

Dengue Bulletin

Volume 36, December 2012



**World Health
Organization**

South-East Asia Region

Western Pacific Region

From the Editor's Desk

During 2011, the incidence of dengue fever (DF)/dengue haemorrhagic fever (DHF) showed a declining trend in the countries of the WHO South-East Asia and Western Pacific regions. Countries in the South-East Asia Region reported 172 053 cases with 871 deaths [case-fatality rate (CFR) 0.5%]. Although all countries showed a marginal decline, Thailand and Indonesia showed a substantial decline. India, Indonesia, Sri Lanka and Thailand reported more than 25 000 cases each with a CFR of 0.87%, 0.63% and 0.09% respectively.

The WHO Western Pacific Region reported a total of 230 408 cases with 811 deaths, with a CFR of 0.35%. Dengue activity in the Region was variable. While Cambodia reported more cases than in 2010, Lao PDR, Malaysia, the Philippines, Viet Nam and Australia showed a declining trend. Singapore remained static.

A mapping of the prevalence of vector-borne diseases (VBDs) in the WHO South-East Asia Region was undertaken during 2011. The maximum burden of VBDs in the Region is borne by India, followed by Bangladesh, Sri Lanka and Thailand. The lowest prevalence is in Democratic People's Republic of Korea. Malaria and dengue are prevalent in all Member States, with the exception of malaria in Maldives and dengue in Democratic People's Republic of Korea. Lymphatic filariasis is prevalent in nine of the 11 Member countries. Bhutan joined the list of endemic countries reporting visceral leishmaniasis (kala-azar) in 2006.

The WHO Western Pacific Region has initiated a number of activities on the clinical management of DF/DHF conforming to the WHO revised guidelines for diagnosis, treatment, prevention and control of DF/DHF. These included holding of an Expert Consultation on Regional Clinical Management, drawing on the expertise of the University of Malaya and using material from the United States Centers for Disease Control and Prevention and Singapore. The Western Pacific Surveillance and Response Journal published by the WHO Western Pacific Regional Office brought out a special issue entitled "Dengue Special" during 2011 which highlighted dengue lessons, challenges and new approaches.

The current volume of *Dengue Bulletin* (No. 36, 2012) contains contributions by authors from the WHO regions of South-East Asia (14), the Western Pacific (5), the Eastern Mediterranean (3) and the Americas (2).

We now invite contributions for Volume 37 (2013). The deadline for the receipt of contributions is 31 May 2013. Contributors are requested to please peruse the instructions given at the end of the *Bulletin* while preparing their manuscripts. Contributions should either be sent accompanied by CD-ROMs to the Editor, *Dengue Bulletin*, WHO Regional Office for South-East Asia, Mahatma Gandhi Road, I.P. Estate, Ring Road, New Delhi 110002, India, or by e-mail as a file attachment to the Editor at dengue@searo.who.int. Readers desirous of obtaining copies of the *Dengue Bulletin* may write to the WHO regional offices in New Delhi or Manila or the WHO Country Representative in their country of residence.

Dr A.P. Dash
Regional Adviser, Vector-Borne and Neglected Tropical
Diseases Control, and
Editor, *Dengue Bulletin*
World Health Organization
Regional Office for South-East Asia
New Delhi, India.

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Contents

1. Dengue in South-East Asia: an appraisal of case management and vector control 1
AP Dash, Rajesh Bhatia and NL Kalra
2. Identifying requirements for targeted risk communication in prevention of dengue transmission in vulnerable areas, Mawlamyaing, Myanmar 14
Pe Thet Zaw, Khin Thet Wai, Tin Oo, Zaw Win, Hlaing Myat Thu, Moe Thida, Phyu Sin Aye and Tin Tin Wai
3. The benefits and challenges of scaling up dengue surveillance in Saudi Arabia from a GIS perspective 26
Hassan M Khormi, Ramze A Elzahrany and Lalit Kumar
4. Trends of dengue infections (1997–2008) in Cebu Province, Philippines 37
Frances Edillo and Susana Madarieta
5. Dengue in the northernmost part of Brazil from 1999 to 2011: characterization of circulating DENV strains 50
Pablo Oscar Amézaga Acosta, Joel da Silva Cordeiro, Fabiana Granja, Thalita Caroline da Silva Siqueira, Francisco Eduardo Gomes Brito, Aline Gondim de Freitas, Débora Dinelly de Sousa, Joel de Melo Lima, George Allan Villarouco da Silva, Raphaela Honorato Barletta-Naveca, Victor Costa de Souza, Vera Margarete Scarpassa and Felipe Gomes Naveca
6. Climatic factors affecting dengue fever incidence in Lahore, Pakistan 64
Hassan Z Baig and Anem J Nawaz
7. Risk assessment and risk maps using a simple dengue fever model 73
José Eduardo Marques Pessanha
8. *Aedes* survey following a dengue outbreak in Lahore, Pakistan, 2011 87
Muhammad Saeed Akhtar, Ayesha Aihetasham, Mehwish Saeed and Ghazanfer Abbass
9. Performance of WHO probable case definition of dengue in Kerala, India, and its implications for surveillance and referral 94
Zinia T Nujum, Vijayakumar K, Pradeep Kumar AS, Anoop M, Sreekumar E, Ramani Bai JT, Dalus D, Lalitha Kailas, Saritha N, Anitha Abraham, Anto Varghese, Raji RT and Sudheesh Kumar TK



