Working groups, chairs, and TDR/WHO secretariat	
Closure day 2		

Wednesday 4 October	Item	Name
Consolidating research needs and setting priorities		
09.00–9.15	Objectives and outcomes for day 3	SWG Chair/Rapporteur
09.15–10.30	Small group to review the overall prioritization, harmonize recommendations, and outline strategic plan Working groups to finalize reports	Small group (meets separately): SWG chair, Rapporteur and TDR/WHO members Working groups (meet individually)
10.30–11.00	Coffee break	
11.00–12.30	Small group: continued Working groups: continued	Small group Working groups
12.30–14.00	Lunch break	
14.00–14.20	Small group: report on overall priorities and draft strategic plan (in plenary)	SWG Chair/Rapporteur
14.20–15.00	Working groups: comments on summary recommendations (in plenary)	SWG Chair/Rapporteur
15.00–16.30	Discussion and amendment of conclusions, recommendations, and draft strategic plan (in plenary)	SWG Chair/Rapporteur
16.30–17.00	Concluding remarks and closure of meeting	SWG Chair/Rapporteur
Closure day 3		

Thursday 5 October	Item	Name
08.30–10.30	SWG and updated dengue guidelines	Guidelines working group
10.30–11.00	Coffee break	
11.00–12.30	Finalization of SWG Report	Chairs, rapporteurs
12.30–14.00	Lunch break	
14.00–15.30	Finalization of SWG Report	Chairs, rapporteurs
15.30–16.00	Coffee break	
16.00–17.30	Finalization of SWG Report	Chairs, rapporteurs
Parallel meeting of TDR/PDVI diagnostic group from 10.30 hrs.		

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Annex 3
WORKING PAPERS: Scientific Working Group on Dengue

EPIDEMIOLOGICAL TRENDS AND DISEASE BURDEN

WORKING PAPER 3.1. **RECENT EPIDEMIOLOGICAL TRENDS, THE GLOBAL STRATEGY AND PUBLIC HEALTH ADVANCES IN DENGUE**

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EPIDEMIOLOGICAL HIGHLIGHTS

We conservatively estimate that, since the last meeting of the Scientific Working Group (SWG) on Dengue in 2000 (TDR, 2000), there has been an increase of 110 million in the number of persons living in urban areas of the world with a high burden of dengue (United Nations, 2002). Approximately 975 million people now live in these urban areas, that is, almost half the global population estimated to be at risk of dengue infection.

By decade, the annual average number of cases of dengue fever or severe dengue reported to the World Health Organization (WHO) continues to grow exponentially. For the first 5 years of the current decade, the annual average number of cases was 925 896, almost double the figure for 1990–1999 (479 848 cases) (figure 1). In 2001, a record 69 countries from the WHO Regions of South-East Asia, Western Pacific, and the Americas reported dengue activity (figure 1). In 2002, the WHO Region of the Americas reported more than 1 million cases for the first time. Although there is poor surveillance and no official reporting of dengue to WHO from countries in the WHO African Region and the Eastern Mediterranean Region, recent outbreaks of suspected dengue have been recorded in Madagascar, Pakistan, Saudi Arabia, Sudan and Yemen, 2005–2006. For reviews of dengue history in these regions, see the paper by Rosemary Sang, in the present report, and EMRO (2005).

Geographical extension of areas with dengue transmission or resurgent dengue activity have been documented in Bhutan, Hawaii (USA), the Galapagos Islands (Ecuador), Easter Island (Chile), Hong Kong Special Administrative Region and Macao Special Administrative Region (China) between 2001 and 2004 (figure 2). All four dengue viruses are circulating in Asia, Africa and the Americas. Perhaps the only comforting news is that reported case-fatality rates have been lower in recent years than in the decades before 2000.

Figure 1. Average annual number of cases of dengue or severe dengue reported to WHO, and average annual number of countries reporting dengue

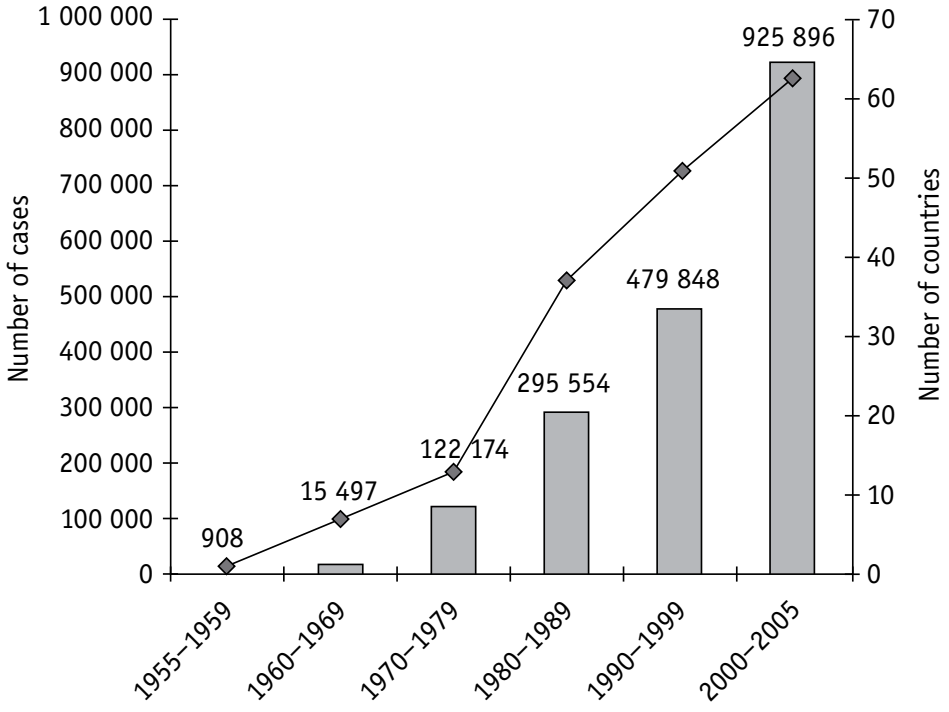
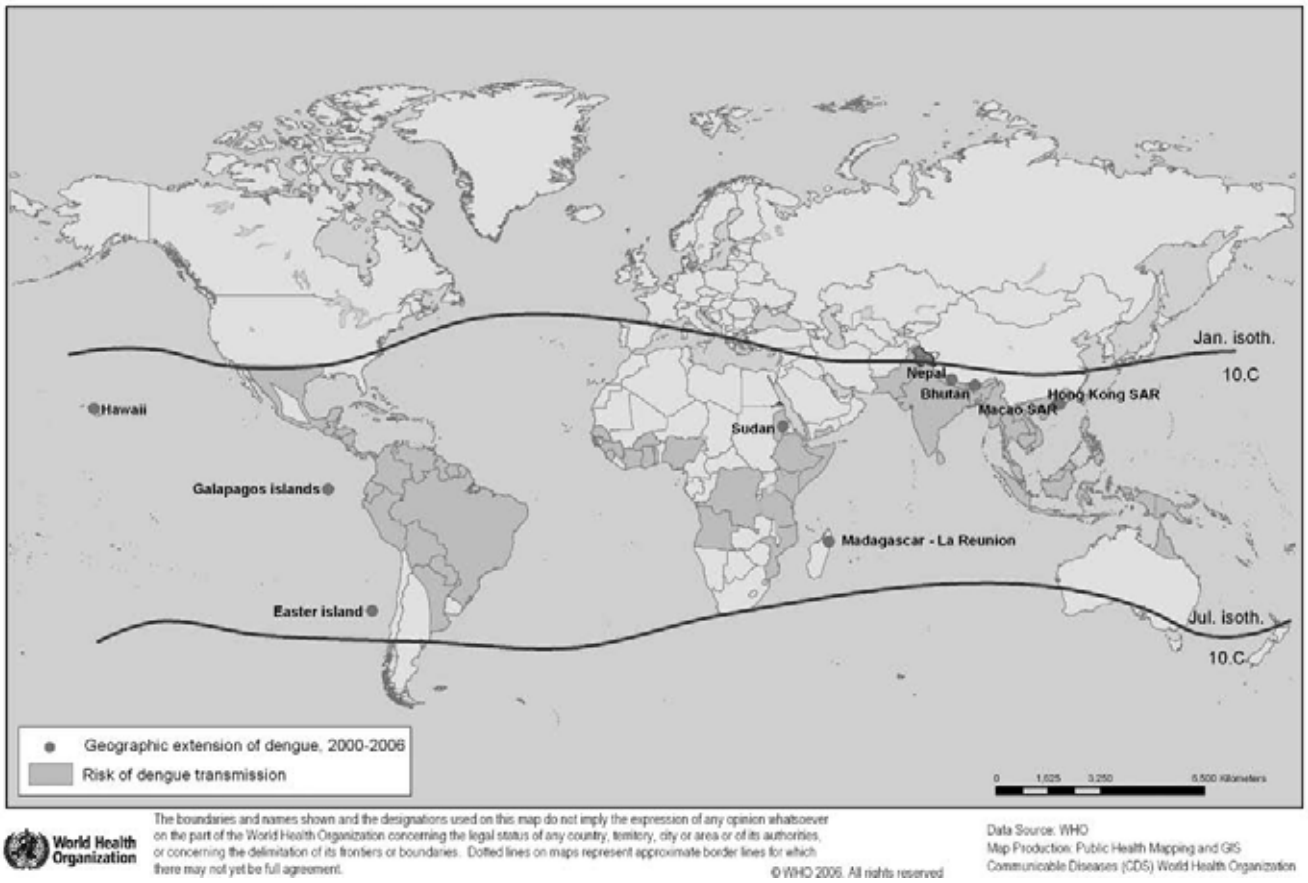


Figure 2. Countries and areas at risk of dengue transmission, 2006



Isoth.: Isotherm; the 10°C January and July isotherms represent the approximate northern and southern geographic limits, respectively, at which *Aedes aegypti* can survive the coolest months of the year. It has been found at higher latitudes (45 degrees N) but has not been able to survive the winter.

Chikungunya virus, *Alphavirus* (Togaviridae), has received considerable attention recently, because of severe outbreaks in areas of known endemicity, notably India and Indonesia, and in islands of the Indian Ocean, where it has seldom or never been reported before. Mention is made of these outbreaks because chikungunya and dengue share common vectors in *Aedes aegypti* and *Ae. albopictus* mosquitoes.

THE GLOBAL AND REGIONAL STRATEGIES

The Global Strategy for Dengue Fever/Dengue Haemorrhagic Fever Prevention and Control is more than ten years old (WHO, 1996), but remains essentially unchanged. It comprises five major elements: selective integrated vector control, with community and intersectoral participation; active disease surveillance based on a strong health-information system; emergency preparedness, capacity building and training; and vector-control research. Efforts have since focused on three fundamental aspects: surveillance for planning and response; reducing the disease burden; and changing behaviours. The Fifty-fifth World Health Assembly in 2002 (resolution WHA55.19) urged greater commitment among Member States and WHO to implement the strategy.

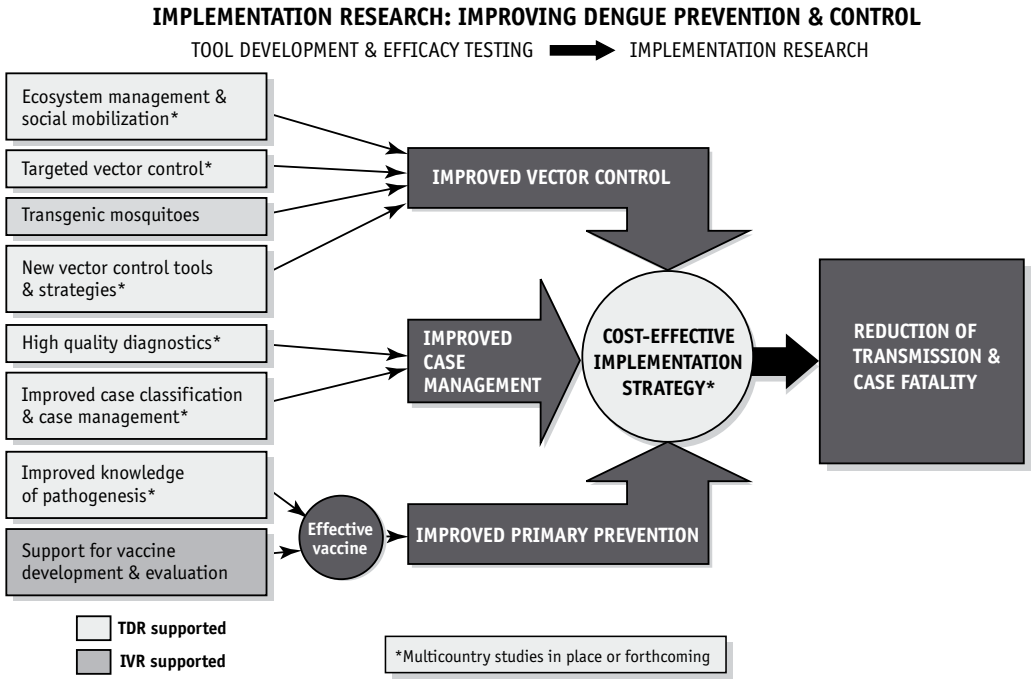
Of particular significance is the resolution (WHO, 2006c), Revision of the International Health Regulations, made by the Fifty-eighth World Health Assembly in 2005 (WHO, 2005), which includes mention of dengue fever (and yellow fever) as an example of a health 'event that may constitute a public health emergency of international concern', and of which, under such circumstances, WHO should be notified under the International Health Regulations.

ADVANCES SINCE THE LAST SWG

Since the last SWG, several new, improved or validated tools and strategies have been developed and are available to public health practitioners and clinicians. They include:

- Rapid commercial diagnostic tests in use in endemic countries.
- *Pocket book of hospital care for children. Guidelines for the management of common illnesses with limited*

Figure 3. Dengue research and training supported and encouraged by the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases or the Initiative for Vaccine Research



*Topics that are currently under investigation, or will soon be under investigation, in multicountry studies.

Modified from Kroeger et al. (2006) *Annals of Tropical Medicine and Parasitology,* 100(Suppl. 1):S98, with the permission of the Liverpool School of Tropical Medicine.

resources (inclusion of dengue in the management of fever) (WHO, 2006b).
- An audiovisual guide and transcript for health-care workers responding to outbreaks (WHO, 2006a).
- Guidelines for planning social mobilization and communication.
- Global strategic framework for integrated vector management (WHO, 2004).
- Dengue CD-ROM, Wellcome Trust, Topics in International Health series (Wellcome Trust et al., 2006).
- Entomological survey technique to identify the most productive container habitats of the vector(s).
- Seven insecticide products evaluated by WHO for mosquito larviciding (five insect growth regulators, two bacterial larvicides), four of which are approved for use in drinking-water (methoprene EC, pyriproxyfen GR, Vectobac DT and GR); three insecticide products evaluated by WHO for space spray applications to control mosquitoes (all pyrethroids).
- The sequence of the *Ae. aegypti* genome.
- Advances in the development and operational deployment of DengueNet[a] for global dengue surveillance.
- International Health Regulations 2005: voluntary compliance in effect.

New initiatives contributing to the development of new tools and strategies include:
- Paediatric Dengue Vaccine Initiative[b] (Bill & Melinda Gates Foundation).
- The Innovative Vector Control Consortium[c] (Bill & Melinda Gates Foundation).
- Asia-Pacific Dengue Partnership.
- DENFRAME[d] and DENCO (European Union).
- Novartis Institute for Tropical Diseases[e] (Novartis and the Singapore Economic Development Board).
- Regional Development Banks (Asian Development Bank, Inter-American Development Bank).

Streams of dengue research and training that have been supported by the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (TDR) and by the Initiative for Vaccine Research, or which will soon be the subject of investigation, are summarized in

[a] DengueNet: http://www.who.int/csr/disease/dengue/denguenet/en/index.html

[b] Paediatric Dengue Vaccine Initiative: http://www.pdvi.org/

[c] Innovative Vector Control Consortium: http://www.ivcc.com/

[d] DENFRAME: http://www.denframe.org/

[e] Novartis Institute for Tropical Diseases: http://www.nitd.novartis.com/

Table 1. Control strategy, major challenges and research needs for the prevention and control of dengue

Main control strategy	Major problems and challenges	Major research needs
Reduction/interruption of transmission through vector control	Lifestyles that provide abundant larval habitats Water infrastructure and solid waste management Sustainability of vector control	Vector-control targets/thresholds to reduce/interrupt transmission Behavioural changes conducive to the prevention and control of dengue
Patient management and supportive treatment	Early diagnosis and treatment	Patient management and supportive treatment
	Vaccine and drug development	Pathogenesis and disease prognosis Vaccine and drug development

Modified from Cattand et al. (2006), reproduced with permission from the World Bank.

figure 3 (from Kroeger et al., 2006). They involve the development and efficacy testing of tools for vector control, case management and primary prevention (vaccine development).

Cattand et al. (2006) perceive a growing consensus that community-based approaches to transmission control are desirable and necessary (see table 1), and that they improve prospects for the sustainability of vector-control programmes, although few such interventions have expanded beyond the pilot stage. However, Cattand et al. suggest that decentralization of budgets and programme operations offer opportunities for strengthening and expanding this integrated vector management approach for transmission control.

The biggest challenge today appears to be how to maximize the cost-effective deployment of tools and interventions that are already available. For this, we need clearly-directed operational research. For tomorrow, we must pursue the development of new tools and strategies, including better diagnostics, treatments and the elusive public-health vaccine.

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WORKING PAPER 3.2. DENGUE: BURDEN OF DISEASE AND COSTS OF ILLNESS

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SUMMARY

This paper provides a framework for considering the global burden of illness attributable to dengue, and its cost, and describes challenges encountered in the estimation of these values. Major challenges include: lack of uniform application of the World Health Organization (WHO) case definition, limited capabilities and standards of dengue laboratories, limited accuracy of rapid tests, misdiagnosis, lack of uniform criteria to report cases of dengue to WHO, limited role of surveillance and reporting systems, under-reporting of fatal and non-fatal dengue, misclassification in reporting, limited public knowledge about major regions at risk and travellers. While the scientific literature contains few studies on the burden of dengue and cost of illness, available results suggest that the actual number of cases of dengue may range from 3 to 27 times the reported number. We propose a conceptual framework for research.

INTRODUCTION

Dengue is a rapidly growing public health problem in tropical and subtropical countries where the majority of the world's population resides and is increasing most rapidly. However, most of these nations are economically disadvantaged and are faced with multiple public health problems, and therefore may not have the resources to combat the continued emergence of dengue.[1-3]

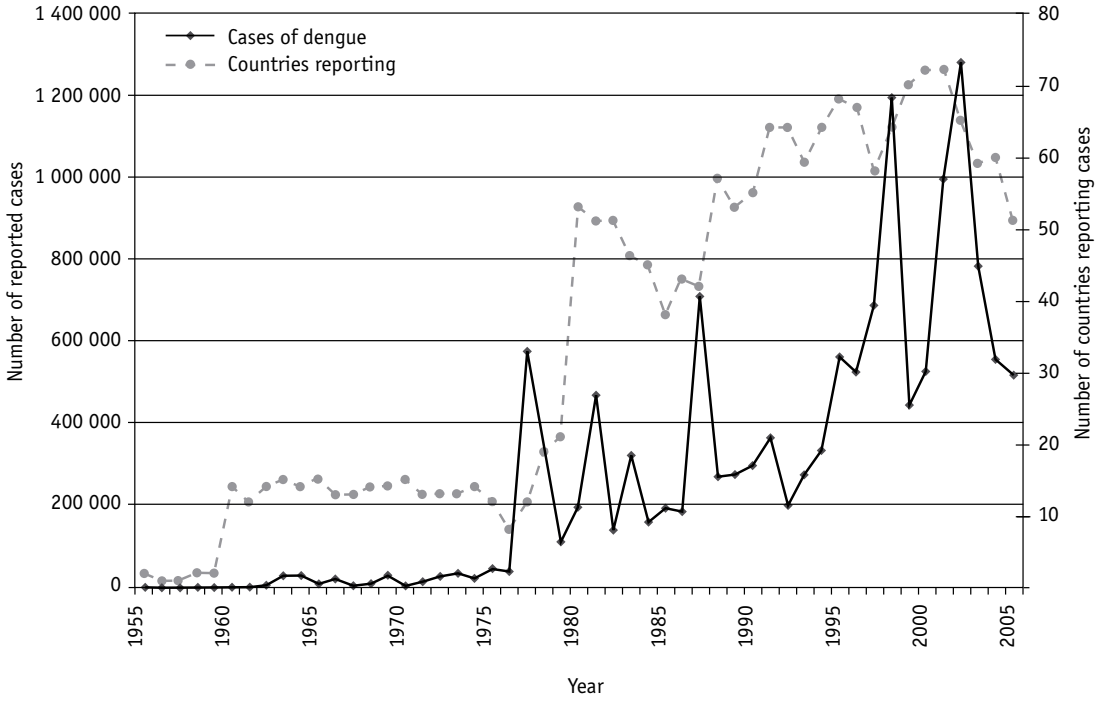
With approximately two billion people living in tropical and subtropical regions of the world, and an additional roughly 120 million people each year[4] travelling to these regions, a large share of the world's population is at risk of contracting dengue. Two estimates have suggested that between 50 and 100 million cases of dengue fever (DF) occur annually,[5-7] corresponding to an incidence rate of 2.5–5.0% of the two billion people worldwide at risk. These cases result in hundreds of thousands of hospitalizations, and about 20 000 deaths each year.[2] The spectrum of dengue infection ranges from asymptomatic infection to death. Clinical presentations of the febrile phase include a milder non-localizing fever syndrome, or influenza-like illness, and classic dengue, or break-bone fever. Immediately after the febrile phase, the disease may progress to the more severe but less common forms of disease, which include dengue haemorrhagic fever (DHF; a febrile illness followed by abnormally low platelet counts, egress of plasma into the pleural and abdominal cavities and haemorrhagic symptoms), and dengue shock syndrome (DSS; DHF with evidence of systemic hypoperfusion). Although death occurs rarely in the febrile phase, it is most commonly the result of hypoperfusion after the development of DHF. Between 250 000 and 500 000 people develop severe dengue each year.[8]

Mildly symptomatic dengue is usually treated in an ambulatory care setting, while the more severe forms require inpatient management. Although more than 90% of patients who develop severe dengue have serological evidence of a previous dengue infection, it is not possible to predict which patients will progress to these more serious forms, complicating the triage and medical management of patients. Given the scope of the disease and the large numbers of persons with symptomatic infection, dengue infection may have a tremendous impact on the health-care systems of the countries involved and on household and labour economies, especially during epidemics.

Quantifying the epidemiological and economic burden of dengue is key to formulating policy decisions on research priorities, prevention programmes, clinical training for management of the disease, and the introduction of new technology. Reliable diagnosis, testing, and reporting of dengue would allow better understanding of the true burden posed by dengue in a world of competing public health issues. Reports should capture seasonal and cyclical (annual) variations in disease incidence. Such reports can be used for multiple purposes. First, they provide the basis for comparisons of the burden of dengue in different geographical locations and time periods. Second, they help country-level, regional, and global public health authorities to make informed decisions on resource allocations. Decisions can be based on a comparison of the burden of dengue with that of other health problems. Accurate estimates of the magnitude of dengue can serve to justify donor funding, setting priorities for research, and accelerating the development of dengue vaccines. Third, such reports will serve as important baselines for assessing the impact of any intervention (e.g. a larvicide campaign or vaccine) that could alter the burden of dengue, and will also provide a key ingredient in cost–effectiveness analyses of a single or multiple interventions and technologies.

The prospect of introducing vaccines against dengue

Figure 1. Dengue cases: global annual number of cases reported and number of countries reporting to WHO by year, 1955 to 2005



makes the importance of understanding the burden of dengue especially important. Substantial investments are required in preclinical research, human testing, manufacturing, distribution, etc. These activities will involve many stakeholders, from donors to manufacturers and governments to consumers. This paper seeks to make these decisions better informed.

THE EPIDEMIOLOGICAL BURDEN OF DENGUE

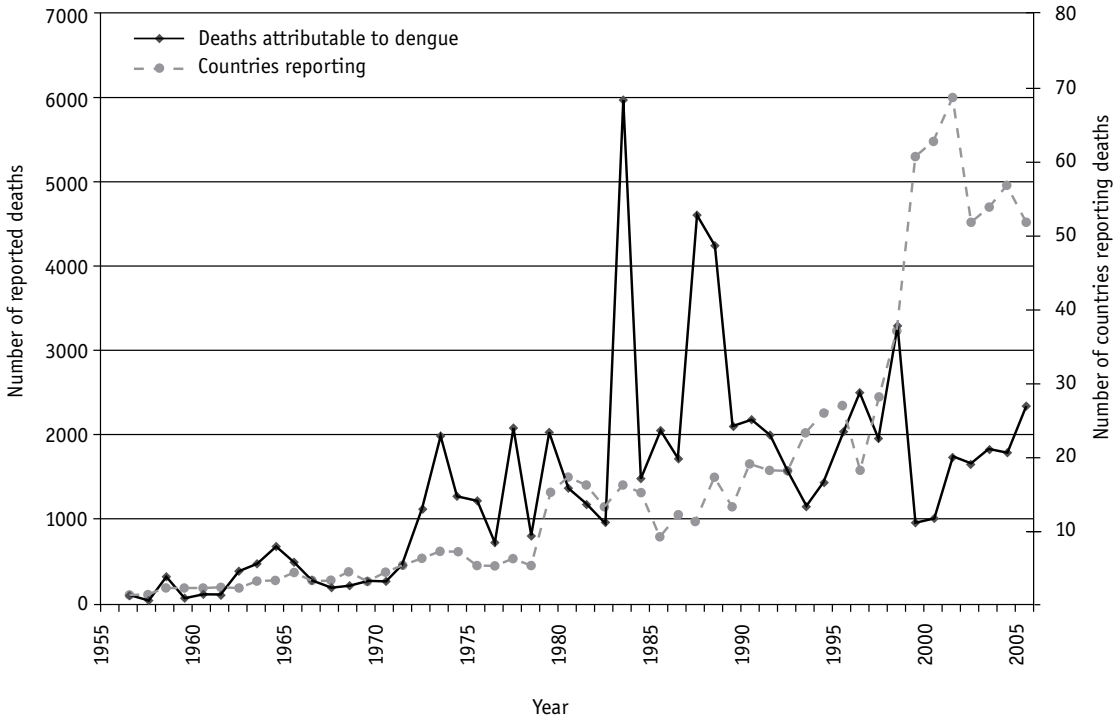
The burden of illness caused by dengue refers to the amount of disease imposed by dengue and measured using a set of epidemiological indicators, such as number of clinical cases classified by severity (DF, DHF, and DSS), duration of the illness episode, quality of life during the illness episode, case-fatality rate, and absolute number of deaths during a period of time. All of these epidemiological indicators can be combined into a single health indicator, such as quality-adjusted life years (QALY)[9] or disability-adjusted life years (DALY).[10] Internationally, DALYs are most often used. The burden imposed by dengue and the potential benefits of any intervention, such as vaccination, can then be expressed in terms of DALYs lost or saved and cost per DALY lost or saved.

Current knowledge of the burden of dengue

Dengue is endemic in all WHO Regions except the WHO European Region.[11] Figure 1 shows the global number of cases reported by year and the characteristic cyclical variations attributable to high epidemic and low post-epidemic years. In each year until 1976, fewer than 40 000 cases from fewer than 15 countries were reported annually to WHO. In 1974, WHO developed the first guidelines for the diagnostic and management of dengue.[8] Figure 1 shows that the lowest number of cases was reported in 1979 and the highest number in 2002, with 110 000 and 1 300 000 cases reported, respectively. Figure 2 shows the annual global number of deaths attributable to dengue and the number of countries reporting. There were similar cyclical variations in the number of deaths coinciding with epidemic and non-epidemic years. After 1977, the year with the lowest reported number of dengue deaths was 1978 (with 807 fatalities) and the year with the highest number was 1983 (with 6031 fatalities). The majority of the cases and deaths were reported from southeast Asia and the western Pacific.

A number of studies have attempted to estimate the burden of dengue in terms of DALYs. For example, the burden was estimated for Puerto Rico in 1984–1994.[12] Estimates were based on the numbers of cases and deaths reported to the national surveillance system, and on age-group expansion factors used to control for under-reporting. The authors estimated an average loss of 658 DALYs per million population per year, and concluded that this burden was comparable to that attributed to meningitis,

Figure 2. Dengue deaths: global annual number of deaths reported and number of countries reporting to WHO by year, 1955 to 2005



hepatitis, malaria, tuberculosis, the childhood cluster of diseases (polio, measles, pertussis, diphtheria, and tetanus), or intestinal helminthiasis, and of the same order of magnitude as tuberculosis in Latin America and the Caribbean. A study in south-east Asia estimated a loss of 420 DALYs per million population per year, comparable to that of meningitis (390 DALYs per million population per year), twice the burden of hepatitis, and one third of the burden imposed by HIV/AIDS in the region.[13] A study for Thailand estimated that country's burden at a loss of 427 DALYs per million population per year for 2001.[14]

The Disease Control Priorities Project has recently published the global burden of disease for 2001 to 2003[15]. It estimated the global burden of dengue as 528 000 DALYs for the year 2001. This corresponds to a burden of 264 DALYs per million population per year for two billion people living worldwide in areas at risk of dengue.

Major gaps in knowledge of dengue burden

Current global estimations of the burden of dengue are considered to be uncertain because of a number of factors, discussed below. The under-reporting of dengue cases (both fatal and non-fatal) is probably the most important barrier to obtaining an accurate assessment.

Lack of uniform application of the WHO case definition

WHO has published guidelines for the diagnosis, classification, and management of dengue, which have been adapted by WHO regional offices.[16] Investigators have reported difficulties in applying the case definition because of its complexity and the limited ability to explain observed patterns of disease.[8, 17, 18] Because of these difficulties and the need to use categories relevant to their own needs for planning and management, some countries have instituted their own case definitions.[8]

Limited capabilities and standards of dengue laboratories

Tests for anti-dengue IgM antibodies are commercially available. Their accuracy may vary with the situation in which they are used. Tests for virus detection by cell culture or nucleic acid detection (e.g. polymerase chain reaction) require the capabilities of sophisticated research laboratories to produce test materials and perform quality testing.[19] Laboratory capabilities, infrastructure, technical expertise and research capacity need to be improved.[20]

Scientists at a recent WHO-sponsored meeting pointed out limitations in laboratory standards, quality control, and dengue serological diagnosis and virus isolation, as well as for reporting and information exchange in south-east Asian and western Pacific countries.[21] Similar considerations may

apply to laboratories in the Americas as suggested by an evaluation of quality control of serological diagnostic tests in major national reference laboratories responsible for dengue surveillance and diagnosis in the region.[22] Although the majority of the participating laboratories had a high level of performance in detecting IgG and IgM antibodies to dengue, only 63% of 86 laboratories that received samples for testing between 1996 and 2001 decided to participate in quality control. This study also highlighted the challenges faced by participating laboratories, such as interruptions in the availability of antigens, the low sensitivity of testing for IgM antibody by enzyme-linked immunosorbent assay, and the lack of alternative tests and techniques for the diagnosis of dengue. There is no information about quality control for the isolation or identification of dengue virus. Regions with imported dengue, such as the USA and Europe, may face additional challenges. Since dengue is not common in these areas, laboratory testing for dengue may not be available and the disease may be overlooked as a cause of symptoms among ill travellers. For example, as part of an external quality assurance evaluation, 20 serum samples were sent in 2002 to 18 European participating laboratories to be tested for the presence of dengue virus-specific IgM and IgG antibodies.[23] Laboratories reported concurrent and correct results for 71% of the IgG-positive samples and 89% of the IgG-negative samples. However, though 97% of the IgM-negative samples showed concurrent and correct results, only 58% of the IgM-positive samples had concurrent and correct results. These findings highlight the need for quality controls and improvements in testing for dengue in countries with imported dengue; worldwide laboratory capabilities and quality control are not adequate.

Limited accuracy of rapid tests

In a recent meta-analysis of 11 studies of rapid diagnostic assays for dengue, the authors evaluated the performance of an immunochromatographic test (ICT) manufactured by a leading company in its field. The meta-analysis showed that these assay had a wide spectrum of sensitivity (0.45 to 1.0) and specificity (0.57 to 1.0), suggesting that such tests may have limited accuracy.[24] Also, the cost of rapid tests is a barrier to their systematic use in developing countries. Little is known about the performance of other rapid diagnostic tests sold in the market.

Misdiagnosis

Despite the available clinical guidelines, dengue can be misdiagnosed (by under-diagnosis or over-diagnosis). Given the lack of specificity of the symptoms of dengue, clinicians can confuse dengue with other infections, such as influenza, enterococus, chikungunya, viral haemorrhagic fevers, leptospirosis, malaria, or typhoid.[25] Moreover, dengue infection as an undifferentiated febrile illness may represent a large proportion of all the symptomatic cases of dengue.[26]

A study reviewing medical records for a period of 6 months in Laredo, Texas, in 1999 showed that only 50% of patients with clinical suspicion of dengue were diagnosed as such.[27] Misdiagnosis is more likely if other febrile diseases with similar clinical characteristics occur concomitantly. For example, in Barbados in 1995 and 1997, the majority of patients with suspected leptospirosis actually had dengue. Conversely, some of the cases of suspected dengue were actually leptospirosis. [28] In another study of an outbreak of dengue in Bangladesh, about 18% of cases of suspected dengue gave negative results in laboratory tests for dengue and proved to have leptospirosis.[29] In a study in a dengue-endemic province in Viet Nam, among 697 patients with acute undifferentiated fever visiting primary care facilities, for whom paired serum samples were collected, acute dengue was diagnosed in 33.6% cases.[26]

Misdiagnosis can be influenced by treatment guidelines. For example, although WHO guidelines for the treatment of febrile children aged 2 months to 5 years are useful to ensure that children with fever and no alternative explanation are empirically treated for malaria, this guideline may contribute to misdiagnosis of dengue, particularly in areas of low malaria transmission or where physicians are not routinely using malaria smears to confirm the diagnosis.[30, 31] In addition, drawing blood and sending it for testing for dengue may not be viewed by the health-service provider as a worthwhile expenditure of time or money. In the absence of rapid testing, the result of the test will not be available for days or weeks, and the provider may be left to diagnose the patient with a viral syndrome or fever of no known source and treat the patient empirically. Rapid testing may improve the situation, but the cost of the test may be a disincentive. Finally, even in those countries with adequate laboratory facilities, cross-reactivity between anti-dengue antibodies and antibodies to other flaviviruses (West Nile virus, St Louis Encephalitis) and the dynamics of the immune response to flavivirus (boosting of antibodies to the primary infecting flavivirus during a second flavivirus infection—'original antigenic sin') further contributes to the problem of confirming diagnosis of dengue in areas of the world where multiple flaviviruses exist.

Lack of uniform criteria to report cases of dengue to WHO

In the WHO Region of the Americas, cases of dengue are reported to WHO stratified by severity: DF and DHF. However, in the WHO South-East Asia Region and the Western Pacific Region, cases are reported without distinction of severity, though most reported cases involve hospitalization for DHF.[11] Additionally, while some countries only report cases of severe dengue, others report all cases, and still others report only confirmed suspected cases.[32] This lack of uniform reporting makes it difficult to perform meaningful international comparisons and aggregations.

Limited role of surveillance and reporting systems

Surveillance systems depend mainly on the capacity of the hospital to record, monitor, and report dengue statistics. Less information is obtained from clinics, and still more limited data are reported by private-sector medical practices. In south Asia, for example, notification is barely enforced and the number of cases of communicable diseases (such as dengue) dealt with in the private sector is usually unknown.[33] In the Americas, surveillance systems are also generally passive and considered to be ineffective in defining the full scope of transmission in a given community.[34]

Underreporting of non-fatal dengue

There are a number of factors that contribute to the under-reporting of dengue. Firstly, notification of suspected dengue to public health authorities (communicable diseases units) is legally required in most of the affected countries, but rarely enforced. Since the results will often not be available for days or weeks after the visit that led to the sample collection, the results may be viewed as having little clinical value to the treating physician. Complicated reporting or lengthy requirements may be additional factors that reduce the motivation of health-care providers to routinely report cases, since reporting may not be of intrinsic value to the patient or busy health-care providers. Surveillance systems usually have logistic and budgetary constraints. Consequently, the reporting of dengue is likely to be fragmented, incomplete, inconsistent, and unreliable. Thus, under-reporting of dengue cases, and probably even of deaths attributable to dengue, is a major concern to be addressed.

Evidence of under-reporting is evident in studies conducted in Brazil, Indonesia, Puerto Rico, and Thailand. In Belo Horizonte, south-eastern Brazil, the level of reporting of hospitalized suspected cases of dengue was estimated to be 63% between 1997 and 2002.[35] As the cases recorded in the reporting system were the more severe, the overall case-fatality rate may have been consequently overestimated. In Indonesia, the number of reported cases was compared with medical records of hospitalized DHF cases admitted in four major hospitals in Bandung during 1994. Only 31% of these cases were captured in the report.[36] Similar under-reporting was found in Puerto Rico, where only 28.4% of hospitalized cases of DHF were detected by any of the surveillance and reporting systems.[37] In another study, the same author tried to measure the burden of dengue in Puerto Rico during 1984–1994.[12] To deal with the existing under-reporting, it was estimated that for every case of dengue reported among children, there were about 10 additional cases not reported. Among adults, it was estimated that for every case reported, 27 cases went unreported. In a recent study in Thailand, under-reporting was recognized and the true number was estimated as 10-fold the number reported.[14]

Misclassification in reporting of dengue

Cases of dengue can be misclassified at the time of diagnosis because of the lack of familiarity of some medical providers with dengue as a disease, or difficulties with using the WHO classification system.[8] Correct classification is important clinically, because death is associated with the more severe form of the disease. Correct classification is important epidemiologically because WHO has suggested that the dengue case-fatality rate can be computed by dividing the number of deaths by the number of cases of DHF.[16] If classification is not uniform, comparisons of case-fatality rate between countries can be misleading. The severity of dengue is also a predictor of the use of health-care services and of the costs of medical care.[38] In a study in Puerto Rico, only 17 DHF and 3 DSS cases were identified among 986 hospitalized cases of dengue reported via the surveillance system in 1990–1991. A review of the hospital records of those patients, however, found that 88 and 14 of them had a clinical diagnosis of DHF and DSS, respectively.[39] Reviews of medical records identified about five times more cases of severe dengue than were reported to the routine surveillance system. If appropriate allocation of resources to address the dengue problem is to occur, better recognition of the severity of dengue and improved reporting is needed. Another review of the medical records of patients with dengue during the 2002 epidemic in Taiwan, China, found that 71% of DHF patients were discharged without such a diagnosis.[40]

A consequence of this misclassification is the under-reporting of severe dengue.

Underreporting of fatal dengue

Reports of deaths caused by dengue are intuitively assumed to be more accurate than reports for non-fatal cases. During the 1998 epidemic of dengue in Puerto Rico, there were 17 000 reported cases of dengue and 19 deaths for which dengue was confirmed or probable. For the same year, however, only five deaths attributable to dengue are shown on WHO DengueNet, the WHO-sponsored internet-based system for the global surveillance of DF and DHF.[41] This single time-point finding indicates a four-fold under-reporting of laboratory-positive dengue deaths. In addition, there were another 37 deaths for which dengue was initially suspected but could not be confirmed because the virus was identified by virus isolation or immunohistochemcial staining of tissue, the patient died before seroconverting, and no other explanation for the death was identified. However, dengue was ruled out in six of these cases.[42]An analysis of paired samples gathered during routine surveillance in Puerto Rico demonstrated that roughly half of the cases that were initially indeterminate based on testing of the acute-phase sample could be reclassified as confirmed owing to seroconversion identified by testing a convalescent sample (Garcia, unpublished data), suggesting that an additional 15 deaths suspected to be from dengue may actually may have been caused by dengue. If correct, this raises the under-reporting factor to seven in Puerto Rico. Difficulties in reporting and classification that occurred in Puerto Rico are likely to occur in other countries where dengue is endemic. Additional factors that may further interfere with confirmation and reporting of dengue deaths to WHO include political and economic disincentives raised by concerns regarding the possible impact on tourism.

Limited public knowledge from major regions at risk

Data on the transmission of dengue is limited for dengue-endemic regions that have a significant portion of the world's population—India, China, and sub-Saharan Africa.

INDIA

One billion people (15% of the world's population) reside in India. India's population is twice that of south-east Asia, the region that currently reports the most dengue-related deaths. Despite comparable environmental risk conditions, the number of reported cases and deaths in India is only a fraction of that reported in south-east Asia,

In many regions of India, an increasing number of suspected cases of dengue are seropositive for IgM and IgG antibodies.[43] The existence of IgG antibodies in a patient demonstrates prior infection with dengue and an increased risk of the severe forms of the disease. Outbreaks of dengue are increasingly reported in rural areas, implying that the population at risk is increasing, since dengue is considered to be a predominantly urban disease.[44-47]

Surveillance for dengue has been very limited in India and reporting to the central government has not been mandatory.[48] A recent study concerning the epidemic of dengue in Chennai in 2001 has suggested that the surveillance system was unlikely to generate proper information on the epidemiology of the disease.[49] In 2004, a WHO initiative called for promoting improvement of dengue surveillance as part of the Integrated Disease Surveillance Programme in India, strengthening laboratory networking and quality assurance, and reviewing case definitions.[50] Although improvements are being made, the current gaps in epidemiological data and surveillance mean that the burden of dengue in India is uncertain. However, dengue is recognized as one of the leading causes of death and hospitalization among children in India.[51]

CHINA

One billion three hundred million people, 20% of the world's population, live in China Roughly one fifth of China's land mass, including some of the more densely populated regions, lies in tropical climes where dengue transmission could occur all year round. Published reports on outbreaks of dengue detailed the re-emergence of dengue in the 1980s and 1990s.[52-54] However, since 2003, public data from WHO does not include cases in China[41], making the documentation of the current burden of dengue in this country very difficult.

SUB-SAHARAN AFRICA

The burden of dengue in Africa remains poorly understood. Travellers and military personnel visiting or stationed in Africa have been identified as having laboratory-confirmed dengue infections, indicating that the virus is circulating.[55, 56] Several studies of seroprevalence and fever in sub-Saharan Africa have identified evidence for the presence of the dengue virus in many sub-Saharan countries, including Cameroon, Djibouti, Kenya, Senegal, the Sudan and Burkino Faso. These studies reported lower seroprevalence rates than those seen in other

tropical countries, such Haiti, Brazil, or Thailand, ranging from 10% to 34% among persons tested.[57-59] As expected, higher levels of prevalence are noted among urban residents than rural residents.[60-65] In addition, periodic outbreaks of DF have been reported in the region.[63-66] Although genetic factors could provide some protection, without systematic surveillance and serosurveys with appropriate sample schemes to give a fair representation of the disease burden in the population, the past and current burden of dengue in Africa may remain poorly understood. Moreover, if dengue is an endemic problem in sub-Saharan Africa, more urbanization will only increase the burden of dengue.

Limited knowledge about dengue in travellers

According to the World Tourism Organization, in 2004, 125.4 million international tourists visited countries where they might be at risk for acquiring dengue infection.[4] Depending on the population studied and the laboratory methods used, serological evidence of recent dengue infection was found in between 7% and 45% of cases of febrile travellers returning from areas where dengue is endemic,[67-69] confirming that dengue is an important cause of fever among returning travellers. The increasing number of cases of dengue creates a significant economic burden owing to working days lost. However, given the spectrum of clinical illness, not all patients may seek medical attention or receive diagnostic testing. As a result, under-reporting of dengue infection occurs even in developed countries. Moreover, of those patients who are diagnosed with dengue not all may be reported to public health authorities. For example, between 1 January 2001 and 31 December 2004 seven residents of the USA were diagnosed with dengue after returning from Thailand.[56] According to the World Tourism Organization, 2 012 077 USA tourists visited Thailand during the same period,[70] giving a rate of 3.5 dengue infections per 1 million visitors to Thailand. However, among a prospective cohort of Dutch travellers, 0.7% of travellers returning from south-east Asia experienced symptomatic, laboratory-confirmed (anti-dengue IgM seroconversion) dengue infections.[71] If the risk of infection is similar for travellers from the Netherlands and the USA visiting south-east Asia, for each case reported to the United States Centers for Disease Control and Prevention there may be 5000 additional unreported clinical dengue infections. This is a conservative estimate since the USA, unlike the Netherlands, shares a border with a country where dengue is endemic—Mexico. As a result, USA residents have a higher potential exposure to dengue.

Personnel deployed in dengue-endemic areas during humanitarian emergencies and conflicts are at a higher risk of dengue infection than are regular travellers, since they usually live in areas without vector-control activities or air conditioning, and usually stay in those areas longer than do tourists. For example, during a 5-month deployment as part of the United Nations Mission in Haiti, 32% of 249 personnel with febrile illness had dengue.[72]

THE ECONOMIC BURDEN OF DENGUE, OR COSTS OF ILLNESS

Terminology

Cost-of-illness calculations generally distinguish 'direct' and 'indirect' costs.

Direct costs are those within the health-care system. They comprise the cost of diagnosis, treatment and prevention of dengue. There are three major direct cost categories—medical care, surveillance and reporting, and prevention. The cost of medical care includes the cost for ambulatory and inpatient care. Surveillance and reporting costs take into account efforts by governments and international organizations to monitor and disseminate information about cases, outbreaks, and deaths. Prevention costs include activities to prevent dengue, such as vector control (e.g. inspections, management of disposables, use of larviciding and fumigation, education, media campaigns, and community mobilization).

Indirect costs are the economic value lost by households and society in general owing to illness and premature mortality of dengue patients and productivity losses of household members and friends affected.

The estimation of direct and indirect costs is complex because it must take into account different 'payers' or economic sectors (public sector, household, third party, employers, society), different levels of government (district, regional, national), different national government agencies (Ministries of Health, Education, Environment; the Armed Forces), and different international organizations (e.g. WHO, United Nations Development Programme).

The system of national health accounts (NHA) provides a framework for examining costs within the health sector (i.e. direct costs).[73] This framework helps countries to assess the totality of financial resources available to the health sector (from the public, private, and donor sectors), to identify the financing agencies through which these funds flow, and to analyse how these funds are used (by type

of provider, function, geographic region or population group). NHA also provides analysts and policy-makers with a tool that not only assists in the analysis of current use of resources, but also helps in the planning of future resource needs and tracking to determine whether resources are reaching the target population. In countries where dengue is endemic, NHA can help analyse current expenditure (public, private, and by donor) on treating dengue. In turn, this information can be used to analyse the cost–effectiveness of any new vaccine and understand who will derive the most benefit. Understanding patterns of health-care use and expenditure may contribute to the development of policies that will improve the allocation of resources to the poorer segments of society, who might not be able to pay for a vaccine or other dengue-related interventions. WHO published the *Guide to producing national health accounts* in 2003.[73]

The most important government activities related to dengue include vector control, educational activities, mass media programmes, and ambulatory and inpatient care. Knowledge about spending on these activities by district, regional or national governments is fragmented. Government-sponsored health-care activities include care at clinics and hospitals. In hospitals, patients can receive ambulatory care (outpatient department and emergency room) or inpatient care (general, intermediate, or intensive care). Information about the use of hospital services can be obtained by following a cohort of people for a given period of time (community-based study), or obtained from a hospital itself (facility-based study). Hospital costs include tests, drugs, supplies, health-care personnel and medical facilities. To estimate the hospital costs of dengue patients, two approaches can be adopted: micro- or macro-costing. Micro-costing consists of a detailed inventory of the different services available and used in the hospital, the quantity used and the unit cost for each of the services. Macro-costing estimates the average unit cost for each output (e.g. hospital day of care or emergency room visit) rather than cost for each of its components (each laboratory test, drug administered, or procedure carried out by medical personnel). Macro-costing is simpler than micro-costing, as it requires access only to the hospital annual budget or spending and its breakdown by departments, and the total number of output units (such as hospitalizations, average length of stay, outpatient visits, emergency room visits, etc).

Productivity losses and school absenteeism as a result of dengue infections have not been accurately evaluated in most countries. Similarly, care-seeking behaviour, household out-of-pocket spending on treatment for dengue, caregiver's time, and family and psychological disruption have not been systematically or consistently measured.

Current knowledge and gaps concerning economic burden

Data on costs (in US dollars) of treatment are limited to the impact of outbreaks in a few countries. A few examples follow.

Costs of dengue in Thailand

In Thailand, a cost study done on DHF in 1994 estimated the weighted average direct patient cost (including travel, food and lodging and opportunity) at US$ 63.60, plus US$ 45.56 borne by the government for routine service costs in hospitals, totalling US$ 109.16. The per-capita cost of vector control in Thailand in that year was US$ 0.081 [74] A more recent study calculated a similar cost per case of US$ 61.[14]

Costs of dengue in South-East Asia

In another study in south-east Asia that assessed the potential cost–effectiveness of a paediatric vaccine for dengue, it was estimated that the societal cost per case of treating dengue (including ambulatory visits, hospitalization, medications, travel expenses and parents' time seeking treatment) was US$ 139 for DHF, and US$ 4.29 for DF, with a baseline cost of treatment equal to US$ 99 per 1000 population per year. For comparison, the population-weighted average gross national income per capita was US$ 1083 for south-east Asia in 2001.[13]

The same study reported the cost of dengue vector control per capita in other Asian countries: US$ 0.015 in Indonesia in 1998, US$ 0.081 and US$ 0.188 in Thailand in 1994 and 1998, respectively, US$ 0.240 in Malaysia in 2002 and US$ 2.40 in Singapore in 2000. On the other hand, per-capita spending on vector control in 14 Latin American countries in 1997 ranged from US$ 0.020 to US$ 3.560, compared with US$ 0.140 to US$ 8.490 in 17 Caribbean islands in 1990.

Costs of dengue in Puerto Rico

According to a study on the impact of an outbreak of DF in rural Puerto Rico, the loss of income attributable to the disease,(either from illness or from loss of time in caring for ill family members) was estimated to be equal to US$ 305 per household or US$ 125 per person.[75]

Worldwide summary by WHO

The UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (TDR) has summarized the costs of epidemic outbreaks of DF/DHF in several countries.[11] In Cuba, for example, the cost per treated case is US$ 299 compared with US$ 44 in Nicaragua, and US$ 44 in Puerto Rico.

Facility-based studies in eight countries

With support from the Pediatric Dengue Vaccine Initiative, a team from Brandeis University is coordinating researchers in eight countries to implement facility-based studies to measure the socioeconomic impact of dengue on households and the local or national health system. These multi-country studies use a common protocol for data collection and analysis. Three of the countries are in south-east Asia (Cambodia, Malaysia, and Thailand), while the other five are in Central and South America (Brazil, El Salvador, Guatemala, Panama, and Venezuela). Each study identified treated cases of dengue via one or more health institutions in the country (hospital, clinic, national laboratory, or public insurance system). For hospitalized patients, the researchers abstracted data from the patient's medical record. One or two rounds of interviews were conducted with the patient or guardian (if the patient was a child). About 60% of patients were interviewed twice (generally once during treatment and again after recovery), and the remainder interviewed once. About 2000 patients from 62 health facilities (both public and private) were recruited. Of the eight studies, five cover adults and seven cover children.

While analysis is still underway, data are feeding into efforts to work with ministries of health and other critical agencies in the collaborating countries to judge the potential benefit of dengue vaccines. For example, the investigators in Malaysia found that a hospitalized patient received an average of 10 visits from household members. Data from Thailand showed that family members invested the equivalent of 23 working days caring for one hospitalized patient. The Cambodian team documented substantial family disruption when a mother had to spend her day beside her sick child in the hospital while the grandmother provided food and other relatives cared for the other children at home. The breadth of involvement by household members illustrates that the impact of each case goes well beyond medical spending.

The data will allow careful comparisons to be made between the cost-of-illness for dengue and other diseases, such as rotavirus and pneumonia, which commonly cause hospitalization of children. The data suggest that the cost to the family is a severe burden and is as high as or greater than that of other diseases.

Models of disease burden and costs

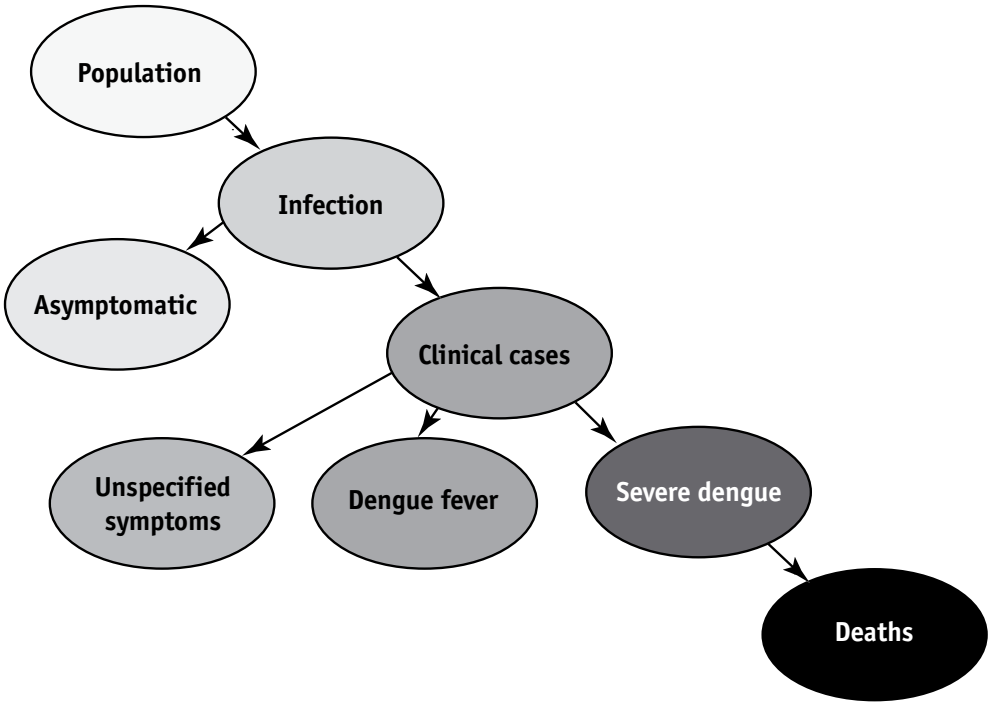
Disease modelling is often used to estimate the burden of disease and costs at the country or regional (multi-country) level. A model for the economics of a disease comprises a group of mathematical relationships among disease states, such relationships being able to provide estimates of infections, clinical cases of varying levels of severity, treatments received, use of health resources, and costs. While an original study may seek to describe the burden of disease in one population at one time (e.g. a specific district), a model can potentially cover a large geographical area and span of time periods. Disease models typically incorporate data from a multitude of sources. By pooling studies, best estimates can be derived and refined to achieve internal consistency. For example, staff at WHO have developed a mathematical model of disease, 'DISMOD', to check the internal consistency of epidemiological estimates of incidence, prevalence, duration and case fatality.[76] Disease models not only describe the current or historical situation, but can also be used to project future situations under current policies, as well as the impact of new policies and technologies for prevention or treatment.

In 1993, Shepard & Halstead published a disease model to examine the benefits and cost-offsets of improved case management of dengue (medical treatment, environmental control, such as reduction of mosquito breeding sites, and the potential development of a vaccine).[77] This study demonstrated that a model can be used not only to examine individual proposed policies, but also on any combination of proposed policies. A study on the cost–effectiveness of a potential paediatric tetravalent dengue vaccine[13] modelled the burden of disease attributable to dengue in south-east Asia in 2000, and projected how that burden would be reduced by the proposed vaccine. Figure 3 shows an updated version of the study's state-transition model of dengue infection and illness. The study's cost projections were based on estimated vaccine costs and projected savings in treatment costs. The net cost per 1000 population was estimated to be only US$ 17 (89% less than the gross cost). Also, the cost per DALY saved by a paediatric vaccine would be US$ 50, making the potential vaccine highly cost-effective.

Estimates of the costs of vector control

Several researchers have developed estimates of the

Figure 3. State-transition model of dengue illness



costs of vector control programmes for dengue. In 1993, a team sponsored by WHO developed guidelines for assessing the cost–effectiveness of vector control, which included procedures for ascertaining the costs of such programmes.[78] They presented case studies on vector-control programmes for malaria and schistosomiasis, but not dengue. In the same year, another study reported the costs of Singapore's intensive vector-control programme.[77]

Two of the authors of this paper recently developed a model for estimating the cost of vector-control programmes at a country level and applied the model to Malaysia.[79] The procedure identifies the two major components of a vector-control programme: inspections and fumigation. Aggregate costs were estimated by determining the volume of each activity per year (inspecting and fumigating premises and neighbourhoods near a location where dengue had been found) and the unit cost of each activity, and deriving the total cost. The study estimated the national cost of vector control in 2002 at US$ 5.8 million or US$ 0.24 per capita. Of this total, 74% of costs were attributed to inspection and 24% to fumigation.

Estimates of population-based costs

Building on selected facility-based studies and studies of the costs of vector control, the costs of hospitalized cases and vector-control activities were estimated for Malaysia using a NHA perspective.80 The study found that these costs (which exclude ambulatory cases) to the health system in Malaysia was US$ 12.8 million or US$ 0.53 per capita, of which 54% was for treatment of illness and 46% was for vector control.

Standardization of protocols

Standardized protocols for the collection of epidemiological and cost data, for analysis and interpretation can make study results both complete and comparable across countries, as recently noted for another disease.[81]

RESEARCH PRIORITIES

Conceptual framework

The challenge in estimating the epidemiological and economic burden of dengue can be encapsulated in an imaginary dialogue between the user and the producer of this information. The user of information, nicknamed 'InfoNeed', is the director of a programme or the developer of a policy around dengue. InfoNeed needs information for his policy-making and managerial responsibilities, such as planning or revising a control programme, developing a treatment plan, or considering the development or

purchase of some new technology, such as a better diagnostic test or a dengue vaccine. The producer of information, nicknamed 'InfoGive', is an analyst with access to the scientific literature, public databases, and possibly additional data. InfoGive knows that research studies may have high precision but limited generalizability, while databases such as reported numbers of cases can be subject to under-reporting, misclassification, and other limitations discussed above. Data may be virtually absent for some regions of the world or times.

As the imaginary dialogue begins, InfoGive may plead that the data do not exist to answer the policy-maker's questions definitively. Continuing the dialogue, the policy-maker acknowledges the problem, but responds that the questions cannot wait until the ideal data become available. Policy-makers need guidance now. Understanding that need, InfoGive seeks to generate the most accurate answer possible based on existing data. For purposes of generating research priorities, this paper also seeks to identify the types of studies which could be done within a few years and with limited resources that would contribute most to strengthening the world's understanding of the burden of dengue.

To address the policy-maker's needs while acknowledging the limitations of existing data, it is helpful to generate a conceptual framework for the burden of dengue. Table 1 describes the three domains of epidemiological and economic burden. For each domain, it is important to describe quantities (numbers of surveillance and prevention activities, and numbers of cases treated) and aggregate costs. The last column, illnesses, comprises both epidemiological and economic burdens.

The cases of illness are the most complicated domain. In view of the earlier discussion in this paper about under-reporting and misdiagnosis, studying dengue illness reported via a surveillance system or diagnosed in a specific health facility is analogous to viewing an iceberg. At first sight, an analyst sees only the part above the water, yet 90% of the iceberg is hidden below the water. In the case of dengue, at first sight, the analyst may study only reported cases. The full burden of dengue includes a spectrum of types of services and reporting:

- Confirmed dengue, seen and correctly labelled by a health professional and confirmed by laboratory diagnosis.
- Suspected dengue, seen by a health provider and classified as suspected dengue, without laboratory confirmation. Note that the absence of laboratory confirmation may be due to many factors: the health provider did not order the test, the cost of testing is high, the time window was not appropriate, or, occasionally, the patient passed away before the biological marker would be applicable. Depending on the location of treatment and the completeness of the reporting system, the case may or may not be reported in the country's epidemiology system.
- Fever treated by a health provider, but attributed to an illness other than dengue. The patient did not receive a laboratory test for dengue because providers did not consider a diagnosis of dengue to be likely, the patient was diagnosed with a disease that is treatable and should not be 'missed' (e.g. malaria) as well as the factors above. Nevertheless, in aggregate these cases are numerous and some are likely to be dengue.
- Fever or other symptoms experienced by the patient and managed by self-treatment. The fear of dengue may cause these undifferentiated fevers to be treated more intensively (and perhaps hospitalized) than they would have otherwise if the risk of dengue infection were not present.

In addition, illness caused by the dengue virus falls within a spectrum of severity, ranging from asymptomatic infection to death. Figure 3, adapted from the authors' earlier model,[13] shows this spectrum of the disease. Conceptually, the epidemiogical burden could be computed by estimating the number of patients who reach each stage in the diagram by

Table 1. Domains for estimating the epidemiologic and economic burden of dengue

Location	Surveillance and reporting	Prevention	Illness (confirmed and suspected cases)
Inside the health system[a]	Laboratory testing and personnel; operation of surveillance system	Costs of vector control by government	Cost of care faced by medical-care providers, out-of-pocket expenses by households, and payments by third parties
Outside the health system	Negligible items	Value of community resources and participation in vector control	Travel expense, time lost, and quality of life lost by patients and their family and friends

[a] Would be included in a country's national health accounts

assigning probabilities to each stage, their duration with dengue, and the associated loss in DALYs per case. Similarly, the economic burden can be obtained by multiplying the number of people at each stage by the cost to the health-care system per person at each stage and the other costs of illness per patient at each stage. The greatest practical challenge is obtaining the number of cases in each branch and their associated unit burdens.

Implications for the evaluation of vaccines

The literature as discussed above indicates how important it is that all stakeholders involved in the development of a dengue vaccine understand the burden of dengue, as well as the reasons why current data substantially underestimate the burden. To some extent, existing data can generate expansion factors that correct for under-diagnosis, misdiagnosis, and other limitations of existing information. More importantly, additional data are needed to fill the gaps. Studies are starting to focus on health facilities in which severe dengue is treated and concentrated, while population-based studies indicate the full spectrum of the disease. As dengue varies by locality, such studies are being conducted in many continents in which the disease is endemic. By linking burden to the cost of treatment and the loss of time and productivity, the economic burden of dengue is assessed together with the human burden. Donors such as the European Commission, the Pediatric Dengue Vaccine Initiative, and WHO are supporting work on these important gaps and contributing to the information base for vaccines and other approaches to controlling the disease.

Specific recommendations for research

Improve knowledge of the burden of dengue

- Standardize the reporting of cases among reporting counties to allow comparability.
- Improve documentation of reporting procedures and sites used by reporting countries to provide clarity in the application of the case definition.
- Develop and offer a system for quality assurance in laboratories in reporting countries, to maintain consistency and comparability in case reporting.
- Temporarily, and if possibly periodically, employ alternative but complimentary methods of case ascertainment in reporting countries to evaluate the effectiveness and representativeness of existing surveillance and reporting systems (e.g. population-based serosurveys and facility-based studies oriented towards capture-recapture methods).
- Promote comparative studies with other diseases to provide points of reference for competing public-health priorities.

Increase knowledge of costs

- Develop and apply standard methods for economic studies.
- Add economic components to epidemiological and clinical studies and to outbreak investigations.
- Measure the costs of vector-control programmes.
- Measure the costs of community efforts for vector control.
- Determine the cost–effectiveness of strategies for the prevention and management of dengue.
- Estimate the reduction in dengue burden, costs and cost–effectiveness of new diagnostic, preventive, and therapeutic technologies.

Generate data for under-studied regions and populations

- Initiate studies of epidemiological and economic burden in India, China, and Africa.
- Initiate studies of epidemiological and economic burden for international travellers.

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WORKING PAPER 3.3. **DENGUE IN AFRICA**

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INTRODUCTION

Although the history of dengue in Africa is poorly documented, it is known that dengue has been on the continent since the start of the 20th century. A retrospective serosurvey by Kokernot et al. (1956) suggests that dengue in Africa existed as far back as 1926–1927, when it caused an epidemic in Durban, South Africa. Despite poor surveillance for dengue in Africa, it is clear that epidemic dengue fever caused by all four dengue serotypes has increased dramatically since 1980, with most epidemics occurring in eastern Africa and to a smaller extent, in western Africa. The threat of the disease in South Africa has been evaluated and documented.

EASTERN AFRICA

Although dengue epidemics are infrequent in eastern Africa when compared with south-east Asia, the Americas and the Caribbean, all four serotypes of dengue have caused outbreaks in this region.

In 1982, an outbreak of dengue fever caused by dengue virus 2 (DEN-2) was reported in the Kenyan coastal towns of Malindi and Kilifi (Johnson et al., 1982); clinical presentation was consistent with classical dengue fever, with no severe dengue reported. Since then there have been sporadic cases of dengue reported in Kenya (unpublished observations), and a serology survey carried out in 2005 (unpublished) revealed the occurrence of dengue transmission in coastal and inland parts of Kenya.

The 1982 outbreak in Kenya is believed to have spread from the Seychelles outbreak that occurred between 1977 and 1979 (Metselaar et al., 1980). The islands of Comoros, in the Indian Ocean, experienced an epidemic in 1993 that affected more than 56 000 people. Serology surveys revealed that there had been previous outbreaks on the islands in 1948 and 1984.

In 1984–1985, an outbreak of dengue was reported in Pemba, Mozambique. Two deaths were reported to be associated with this epidemic. During this outbreak, most patients appeared to be experiencing secondary infection with flavivirus, and the two deaths were attributed to severe dengue (Gubler et al., 1986). Another outbreak of dengue caused by DEN-2 was reported in the city of Djibouti, on the horn of Africa, in 1991–1992, with cases of severe dengue being reported mainly among tourists and expatriates (Rodier et al., 1996). In 1992–1993, an outbreak of dengue was reported among United States troops engaged in the mission Operation Restore Hope in Somalia, which lies to the north of Kenya. It was found that 2% of cases were caused by DEN-3 and 41% by DEN-2 (Sharp et al., 1995). During the outbreak, only cases of classical dengue were seen, and there were no cases of severe dengue.

A dengue outbreak attributed to DEN-2 was reported to have occurred in Sudan, also north of Kenya, in 1986. The clinical presentation of patients seen during the outbreak was consistent with dengue fever and there was no evidence of severe dengue. One infection with DEN-1 and 17 infections with DEN-2 were diagnosed by viral isolation (Hyams et al., 1986).

The available evidence so far indicates that DEN-1, -2 and -3 appear to be a common cause of acute fever in eastern Africa, and that the frequency of epidemics continues to increase, with emergence of other serotypes since the Seychelles outbreak in 1977 (see table 1).

Table 1. Past epidemics and reported cases of dengue and severe dengue in Eastern Africa

Epidemic/ detection	Country	Dengue serotypes	Reference
1977–1979	Seychelles	DEN-2	Metselaar et al. (1980)
1982	Kenya	DEN-2,	Johnson et al. (1982)
1984–1985	Mozambique	DEN-3	Gubler et al. (1986)
1985–1986	Sudan	DEN-1, DEN-2	Hyams et al. (1986)
1991–1992	Djibouti	DEN-2	Rodier et al. (1996)
1992–1993	Somalia	DEN-2, DEN-3	Kanesa-Thesan et al. (1994); Sharp et al. (1995)
1948, 1984 and 1993	Comoros	DEN-1, DEN-2	Boisier et al. (1994)
2005	Eritrea	Not determined	Unpublished

Although outbreaks and sporadic cases of dengue have continued to occur in eastern Africa, little effort has been made to identify vectors and transmission cycles (sylvatic, periurban or urban). It has been assumed that the outbreaks are most likely to be transmitted by *Aedes aegypti*, which is widely

distributed in the region. Most of the reports on dengue in eastern Africa arise from outbreak investigations that are carried out by visiting scientists and, in most instances, no entomological studies are performed, and no serology surveys are done to determine the extent of the outbreaks.

WESTERN AFRICA

In the 1960s, DEN-1, -2 and -3 were isolated for the first time from samples taken from humans in Nigeria (Carey et al., 1971). Subsequently, dengue has been found to occur in Senegal and Burkina Faso (predominantly being transmitted in sylvatic cycles), and possibly in other tropical rainforests in western Africa (see table 2).

Table 2. Past epidemics and reported cases of dengue and severe dengue in western Africa

Epidemic/ detection	Country	Dengue serotypes	Reference
1964–1968	Nigeria	DEN-1, DEN-2	Carey et al. (1971)
1974–85	Senegal	DEN-2	Saluzzo et al. (1986)
1983–86	Burkina Faso	DEN-2	Robert et al. (1993), Hervy et al. (1984)
1982	Burkina Faso	DEN-2	Gonzalez et al. (1985)
1980, 1990	Senegal	DEN-2, DEN-4	Saluzzo et al. (1986), Traore-Laminaza et al. (1994)
1999–2000	Senegal	DEN-2	Diallo et al. (2003)

Fewer outbreaks have been documented than in eastern Africa. Most reports are associated with transmission in vectors and some sporadic cases in humans. A number of entomological surveillance activities (motivated by the ambition to identify sylvatic cycles of dengue and the evolutionary relationship between sylvatic dengue and endemic/epidemic dengue) have been undertaken. Through virus isolation, a number of *Aedes* species have been associated with dengue transmission in western Africa; these include *Ae. taylori, Ae. furcifer, Ae. luteocephalus, Ae. vittatus* and *Ae. aegypti* (Diallo et al., 2003). Interestingly, there are more outbreaks of yellow fever in western Africa than in eastern Africa, although these two viruses have common vectors.

SOUTHERN AFRICA

Since the first outbreak of dengue in South Africa in 1926–1927, cases of the disease imported from India have been detected in the 1980s (Blackburn & Rawat, 1987). The threat of dengue in South Africa has been evaluated in vector studies, and competent vectors have been identified (Jupp & Kemp, 1993). This, together with the occurrence of imported cases, led to recommendations for continuous surveillance to obvert outbreaks in the country.

CONCLUSION

Although dengue appears to be spreading in Africa, the funding received for surveillance and other research activities pertaining to dengue has been very limited. This has been mainly owing to the assumption that dengue is not a significant health problem on the continent, and this is largely attributed to the fact that severe forms of dengue illness are rarely reported. As most dengue infections are subclinical or present as dengue fever, they go undiagnosed and are commonly treated as malaria or other endemic fevers, such as typhoid and leptospirosi,. This has resulted in an underestimation of the magnitude of the dengue problem in Africa.

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Annex 4
WORKING PAPERS:
Scientific Working Group on Dengue

PATHOGENESIS, VACCINES, DRUGS, DIAGNOSTICS

WORKING PAPER 4.1. UNDERSTANDING PATHOGENESIS, IMMUNE RESPONSE AND VIRAL FACTORS

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THE PROBLEM

Dengue is the most important human viral disease transmitted by arthropod vectors, yet no effective and sustainable control measures exist. The recognition of severe dengue in Bangkok and Manila in the 1950s, and the spread of the disease to the Asian and Pacific regions, and more recently to the Americas, represents a major public health challenge posed by a disease previously characterized as a debilitating but mild illness. Currently, dengue fever and severe dengue are important causes of morbidity in many tropical and subtropical regions of the world. More than half of the world's population lives in areas at risk of infection and the incidence of the disease has grown 30-fold in the past 50 years.[1] It has been estimated that the baseline burden of disease in south-east Asia is 0.42 disability-adjusted life years (DALYs) per 1000 population, of which 52% was attributable to premature mortality and 48% to acute morbidity, comparable to that attributable to meningitis in the Western Pacific, twice the burden of hepatitis and one third that of HIV/AIDS.[2] Case-fatality rates for dengue shock syndrome range from 0.1% to 10%, depending on the level of clinical expertise for fluid management.

The Scientific Working Group on Dengue Research organized by the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (TDR)/WHO from 2–4 October 2006 discussed the global agenda for future research on dengue and identified priority research areas for future years. Dengue pathogenesis was one of the topics widely discussed, given its implications for case management, and the development of vaccines and drugs.

The objectives:

- To briefly summarize the state-of-the-art of research on the pathogenesis of dengue.
- To identify avenues of research with implications for the treatment, control and prevention of dengue.

PATHOPHYSIOLOGY OF DENGUE

The definition of severe dengue is currently under review.[3] Nevertheless, there is a consensus that mild and severe dengue syndromes generally represent a disease continuum that is differentiated physiologically by the degree of vascular permeability. Vascular leakage begins during the febrile phase but at a time when the dengue virus (DENV) load is in steep decline. Systemic leakage often becomes clinically apparent around the time of defervescence, when haemoconcentration, ascites, pleural effusion or cardiovascular hypotension are present. In children and adults, severe dengue is most often (although not exclusively) associated with anaemnestic immune responses (secondary DENV infections). Severe dengue can also occur during primary DENV infection of infants born to dengue-immune mothers. An immunological basis for the pathogenesis of endothelial permeability and vascular leakage is widely accepted, but definitive data that explain the mechanism of endothelial permeability are lacking. Thus, the biggest challenge in the field is to understand the molecular mechanisms of vascular leakage. In addition to plasma leakage, haemostatic abnormalities including marked thrombocytopenia and bleeding are observed. It has been suggested that depression of platelet synthesis resulting in thrombocytopenia, and the production of anti-platelet auto-antibodies occur during dengue

infection. Also, cross-reactivity of antibodies to the envelope glycoprotein of the virus with plasminogen has been associated with bleeding. Finally, liver involvement with mild elevation of serum transaminase activity is common.

Research priorities:

1. To acquire a better understanding of fluid physiology during severe dengue, and thus support interventions based on rational fluid selection and resuscitation in severe dengue.
2. To compare the pathophysiology of severe dengue during primary and secondary DENV infections among children and adults.
3. To identify changes in the endothelial layer that are associated with severe dengue, e.g. through biopsy studies or appropriate animal models.
4. To identify molecular mediators of endothelial permeability and vascular leakage during severe dengue.
5. To acquire a better understanding of thrombocytopenia and bleeding manifestations.
6. To define the role of molecular mimicry between dengue virus and endogenous host proteins in pathogenesis.
7. To study the possible hepatotropic capacity of DENV.

DENV GENOTYPES AND VIRULENCE

Humans are the major host of DENVs, with *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus,* being the principal vectors. At the genetic level, DENVs exist as four antigenically distinct serotypes that exhibit up to 30% divergence across their polyproteins. There is also considerable genetic variation within each serotype in the form of phylogenetically distinct 'subtypes' or 'genotypes'.[4,5] Currently, three subtypes can be identified for DENV-1, six for DENV-2 (one of which is only found in non-human primates), four for DENV-3 and four for DENV-4, with another DENV-4 being exclusive to non-human primates. DENV subtypes often have differing geographical distributions, with some being more widespread than others, indicating that both population subdivision and gene flow are important in structuring genetic diversity. Phylogenetic analyses have also revealed that (i) subtypes frequently co-circulate within the same locality; (ii) south-east Asia harbours the greatest amount of DENV genetic diversity, suggesting that it acts as a viral 'source' population; and (iii) there are periodic fluctuations in genetic diversity, including lineage extinction.[6] Sequence surveillance of DENV genotypes has suggested that some subtypes are more commonly associated with severe dengue. For example, DENV-2 and DENV-3 with Asian origins have been associated with epidemics (or cases) of severe dengue.[7,8] These genotypes have established endemic cycles in other continents, and in some cases, seem to have displaced the autochtonous genotypes that did not cause severe dengue. More recent studies, in both human primary targets of infection (e.g. dendritic cells) and in whole mosquitoes, have also shown that these viruses produce higher viral titre.[9] It is not well understood why some viruses have greater replicative fitness than others. Therefore, it is important to more broadly monitor the transmission of these potentially 'virulent' genotypes, to define if these associations hold up in diverse human genetic backgrounds and immune histories. The evolution of DENV during an epidemic and its association with epidemic severity also warrant further study.[10] Finally, the sequence of primary and secondary infection may be important, with sequential infection, e.g. DENV-1/DENV-2 and DENV-1/DENV-3, suggested to give a high risk of severe disease.[11,12] A technical manual, prepared by the Pan American Health Organization (PAHO), outlines protocols that could be used in many laboratories to standardize approaches to sequencing and phylogenetics and obtain comparable results.

Research priorities:

1. To move towards genotyping, in addition to serotyping, of epidemic/case samples.
2. To acquire more quantitative measures of virulence factors to use in new, in-silico models of dengue population dynamics.
3. To investigate the potential role of intrahost diverstiy ('quasispecies') in viral evolution and disease severity.
4. To understand whether some sequences of DENV are associated with greater severity of disease.

VIRAL PATHOGENESIS

Current evidence points to skin dendritic cells, tissue macrophages, peripheral blood monocytes and hepatocytes, but not endothelial cells, as host cells for DENV replication,[13,14] although more work is needed to define all target cells for DENV infection in humans. The dominant host cell receptor(s) for virus entry into these cell types has not been identified, although co-receptors such as DC-SIGN on dendritic cells have been reported. In the monkey model, DENV inoculated into skin rapidly moved to macrophages in regional lymph nodes and other lymphatic organs including spleen and liver.[13] The magnitude of viraemia and NS1 antigenaemia has been associated with disease severity, including

complement activation, although these factors alone may not explain all aspects of disease pathogenesis or clinical outcomes.[15,16] Antiviral drugs to inhibit DENV replication or cell adherence are being developed by several groups, and if administered early enough, might modulate the course of infection and possibly improve or shorten clinical illness. Cytokines and chemokines generated by immune activation correlate with disease severity.[17] Animal models (not all dengue-related) have established the potential for these cytokines to contribute to the clinical manifestations of dengue, e.g. vascular permeability. Inflammatory cytokines alone may account for systemic vascular leakage in severe dengue, but unequivocal evidence for this is lacking.

Research priorities:

1. To identify early prognostic markers of disease severity (host or viral) for use at point of care, e.g. antigen detection in urine or blood.
2. To understand the very early events in DENV infection, from mosquito inoculation (dose, site) to peak viraemia.
3. To understand the role of NS1 in complement activation, immune evasion and anti-NS1 responses in immunity.
4. To identify the human cells infected after mosquito inoculation and the cellular receptors.
5. To identify cell types infected by DENV in humans, both early in infection (peripheral blood mononuclear cells, biopsies) and late in infection via autopsy studies.

ANTIBODIES INVOLVED IN THE DEVELOPMENT OF IMMUNITY

There is a consensus that antibodies (IgM and IgG) are likely to be critical effectors in the resolution of dengue viraemia and long-term immunity. Antibodies may provide immune protection by blocking cellular attachment, viral fusion or by antibody-dependent cellular cytotoxicity (ADCC). ADCC has been associated with severe dengue.[18] It is not certain that existing in-vitro assays accurately characterize the anti-dengue activity of antibodies as they exist in vivo. For example, some studies have suggested that pre-infection neutralizing antibody titres measured in the conventional plaque-reduction neutralization assay (PRNT) are poorly predictive of subsequent viraemia or disease severity caused by certain DENV serotypes.[19,20] These are important observations given that neutralizing antibody in PRNT-type assays is being used as a primary end-point in trials of dengue vaccines. Investigations into the structural organization and folding of DENV and West Nile virus Env proteins have identified epitopes and protein domains targeted by neutralizing monoclonal antibodies, and in some cases have described their mechanisms of action.[21,22] A better understanding of the relevance of anti-NS1 antibodies in the resolution of viraemia and immunity is also an important goal. The characterization of DENV-specific human monoclonal antibodies from immune donors will provide further insights into the molecular basis of antibody function in neutralization or infection enhancement. The Pediatric Dengue Vaccine Initiative* is providing leadership and funding for basic science aimed at improving our understanding of the role of antibody in immunity to and pathogenesis of dengue.

Research priorities:

1. To acquire a better understanding of in-vitro 'neutralizing' antibodies as correlates of protection against different DENV serotypes and genotypes.
2. To understand the role of ADCC during dengue infection.
3. To develop better assays to measure antibody-mediated immunity and assess their predictive value in prospective field studies.
4. To assess the memory B-cell response over time in vaccinated individuals versus in natural infections.

ANTIBODY ENHANCEMENT OF INFECTION

Classical severe dengue accompanies a first DENV infection in some infants aged less than 1 year who are born to mothers who are immune to dengue.[23,24] Severe dengue also occurs in second (occasionally third) heterotypic DENV infections in older individuals, but the overall incidence is low given the total number of heterotypic infections.[25] Severe dengue can occur after a long interval between primary and secondary DENV infection (20–25 years), and indeed might be more severe after long intervals.[26,27] Infants born to dengue-immune mothers and previously-infected children or adults have in common a single immune factor—IgG dengue-reactive antibodies. The phenomenon of antibody-dependent enhancement (ADE), whereby dengue antibodies at subneutralizing concentrations enhance DENV infections in Fc-receptor bearing cells,[28] provides a unifying basis for these epidemiological findings. One explanation for the low incidence of severe dengue given the total number of heterotypic infections is that cross-reactive dengue-neutralizing antibodies may block productive infection.[29] Heterotypic

* Pediatric Dengue Vaccine Initiative: www.pdvi.org

neutralization may explain why American genotype DENV-2 viruses failed to produce severe dengue in DENV-1-immune individuals.[30] However, the clinical significance of ADE in potentiating disease severity remains controversial. In part, this is because the in-vitro infection-enhancing activity of pre-infection plasma has not always correlated with subsequent clinical severity of disease.[20,29] The natural history of the antibody response to dengue, and particularly the characteristics of homotypic and heterotypic neutralizing antibodies, e.g. avidity, cross-reactivity to different genotypes and their role in protection or pathogenesis, remain poorly studied. A better understanding of the clinical importance of ADE and a comprehensive study of human antibodies raised by dengue infection will be crucial as candidate dengue vaccines advance into larger clinical trials.

Research priorities:

1. To thoroughly characterize the natural history of the antibody response to dengue infection.
2. Large prospective studies are required to determine whether in-vitro assays that measure infection enhancement can predict subsequent clinical severity of disease.
3. Studies in infants are needed to understand the pathogenesis of severe dengue in hosts possessing anti-dengue antibody, but not cellular immunity.
4. Acquire a better understanding of the role of ADE after short and long intervals between infection with different DENV serotypes/genotypes.

T CELL-MEDIATED IMMUNITY AND PATHOGENESIS

The similarity between DENVs accounts for cross-reactivity in the humoral and cellular immune response. In Thai children with acute secondary dengue, there is massive activation, proliferation and programmed cell death of dengue-specific T cells.[31] There is also evidence that the response is dominated by cross-reacting memory T-cell clones generated during previous infections ('original antigenic sin').[31] Finally, there is a correlation between the magnitude of the peripheral blood T-cell response and disease severity, although in many cases this association is observed well after the acute symptoms have resolved.[31,32] In vitro, many serotype-cross-reactive T-cell clones respond in a different fashion to stimulation in vitro by antigens from different DENV serotypes. This effect results in a modified profile of cytokine production.[33] Collectively, these observations have led to the suggestion that profound T-cell activation, cytokine release and cell death may contribute to the systemic disturbances leading to severe dengue, and original antigenic sin in the T-cell responses may suppress/delay viral elimination, leading to higher viral loads and increased immunopathology. These events may act in concert with phenomena such as ADE. Pathogenic memory T-cell responses cannot explain severe dengue in infants with primary infections. However, it is conceivable that severe dengue in infants occurs via a different mechanism than in more immunologically experienced subjects like children and adults with secondary DENV infections. A challenge for the interpretation of T-cell responses during dengue haemorrhagic fever (DHF) is that often the flavivirus-infection history of the patient is unknown. Furthermore, dengue-specific T cells are not usually detectable in the peripheral blood during the febrile phase of the illness.

Research priorities:

1. To determine how the primary and secondary T-cell responses to dengue differ.
2. To identify the predominant site of T-cell reactivity in DENV infection.
3. To assess whether T-cell responses can be protective in dengue.
4. To define the roles of CD4 versus CD8 T-cell responses in immunopathology in dengue.
5. To explore whether dengue vaccines should aim to induce T-cell responses to nonstructural antigens or whether they should be tailored to concentrate purely on antibody responses to envelope determinants.
6. To study the short- and long-term T-cell response against DENV serotypes/genotypes.

INNATE IMMUNITY

Host and viral events during early DENV infection remain poorly understood. Type I and type II interferons can contribute to control of viral replication in vitro and in laboratory mice.[34,35] Studies in vitro suggest that DENV nonstructural proteins can attenuate the antiviral effects of type I interferon.[36] Natural killer cells are activated in acute dengue and may contribute to killing of infected cells by cytokine release or ADCC. Complement is also activated in acute dengue and soluble NS1 may be important in this process.[37] Dendritic cells play significant roles in antigen presentation and the regulation of acquired immune responses. Dendritic cells, either in the dermis or in lymphoid tissue, may be receptive to DENV replication in vivo.[13] CD209, or DC-SIGN, a dendritic cell-expressed lectin, is an efficient coreceptor for viral entry into dendritic cells.[38]

DENV infection modulates dendritic cell maturation, but is associated with secretion of inflammatory and immune-modulating cytokines.[39] Mice that are transplanted with human haematopoeitic cells and that develop functional dendritic cells display clinical parameters (viraemia, fever, thrombocytopaenia, rash) that reflect similar events in dengue fever in humans.

Research priorities:

1. Animal models and studies of skin explant infection could accelerate our understanding of early host–virus interactions.

GENETIC SUSCEPTIBILITY AND RESISTANCE

A genetic basis to the regulation of disease expression is likely, a remarkable example being resistance to DHF observed in Cubans of African descent.[40] More recently, the prevalence of Negroid anthropometric characteristics was associated with a lower incidence of DHF (2%), while individuals with a predominance of Caucasoid anthropometric characteristics had a higher incidence of DHF (30%).[41] Numerous small case–control studies have identified disease associations, e.g. in human leukocyte antigen (HLA) alleles, the vitamin D receptor and FcγIIa. A functional mutation in the promoter region of DC-SIGN has been associated with susceptibility to mild dengue, but not DHF.[42] Surprisingly, this data suggested that dengue fever and DHF were not a continuum, but discrete clinical entities. With the exception of studies on DC-SIGN, case–control association studies in dengue have been small, have not been replicated in multiple populations, and lack functional data to support the genetic association. Interpretation of these existing data therefore requires caution.

Research priorities:

1. Large, well-powered case–control or family trio studies that investigate genome-wide polymorphisms that are associated with the clinically important events in dengue, e.g. dengue shock syndrome versus DHF, symptomatic versus asymptomatic, that can be replicated in different populations.
2. Functional investigations that support and help explain genetic associations.

CONCLUSIONS

For simplicity, we have presented brief research backgrounds and priorities in digestible segments. However, we recognize that the outcome of a DENV infection is dictated by a complex interaction between host and viral factors. This implies that, wherever possible, future research investigations should take a balanced approach by comparing the infection outcome in symptomatic cases as well as in asymptomatic individuals, and considering the afferent (phenomena that promote the survival of the virus) and efferent (phenomena that promote the elimination of the virus) mechanisms. Large, longitudinal studies of populations at risk in areas where dengue is endemic could provide the foundation for this 'balanced' approach to dengue pathogenesis.

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WORKING PAPER 4.2. OPPORTUNITIES IN THE DEVELOPMENT OF DENGUE VACCINES

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INTRODUCTION

It has been said that no modality, with the exception of clean drinking-water, has had a greater effect on the control of infectious diseases than vaccines. Dengue vaccine research was initiated in the 1940s with Sabin and colleagues (Sabin & Schlesinger, 1945) working on the development of a live attenuated vaccine. In the mid 1970s, many stakeholders came together to develop a dengue vaccine; it was stated that this objective would be achieved within 10 years. Although great strides have been made in our understanding of the virus and disease, no vaccine has yet been licensed and no candidate vaccine has progressed beyond phase II clinical trials. While some manufacturers remain optimistic that a dengue vaccine will be launched within the next 5 years, it should be remembered that it takes an average of at least 15 years for a candidate vaccine to advance from the discovery phase to licensing. There are multiple reasons why the development of a dengue vaccine is complex, and these are discussed below.

THE COMPLEXITY OF DENGUE VACCINE DEVELOPMENT

Dengue disease is caused by four serologically related flaviviruses designated DEN-1, DEN-2, DEN-3 and DEN-4. The first major problem in developing a dengue vaccine is thus the need to develop not just one but four immunogens that will give a balanced response such that a protective immune response is induced against all four viruses simultaneously, i.e. the vaccine has to be tetravalent.

Another factor behind the need for a tetravalent vaccine is the problem of immune enhancement, including antibody-dependent enhancement. It is clear that infection by one dengue virus leads to lifelong protective immunity to the infecting virus, i.e. homotypic immunity; however, many studies have demonstrated that secondary infections (i.e. infection by one dengue virus followed by infection by a second dengue virus, e.g. DEN-2 followed by DEN-3) can lead to severe and occasionally fatal dengue. Thus, there is the theoretical danger that a dengue vaccine would have the potential to cause severe dengue in vaccinees, if solid immunity was not established against all four viruses. However, it should be emphasized that, to date, there is no evidence that any vaccinee receiving a candidate vaccine has subsequently succumbed to severe disease. It is rather the case that vaccinees have shown evidence of immunity for varying lengths of time depending on the candidate vaccine administered (Pengsaa et al., 2006). Nonetheless, the potential risk of immune enhancement by a candidate vaccine must be evaluated.

The third problem is that the mechanism by which protective immunity against dengue infection is induced is poorly understood. At the present time, vaccines are licensed for use in humans against three diseases caused by other members of the genus *Flavivirus*: yellow fever, Japanese encephalitis and tick-borne encephalitis. All the available data indicate that neutralizing antibodies are the major contributor to protective immunity; it is thus assumed that neutralizing antibodies are the main effector of protection against infection by dengue viruses. Evidence in support of this hypothesis comes from studies on passive transfer and maternal antibodies (Pengsaa et al., 2006). However, this does not prove that neutralizing antibodies are the only mechanism of protective immunity against infection by dengue. Further studies are necessary to establish correlates of protection to demonstrate that candidate vaccines induce a protective immune response. Evaluating immunity is difficult as it must be demonstrated (via clinical data or immunological correlates) that each of the four components of the tetravalent vaccine is able to induce immunity. The situation is complicated by the fact that dengue viruses co-circulate in geographical areas where other flaviviruses are found; distinguishing immunity to disease caused by a particular dengue virus is consequently a difficult and complex problem.

The fourth major problem is the lack of a suitable animal model in which candidate vaccines can be evaluated. The lack of animal models has severely hindered progress in identifying the determinants of attenuation, virulence and immunogenicity of dengue viruses that can be applied to vaccine development. The dengue viruses are also arboviruses ('arthropod-borne viruses') and normally have a transmission cycle involving mosquitoes (predominantly *Aedes aegypti*), with humans as the vertebrate

host and, unlike most other arboviruses, they have lost the need of an enzootic cycle for maintenance. Clearly, it is not ethical to undertake experiments on humans unless they are justified. Two animal models (mice and non-human primates) are used to evaluate candidate vaccines but, unfortunately, neither displays the clinical symptoms seen in humans.

Mice are often used as a small-animal model to make an initial evaluation of the ability of candidate vaccines to induce a protective immune response. Although this information is useful, all strains of dengue virus investigated to date cause encephalitic disease in the mouse model, which is not representative of the disease in humans. Furthermore, the majority of strains of the four viruses are only able to cause disease in newborn or very young mice. Some strains cause disease in weanling mice, but only when inoculated directly into the brain. Not surprisingly, it has been found that the mouse model is not always predictive of the situation in species of higher animals, i.e. a candidate vaccine that protects mice may not be effective in another animal. The second animal model is the non-human primate, of which several species have been used to evaluate candidate dengue vaccines. Non-human primates do not get clinical disease but do demonstrate viraemia (originally measured as 'infectivity', but now normally measured by real-time reverse-transcriptase polymerase chain reaction, RT-PCR, to determine genomic equivalents of virus, and termed 'viral load'), and immunological parameters are used as a proxy to measure efficacy. Clearly, the mouse and non-human primate models must be used to evaluate candidate vaccines before they are tested in humans. However, the only true model for dengue disease is in humans and, not surprisingly, candidate vaccines fail at different stages of evaluation in mice, monkeys or humans.