10. Early predictors of dengue infection in adults (EPOD) – a Malaysian outpatient experience..... 105
Emer Breen, Indrani Pemmulu, Daisy SH Ong, Shamala Devi Sekaran, Praba Appana, Thien Hoong Tew, EM Khoo, Lesley Pillans, Noor Azina Ismail, Sharifah FS Omar, Sasheela Ponnampalavanar, R Vhimaleshwari and Lucy CS Lum

11. Levels of serum transaminases in patients of dengue fever during the 2011 outbreak in Lahore, Pakistan..... 116
Sabrina Rashid and Farhana Shahzad

12. Dengue-specific IgA in different body fluids: a prospective alternative for dengue diagnosis in resource-poor settings 124
Rabeeya Sharmin, Shahina Tabassum, KZ Mamun, Afzalun Nessa and Munira Jahan

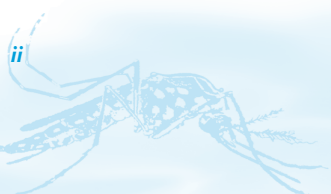
13. A clinicroadiological and laboratory analysis of dengue cases during an outbreak in central Nepal in 2010 134
Arun Sedhain, Shital Adhikari, Gandhi R Bhattarai, Sudhir Regmi, Lok R Subedee, Sunil K Chaudhary, Manoj Shah and Barun Shrestha

14. Evidence for the use of intravenous rehydration for treating severe dengue with plasma leakage in children and adults: a systematic review 149
Usman HB, Safitri I, Lum L, Martinez E, Kroeger A, and Horstick O

15. Relationships between *Aedes* indices and dengue outbreaks in Selangor, Malaysia 166
Shamsul Azhar Shah, Jamiatul Aida Mohd Sani, Mohd Rohaizat Hassan, Nazarudin Safian, Azimatun Noor Aizuddin and Rozita Hod

16. Water quality and *Aedes* larval mosquito abundance in Caloocan city, Philippines 175
Glenn Sia Su, Abigail Beronilla and Kathleen Beatrice Yao

17. Baseline and key container survey for *Aedes aegypti* and *Aedes albopictus* in Albay Province, Philippines..... 182
A Schapira, C Garcia, M Asor, I Riquentino, L Bahillo, M Palattao, P Morano, T Mirandilla, K Discaya, M Banzuela, R Berango, F Buban, R Caldino, M Cedeno, D Dayandante, R Delfin, L Grageda, S Oguan, E Olitan, A Quitasol, S Rabano, S Sabareza, MR Sy, B Tapit, L Vasquez, L Vicera, J Aguilar, Coper, S Candila, G Flores, J Garcia, L General, A Gutierrez, L Lazona, JA Balangat, K Rodriguez, K Almiñe, MM Camata, C Aquino and L Mendoza



18. Knowledge of dengue and related preventive attitude and practices among urban slum dwellers of Jaipur city, Rajasthan, India..... 197
Vivek Lal, Dinesh Chandra and Shiv Dutt Gupta
19. Community-based dengue source reduction interventions in two townships of Yangon region that significantly reduced entomological indices..... 206
Zaw Lin, Tun Lin W, Tin Oo, Khin Thet Wai, Hla Myint and Monti F
20. Comparative field efficacy of different formulations of trypsin modulating oostatic factor-*Bacillus thuringiensis israeliensis* (TMOF-Bti) against *Aedes aegypti* Linnaeus by manual application and Ultra-Low Volume (ULV) Spray at Setapak high-rise flats in Kuala Lumpur, Malaysia..... 221
Saiful Azlan Nordin, Lau Ming Seong, Sallehudin Sulaiman, Paramessavathy Ramanathan, Zainol Ariffin Pawanchee, Sallehudin Abu Bakar and Hidayatulfathi Othman

Short Notes

21. Guillain-Barré syndrome as a rare complication of dengue fever: a report of two cases 231
Dinesh Srivastava, Kailash Kumar Goyal, Sudeep Vaniyath and Dhananjaya Melkunte Santhaiah

Book reviews

22. Dengue and severe dengue factsheet (Revised January 2012) 236
23. Global strategy for dengue prevention and control 2012–2020..... 240
24. Handbook for clinical management of dengue..... 242
25. Handbook for integrated vector management..... 244