Overall, there is limited understanding concerning which parameters can be used to demonstrate the development of protective immunity (although it is assumed that seroconversion of antibodies is important). Owing to the limitations of current animal models, clinical trials are a critical component of vaccine development in terms of the information they provide on immunity and reactogenicity, while long-term evaluation of volunteers is required to demonstrate lack of evidence for immune enhancement/severe disease.

In spite of the limits of our knowledge, there has been significant progress in the development of candidate dengue vaccines in the past 5 years, indicating promise for a commercial vaccine in coming years. Two candidate vaccines (WRAIR/GSK and Acambis/Sanofi Pasteur) are in phase IIb clinical trials, while other candidates are in phase I or advanced preclinical development.

OVERVIEW OF CANDIDATE VACCINES

Live attenuated vaccines

Since the 1940s, several live, attenuated vaccine candidates have been developed on the basis of classical virology techniques, of which one, developed by the Walter Reed Army Institute of Research (WRAIR), has involved passage of dengue virus isolates in primary dog kidney (PDK) and primary African green monkey kidney cells for attenuation, and the candidate vaccine is produced in fetal rhesus lung (FRhL) cells. Although monovalent candidates have proved to be safe and immunogenic in studies in humans, interference was seen when the candidate strains were combined into tetravalent formulations. In collaboration with GlaxoSmithKline (GSK), a total of 18 different tetravalent formulations have been empirically derived, to select for high and balanced immunogenicity and low reactogenicity, and evaluated in human volunteers (Edelman et al., 2004). At present, formulation '17' is undergoing phase II clinical trials.

Recombinant vaccines

A number of research groups have used genetic engineering to develop a dengue vaccine; site-directed mutagenesis or exchange of genes from different sources is used to make a full-length complementary DNA (cDNA) of the virus genome in a plasmid that can be transcribed in vitro to generate viral RNA that is transfected into cells from which the virus can be recovered. The most advanced candidate to be based on this approach is the ChimeriVax platform, which uses the yellow fever 17D vaccine as a backbone. The development of chimeric yellow fever vaccine–dengue vaccine viruses is being undertaken by Acambis in collaboration with Sanofi Pasteur. In this case, the yellow fever 17D vaccine virus is used as a vector for insertion of the DEN prM and E structural genes to replace the corresponding yellow fever genes. Four candidates have been developed, one for each DEN virus (Guirakoo et al., 2004). Like the WRAIR/GSK candidate described above, the Acambis/Sanofi Pasteur candidate is in phase IIb clinical trials.

Of the other candidate vaccines being investigated, one is produced using a platform technology developed by a group at the United States National Institutes of Health and is based on a recombinant DEN-4 virus backbone that contains a 30 nucleotide

deletion in the 3′ non-coding region of the viral genome (termed rDEN4Δ30 3′NCR), which attenuates dengue viruses The production of the candidate vaccine involved two approaches: (1) introduction of the Δ30 3′NCR deletion into wildtype DEN-1, DEN-2 and DEN-3 viruses i.e. resulting in rDEN1Δ30, rDEN2Δ30, and rDEN3Δ30 attenuated viruses; and (2) construction of chimeric DEN-4/DEN-1, -2, -3 viruses, which expressed the prM/E gene region of DEN-1, -2, or -3 virus in the attenuated genetic background of the rDEN4Δ30 virus (Blaney et al., 2006). These vaccine candidates are currently in phase I and phase II clinical trials.

A group at the United States Food and Drug Agency is using a similar approach. This involves introducing several attenuating mutations into the stem-loop structure at the 3′ terminus of the dengue virus genome (termed 'MutF'). This group has successfully tested a DEN-1 candidate vaccine in monkeys (Markoff et al., 2002).

The United States Centers for Disease Control and Prevention has developed a platform based on the empirical attenuated DEN-2 PDK53 virus developed by researchers at Mahidol University in Thailand. The significance of this platform is that it is based on attenuating mutations in the 5′ non-coding region and nonstructural proteins NS1 and NS3 (Huang et al., 2003), with no mutations in the envelope E protein, making it a potential platform for tetravalent vaccine development. Chimeric recombinant DEN-2 PDK53 with substitution of membrane protein precursor (prM) and E protein genes from wildtype DEN-1, -3 and -4 viruses have been generated and produced promising results in non-human primates.

Alternative approaches

Although the development of recombinant viruses generated by genetic engineering is a very promising approach, potential complications remain: the molecular basis of attenuation of dengue viruses is poorly understood and it is difficult to evaluate the attenuated phenotype of these candidate vaccines without studies in humans. This has encouraged other research groups to investigate alternative approaches. A number of groups have investigated candidate DNA vaccines, the most promising candidate having been developed by the United States Naval Medical Research Center. Using DEN-1 for proof-of-principle, this DNA candidate vaccine is based on the prM and E protein genes; it has been shown to induce immunity in non-human primates, and is currently undergoing phase I clinical trials. The most advanced subunit vaccine is being developed by Hawaii Biotech Inc., and is based on recombinant E and NS1 proteins produced in the *Drosophila* S2 expression system and purified by immuno-affinity. The vaccine contains the ectodomain of the E protein (called 80%E, i.e. it comprises N-terminal 80% of the E protein) plus the DEN-2 NS1 protein, and has been formulated in a number of proprietary adjuvants. The rationale for including DEN-2 NS1 was the aim of induction of a cell-mediated immune response in addition to the induction of neutralizing antibodies by the E protein. This candidate vaccine has been demonstrated to show promise in non-human primates.

A purified inactivated virus (PIV) vaccine formulation, similar to the successful inactivated Japanese encephalitis and tick-borne encephalitis vaccines, and using DEN-2 virus, has been investigated by the WRAIR group on a 'proof-of-principle' basis. This vaccine demonstrated promise in non-human primates, but was not as efficacious as live DEN-2 vaccine (Putnak et al., 2005).

Although the DNA, 80%E and PIV approaches have their limitations, there is currently much interest in 'prime–boost' approaches (i.e. the sequential use of different immunogens) for a candidate DEN vaccine. It is thus possible that live vaccines used in conjunction with the one or more of the alternative approaches may yield a vaccine with improved safety and immunogenicity profiles. Finally, there are recent developments in adjuvant technology that may have applicability to improved immunogenicity of subunit vaccines.

PROSPECTS AND PROBLEMS

The current candidate vaccine pipeline appears to be sufficiently advanced such that licensing of one or more dengue vaccines may be expected within 5–7 years. We note that the most advanced candidates are live vaccines. These have the advantages of inducing a broad immune response that has the highest chance of being protective and long-lasting, while also being relatively cheap to produce. However, the issues involved in licensing and use of live vaccines are well-known, relating to safety follow-up and use in special target groups (people infected with HIV, pregnant women, etc). Accordingly, there is value in continuing research on alternative strategies, such as subunit vaccines, which may become the product of choice for travellers or other special target groups, such as those for whom live vaccines are not appropriate.

A major challenge in the future concerns the criteria that should be used to evaluate candidate vaccines

in population-based efficacy trials in exposed populations. This will require consideration of a number of technical, operational and regulatory issues. Methodological obstacles to the addressing of these issues remain considerable, particularly in the context of a tetravalent response (i.e. the need to discriminate between the four different dengue viruses simultaneously), and neutralization needs to be correlated with protection in vivo. The role of heterotypic antibody in protection appears to be limited, but further studies are desirable. These antibodies, however, might be less relevant for vaccines, but current methods do not allow for discrimination between homotypic and heterotypic antibodies in a tetravalent response. The demonstration of tetravalent priming remains therefore an important goal in vaccine evaluation. In the case of dengue, particular attention needs to be given to the definition of 'protection', which should be scientifically sound and reasonable from a public health perspective. Apart from clinical definitions, virological parameters—in particular, viraemia—need careful consideration. Also to be considered are means to demonstrate efficacy in areas in which dengue activity and circulating dengue virus vary, and in a background of other circulating flaviviruses that will complicate measurement of the immune response in vaccinees. Multicentric vaccine trials will be required in different geographical settings, including Asia and the Americas. Vaccine developers and the Pediatric Dengue Vaccine Initiative (PDVI)* are establishing such field sites.

How will vaccine efficacy be measured? Clinical end-points need to be defined for DEN vaccine efficacy trials. This could be dengue fever, severe dengue, or another disease entity. The WHO Initiative for Vaccine Research has set up a study group to address this and other considerations, in order to produce a guidance document for the planning of efficacy trials. This will include directions for safety assessment of dengue vaccines.

As stated above, there is currently no established immunological correlate for protection from dengue, although there is evidence that points towards the protective role of neutralizing antibodies. The availability of a correlate would greatly facilitate the evaluation of candidate vaccines, and WHO has recommended that comprehensive immunology studies to be done in conjunction with future trials for the definition of correlates (Hombach et al., submitted). In this context, there is also need for harmonization and validation of key immunological assays, in particular for the assessment of functional, neutralizing antibodies. WHO, in collaboration with PDVI, is developing tools and reagents for this purpose. PDVI is also investing in research on tools to enable the exact characterization of antibody responses, with the aim of distinguishing neutralizing antibodies from potentially enhancing antibodies. Such a tool would be of immense value for the safety characterization of candidate vaccines, and would complement long-term safety follow up.

In addition, immunological correlates will be of critical utility in facilitating interpretation of immune response data in relation to protection of an immunized individual; facilitating bridging clinical studies; enabling comparison between vaccine candidates; defining the relevant parameter to establish vaccine potency tests; enabling comparison of the efficacy of components of candidate tetravalent vaccines, and supporting the assessment of vaccine efficacy in different settings and population groups.

In conclusion, we are living at an exciting time with regard to the development of a dengue vaccine, but much has yet to be achieved before such a vaccine will be licensed.

* PDVI: http://www.pdvi.org/default.asp

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WORKING PAPER 4.3. OPPORTUNITIES IN THE DEVELOPMENT OF ANTI-DENGUE DRUGS

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OBJECTIVES

The aim of this paper is to review opportunities in the development of anti-dengue drugs. First, the timeliness and potential use of such drugs will be addressed in the context of the growing dengue problem, evolving diagnostic technologies, and the difficulties of the task. Second, an analysis of current sources of drugs will be provided in order to position future treatments for dengue in a realistic economic context. Finally, dengue virus (DENV) drug targets will be considered, with a particular emphasis on nonstructural (NS) viral proteins. We will list and describe state-of-the-art methods that are expected to lead to the discovery, design, and characterization of drugs that are effective against the four DENV serotypes.

DRUG THERAPY FOR DENGUE

There are three main ways to address the problem of dengue disease control. The first and currently only available strategy is to eradicate the mosquito (*Aedes* spp.) vector. Although appropriate vector control is effective, it may be difficult for political, geographical and logistical reasons, as evidenced by the resurgence of both the mosquito vector and DENV in areas where both had been previously eradicated. The second is vaccination, as exemplified by the success of the vaccine against yellow fever virus. The development of vaccines against dengue is an active area of research complicated by the presence of four DENV serotypes and the previous lack of a suitable animal model for dengue disease. The third way, drug therapy, is complementary to the other approaches. There are currently no anti-DENV drugs available.

The timeliness of anti-DENV drug discovery

Successful antiviral drug design and chemotherapy depends on answers to several questions such as:

- Is the disease detectable and treatable in a timely fashion?
- Are there enough patients to conduct clinical trials?
- Does a drug-mediated decrease of viral load affect a viral disease outcome?
- Are drug-discovery and drug-design technologically and scientifically feasible?
- Can cost-effective drugs be made available in afflicted countries and regions?

Today, based on our current understanding of DENV and related viruses, and the diagnostic methods currently available and under development, the answers to these questions argue positively in favour of dengue drug discovery and design.

The appropriateness of antiviral chemotherapy

Several lines of research or recent developments argue in favour of the development of drugs to treat dengue. First, it has been demonstrated that there is a direct correlation between high viral load and the development of the more severe, life-threatening form of the disease. Thus, a drug able to reduce viral load at an early stage would potentially prevent dengue fever and the life-threatening severe form of dengue. Second, diagnostic tests to rapidly detect DENV infection at an early stage are currently under development by a number of research organizations, and should soon be available. Third, viral replication may be occurring in cell reservoirs, tissues, and body compartments other than the circulatory system where an antiviral could reach and target them. Fourth, in endemic outbreaks, prophylactic mass treatment around index cases would be essential. Rapid diagnostics would detect infected yet asymptomatic people. Decreasing viraemia in humans should result in a decrease in the numbers of infected vectors and thus interruption of the transmission chain. Therefore, an efficient and safe drug, delivered early in the course of the disease, might not only save lives but also curb potential epidemics. Fifth, an on-the-shelf drug would allow a rapid response in the case of a sudden outbreak, and should not require cold storage (unlike current prospective vaccines), a benefit for use in developing countries. Sixth, it is also possible, at a very early stage in drug design, to guide research on molecules that will be cheap to produce. Cost–effectiveness is a crucial issue for this poverty-linked disease. Cost–effectiveness can be achieved by selecting appropriate, easy-to-synthesize chemical scaffolds or, once active natural compounds are found, by pursuing research on those that represent major chemical constituents of appropriately available plants. This last point has been totally neglected for a number of

reasons, among which is an excessive faith in high-throughput screening (HTS) techniques and the fact that the success of the natural-product screening approach in cancer is not translatable to drugs to combat infectious agents, as described later in this paper.

Novel scientific and technological developments promote modern drug design

Twenty years of research on the human immunodeficiency virus (HIV) and, more recently, hepatitis C virus (HCV) have boosted the antiviral chemotherapy field.[1–3] Viral polymerases and proteases are targets par excellence, as validated by the use of inhibitors of HIV reverse transcriptase and protease, hepatitis B polymerase and herpes virus polymerase as antiviral drugs. Anti-HCV protease inhibitors are in various stages of clinical trials. HCV belongs to the *Hepacivirus* genus within the Flaviviridae family. Knowledge and strategies gained from the successful three-dimensional structure-based drug-design process for HCV can now be translated to the DENV research field.

Research on *Flavivirus* and Flaviviridae has led to the characterization of an increasing number of virus-encoded proteins and enzymes, including envelope and capsid proteins, polymerases, helicases and proteases. Processes involved in the entry of DENV into cells (virus–receptor binding, E protein conformational changes, virus internalization and membrane fusion) are becoming better understood at the molecular level. For DENV, whose RNA genome is decorated by a type-1 cap structure, enzymes involved in cap formation such as the RNA triphosphatase, guanylyltransferase (still unknown) and methyltransferase are additional potential targets; considerable progress has been made in their characterization. Chemical libraries containing molecules of natural and synthetic origins can now be screened against these novel pathways and targets. In this review we intend to give an overview on the current situation of dengue drug development.

Screening and design of anti-DENV drugs

HTS techniques allow large numbers of compounds to be screened against a given target, on a faster-than-ever timescale. Computer-aided studies on structure–activity relationships facilitate a responsive and efficient management of research results and programmes. Drug resistance must be considered as part of the drug-design process, as drug-resistance mechanisms are being increasingly characterized and drug combinations optimized, in order to avoid or delay resistance. Perhaps the first large-scale effort to discover anti-DENV drugs is to be credited to researchers at the Novartis Institute of Tropical Diseases, Singapore, who conducted a complete screen of their proprietary library against the DENV protease domain from nonstructural protein NS3 (see below).

The advent of drug discovery and design in an academic environment

The availability of molecules of potential therapeutic interest has long been hampered by their proprietary origin. Pharmaceutical companies have developed their own chemical libraries over the years. Access to these libraries is either restricted or difficult for academic laboratories; this represents a major problem for research into neglected diseases, as academic and industrial interests may not coincide. Several countries have generated libraries of synthetic chemicals that are fully accessible to academic researchers working in drug discovery. The National Cancer Institute in the USA has set up a programme to freely distribute chemical libraries for screening purposes, with an agreement on subsequent potential intellectual properties. Several similar free-access chemical libraries exist.[a] The existence of such libraries renders drug discovery and design possible for academic groups that do not have strong collaborations with chemists, or an internal source of compounds. In such academic environments, a network of international academic institutions would thus control the whole chain of events and processes required, from the discovery of a drug up to its preclinical evaluation.

New challenges from natural products can be met in a sustainable manner

In the last 20 years, the advent of HTS techniques has led to decreased emphasis on natural products as drug sources, with a subsequent decrease in the discovery of new active substances. However, the Convention on Biodiversity signed in Rio de Janeiro, 1992,[b] facilitates fair, reciprocal, and legal collaborations to use natural resources for research purposes. The ongoing progress in structure determination of viral targets presents novel avenues for research. One can now infer how a crude extract of natural substances should be pre-cleaned so as to select and bulk pre-fractionated extracts, ensuring that their molecular diversity will be fully usable. For example, examination of the surface potential of a protein target discriminates the chemical characteristics of a drug target site (e.g. active site) from secondary sites that are of no use for drug selection

[a] Free-access chemical libraries are listed in: http://www.univ-orleans.fr/icoa/eposter/eccc10/monge.

[b] The Convention on Biological Diversity: http://www.biodiv.org/convention/default.shtml

(e.g. positively-charged grooves to which bind sulfated polysaccharides that non-specifically inhibit the reaction assay). It is thus possible to exploit the as-yet unknown, potentially great, antiviral properties of natural products.

Natural products have previously been neglected in antiviral research because of difficulties that can now be overcome. Many plant-derived compounds are cytotoxic. From the antiviral point of view, cytotoxicity is not desirable and complicates screening efforts. By jeopardizing selectivity, cytotoxicity has stopped many compounds or extracts on their way towards antiviral preclinical trials. It masks the putative antiviral property of a plant extract. Such an extract, that is both cytotoxic and contains a potent antiviral molecule, cannot be easily selected using infected cell-based assays. This difficulty can obviously be circumvented by assays making use of isolated (or purified) viral targets.

The chemical space provided by natural products is nearly infinite. It has been evaluated that up to 10^{60} molecules with the potential to be used as drugs exist in nature,[4] and could be made available, provided that the current biodiversity (much present in tropical, dengue-afflicted countries) is not lost but wisely used. This astonishing number is to be compared to the current number of synthetic, pure chemical compounds, approximately 2×10^{10}, present in laboratories around the world, most of them in proprietary libraries unavailable to most scientists.

Finally, plants are natural and cheap factories for the production of costly drugs. Basing the design of an anti-DENV drug on natural active substances has the potential to provide and disseminate knowledge, growth, and economic benefits in a more sustainable way, to countries afflicted by a specific disease such as dengue.

Screening Federal Drug Administration-approved drugs

We believe that a screening facility making use of the more than 1000 Federal Drug Administration-approved drugs should be established. These drugs should be tested as first-line strategy, for dengue as for any other emerging virus, because one might find a cheap and already approved drug that is active against dengue, a discovery process that would greatly speed-up approval. If we draw experience from the chikungunya epidemics in the Indian Ocean in 2006, a cheap antimalarial drug has shown potent anti-chikungunya virus in vitro, and is currently ongoing clinical trials (X. de Lamballerie, Université de la Méditerranée, Marseille, France, personal communication). The Federal Drug Administration-approved status of this drug against malaria has certainly greatly accelerated and facilitated clinical trials. Such an investigation has not so far been performed for dengue, and should be a priority.

DENV TARGETS

The NS3 protein

The protease domain

Initial work on enzymatic and structural characterization of the N-terminal serine protease domain of NS3 (NS3pro) was carried out by groups led by Radhakrishnan Padmanabhan (Georgetown University, Washington, USA), Paul Young (University of Queensland, Brisbane, Australia), and Krishna Murthy (University of Alabama Birmingham, USA). They defined a minimal active protease domain,[5] determined the structure of NS3pro[6,7] and revealed that a conserved hydrophilic domain of 40 amino acids within NS2B conveys full activity to NS3pro.[8] They, and in parallel, the groups of Paul Young and of David Farlie (University of Queensland, Australia) were able to generate soluble, active single-chain NS2B/NS3 constructs allowing studies on protease/substrate and protease/cofactor interactions conducted by these groups[9,10] and the group of Gerd Katzenmeier (Mahidol University, Nakornpathom, Thailand.[e.g.11] These studies in turn paved the way for the identification and characterization of inhibitors. Non-substrate-based inhibitors were identified that bind into the P1 sub-pocket of the catalytic site of the dengue NS3 protease and it was found that they are of equal potency in West Nile virus (WNV) NS3 protease.[12] groups headed by Thomas Keller (Novartis Institute for Tropical Diseases, Singapore), and Gerd Katzenmeier explored substrate- or transition-state-based synthetic peptide inhibitors.[13–15] Small-molecule inhibitors purified from a plant were identified[16] by a group led by Noosaadah Abd Rahman (University of Malaya, Kuala Lumpur, Malaysia. Novartis launched HTS with 1.4 million compounds and a lead compound was identified.[c] Evaluation work with proteases from all four DENV serotypes[17] is in progress. Novartis is complementing this approach by virtual docking, speed-screen and fragment-based screening.[d] The most recent contribution to dengue anti-NS3pro drug research is the release of the three-dimensional structures

[c] See *Dengue Digest* 3-1, March 2006 (http://www.nitd.novartis.com/includes/teasers/teaser_attaches/dengue_diguest/Dengue%20Digest%20vol%203%20no%201.pdf)

[d] See *Dengue Digest* 2-1, April 2005 (http://www.nitd.novartis.com/downloads/dengue_digest_vol_2_no_1_april.pdf)

of the NS2b/NS3 construct of DENV2 and WNV,[18] the latter in complex with a peptide inhibitor, by the group of Ulrich Hommel (Novartis Institutes of Biomedical Research, NIBP, Basel, Switzerland). The Hommel group showed that DENV NS3pro in complex with NS2B deviates substantially from the uncomplexed structure. The cofactor is stabilizing the NS3pro structure by contributing additional β strands, completes the substrate-binding site and is thus involved in substrate–inhibitor binding. This was evidenced by the WNV NS2b/NS3–inhibitor complex, which explained the strong activation of NS3pro by NS2b and revealed key interactions for substrate recognition that accounted for structure–activity relationships for substrate-based inhibitors.

The helicase/RNA triphosphatase domain

Research on the molecular characterization of the helicase/NTPase/RNA triphosphatase domain (NS3hel) of DENV and WNV was initiated mainly by groups led by Padmanabhan and by Gerd and Gisela Wengler (Institut für Virologie, Justus-Liebig-Universitat Giessen, Germany). The latter demonstrated the existence of NTPase (providing the necessary energy for the unwinding helicase activity) and RNA triphosphatase (RTPase) activities in NS3hel of WNV.[19,20] Padmanabhan and colleagues demonstrated helicase activity and defined a minimal helicase domain.[21] They and our own group showed evidence that all three activities share the same active centre, including the characteristic Walker A (P-loop GxGK) and B (DExH) motifs.[21,22] Accordingly, all activities are strictly dependent on magnesium ions.[22]

The RTPase of DENV represents a new class of RTPases that use a helicase scaffold to exert their activity. Additional elements for RTPase activity might in part be provided by the C-terminal subdomain. This was evidenced by the recent determination of the three-dimensional structure of DENV NS3hel[23] by Julien Lescar and colleagues (Nanyang Technological University, Singapore), which shows the same features as yellow fever virus (YFV) NS3hel[24] determined in parallel by a group led by Richard Kuhn and Janet Smith (Purdue University, West Lafayette, USA). *Flavivirus* NS3hel shares the fold of the 'NTP-hydrolysing molecular motor', the catalytic core, consisting of a tandem repeat (subdomains I and II) of a RecA-like fold with HCV NS3 helicase devoid of the RTPase activity. The C-terminal subdomain (III) is completely different. A great deal of research is still required to elucidate the molecular mechanism of RTPase activity and the contribution of the C-terminal subdomain. A HTS-adaptable assay of RTPase activity has not yet been described.

The helicase catalyses the unwinding of double-stranded DNA and RNA substrates with overhanging 3′ and 5′ ends in vitro.[21–23] Full-length NS3 showed an activity that was 30 times higher than that of the isolated NS3hel domain. A mutation study by the group of Subhash Vasudevan (NITD, Singapore) used therefore full-length NS3 to elucidate critical residues for helicase activity.[25] Interestingly, they found that the decrease of the energy-supplying NTPase activity does not always lead to a concomitant decrease of the helicase activity. Thus NTPase inhibitors might not necessarily inhibit helicase activity. Additionally, they proposed the existence of a surface pocket on subdomain II that might act as a 'helix opener' and thus be an attractive target for small molecules. Consequently, Novartis is currently using an assay for helicase rather than NTPase to identify a lead compound by HTS of small-molecule libraries.[c] Other strategies are to try ATP-competitive or nucleic acid-competitive inhibitors. The groups of Peter Borowski (Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany) and Ramachandra Hosmane (University of Maryland, Baltimore, USA) have identified ring-expanded nucleoside analogues as inhibitors of the helicase but not NTPase activity of WNV NS3.[25]

The NS5 protein

The methyltransferase domain

In 1993, sequence analysis by Eugene Koonin[27] predicted the presence of a binding site for *S*-adenosyl-L-methionine (AdoMet), the cofactor of AdoMet-dependent methyltransferases (MTase) in the N-terminus of *Flavivirus* NS5. In 2002, our group was able to decipher the three-dimensional structure and activity of an N-terminal methyltransferase domain of DENV NS5.[28] The DENV NS5MTase domain acted as a (nucleoside-2′-*O*)-MTase (2′OMTase) on small RNA GpppAC$_5$ substrates, thus being apparently responsible for the ribose methylation of the conserved adenosine of *Flavivirus* cap-1 structure (^{7Me}Gppp$_{2'OMe}$AGU). NS5MTase adopts the conserved fold of a vast family of AdoMet-dependent MTases being complemented by a subdomain that seems to be responsible to bind the cap guanosine of the RNA substrate during 2′*O*-methylation. A structure of the complex of DENV NS5MTase with weak inhibitor ($IC_{50,}$ approximately 101 µmol/l]) ribavirin-triphosphate demonstrated binding to this site, which thus represents an inhibitor-binding site that should be explored further. Another site is the AdoMet-binding site itself. The structure of the complex of DENV NS5MTase with the coproduct methyltransfer *S*-adenosyl-L-homocysteine[28] is thus a starting point for drug design.

Very recently, the group of Pei-Yong Shi (State University of New York, Albany, USA) disclosed that the NS5MTase domain of WNV can act as the 2'OMTase but also as the (guanine-N7)-MTase (N7MTase) on specific RNA substrates with the sequence of the 5' of the WNV genome.[29] Thus, the active centre of the WNV MTase seems to be fairly flexible, being able to support the methylation of two chemically very different acceptor groups. If this holds true for DENV MTase, inhibitors binding to the cofactor-binding site should be able to inhibit the two methylation steps within DENV cap formation. They also showed that the presence of the polymerase domain did not show any influence on the MTase activites in vitro. Thus the MTase domain alone can be used for HTS. Novartis is currently setting up an HTS-adaptable assay to explore the MTase as a target.[c] Within the European academic collaborative project VIZIER* on structural genomics of RNA viruses, our group in collaboration with the group of Alwyn Jones (Uppsala University, Uppsala, Sweden) have been using the DENV NS5MTase in a virtual screening process of AdoMet analogues that led to the discovery on one active molecule as confirmed in biochemical tests (unpublished results).

The polymerase domain

The C-terminal domain of NS5 bears the primer-independent (de novo) RNA-dependent RNA polymerase (RdRp) activity. This is a key activity within viral replication, and DENV RdRp is thus a prime target for drug discovery. Nevertheless, its exploitation has been hampered by difficulties in defining the limits of a functional RdRp domain and producing sufficient amounts of protein for HTS and structure studies. Concerning the characterization of the RdRp activity of recombinant full-length DENV NS5, important contributions were made by the group of Padmanabhan.[30,31] They presented evidence for conformational changes of NS5 necessary to initiate de-novo synthesis and elongate nascent RNA. This invites the investigator to search for allosteric non-nucleoside inhibitors (NNIs) that trap the enzyme in one of these conformations and in this way inhibit RNA synthesis. There are many examples of such inhibitors for HCV RdRp that might be used as a base for inhibitor discovery and design. Nevertheless, the elucidation of the three-dimensional structure is a vital point. One step in this direction was the definition and high-yield expression of a functional RdRp domain (NS5Pol) by our group.[32] The functional comparison with full-length NS5 showed that the presence of the MTase domain does not significantly influence RdRp activity. DENV NS5Pol has been used in a recent study by the group of Andrea Gamarnik (Institut Leloir, Buenos Aires, Argentina) who explored RNA synthesis on specific templates, especially minus strand synthesis, and the interaction of NS5Pol with a newly defined, essential promoter element in the 5' end of the genomic RNA template.[33] Since NS5Pol by itself is suitable for HTS, we have adapted a radioactive assay on homopolymeric template poly(rC)[32] for the screening of several chemical libraries (20 000 compounds) accessible to academic laboratories (French National Chemical Library, Prestwick library, NCI diversity library) giving rise to various hits (unpublished results). Novartis has launched HTS of DENV NS5 and identified various hits that are in the process of being evaluated.[c]

The structure of DENV NS5Pol has been studied intensively, and has now been determined at 2.4 Å resolution by Julien Lescar's group in Singapore. It will soon be released thanks to the collaboration between this group, Novartis, and our own group, and thus will give a boost to DENV anti-RdRp drug design. The structural elucidation was greatly facilitated, through molecular replacement techniques, by recently available crystal structures of cognate WNV RdRp domains, obtained by our group at 2.4 Å resolution (Malet et al., 2006, submitted, pdb codes 2HFZ, 2HCN and 2HCS).

The replicative complex in its cellular environment

Protein–protein interactions within the DENV replicative complex have been proposed as potential drug targets.[34] Our knowledge on the formation and composition of the replicative complex of DENV and the actual interactions between the NS proteins as well as cross-modulations of their activities is still rudimentary, but progress is being made. It was shown in a recent paper from the group of Padmanabhan that the interaction with protein NS5 was important for nucleoside triphosphatase/RNA helicase and 5'-RNA triphosphatase activities of NS3.[35] Additionally, the group of Subash Vasudevan showed that NS4B modulated NS3 helicase activity.[36] Further insight into the structural organization of the NS proteins and putative cellular partners within the replicative complex will provide new possibilities in the control of DENV replication. The induction of a number of cellular proteins during DENV infection has been reported by several authors.[37–39] A key step in the evolution of the infection towards a disease is the down-regulation of the interferon (IFN) response. NS protein 4B and possibly 2A and 4A were identified as candidate IFN antagonists.[40] Research on drug therapies aiming at boosting the IFN response or other yet-to-

* See http://www.vizier-europe.org/

be discovered mechanisms governing innate immunity will certainly blossom in the near future.

Other targets, and inhibitors without identified target

In addition to the NS3 or NS5 enzymes described above, the DENV envelope protein represents an alternate target whose crystal structure, published by the group of Stephen Harrison[41,42] revealed a ligand-binding pocket that has not yet been targeted in structure-based studies of drug design.

A 'blind' screening assay of drugs is based on infected-cell or replicon assays. For example, inhibition of DENV replication by 5-Aza-7-deazaguanosine was demonstrated in an infected-cell-based assay[43] by the group of Robert Sidwell (ZymeTx Inc., Oklahoma City, USA). Moreover, a group from the Pasteur Institute in Noumea (New Caledonia) has shown that natural metabolites isolated from marine invertebrates were potent anti-dengue agents.[44] The search for anti-flavivirus compounds is currently also focusing on WNV. Recent reports by the groups of Pei-Yong Shi and David Ferguson (University of Minnesota, Minneapolis, USA) described new inhibitors of WNV RNA synthesis identified by luciferase-expressing WNV-infection assay in HTS format,[45,46] which also showed activity in a DENV replicon system. The group of Timothy Block (Drexel University, Dylestown, USA) found that screening using a subgenomic-replicon system allowed the identification of new compounds to combat WNV.[47]

CONCLUSION AND RESEARCH PRIORITIES

The design of drugs to treat dengue is at a very exciting crossroads, not only because scientific progress on DENV targets has been tremendous in the last five years, but also because the dengue problem is huge and is increasing, such that dengue might soon join the list of diseases with economically viable drug markets. Lessons from the arboviral disease chikungunya in the Indian Ocean indicate that, in addition to the morbidity issue, economies can be harshly affected by sudden epidemics, and that antivirals will certainly play a role complementary to that of vaccines and vector control in the near future. The problem of anti-DENV clinical trials will probably remain a challenge in terms of patient recruitment and follow-up, but the field of antivirals against dengue is expected to be the first of its kind focusing on neglected diseases, paving the way for other neglected or unexpected potentially pandemic diseases.

We would like to propose the following research priorities:

1. To continue structural elucidation, for the four DENV serotypes, of major players in the replication complex (mainly full-length NS3 and NS5, and their assembled complex), and envelope proteins. Together with screening programmes, accelerated drug design will obviously and crucially depend on these structural models.
2. To unravel the intracellular traffic and interactome of DENV proteins in the infected cell, in order to provide novel cellular and/or viral interactors, or to characterize antiviral innate responses suitable as targets for drug design.
3. To support the use of natural-product chemical libraries in drug discovery for several key reasons: sustainability, access to substances in dengue-afflicted countries, independent economic development provided by natural substance cultivation and exploitation, and most importantly, great chances for successfully discovering active drugs.
4. To support the development of suitable animal models for preclinical evaluation of drugs.
5. To support global reflection on the prequisites and settings of clinical trials for seasonal, emerging/re-emerging, or pandemic diseases for which it is now beyond doubt that antivirals must be developed in the margin of the current market- and corporate-driven initiatives.

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WORKING PAPER 4.4. LABORATORY TESTS FOR THE DIAGNOSIS OF DENGUE VIRUS INFECTION

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1. INTRODUCTION

Dengue, a mosquito-transmitted viral disease that produces variable symptoms, ranging from asymptomatic infection to life-threatening disease, is present in about 110 tropical and subtropical countries. As dengue is increasing in incidence, improved diagnosis, early detection of severe cases, and efficient medical management are of primary importance in all areas where dengue is endemic. Traditionally, dengue has been diagnosed by virus isolation or serological methods, but with recent advances in molecular techniques and in rapid detection technology, a range of novel diagnostic tests will soon be commercially available that will improve case management and aid disease control efforts.

The goal of this paper is to review the diagnostic tools that are currently available or in development and their potential role in case detection, identification of prognostic markers of severe disease, surveillance and outbreak investigations.

2. THE IMMUNOLOGICAL RESPONSE TO INFECTION WITH DENGUE VIRUS

The acquired immune response to infection with dengue virus consists of the production of IgM and IgG antibodies primarily directed against the virus envelope proteins. The immune response varies depending on whether the individual has a primary or a secondary infection (Vorndam & Kuno, 1997). In general, serodiagnosis of dengue is dependent on the stage of the infection. Figure 1 depicts the general time-line of a primary infection from virus isolation/identification to detection of IgM and IgG.

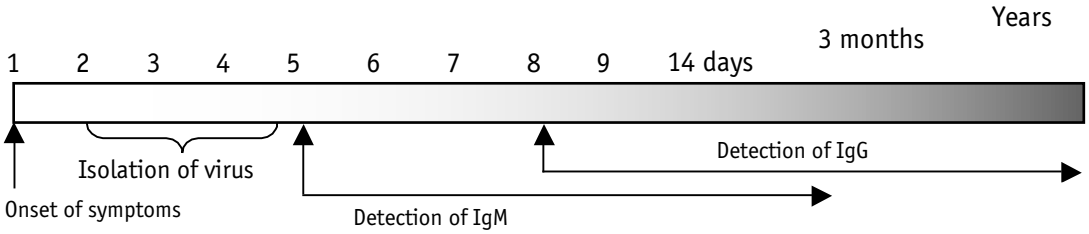
A primary infection with dengue is characterized by a slow and low-titre antibody response. IgM antibody is the first immunoglobulin isotype to appear. Anti-dengue IgG at low titre is detectable at the end of the first week of illness, increasing slowly thereafter. In contrast, during a secondary infection (a dengue infection in a host that had been previously infected by a dengue virus or other flavivirus) antibody titres rise extremely rapidly and antibody reacts broadly with many flaviviruses (Innis et al., 1989). High levels of IgG are detectable even in the acute phase and they rise dramatically over the following 2 weeks. The kinetics of the IgM response are more variable. Since IgM levels are significantly lower in secondary dengue infections, some false-negative results in tests for anti-dengue IgM are observed during secondary infections. According to the Pan American Health Organization (PAHO) guidelines (PAHO, 1994), IgM antibody is detectable by day 5 of illness in 80% of all dengue cases, and by day 6–10 of illness in 93–99% of cases, and may then remain detectable for more than 90 days. IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) has become an important tool in the routine diagnosis of dengue; this technique has a sensitivity and specificity of approximately 90% and 98%, respectively, but only when used 5 or more days after the onset of fever. Different formats, such as capture ELISA, capture ultramicroELISA, dot-ELISA, AuBioDOT IgM capture and dipsticks, have been developed. Serum, blood on filter paper, and saliva, but not urine, can be used for detection of IgM if samples are taken within the appropriate time frame (5 days or more after the onset of fever) (Vasquez et al., 2006). The different commercial kits available have variable sensitivity and specificity (Guzman et al., 2004; Blacksell et al., 2006). A further challenge in the diagnosis of dengue is the fact that anti-dengue IgM antibodies also cross-react to some extent with other flaviviruses, such as Japanese encephalitis, St Louis encephalitis and yellow fever.

3. A REVIEW OF DIAGNOSTIC METHODS FOR THE DETECTION OF DENGUE INFECTION

3.1 Techniques for virus isolation and identification

In the early stages of infection, isolation and identification of dengue virus is traditionally the only way to diagnose a current dengue infection. In this

Figure 1. General time-line of a primary infection with dengue virus, from identification and isolation of the virus to detection of IgM and IgG.



technique, serum from patients is applied to mosquito cell lines. After amplification of the virus in infected cells, the serotype is identified using monoclonal antibodies specific to each dengue serotype. This technique is only sensitive when there is a relatively high level of infectious particles in the serum. Dengue viraemia is short, typically starting 2 or 3 days before the onset of fever and lasting until day 4 or 5 of illness. Serum is the sample of choice for routine diagnosis by virus detection, although dengue virus can also be detected in plasma, leukocytes and in some tissues obtained at autopsy.

The intrathoracic inoculation of mosquitoes (*Ae. aegypti*, *Ae. albopictus*, *Toxorhynchites splendens*, *Tx. amboinensis*) is the most sensitive system for the isolation of dengue virus, but because of the particular technical skill and special containment facilities required for direct inoculation of mosquitoes, cell culture is preferable for routine diagnosis. The mosquito cell line C6/36 (clone obtained from *Ae. albopictus*) has become the host cell of choice for routine isolation of dengue virus, although the *Ae. pseudoscutellaris* cell line AP61 has also been used successfully (Singh et al., 1968; Race et al., 1979). Mammalian cell cultures such as Vero cells, LLCMK2 and others have also been employed, with less efficiency.

Identification of the dengue virus is generally accomplished using immunofluorescence techniques with serotype-specific monoclonal anti-dengue antibodies on mosquito head squashes or infected cells (Henchal et al., 1983). Some strains are not easily identified because of a low concentration of virus. Plaque assay is the gold standard methodology for the quantification of dengue virus. An indirect immunofluorescence assay was proposed by Payne et al. (2006) as an alternative to this test. Flow cytometry has recently been reported as a useful method for the identification of dengue virus 1 (DEN-1), and allows the virus to be identified 10 hours earlier than with an immunofluorescence assay, using an anti-nonstructural glycoprotein (NS1) monoclonal antibody (Kao et al., 2001)

3.2 Serological methods

3.2.1 MAC ELISA

Classic serological testing for dengue includes MAC-ELISA. This assay uses dengue-specific antigens from all four serotypes (DEN 1–4) for the capture of anti-dengue IgM-specific antibodies in serum samples. Most of the antigens used for this assay are derived from the dengue virus envelope protein. The limitations of this test include the specificity of these antigens and cross-reactivity with other circulating flaviviruses. These limitations have to be taken into account when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for the determination of dengue serotypes owing to cross-reactivity of the antibody, even during primary infections.

3.2.2 IgG ELISA

The classic IgG ELISA used for the detection of a past infection with dengue uses the same antigens as the MAC-ELISA. The assay is usually performed with multiple dilutions of the sera tested to determine an end-point dilution. This assay correlates with the haemagglutination assay used in the past. The higher the end-point dilution, the more robust the response obtained after the infection. In general, IgG ELISA lacks specificity within the flavivirus sero-complex groups; however, Cardosa et al. (2002) demonstrated that the IgG response to premembrane protein is specific to individual flaviviruses. No cross-reaction was observed when sera were tested from individuals infected with dengue virus or Japanese encephalitis virus. An excellent specificity of anti dengue-specific IgG was obtained by Baretto Dos Santos et al. (2004) in an assay using a recombinant polypeptide located in the N-terminal portion of the envelope protein. Although the detection of specific IgG has been superseded in the diagnosis of acute infection, seroepidemiological studies are best carried out using ELISAs to detect specific IgG. IgG avidity ELISAs can be used to determine whether an infection is primary or secondary, and can be more useful than the haemagglutination inhibition test for this purpose (Matheus et al., 2005).

3.2.3 IgM/IgG ratio

The IgM/IgG ratio is also used to distinguish primary from secondary infections with dengue. A dengue virus infection has been defined as primary if the capture IgM/IgG ratio is greater than 1.2, or as secondary if the ratio is less than 1.2. This ratio testing system has been adopted by commercial vendors such as PanBio. However, Falconar et al. (2006) have recently shown that the ratios vary depending on whether the patient has a serological non-classical or a classical dengue infection, and redefined the ratios, taking into consideration the four subgroups of classical infection with dengue. The adjusted ratios of greater than 2.6 and less than 2.6, established by these authors, correctly classified 100% of serologically classical dengue infections and 90% of serologically non-classical infections.

3.2.4 Plaque reduction and neutralization test (PRNT) and the microneutralization assay

PRNT is the most specific serological tool for the determination of dengue antibodies (Calisher et al., 1989) and is used to determine the infecting serotype in convalescent sera. This assay measures the titre of neutralizing antibodies in the serum of the infected individual and determines the level of protection the individual had against the infecting virus. The assay is based on the principle of interaction of virus and antibody, resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture. Some of the variability found in this assay is attributable to differences in interpretation of the results. The cell lines and virus seeds used as well as the dilution of the sera accounts for these differences.

The microneutralization assay is based on the same principle as PRNT; however, instead of counting the number of plaques per well, this assay uses a colorimetric measurement of virus-induced cell lysis to determine the end-point dilution. This assay was designed to use small amounts of reagents and to be suitable for the high-throughput testing of large numbers of samples. Some of the limitations of the assay include a poor correlation with PRNT results with samples from people with secondary infections.

3.3 Molecular methods

3.3.1 Reverse-transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR

The PCR assay routinely used by some laboratories for the identification of dengue virus is the nested RT-PCR assay developed by Lanciotti et al. (1992). This comprises a two-step PCR reaction involving an initial reverse transcription and amplification step using universal dengue primers targeting a region of the virus genome (C-prM) followed by a second amplification that is serotype specific. The products of these reactions are separated by electrophoresis on an agarose gel, and the different-sized bands observed are compared with a standard marker for the relative molecular mass of nucleic acids. Dengue serotypes are identified by the size of their bands.

The real-time RT-PCR assay is a one-step assay system using primer pairs and probes that are specific to each dengue serotype. The use of a fluorescent probe enables the detection of the reaction products in real time without need for electrophoresis. Many real-time RT-PCR assays have been developed either as 'singleplex' (only detecting one serotype at a time) or 'multiplex' (able to identify all four serotypes from a single sample). The multiplex assays have the advantage that a single reaction can be used to determine all four serotypes without the potential for introduction of contamination during manipulation of the sample (Johnson et al., 2005b; Chien et al., 2006). The fourplex real-time RT-PCR assays are often less sensitive than nested RT-PCR assay but are faster. An advantage of this assay is the ability to determine viral load in a given sample, which is believed to be important in determining the severity of dengue disease (Vaughn et al., 2000).

4. INNOVATION IN THE DEVELOPMENT OF DENGUE DIAGNOSTICS

4.1 NS1 assays

The NS1 gene product is a glycoprotein produced by all flaviviruses and is essential for viral replication and viability. During viral replication, NS1 is localized to cellular organelles. The protein is secreted by mammalian cells, but not by insect cells. The secreted form of the protein is a hexamer composed of dimer subunits. Glycosylation of this protein is believed to be important for secretion. NS1 antigen appears as early as day 1 after the onset of fever and declines to undetectable levels after day 5–6. NS1 is also a complement-fixing antigen and it produces a very strong humoral response. Because this protein is secreted, many studies have been dedicated to the utility of NS1 as a tool for the diagnosis of infection with dengue virus. These studies focus on various aspects of diagnosis, including antigen-capture enzyme-linked immunosorbent assay (ELISA), and NS1-specific IgM and IgG responses.

In the last 6 years there have been several studies addressing the use of NS1 antigen and anti-NS1

antibodies as a tool for the diagnosis of dengue. An antigen-capture ELISA test was described, with sensitivities ranging from 4 to 1 ng/ml (Young et al., 2000; Libraty et al., 2002). These studies identified a correlation between disease severity and the quantity of NS1 antigen in the serum. However, another study did not find this correlation and in fact could not differentiate between a primary and a secondary infection (Alcon et al., 2002). Recently, a serotype-specific monoclonal antibody-based NS1 antigen-capture ELISA that showed good serotype specificity has been developed (Xu et al., 2006). Shu et al. (2003) have standardized an NS1 serotype-specific IgG indirect ELISA to differentiate primary and secondary dengue virus infections and demonstrated a good correlation between anti-NS1 serotype-specific IgG (determined by ELISA) and PRNT results. The NS1 serotype-specific IgG ELISA worked reliably for the serotyping of dengue virus in convalescent-phase sera from patients with primary infection and in acute-phase sera from patients with secondary infection (which would detect the serotype that caused the first infection), but not so with convalescent-phase sera from patients with secondary infections. Because the results of these studies were varied, results correlating with IgM and IgG assays as well as disease severity and predictors of viraemia, further evaluation of this assay should be performed to determine the main differences between each study.

Commercial kits for the detection of NS1 antigen in serum samples are available. These assays do not differentiate between the serotypes. As NS1 antigen appears early in infection and before the appearance of antibody, such assays are useful for early case detection and for outbreak investigations. Evaluations of these assays should be performed to assess their utility and cost–effectiveness.

4.2 The DENFRAME project

The DENFRAME project, funded by the European Economic Community in 2006, proposes to develop novel tools for the diagnosis of dengue based on the use of chemiluminescent optical-fibre biosensors to detect virions, genome and anti-dengue antibodies. Dengue-specific recombinant antigen and new monoclonal antibodies are also used to produce new ELISA tests for the detection of anti-dengue IgM, IgG, IgA and IgE in the serum and saliva of infected patients, in order to increase the sensitivity and specificity of these assays and to facilitate their standardization and automation.

4.2.1 Luminescence-based optical fibre biosensor

Luminescence-based techniques are becoming increasingly popular owing to their high sensitivity, low background, wide dynamic range and relatively inexpensive instrumentation. Luminometry is up to five orders of magnitude more sensitive than absorption spectroscopy and more than 1000 times more sensitive than fluorometry (Salama et al., 2004). A state-of-the-art luminometer can detect as little as 0.6 pg of ATP or 0.1 fg of luciferase (approximately 1100 molecules), two common luminescent analytes (Turner et al., 1985). Compared with fluorescence, luminometry does not need an excitation source or interference filters, luminescent analytes do not undergo photobleaching, and remains the technology that is most suitable for use on a DNA microarray, or 'chip'. Successful assays have already been developed for monitoring of water (Polyak et al., 2001) and diagnosis of bacterial (Leshem et al., 2004) and viral (Konry et al., 2003) infections, thanks to proprietary immobilization technologies (Marks et al., 2002) and a photon-counting device based on a photomultiplier tube.

4.2.2 Monoclonal antibody mAb4E11

This antibody is directed against the DEN-1 virus. It binds domain III (residues 296–400) of the viral envelope glycoprotein E and neutralizes the virus (Bedouelle et al, 2006). This monoclonal antibody recognizes and neutralizes the four serotypes of the virus and does not cross-react with other flaviviruses (Megret et al., 1992). The Fab and Fv fragments of mAb4E11 and domain EDIII can be expressed in *Escherichia coli* (Renard et al., 2003).

4.2.3 Natural cytotoxicity receptor immunoglobulins (NCR-Igs)

Natural cytotoxicity receptors (NCRs) are expressed by natural killer (NK) cells. Different NCRs recognize viral haemagglutinins from different virus families, including orthomyxoviruses, paramyxoviruses, pox viruses and flaviviruses. NCR-Igs are recombinant molecules comprising the extracytoplasmic part of the NCR fused to the Fc portion of human IgG1.

4.2.4 Recombinant proteins

A recombinant soluble form of E glycoprotein (sE) of dengue virus can be expressed in *Drosophila melanogaster* S2 cell lines. S2 cell clones grow well in serum-free medium at room temperature, without the need for a carbon dioxide incubator (Muerhoff et al., 2004). sE secretion is easily induced by addition of copper sulfate. Large amounts of purified sE

for each of the four serotypes of dengue virus can be easily produced (Després P, personal communication). *D. melanogaster* S2 cell clones expressing the domain III (EDIII) from glycoprotein E have been developed. Purified soluble EDIII is immunoreactive with neutralizing anti-E monoclonal antibodies.

An optical fibre immunosensor has been generated by modifying the fibre tip with recombinant proteins or with a phage-display library of selected epitopes/mimotopes. This methodology has been shown to be highly sensitive, allowing the detection of antibodies in a serum titer of as low as 1:2 621 440 (Marks et al., 1997). In the West Nile virus (WNV) model, the optical-fibre immunosensor was found recently to be at least 100 times more sensitive than the typical ELISA methodology, and allowed the assay time to be reduced to only 30 minutes (Marks RS, personal communication). This assay will be adapted for the detection of anti-dengue IgM, IgG, IgA and IgE. Other samples (whole blood, plasma and saliva) from patients infected with dengue will also be tested.

A biosensor based on modified optical fibres designed to detect the viral genome as well as an optical fiber tip modified with monoclonal antibodies or NCR-Igs for virions capture will be built and evaluated for the DENFRAME project.

Since the use of either monoclonal antibodies (Nawa et al., 2001) or recombinant proteins (Cardosa et al., 2002; Barreto Dos Santos et al., 2004; Videa et al. 2006) significantly improves the quality (sensitivity and specificity) of IgM- and IgG-specific ELISA assays, we propose using both approaches simultaneously. The use of recombinant antigen eliminates the problems and avoids the laborious procedures associated with the standardization of dengue virus antigen prepared in mouse brain or cell culture. By using 'classical' ELISA and MAC-ELISA approaches, new assays for the detection of anti dengue-specific immunoglobulins (IgM, IgG, IgA, IgE) using novel recombinant antigens and several appropriate antibodies are being developed. Serum, plasma and saliva from infected patients will be screened in parallel in different laboratories and results will be compared with the 'gold standard' diagnostic methods. Such ELISA tests can eventually be automated, thus allowing large-scale screening during dengue outbreaks or its use in sero-epidemiological studies.

4.2.5 Microsphere-based immunoassay (MIA)

The traditional serodiagnostic methods use the MAC-ELISA and IgG-ELISA as principal tests. Depending on the sample, this testing is followed by a confirmatory PRNT for positive samples; however, this confirmation can only take place in laboratories with this capability. The MAC-ELISA is a 2-day test that requires about 4 hours of a technician's time. Therefore, a more rapid yet equally sensitive, single test to replace the dengue MAC-ELISA would be of benefit.

Microsphere-based immunoassays (MIAs) are becoming increasingly popular as a serological option for the laboratory diagnosis of many diseases (Kellar et al., 2001). This technology is based on the covalent bonding of antigen or antibody to microspheres or beads. The detecting instrument is a simplified flow cytometer. The lasers simultaneously identify the microsphere sets (bead sets) and measure the fluorescence associated with the reaction. This methodology is particularly attractive because it is faster than the MAC-ELISA and can be used to identify many different antibody responses to multiple viruses. MIAs have the potential to be especially useful in arbovirus serology because tests for infection by viruses of the same genus can share similar formats.

At the Centers for Disease Control and Prevention (CDC), USA, dengue-specific antigens are being developed that could be combined with the current WNV and St Louis Encephalitis (SLE) platform previously developed by Johnson et al. (2005a). In this assay system, unique beads will contain covalently-bonded flavivirus-reactive antibody. The dengue-specific antigen is allowed to bind to these beads and the sample from the patient is then mixed with the dengue antigen-coated beads. Using flow cytometry, the microspheres or beads are sorted to identify a sample as WNV, SLE or DEN1–4 in a single reaction.

4.3 Biosensor technology using mass spectrometry

Rapid advances in biosensor technology using mass spectrometry have led to the development of powerful systems that can provide rapid discrimination of biological components in complex mixtures. The mass spectra that are produced can be considered to be a specific fingerprint or molecular profile of the bacteria or virus analysed. The software system built into the instrument identifies and quantifies the pathogen in a given sample by comparing the resulting mass spectra with those in a database of infectious agents, and thus allows the rapid identification of many thousands of types of bacteria and viruses. Additionally, these tools can recognize a previously unidentified organism in the sample and describe how it is related to others previously

encountered. This could be useful in determining not only dengue serotypes, but dengue genotypes during an outbreak. The infectious-agent identification kits can be designed to meet specific needs and come in a 96-well format. Processing the samples involves four main steps of DNA extraction, PCR amplification, mass spectrometry and computer analysis of results.

DNA extraction

The profile of the pathogen can be identified from human or animal clinical samples (blood, throat swabs, skin wipes, hair). Before amplification of any genetic material present, a lysis/purification protocol removes PCR inhibitors and concentrates nucleic acids.

PCR

The sample is applied to a microtitre plate containing a pair of broad-range PCR primers. The broad-range primers are designed to amplify the DNA/RNA of an individual viral family or families, typically resulting in a mixture of amplicons of about 100 base pairs in length that reflects the complexity of the original mixture of virus present in the starting sample.

Analysis by electrospray mass spectrometry

The PCR products are desalted and electrosprayed into a mass spectrometer, the fundamental component of the system. The mass spectrometer measures the precise weight of each nucleic acid present. Signals, which appear as peaks, are obtained for each of the amplified regions and for a calibrant molecule.

Signal processing and identification of organisms

Signal-processing software is used to process the spectral signals to determine the mass of each of the PCR products present with sufficient accuracy such that the base composition of adenosines, guanosines, cytidines, and thymidines can be established. Using the unique 'fingerprint' represented by combined base compositions from multiple PCR reactions, it is possible to identify the organisms present in the starting sample.

This technology has been successfully used to identify 24 bacterial contaminants in food (Mazzeo et al., 2006). One of the major advantages of this system is the ability to identify the pathogen in a relatively short period of time compared with standard methods, and to develop an arbovirus-testing algorithm that could be specifically designed for the needs of each country. In addition, identification of the agent responsible for an outbreak of an emerging infectious disease could be determined more rapidly than by conventional methods.

5. EVALUATION OF DIAGNOSTIC TESTS FOR DENGUE

5.1 UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR)

5.1.1 Background

The mission of the Diagnostics Research and Development unit within TDR is to promote and facilitate the development, evaluation and deployment of diagnostic tools for the control of tropical diseases.

Accurate diagnostic tests have a key role in patient management and the control of most infectious diseases. Unfortunately, in many developing countries clinical care is often critically compromised by the lack of regulatory controls on the quality of these tests. As a result, in many developing countries diagnostic tests are sold and used without evidence of effectiveness. There is an urgent need for evaluation of commercially available diagnostic tests for dengue.

5.1.2 Identifying priorities

TDR, in collaboration with the Pediatric Dengue Vaccine Initiative (PDVI), convened a meeting of experts to determine the ideal test specifications for diagnostic tools for case management, epidemiological surveillance or vaccine efficacy trials. An inventory of antigen- and antibody-detection tests for the diagnosis of dengue was compiled. A consensus was reached that the highest priority was to evaluate assays for the detection of anti-dengue IgM, in either a rapid test or ELISA format. The next priority was the evaluation of antigen detection tests, such as the NS1 assays, for early case detection.

The group also identified the need to establish a bank of well-characterized specimens from different endemic areas for facilitating test evaluations.

5.1.3 Establishing a dengue specimen bank/ evaluation network

TDR/PDVI identified a network of laboratories in Latin America and Asia that have the capacity and expertise to perform diagnostic evaluations as well as participate in the collection of well-characterized specimens. A reference laboratory has been identified in each region to which specimens from

participating laboratories in the region are sent and stored.

In preparation for the evaluation, standardized evaluation protocols are developed and panel composition is determined according to the type of evaluation required. Evaluation panels are drawn from the two regional specimen banks and are validated by the reference laboratories. The two regional reference laboratories collaborate in the formation of final panel that will be used for the evaluations.

Table 1. TDR/PDVI dengue specimen bank/evaluation sites

Selected site	WHO Region	
	South-East Asia	Americas
Reference centre	*Dr Sutee Yoksan* Centre for Vaccine Development, Mahidol University, Bangkok, Thailand	*Dr Elizabeth Hunsperger* Dengue Branch, Centers for Disease Control and Prevention (CDC), San Juan, Puerto Rico
Evaluation laboratories	*Dr Vinh Chau Nguyen* Hospital for Tropical Medicine (Cho Quan Hospital), Ho Chi Minh City, Viet Nam	*Dr Susana Vázquez* Instituto Medicina Tropical 'Pedro Kouri', Havana, Cuba
	Dr Philippe Buchy Institut Pasteur in Cambodia, Phnom Penh, Cambodia	*Dr Pedro Vasconcelos* Instituto 'Edvandro Chagas', Belem, Brazil
	Dr Shamala Devi Sekaran Department of Medical Microbiology, Faculty of Medicine, University of Malaya. Kuala Lumpur, Malaysia	*Dr Delia Enria* Instituto Nacional Enfermedades Virales Humanas 'Dr Julio I Maiztegui' Pergamino, Argentina

Names of the principal investigators are given in italic type

5.1.4 Engagement with industry

A letter was sent from TDR to manufacturers of commercially available dengue IgM tests describing the evaluation process, to explore their interest in participating in the evaluation. Companies that indicated interest were sent a copy of the standard WHO Confidentiality and Material Transfer Agreement that describes in detail their obligations in providing a sufficient number of tests for the evaluation and explains that they would be given courtesy review of the evaluation results before publication. Companies can visit the evaluation sites and send queries but are not in a position to alter or block the publication of the evaluation results. All results will be available to WHO Member States. They will also be posted on the TDR website and published in peer-reviewed journals. Companies that agree to the terms of the evaluation will be included in the evaluation. Table 2 describes the operational characteristics of the IgM tests that are currently under evaluation.

5.2 Evaluation of dengue IgM tests

5.2.1 Tests under evaluation

The operational characteristics of the IgM tests under evaluation are described in Table 2.

Table 2. Dengue IgM tests under evaluation

Description	ELISA tests	Rapid tests
Country of origin	Australia, USA, UK	Australia, Japan, India, Republic of Korea, USA
Antibodies detected	IgM/IgG or IgM only	IgM/IgG or IgM only
Solid phase	Antigen adsorbed into plastic wells sold as 8-well strips × 12 set into a plastic tray	Nitrocellulose strips sold as dipsticks or encased in plastic cassettes
Specimen type	Sera	Whole blood, sera or plasma
Number of tests, by package	96- well or 192	10–96
Antigen	Purified virus or recombinant antigen	Purified dengue antigen or recombinant antigen, four serotypes
Volume of sample required	1–10 µl	1–5 µl
Supplies required but not provided	ELISA reader, pipettes	Some may require a micropipette
Time taken to obtain results	1–4 hours	15–90 minutes
Price per kit (US dollars)	Depends on volume of order	Depends on volume of order
Storage (°C)	2–8 °C	2–8 °C or 4–30 °C

ELISA, enzyme-linked immunosorbent assay; UK, United Kingdom; USA, United States of America

5.2.2 Composition of the IgM evaluation panel

A panel of 350 well-characterized serum specimens will be used for the performance evaluation. The specimens will be assembled by the reference laboratories from archived collections from geographically diverse areas, provided by the evaluating laboratories. These samples will be validated by the reference laboratories and assigned panel codes.

The serological performance panel is intended to offer a comprehensive assessment of the performance of existing diagnostic laboratory tests, to ensure that such tests are specific to dengue viruses and do not give false positive results when tested against sera from patients infected with a related flavivirus or with other etiological agents causing acute febrile illness. The inclusion of dengue sera at different titres is intended to determine whether the kits being evaluated are capable of detecting sera with medium and low titres of antibody.

To evaluate sensitivity, a total of 200 serum specimens from patients with primary and secondary infections and by different serotypes are tested, as described in Table 3.

For the evaluation of specificity, the panel contains samples of sera that are negative for dengue, as described in Table 3, including those from patients infected with other flaviviruses, with acute febrile illness attributable to other causes, with clinical conditions such as rheumatoid arthritis that may interfere with the assay causing false positives.

Table 3. Proposed composition of a serological panel to be used for evaluating the performance of diagnostic tests for dengue

No. of samples	Element	Source of sera
200 combined	Dengue types	Primary infection – serotypes 1, 2, 3, 4
		Secondary infection – serotypes 1, 2, 3, 4
	Antibody titre	Primary infection – high, medium, low titre
		Secondary infection – high, medium, low titre
Up to 50	Cross-reactive flavivirus (acute or convalescence)	West Nile virus, yellow fever, Japanese encephalitis, St Louis encephalitis, tick-borne flavivirus
Up to 100	Syndromic – acute febrile illnesses	Leptospira, malaria, scrub typhus, mayaro, enterviruses
Up to 25	Interference panel	Rheumatoid arthritis, myeloma, hypergammaglobulinaemia, medications (e.g. steroids)
20	Negatives	Negative for dengue antibodies

5.2.3 Prospective sample collection for serum banking

Since a reasonably large amount of serum is needed to meet the panel requirements, guidelines were developed for the collection process. Institutional review board (IRB) approval details were summarized as well as possible sources to locate specific panel components. A simple questionnaire and tracking records for specimen management will be developed, which should include a unique specimen number, date and site of specimen collection, demographic and epidemiology information. After receiving informed consent from the blood donor, samples can be collected by venepuncture or by plasmaphoresis. The sera should be coded in such a way that they cannot be traced to the donor.

5.2.4 Reference standard test

The CDC ELISA and the Armed Forces Research Institute of Medical Sciences (AFRIMS) ELISA are the reference methods for the IgM test evaluation and the results for all test kits will be compared to those for these assays.

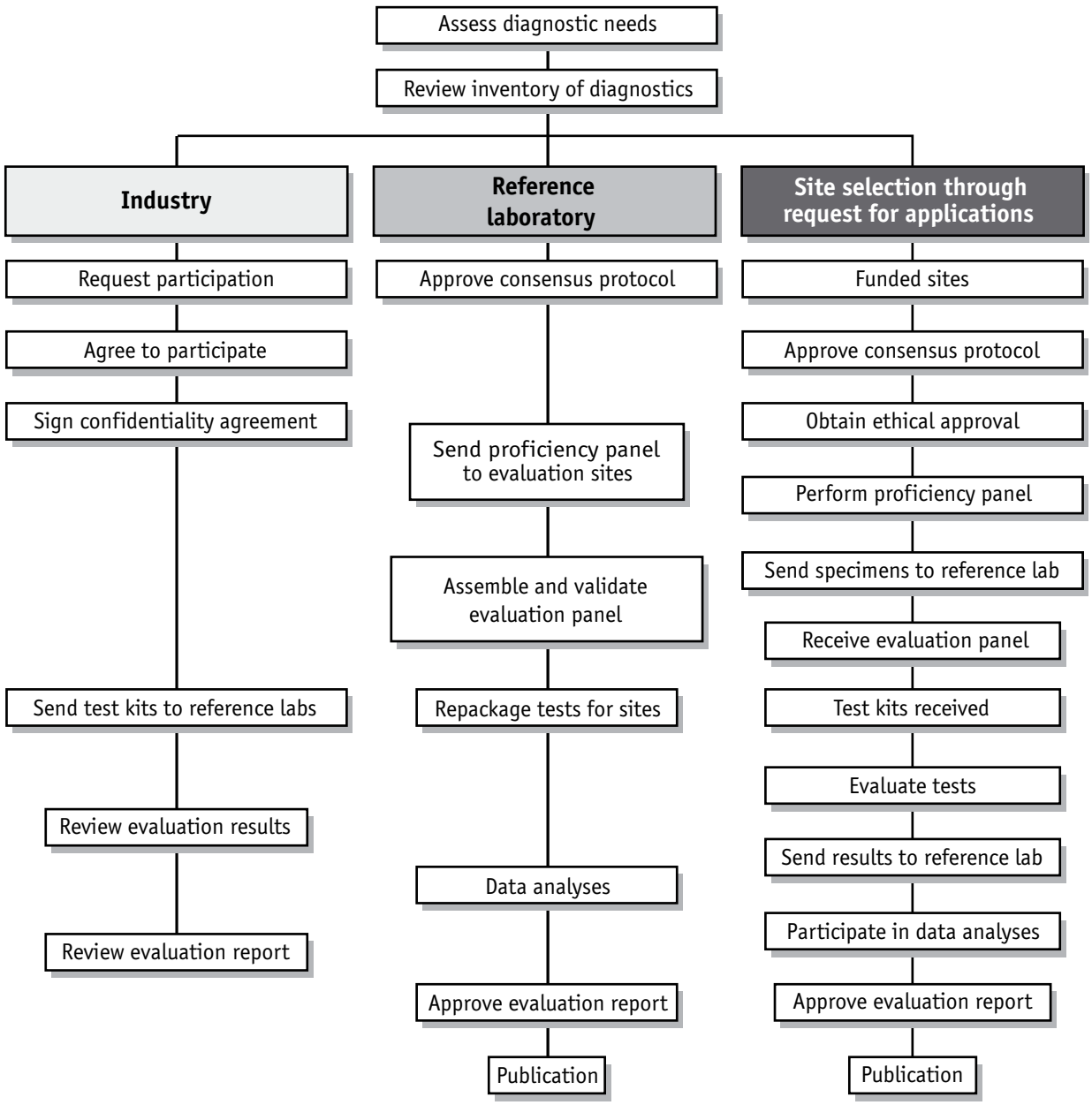
5.2.5 Summary of the evaluation scheme

A schematic of the evaluation scheme is shown in Figure 2. The roles and responsibilities of the reference laboratories, the network laboratories and industry are outlined for every stage of the evaluation process.

6. CONCLUSIONS AND RECOMMENDATIONS

To improve case management, surveillance, outbreak investigations and to ensure the success of dengue vaccine trials, quality diagnostic tools are essential. However, current diagnostic tools available for dengue are not practical for point-of-care use or during the febrile phase of the disease. Many tools are commercially available but their performance and operational characteristics have not been widely evaluated. More novel diagnostic techniques need to be developed for patient management. The goal of a new diagnostic tool would combine antigen (e.g. NS1 antigen) and IgM/IgG detection in a single test and ideally prognostic markers of disease severity would be paired with etiologic diagnosis. The recommended new tools, reference material collection and specimen banks discussed within this document address these needs.

Figure 2. Schematic for laboratory-based evaluation of dengue diagnostics



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Annex 5
WORKING PAPERS:
Scientific Working Group on Dengue

CLINICAL MANAGEMENT

WORKING PAPER 5.1. RESEARCH NEEDS RELATED TO DENGUE CASE MANAGEMENT IN THE HEALTH SYSTEM

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OBJECTIVES

The objectives of this paper are to summarize the current state of dengue management, and to identify areas of clinical management needing further improvement and research, in order to improve the clinical outcome of dengue.

The mechanisms of pathogenesis in severe dengue are complex and remain incompletely understood, but it is clear that the critical abnormality that differentiates severe dengue from its mild form is the presence of increased vascular permeability. This phenomenon begins during the febrile phase, but at a time when the viral load and body temperature are declining. This period, known as defervescence, is defined by an axillary temperature of less than 38 ºC, and usually occurs between day 3 and day 5 of fever. An early sign of increased vascular permeability is haemoconcentration but, with continued plasma leakage, pleural effusion, ascites and depletion of the intravascular volume leading to hypovolemic shock become apparent. It is at the time of defervescence that the disease manifests its severity, unlike other viral illnesses for which a clinical improvement is to be expected with a decline in body temperature. Treatment that is focused on the early recognition of plasma leakage and shock, and replacement of intravascular fluids and restoration of haemodynamic stability is associated with a good clinical outcome (WHO, 1997; Bridget, 2005). In contrast, when the early state of shock is not diagnosed and the consequent delay in administration of intravenous fluid therapy leads to prolonged shock, multi-organ dysfunction and significant haemorrhage set in to complicate the clinical picture (Deen, 2000; Lum et al., 2002). Thus, in order to reduce mortality and morbidity caused by dengue, the goals of dengue management should be:

- To recognize dengue infection at an early stage;
- To detect the early onset of plasma leakage in these patients; and
- To appropriately manage dehydration and hypovolemia.

RECOGNIZING INFECTION AT AN EARLY STAGE

There are no currently accepted guidelines for the recognition of early dengue infection. Kalayanarooj et al.(Kalayanarooj et al., 1997) have demonstrated that when children were recruited having had a fever for less than 72 hours, those with dengue infection were more likely to have marked gastrointestinal symptoms of nausea, vomiting and anorexia, a positive tourniquet test and leukopenia than those with non-dengue viral illnesses. Additionally, children with raised levels of liver enzymes were more likely to have severe dengue than those whose levels of liver enzymes were normal at recruitment. The mean age of the children in this study was 6–8 years.

How applicable are these clinical features in distinguishing dengue from non-dengue infection in a wider population including adults, who are increasingly bearing the burden of illness? Current laboratory confirmation of dengue is largely by dengue IgM serology. In most patients with dengue, IgM serology begins to become positive at the time of defervescence, and hence is not helpful in identifying an early infection.

Research priorities:

1. To acquire a better understanding of the clinical and laboratory features of the early stage of infection with dengue in both children and adults.
2. To determine the early indicators of severe disease.
3. To validate the early warning signs and symptoms (for clinician and caregivers).
4. To identify criteria for hospital admission.
5. To identify high-risk populations—infants, patients with obesity, bronchial asthma, underlying diseases, adults with comorbid conditions.
6. To identify early viral markers of dengue infection that can be applied in the field (Libraty, 2002).
7. To characterize the spectrum of dengue infections and unusual manifestations, such as myocarditis, encephalitis (Nimmannityav et al., 1987; Lum et al., 1996; Solomon et al., 2000).

OUTPATIENT MANAGEMENT OF PATIENTS WITH DENGUE

Once a clinician suspects or is able to confirm that his patient is infected with dengue, what is the best way to provide the care that will determine a good outcome?

Early dengue

An international study on the economic burden of dengue has shown that about 80% of patients with dengue in Cambodia, Malaysia and Thailand make the first visit to a medical doctor within the first 2 days of fever (LCS Lum, personal communication). This early contact could be provoked by generalized body pains or marked gastrointestinal symptoms that may cause dehydration.

Research questions:

1. To validate the early warning signs and symptoms of severe disease.
   What is the best system for caring for outpatients with dengue? A patient could be given a follow-up card that has information including the diagnosis of suspected dengue, serial full-blood-count results (to include, at least, haematocrit [erythrocyte volume fraction]) and indicators for admission, at the first medical contact. This card would be shown to the doctor providing the subsequent care, thus providing some degree of continuity. Would this practice reduce the incidence of patients being turned away from admission, resulting in late presentation of shock?
2. Would this follow-up card system result in a more effective triage system at the emergency department?
   Physicians at the emergency department who may not be familiar with the disease presentation might be alerted to the possibility of a severe disease and thus initiate prompt referral and treatment.
   Harris and colleagues (Harris et al., 2003) have demonstrated that ingestion of fluids such as water, fruit juices and lemonade in the 24 hours before being seen by a clinician is protective against hospitalization. What is the best fluid that could be tolerated by patients with marked gastrointestinal symptoms? A possible candidate is the reduced-osmolarity oral rehydration solution (sodium, 75 mEq/l; chloride, 65 mEq/l; potassium, 20 mEq/l; citrate, 290 mg/l; glucose or rice powder as carbohydrate) recently recommended by WHO and the United Nations Children's Fund (UNICEF) (Alam, 2006). This formulation has been demonstrated to be associated with an extremely low incidence of symptomatic hyponatremia in patients with dehydrating diarrhoeal diseases, irrespective of their age and the cause of diarrhoea.
3. Would this reduced-osmolarity oral rehydration solution be tolerated by ambulatory or inpatient dengue patients? Would it prevent dehydration and hyponatremia?
4. Would the reduced-osmolarity oral rehydration solution reduce the severity of shock and avoid hyponatremia and metabolic acidosis?

Patients in the critical phase of dengue

Patients with severe dengue do present for the first time in a busy emergency department and are attended by a junior physician who may not be familiar with the disease (Simoes et al., unpublished data). In the context of the Integrated Management of Childhood Illness, most countries in south-east Asia have adopted guidelines to recognize severe dengue in first-level health facilities (WHO, 2005).

Patients may be triaged based on certain parameters, such as temperature, heart rate and blood pressure. Patients in whom blood pressure is thought to be normal and the temperature normal or below normal, as would be the case in dengue shock syndrome, would receive the lowest priority in a triage based on presence or absence of fever. Furthermore, in patients in a state of shock, blood pressure measurements using the automated oscillometric method, in which the systolic and diastolic pressures and pulse rate are displayed digitally, have been found to be higher than measurements with the conventional

sphygmomanometer. A literature search suggests that this technology has been validated in children and adults with haemodynamic stability (Gurdial et al., 2004; Ni et al., 2006). There has been no study to compare blood pressure readings taken with the oscillometric method and those taken using the conventional sphygmomanometer in patients in different states of haemodynamic instability.

Research questions:

1. To compare the automated oscillometric blood pressure method with the conventional sphygmomanometer in patients with severe dengue in various states of haemodynamic instability.
2. To compare capillary refill time, quality of pulse, cold extremities and other signs of shock with blood pressure and pulse pressure as early signs of shock, to monitor progress and fluid therapy.
3. What are the clinical features of shock in children and adults?
4. Will early oral fluid therapy while waiting in the outpatient department reduce severity of shock?
5. Will intravenous fluid therapy prevent dengue shock and when should it be started?
6. Will the prescription of 0.9% saline solution at a dose of 5 to 10 ml/kg body weight given intravenously during 1 hour to all patients with a history of more than 3 days of fever and any one of the following signs—repeated vomiting, severe abdominal pain, lethargy (regardless of the intravascular status)—reduce the severity of subsequent shock, i.e. reduce the need for subsequent fluid replacement?
7. How useful is ultrasound examination of the chest and abdominal cavity in detecting subclinical plasma leakage? To validate this against clinical signs of significant plasma leakage. Gall bladder wall-thickening and or fluid perigall bladder wall as measured by ultrasound (Srikiatkhachorn et al., 2005, and Srikiatkhachorn et al., in press).
8. Additional laboratory markers, such as cholesterol, albumin, aspartate amino transferase, and alanine aminotransferase, could be useful for differentiating dengue and severe dengue and for guiding management, e.g. where baseline haematocrit [erythrocyte volume fraction] is not known, or cases of bleeding where erythrocyte volume fraction may not be elevated, a low albumin level may alert the physician to possibility of dengue. Would any of the markers apart from haematocrit alert the physician to the possibility of increased capillary permeability and hence prompt the early initiation of fluid therapy?

Treatment of severe dengue that is entirely orientated towards prompt assessment and replacement of fluid needs is live-saving. Comprehensive guidelines for dengue case management published by the WHO Regional Office for South-East Asia have shown that early volume replacement of lost plasma with isotonic salt solution can modify the severity of disease and prevent shock.

Indications for intravenous fluid therapy for dengue haemorrhagic fever grade I and II are developed in Viet Nam for one or more of the following signs/symptoms:

- Repeated vomiting;
- Acute, severe abdominal pain; rapid liver enlargement;
- Haematemesis, melaena, frank gingival bleeding, severe epistaxis;
- Lethargy;
- Rapid pulse;
- High degree of haemoconcentration, rapidly rising haematocrit.

Among thousands of patients treated each year in Viet Nam, around one quarter of the patients with dengue haemorrhagic fever grade I and II need intravenous fluid therapy during 24–48 hours in the critical phase of the illness. Early volume replacement of lost plasma by intravenous fluid therapy in these patients can modify the severity of disease.

Haemorrhagic manifestations in severe dengue are to be expected but are usually minor. Severe and clinically significant haemorrhages are however, unusual, despite the severe thrombocytopenia and prolonged coagulation profile. In severe dengue, significant haemorrhage is a complication of the disease rather than an integral to it and usually accompanies prolonged shock. However, there have been several reports of severe bleeding in patients who did not have or had minimal evidence of plasma leakage (Sumarmo et al., 1983; Chan, 1987; Hayes et al., 2000; Qiu et al., 1991; Pushpa et al., 1998). The latter phenomenon has a high morbidity and mortality and pathophysiology is still poorly understood.

There are no studies on the management of severe bleeding in severe dengue. The administration of prophylactic transfusions of platelets or blood products is still widely practiced. Although there is evidence that these practices are not useful in the prevention of significant haemorrhage (Lum et al., 2003), demonstrating their harmfulness to the patient with dengue might further deter the use of prophylactic transfusions of platelets and fresh frozen plasma. How useful is platelet transfusion in a patient with severe dengue and significant haemorrhage?

Research questions:

1. Are platelet transfusions harmful for patients with non-bleeding severe dengue?
2. How useful is platelet transfusion in a patient with severe dengue and significant haemorrhage?
3. What is the optimal method of management of bleeding in a patient with severe dengue—fresh whole blood or packed cells, with or without platelets, etc?
4. How can significant but occult haemorrhage be detected? Is there a formula that suggests that significant haemorrhage has occurred?
5. Is hormonal therapy useful in reducing the severity of per-vaginal bleeding in menstruating patients with dengue?
6. To identify the cause of death in dengue—e.g. delayed recognition of dengue, fluid overload.

Severe dengue in adults

Previously an almost exclusively childhood disease, severe dengue is now increasingly being observed in adults, especially young adults, in countries with intermediate economies, such as Malaysia, Singapore and some parts of Thailand. While childhood dengue is well described, severe dengue in adults is a relatively unexplored area, and nowhere is the challenge greater for physicians than in adults during pregnancy or with comorbid conditions such as diabetes mellitus, hypertension, and asthma.

TRAINING IN CASE MANAGEMENT OF DENGUE AND SEVERE DENGUE

Successful management of severe dengue demands the highest clinical acumen from the physician. Reorganizing the delivery of care to patients with dengue may enhance the acquisition of knowledge and skills. Teams dedicated to the case management of dengue have been successfully formed in countries such as Thailand (Kalayanarooj, 2000), Viet Nam and, more recently, Malaysia. If reorganizing delivery of care to patients can have a positive impact on the outcome (e.g. reducing length of stay, reducing use of blood products, more uniform care, economic burden, morbidity and mortality), perhaps hospital management will be motivated to support the establishment of such a team.

Dr Suchitra Nimmannitya and Dr Siripen Kalayanarooj (Queen Sirikit National Institute of Child Health, Children's Hospital, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand) have conducted an effective training programme on case management for medical staff in Thailand and other countries for many years.

In Viet Nam; the National Control Program for dengue has developed and organized a training programme on the standard case management of dengue and severe dengue for medical staff, including physicians, nurses, medical students, and health workers at all levels of the health-care system. The programme also focuses on health education on dengue for mothers/caregivers. Training of trainers has been organized and on-site intervention teams have been set up in provincial and referral hospitals. A dengue-management team has been set up in each hospital. Staff of the teams can consult together regarding the management of severe cases. They can also discuss directly with experienced teams in regional and central hospitals via a hotline connecting all health-care facilities by telephone, fax and e-mail, which has been set up in order to exchange information and experience on the case management of severe dengue. These measures have a good impact on reducing the fatality of severe dengue in southern Viet Nam (Hung & Lan, 2003). Such programmes should be extended to countries where case fatality for severe dengue is high.

EVALUATION OF IMPACT OF TRAINING (TRANSLATIONAL RESEARCH)

Evaluating the impact of training will require research into cost–effectiveness, better-trained nurses, capacity building, how best to deliver health care via the system, organization of health care, implementation research in the context of a health-care system. How can the medical knowledge acquired in Malaysia, Nicaragua, Thailand, and Viet Nam be transferred to other countries with less experience in dengue management?

CONCLUSION

In summary, research priorities in clinical dengue research include studies on optimization of clinical management in the outpatient system, clinical and laboratory indicators of early dengue infection, plasma leakage and shock, as well as a safe method of managing severe bleeding, dengue in pregnancy and patients with comorbidity. The impact of existing and future training programmes should be evaluated.

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Annex 6
WORKING PAPERS:
Scientific Working Group on Dengue

TRANSMISSION DYNAMICS AND VECTOR CONTROL

WORKING PAPER 6.1. DENGUE TRANSMISSION DYNAMICS: ASSESSMENT AND IMPLICATIONS FOR CONTROL

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ABSTRACT

This paper is essentially a mathematical treatment of the epidemiology of dengue with a view to control. The paper begins with two important mathematical insights central to the development of mathematical epidemiology: *the mass action principal* – the course of an epidemic is dependent on the rate of contact between susceptible hosts and infectious vectors, and *threshold theory* – the introduction of a few infectious individuals into a community of susceptible individuals will not give rise to an outbreak unless the density of vectors exceeds a certain critical level. These insights lie at the heart of two mathematical, mechanistic, and weather-driven models (CIMSiM and DENSiM) used to elucidate the non-linear relationships influencing the dengue system. Transmission thresholds in terms of *Ae. aegypti* pupae per person are discussed in the context of dengue and control. A method, the pupal demographic survey, is described whereby the productivities of various classes of water-holding container can be measured, permitting the development of targeted control strategies that have estimates of endpoints in terms of transmission thresholds, e.g. *Ae. aegypti* pupae per person.

Next, the role of weather is discussed noting that daily, seasonal, and interannual variability in temperature, atmospheric moisture, and rainfall all influence the dengue system in a variety of ways. Whether a particular aspect of weather can exert a controlling influence depends on the state of the system. Several cities are contrasted in terms of rainfall being or not being a driver of the dynamics of *Ae. aegypti* and dengue. Atmospheric moisture is shown under rare conditions to adversely influence egg and adult survival, and transmission dynamics. Under the heading of temperature, the influence of temperature-driven variation on the extrinsic incubation period and gonotrophic cycle length is discussed and examples are given where these two temperature-driven variables are responsible for much of the interannual variability in transmission. Finally, the influence of weather anomalies associated with El Niño/Southern Oscillation (ENSO) is discussed. The section concludes with a discussion of the possible use and potential of early warning systems (EWS) for dengue control.

The section on lags between factors favouring transmission and cases presents examples of how increasingly high initial values of R_0 in the months preceding an epidemic can result in substantially more infections in the subsequent epidemic phase when conditions may have actually moderated and R_0 values are lower. This phenomenon produces a *lag* (temporal autocorrelation) between conditions promoting transmission and the subsequent realization in the epidemic when the number of infections is high.

The final section, viral factors, investigates the role that virus titre and variation in viraemic periods play in transmission dynamics. Also covered is the often underappreciated role that stochastic events play in the dengue system. The section on co-circulation of multiple serotypes includes the following topics: 1) spatial and temporal variation in serotype abundance; 2) the founder or stochastic effect; 3) the influence of herd immunity on serotype abundance; 4) the interaction of different serotypes through the mechanism of heterologous immunity; and 5) the potential influence of antibody-dependent enhancement on the dynamics and persistence of multiple serotypes of virus.

INTRODUCTION

A cursory scan of the chapter titles in a text on dengue epidemiology highlights the many facets of the dengue story. Similar to work in other vector-borne infectious systems, as the understanding of the biology became more detailed, our reductionist efforts have necessarily involved a growing variety of highly specialized researchers working on particular problems ranging from molecular to cellular to the whole animal on three entities, the virus, the insect vector, and the human. This has led, on a larger scale, to studies on the dynamics of the dengue system as also influenced by human behaviour, climate, and the movement of viruses and humans. Epidemiology, the branch of medicine that investigates the causes and control of epidemics, involves, implicitly or explicitly, all of the elements contributing to the occurrence or non-occurrence of a disease in a population – in a word, epidemiology deals with the ecology of the disease. Given the many aspects of dengue which interact directly or indirectly and at different temporal and spatial scales and usually in a nonlinear fashion, it is not surprising that it is difficult to identify a single key factor responsible for the particular dynamics of the system as a whole. Yet it is understandable that each specialist tends to see the overall behaviour of the disease from the perspective of his/her discipline. The virologist sees variation in viral virulence as an important determinant of dynamics. Some entomologists are fairly certain that the density and dynamics of the vector population are major influences, yet others uncritically believe that any density of the vector is sufficient for epidemics and causes are to be sought elsewhere. Climatologists suspect that interannual climate variability is a facet that is underappreciated by others in understanding epidemics. Herein, then, lies the utility of mathematical epidemiology—the building of models of infectious

diseases to integrate the interacting components of the system—so that its behaviour and causes can be understood. It is simply another tool, a tool potentially and historically useful in understanding the dynamics and control of infectious disease.

Mathematical epidemiology, the application of mathematics to the investigation of infectious disease, was probably begun by Daniel Bernoulli in 1760; he used mathematical techniques to evaluate the effectiveness of variolation against smallpox in an attempt to influence public policy.[1] Today, mathematical epidemiology has evolved, from simply providing quantitative tools useful in the description of incidence of infectious diseases, to statistical models attempting to correlate incidence with various determinants, and more recently, to dynamic, mechanistic, first principal models that serve as tools to investigate the role of one variable against the background of other factors, which in combination, are involved in the dynamics and control. Two important mathematical insights were central to the development of mathematical epidemiology. In 1906, Hamer postulated that the course of an epidemic depended on the rate of contact between susceptible and infectious individuals;[2] this notion, *the mass action principal*, has become a central concept in mathematical epidemiology—the rate of spread of an infection within a population is proportional to the product of the density of susceptible and infectious people. Ross used this principal in his pioneering work on the dynamics of malaria transmission.[3] The insight of Hamer and Ross was further developed by Kermack and McKendrick in 1927 into an understanding of the concept of thresholds.[4] Anderson and May consider this *threshold theory*, coupled with the mass action principal, to be the cornerstone upon which modern epidemiological theory is built.[1] This notion of thresholds indicates that the introduction of a few infectious individuals into a community of susceptibles will not give rise to an epidemic outbreak unless the density of susceptibles (or vectors) is above a certain critical level. Threshold theory has important control ramifications. More recent advances in the rapid growth of mathematical epidemiology have recognized that spatial aspects cannot be ignored and that variation and the elements of chance are important determinants of the spread and persistence of infection.

It is unfortunate that little use of epidemiological models has been made in empirical studies and in the development of public health policy regarding infectious human diseases – Anderson and May relate this directly to the abstract nature of much of the theoretical work and the lack of ties to field data.[1] They find that "in view of the successes achieved by combining empirical and theoretical work in the physical sciences, it is surprising that many people still question the potential usefulness of mathematical models in epidemiology".

This paper on the epidemiology of dengue differs a bit from the traditional approach of documenting epidemics and their spread by use of insights gained from a pair of weather-driven simulation models that were developed to provide insight into dengue dynamics and control, CIMSiM (Container-Inhabiting Mosquito Simulation Model) and DENSiM (Dengue Simulation Model). The models incorporate the theoretical principals outlined above, but in a computer simulation environment that permits use of site-specific information on human demographics, herd immunity, and the breeding habitat of *Ae. aegypti* and related mosquitoes. Descriptions and validation studies of these models have been presented earlier.[5,6,7] CIMSiM is used to integrate a host of factors pertaining to vector dynamics and provides the entomological inputs to DENSiM. Site parameterization of CIMSiM requires conducting a pupal and demographic survey described below. Whereas CIMSiM is essentially an accounting program of vector dynamics, DENSiM is the corresponding account of the dynamics of human population and virus transmission between hosts and vectors. Both models are weather-driven and stochastic with a daily time step.

Specifically, the models take into account the following aspects of the dengue system: the development rates and survival rates of *Ae. aegypti* eggs, larvae, pupae, and adults are functions of temperature and atmospheric moisture (saturation deficit); the extrinsic incubation period of the virus within the mosquito is a function of temperature and the titre of virus within the host, the titre being a characteristic of the particular type of virus circulating; human age structure and density are dynamic, reflecting country-specific demographic patterns in age-specific birth, fecundity, and death rates; the type-specific immune status of individuals is maintained with maternally-acquired antibody of newborns reflecting the mother's immune status; age-specific and type-specific ratios of cases to infections or of DHF/DSS to cases, if known, can be used to model incidence of clinical illness in addition to infection.

TRANSMISSION THRESHOLDS

We begin with a brief discussion of transmission thresholds as they pertain to the *Ae. aegypti*/dengue system because they will be a useful measure in subsequent sections; a fuller description was published earlier.[8] The phenomenon of thresholds is

based on two concepts: *the mass action principal* – the course of an epidemic is dependent on the rate of contact between susceptible hosts and infectious vectors, and *threshold theory* – the introduction of a few infectious individuals into a community of susceptibles will not give rise to an outbreak unless the density of vectors exceeds a certain critical level. In practice, both of these concepts require knowing the ratio of humans to vectors in absolute numbers. In contrast to other mosquito-borne systems such as malaria where it is essentially impossible to quantify adult production or density, the strict preference of *Ae. aegypti* for artificial containers in the domestic and peridomestic environment allows estimation of the required ratio with a high degree of accuracy. Before presenting estimates of dengue thresholds, we need to look at the quantification tool, the pupal and demographic survey, and at a definition of what constitutes an epidemic.

Pupal and demographic survey

Dengue control programmes today are most commonly based on the suppression of *Ae. aegypti* and not on eradication. With the trend away from a strict reliance on insecticides, current efforts largely focus on reducing the number of larval breeding habitats.[9,10] Several authors have recently made the case that the traditional *Stegomyia* indices, as epidemiologic indicators of dengue transmission risk, should be abandoned as they have a number of serious shortcomings.[11] These authors instead argue that a pupal and demographic survey, providing an estimate of the number of pupae per person in a community by type of container, e.g. drums, flower vases, pots, cisterns, discarded tyres, is more appropriate for assessing risk and directing control operations.[11] This method uses the ratio of pupae per person for several reasons: 1) unlike any of the other life stages, it is possible to actually count the absolute number of *Ae. aegypti* pupae in most domestic environments; 2) container-inhabiting *Stegomyia* pupae are easily and inexpensively separated from other genera and identified to species as emerged adults or pupae; 3) because pupal mortality is slight and well-characterized, the number of pupae is highly correlated with the number of adults; 4) the statistic of pupae per person can be related to transmission risk and provide target levels of reduction required in control efforts.

In practice, conducting the pupal and demographic survey involves visiting 50 or more residences, usually with a pair of inspectors equipped with nothing more than a few litres of clean water, a sieve,* some large-mouth pipettes, a white enamel pan, and small shell vials. The inspectors request permission to examine the water-holding containers and enquire as to the number of people living at the house (or sleeping there the previous night). With permission, they proceed to strain each container at the location, re-suspending the sieved contents in a small amount of clean water in the enamel pan, from where the container's pupae are pipetted into a labelled vial. If there are other container-inhabiting species in the area besides *Ae. aegypti*, the contents of each vial are transferred to small cups covered with bridal veil secured with a rubber band; these are held in the lab (or hotel room) until adult emergence occurs and identification can be made.[11] A key for identification of container-inhabiting mosquito pupae from South-East Asia has recently been published.[12] Data are usually summarized by container type in a spreadsheet.

Definition of epidemics

A definition of an epidemic that was arbitrary but useful from a public health point of view was used in defining transmission thresholds: any single year where seroprevalence rises by at least 10% was to be considered to be an epidemic year. Ten per cent was selected because any disease involving that proportion of the population would be considered an epidemic and this level of transmission would result in slightly more than 1% of the population being infected during the peak of the epidemic – a minimum value that has been suggested as sufficient for the detection of transmission.[13] Just how many mosquitoes per person are required to support this level of transmission is a function of many factors, but the ones considered key determinants are seroprevalence of dengue antibody and temperature.[8] In these assessments, several important assumptions that are likely to be true in most tropical locations were made: 1) vector competence is adequate; 2) blood feeding by *Ae. aegypti* occurs primarily (>90%) on humans; 3) essentially all hosts are at risk of being bitten. The conditions in the southeastern United States are an obvious exception to these assumptions. The catholic feeding preferences of some of the other *Aedes* dengue vectors, e.g. *Aedes albopictus*, would preclude using the thresholds developed specifically for *Ae. aegypti* presented here.

Transmission thresholds

The dengue models were used to estimate thresholds as a function of pre-existing antibody levels in human populations, ambient air temperatures, and size and frequency of viral introduction (table 1).[8] Because the dengue system (both models and biology) is stochastic, at threshold, as defined above,

* USA Standard Sieve Series Number 30 sieve (equivalent to ASTM designation E11, 600 µm (0.0243") opening)

Table 1. Transmission thresholds in terms of *Ae. aegypti* pupae per person as a function of ambient temperature and prevalence of dengue antibody.

Specifically, this table contains the estimated number of *Ae. aegypti* pupae per person required to result in a 50% probability of a 10% or greater rise in seroprevalence of dengue antibody during the course of a year resulting from 12 monthly viral introductions of a single viraemic individual.[8]

Temperature (°C)	Transmission threshold by initial seroprevalence of antibody		
	0%	33%	67%
22	7.13	10.70	23.32
24	2.20	3.47	7.11
26	1.05	1.55	3.41
28	0.42	0.61	1.27
30	0.10	0.15	0.30
32	0.06	0.09	0.16

the probability of a viral introduction leading to an epidemic is 50%. And obviously, therefore, 50% of introductions would lead to situations ranging from the complete loss of virus to situations where there was less than a 10% rise in seroprevalence. In other words, the threshold is the break or tipping point. Threshold levels were estimated to range between about 0.5 and 1.5 *Ae. aegypti* pupae per person for ambient air temperatures of 28°C and initial seroprevalences ranging between 0% to 67%. The size of the viral introduction used in these studies, ranging between 1 and 12 infectious individuals per year, was not seen to significantly influence the magnitude of the threshold. The development of transmission thresholds has given us a new and important tool for monitoring targets for source reduction/control efforts. Moreover, in terms of risk assessment, transmission thresholds provide estimates of the level of elimination or control that are necessary to preclude transmission (table 2).

It should go without saying that we see exceeding threshold as being a necessary but not sufficient cause of transmission. Using a table of transmission thresholds (table 1) takes into account the degree of susceptibility in the human population (and can give you an appreciation of the possible consequences of inadequately knowing herd immunity levels), but there is obviously no statement about the presence or type of viruses that may or may not be circulating or introduced. As presented below, transmission thresholds are useful for risk assessment and risk reduction. In the absence of virus and a control programme, they speak of receptivity to virus; in the endemic state, they provide targets and end points for targeted source reduction/control programmes. Because for a given level of herd immunity transmission thresholds are so strongly influenced by temperature (table 1), there is the possibility of developing early warning systems for dengue in regions such as parts of South-East Asia where predictable El Niño/Southern Oscillation (ENSO) events are associated with known temperature anomalies.

Table 2. Comparison of observed numbers of *Ae. aegypti* pupae per person in various dengue-endemic or dengue-receptive locations with estimated transmission thresholds based on average summertime temperatures and an initial seroprevalence of 33%.[8]

Location	Temp (°C)[a]	Pupae per person[b]	Threshold[c]	Ratio[d]	% Control[e]
Reynosa, Mexico[f]	29.4	2.75	0.26	10.4	90
Mayaguez, Puerto Rico[f]	26.6	1.73	1.05	1.7	40
Trinidad (20 sites)[11]	27.0	22.7[g]	0.86	26.4	96
El Progreso, Honduras[7]	29.1	0.34	0.31	1.1	10
San Juan, Puerto Rico[f]	27.8	2.75	0.58	4.7	79
Bangkok, Thailand[6,20]	29.2	1.69	0.29	5.8	83

[a] *Temp* refers to average temperature during the months of June–August or December–February in locations above and below the equator, respectively.
[b] *Pupae per person* refers to the average number of *Ae. aegypti* pupae per person observed in survey.
[c] *Threshold* refers to the estimated transmission threshold for 12 monthly introductions, assuming an initial seroprevalence of 33%.
[d] *Ratio* is the ratio of observed pupae per person and the estimated temperature and seroprevalence-specific threshold.
[e] *% Control* is the degree of reduction in pupae per person necessary to reduce observed field level to that of the threshold.
[f] Unpublished studies conducted by Focks in collaboration with others. Surveys in Puerto Rico and Mexico were limited and preliminary.
[g] Observed range: 1.4–63.4 pupae per person; the island-wide average is used for calculation.[11]

IMMATURE HABITAT

The primary habitat of immature *Ae. aegypti* in the domestic and peridomestic environment is man-made containers. Breeding in natural containers such as leaf axils in the domestic environment is thought possible only in so far as adults from nearby artificial containers can supply oviposition. For surveillance and control programmes, containers have been classified by a number of schemes: indoors/outdoors, essential/non-essential, presence of active immatures, etc. However, the notion of using the product of productivity and abundance of each type of container has been shown to more useful from the perspective of adult dynamics, risk assessment and control.[8,11]

Productivity

Initial efforts based on counting positive containers

During the initial efforts to control urban yellow fever in South America, control specialists discovered that a substantial reduction in the number *Ae. aegypti* breeding sites would often eliminate transmission. This observation became the basis of efforts, organized in 1923 by the Rockefeller Foundation, to eradicate yellow fever in coastal cities of northern Brazil.[14] Improved methods developed subsequently under Fred Soper resulted, quite unexpectedly, in the eradication of *Ae. aegypti* in several cities in 1933. The goal of vector eradication arose later in Brazil, not as a requirement for yellow fever eradication but rather from a desire to protect *Ae. aegypti*-free zones from reinfestation.[15] To monitor vector control progress and to determine if prophylactic levels had been achieved, *Stegomyia* indices were developed.[16,17] The initial indices, described in 1923, were the House (Premises) Index (HI) – the percentage of houses infested with larvae and/or pupae, and the Container Index (CI) – the percentage of water-holding containers infested with active immatures; 30 years later, the Breteau Index (BI) – the number of positive containers per 100 houses – became a common measure.

Inadequacy of traditional measures

Today, most dengue control efforts are based on suppression of *Ae. aegypti* and not eradication.[18,19] The *Stegomyia* indices, as epidemiologic indicators of dengue transmission, have recently been shown to be inadequate. The traditional indices have a number of serious shortcomings. The CI is probably the poorest since it reflects only the proportion of containers positive in an area and does not take into account the number of containers per area, per house, or per person. The HI is perhaps better, but this index fails to give the number of positive containers per positive house or person. Of the indices, the BI is arguably the best, combining information on containers and houses. Ostensibly these measures are, in some undefined sense, related to risk of transmission; surprisingly, however, the statistics HI, CI, and BI do not correlate well with one another.[11] Moreover, all three indices fail to take into account the fact that containers vary in the production of adult *Ae. aegypti*. For example, two very different containers, an indoor flower vase, commonly found with larvae but seldom producing an adult because of frequent water changes and, say, an uncovered, outdoor 55-gallon (207-litre) drum under a fig tree which supports a standing crop of 10 or 20 or 50 pupae, are for the purposes of calculating the indices, equally positive. Field observations bear this out: Southwood et al. reported, for a temple area in Bangkok, about a 23-fold difference in the most and the least productive types of container.[20] A six-fold difference was seen in Honduras.[8] Connor and Monroe, in their original paper on indices, recognized these shortcomings and, in 1923, pointed out that herd immunity was an additional and important epidemiologic factor not considered by the *Stegomyia* indices.[16] We would add an additional shortcoming – the indices fail to adequately provide information about *Ae. aegypti* density on a per area or, more importantly, a per person basis. This latter statistic, *Ae. aegypti* pupae per person, can be used to estimate, for each type of container (drum, tyre, vase, etc.), what proportion of the transmission threshold it accounts for. Pupae per person, through the use of the table of transmission thresholds (table 1), permits specifying the epidemiological significance if that particular type is eliminated or controlled (table 3).

Threshold estimates in risk assessment and targeted source control programmes

The underlying notion of targeted source reduction is one of selectively attacking the most important types of container. Field observations cited above suggest the rationale is sound in that containers vary significantly in their production of *Ae. aegypti*. The actual epidemiologic significance of any particular type of container, say discarded tyres, is a function of the average standing crop of pupae found in that type and the abundance of that container. Table 3 is an example of how transmission thresholds and the pupal and demographic survey could provide guidance to a targeted source reduction effort. The estimate of the transmission threshold provides an overall target, an upper limit to the number of pupae per person for the environment that ensures viral introductions would result in very little or no transmission. The survey permits estimating the contribution of each type of container and allows, using nothing more than a spreadsheet, conducting what-if analyses of various strategies designed to selectively attack different types of containers at various rates of elimination or control based on their epidemiologic importance and how amenable they are to elimination and/or control.

Our example is based on surveys conducted during June 1995 in urban areas of central St. George County in northern Trinidad.[11] Based on average temperatures for this period (27.8ºC) and assuming a seroprevalence rate of 33%, the estimate of the transmission threshold is ca. 0.71 pupae per person (interpolation of table 1). The surveys estimated human densities to be ca. 160 per hectare and provided data on the nine major types of breeding

Table 3. An example of pupal/demographic survey results from urban sites in St. George County of Trinidad conducted during the rainy season of 1995 and incorporating a transmission threshold estimate of 0.71 pupae per person.[11]

The threshold estimate is based on interpolating values in table 1 using an average June temperature of 27.7°C and an overall seroprevalence of 33%. *Pupae per ha* is the product of *containers per ha* and *pupae per container*. *Pupae per person* is the ratio of *pupae per hectare* and the average human density of 160 per ha. *Portion of threshold* is the ratio of *pupae per person* and the threshold estimate. *Relative importance* is the ratio of *pupae per person* for each container type and the total number of pupae per person, 1.307. In the dry season, the rain-filled containers dry out and cease to produce adult mosquitoes.[a]

Container type[a]	Containers per hectare	Pupae per container	Pupae per hectare	Pupae per person	Portion of threshold	Relative importance
Saucer	3.9	0.20	0.8	0.005	0.007	0.004
Tyre	0.8	1.00	0.8	0.005	0.007	0.004
Small miscellaneous	1.2	1.10	1.3	0.008	0.012	0.006
Indoor vase	40.0	0.05	2.0	0.013	0.018	0.010
Tank	9.5	0.40	3.8	0.024	0.034	0.018
Bucket	1.1	10.90	12.0	0.075	0.106	0.057
Tub	13.5	3.80	51.3	0.321	0.452	0.245
Outdoor drum	8.3	6.70	55.6	0.348	0.490	0.266
Indoor drum	19.4	4.20	81.5	0.509	0.719	0.390
Totals	97.7	-	209.1	1.307	1.844	1.000

[a] Container names in bold indicate rain-filled containers.

container, their abundance and average standing crop of *Ae. aegypti* pupae (table 3). In this environment, there was an average of ca. 98 water-filled containers and 209 pupae per hectare; the number of pupae per person was 1.31 or 184% of the threshold. Numerically, the two most common types were indoor containers, the flower vase and water storage drum. Notice, however, that because these types differed significantly in productivity, the epidemiologic significance of the indoor drum, based on contribution to the number of pupae per hectare or per person, is some 40-times that of the vase. Dividing the estimate of pupae per person for each type by the threshold of 0.71 yields an estimate of what proportion of the threshold is contributed by each; this indicates the vases contribute <2% of the threshold whereas the indoor drum accounts for >70%. Obviously, as eradication is not in mind, targeting the more important types based on this logic would suggest a focus on indoor and outdoor drums and perhaps on tubs. If table 3 is put into a spreadsheet, evaluating various targeted strategies becomes easy. We see that an overall reduction of ca. 50% of all containers, the control or elimination of about 50 of the 100 containers, would result in the number of pupae per person being about 92% of the threshold. We also can see that a targeted approach that eliminated about 55% of the three most important types, the drums and the tubs, would put the population at about 93% of threshold, and would require the control or elimination of only about 23 containers per hectare. This approach would also take into account specific container types for the required reduction in pupae, given some types were uncontrollable by virtue of their location, ownership, use, etc. Below, we return to this site, and will look at the consequences of lack of rain during the dry period of March–May.

WEATHER

Daily, seasonal, and interannual variability in temperature, atmospheric moisture, and rainfall all influence the dengue system in a variety of ways. Whether a particular aspect of weather can exert a controlling influence depends on the state of the system.

Rainfall and the immature habitat

The response to seasonal and interannual variation in amount of rainfall is a function of the proportion of the transmission threshold that arises from rain-filled containers. The following examples illustrate the role of rainfall in the dynamics of vector and transmission, and how precipitation seasonality can interact with other parameters.

Bangkok, Thailand

In response to the emerging problem of epidemic dengue haemorrhagic fever (DHF) in South-East Asia in the late 1950s, the World Health Organization, at the request of the government of Thailand, set up the *Aedes* Research Unit (ARU) to study the ecology and control of *Ae. aegypti*. At the time it was fairly well established that dengue viruses were responsible for DHF and that *Ae. aegypti* was the epidemic vector.[21] Moreover, the initial hypothesis was

that seasonal changes in the density of the vector and the incidence of DHF were correlated, primarily because the disease was associated strongly with the wet season, when rainfall would presumably increase the number of breeding sites and/or increase adult survival.[22,23] In light of these conjectured relationships, a series of year-long studies was conducted between 1966 and 1968 on the larval habitat,[23] survival, density, and dynamics of immature[20] and adult populations,[24] and on the gonotrophic cycle of *Ae. aegypti*.[25]

With one exception, these studies were conducted in the residential compound of a Buddhist temple, the Wat Samphaya. The Wat was chosen because of its convenient size and because the type of housing and human density were representative of much of Bangkok. Also typical was the water supply of standpipes and the types of water-filled containers present, primarily large 100–200-litre water-storage jars, flower pot plates, and ant traps located under the legs of food cupboards, tables, etc.[24] Southwood et al. and Tonn et al. reported finding that in the Wat, as in the rest of Bangkok, *Ae. aegypti* was the only mosquito breeding in the great majority of containers.[20,23] Throughout the study period there were ca. 100 jars, 50 flower plates, and 50 ant traps present in the Wat and ca. 53% of these were occupied by *Ae. aegypti*; the number of water-filled containers and the proportion with mosquitoes were remarkably constant, and with the exception of a portion of the ant traps, all containers were filled manually and not influenced by rainfall.[20,23] The initial conclusion from the larval studies was that the key factor(s) responsible for the seasonality of DHF in Bangkok was *not* fluctuation in adult production and density in response to rainfall. The models demonstrate this as well: in 20-year runs, there is only a slight, and not statistically significant, variation in the number of *Ae. aegypti* females per person and this and its lags going back several months do not correlate with cases. The initial field results led to the next hypothesis, that seasonality in transmission was due to seasonality in adult survival due to temperature and/or atmospheric dryness associated with the hot season. We will come back to the Bangkok story when the role of these variables is discussed.

South-western Puerto Rico

In contrast with Bangkok, longitudinal studies in southwestern Puerto Rico show a positive correlation between rainfall and vector abundance, with the correlation being strongest in the dry, south coastal portions of the island.[26] Also in marked contrast with Bangkok is the fact that most breeding occurs outdoors and in rain-filled containers, the primary ones being animal watering dishes and discarded tyres. Moore describes the relationship between rainfall, vector abundance, and transmission as follows: "At least in southern Puerto Rico, *Ae. aegypti* densities rise quickly with the onset of rains in July and August. This relationship further leads to a rather close correspondence between seasonal rainfall and dengue fever incidence, the peak of which occurs about six or eight weeks after the peak in rainfall. In 1973 the rains began in June, and dengue therefore also appeared earlier than usual. In 1974, however, when the rains began later than usual, the peak dengue fever incidence did not occur until December 1974 and January 1975".[26] The authors concluded under their conditions of principally outdoor breeding that "rainfall patterns seem to be a reasonably effective predictor of time of peak dengue transmission." That is not to say that they understood only rainfall to be necessary, as they certainly appreciated the need for viruses and susceptible human populations. So here we have an example, especially in the south coastal areas, of where rainfall is the key factor influencing temporal and spatial patterns of transmission.

St. George County of Trinidad

To put the influence of seasonal variation in rainfall into a more quantitative context, that of pupae per person, contrast the total number of pupae per person in table 3 (wet season) with that of table 4 representing the dry season. Note that the rain-filled containers (tyres, small miscellaneous, and outdoor drums) cease to support breeding and the total number of pupae per person falls from 1.31 to 0.95; in the uncontrolled situation, the respective proportions of the transmission threshold are 1.84 and 1.33 respectively. Note also that in the dry season when the populations are a bit lower, only a single class of container, either the tub or the indoor drum, would have to be controlled or eliminated to bring the community below threshold.

Atmospheric moisture

The drying power of the atmosphere, as measured in saturation deficit (mBars pressure), reflects the combined influence of temperature and relative humidity. The dengue system (and models) are influenced by saturation deficit in several ways. In CIMSiM, atmospheric moisture influences evaporation rates from containers along with certain characteristics of the container, including their size, shape, and exposure to direct sunlight. Also, deficits greater than ca. 10 mBars progressively reduce survival of newly-laid eggs and adults. The impact on egg survival under very dry conditions is minimal and, with the possible rare exception of breeding in exposed lime

Table 4. Pupal/demographic survey results from urban sites in St. George County of Trinidad, conducted during the dry season (March–May) and incorporating a transmission threshold estimate of 0.71 pupae per person.[11]

Container type[a]	Containers per hectare	Pupae per container	Pupae per hectare	Pupae per person	Portion of threshold	Relative importance
Saucer	3.9	0.20	0.8	0.005	0.007	0.005
Tyre	-	-	-	-	-	-
Small miscellaneous	-	-	-	-	-	-
Indoor vase	40.0	0.05	2.0	0.013	0.018	0.013
Tank	9.5	0.40	3.8	0.024	0.033	0.025
Bucket	1.1	10.90	12.0	0.075	0.106	0.079
Tub	13.5	3.80	51.3	0.321	0.452	0.339
Outdoor drum	-	-	-	-	-	-
Indoor drum	19.4	4.20	81.5	0.509	0.717	0.538
Totals	87.4	-	151.4	0.946	1.332	1.000

[a] Container names in bold indicate rain-filled containers.

rock solution holes adjacent to beaches, is easily compensated by subsequent density-dependent larval survival. Based on CIMSiM, only at particularly hot and dry continental locations such as Ouahigouya, Burkina Faso, are conditions such that adult survival is reduced by excessive temperatures and high saturation deficits. Here, the dynamics and abundance of adults and immatures would not be materially different under milder conditions, again, primarily due to resilience in the entomological system from density-dependent larval survival. However, the shortened adult lifespan significantly reduces transmission in simulation studies (not shown).

Temperature

Vector dynamics

In temperate locations, *Ae. aegypti* overwinters in the immature stages, and seasonal variation in adult abundance clearly reflects the key role of temperature on the development of immature stages. However, under tropical conditions, adult abundance varies not with temperature but with variation in the abundance and productivity of water-holding containers; container productivity is limited, not by temperature or oviposition, but by density-dependent larval survival which is ultimately driven by the amount of food falling into or formed photosynthetically within the container. This is consistent with both CIMSiM's rather constant estimates of adult abundance from manually-filled containers under conditions of constant temperatures of 22°C to 32°C (not shown). In light of this, going back to the Bangkok story of the investigation into the cause(s) of seasonal variation in incidence of dengue, we should not be surprised to read that the field work of Sheppard et al. indicated no seasonal trends in adult survival.[24]

The influence of temperature-driven variation on the extrinsic incubation period and gonotrophic cycle length

Under moist, tropical field conditions, for example Bangkok, where the major mortality sources are accidents such as encountering a spider's web, the probability of surviving a single day (Sa) is constant and independent of temperature. Experiments to measure this parameter in the field are notoriously noisy but a consensus value is somewhere between 0.87 and 0.91 per day under conditions without temperature or moisture deficit extremes such as for most locations in dengue-endemic regions, e.g. South-East Asia.[5] The integral of Sa^t provides the average lifespan of the female; for Sa = 0.89, the average lifespan is ca. 8.6 days. Keeping in mind that the resulting age distribution declines exponentially with age, it is easy to see that numerically, while most emerging females die at an early age, the tail of this age distribution contains the rather rare but older individuals with the potential to transmit.* The length of time required for a newly infected female to become infectious, the extrinsic incubation period (EIP), is a non-linear function of temperature; the same can be said regarding the length of the gonotrophic cycle (table 5). Notice that if a female takes an infectious bite on her first day of life, the length of time required for her to have a disseminated infection is EIP plus one day—a substantial portion of the average lifespan, so most will not pass on virus before death. Moreover, once disseminated, the probability of transmitting virus will vary with how often the mosquito bites, which is related to the duration of gonotrophic development.

* $\int_{t=1}^{t=\infty} Sa^t td$

Figure 1 presents an estimate of the average number of potentially infectious replete feeds per newly-emerged female as a function of temperature and daily survival probability. This figure makes, for the purpose of comparisons, the unrealistic assumption that all mosquitoes take an infectious blood at one day of age. The actual number of potentially infectious bites per replete feed is unknown and may be as high 2 or 3 or more interrupted feeding attempts with resumption on the same or different host.[7] From an epidemiological perspective, it is important to realize that a temperature-related doubling of expected number of potentially-replete feeds, the consequence of 2 or 3 degrees warmer temperatures, is equivalent to a doubling of the density of *Ae. aegypti*. While temperature plays a role in most facets of transmission dynamics, its influence on the speed of viral dissemination and frequency of biting is a key regulating force entraining seasonal variability. And indeed, the field work of Pant et al. allowed them to conclude that the source of the seasonality seen in dengue in Bangkok was due not to rainfall variability leading to adult abundance seasonality, nor to excessively high temperatures and/or dryness leading to reduced adult survival, but to temperature-related variability in the infectiousness of *Ae. aegypti* females through the agency of EIP and gonotrophic development rates.[25] This is the same conclusion reached through an analysis of the Bangkok situation made with CIMSiM/DENSiM.[6]

Table 5. Lengths and daily rates of the extrinsic incubation period of virus within *Ae. aegypti* and the gonotrophic development cycle.[5]

Temperature	Extrinsic incubation period		Gonotrophic cycle	
	Rate per day[a]	Days	Rate per day[a]	Days
22	0.04	24.0	0.14	7.3
24	0.05	20.0	0.17	6.1
26	0.07	14.0	0.24	4.2
28	0.08	11.8	0.29	3.5
30	0.10	9.9	0.34	2.9
32	0.12	8.4	0.41	2.4

[a] The 'daily rate' is that proportion of total development occurring on a particular day at the specified temperature

The influence of interannual climate variation

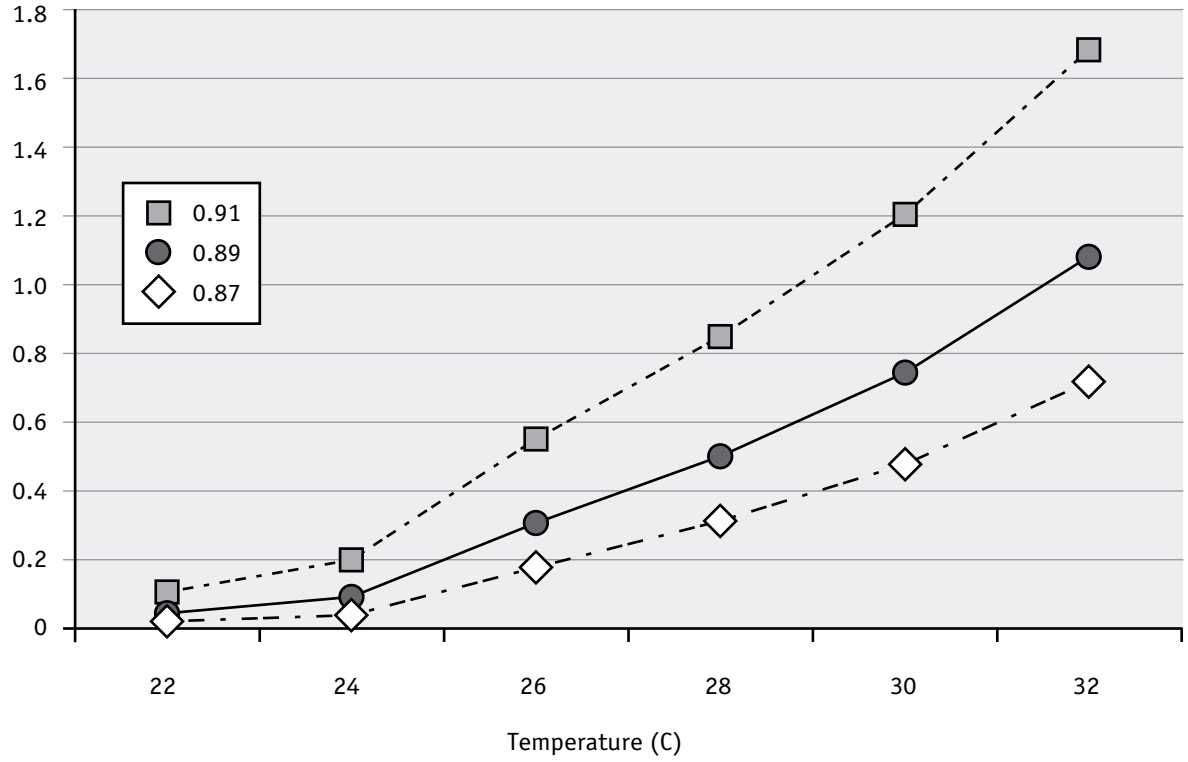
The El Niño Southern Oscillation (ENSO) is an atmosphere–ocean coupled system which produces quasi-periodic short-term climate and sea surface temperature changes over the Pacific region with impacts on weather worldwide including many countries in the Americas, Africa, and Asia. The system oscillates between two extremes known as El Niño and La Niña, which are associated with approximately opposite disturbances to climate worldwide. A chief phenomenon of an El Niño phase is eastward extension of warm surface waters situated off northwestern Australia towards the west coast of equatorial South America. During the cool phase, La Niña, equatorial westerlies result in an upwelling of cold abyssal water which is transported to the west creating a tongue of abnormally cool surface waters extending towards Indonesia. Because convection rainfall in this region is limited to sea surface temperatures greater than ca. 26–27°C, the spatial distribution of rainfall is associated with equatorial sea surface temperature anomalies associated with ENSO state. The 'Southern Oscillation' refers to the oscillation of atmospheric pressures between the eastern and western Pacific. One of the summary or indicator statistics of ENSO state is the southern oscillation index (SOI), the normalized difference in pressure between Darwin and Tahiti. El Niño and La Niña are associated with negative and positive values of the SOI respectively. It is not surprising that much innerannual variability in climate in the central Pacific is attributable to the state and intensity of ENSO.

Because many infectious disease systems are influenced by weather, ENSO state and associated anomalies in rainfall, atmospheric moisture, and temperature have become topics of considerable interest. An excellent review of ENSO and health has been provided by Menno Bouma and others.[27] Largely through the use of numerical simulation models of the ocean and atmosphere, forecasts of anticipated ENSO state are increasingly skilful, such that it is reasonable to expect that useful early warning systems (EWSs) will be developed.[28] Initial efforts involved nothing more that simple attempts to demonstrate correlations between ENSO state and outbreaks. More sophisticated development of ENSO-based EWSs will require addressing two related problems. The first of these is that, while skill in forecasting an ENSO event is currently adequate, predicting the strength of the oscillation is problematic and leads to lack of skill in predicting regional weather anomalies. The second area in need of much attention is elucidation of the mechanisms whereby weather anomalies lead to anomalies in the disease system.[29] Several recent studies have shown temporal correlations between malaria epidemics and various indices of ENSO state.[30,31,32]

Given that dengue incidence is a function of interaction of the many factors outlined above, it is not surprising that dengue activity has been correlated

Figure 1. Average number of potentially infectious replete feeds per newly-emerged female as a function of temperature and daily survival probability.

This figure makes, for the purpose of comparisons, the unrealistic assumption that all mosquitoes take an infectious blood meal at one day of age. The actual number of potentially infectious bites per replete feed is unknown and may be as high 2 or 3 or more interrupted feeding attempts with resumption on the same or different host.[7]



with ENSO state or one of its statistics, SOI, in regions (most clearly in the South Pacific) where ENSO or SOI is correlated with temperature and/or rainfall anomalies.[28,33] Unfortunately this study does not identify the environmental risk factors unequivocally.

At a recent World Health Organization dengue workshop in the South-East Asian region, directors of national anti-dengue programmes in Thailand, Vietnam, and Indonesia expressed the operational need for an early warning system (EWS) that would provide sufficient lead time (one to three months) to permit mobilization of control operations. In response, Focks et al. have attempted to develop practical EWSs for Yogyakarta, on the island of Java in Indonesia, and Bangkok, Thailand, based on logistic regression.[34] The predictor variables are sea surface temperature (SST) anomalies (a five-month running mean of spatially averaged SST anomalies over the tropical Pacific: 4°S–4°N, 150°W–90°W as measured by the Japanese Meteorological Association) and past monthly cases of dengue in each city. Previous incidence of anomalously high or low cases is an indication of interaction between the types of virus currently circulating and the nature of the immune status of the human population. SST anomalies are highly correlated with subsequent surface air temperature anomalies and may be correlated with atmospheric moisture as well. The predicted variable is the probability of an epidemic year forecast one to three months before peak transmission season. The skill level for three-month predictions for Bangkok were inadequate for an operational system (6 errors in 35 years); the two and one-month forecasts had error rates of 3 and 2 per 35 years, respectively. The Java EWS, however, was sufficiently skilful to be put into use in Yogyakarta, Indonesia; one, two and three-month forecasts were without error for the 14-year period of record. Note that this system does not use ENSO state directly, but rather, one of its indicators, sea surface temperature anomalies.

A recent National Research Council report on the subject of early warning systems is cautiously optimistic but concludes that substantially more research is needed to understand the relationships between climate, human behaviour, and infectious diseases. The report states that one goal of such research should be to support a transition from the current practice of "surveillance and response towards a more proactive 'prediction and prevention' strategy."[29]

LAGS BETWEEN FACTORS FAVOURING TRANSMISSION AND CASES

Dengue epidemics obviously involve one person's infection leading to another's; the number of infections resulting in the next cycle from a single individual is commonly referred to as R_0. As long as R_0 is greater than one, the epidemic grows exponentially at a rate proportional to this ratio. The magnitude of R_0 is dynamic, reflecting integration of the host of factors influencing dengue dynamics. Higher temperatures shorten EIP and the gonotrophic cycle and are thus a factor tending to increase R_0, as would rainfall lead to more *Ae. aegypti* in the case of southwestern Puerto Rico. High levels of herd immunity, effective spraying that shortens adult survival, or window screens limiting host access would favour reductions in R_0. Dynamically accounting for the influences of the various factors through time is a chief activity of accounting software such as CIMSiM/DENSiM. Table 6 and figure 2 present three examples of how increasingly high initial values of R_0 in the months preceding an epidemic can result in substantially more infections in the subsequent epidemic phase when conditions may have actually moderated and R_0 values are lower. This produces a *lag* between conditions promoting transmission and the subsequent realization of the epidemic when the number of infections is high.

Table 7 provides correlation coefficients between monthly cases and lagged monthly cases, average temperature, rainfall, length of gonotrophic cycle, and EIP for dengue in Bangkok, Thailand, 1966–1994. It is not surprising that cases are highly auto-correlated going back at least four months; anomalously high (or low) prevalence this month reflects unusually large (or small) prevalence last month through the agency of *Ae. aegypti* giving rise to subsequent cases. The lack of substantive correlation between current cases and current weather, temperature and rainfall and their lags going back one, two or three months may come as a bit of a surprise to people who are not acquainted with dengue data. This phenomenon reflects the fact that epidemics take several months to develop to a level where they are recognized to be a result of antecedent conditions as described above.[13,35] To be truthful, there are some non-trivial correlations between cases and the preceding two months' rainfall, suggesting that, in contrast to the Wat Samphaya temple area, not all containers are manually-filled in the metropolitan area.[20] With regard to collaborating the story of antecedent conditions being key determinants of epidemics, the peaks in correlations between cases and temperature, gonotrophic cycle length, and EIP (as estimated with CIMSiM and DENSiM using historical weather data) three and four months earlier, are important. Epidemics, under these conditions of constantly endemic virus, are entrained by environmental determinants at play months before the health community is aware that a nascent epidemic is building. And, epidemics can and do occur under weather conditions less than optimal for intense transmission.

VIRAL FACTORS

Virus titre and variation in viraemic periods

The size of the virus inoculum, that is, the product of viral titre and quantity of blood, influences the probability of the vector subsequently developing a disseminated infection with virus in the salivary glands.[7] It has been suggested, and there is some evidence to support the notion, that the titre of virus in the blood meal alone could influence the probability of subsequent infection.[36,37,38] Moreover, duration of the dissemination period, EIP, can vary with titre. Watts et al. reported that the EIP for dengue in *Ae. aegypti* at 30°C was 12 and 25 days for mosquitoes infected with *high* and *low* doses respectively.[39] Provision has been made in DENSiM to allow evaluation of the consequences of these relationships.

Simulation studies regarding the nature of epidemic and endemic transmission

Studies comparing the consequences of viral titre on the dynamics of endemic dengue suggest that titre, through the agency of probability of dissemination and EIP, does indeed play a role. In studies by Focks et al,[7] the titres evaluated were 10^5 (low) and 10^6 (high) median infective dose (MID_{50}), the human population was low, and the number of *Ae. aegypti* pupae per person was ca. 150% of threshold. Using 10^5 MID virus, the initial virgin soil epidemic was acute leaving only 20% of the population uninfected. For the next five or six years after the initial epidemic, additional introductions resulted in few locally-contracted infections due to herd immunity and the relatively low abundance of vector. As the immune population aged, the younger age classes progressively became more susceptible and, as a consequence, most of the subsequent infections occurred primarily in these classes. If this scenario is run for decades, the age-specific distribution of seroprevalence settles down to one of rising prevalence with age, with only small epidemics involving at most a few hundred (primarily young) individuals, and with the overall prevalence of antibody averaging ca. 70%. If this scenario is run again with the titre of the introduced virus increased from 10^5 to 10^6 MID_{50}, the initial epidemic is more acute and

Table 6 and Figure 2. Projected numbers of infections over time as a function of R_o.

For illustration, we assume the periods of time between the onset of viraemia in the first and subsequent infection cycles are multiples of 17 days. In each example, the epidemic is initiated with a single viraemic individual. During the first four cycles, the pre-epidemic period (up to day 81), Ro is set to a constant value of 1.5, 2.0, or 2.5 for lines labelled infections (1.5, 1.5), infections (2.0, 1.5), and infections (2.5, 1.5), respectively. For cycles 5–10, Ro is set to a constant 1.5 in each case. The purpose of this illustration is to demonstrate that conditions several months before the appearance of a large number of cases (the epidemic) significantly affect the magnitude of the event. Note in each example that the ratio of new infections in each cycle after day 81 is the same, 1.5, but the absolute numbers of infections after additional cycles in the epidemic phase is larger as a function of the number of infected in the pre-epidemic period. This then is a mechanism whereby environmental conditions that promote increased intensity of transmission but before there are large numbers of infections can become manifest months later as an epidemic under conditions that are less conducive to transmission.

Cycle	*Days*	*Months*	Example 1		Example 2		Example 3	
			Ro	*Infections (1.5, 1.5)*	*Ro*	*Infections (2.0, 1.5)*	*Ro*	*Infections (2.5, 1.5)*
1	1	0.03	1.5	1	2.0	2	2.5	3
2	17	0.56	1.5	2	2.0	4	2.5	8
3	33	1.08	1.5	2	2.0	8	2.5	19
4	49	1.61	1.5	3	2.0	16	2.5	47
5	65	2.14	1.5	5	2.0	32	2.5	117
6	81	2.66	1.5	8	1.5	48	1.5	176
7	97	3.19	1.5	11	1.5	72	1.5	264
8	113	3.72	1.5	17	1.5	108	1.5	396
9	129	4.24	1.5	26	1.5	162	1.5	593
10	145	4.77	1.5	38	1.5	243	1.5	890

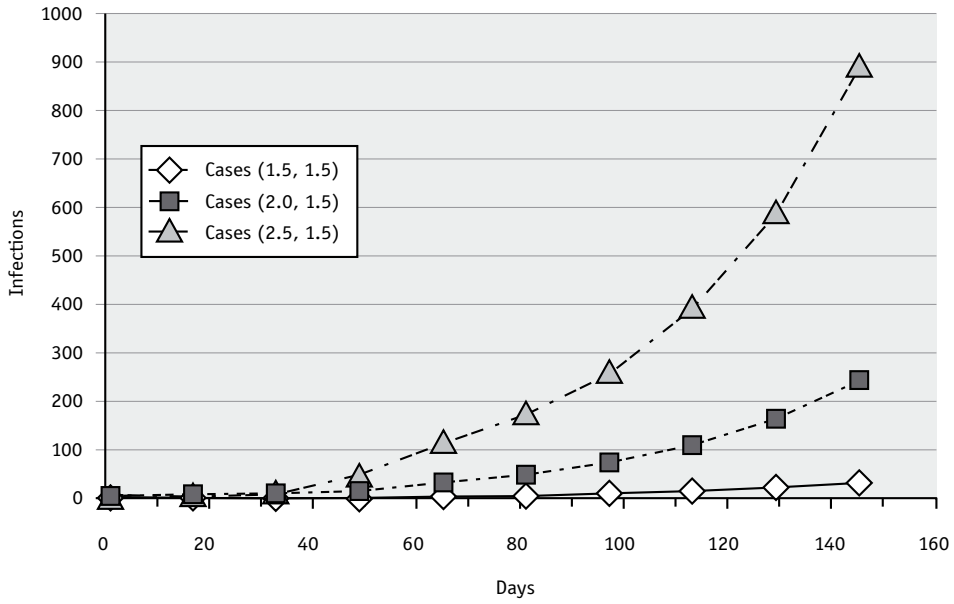


Table 7. Correlations between monthly cases and lagged cases, monthly average temperature (°C), rainfall, length of gonotrophic cycle (Gono) and extrinsic incubation period (EIP) for dengue in Bangkok, Thailand, from 1966–1994.

The length of the gonotrophic cycle and EIP were estimated using CIMSiM and DENSiM with historical weather data from metropolitan Bangkok. Correlations greater than ± 0.30 are highlighted.

Lag (months)	Correlations between current and past monthly averages				
	Cases	Temperature	Rain	Gonotrophic cycle	EIP
0	1.00	0.07	0.22	-0.06	-0.09
1	**0.91**	0.18	0.28	-0.16	-0.20
2	**0.74**	0.29	0.24	-0.25	-0.29
3	**0.57**	**0.37**	0.09	**-0.32**	**-0.36**
4	**0.41**	**0.37**	-0.04	**-0.31**	**-0.35**
5	0.27	0.26	-0.14	-0.21	-0.25
6	0.17	0.08	-0.22	-0.05	-0.07

shorter in duration and involves ca. 95% of the population. The nature of transmission following the primary is different as well, with transmission being more intense, the small ensuing epidemics sporadic and more frequent, and with fewer people involved but producing higher levels of immunity than those associated with the lower-titre virus.

Simulation studies evaluating combinations of titres and viraemic periods clearly indicate that combinations favouring transmission, e.g. higher titres and longer periods, lead to more acute initial epidemics followed by more frequent smaller epidemics that ultimately involve a larger portion of the population and higher seroprevalences.

Simulation studies on the role of stochastic events

The dengue models have been used to estimate the probability of an epidemic following a single introduction.[7] Obviously, any number of factors combine to determine the fate of introduced virus – temperature, herd immunity, virus and vector characteristics, to name a few. However, under conditions near transmission threshold, the outcome of an introduction is highly unpredictable for stochastic reasons. An interesting question could be: how receptive is a small village to a single introduction occurring at various times of the year? Would this be modified by titre of the virus, given the influence of titre on the probability of infection and EIP in the mosquito? Less ambitiously, we could frame the questions in terms of parameter sensitivity – if conditions are near threshold, would factors such as seasonality in mosquito abundance, size, and temperature be sufficiently influential against the backdrop of other factors to significantly alter the probability of an epidemic, and would we expect this to be substantially modified by the titre of introduced virus?

Simulation results for the eastern coastal region of Honduras indicate that, at a low titre (10^5 MID_{50}), seasonal changes in weather result in an almost three-fold difference in probability of an epidemic resulting from a single introduction (30%–35% in December and January vs. 80% in April–May). That is to say, a wintertime introduction is about one-third as likely to cause an epidemic as one occurring in the spring or summer. The results, while suggesting that many introductions into a naive population would be lost and not produce an epidemic, also indicate that a single introduction is capable of producing an epidemic at any time of the year. Simulations also indicate that introductions of high-titre virus more frequently lead to epidemics than introductions of the lower-titre type. Associated with the higher-titre virus is a reduction in magnitude of the role played by seasonal influences – summer introductions are only ca. 1.5 times more likely to cause an epidemic than wintertime introductions of the same virus. The difference between the ability of the two viruses to cause an epidemic is most pronounced during the cooler months when the high-titre virus is about two times more likely to result in an epidemic than introduction of the lower-titre type. These results are typical of others (unpublished), where different factors or combinations of factors become key regulatory factors under different conditions of weather, antibody presence, demographics, and mosquito characteristics.

CO-CIRCULATION OF MULTIPLE SEROTYPES

The current pandemic of dengue and DHF/DSS originated in the Pacific and South-East Asia in the 1940s, and has subsequently spread to the Americas and Africa. Today, most urban centres of South-East Asia and many in Central and South America are hyperendemic for dengue, frequently with all four serotypes circulating simultaneously.[40] Given the significance of sequential infections in developing serious illness via antibody-dependent enhancement, factors regulating or influencing the spatial and temporal distributions of dengue serotypes may be important in regulating or influencing the age-specific dynamics of infection and illness.[41]

An example of spatial and temporal variation in serotype abundance

Figures 3 and 4 are presented as examples of serotype variability on a country scale; the figures are based on ca. 1200 virus isolations from human sera in North and South Viet Nam between 1990 and 1999.[42] The data provide an indication of the relative frequency and dynamics of dengue serotypes in circulation over a ten-year period. These estimates are not necessarily unbiased or highly correlated with the real picture, considering the possible differences in virulence among serotypes and low number (<30–40) of isolations in several years. Keeping in mind the nature of the data, and that dengue activity is usually confined to roughly June–November in the North and is continuous but seasonal in the South,[43] what can be said regarding some of the possible factors influencing the dynamics of serotypes spatially and temporally?

Founder or stochastic effect

In some regions, the mix of serotypes found each year simply reflects the mix in other endemic areas.

It is likely that dengue virus is lost during the cool season in the North and is annually reintroduced from more southerly locations each year;[43] hence it is not unexpected that the mix of serotypes circulating in the North bares some correspondence to those in the South. A similar situation is reported for small relatively isolated Pacific islands that are too small to remain endemic; when virus does arrive, the introduced strain was usually active earlier on other larger islands.[33]

The influence of herd immunity on serotype abundance

Another factor influencing the distribution of serotypes is the nature of herd immunity. During the course of an epidemic not influenced by control efforts or cooling temperatures, R_o ultimately falls to less than one as a function of the rising proportion of immunes – increasingly, potentially infectious bites fall on refractory individuals and the epidemic dies out. The notion of herd immunity,

Figure 3. Circulation of dengue serotypes in North Viet Nam between 1990 and 1999 based on virus isolation from febrile patients.[42]

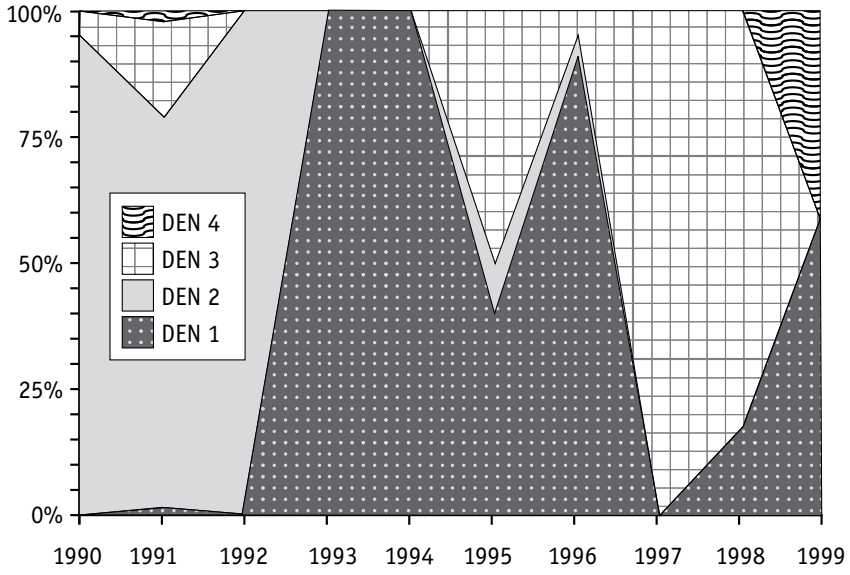


Figure 4. Circulation of dengue serotypes in South Viet Nam between 1990 and 1999 based on virus isolation from febrile patients.[42]

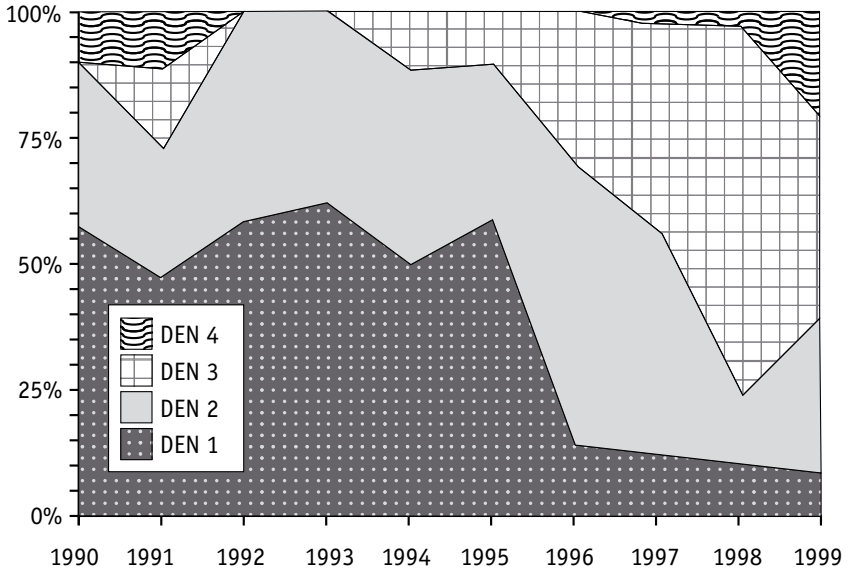
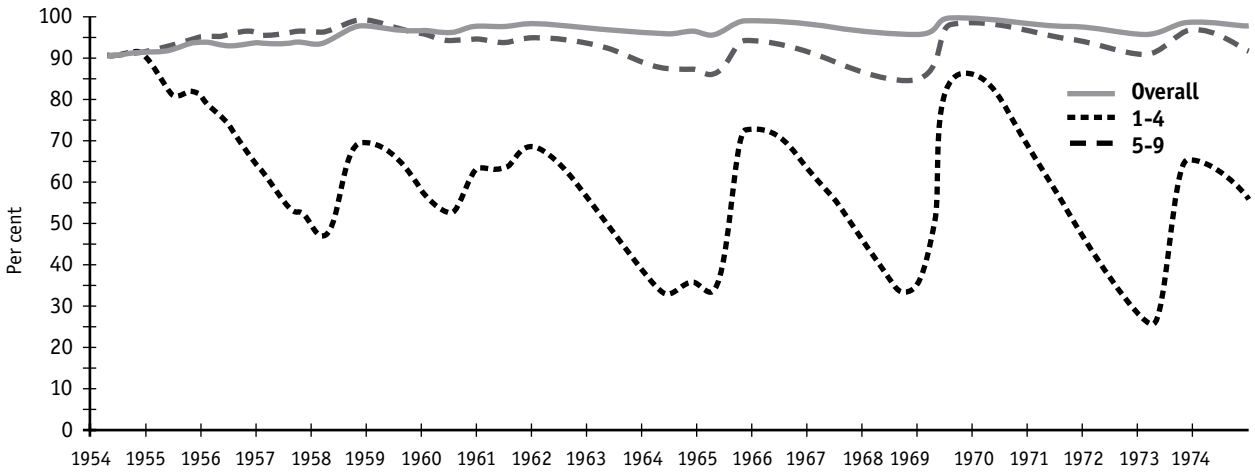


Figure 5. Output from DENSiM for a hypothetical site where dengue is endemic (see text for explanation).

The solid line is overall prevalence and mirrors the different age classes closely except for those of 1–4 and 5–9 years.



the proportion of individuals immune to a particular serotype of virus, therefore is a useful concept. In acute, virgin soil epidemics, such as the DEN-1 outbreak in Cuba of 1977–1979 where some 44.5% of the urban population experienced infection in a single year, the level of herd immunity was roughly identical with the prevalence of antibody in each age class.[44] However, in endemic areas where the norm is ongoing circulation of multiple serotypes, there is a general trend of increasing seropositivity with age. As a result, not only is the nature of illness and the age-specific distribution of serious illness a function of current and previous dengue activity, but the dynamics in abundance of dengue serotypes is a function of previous dengue activity through the proxy of herd immunity. Here past activity (or lack of it) can influence the innate R_o of the same serotype through the agency of herd immunity.

Figure 5 is a plot of output from DENSiM for a hypothetical site where dengue has historically been endemic and seroprevalence to all four serotypes is high. During the 20 years displayed, virus was not introduced from the outside but remained endemic in the ca. 150 000 people in the simulation. The ca. 3–4 year periodicity of epidemics is driven by waxing and waning herd immunity in the 0–9 year age classes. The volatility in prevalence of antibody in the 1–4 year age class, and to a lesser extent among the 5–9 year age class, reflects two sources. Reductions in prevalence through time come about by children moving from the 1–4 year class to the 5–9 year class, with replenishment from predominantly uninfected infants from the 0–1 year age class during years of low transmission. Prevalence in the 1–4 year age class goes up sharply in epidemic years because infection occurs in the age class and the recruits from the infants' class are more likely to already be positive in epidemic years. In this example of hyper-endemicity, the variability in seroprevalence directly influences abundance of the associated virus serotype. Note that in this example it is ultimately the human birth rate that entrains the 3–4 year epidemic cycle.

The interaction of different serotypes through the mechanism of heterologous immunity

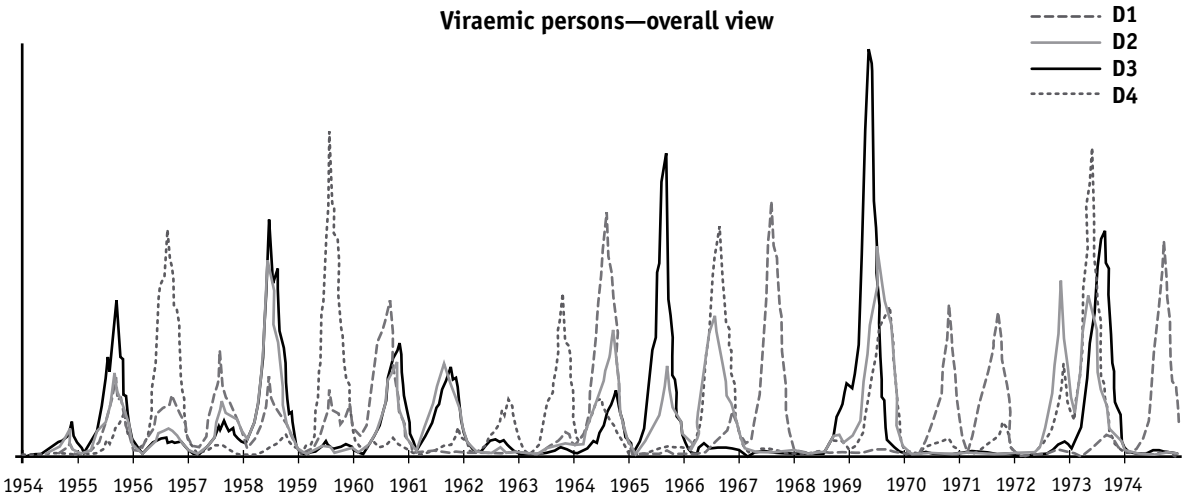
On the basis of simulation studies with DENSiM, abundances of the different serotypes can influence each other through the phenomenon of short-lived heterologous immunity following infection. In essence, an ongoing epidemic of a particular serotype temporally raises the effective herd immunity to the other three serotypes by producing heterologous and cross-reacting titres in those recently infected; once one epidemic is under way, it is somewhat less probable that a second epidemic (with similar force of infection) will begin. This is a factor in the commonly observed phenomenon of asynchrony of epidemics of different serotypes (figure 6). But regarding the question of relative abundance by serotype for any particular month, the proximate determinant of abundance of each virus type in mosquitoes is simply a linear function of the frequency of human infection of that type.

The potential influence of antibody-dependent enhancement on the dynamics and persistence of multiple serotypes of virus

Recently, another interesting hypothesis regarding the interaction of serotypes was made by

Figure 6. Output from DENSiM for hypothetical site where dengue has historically been endemic for all four serotypes.

Simulation begins with all age classes being 90% positive for antibody and an initial human population of 100 000; the annual population growth is ca. 3.2%. Virus was introduced only during the year preceding the results shown here, i.e. the results depict a situation where the viruses are not lost between epidemics.



Ferguson et al.[45] The authors note that antibody-dependent enhancement (ADE) of dengue infection involves cross-reactive antibodies from a previous infection that serve to facilitate virus replication within the host. They posit that the phenomenon of 'enhanced' infections in a subset of cells can result in a higher probability of transmission of the virus causing the secondary infection. Using a simple set of differential equations, they demonstrate that this linkage between serotypes via ADE can result in persistent and complex cyclical patterns in the relative abundance of serotypes of virus given the assumption regarding ADE leading to a change in transmission probabilities. The results of this study suggest that this phenomenon of linkage via ADE theoretically makes possible the co-existence of multiple serotypes, whereas without such linkages, one or more serotypes would be expected to be lost due to drift.

The recent work by Vaughn et al. has clearly demonstrated that peak viraemia is increased in at least some secondary infections in humans.[at] Given that virus titre is thought to influence both the probability of disseminated infection and the duration of EIP within the female mosquito, a hypothesis linking ADE and increased probability of transmission seems plausible.

Acknowledgment

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WORKING PAPER 6.2. **CONTROL OF DENGUE VECTORS: TOOLS AND STRATEGIES**

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INTRODUCTION

Dengue is a vector-borne disease of tropical and subtropical human populations, which occurs predominantly, but not only, in urban areas. The global increase in urbanization, such that the world's urban population of 1.7 billion in 1980 is expected to double by 2010 (UNPP, 2006), is likely to lead to an increase in dengue in the future. Control of peridomestic vectors is the only effective preventive measure currently available. With dengue occurring in a range of different geographic, socioeconomic and cultural contexts (high-density urban to lower-density periurban and rural situations), and with geographic variation in vector (sometimes two vectors) and host behaviour, one intervention strategy is very unlikely to suit all situations, and a range of control methods and strategies is needed.

Dengue is transmitted by *Aedes sp.* mosquitoes that breed in container habitats. The main vector, *Aedes aegypti*, is a cosmotropical species that proliferates in water containers in and around houses. Secondary vectors include *Ae. albopictus*, an important vector in south-east Asia and that has spread to the Americas, western Africa and the Mediterranean rim, *Ae. mediovittatus* in the Caribbean, and *Ae. polynesiensis* and *Ae. scutellaris* in the western Pacific region. Although zoonotic cycles involving monkeys occur in some forest areas of western Africa and south-eastern Asia, there is no evidence that these play any role in epidemics of human disease. Consequently, control can be directed at *Ae. aegypti* breeding and biting in the household and immediate vicinity. *Ae. aegypti* breeds in many types of household containers, such as water storage jars, drums, tanks and plant or flower containers. They do not fly far, dispersing probably no more than 100 m beyond the emergence location (Reiter et al., 1995; Muir & Kay, 1998; Honorio et al., 2003; Harrington et al., 2005) and are highly anthropophilic, rarely feeding on non-human hosts. Given these limits, control or indeed elimination of peridomestic vector populations might appear feasible, but experience has proven otherwise. Many eradication attempts have failed, for various reasons: vertical programmes were inefficient and unsustainable, outdoor space spraying was ineffective; larviciding was often rejected by communities and educational messages to the population were often unsuccessful (Slosek, 1986; Winch et al., 1991; Gubler & Clark, 1994; Parks & Lloyd, 2004). Today it is not unusual to find that more than 50% of houses in many endemic areas are infested with *Aedes* larvae, and the risk of epidemics is high (Nathan & Knudson, 1991; PAHO, 1994; WHO, 2000). Eradication of *Ae. aegypti* populations may be achievable, but it is rarely sustainable. The present aim is to control, to below certain threshold levels, rather than eliminate vector populations.

The previous UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (TDR) Scientific Working Group on Dengue (Scientific Working Group on Dengue, 2000) stated the need for the "development and evaluation of new tools to reduce mosquito populations, including source reduction" and "to increase evidence based vector control programmes and to support state-of-the-art research on human behaviour and behaviour change in relation to mosquito breeding". Partly in response to this call, a range of promising intervention tools are now ready for further investigation for prevention of oviposition or biting or suppression of vector populations, while developments in molecular genetic research have increased the likelihood that novel methods will be developed.

CONTROL OF IMMATURE STAGES

Vector control, either by elimination of breeding sites ('clean-up' campaigns) or by larviciding with insecticides, has led many dengue programmes in the past. Although still widely used and promoted, and successes have been achieved in many contexts, clean-up campaigns are limited by the level of compliance by the community and by the fact that often the most productive containers (i.e. those from which most *Ae. aegypti* emerge) cannot be disposed of or emptied (e.g. drinking-water stores). Larvicidal treatment of such water is possible. Only temephos, permethrin, Bti (see below) and pyriproxyfen are approved by WHO for use in drinking-water (Chavasse, 1997). The organophosphate temephos (Abate) has been most widely used and, although it is effective, acceptance levels are often low and coverage poor. Placing additives with persistent flavour into domestic water, particularly drinking-water is, understandably, often viewed with suspicion and not well accepted. Additionally, regular water usage or emptying of containers (often

encouraged in simultaneous 'clean-up' campaigns) can reduce or negate the intended effect. The situation would be improved, first, if attention could be limited to known productive, rather than all potential, breeding sites and secondly, if more acceptable larvicides were available. A major new multi-country study using pupal/demographic surveys has demonstrated that accurate identification of epidemiologically important breeding sites/containers is possible and should enable targeting of control (Focks & Alexander, 2006; Nathan et al., 2006). This is a very important development as, although initial surveys will have to be more intensive, the potential increase in effectiveness gained by directing control at productive containers is very significant. With this strategy in mind, we have looked at the potential for a range of new larval control tools.

Insect growth regulator (IGR) — pyriproxyfen

Pyriproxyfen is an insect juvenile-hormone analogue, which is active against many arthropods and which has been in use for agricultural pest control for about 15 years. It is extremely effective against mosquitoes and can prevent the emergence of *Ae. aegypti* at concentrations as low as 1 ppb or less, while extremely high concentrations do not inhibit oviposition (Itoh, 1994; Sihuincha et al., 2005). Treated water does not taste tainted. Interestingly, very low doses of pyriproxyfen can also sublethally affect adults by decreasing fecundity or fertility, and the contaminated adult female can transfer effective doses to any breeding site she subsequently visits (Itoh, 1994; Dell Chism & Apperson, 2003). New pyriproxyfen formulations can retain efficacy for 6 months (Seng et al., 2006), reducing the need for frequent reapplication, and new studies (Sihuincha et al., 2005) are indicating its high level of efficacy. Clearly further trials are now called for. Worryingly, however, this intervention was not accepted by communities in Mexico (Kroeger et al., 2006), suggesting that suspicion of domestic-water treatments for dengue control remains an obstacle to larval control. Moreover, since pyriproxyfen only prevents eclosion, larvae and pupae remain visibly active in breeding sites, conveying the false impression of a lack of efficacy and further compromising acceptance. A current trial in Thailand is investigating these areas further.

Predatory copepods

Various predatory *Mesocyclops spp.* (Crustacea; Eudecapoda) have been studied for their potential to control mosquito larvae. Two species in particular, *M. thermocyclopoides* and *M. aspericornis*, have proven effective against dengue vectors (Riviere et al., 1987; Brown et al., 1991; Kay et al., 1992; Marten et al., 1994; Mittal et al., 1997; Schaper 1999; Nam et al., 2000). However, although copepods can survive up to 6 months in containers, they are often lost when water is removed, containers are cleaned or (copepod) food is limited, and reintroduction may be necessary for sustainable control (Lardeux, 1992; Marten et al., 1994; Schaper, 1999; Chansang et al., 2004). However, in certain contexts *Mesocyclops* can be very effective. A large-scale vector-control programme using copepods and clean-up campaigns in Viet Nam successfully controlled dengue transmission for a number of years and, in this region at least, appeared to be sustainable (Nam et al., 1998; Kay et al., 2002; Nam et al., 2005). Attempts should now be made to investigate the potential of this method in other countries where vector-productive containers could sustain copepod populations, and where communities might be willing to accept such a (depending on their experience) novel intervention. Furthermore, the efficacy of this method might be improved by combining it with other compatible tools, such as fish (Lardeux, 1992) or other methods (see next section).

Bti toxins

The endotoxin produced by the entomopathogenic bacteria *Bacillus thuringiensis* var. *israelensis* (Bti) has high larvicidal activity in mosquitoes, but is non-toxic to important beneficial organisms. Various formulations (i.e. wettable powders, granules and briquettes) are effective, and newer slow-release long-lasting formulations may reduce the need for frequent reapplication. One recent field trial of slow-release long-lasting Bti products in Thailand demonstrated that the treatments could persist for 3 months in utility water containers in rural villages (Wiwat et al., personal communication).

Combination strategies have often yielded satisfactory results in terms of potency and long-term efficacy (Riviere et al., 1987; de Andrande & Modolo, 1991; Tietze et al., 1994; Neri-Barbosa et al., 1997). When a combination of *M. longisetus* and Bti, *Bacillus sphaericus*, or methoprene was evaluated (Tietze et al., 1994), the combined agents were better than either alone. The compatibility of the commercial formulation Vectobac 12AS with certain chemical insecticides for controlling *Aedes* larvae and adults was demonstrated in Malaysia (Seleena et al., 1999). A mixture of Vectobac 12AS and Actellic 50EC (pirimiphos-methyl) showed promise as a larvicide in the laboratory (Chung et al., 2001). These combinations clearly warrant follow-up. An experiment to integrate Bti with *Mesocyclops* and mosquito densovirus (*ATh*DNV; see below), conducted in semi-natural conditions, demonstrated that this complex

combination might also be effective (Wiwat et al., personal communication).

In Thailand it has been demonstrated that reductions of *Ae. aegypti* larvae of more than 90% could be achieved for 3-month periods with combined treatment of copepods and Bti, although supplementary food for the copepods (community-supplied products such as rice and leaves) was required (Kosiyachinda et al., 2003; Chansang et al., 2004). A pilot community-based intervention using integrated physical and biological control, including a combination of copepods and Bti, in eastern Thailand was successful and showed potential for expansion into other areas (Kittayapong et al., 2006).

Mosquito densonucleosis viruses

Densonucleosis viruses or densoviruses (DNVs) are invertebrate viruses in the genus *Brevidensovirus* of the family Parvoviridae (Bergoin & Tijssen, 2000). The virions of the DNVs contain a 4 kb single-stranded DNA genome with terminal hairpins packed in a non-enveloped particle (Bando et al., 1990; Berns et al., 1996). Five strains of *Aedes* densoviruses have been identified to date (Buchatsky, 1989; Jousset et al., 1993; Barreau et al., 1994, 1996; Kittayapong et al., 1999; Afanasiev & Carlson, 2003; Chen et al., 2004). Current experiments show that the efficiency in vector control could vary greatly depending on both viral strains and geographic origins of the mosquito vectors (Wiwat et al., personal communication). Application could involve either direct lethal effects on treated populations, like a biological insecticide, or shortening of adult lifespan, much as described below for *Wolbachia*. A recent finding has reported improved survival of *Ae. aegypti* larvae from 15% to 58% after infecting successive generations of mosquitoes with *ATh*DNV, the Thai strain densovirus (Roekring et al., 2006). Future research to study interactions between the pathogenic viruses and their mosquito hosts, with respect to resistance and mode of action, will generate useful data. Field trials should be encouraged in due course.

VECTOR CONTROL OF ADULT MOSQUITOES

In the past, control directed at adult mosquitoes has been limited to the use of ultra-low-volume (ULV) application of insecticides, usually by vehicle-mounted apparatus. There is controversy regarding the efficacy of this type of control, with a number of studies indicating that its effect is rarely, if ever, significant (Reiter & Gubler, 1997; Perich et al., 1990, 2000). This is likely to be partly the result of a failure of outdoor sprayed insecticide to reach indoor populations of mosquitoes and failures of vertical programmes to deliver at community level. Despite doubts about efficacy, such interventions remain the last resort in combating epidemics. However, new tools open the way to adult mosquito control at community level.

Insecticide-treated materials

Insecticide-treated materials (ITMs; typically deployed as insecticide-treated bednets [ITNs]) have proven highly effective in preventing diseases transmitted by nocturnally-active vectors. Their efficacy in controlling diurnally-active *Ae. aegypti* is now being evaluated. A cluster-randomized trial in Latin America has demonstrated that insecticide-treated window curtains and/or insecticide-treated domestic-water container covers can reduce dengue vector densities to low levels and potentially have an impact on dengue transmission (Kroeger et al., 2006). ITMs, particularly curtains, were well accepted and their efficacy reinforced by the sight of dead insects (cockroaches, houseflies and other pests, as well as mosquitoes) beneath the treated curtains. Importantly, a spill-over effect, whereby the intervention reduced vector populations in neighbouring control clusters, also occurred, such that houses without ITMs that were located close to treated houses were less likely to have infestations than those further away. Presumably, as with other vectors, the risk of a host-seeking mosquito contacting an ITM at some point during its frequent visits to houses to blood-feed, means that its life expectancy is reduced; in effect, the age structure of the vector population is altered and few individuals are likely to live long enough to become infective with dengue. This mass effect will reduce vectors throughout the community (as shown for ITMs and malaria vectors; Gimnig et al., 2003; Hawley et al., 2003) and is an excellent outcome, given that coverage with any intervention tool is always less than 100%, sometimes markedly so. New trials are now underway in Venezuela and Thailand to examine these interventions further and additional trials in yet more locations and contexts should be encouraged.

Indoor residual treatments are known to kill *Ae. aegypti*, although such methods have rarely been used, nor are they recommended today (WHO, 2006). Indeed, such is the strong endophagic/endophilic tendency of this vector (Christophers, 1960; Perich et al., 2000) that the use of insecticidal aerosol cans in the household can also markedly affect dengue transmission (Osaka et al., 1999). Could other 'indoor treatments' also work? The question of whether ITNs might also affect *Ae. aegypti* has also

been considered in a pilot study in Haiti (Wiwat et al., personal communication). Although not a fully controlled trial, results indicated that bednets reduced peridomestic dengue vector breeding (as measured by standard indices), and may have helped reduce seroconversion rates over 12 months. A community-wide effect was again observed, as dengue-vector breeding also appeared to be reduced in neighbouring control areas. As dengue vectors bite during the day, how could ITNs exert an effect? Either the presence of the insecticide reduced entry into houses by repelling incoming mosquitoes or, most likely given the community-level effect observed, the ITNs functioned as 'residually-treated resting surfaces' with which contact was made resulting in killing; an effect that one might expect to be enhanced inside the smaller houses typical in poorer districts. Effects on *Ae. aegypti* of ITNs deployed widely for malaria control have not been recorded despite the many trials that have been undertaken (presumably because routine sampling for nocturnal malaria vectors would not record *Ae. aegypti*), although low-level *Ae. aegypti* biting was reduced to zero after introduction of ITNs in a village in the Democratic Republic of the Congo (Karch et al., 1995). Sustainability of many interventions against all mosquito vectors is a problem in many contexts. However, use of ITNs is invariably higher in urban areas (Gimnig et al., 2005; Lindblade et al., 2005), precisely where dengue is the greatest problem. That such an appropriate and widespread intervention might also reduce dengue transmission should be evaluated.

In considering ITM-based strategies for dengue control, new tools primarily developed for malaria control can also have applications for dengue control. In the case of long-lasting insecticide-treated netting (LLIN), the netting is loaded with sufficient insecticide during manufacture to avoid the need for re-impregnation. Both LLIN window curtains and water-container covers are effective (Kroeger et al., 2006). The long-lasting formulation 'KO-Tab 1-2-3®', which can be applied in the community to any material, renders it as wash-resistant as LLIN (Yates et al., 2005).Treatment of the existing window curtains in a house might be possible and should be investigated, although loss in efficacy over time, resulting from the degradation of the insecticide by ultraviolet rays in sunlight may be a problem. Renewed interest from industry partners that perceive potential markets in this field, particularly for malaria control, is likely to result in new products to meet control needs in the coming years.

The mode of action of ITMs should be investigated: do they repel *Ae. aegypti* (curtains, bednets or jar covers), attract and kill them (during hostseeking or oviposition) or exert their effect in some 'passive' or as yet unknown way? Finally, as is always the case with insecticide-based strategies, resistance to existing insecticides is inevitable and the current global state of insecticide resistance in *Ae. aegypti* is worrying (see Hemingway, in the present report). This could be resolved by using alternatives to insecticides, as described in this article, by using different or by rotating insecticides—a real possibility, as some of the ITMs that are proving effective in dengue control can be used with insecticides other than the pyrethroids, which, because of their low mammalian toxicity have been first choice in the past. Eventually, new insecticides will be needed, an approach that is fundamental to the Gates-funded 'Innovative Vector Control Consortium'* which aims to develop a portfolio of chemical and technological tools suitable for vector control (Hemingway et al., 2006).

Lethal ovitraps

The ovitrap or oviposition trap was first recommended by WHO for surveillance of *Aedes* vectors (WHO, 1972; WHO, 1975), then modified to render it lethal to adults or larvae of *Ae. aegypti* (Chan et al., 1973; Chan, 1977; Zeichner & Perich, 1999; Perich et al., 2003; Sithiprasasna et al., 2003; Kittayapong et al., 2006). Notably, they were used to eradicate *Aedes* vectors from Singapore International Airport (Chan, 1973). The autocidal ovitrap was designed and developed for the control of *Aedes* vectors in urban areas with a high density of *Aedes* and a high incidence of dengue haemorrhagic fever in Singapore (Chan et al., 1977). In principle, ovitraps could kill adult mosquitoes if the ovistrip was treated with insecticide (Zeichner & Perich, 1999) or destroy progeny by using fine nylon netting for trapping the larvae (Lok et al., 1977). In Brazil, lethal ovitraps with deltamethrin-treated ovistrips killed 89% of *Ae. aegypti* adults and produced more than 99% larval mortality during 1-month field trials (Perich et al., 2003). The advantages of lethal ovitraps for controlling *Aedes* vectors include their simplicity, their specificity for and effectiveness against container breeders like *Ae. aegypti* and their potential for integration with other chemical or biological control methodologies. Studies are still at an early stage (Perich et al., 2003; Sithiprasasna et al., 2003) and should be encouraged.

* Innovative Vector Control Consortium: http://www.ivcc.com/

THE FUTURE: STRATEGIES BASED ON GENETIC MODIFICATION

In the search for novel tools for vector control, genetic modification of *Aedes* to resist infection with dengue virus is one of the most seductive long-term approaches. Although some methodology has already been shown to be effective in the laboratory (Kokoza et al., 2000), the challenges here are technical (and potentially independent of any kind of community participation in vector-control activities, although initial acceptance of genetically-modified mosquitoes may not always be certain) requiring development of mosquito lines resistant to or unable to transmit dengue and replacement of the natural populations with these non-vector lines.

A major obstacle however, has been the slow development of strong driving systems to deliver dengue virus-resistant genes into natural vector populations (Scott et al., 2002), although much research has been devoted to identifying candidates such as transposable elements, meiotic drive or endosymbiotic *Wolbachia* bacteria (Braig & Yan, 2001; James, 2005; Sinkins & Gould, 2006). Transposable elements have been shown to be efficient for transformation of mosquitoes using an external transposase source, but further research is needed to investigate the autonomous transposable elements in which the effector gene is linked to the source of transposase (Christophides, 2005). The use of meiotic drive in genetic control also requires a greater understanding of the molecular mechanism, especially in mosquitoes. One of the proposed gene driving systems which has potential is the use of maternally inherited *Wolbachia* endosymbionts (Sinkins & Godfrey, 2004). An advantage is the possibility for repeated spread and invasion of naturally *Wolbachia*-infected populations via superinfections of different *Wolbachia* strains. Much research is still needed.

Development of strategies based on genetic modification of *Ae. aegypti*

One of the global collaborative Grand Challenges in Global Health (GCGH)-funded projects aims to use genetic-based strategies to prevent *Ae. aegypti* from transmitting dengue viruses either by reducing densities of mosquito populations or by eliminating their ability to transmit dengue viruses (James, personal communication). The main challenges and objectives of this project are summarized in three topics: first, the effector genes for population replacement and reduction will be optimized; secondly, safe and efficient drive systems for introgressing effector genes into mosquito populations will be developed; and lastly, a field site for genetic control trials will be established in a suitable developing country. Different directions are being pursued. Two approaches aim to reduce or eliminate natural populations of *Aedes* vectors: densoviruses could be developed as biological control agents of *Ae. aegypti* larvae (Buchatsky, 1989), while release of insects carrying dominant lethal mutations (RIDL) are designed to reduce or eliminate mosquito populations, especially those that are infected with dengue viruses (Heinrich & Scott, 2000; Thomas et al., 2000; Horn & Wimmer, 2003). Other methods—synthetic transposable elements (TEs) (Adelman et al., 2002), meiotic drive (Mori et al., 2004) and underdominance (Davis et al., 2001)—aim to modify vector populations by introgressing genes that eliminate vector competence. These methods are currently at various stages in development and readiness for field-testing.

A different approach to is being taken via the development of a novel gene-based sterile insect technology (SIT) whereby genetically altered, rather than irradiated, males are released into the environment to mate with wild females, thereby reducing population numbers and ultimately preventing the transmission of disease (Coleman & Alphey, 2004). This strategy is very well advanced and likely to lead to field trials in the very near future (Alphey, personal communication).

Development of *Aedes* vectors with a short lifespan

Another GCGH project aims to modify the age structure of the population of dengue vectors such that lifespan is reduced and mosquitoes die before reaching the age when they could transmit dengue (O'Neill et al., personal communication). The strategy is based on introducing endosymbiotic *Wolbachia* bacteria into wild vector populations, these bacteria being capable of significantly shortening the adult mosquito lifespan (Min & Benzer, 1997; McGraw et al., 2002). These bacteria are present naturally in most insects and widespread in mosquitoes (Werren et al., 1995; Kittayapong et al., 2000). Both single and double strains of *Wolbachia*, naturally found in *Ae. albopictus*, have already been successfully transferred into *Ae. aegypti* (Xi et al., 2005; Ruang-areerate & Kittayapong, 2006). Dengue viruses need an extrinsic incubation period of about 12–14 days in the vector before being transmitted into humans; the 'Popcorn' strain of *Wolbachia* could shorten the life of its insect hosts to about half this period. Theoretical models with realistic parameter estimates suggest that an intervention using release of Popcorn-transinfected vector lines could reduce

dengue transmission by 90–100% (Brownstein et al., 2003; Rasgon et al., 2003).

The primary objective of this project is to generate stable lines of *Ae. aegypti* and *Ae. albopictus* that are infected with the Popcorn *Wolbachia*. Once established, these *Wolbachia*-transinfected lines could be characterized and tested in experimental cages to determine virulence, transovarial transmission, effect on host age and potential to spread in natural vector populations via cytoplasmic incompatibility. The project is ongoing and, if successful, a field trial in large, confined field cages will be attempted in an endemic country. Importantly, this strategy has the potential to be implemented without the need to release transgenic mosquitoes into the environment.

Any studies on post-genomic relationships between mosquito hosts and their endosymbionts will benefit further development of genetic strategies to control dengue vectors. Application of the population-replacement approach in field situations will require detailed understanding of *Wolbachia*-transfected laboratory-generated strains compared with the naturally uninfected strains of dengue vectors in term of their expressed physiological and behavioural parameters at the post-genomic level. The recently-completed sequencing of the *Ae. aegypti* genome should facilitate identification of new effector genes, as well as potential gene-driving mechanisms.** Future research should include studies related to novel genetic markers to investigate gene flow and genetic diversity among different geographic populations, which will be crucial to the success of population replacement strategies. Also essential, in due course, will be the fostering of public understanding and support of such tools.

PRIORITIES AND OPPORTUNITIES FOR RESEARCH

A number of major initiatives have brought together scientists from many disciplines. Current programmes include the WHO/TDR/International Development Research Centre (IDRC) Research Initiative on Eco-Bio-Social Research on Dengue in Asia, a research initiative aimed at improving the prevention of dengue by understanding its multifarious determinants and how they affect transmission (and therefore influence the likelihood of successful control) at community level. The European Union is funding two major multidisciplinary studies on dengue, one of which, 'DENCO', includes two large cluster-randomized trials of ITMs and pyriproxyfen. The Bill & Melinda Gates Foundation have funded a range of initiatives in vector-borne diseases, three of which at least directly target dengue (see above).

But many areas still require work. In addition to those directions already mentioned, further studies are required to confirm the efficacy, acceptance, cost–effectiveness and sustainability of ITMs in controlled trials; trials of slow-release formulations of Bti and pyriproxyfen; densovirus trials; the efficacy of tools against *Ae. albopictus* should be investigated wherever this is an important vector; the long-term effects of interventions on mosquito populations and the long-term effects and benefits to communities should also be investigated.

Ideal tools should be 'user-friendly', requiring little additional work or behaviour change by householders. They should be affordable, safe and effective in reducing vector densities below threshold levels estimated by the 'pupae per person index'; indeed, as might be the case with insecticide-treated curtains in many societies, they could even be viewed as desirable by householders. The ideal situation that we must strive for is to have a suite of proven effective, safe and environmentally friendly tools available for intervention, from which the most appropriate tool or combination of tools can be selected to suit the specific biological and cultural needs of each community.

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** A special issue of *Science* will be devoted to the *Aedes* genome in early 2007.

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WORKING PAPER 6.3. INSECTICIDE RESISTANCE IN *AEDES AEGYPTI*

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INTRODUCTION

Insecticide resistance in the vector *Aedes aegypti* is an important but under-researched and poorly understood phenomenon. Several early reports of DDT resistance, in the 1960s to 1980s, reported cross-resistance between DDT and pyrethroids. Later literature suggests that organophosphate resistance is also developing in some areas. The impact of this resistance on operational activities such as larviciding and space spraying is largely unknown.

RESISTANCE DISTRIBUTION

Resistant populations of *Ae. aegypti* have been detected in several countries throughout the geographical range of this species and, in some areas, the evolution of insecticide resistance has been linked to failure of the dengue control programme. However there has been no systematic review of the resistance status or of the impact of resistance on insecticide-based vector control activities. The bulk of the data available are from simple WHO susceptibility assays using insecticide impregnated papers; very little is known about the molecular or biochemical basis of this resistance and yet such information is needed to identify the origin of resistance and develop strategies to reduce the spread and minimize the impact of insecticide resistance mutations.

THE BIOCHEMICAL BASIS OF RESISTANCE IN *AEDES*

There are two major routes by which insecticide resistance develops. The insect can either change the speed at which the insecticide is detoxified or it can alter the target site in order to reduce sensitivity to poisoning. Both types of resistance have been reported in *Aedes*. A number of simple biochemical and molecular assays can be used to ascertain which form of resistance occurs in any individual; further work then needs to be undertaken on the implicated metabolic class of enzymes or nervous system target site.

Three enzyme families are primarily implicated in effecting increased levels of insecticide degradation: the cytochrome P450 monooxygenase (P450), glutathione transferase, and esterase enzyme families, which catalyse a wide range of detoxification reactions. These enzymes provide the first line of enzymatic defence against xenobiotics in most organisms; the different classes are large and diverse in function, and consist of mixtures of highly specialized enzymes, often with specific substrates and strictly regulated expression profiles, and more generalist, ubiquitously expressed enzymes. Many insect species show an amazing diversity of detoxification enzymes. As insect genomes have been sequenced, and the detoxification genes annotated, it has become apparent that these gene families are evolving very rapidly and that each insect has a unique complement of detoxification genes, with very few orthologs across insect species.

In *Ae. aegypti*, the best understood of the metabolic enzyme families are the glutathione S-transferases. Grant and Hammock in the 1980s[1] looked in detail at a DDT-resistant strain of *Ae. aegypti*; the resistance was DDT specific and due to an elevation in glutathione S-transferase (GST) activity. They isolated a single up-regulated GST from the strain. Groups in Liverpool and Thailand took a more holistic approach to looking at this enzyme class and their pioneering work was later complemented and extended when the draft *Ae. aegypti* genome sequence became searchable.

Data from the draft genome sequence show that there has been no expansion of the GST family in *Ae. aegypti*. Indeed mosquitoes have considerably fewer GST genes than *Drosophila*, although this deficit is partially rectified by alternative splicing of two mosquito GST genes, which increases the number of *Ae. aegypti* GST transcripts by three, from 26 to 29. Each of the GST classes found in *An. gambiae* is represented in *Ae. aegypti,* including the two classes which so far appear to be unique to mosquitoes. Over half of the GSTs belong to two insect-specific classes, the Delta and Epsilon classes, which include the vast majority of GST enzymes with a defined role in insecticide metabolism.

MOLECULAR ANALYSIS OF TARGET SITE RESISTANCE

The clade of esterases associated with organophosphate (OP) resistance is the actetylcholinesterase (ace) clade. These esterases provide the target sites for both OP and carbamate insecticides; the toxins act as irreversible inhibitors of the enzymes, blocking hydrolysis of the neurotransmitter acetylcholine. Mutations in ace can reduce the binding of insecticides, resulting in resistance. As in *An. gambiae, Ae.*

aegypti contains two ace genes. Mutations in ace-1 have been associated with resistance to insecticides in other mosquitoes, but no such mutations have been reported in *Ae. aegypti* to date.

NEW INITIATIVES

Research in this area received a boost in the last 12 months with the provision of funding for the Innovative Vector Control Consortium from the Bill and Melinda Gates Foundation. This Consortium is supporting three large-scale projects that should positively impact on dengue control. The team, which involves staff from Colorado State University, the University of California Davis, and the Liverpool School of Tropical Medicine, together with collaborators in many parts of the world, is developing:

- A dengue decision support system to help operational staff at municipality, district or country level to make rational decisions on where, when and how to implement vector control.
- Improved threshold modelling of dengue vector control to help estimate the level of control that will need to be achieved operationally to effect control in different epidemiological settings (this is reported in more detail in Focks and Barrera, working paper 6.1).
- Simpler and more accurate methods for operationally monitoring insecticide resistance in *Ae. aegypti* in the field.

Extensive work has already been undertaken to underpin this latter project, building on work initiated in Liverpool and funded by the Wellcome Trust and others. The sequencing of the *Ae. aegypti* genome, and the development of a robust microarray platform for detoxification genes, have greatly facilitated the monitoring of insecticide-based control programmes and will enhance our ability to control this major disease vector in future. Searches of the draft *Ae. aegypti* genome sequence, and comparison with other insects, have revealed that *Ae. aegypti* has far more potential detoxification genes than any other insect studied at the genome level to date.

Why *Ae. aegypti* has such an abundance of detoxification genes compared to other insect species is unknown. The approximately one third higher gene count in *Ae. aegypti* than in *An. gambiae* cannot be readily accounted for by differential exposure to xenobiotics. Both species have a preference for breeding in clean water (as opposed to *Culex* mosquitoes, which readily breed in water heavily contaminated with organic material), both are highly anthropophilic and hence exposed to man-made pollutants, and both are frequently targeted with insecticides.

GROWING EVIDENCE OF RESISTANCE INVOLVING CYTOCHROME P450S

Ae. aegypti contains a total of 158 full length, putatively catalytic P450 genes. This represents an expansion of approximately 55% compared to *Anopheles gambiae*, and 86% compared to *Drosophila melanogaster*. Several large clusters of P450s are found in the *Ae. aegypti* genome, the largest being a cluster of 18 CYP6 genes and a cluster of 16 CYP9 genes.

A total of thirty-seven CYP9 genes are present in the *Ae. aegypti* genome compared with just eight in *An. gambiae*. Why the CYP9 family is so abundant in *Ae. aegypti* is unclear but a large subset of these genes are over-expressed in one or more insecticide-resistant strains, suggesting that this recent expansion may at least partially reflect an adaptation to insecticide exposure.

THE POTENTIAL ROLE OF ESTERASES

The lack of clear orthology between *Anopheles* and *Aedes* α-esterases suggests that this is a rapidly evolving enzyme group. Alpha-esterases in many other species are involved in metabolic resistance to insecticides; these include the *Lucilia cuprina* α-E7 and the *Culex quinquefasciatus* 'α' and 'β' genes (named according to substrate specificity as opposed to phylogenetic relationships). These two *Culex* genes are arranged in a head-to-head orientation, and amplification of one or both of them is a major cause of OP resistance in *Culex* populations worldwide. Interestingly, in contrast to the rapid radiation of other α-esterases, the two genes are well conserved across *Culex, Anopheles* and *Aedes*, and the head-to-head orientation is maintained in all species. It remains to be seen whether OP resistance in *Ae. aegypti* (reported by Mourya[2]) is associated with amplification of this genomic locus.

WHICH GENES ARE ASSOCIATED WITH INSECTICIDE RESISTANCE?

A small-scale microarray, the *Aedes Detox Chip*, containing unique 70mer probes for 204 of the 233 *Ae. aegypti* detoxification genes, has been constructed in Liverpool and used to compare expression profiles in susceptible and resistant populations. Twenty-five genes over-expressed in a resistant strain from Thailand and 14 over-expressed in a resistant strain from Mexico. Five of these genes, three CYP9 P450s and two Epsilon GSTs, were over-expressed in both resistant strains. Results of this preliminary analysis will be reported in *Science* in 2007.

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Annex 7
WORKING PAPERS: Scientific Working Group on Dengue

SURVEILLANCE AND DELIVERY ISSUES

WORKING PAPER 7.1. DENGUE RESEARCH NEEDS RELATED TO SURVEILLANCE AND EMERGENCY RESPONSE

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INTRODUCTION

Dengue fever/dengue hemorrhagic fever (DF/DHF) is a disease that is endemic in the tropics, has re-emerged to become the most common and most important mosquito-borne viral disease in the world. The current trend is a 4- to 6-yearly cycle of dengue epidemics, with each cycle becoming larger in magnitude (Gubler, 2004). In the absence of an approved vaccine, prevention of viral transmission through public-health measures directed at controlling the density of the vector mosquito population remains the only viable preventive strategy. Effective prevention depends on a well-planned and operated public-health, laboratory-based surveillance programme (Gubler, 1989; Gubler & Casta-Velez, 1991; Rigau-Perez & Gubler, 1997).

A primary goal of public health surveillance in dengue is to monitor transmission to facilitate prevention of the occurrence and spread of disease (Rigau-Perez & Gubler, 1997). Other goals for surveillance include defining disease severity, determining the cost–effectiveness of public-health prevention programmes, and estimating the burden of disease in the community (Teutsch, 2000). The ideal surveillance programme should thus be able to monitor dengue cases accurately and predict impending epidemics from a background of endemic disease and trigger the necessary preventive measures.

RECENT RESEARCH FINDINGS

In recent years, new findings have shed light on factors, other than incomplete immunity, that contribute to epidemic transmission for all four dengue virus serotypes.

Viral factors

Phylogenetic studies have yielded interesting insights into the selection and evolution of the dengue viruses, both temporal and geographical (Lewis et al., 1993; Rico-Hesse et al., 1998; Twiddy et al., 2002; Zhang et al., 2006; Imrie et al., 2006). Such studies may also enable us to gain a better understanding of the viral factors that contribute to dengue virus virulence and epidemic potential (Gubler et al., 1978; Gubler et al., 1981; Bennett et al., 2003; Myat Thu et al., 2005). However, although current technology allows us to monitor genetic changes in viruses, the biological effect of these mutations has yet to be elucidated. It is often difficult to attribute genetic variation to specific phenotypic expression, since dengue epidemics are associated with a host of factors that could confound the analysis, and there is no good animal model for dengue. Nonetheless, further investigations in this field may yield fruitful results as genetic changes are likely to contribute to increased or decreased viral fitness and thus epidemic potential and virulence. This includes infectivity and ability to replicate in humans and mosquitoes, thus resulting in increased or decreased transmission. Genetic change in the virus could also influence disease severity, and thus result in more or less clinically overt disease. A critical area of research, therefore, is to determine the influence of viral factors on disease transmission dynamics and disease severity.

Vector control

Various reports have shown that the inclusion of community participation along with the use of old and new vector-control tools (Chang et al., 2006; Kroeger et al., 2006) as well as biological control (Kay & Nam, 2005; Nam et al., 2005) have had positive effects on preventing disease transmission. However, sustainability remains a problem, and there is a need to establish proactive, laboratory-based disease- and vector-surveillance programmes that provide a trigger for intensified vector control to prevent epidemic transmission.

Experience gained in Singapore suggests that keeping the density of the vector population below a threshold for epidemic transmission is a moving target. Lowered herd immunity after implementation of effective control measures may paradoxically lead to a rise in the number of cases of dengue, which in turn, requires more intensive vector-control measures to prevent an epidemic (Chan, 1985; Goh, 1995; Ooi et al., 2006). Identification of predictive entomological thresholds or markers for epidemic dengue, in combination with population susceptibility would thus be a useful area of research. In addition,

experience gained recently in Viet Nam (Nam & Kay, 2005) reinforces the lessons learned decades ago in Singapore by Chan (1967), that without community involvement, vector control cannot be sustained. Subsequent experience in Singapore shows that sustainability of the vector-control programme is challenging, despite continued public education and law enforcement.

While the role of *Aedes aegypti* is obvious, the role of *Ae. albopictus* in maintaining dengue endemicity is less clear. Although *Ae. albopictus* is an excellent host and experimental vector for dengue viruses (Gubler & Rosen, 1976; Rosen, et al., 1985), it has not been frequently associated with epidemic dengue (Gubler, 2004). The reason for this is thought to be related to this species' ecology and blood-meal seeking behaviour; *Ae. albopictus* has a broader host range, and usually bites only once in obtaining a blood meal. However, in most countries where dengue is endemic, both species of vectors can be found. Furthermore, in many places such as Singapore, *Ae. albopictus* outnumber *Ae. aegypti* and are more widespread geographically. Although *Ae. albopictus* may not be an efficient vector for epidemic dengue, it may play an important role in endemic transmission, maintaining dengue viruses in the population until the population immunity is sufficiently lowered and the *Ae. aegypti* population is sufficiently dense to support epidemic transmission (Rudnick, 1965; Gubler, 1987, 2004). Studies on the role that *Ae. albopictus* plays in dengue transmission may thus be useful in our understanding of the epidemiology of dengue disease.

Population immunity

Several reports of large-scale serological surveys have been published in the last 5 years (Ooi et al., 2001; Darcy et al., 2001; Hayes et al., 2003; Tuntaprasart et al., 2003; Yamashiro et al., 2004; Thai et al., 2005; Van Benthem et al., 2005; Balmaseda et al., 2006). Although such studies may aid understanding of overall dengue activity in the area, few have attempted to use these data to guide vector-control operations. Serological surveys could be useful in elucidating the roles played by *Ae. aegypti* and *Ae. albopictus* during epidemic and inter-epidemic periods. Such a study would be entirely feasible in places like Singapore where the geographical distribution of *Ae. aegypti* is different from that of *Ae. albopictus*. With a combination of active virological and entomological surveillance, such a study may improve our understanding on the dynamics of dengue transmission and how these factors in turn contribute to endemic versus epidemic transmission.

SURVEILLANCE SYSTEMS

A common theme that appears in reviews of the areas where research on dengue is needed is that of surveillance. Here, the literature suggests that much could yet be done to improve on the sensitivity and specificity of our surveillance programmes (Gubler & Casta-Valez, 1991; Rigau-Perez & Gubler, 1997; Gubler, 2002). Most countries continue to monitor dengue cases by using a passive surveillance approach. An update of the table first published by Gubler in 2002 (table 1) subjectively summarizes the surveillance systems in countries where dengue is endemic. Passive surveillance relies on disease notification by health-care professionals who have a duty to report all suspected cases to public health authorities. However, passive surveillance systems are uniformly insensitive because of the low index of suspicion for dengue during inter-epidemic periods (Gubler, 1989; Rigau-Perez & Gubler, 1997).

Limitation of passive surveillance

Two main problems are encountered in passive surveillance for dengue. These are:

Dengue infection leads to a wide range of disease manifestations

Dengue infection results in a spectrum of clinical outcomes: completely asymptomatic, undifferentiated viral syndrome, DF, DHF, dengue shock syndrome, and other severe manifestations such as neurotropic disease and hepatic failure (George & Lum, 1997). Passive surveillance using case definitions lacks specificity since many other infectious diseases, such as influenza, chikungunya fever, the viral haemorrhagic fevers, enterovirus infections, leptospirosis, malaria, typhoid fever, etc., all present with symptoms and signs that are similar to those seen in patients with dengue in the acute phase of illness (Halstead, 1997; George & Lum, 1997).

The use of passive surveillance alone also ignores patients who present with undifferentiated febrile illness or viral syndrome. This group of patients may represent a large proportion of those with symptomatic dengue infection, depending on the age of the patient and the strain of infecting virus (Hoang et al., 2006). Any attempt to carry out passive surveillance among this group of cases will not be feasible. However, mild viral syndrome may be of particular use in monitoring dengue transmission during inter-epidemic periods when the incidence of classical DF and DHF is low (Gubler, 1998; Gubler & Casta-Valez, 1991). In countries where dengue circulates hyperendemically, it is likely that emergence of genetic variants with greater epidemic potential may

Table 1. Surveillance capabilities in the main countries where dengue fever or dengue haemorrhagic fever is endemic

Country/ location	Surveillance			Laboratory capability		Epidemic prediction
	Passive		Active			
	DF	DHF	DF/DHF	Serology	Virology	
WHO South-East Asia Region						
Bangladesh	-	++	-	+	+	-
India	+	+	-	+	+	-
Indonesia	-	+++	-	+	+[a]	-
Maldives	-	++	-	-	-	-
Myanmar	-	++	-	+	+	-
Sri Lanka	-	++	-	+	+	-
Thailand	-	+++	-	++	++[a]	-
WHO Western Pacific Region						
Australia	+++	+++	++	+++	+++	++
Cambodia	+	++	-	+++	++	-
China	-	+	-	+	+	-
Lao People's Democratic Republic	+	+	-	-	-	-
Malaysia	++	+++	+	+++	+++	+
New Caledonia	++	++	-	+++	+++	-
Other South Pacific islands	+	+	-	-	-	-
Philippines	-	+	-	++	+	-
Singapore	+++	+++	+	+++	+++	+
Tahiti	++	++	-	+++	+++	-
Viet Nam	-	+++	-	++	+++	-
WHO Region of the Americas						
Argentina	+	+	-	++	++	-
Barbados	+	+	-	+	+	-
Belize	+	+	-	+	+	-
Bolivia	+	+	-	+	+	-
Brazil	++	++	+	+++	+++	+
Colombia	+	+	-	++	++	-
Costa Rica	+	+	-	++	++	-
Cuba	+++	+++	+	+++	+++	+
Dominican Republic	+	+	-	+	+	-
Ecuador	+	+	-	+	+	-
El Salvador	+	+	-	+	+	-
French Guiana	+	+	-	+	+	-
Grenada	+	+	-	+	+	-
Guatemala	+	+	-	+	+	-
Haiti	-	-	-	-	-	-
Honduras	+	+	-	+	+	-
Jamaica	+	+	-	+	+	-
Lesser Antilles	+	+	-	+	+	-
Mexico	++	++	-	++	++	-
Nicaragua	+	++	++	+++	+++	-
Panama	+	+	-	-		-

(continued on next page)

Table 1 (continued)

Country/ location	Surveillance			Laboratory capability		Epidemic prediction
	Passive		Active			
	DF	DHF	DF/DHF	Serology	Virology	
Paraguay	+	+	-	-		-
Peru	+	+	-	-		-
Puerto Rico	++	++	-	+	+	-
Suriname	+	+	-	-		-
Trinidad	+	+	-	-		-
United States of America	+	+	-	+++	+++	-
Uruguay	+	+	-	-		-
Venezuela	++	++	-	++	++	-
WHO African/Eastern Mediterranean Regions						
Kenya	-	-	-	+	+	-
Senegal	-	-	-	+	+	-
Other African countries	-	-	-	-	-	-
Pakistan	-	-	-	-	-	-
Saudi Arabia	+	+	-	+	+	-
Others						
Province of Taiwan (China)	+++	+++	+	+++	+++	+

Modified from Gubler (2002), with permission from SEARO/WPRO *Dengue Bulletin*.

DF, dengue fever; DHF, dengue haemorrhagic fever

The efficacy of the surveillance system and laboratory capability is rated as follows:
- does not exist ; + exists; ++ good; +++ best.

[a] Does not include United States military, Centers for Disease Control, Institute Pasteur or WHO laboratories.

be partially responsible for the cyclical outbreaks (Gubler et al., 1979; Gubler, 1987; Harris et al., 2000; Gubler, 2004) since certain viral clades appear to be more closely associated with increased transmission and severe disease outcomes (Rico-Hesse et al., 1997; Wang et al., 2000; Bennett et al., 2003; Bennett et al., 2006). Virological surveillance on cases that present with mild viral syndrome may yield such pre-epidemic isolates for comparative analysis. Although more work will need to be done before such data can be used for epidemic prediction, the key to understanding dengue epidemiology lies in better virological surveillance during the inter-epidemic periods (Gubler, 1989; Gubler & Casta-Valez, 1991; Gubler, 2004).

Variation in the case definitions used

The usefulness of the existing scheme for the classification of dengue and case definitions established according to the WHO guidelines has also come under scrutiny (Sumarmo et al., 1983; Deen et al., 2006). Experiences from various parts of the world suggest that the usefulness of the case definition is not universal (Sumarmo et al., 1983; Dietz et al., 1990; Harris et al., 2000; Balmaseda et al., 2005). Perhaps more importantly, the WHO case definition underestimates the number of cases of severe dengue among adults (Hammond et al., 2005). This is a problem that needs to be addressed as dengue infection among travellers (Wilder-Smith & Schwartz, 2005) and even in endemic countries like Singapore, primarily affects the adult population (Ooi et al., 2001; Ooi et al., 2006). Notwithstanding the current debate over the WHO case definition, there is also no consistency in the way these definitions are applied between countries where dengue is endemic. Different countries classify DF/DHF differently, and there is variation in the types of dengue cases that are included in surveillance reports, countries adopting different criteria for classifying dengue cases (Gubler, 2002; Deen et al., 2006). Some countries report only DHF while others include DF in their surveillance (Gubler, 2002). The existence of all these different practices contributes to underestimation of the true extent of dengue transmission and limits the ability to compare surveillance data among countries and regions.

WHO and others have advocated active surveillance since the 1980s (Gubler, 1989; PAHO, 1994; WHO, 1999). As previously recommended, virological surveillance should be conducted on patients that present with nonspecific viral syndrome, classical DF, with haemorrhagic or neurological manifestation and on all patients with a fatal outcome following viral prodrome (Gubler et al., 1979; Gubler, 1989; Gubler & Casta-Valez, 1991; Rigau-Perez & Gubler, 1997; Gubler, 1998). This approach, using sentinel physicians, clinics, and hospitals, would result in a more comprehensive surveillance for the transmission of dengue virus in the population. Yet, in southeast Asia where DF/DHF epidemics are reported every 3 to 6 years, only Malaysia and Singapore have an adequate laboratory capacity (Table 1). Most other countries continue to rely on passive surveillance systems for DHF alone.

The long experience with dengue surveillance and vector control in Singapore has recently been reviewed (Ooi et al., 2006). One of the lessons learned is the need for surveillance and vector control to be carried out at the regional level (Ooi et al., 2006). If it is not, countries that attempt to prevent this viral disease are doomed to failure owing to re-importation of both virus and vector because of the rising trend in global trade and travel.

In the Americas, proportionately more countries report both DF and DHF, although good laboratory support is still only available in Cuba, Brazil, Puerto Rico, Nicaragua, and the USA (Table 1). However, few countries carry out active surveillance for dengue disease. This is despite the efforts of PAHO in the 1980s and 1990s to encourage Member States to develop plans for disease prevention and control of DF/DHF (Gubler, 2005; Panagos et al., 2005).

The situation in the Pacific has not changed since 2002; only Australia, Tahiti and New Caledonia have good laboratory support for surveillance, although active surveillance with epidemic prediction is carried out only in the state of Queensland, Australia. This north-eastern state remains prone to dengue outbreaks (Hanna et al., 2006), although very active vector surveillance and control is in place to complement the existing epidemiological surveillance (Montgomery & Ritchie, 2002; Ritchie et al., 2004).

To establish an active, laboratory-based surveillance system, coupled with effective community-based, integrated vector control requires both the necessary public funds and political will. Unfortunately, most countries where dengue is endemic have developing economies, and resources that could be channelled to prevention of disease transmission have been directed to other more highly visible public-health programmes. Although, the benefits derived from an effective public-health approach to prevention and control (Gubler and Casta-Velez, 1991) are significant, many countries have preferred to adopt a spend-only-when-needed approach to vector control. This approach is often too little, too late, since most emergency controls are only implemented at the height of the epidemic (Gubler, 1989; Reiter & Gubler, 1997; Reiter, 1998) and thus represent a waste of public funds.

Solutions and issues to be addressed

Active surveillance

Given the type of information needed for dengue surveillance, it is apparent that passive surveillance alone will not generate sufficient information needed for the prediction of outbreaks. An active, laboratory-based surveillance system and a better understanding of dengue epidemiology are needed for a more cost-effective prevention (Gubler 1989, Gubler & Casta-Velez 1991, Rigau-Perez & Gubler, 1997). The universal use of the WHO case definitions, in the absence of new developments in this field, is essential to enable the surveillance data to be compared across countries and regions. In addition, the following will also be useful:

STANDARDIZATION OF THE DENOMINATOR

While the isolation of dengue virus has been attempted in some parts of the world, the case definition used to select patients for virological surveillance varies from country to country. Furthermore, there is often a lack of denominator, or the extent to which the cases that fit the clinical entities are sampled for dengue virus. This lack of denominator limits the ability to make quantitative assessment of dengue transmission and thus compare the effectiveness of various preventive measures when these are applied in different places. As recommended by Gubler (1979, 1989, 1991, 1998), virological surveillance should include patients that present with nonspecific viral syndrome, in addition to classical DF, with haemorrhagic or neurological manifestation and on all patients with a fatal outcome following viral prodrome. This approach would result in a more comprehensive surveillance on dengue virus transmission in the population.

FOCUS ON URBAN CENTRES

Since all public health authorities operate within certain budget constraints, it would be important to focus the surveillance effort on places where outbreaks are likely to emerge. The work by Cummings et al. (2004) has provided good data to support

previous epidemiological observations that dengue epidemics emerge from urban environments and then spread to new areas (Gubler et al., 1979; Gubler, 2004). Thus, despite limited resources, focus on tropical urban centres, may enable a surveillance system to be predictive of outbreaks.

Laboratory support for dengue virus surveillance

Laboratory support is a critical component in surveillance (Gubler & Casta-Velez, 1991, Rigau-Perez & Gubler, 1997). In particular, the laboratory should be able to identify not only the presence of dengue virus, but also its serotype, the severity of illness, and whether the patient is experiencing a primary or secondary infection. Furthermore, information on the genetic sequence of the viruses circulating, both during and between epidemics, will be of great value to our eventual ability to predict epidemics.

STANDARDIZATION OF LABORATORY METHODS

Currently, many countries where dengue is endemic lack laboratory support for dengue surveillance. Among those that do have laboratory support, there exists variation in laboratory methods used for virological surveillance. This is especially true for molecular methods; the literature reports a large number of real-time or end-point reverse-transcriptase polymerase chain reactions (RT-PCRs) for dengue virus. These assays vary in their sensitivity and specificity. Importantly, many of the new serological assays have not been tested for cross-reactivity to other viruses, especially co-circulating flaviviruses like Japanese encephalitis, yellow fever and West Nile viruses. Standardization of the laboratory methods used for virological and serological surveillance, along with the establishment of an international quality assurance programme for such laboratories would yield clear benefits.

Besides methods for the isolation or detection of dengue viruses, standardization of the genes to be sequenced for phylogenetic analysis should also be determined and agreed upon such that the data can be shared and compared within and between regions. Currently, such studies have ranged from partial to full genome sequences. Obviously full genome sequences will be more informative, although cost prohibits this from being done on a large scale. New developments on mass spectrometry technology-based genomic sequencing (Liu et al., 2005), chip-based high-throughput resequencing arrays (Wong et al., 2004) may lower the cost of genetic sequencing when compared with the use of capillary sequencers, and thus allow more viruses to be analysed in this manner. If not, an international agreement and standardization of the region of the genome to be analysed will be useful in making the sequence data more amenable to comparison across countries and regions. Before this can be done, however, studies need to be conducted on the influence of genetic changes on phenotypic expression (see section on viral factors).

GLOBAL LABORATORY NETWORK FOR DENGUE SURVEILLANCE

It would be beneficial to establish WHO Collaborating Centres for dengue virus surveillance. This would complement and expand the DengueNet system, where morbidity and mortality data are shared among countries. Following the lead of the global influenza and polio surveillance systems, establishing reference laboratories in different regions of the world, which are responsible for more detailed analysis of the dengue virus isolates (such as phylogenetic analysis) may overcome the limitation posed by the lack of public funds for such high-technology laboratory support in countries where dengue is endemic. Countries with the necessary financial and laboratory resources, such as Australia, Japan, Singapore and the USA, could play such an important regional role. This, however, does not negate the fact that every country where dengue is endemic should have at least one laboratory that can support virological surveillance.

Entomological surveillance

The clear need in entomological surveillance is an index or a measure of vector population density that may be predictive of epidemic dengue transmission. Since eradication is not feasible, the goal of public-health preventive measures, in the absence of a vaccine, is to maintain a vector population density that is too low to support sustained viral transmission. It was thought from experience in Singapore in the 1970s that a premises index (the percentage of premises where *Ae. aegypti* larvae are found) of less than 5% was sufficient to prevent epidemic dengue (Chan, 1985). However, since the 1990s, it is obvious that in Singapore, dengue incidence has increased dramatically, despite an overall premise index of 2% and below (Ooi et al., 2006). This, however, may be owing to the insensitive nature of a national premise index; despite the low national index, there are places in Singapore where the density of the *Ae. aegypti* population is high. Likewise, similar reports of limited ability to predict outbreaks have also been associated with the use of Breteau and container indices. A complicating factor is the role of herd immunity. Clearly, the vector-population densities required for epidemic transmission are lower in regions with low herd immunity (Newton & Reiter, 1992).

EMERGENCY CONTROL

While vector population control in several instances, such as in Singapore and Cuba, has clearly been successful although not completely sustainable, the effectiveness of emergency control, as practiced in most countries, is highly questionable. Most emergency control makes use of a combination of reducing the availability of larval habitats as well as chemical adulticide . Chemical control, other than using those chemicals with residual effect, has only limited usefulness in either preventive or emergency control. Yet, they continue to be used in many places despite the lack of evidence of their effectiveness (Gubler, 1989; Reiter & Gubler, 1997; Reiter, 1998). In addition, their indiscriminate toxic activity may also remove the natural predators of the dengue vectors.

In recent years, the development of powerful mathematical and computer tools allows for more sophisticated modelling of outbreaks of infectious disease. Such models also allow for a theoretical assessment of the ecological determinants of epidemic transmission, effectiveness of disease control and preventive measures (Anderson & May, 1991; Focks et al., 1995; Wearing & Rohani, 2006). Emergency control measures could perhaps benefit from the use of such a tool to assess their efficacy. They might allow for various control modalities to be assessed for their effectiveness in reducing virus transmission, given a range of likely scenarios. However, for such a tool to be practically useful, validation of the mathematical assumptions need to be carried out with actual epidemiological and entomological data.

A major problem with emergency control operations is that, because of poor surveillance, they are usually implemented near peak epidemic transmission, too late to have any impact on the epidemic (Gubler 1989, 1998). The reason for this is that implementing emergency control is a political decision, not a public-health decision. To be fully effective, early warning surveillance systems must have built-in triggers to automatically initiate the emergency control programme.

In conclusion, DF/DHF has emerged as the most important vector-borne viral illness in the tropical world and this is likely to be the case well into the 21st century. In the absence of an approved vaccine, the only means to control this disease is to interrupt the transmission of the virus. This will require a sensitive and cost-effective disease- and vector-surveillance, coupled with a community-based larval-control programme. Few countries where dengue is endemic have such public-health infrastructure in place (Gubler, 2002). The challenge that lies ahead is to put into operation these surveillance and vector-control systems, while exploring how new technologies in genetic sequencing can aid in our understanding of dengue epidemics and capability to predict outbreaks.

Research priorities:

- To establish an active laboratory-based multicentre surveillance system for dengue infection in selected countries where dengue is endemic.
- To establish a regional laboratory network to support virological surveillance and information exchange.
- To establish a system, coordinated by reference centres, to share and genetically characterize viruses isolated during epidemics and inter-epidemic periods.
- To initiate research to understand the role of *Ae. albopictus* in dengue transmission during epidemic and inter-epidemic periods.

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WORKING PAPER 7.2. GEOGRAPHIC INFORMATION SYSTEM FOR DENGUE PREVENTION AND CONTROL

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INTRODUCTION

The design and implementation of a sound information system is essential for the prevention and control of dengue. The purpose of the information system is to provide data and information needed in any effort to prevent and control the transmission of dengue, including in decision-making, planning, evaluation and research. Owing to the complexity of dengue transmission, access to data and information from all components of the control strategy, including vector control, environmental and social determinants, community behaviour, attitude and practices, disease surveillance, the laboratory network, and health services at every level, is required.

Geographic information systems (GIS) and related technologies have emerged as a new generation of information systems with the capability to manage spatial dimensions together with time, people and other dimensions of interest, which is not possible with the former information systems. GIS are currently recognized as a set of strategic and analytic tools for public health, so the design and implementation of an information system for dengue control with GIS capacity should be considered.

In this paper, a definition of GIS and some potentialities and limitations of GIS as components of the information system for dengue prevention and control are presented.

DEFINITION

A generally well accepted definition of geographic information system is 'an organized set of hardware, software, spatial and non-spatial data, methods and procedures, and personnel designed to input, store, update, manage, analyse geographically referenced data, and display information in a synthetic and comprehensive way' (Longley et al, 1999; Castillo-Salgado et al, 2000). From a public health perspective, GIS and related technologies such as global positioning systems (GPS) and remote sensors (RS) facilitate: the locating of health events and associated factors in space and time, the monitoring of risk factor behaviours and their relation to health events, the identification and description of distribution patterns of risk factors and health outcomes in time and space, health needs assessment, the identification of vulnerable geographical areas and population groups, priority-setting and monitoring and evaluation of the impact of health interventions, and assessment of health service response (Castillo-Salgado et al, 2000).

The inclusion of 'people' and 'procedures' as part of the definition is essential for GIS applications in a public health context, given the need to link the science and methods of epidemiology to GIS mapping. Without trained staff, one scenario is that GIS software will not be used at all, given the time and staff constraints that exist in many public health agencies and organizations. Alternatively, without trained staff and standardized procedures, the technology may be used to develop maps that are invalid or misleading.

POTENTIALITIES OF GIS FOR DENGUE PROGRAMMES

GIS technology has potential for dengue prevention and control programmes as follows:

- ***GIS technology improves the ability of programme staff, planners, decision-makers and researchers to organize and link datasets (e.g. by using geocoded addresses, geographic boundaries, or location coordinates) from different sources.*** Geography provides a near-universal link for integrating records from multiple information sources into a more coherent whole. This ability to link datasets can help dengue programmes integrate data from the five essential components (epidemiology, entomology/vector control, community participation, laboratory, case management) and plan more cost-effective interventions. For example, suppose that a dengue programme could access the socio-demographic database of the city, which is maintained by the local statistics agency, and also the epidemiological dataset from epidemiological surveillance, and the entomological dataset. Using GIS technology, the dengue programme can combine these databases, mapping the demographic and social indicators by block, pinpointing the location of breeding sites and related entomological indicators, and including on the map the location of cases by residential address to identify populations at higher risk of dengue transmission and plan focalized action in an efficient way. To take advantage of this potential, any dengue initiative and programme should

establish organizational changes and new collaborative links with institutions and departments in the health sector, units of other sectors, governmental departments at regional, municipality and community levels, and community organizations. A review of the scope and necessary relationship of the dengue programme information system with other information systems based on a conceptual framework for reduction of dengue transmission is an essential step in improving it.

- ***GIS, GPS and RS technologies provide dengue programme staff with new types of data.*** For example, with these technologies and when cartographic files (geo-referenced data) are not available, local programme staff and health workers can use a GPS receiver to determine latitude–longitude coordinates for the locations of breeding sites, cases and transmission sources, according to house lot, block and neighbourhood in the city. Programme staff can also use digital imagery from satellites and aerial photos to add details to the map and improve the accuracy of information, and help create/update cartographic databases. For examples of digital imagery, see www.terraserver.com, www.spaceimaging.com and www.ikonos.com. If a sequence of digital images for a small area of interest is available, this can be used to observe changes over time, such as in the development of housing, water bodies, roads, and landfills and other changes in land use and land cover. GIS provides functions and procedures to facilitate the input of these types of data.

  GPS receivers and personal data assistant (PDA) technology used together can improve the collection of data in the field. For example, the entomological survey could be carried out using a digital questionnaire implemented in a PDA. To locate the breeding sites, a GPS receiver linked to the PDA can feed in the longitude–latitude coordinates. Depending on the type of connection and communication implemented, the collected data can be entered into the GIS on return to the office or can feed directly into the database using internet services (web-based system). For a programme with fewer resources, the survey could be carried out using paper forms to record, manually, the location coordinates read from the GPS receiver. In this case, once in the office, each completed questionnaire should be entered into a digital format, spreadsheet or database table using any well known and simple software. Experience in implementing vector control information systems at local level in Central American countries shows that vector control programme staff can learn, in a one-hour demonstration, how to use a GPS receiver as well as the complete procedure for feeding collected data into the GIS.

- ***GIS technology encourages the formation of data partnerships and data sharing at the community level.*** For example, to develop a map of coverage by, and frequency of, drinking water supply to identify those areas in the city with no drinking water supply or with less frequent supply, where people need to accumulate water for several days, the local dengue programme could develop data partnerships with the local department in charge of drinking water supply. This is only a simple example, but the same situation pertains to obtaining data produced by other sectors. It is well recognized today that the inter-sectoral approach is key to success in controlling dengue transmission. GIS technology facilitates the linking of datasets from different sources, but more important are the organizational aspects and inter-sectoral agreements that guarantee access to data from other organizations and help the processes of analysis, planning and decision-making in dengue programmes.

- ***Spatial analysis capability of GIS (distance, proximity, containment measures) can be used to improve entomology/vector control activities and interventions such as focal treatment, and to search for and destroy transmission sources.*** For example, suppose the control programme, as part of its continuous analysis, produces a map of the city by block (the block as the unit of analysis). The socio-demographic dataset is linked (geocoded) to blocks, including the number of houses and population by age group. As a result of an entomological visit, some *A. aegypti* breeding sites are found and their locations pinpointed on the map. From the epidemiological component, the dengue cases are reported and their residence locations plotted on the same map. Creating a buffer zone at a radius of 100 metres to the location of breeding sites and dengue cases, programme staff can determine the areas at higher risk of dengue transmission and can answer questions such as how many houses are within the high risk areas, how many houses are within 100 metres of the breeding sites, how many houses are within 100 metres of a house that has a dengue case, how many houses are within an area close to the breeding sites and the house of the dengue case. The answers provide information for deciding the type of action and resources needed. How many children are in these areas, and how many housewives live in the areas? The answers to these questions provide information about the people at risk, and help to determine how soon action should

be taken. To complete this scenario, behavioural indicators, community knowledge, attitudes and practices, and availability of health services need to be included. This leads to more evidence-based decision-making in the dengue programme.

- ***GIS technology enables work on multiple scales in space and other dimensions (time, individual and aggregated data).*** GIS allows the linking of data for an individual with contextual information aggregated at a variety of geographic levels (e.g. household, block, neighbourhood, city, municipality, state/department/province). This capability enables the preparation of multi-level spatial models to better evaluate and distinguish biologic, contextual, and ecologic effects. For each factor considered, a multi-level model can include both individual predictors (data for each individual) and ecologic predictors (average or aggregate measures) (Morgenstern, 1998). The information system for the dengue programme should be implemented as a multi-level approach, which at the very least includes municipality, city/locality/community, block, and, if possible, house lot and individual dengue cases.

- ***GIS capabilities for spatial and spatial–temporal statistical analysis can improve the information system, giving better support to planning, monitoring, evaluation, and decision-making in the dengue control programme.*** For example, in exploratory data analysis to assess the excess density of *Aedes aegypti* larvae across neighbourhoods, blocks or vector-source sites in the city, tests for spatial randomness could be used to evaluate if a cluster is present, which would indicate there are underlying factors (e.g. contextual, environmental, community practices). In a study in two neighbourhoods in Iquitos, Perú, spatial statistics analysis was used to determine the spatial pattern of *Aedes aegypti* and the containers in which they develop (Getis, 2003). Spatial smoothing methods could be used to reveal the areas with high and low risk of dengue transmission, producing maps of 'smoothed' dengue incidence rates. This method could also be used to stabilize the rate when using small analytic units, a situation in which unreliable estimations are due to small numbers and/or small denominators.

- ***GIS capability to synthesize and visualize information in maps.*** Compared with tables and charts, maps developed using GIS technology can be an extremely effective tool to help dengue programme technical staff synthesize, visualize and understand the problem. In addition, action is more likely when the decision-maker can see on a map that a problem is occurring in his/her area of responsibility. GIS technology enables detailed maps to be generated with relative speed and ease. In turn, maps provide health workers, dengue programme staff and community health volunteers with useful information to advise community members, and health and other sector decision-makers, about dengue as a health problem. For example, at a meeting with community members including community health volunteers and leaders, a map can be used to display the neighbourhoods with high densities of *Ae Aegypti* larvae, pupae and adults. The map can include the location of potential vector sources (e.g. sites with an accumulation of solid waste and containers, houses identified as having domestic breeding sites). With each click of the mouse, a point on the map is selected and a box appears on the screen showing a picture of the site and displaying the conditions of this specific site. This dynamic feature of geographic information technology (the ability to display information linked to a map) can be very useful to programme staff and community members in identifying problems and searching for solutions. This GIS feature has been used successfully by the dengue control programme in Puntarenas, Costa Rica, in the process of advocacy with community leaders and health decision-makers at local, regional and national levels.

LIMITATIONS OF GIS TECHNOLOGY FOR DENGUE PROGRAMMES

Some of the current limitations of GIS technology from a dengue prevention and control programme perspective are as follows:

- ***GIS technology is not yet a common tool in vector control programmes. In fact, few GIS applications can be found for prevention and control of dengue and other vector-borne diseases.*** In spite of the inclusion of a GIS component in many health projects, few of these applications have remained functional after the end of the project. GIS being a young technology and the users in need of training makes it difficult to integrate GIS technology into health programmes at local/community level. Although there has been enormous development of GIS software in the last two decades, considerable room remains for improvement and development of public health applications. To this end, some national and international institutions such as the US Centers for Disease Prevention and Control (CDC) have developed the mapping software EpiMap as part of EpiInfo (Dean, 1999; CDC, 2006), the World Health Organization (WHO) has developed the software HealthMapper (WHO, 2006[a]), and the

Pan American Health Organization (PAHO) has developed the software SIGEpi (Martinez-Piedra, 2001; PAHO, 2006). More details about these and other softwares can be found in Martinez-Piedra, 2004. GIS technology could be linked with current information systems and specific information tools at all levels, and specialized GIS software products could be designed to support the new challenges in prevention and control of dengue.

- ***Accurate, low-cost street maps and other cartographic databases such as of neighbourhood, block and house lot boundaries, are essential for dengue control programmes.*** Without an up-to-date street map, for example, control programme staff will need to spend a lot of time and effort digitizing the location of cases, or may not be able to map all reports of cases and/or *Ae. Aegypti* breeding sites. Accurate street maps are especially needed for urban areas with fast growth. It is recommended that dengue control programmes establish a coordination mechanism with the national institution that produces standard cartographic data. It is also recommended that control programmes develop basic capabilities for cartographic editing, including using GPS receivers and inputting GPS data into GIS systems. Some countries do not have a good structure or standard system of addresses, even in urban areas, which makes the geocoding process using residential address or other references all the more difficult. In this case, a specific standard coding scheme should be created (e.g. coding house lots and blocks).

- ***Professionals, planners, technicians, and especially state/departmental/provincial and local dengue prevention and control programme staff, need training and user support in GIS technology, data, and epidemiologic methods in order to use the technology appropriately and effectively.*** The cost of training programmes offered by commercial GIS vendors can be a financial burden for any dengue programme, while GIS training programmes specifically custom designed for public health professionals are still relatively limited. The GIS team at the Pan American Health Organization (PAHO) has developed a course/workshop on GIS applications in public health: on each of four days, participants solve a public health problem using different epidemiological methods, including exploratory data analysis and spatial analysis. This course/workshop has been held in ten countries of the America's Region in the past two years as part of PAHO technical cooperation to Member State institutions and professionals in epidemiology and analytic capacity. Other successful training experience includes the workshops for capacity building of vector control programme staff at local level and for technology transfer of the GIS model for malaria transmission control as part of the Regional programme of sustainable alternatives for malaria vector control without DDT in Mexico and Central America.

- ***The cost of commercial GIS software is a barrier to extending the use and development of GIS applications in public health and, particularly, in dengue control programmes.*** GIS software for free and/or low-cost distribution should be evaluated under conditions of funding shortage, which is the most frequent case in dengue endemic countries.

ADDITIONAL CONSIDERATIONS

Development of the information system for a dengue programme should take into account the requirements derived from the Integrated Dengue Management Strategy (EGI-Dengue) and its implementation in each specific country.

Every level of a dengue programme has its specific information needs. A significant number of countries, at least in the America's Region, have a decentralized health system where more responsibility, in terms of resources and decisions for action, is assigned to local level. The information system should take these elements into consideration so that the requirements of each level, including the global initiatives, can be identified and redesigned if necessary to ensure data flow between levels according to the requirements and availability of new information technology. As an example, the information system of the dengue programme should be articulated with the global information system DengueNet (WHO[b], 2006) and, at the same time, the experience and lessons derived from the implementation of DengueNet should help in the design of information systems at national level.

The information system for dengue control should provide solutions for increasing interoperability among the information subsystems of each component (epidemiology, laboratory, entomology/vector control, community participation). Similar solutions should be implemented in the accessing of data from the information systems of other sectors as needed. This will require coordination and agreements with institutions of the other sectors.

The information system/GIS and data model should be multi-dimensional and provide a multilevel approach for responding to the complexity of dengue transmission. As mentioned above, the data model should allow analysis at the level of

municipality, city/locality/community, block, house lot if possible, and the individual dengue case. With micro (disaggregated) data, it is possible to identify heterogeneity in the magnitude and frequency of any measure across the area of analysis. The information system should help to create indicators at all levels using standard procedures and methods.

Organizational, behavioural (motivation) and technical determinants of the performance of the information system should be taken into account to guarantee its sustainability, data quality and information use for decision-making. Usually it takes a lot of effort and resources of programme staff at local level to capture data. Tons of papers (data forms) are accumulated in health facilities/units of vector control, but transforming those data into useful information for analysis is very poor. A high proportion of staff at local level even don't know the importance of correctly filling in a column of the weekly aggregated report, nor have they ever seen guidelines for completing the data forms. Technical staff are limited in their ability to analyse data, including plotting/creating charts or calculating simple measures/indicators. The limited empowerment and accountability of staff also contributes to their non-motivation. More emphasis should be given to data quality and the use of information for decision-making. At the same time, an effort is needed to build the analytical capacity of staff at local and intermediate levels. Improvement of the current information systems should help to make dengue programme procedures more efficient and cost effective.

EPIDEMIOLOGICAL STRATIFICATION

To be more cost effective in dengue control interventions, epidemiological stratification based on epidemiological, entomological and behavioural risk indicators is needed. The information system should help to provide data and/or indicators from these components, as well as methods and procedures for epidemiological stratification and priority-setting. The methods should be implemented in a simplified way and driven by the end-user in order to be used on a continuous basis. Epidemiological stratification has the main purpose of identifying groups of areas (units of analysis) or strata which share the same hierarchy/priority of risk factors, and consequently, of applying specific and focalized interventions to each stratum. Epidemiological stratification followed by priority-setting would be one solution to staffing shortage and the impossibility of covering large areas of housing when carrying out entomological surveys/inspections or source reduction/elimination by limited vector control staff.

PRIORITY RESEARCH QUESTIONS

1. Does the current information system of the dengue control programme respond to the information needs and support planning, monitoring, evaluation, and decision-making?
2. How should an information system for the dengue control programme be implemented/operationalized with the participation of all levels, including the local/community level?
3. How can GIS, GPS and RS technologies be transferred to local/community level staff? How can capacity be created at this level?
4. How can an information system be designed taking into consideration the need to interoperate among the five essential components of dengue programmes?
5. What are the core indicators of each component of the dengue programme that the information system should produce?
6. What indicators or risk factors needed by the dengue control programme come from other sectors (e.g. water supply frequency and coverage, water quality, climate measurements, land cover, soil use)?

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WORKING PAPER 7.3. ACHIEVING BEHAVIOUR CHANGE FOR DENGUE CONTROL: METHODS, SCALING-UP, AND SUSTAINABILITY

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INTRODUCTION

With the global resurgence of dengue and its more severe form, dengue hemorrhagic fever (DHF), the disease has re-emerged as a major threat to public health. Typical approaches to dengue control and vector control involve vertical programmes to reduce the source of transmission. Physical (e.g. destruction or other physical manipulation of water-holding containers), biological (e.g. use of fish), and chemical (e.g. use of larvicides, spraying with systemic insecticides) control methods can be successful if substantial administrative and political support is provided. However, such efforts often result in short-term control as the areas become reinfested in a fairly short period of time. Vertical vector control programmes may be ineffective because communities are not active partners in the control actions but rather are passive participants or recipients of the control efforts (Gubler, 2002). In light of the restructuring efforts by ministries of health to decentralize services, and of the generalized chronic underfunding of dengue control programmes, and in order to provide effective control measures, it is critical to address issues such as: (1) how to maintain quality of control in a decentralized system where decision-making takes place at regional, state, provincial or municipal levels; (2) how to ensure that funding is adequate to maintain programme infrastructure; and (3) how to ensure, where traditionally staff have been under the purview of the ministry of health (e.g. communications, entomology) rather that the regional or municipal health department, that there are trained staff in technical areas at the local level.

Dengue may present as a mild illness episode, leading many people to underestimate its seriousness and therefore the importance of controlling the mosquito vector. Some residents may be unaware of how dengue is transmitted, and some may be unaware of the source of the vector mosquito; others however may know where the *Ae. aegypti* mosquito is produced and how the breeding sites can be controlled or eliminated but are not motivated to take preventive action. Even those who do follow the recommended actions may still have *Ae. aegypti* or other mosquitoes in their houses and, worse still, may suffer dengue infections if their neighbours do not participate in controlling domestic breeding sites, or they may get bitten by an infected mosquito at their place of work or study. Therefore, the issue for vector control is not whether source reduction is effective, but whether and how community participation can be a part of that source reduction effort (Gubler and Clark, 1996; Lloyd et al. 1994). Regardless of whether the dengue control efforts take place through a centralized or decentralized system of care, the issues are (1) how to meaningfully engage residents in sustained control actions; (2) how to effectively communicate with residents in ever-expanding urban and semi-urban areas in light of reduced vector control staffing and chronic budget shortfalls; and (3) how to measure the impact of residents' actions on *Ae. aegypti* breeding sites. This paper is divided into two sections. The first will examine behaviour change and dengue control efforts and the second will examine delivery mechanisms for behaviour change interventions in the community.

Behaviour change and dengue control

Although experts agree that community participation and modification of human behaviour at the household level are crucial to effective control of *Ae. aegypti*, the specific form that control efforts should take continues to present a challenge to public health officials. As the context for the present paper, a review was conducted of research studies in community-based dengue control efforts published since 1995. This review was carried out through Internet search engines (PubMed, Google Scholar, etc.) and through reviews of existing paper files and library-housed journals. Given the nature of these searches, most literature identified was published in English.

All behaviour change and/or health communication-oriented papers were reviewed with respect to the following characteristics or variables:

- country/setting
- planning tool or approach used
- level at which the intervention was directed (i.e. household, school and other organizations, or entire communities or regions)
- person(s) who was(were) the source of communication or agent of change
- dependent measure or outcome variable
- research or evaluation design of the study or programme evaluation
- results, and conclusions drawn by the authors.

The studies presented here were those for which most of this information was evident in the article, especially with regard to whether any evaluation of the dengue control effort had been undertaken. A summary of these studies (or programme evaluations) is presented in table 1.

In 2005, an evaluation of 11 WHO-supported dengue communication and mobilization programmes using the communication for behavioural impact (COMBI) planning tool (Parks and Lloyd, 2004) was conducted in six South Asian and Latin America/ Caribbean countries (Elder, 2005). The conclusions below are derived from this evaluation, as well as from the review of recent programmes (table 1).

PROGRESS AND CHALLENGES IN COMMUNITY-BASED BEHAVIOUR CHANGE EFFORTS

Multi-level behaviour and community change

As can be seen in table 1, all programmes included behaviour change efforts at the household level, and some targeted the broader community and other partners (schools were the most common partner). However, vector control cannot be effective (or at least, very effective) if carried out only on an individual basis. Thus, if mosquito breeding sites are eliminated in one household but not in a neighbour's or in public areas, the individuals in that cleaner household are likely to receive some but little added protection against dengue. At some point, however, a critical mass may be reached where a sufficient number of vectors are eliminated in an area or region, thereby reducing everyone's risk for contracting dengue. Thus, multi-level, vertically and horizontally integrated programmes offer the best solution to dengue control. For optimal effects, such programmes would include not only community-wide (e.g. mass media) and house-by-house efforts, but also those efforts of schools, worksites and other organizations within the community.

At the community and regional levels, responsible agencies may need to identify 'programme champions' in order for their efforts to succeed, while at other levels, groups of individuals may share responsibility for maintaining programme momentum and integrity. An assessment of different leadership modalities from the WHO evaluation revealed that roughly half of the programmes were led by strong, forceful individuals, while the others seemed to be more 'committee driven', with two to several individuals sharing responsibility and decision-making. Neither model seemed to have an advantage over the other. In one case, the group of individuals responsible for the effort seemed not to agree on its key aspect, impeding progress towards COMBI goals. In another, the programme champion was such a strong individual that one would worry about the future of the programme after leadership turnover. But in most other cases, the model that was chosen by the country or community seemed to be the best one for them, a phenomenon consistent with recent research on tailoring health communications. The advantages of a programme champion are that their investment of energy and enthusiasm will often achieve more results in the short term, while the downside relates to the unclear implications for longer term sustainability and generalizability to regions without such individuals.

In any case, enthusiasm will at some point die out, especially among non-paid volunteers, thus threatening sustainability. Setting limited periods of commitment or allowing the workers to move on at some point to other health issues or even other communities (perhaps helping new neighbourhoods to start their own front-line worker teams) could be among the methods used to optimize commitment. Second, plans must be developed to fade an effort out once progress has been sufficient or nearly so. Booster (or spot-check) home visits, for example, should be increasingly infrequent, thus avoiding both health worker and homeowner burn-out. Should entomological, epidemiological or behavioural data indicate a need to renew a full intervention, this could then be accomplished on a shorter-term scale, reverting to spot-checks when needed.

While much has been written about social marketing and many examples of successful social marketing have been projects that took place over several years, there are few examples of incorporation of social marketing principles in dengue prevention and control programmes. According to the UK National Social Marketing Centre (2006), social marketing is 'the systematic application of marketing concepts and techniques to achieve specific behavioural goals, for a social or public good'. In a comprehensive white paper on how to create a 'people-centred' public health strategy, the authors examine how to improve disease prevention and health promotion within the British National Health System, and they assess the potential of social marketing to move beyond the prevention models currently in practice. The authors state that social marketing 'can support efforts to achieve an appropriate and effective balance between the role of individuals and the role of the state and relevant bodies'. Although an assumption might be that, in resource-rich countries, such approaches would be systematically developed and

Table 1

Ref.	Country/ setting	Planning tool	Level	Intervention/change agent	Dependent measure/ outcome	Evaluation design/ time period	Results and comments
i	Cambodia: three rural villages	Selection of polypropylene hoop with netting after initial field test of 19 different jar covers	H	Professional staff distributed deltamethrin-treated polyester net covers for water storage jars	Density of immature stages and adult mosquitoes	Pre- and post-intervention cross-sectional tests over 12 weeks	Effective at preventing growth in larval stages; little impact on adult mosquitoes
ii	Bucaramanga, Colombia	Stages of change in housewives	C,O,H	Mandatory community service for 11th grade students with primary focus on door-to-door campaign targeting housewives Multi-level social marketing (print, theatre, radio, social events)	Knowledge and awareness among students; Household Index of mosquitoes in various stages	Pre- and post-intervention: 8-month knowledge test among students; 5-year follow-up of households using House Index	Knowledge increase House Index dropped from 18% in 1998 to 5% in 2003 Recommend 3+ year intervention to achieve impact
iii	Dominican Republic	Formative studies with female heads of household revealed a desire for 'clean water'	C, H	Breeding site reduction Use of biological control (i.e. fish) to reduce larval stages of the mosquito Information transmission via national & local sources Direct counselling from health educators	Not indicated	Not indicated	Simple and economical health education messages very beneficial
iv	Fiji	National KAP survey + formative research to understand household mosquito behaviours	C,O,H	Multi media social mobilization to reduce breeding sites - focus on control of tyres and drums	Larval indices; no. of tyres in yard and whether they were controlled	Pre- and 10-month post-intervention test on primary outcomes among 100 randomly sampled houses	Increased control of tyres (from 34% to 61%) Recommendations: - Focus on behaviours rather than only on knowledge; keep behavioural targets simple - Time-series evaluation rather than pre- and post-intervention basis
v	El Progreso, Honduras	Examination of existing cleaning behaviours	C,O,H	Multi media campaign & community mobilization Household visits by volunteers teaching effective scrubbing of concrete basins, the primary water storage device Mass media component to support community effort	House Index, incidence of dengue cases, morbidity rates, exposure to and understanding of key messages; no. of households visited; media exposure surveys Entomological information, epidemiological information, household visits	Pre- and post-intervention tests	Surveys in 2002–2003 revealed awareness among 35% of housewives; need for reinforcement of behaviour.
vi	Purwokerto City, Central Java, Indonesia	KAP survey: revealed knowledge, but lack of action.	C,O,H	Social mobilization and communication via community meetings, training, inspection role-modelling, and identification/ endorsement of key stakeholders *Dasawisma*: Ten-house alliance where house occupants rotate inspection responsibility Dissemination of 'source reduction kit' with emphasis on '3Ms': *cleaning, covering, burying*	Monthly dasawisma evaluation to measure level of preventive behaviour (*active, less active, not active*). Quarterly larval surveys Tracking of dengue hemorrhagic fever (DHF) hospital cases	Dasawisma activity ratings every month Pre- and post-intervention larval surveys	House Index decreased from 20% pre-intervention to 2% with intervention

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Table 1 (continued)

Ref.	Country/ setting	Planning tool	Level	Intervention/change agent	Dependent measure/ outcome	Evaluation design/ time period	Results and comments
vii	Johor Bahru, Johore, Malaysia	Minimal success with other strategies	C, H	COMBI approach: 1) Social mobilization (public relations campaign, volunteers, youth) 2) Communication (buntings, self-evaluation checklist, radio and newspaper advertisements, point-of-service promotion, dengue-related radio advertisements and talk shows) Main emphasis on vector inspection/prevention and early fever detection	KAP survey results from household heads or age 18+ Treatment-seeking behaviours in hospital admitted dengue patients	Pre- and post-intervention surveys using multistage stratified sample (926 of 1712 were paired) Treatment-seeking survey	99% of respondents in post-intervention survey self-reported Sunday household inspections vs. 71% in pre-intervention survey 59% of dengue-related hospital admissions occurred within 24 hours of fever onset (42% in control areas)
viii	Colima, Mexico	Pre-intervention KAP and entomological survey	C, H	1) Educational campaign to encourage community participation in breeding ground elimination; presentation, video, socio-drama; reinforcement with prevention-related small gifts and printed materials 2) Intervention with malathion spraying alone 3) Combination education & chemical spraying treatment	Prevalence of *Aedes aegypti*; KAP levels regarding dengue and vector	Prospective evaluation Pre- and post-intervention KAP and entomological surveys (post-intervention survey = after 6 months)	Reduction in Basal House Index, Basal Container Index, and Basal Breteau Index No significant changes in KAP after the intervention treatment
ix	Mérida, Yucatán, Mexico	Examination of failed prior attempts In-depth interviews, focus groups, observation of household waste and water management, KAP and entomological survey	H, O	Communication/education campaign (interpersonal/ mass media): - slogans - strategically scheduled radio and television broadcasts - use of a 'spokes-puppet': Lela - new behaviour introduced every 4–6 weeks (from May–Oct 1995), depending on complexity Interpersonal, through activities in the home and school environment (primarily focused on 4th graders).	KAP and entomological surveys Composite behaviour score	Containers positive for *Ae. aegypti* (requirement for study participation) marked for follow-up and sampled over a ten-month period from June 1995 to March 1996	Decline in House, Container, and Breteau indices Positive increase in behaviour scores after intervention Significant increase in self-reported tyre behaviour associated with no tyre-based mosquito breeding.
x	Managua, Nicaragua	Pilot efforts and focus groups Beyond-KAP questionnaires Entomological surveys Dengue virus infections in children 3–9 years old	C	Stratified cluster sample: 30 sentinel sites Intervention: seven barrios in year 1, three more added in year 2 Control: 20 barrios Socializing Evidence for Participatory Action (SEPA) communication strategy Use of volunteers and brigadistas to encourage further action	Entomological indices to measure control of vector *Aedes aegypti* Serological surveys among young children Beyond-KAP survey	External evaluation conducted at 25 months Utilization of evidence cycles	By year 2, monitoring and elimination of vector larvae significantly more likely in intervention barrios compared to control barrios Intervention barrios: - Decreased use of insecticides, increased knowledge sharing and community leadership - Decrease in entomological and serological indices Reported sense of personal development in intervention community

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Table 1 (continued)

Ref.	Country/ setting	Planning tool	Level	Intervention/change agent	Dependent measure/ outcome	Evaluation design/ time period	Results and comments
xi	Puerto Rico	Increases in DHF incidence prompted need to determine impact of pilot community-based programmes	C,O,H	Community based prevention programmes: *Head Start*: - Children: mosquito-related activity book - Parents: video on dengue fever prevention at the Head Start centre and visit from Head Start personnel Public school programme: 4th grade science component Posters and televised public service announcements (PSAs): 'Dengue-Free Zone' Children's museum interactive exhibit on Ae. aegypti with guided tour	Qualitative data: interviews and focus groups Impact of programmes on overall infestation levels and on four specific behaviours: - Elimination of refuse - Protecting used tyres from the rain - Maintaining larvae-free water storage containers - Use of commercial indoor aerosol insecticides Behavioural outcome variables: House Index, Breteau Index, and container-specific index	Comprehensive cross-sectional evaluation	Higher levels of correct overall knowledge on dengue and decreased incorrect knowledge among parents regarding the mosquito life cycle Greater impact on children's dengue knowledge than on behaviour change and prevention
xii	Vanuatu	Community-based formative research & audience pre-tests	C, H	Manples Community Project (est. 1998): Establish community committees; workshop and community meetings held to increase dengue fever knowledge: - *Wan Smol Bag* theater company model dengue-related preventive behaviours during performances at community & school events. Household donations to purchase plastic bags for small water container disposal Tyres: collection, disposal, and education (how to fill with soil), mobilization of young people to drill water drainage holes in immobile tyres Volunteers apply temephos to larger water storage containers – not well received Local manufacturer commissioned to create mosquito-proof water-storage containers	Larval surveys measuring House and Breteau indices; no formal evaluation of behaviour change	Frequent indices recording throughout programme (1998–2001)	Small outbreak of dengue in 1998 – first test of the intervention: results = 100 cases recorded with 0 mortality Consultations with the community were helpful in tailoring intervention, especially with respect to tyres
xiii	Viet Nam	Vector surveys, KAP surveys (100 households in each programme commune)	C, H	Vector control activities: - Use of *Mesocyclops* in water storage containers to prey on *Aedes aegypti* larvae. - Institution of community clean-up campaigns to collect and remove empty water containers - Prevention education campaigns and interpersonal reinforcement and motivation: church and community meetings, media broadcasts, loudspeaker announcements, drama performances, dengue football competition, and print campaign - Monitoring household mosquito prevention practices & prevalence of mosquitoes	Presence of mosquitoes, *Mesocyclops*, and key breeding containers Measurement of level of prevention practices adoption through standardized ratings KAP survey results	Annual evaluations to monitor and adjust activities Quarterly vector surveys Analysis of dengue prevention behaviours in cross-sectional sample of households	First project (2001) accomplished full *Ae. aegypti* control in five out of six communes Second project (2003) reduced larval populations by 99.6%–100%

C = community or regional; O = school, worksite or other organization; H = home; KAP = knowledge, attitudes and practices

i. Socheat et al., 2004.
ii. Luna et al., 2004.
iii. Leontsini et al., 2004; Leontsini, 2000; Chan et al., 1998; Gordon, 1988; Gordon et al., 1990; Whiteford, 1997.
iv. Bera et al., 2004.
v. Fernández, Martinez and Sherman, 2004.
vi. Kusriastuti et al., 2004.
vii. Suhaili et al., 2004.
viii. Espinoza-Gómez, Hernández-Suárez and Coll-Cárdenas, 2002.
ix. Galván and Gutiérrez, 2004.
x. Arosteguí J et al., 2006; UBS Optimus Foundation, 2006.
xi. Winch et al., 2002; Clark et al., 2004; Clark, 1992.
xii. Toaliu, Taleo, 2004.
xiii. Nam et al., 2004.

supported, in reality the public health and the health care systems generally function as two separate entities with differing views on what prevention is and who the target audience should be. It is important to clarify that a national media campaign is *not* social marketing, although it might be part of a social marketing programme. Regardless of the framework, the following issues have been identified as essential to understanding prior to the development of any community-based approaches.

Operationalization of behaviours

Few programmes provide clear definitions of specific human actions that can improve control as part of the planning phase of community programmes, and those that do often leap ahead to an examination of indications of mosquito breeding in the evaluation effort (table 1). A key element in any health behaviour change or disease prevention programme comprises the initial step of 'operationalization' of target behaviours. Operational definitions of behaviours or the environments surrounding them emphasize an objective observation of the physical aspects of the behaviours. Thus, a behaviour can be observed directly and reliably by examining the frequency, duration, or strength of the behaviour (e.g. the frequency of applying larvicide, the duration of cleaning used to reduce algae in a 55 gallon drum, the intensity with which an individual appears to scrub a cement basin), or the physical by-product of that behaviour (e.g. the number of tyres left unprotected in a backyard vs. the number filled with dirt or placed under a roof). Operational definitions, therefore, go hand in hand with the nature of the assessment used to arrive at those descriptions of behaviour. Thus, phenomena such as 'knowledge of breeding cycles' or 'fear of mosquito contamination' are not behaviours per se but inferred inner causes of these behaviours. In practice it is often difficult for health education or vector control professionals to arrive at specific operational definitions of behaviours, as they have frequently been trained to emphasize these internal mechanisms.

In *Ae. aegypti* control efforts, it may be difficult to observe the nature of the behaviour, and therefore for monitoring purposes it is often necessary to select physical by-products of the behaviour rather than an observation of the behaviour. Nonetheless, behaviours must still be operationally defined even if their direct performance cannot be observed. It is only through the operationalization of each behaviour that indicators can be developed to measure whether or not the behaviour has taken place and to what extent it has been carried out.

The operationalization of behaviour starts with the selection of one or more specific target behaviours. These behaviours are selected on a variety of criteria, the most important of which is whether the behaviour itself seems to have an impact on the specific health problem. Nevertheless, many different behaviours may potentially have such an impact and the target behaviour must be narrowed down to a manageable single or small group of behaviours. Therefore, programme planners should also address the following in the selection of target behaviours:

- ***Feasibility***: To what extent would the performance of the target behaviour result in negative consequences for the individual performing it (e.g. changing the taste of drinking water by adding temephos or fish)? Is the behaviour compatible with the person's current practice and with sociocultural norms in the community? Does the potential target behaviour require an unrealistic rate or frequency or duration in order to be sufficient? What are the costs of the target behaviour in terms of time, energy or other community-identified expenditures?
- ***One step at a time***: Are there any existing 'approximations' to the target behaviour? Is the behaviour already being performed perhaps at a substandard but detectable level? Can this behaviour be 'shaped' to meet criteria? Are there monitoring systems (see below) in place that could be used to provide feedback to household residents, health staff, and others who may gradually provide evidence of improvement in behaviour (Graeff, Elder and Booth, 1993)?
  Some confusion about how to operationalize target behaviours derives from an inability to distinguish between whether the target behaviour exists at all, or whether it does not exist in adequate strength due to 'performance deficit' or 'skill deficit'. 'Performance deficit' refers to a situation in which individuals may actually possess an existing skill but either do not receive the reinforcement necessary for performing the behaviour or receive inadequate reinforcement and hence do not engage in adequate practice. As part of the performance deficit analysis, understanding the functions served by containers (not just their type and capacity) that are potential breeding sites in the home is key to determining the actions that can be implemented (Lloyd et al., 1992; Galván & Gutiérrez, 2004). In contrast, a 'skill deficit' is simply that the individual does not have the knowledge and practice associated with adequate performance of a skill regardless of whether he or she is motivated to engage in that behaviour. Health communication and social mobilization efforts take very different forms

depending on whether the bulk of the population evidences skill or performance deficit with respect to the control of mosquito breeding.

- ***Context of the behaviour***: To further complicate the definition and selection of target behaviours, general community needs and capacity must be examined simultaneously. In most studies, both the target behaviours and the communities selected evidence a range of 'difficulty'. When using the COMBI planning tool, programme planners must focus on target behaviours that will have a measurable impact on the specific component of dengue prevention and control being addressed through the communication/social mobilization plan; the target behaviours, however, are the result of a community-based process through which the target population and the programme planners identify and test behaviours for feasibility and effectiveness. Other programmes have used other models or processes to define behaviours within a participatory community process (table 1). The target areas also evidence a range of characteristics in socioeconomic status and accessibility, ranging from communities enjoying schools, roads in good condition, utilities, general municipal services, and employment, to others with high crime and low employment, and buildings and roads in poor physical condition. Some communities may be within a few kilometres of their health centres while others are in remote locations. In other words, some communities are relatively easy to work with, while others are more difficult.
  These independent dimensions of behavioural and community 'difficulty' may lead one to conclude that generally, when planners select a difficult community (e.g. poorer, with higher crime), they may want to begin with an 'easier' target behaviour (e.g. hermetic covering of containers). Should more accessible and prosperous communities be selected, planners can be more ambitious with respect to the choice of target behaviours (e.g. frequent emptying and scrubbing of containers). It is axiomatic that poorer communities need more resources to achieve an equivalent result. Planners should focus on what is truly practical for modest or resource-poor environments (resource-poor referring to both the programmatic environment and the target community).

The monitoring–feedback loop

Related to the integration of efforts and the operationalization of specific, observable target behaviours is the need for an emphasis on information sharing and feedback loops through monitoring and evaluation. Few studies reported in the literature (table 1) indicate that systematic monitoring and evaluation have been carried out, and perhaps only a few have used ongoing monitoring to improve or reinforce efforts. Vector control and health education/communications staff seem to understand in general what evaluation is, but how to conduct routine programme monitoring, and how to use those data for programme adjustments throughout the year, do not seem to be clearly understood. While many national programmes can show that data are collected for calculating entomological indices, few can describe how these data are used during household visits since *Aedes* breeding sites are not prioritized, leading to the ongoing promotion of general behavioural messages that have limited impact on mosquito breeding as evidenced in continued high larval indices.

Understanding of and enthusiasm for the COMBI interventions among residents seems to be largely a function of health workers giving individual, specific feedback at the household level. In fact, health workers and volunteers seem to be more cognizant of and capable when they use specific behaviourally-based feedback rather than more general exhortations to the community. Monitoring and evaluation data must be accessible and apparent at higher levels as well. In the Nicaragua programme, maps with colour-coded pins used to track neighbourhood outbreaks of dengue and malaria provided feedback to all staff and community health volunteers regarding epidemiological markers for programme progress, and pinpointed specific blocks in the neighbourhoods where more intensive education and behaviour change work were needed. Staff and volunteers met each month to discuss the neighbourhoods and specific challenges, so that staff and volunteers received continuous feedback and reinforcement for their work, just as residents had received through the self-retaining of records.

A weakness of all programmes examined to date is the lack of behavioural indicators that have been tested and validated for routine field use within the context of national dengue programmes. Although indicators have been created and tested in some studies (e.g. in Mexico, Honduras), these indicators have not been operationalized within a dengue prevention and control programme setting. There is lack of staff with specific expertise in this area within ministries of health, leading to ongoing, inappropriate use of entomological indicators as proxies for human behaviours.

Delivery of behavioural interventions to target populations

To date, special community-based projects may use ministry of health staff, a combination of ministry of health/externally funded staff, or may be completely externally funded. The ongoing challenge is how to take promising results from a special project and deliver them on a national scale, taking into consideration differences in vector ecology and in local level capacity to manage programmes, lack of local level staff with behaviour change expertise, political changes that impact programme services from national to municipal level, staffing changes at all levels, and chronic funding and staffing shortages.

Because most behaviour interventions have been delivered through the existing structure of dengue programmes, for the most part, after a certain period, the programme reverts to its original focus and programming, that is, to entomological surveying and source reduction conducted by vector control staff. This is not only the case for behavioural interventions, but laboratory and case management also tend to function independently, even though the need for integration of the five essential components has been highlighted over the past years (PAHO, 1994 and 1999; WHO, 1999). In order to address this issue, the Regional Program Office for Dengue Prevention and Control of the Pan American Health Organization developed a Strategy for Integrated Dengue Management (EGI-Dengue), a process by which countries functionally integrate the five key components of a dengue prevention and control programme (epidemiology, entomology/vector control, community participation, laboratory, case management) (PAHO, 2003). The EGI-Dengue process convenes a national technical expert group with two to three experts in each of the five components to prioritize actions for each component area and then to prioritize actions across the five areas. The national group of experts monitors the implementation of the national integration strategy via the logic framework (marco logico) developed as part of the process. The EGI-Dengue process has been under way in the Americas since 2004.

BEHAVIOURAL RISK INDICATORS

Good programme planning is based on understanding *who* needs *what* service(s), *when*, and *where*. Unfortunately, we do not have indicators by which we can measure dengue behavioural risk, such as blood pressure is used to indicate heart disease and blood sugar to indicate diabetes. We need to be able to stratify areas using epidemiological, entomological and behavioural risk indicators in order to develop and then deliver an intervention mix that will respond to the priority risk indicators of that area.

KEY ISSUES FOR CONSIDERATION IN BEHAVIOUR CHANGE INTERVENTIONS

- Programme leadership and planning for sustainable community participation and involvement.
- Transfer of technical knowledge and skills in planning participatory behavioural interventions to health workers, community volunteers and other partners at the local level.
- Creation and maintenance of monitoring and feedback systems at the local and national levels, including the development of behavioural indicators.
- Judicious mix of communication channels (interpersonal, mass media, publicity, etc.) to support programme behavioural goals over time, based not just on available funding but also on effectiveness for the local context.

PRIORITY RESEARCH QUESTIONS

- How can indicators that measure behaviour change, and the extent of this change, be operationalized?
- What are the indicators of behavioural risk and how can these indicators be part of a stratification process based on epidemiological, entomological and behavioural risk indicators?
- Can the current, entrenched programme delivery model, which is not, for the most part, achieving the goals and objectives of controlling dengue fever/DHF, be revamped, or do we need a new programme model?
- How can cost effectiveness be measured? Do we need to measure the added benefit of each individual component since we don't have a fully integrated model that can be used as a reference point?
- How can we go to scale from pilot models of community-based communication/mobilization efforts?

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WORKING PAPER 7.4. DELIVERY ISSUES RELATED TO VECTOR CONTROL OPERATIONS: A SPECIAL FOCUS ON THE AMERICAS

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INTRODUCTION

Vector control is an essential part of the control of vector-borne diseases and effective preventive measures to reduce or interrupt their transmission. It also plays a critical role in the prevention and containment of epidemics. With the gradual abandonment of programmes for the eradication of malaria and *Aedes aegypti* in the 1960s and 1970s and the decentralization of most vector control programmes, capacity has diminished dramatically in many countries, although expenditure associated with vector control is still responsible for a large share of the budget of vector-borne disease control programmes (Rodríguez Cruz, 2002).

While vaccines have been developed for other flaviviruses, such as yellow fever and Japanese encephalitis, the development of vaccines for dengue is complicated by the need to incorporate all four virus serotypes into a single preparation. An approved vaccine is not likely to be available for 5 to 7 years; the only way to prevent dengue transmission, therefore, is to reduce the population of its principal vector, *Ae. aegypti* (Ooi et al., 2006).

In many countries, health-sector reform poses new challenges for programme delivery, including decentralization and issues of selection, purchase, procurement, and use and monitoring of insecticide application. Moreover, a limited number of new, cost-effective chemical pesticides suitable for public-health use have been developed in recent years. This problem is particularly acute with regard to larvicides suitable for use in stored water for domestic consumption (Ooi et al., 2006). For these reasons, better strategies for programme delivery are needed.

BACKGROUND

Dengue has been successfully prevented via vector control in at least three instances. The first was the highly successful, vertically-structured paramilitary hemispheric eradication campaign directed by the Pan American Sanitary Board (Ooi et al., 2006). Campaigns to eradicate *Ae. aegypti* were successful between 1948 and 1972, when complete vector eradication was achieved in 21 countries of the Americas (Rodríguez Cruz, 2002). The second was also a rigorous, top-down, military-like vector control operation in Cuba that was based on intensive insecticidal treatment followed by reduction of available larval habitats (source reduction) in 1981 (Kouri et al., 1989). The third successful programme was in Singapore. However, none of these programmes was sustainable, with consequent reinfestation and a loss of the progress made in previous years (Ooi et al., 2006).

Since the early 1970s, the World Health Organization (WHO) has been actively involved in developing and promoting strategies for the treatment and control of dengue. In resolution WHA46.31, the Forty-sixth World Health Assembly in 1993 confirmed that dengue prevention and control should be among the priorities of WHO. In 1995, the WHO Global Strategy for Prevention and Control of Dengue Fever and Dengue Haemorrhagic Fever was developed (WHO, 1999). It comprises five major components: selective integrated vector control, with community and intersectoral participation; active disease surveillance based on a strong health information system; emergency preparedness, capacity building and training; and vector control research (WHO, 2000). Global and regional strategies emphasizing the need for effective prevention, active surveillance and outbreak preparedness have since been developed in the Regions of the Americas, Western Pacific and South-East Asia.

The Pan American Health Organization (PAHO) developed regional guidelines for dengue prevention in 1994 (PAHO, 1994) and, during the meeting of its Directing Council in 2001, adopted Resolution CD43.R4, which is a political declaration concerning the alarming situation and regarding support for a new generation of dengue programming (PAHO, 2001a). The new generation of programmes for the prevention and control of dengue aims to strengthen prevention and control through community participation and health education (PAHO, 2001b). In 2003, the 44th Directing Council of PAHO/WHO approved Resolution CD44.R9, promoting the adoption of the Integrated Management Strategy for Dengue Prevention and Control (PAHO, 2003).

The WHO Regional Office for South-East Asia developed a regional strategy for the prevention and control of dengue fever/dengue haemorrhagic fever in 1995, revising it in July 2001. Different countries formulated control programmes according to their own priorities, infrastructure capacity, and resources (e.g. Thailand, Indonesia, Myanmar, Sri Lanka). The countries of this region have developed various models of community-based control programmes-based source reduction, which have met with varying degrees of success (WHO, 2006).

Dengue fever is also a growing problem in the WHO Region of the Western Pacific; more than 160 000 cases of dengue and dengue haemorrhagic fever were reported in this region in 2004. Despite the significance of dengue, activities for the prevention and control of dengue are under-funded in many countries of this region (WHO, 2005a).

PROGRAMME DELIVERY: THE TRADITIONAL MODEL OF ERADICATION VERSUS CONTROL

Control and eradication are two different strategies, with different methodologies and targets. The eradication strategy implies universal coverage of every breeding site of the mosquito in every house of every locality infested in the entire country, for the total elimination of the vector and subsequent permanent surveillance to detect reinfestation. The up-front cost of this strategy is high, but once the mosquito is eliminated, the cost of surveillance to detect reinfestation is much smaller and the transmission of dengue and urban yellow fever is totally prevented (Rodríguez Cruz, 2002).

The first eradication campaigns had great success in the 1950s and 1960s primarily because there was great political will for the implementation of the strategy, which was reflected in internal and external financing for personnel, insecticides and equipment. Great emphasis was placed on the reduction of sources of vector breeding; development and implementation of policies for adequate use of insecticides, including residual insecticides; and management through vertical, centralized and well-organized programmes based on strict discipline.

However, from the 1970s onwards, these results were not maintained and receded notably; the programme lost political importance and priority in the majority of the countries that had achieved eradication. Once reinfestation was detected, government response was very late; high costs were associated with providing materials, equipment, salaries and benefits for the workers that were not kept in their positions, and reinfestation was concomitant with the appearance in *Ae. aegypti* of resistance to organochlorated insecticides and the fast and rampant growth of surban centres. Currently, few countries in the world maintain a strategy of eradication, for example, in the WHO Region of the Americas only Cuba maintains these principles of work (Rodríguez Cruz, 2002).

A control strategy is based on preventing or reducing dengue epidemics and deaths caused by severe dengue; a secondary focus is on the prevention of urban yellow fever. This strategy identifies areas at greater risk and concentrates efforts on these areas in order to reduce, but not eradicate, the vector (Rodríguez Cruz, 2002). The cost of the control strategy is less than the cost of the attack phase of the eradication strategy, but higher than the maintenance phase of the eradication strategy (surveillance against vector reinfestation).

An intermediate strategy between control and eradication, especially when there are insufficient resources for universal coverage, would be the total elimination of the vector in limited high-risk areas, with a progressive expansion of these areas as funds permit, and with surveillance against reinfestation (Rodríguez Cruz, 2002).

National programmes, especially in the Americas, have been predominantly vertically structured; however, there is a growing trend in recent years towards decentralization of dengue control programmes. Unfortunately, this decentralization has often been applied indiscriminately and with little decentralization of financial and human resources, with a consequent loss of control capacity.

Current status of vector control programmes

Currently dengue is presented as a health problem whose magnitude exceeds the borders of the health sector; the prevention and control of dengue is the responsibility of not only the health sector but also of other government sectors.

There are several barriers to addressing the shortcomings of dengue programmes. These obstacles are very similar to those encountered in the past, but current working models are not sufficiently comprehensive and participatory to address service delivery problems in all its magnitude and dimensions. We highlight some elements that make this relevant:

- *Macrofactors related to dengue* – environmental, socioeconomic, political and social factors have a strong impact on dengue, and are associated

with the re-emergence of dengue as a serious issue. Climate change and ecosystem alterations have provided ideal conditions for expanding the geographical distribution of pathogens and vectors, and increases in migration and international traffic favour the spread of the vector and the disease.

- *Unprecedented population growth* – the world's population has tripled in the last 70 years – is also contributing to increasing the number of vector breeding sites. Also, *the presence of dengue in large urban centres, and especially in 'megacities'* (e.g. Rio de Janeiro, São Paulo, and Caracas), associated with urbanization that is neither planned nor controlled and poverty, with the absence of basic services (electricity, running water, sewer systems, refuse collection), poses new challenges and requirements for prevention activities and control. Such activities are expensive and require great coordination and synchronization, and the incorporation of extrasectorial actors, such as the tyre industry.
- The *local health services*, now politically and administratively responsible for disease prevention and control programmes, are generally not sufficiently prepared for the management of dengue control programmes, and resources are usually insufficient. The lack of human resources to cover the large number of houses reduces the quality of work, programme managers do not know how to prioritize areas of high complexity, and the work is converted into a routine household inspection with standard container-control messages offered to homeowners and businesses. The sustainability and continuity of control actions are always given a lower priority than other health demands and policy, with which they compete.
- Elements such as employment instability of the *workers* (i.e. vector control inspectors), training methods that continue to employ curricula content that does not lead to participatory models for vector control, and use of old control/eradication models in which the vector control inspector carries out control actions during his household visit prevent the transfer of responsibilities and creation of abilities to prevent and control *Aedes* breeding in the household and surrounding areas.
- In general, ministries have very few *external partners* and little ability to negotiate partnerships. There exists little communication, collaboration or integration between key components within ministries of health, (epidemiology, entomology, environment, health promotion, laboratory, etc.), as well as with other ministries, and governmental, nongovernmental and community agencies. Establishment of partnerships, traditional and non-traditional, may help to address the problem in all its magnitude and dimensions.
- Countries carry out *vector control primarily using insecticides.* Frequently, larvicides are applied to containers that could be destroyed or better managed; there is excessive use of ultra-low-volume application of adulticides in areas where there is no transmission of dengue. This method is useful as a support for the suppression of epidemics, but not for routine control (Rodríguez Cruz, 2002**).**
- *Participation of the community in the prevention and control of dengue* – the community has transferred the responsibility for *Ae. aegypti* control to the health sector as a result of the long-standing traditional vertical model (Toledo-Romani et al., 2006b). It is limited to response to official demands and control actions, and is not viewed as an empowerment process for the community. The work dynamic of the vector control inspectors and their interactions with families can be paternalistic; their focus is the destruction of the containers in which mosquitoes breed, with little ability to motivate residents towards ongoing environmental management of their premises. There is an evident need for matching the interests of residents and health-care providers in order to attain a significant social mobilization (Toledo-Romani et al., 2006b).
- Incorporation of the *Communication-for-Behavioural-Impact (COMBI)** planning methodology is opening new roads; in contrast to intensified routine control activities, a community-based intervention approach promises to be sustainable (Mosquera et al., 2006; Toledo-Romani et al., 2006a). There is still a need for monitoring and impact assessment of this planning instrument, and we cannot say that has been introduced and generalized in all programmes in the Americas.
- *Water-supply and waste-management systems* are limited in many high-risk areas; this facilitates vector proliferation and persistence. We point out that the high presence of plastic containers that can contain water and that are not biodegradable also facilitates vector persistence, because these containers remain for long periods in the environment and must be eliminated properly by man.
- *Operational research* on new approaches and control strategies has not been sufficient to investigate and monitor its impact.

* COMBI: http://www.paho.org/english/ad/dpc/cd/den-step-by-step.htm

The role of the vector control inspectors: what do we expect from them?

Any strategy or programme plan that is adopted may need the presence of field inspectors, employed either by the public or the community. The household visit is important as a preliminary and basic prevention activity for health promotion. These visits are a great opportunity (particularly in countries at risk of dengue, where large groups of the population have a low level of education), to review and determine the application of control actions.

However, the function of vector control personnel should be analysed seriously; traditional programmes do not have a sufficient impact in disease control owing to severe and ongoing reductions in personnel. A significant programmatic change is needed, and health services must have personnel who are able to interact with residents and who can assume a greater role as a health promoters and evaluators, without losing the point of entomological surveillance and vector control. These personnel should be part of epidemiological surveillance teams and the actions that they recommend or take should not be routine, but should be based on an analysis of the situation.

The great challenge is to provide these field staff with good communication skills, thus training is very important. Given that residents must also assume some responsibility and capacity for self-care, it is hoped that having better relationships with householders will improve the development of practical prevention actions, taking into account that the residents may need not only increased knowledge related to health, but also skill-building to carry out the recommended behaviour. Changing the current passive nature of the house visits by emphasizing communication and interpersonal contact can help transmit more appropriate messages that may modify behaviours related to breeding sites of *Ae. aegypti*. For this, the system has to provide adequate tools and materials for the inspectors that respond to this objective.

Dengue prevention and control programmes need to work with the community, women, young people and children directly; using organized networks that exist in the community is one way to achieve this. This may be a means to create comprehensive control with co-responsibility that is led jointly by the residents and municipalities; the programmes will have to change from the traditional model toward a participatory model, giving a comprehensive nature to the control measures. To achieve this end-point, models of mass interactive community–institution communication may need to be developed and tested.

STRATEGIC PARTNERSHIPS FOR VECTOR CONTROL

Strategic partnerships for dengue prevention and control have been identified as an important source of support for vector control programmes. These partnerships can promote the coordination of actions among the government, health sector and other social and economic sectors, volunteer and nongovernmental organizations, churches, local authorities, industry and mass media. Furthermore, the importance of adapting the programmes to the realities and local needs is recognized, taking into account social, cultural and economic differences.

State–industry–community partnership

Environmental management that promotes the elimination of vector breeding sites should be a priority in control programmes. Programmes that involve the creation of strategic partnerships should include intersectoral participation of public and private corporations with a strong component of community participation, as well as participation of different ministries and institutions with a greater direct relationship to the various components that lead to continued dengue transmission (e.g. ministry of health, of protection of the environment, of finance, of construction, of transportation, of sports), universities, nongovernmental organizations, importers of tyres, tyre repair shops, municipal government, among others. There could also be partnerships between the ministries of health and education, promoting dengue prevention during the teaching process among elementary-school students.

These partnerships can be promoted by the state, through the promulgation and implementation of laws that serve as a framework. For example, Puerto Rico, the United States of America, Spain, Costa Rica, Israel and Brazil have established decrees or laws for the adequate control and management of used tyres—the habitual breeding site of the vector in many countries and for which few or no adequate mechanisms exist for final disposal. Experience gained in Brazil is a positive example. In Brazil, the tyre-recycling industry employs more than 20 000 people directly, and involves nearly 15 companies and 21 factories. To date, 18 municipalities in 8 states are promoting tyre recycling. Other models of application of this have been observed such as the creation of artificial reefs (Colombia, Malaysia, Thailand, the Philippines), use of tyres in the cement industry (Brazil, Barbados), and use of tyres in

construction, lamination and for exportation. Used tyres also have uses in the construction of athletic fields, as roofing materials, vibration insulation and carpets, among others.

Ecoclubs

Ecoclubs are democratic organizations, with more than 15 000 volunteers distributed in 600 networks around the world (International Network of Ecoclubs, INE**). These networks link actions to various institutions of the community, visualizing an improvement of the quality of life. Ecoclubs promote actions in the health–environment axis, such as strategies for the rational use of water, dengue prevention, and waste management, among other topics. With sensitization campaigns coordinated with other institutions and communities, Ecoclubs involve neighbours via the use of participatory strategies and actions in the implementation of programmes that are characterized by their sustainability and that can be evaluated practically.

These experiences have demonstrated that large budgets are not necessarily needed to implement community programmes for the prevention and control of dengue; it is this philosophy, including different social actors for a common cause that Ecoclubs promote. But management guidance is needed and this is a role that should be played by health workers. However, there still are large gaps in information on the overall impact of the work of these associations.

OTHER PERSPECTIVES AND NEW TOOLS FOR VECTOR CONTROL

Integrated vector management

Vector control has mainly relied on the use of chemical insecticides and has not been very successful owing to human, technical, operational, ecological, and economic factors. Problems of insecticide resistance, costs and environmental concerns have resulted in a reduced reliance on insecticides, and an emphasis on the need for other vector control measures involving environmental management, biological control and personal protection. In addition, the Stockholm Convention on Persistent Organic Pollutants (POPs) adopted in 2001 (UNEP, 2001) requires a reduced reliance on, with a goal to eliminate, the use of DDT and other intentionally produced POPs and the promotion of research and development of safe alternative products, methods and strategies. The WHO Global Strategic Framework for Integrated Vector Management provides a basis for strengthening vector control in a manner that is compatible with national health systems (WHO, 2000a).

The integrated vector management (IVM) process aims to be effective and efficient. It uses indicators of impact on vector populations and disease transmission, and employs approaches compatible with local health systems. It is also robust enough to allow for effective planning and decision-making to take place at the lowest possible administrative levels (e.g. community level). It encourages a multi-disease and multi-strategy control approach whenever possible, and efficient integration with other disease control measures as well as the application of a range of interventions. Such a commitment requires an approach that effectively integrates the roles of the various sectors, including health, within a strategic management framework. Finally, IVM can also strengthen the rational use of insecticides, increasing their efficiency and impact and for the achievement of the Millennium Goals (WHO, 2004b).

IVM has been effectively applied in several regions and steps towards its implementation have been taken in the WHO South-East Asia, Western Pacific, Americas, Eastern Mediterranean and African Regions (WHO, 2005a, 2005b, 2004b). Good examples of its application have been provided by researchers in Viet Nam (Kay & Vu, 2005; Vu et al., 2005) and in Africa (Mukabana et al., 2006). IVM is based on the premise that effective control is not the sole preserve of the health sector but requires the collaboration of various public and private agencies, and community participation. The engagement of communities is a key factor in assuring sustainability, but further operational research is required to develop surveillance systems that are practical, affordable, effective and acceptable so that community-based IVM can be implemented (Vanek et al., 2006).

Ecohealth approach for dengue control and prevention

The aim of the Ecosystem Approach to Human Health (Ecohealth)*** is to improve community health through a holistic approach to the management of complex socio-ecological ecosystems. The International Development Research Centre (IDRC) of Canada has made an emphasis on assessing the potential of the Ecohealth approach to contribute to the prevention of vector-borne diseases, and more specifically with dengue (Lebel, 2003).

** Ecoclubs International: http://www.ecoclubes.org/DENGUE/ingles/dengue.asp

*** Ecohealth: http://www.idrc.ca/in_focus_health/

The Ecohealth approach is being supported by the Special Programme for Research and Training in Tropical Diseases (TDR), an independent collaborative programme financed jointly by the United Nations Development Programme (UNDP), the United Nations Children's Fund (UNICEF), World Bank and WHO. With support from IDRC, TDR is applying the Ecohealth approach in two research programmes in South America. Furthermore, the Pan American Health Organization (PAHO) provides support for the implementation of this approach in two projects on dengue in Central America and the Caribbean. These projects are also supported by the United Nations Environment Programme (UNEP). In Guatemala, researchers are developing a 'Community strategy for the reduction of dengue and diarrhoeal diseases in urban ecosystems'; on the border of Guatemala and Mexico the 'Development and validation of a community strategy for the reduction of the risk of dengue and diarrhoea in urban ecosystems' is being carried out; and in the City of Havana, Cuba, a 'model for sustainable development and healthy municipal environments in an approach to ecosystem in human health for the prevention of dengue at the local level' is being tested (Lebel, 2003). In these large agglomerations, many groups with diverse interests interact: the private sector, civil society, municipal authorities, different ethnic groups, castes, and social classes, men and women. All play a role in the management of the urban ecosystem.

Integrated Management Strategy for Dengue Prevention and Control (EGI-Dengue)

The Integrated Management Strategy for Dengue Prevention and Control in the Americas (EGI-Dengue) addresses the issue of how to achieve effective programmatic integration of prevention and control actions. This introduces a new form of technical cooperation between PAHO and member countries through the 'dengue task force' (known by its Spanish abbreviation 'GT-Dengue International'). The GT-Dengue task force is a group of technical experts from across the region who, starting with a regional analysis, works with the dengue technical teams in each country to develop a national strategy for integrated operations. From these initial work plans, efforts are made in consultation with other countries to change existing programme practices and implement the new integrated strategy for dengue prevention and control. The new integrated management strategy is horizontal, intersectoral, inter-programmatic, and seeks changes in behaviour at all levels to reduce the risk factors for dengue.

The purpose of this strategy is to achieve a sustainable national strategy that allows a functional integration of actions among its key components (social communication, epidemiological surveillance, entomology, patient care, laboratory and environment), designed by the country with technical cooperation from the GT-Dengue, using a multisectoral, intersectoral, and interdisciplinary (integrated) approach, based on new practices that permit the evaluation and continuity of the activities, with national resources (PAHO, 2003).

The Integrated Management Strategy for Dengue Prevention and Control demands research on new indicators that better measure the risk of transmission, and environmental and behaviour indicators in order to know what the behavioural impact has been. Indicators are also needed to investigate new or modified existing practices both for surveillance (e.g. MosquiTrap, LIRAa), control (e.g. impregnated curtains, dabbed bleach), and management and integration processes that each country prepares using a log-frame matrix (EGI-Dengue).

Communication for Behavioural Impact (COMBI)

COMBI is a novel approach in the design and implementation of behaviourally focused social mobilization and communication actions for the control of communicable diseases. It is a planning methodology for programme managers to prepare, implement and evaluate the social mobilization and communication interventions developed as part of the integrated plans (Mosquera et al., 2006).

The general strategy for preventing and controlling dengue and dengue haemorrhagic fever is based on promoting behaviour changes that lead to involving the community as a partner in controlling the disease, particularly the vector. In order to achieve this, dengue communication programmes should have two primary aims: converting information into practice and working with the community to adopt and maintain appropriate and relevant prevention and control measures. The new generation of programmes should be designed taking into account the local sanitation structure (water distribution and waste disposal) as well as information on community organizations and the roles of different family members. Furthermore, new vector control models should incorporate all ten components of an integrated programme (PAHO, 2001b): epidemiological surveillance, intersectoral actions, community participation, managing the environment and basic services, patient care, case reporting, education, rational use of insecticides and vector control, training, and preparing for emergencies. Communication

should be aimed at supporting positive mosquito-control behaviours among individuals and the community, and their empowerment to identify and carry out community-relevant prevention and control measures.

Geographic information systems

While investigating the spatial patterning of health events and disease outcomes has a long history, the development of geographic information systems (GIS) has facilitated the inclusion of a spatial component in epidemiological and entomological studies. GIS is a computer system that allows the collection, storage, integration, analysis, and display of spatially referenced data. In the field of health, GIS has been widely used for disease mapping of different pathologies, in analysis of space and space–time distributions of disease data, in identifying risk factors, and in mapping risk areas. In most studies, each patient or person exposed to a disease is located at the residential address, and these locations are integrated into GIS for mapping and analysis. Because GIS allows epidemiologists to map environmental factors associated with disease vectors, it has become especially relevant for the surveillance of infectious and vector-borne diseases such as dengue and malaria (Napier, 2003; Tran et al., 2004).

Examples of the use of this technology include the geographic analysis conducted for the 2001–2002 outbreak of dengue fever in the state of Hawaii (Tran et al., 2004). In another study, a GIS spatial/temporal analysis depicting the spread of the disease and a spatial dengue threat model (DTM) were created. In addition, GIS case-clustering and mean/median distance comparison analysis of homes in rural and semi-urban areas was conducted. This model may be adapted for use as a predictor in other arbovirus (arthropod-borne virus) outbreaks in various geographic locals.

Rapid Survey Index for *Ae. aegypti* for estimating the Breteau and house indices (LIRAa)

Simpler methods for sampling have been proposed, with the objective of facilitating the acquisition of information that contributes to the evaluation of health-services programmes through the conduct of systematic and periodic research. There are simplified methods to estimate entomological indices, associated with acceptable errors of margin that are also rapid and economical. Such is the example of the Rapid Survey Index for *Ae. aegypti* for estimating Breteau and house indices developed in Brazil (LIRAa in Portuguese). The implementation of this system permits the dengue programme manager to target control measures to the areas of highest risk, thereby permitting better use of human resources and of available materials not only during routine control activities but also in critical periods with higher numbers of cases that might indicate an outbreak. The National Program for the Control of Dengue (PNCD) of Brazil, launched in July 2002 by the Ministry of Health, uses this methodology as a component of epidemiological surveillance (Ministério Da Saúde, 2005).

MosquiTRAP

MosquiTRAP is a novel, simple, easy-to-use, low-cost, and efficient trap developed to catch *Aedes* mosquitoes. It relies on visual cues and synthetic oviposition attractants (AtrAedes), based on volatile substances identified from grass infusions. Compared with ovitraps, the MosquiTRAP allows the identification of mosquito species in the field, thus saving time and avoiding laboratory routines such as counting eggs and larval identification. Trapped mosquitoes can also be used for virus diagnosis. New entomological indices are: (a) the positive MosquiTRAP index (PMI), which is the percentage of positive traps; and (b) the adult density index for *Ae. aegypti* and *Ae. albopictus*. Field data can be collected using hand-held PDAs (personal digital assistants) and then loaded directly into a GIS program, for an efficient determination of local entomological indices. At the moment, a national monitoring programme in Brazil using this technology is being established (Eiras et al., 2004).

The new technology for the monitoring and generation of indices for entomological surveillance, composed of MosquiTRAP, AtrAedes for oviposition, and a system of computerized monitoring is promising and should be considered for possible future use as results on efficacy and efficiency are published in the literature.

RESEARCH AND DEVELOPMENT: OBSERVATIONS

Efforts based solely on chemical vector control have been insufficient in modern times. Moreover, evidence demonstrates that educational measures do not modify the behaviours or habits of the population (Texeira et al, 2005). Thus, as a vaccine is not available, further dengue control depends on potential results from basic interdisciplinary research and intervention studies, integrating environmental change, community participation and education, epidemiological and virological surveillance, and strategic technological innovations aimed at stopping transmission. Some examples of these research efforts are:

- The Innovative Vector Control Consortium (IVVC) will address the market for new insecticides by developing a portfolio of chemical and technological tools that will be directly and immediately accessible to populations in the developing world (Hemingway et al., 2006).
- Searching for new bioactive, environmentally friendly and biodegradable natural insecticides and repellents, particularly from botanical sources in Thailand, China, Libya, Burkina Fasso, India and other countries (Choochote et al., 1999, 2005; Bassole et al., 2003; Tuetun et al., 2005; Kamsuk et al., 2006; Chaiyasit et al., 2006; Ravi Kiran et al., 2006; Amer et al., 2006).
- *Ae. aegypti* population replacement: A proposed strategy to aid in controlling the growing burden of vector-borne disease is population replacement, in which a natural vector population is replaced by a population with a reduced capacity for disease transmission. Endosymbiotic *Wolbachia* bacteria are potential transgene drivers. Stable infections of wAlbB *Wolbachia* were established in *Ae. aegypti* and caused high rates of cytoplasmic incompatibility (that is, elimination of egg hatching). Laboratory cage tests demonstrated the ability of wAlbB to spread into an *Ae. aegypti* population after seeding of an uninfected population with infected females, reaching infection fixation within seven generations (Xi et al., 2005).
- A web-based multimedia spatial information system was used to support a study of the re-invasion of *Ae. aegypti* in the deserts of the south-west United States/north-west Mexico. The system was developed by applying open geospatial consortium and worldwide web consortium open specifications and using open source software. The system creates a sensory-rich environment, one that allows users to interact with the system to explore connections among data (maps, remotely sensed images, text, graphs, 360 degree panoramas and photos), visualize information, formulate their own interpretations, generate hypotheses and reach their own conclusions. (Moreno-Sanchez et al., 2006).
- Evaluating the practicality of a survey method based on the rationale that certain water containers are particularly productive of the dengue vector, *Ae. aegypti* and whether this can consistently identify and classify particularly productive classes of container, and so provides guidance on the development of targeted control strategies. This was done as study involving nine Latin American, Asian, and African countries (Focks & Alexander, 2006).

The time has come to restore vector control to its key position in the prevention of disease transmission, albeit with an increased emphasis on multiple measures, which may include use of pesticides and environmental modification, and with a strengthened managerial and operational capacity (Townson et al., 2005). Today, prevention and control of dengue require consideration of a wider perspective than simply tropical disease. Many of the affected countries are also some of the poorest. Approaches that are realistic for limited infrastructures need to be urgently developed. A systematic approach and a clear international research agenda can quickly bring forward the frontiers of knowledge. Better understanding of the above will not only feed into operational policies for dengue control, but also provide fertile terrain for vaccine application strategies in the future. Accelerating the research programme, with emphasis on mechanisms of transmission dynamics, validation and improvement of existing or new vector control methods and their application, partnership building, and formulation of guidelines for research will help in these strategic areas (Guha-Sapir et al., 2005).

Based upon and guided by scientific knowledge and operational research, and subject to routine monitoring and evaluation of control activities, the strategies and interventions need to be adapted to local vector ecology, epidemiology and resources. Well-targeted operational research is urgently needed to make progress in dengue prevention and control.

PRIORITY RESEARCH RECOMMENDATIONS FOR THE NEXT FIVE YEARS (2007–2011)

- Assessment of the impact of dengue prevention and control activities that have incorporated the use of new methodological instruments, strategies, technologies etc.
- Investigate potential indicators for risk of transmission with greater sensitivity than the current entomological indicators.
- Development of mathematical prognostic models, geographic or others, which consider different levels of risk of transmission.
- Studies of cost–effectiveness of the new tools, strategies, and instruments being developed and incorporated into programmes.

CONCLUSIONS

In this document we have summarized current approaches and the status of recent ideas and technologies that are being tested, in particular in the Americas, in response to the broader question of how dengue prevention and control interventions are currently being delivered and/or developed. Nevertheless, some questions still do not have a conclusive answer:

1. What do we expect from vector control services, particularly from vector control inspectors during household visits? Should they continue these visits or does this component need to be changed? Do we need to seek other associations in order to transfer the responsibilities of what they currently do to a more appropriate group locally?
2. Can we change the current control services in other ways? How can we work with the population to change attitudes toward control strategies?
3. Which are the most cost-effective strategies, comparing traditional vector control with new tools and managerial and organizational strategies? If the new tools are effective, (COMBI, LIRAa, GIS, among others) can they be generalized? What operational research is needed to strengthen vector control service delivery?

We look forward to a rich scientific exchange that will contribute new ideas and knowledge to these issues.

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