Obituary

26. In memory of the late Professor Susumu Hotta 246
Eiji Konishi
27. Instructions for contributors 249



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1. Ataru Tsuzuki
Department of Vector Ecology &
Environment
Institute of Tropical Medicine,
Nagasaki University
Nagasaki City
Japan
E-mail: atarutsuzuki@hotmail.com
2. Audrey Lenhart
Entomology Branch
Division of Parasitic Diseases and Malaria
Center for Global Health
Centers for Disease Control and Prevention
Atlanta, GA, USA
Email: AJL8@cdc.gov
3. Brian Kay
Queensland Institute of Medical Research
Royal Brisbane Hospital
Brisbane, Australia
E-mail: Brian.Kay@qimr.edu.au
4. Bruno Guy
Research and Development
Sanofi Pasteur
Marcy l'Etoile, France
E-mail: bruno.guy@sanofipasteur.com
5. Catherine Laughlin
Virology Branch
Division of Microbiology and Infectious
Diseases
National Institute of Allergy and Infectious
Diseases
Bethesda, MD, USA
E-mail: cloughlin@niaid.nih.gov
6. Cox van de Weg
Department of Virology
Erasmus Medical Center
Netherlands
E-mail: c.vandeweg@erasmusmc.nl
7. Duane J Gubler
Program on Emerging Infectious Diseases
Duke-NUS Graduate Medical School,
Singapore 169857
E-mail: duane.gubler@duke-nus.edu.sg
8. Ekpereonne Esu
Department of Public Health
Faculty of Allied Medical Sciences
College of Medical Sciences
University of Calabar
Calabar, Nigeria
E-mail: ekpereonneesu@gmail.com
9. Florence Fouque
Institut Pasteur de la Guadeloupe
Laboratoire d'Entomologie Médicale
Unité Environnement et Santé
Morne Jolivière,
Les Abymes cedex
E-mail: ffouque@pasteur-guadeloupe.fr;
flfouque@gmail.com
10. Guey Chuen Perng
Emory Vaccine Center
Emory University School of Medicine
Atlanta, USA
E-mail: gperng@emory.edu;
pernggc@gmail.com
11. Hassan M. Khormi
Ecosystem Management
School of Environmental and Rural Sciences
Faculty of Arts and Sciences
University of New England
Armidale, NSW, Australia
E-mail: hkhormi@myune.edu.au;
khormi10@hotmail.com
12. Ichiro Kurane
National Institute of Infectious Diseases
Tokyo, Japan
E-mail: kurane@nih.go.jp



13. Jared Aldstadt
Department of Geography
University at Buffalo
Buffalo, New York, USA
E-mail: geojared@buffalo.edu
14. Jeffrey L. Lennon
Liberty University
Lynchburg, VA, USA
E-mail: jeffchona2@yahoo.com
15. Jennifer Duncombe
School of Population Health
University of Queensland
Brisbane, Queensland, Australia
E-mail: j.duncombe@uq.edu.au
16. Jerry M. Spiegel
University of British Columbia (UBC) –
Global Health Research Program
School of Population and Public Health
Vancouver, British Columbia, Canada
E-mail: jerry.spiegel@ubc.ca
17. Kendra Mitchell-Foster
Interdisciplinary Studies Graduate Program
Global Health Research Program
University of British Columbia
Vancouver, BC, Canada
E-mail: foster5@interchange.ubc.ca
18. Lars Eisen
Colorado State University
Department of Microbiology,
Immunology and Pathology
Fort Collins, CO, USA
E-mail: lars.eisen@colostate.edu
19. Linda Lloyd
443 Whittier St.
San Diego, CA, USA
E-mail: lindalloyd01@gmail.com
20. Linda M. Kaljee
Wayne State University
Carmen and Ann Adams Department of
Pediatrics
Pediatric Prevention Research Center
Detroit, MI, USA
E-mail: lkaljee@med.wayne.edu
21. Lourdes Esteva
Departamento de Matemáticas
Facultad de Ciencias
Universidad Nacional Autónoma de México
México, DF, Mexico
E-mail: lesteva@matematicas.unam.mx
22. Mullica Jaroensutasinee
Centre of Excellence for Ecoinformatics
School of Science
Walailak University
Nakhon Si Thammarat, Thailand
E-mail: jmullica@gmail.com
23. Rafael Maciel de Freitas
Oswaldo Cruz Institute
Rio de Janeiro Brazil
E-mail: macieldefreitas@gmail.com
24. Roberto Barrera
Entomology and Ecology Activity
Dengue Branch
Centers for Disease Control and Prevention
San Juan, Puerto Rico
E-mail: rbarrera@cdc.gov
25. Rosanna Peeling
Department of Clinical Research Unit
London School of Hygiene & Tropical
Medicine
London, United Kingdom
E-mail: rosanna.peeling@lshtm.ac.uk
26. Siripen Kalayanaroj
Queen Sirikit National Institute of
Child Health
Bangkok, Thailand
E-mail: siripenk@gmail.com
27. Tassanee Silawan
Department of Tropical Hygiene
Faculty of Tropical Medicine
Mahidol University, Bangkok, Thailand
E-mail: tsilawan@gmail.com



28. Vanessa Racloz
School of Population Health
University of Queensland
Brisbane, Queensland, Australia
E-mail: v.racloz@uq.edu.au
29. Van-Mai Cao-Lormeau
Infectious Diseases Unit
Institut Louis Malardé
Papeete, Tahiti
French Polynesia
E-mail: mlormeau@ilm.pf;
m.lormeau@gmail.com
30. Veerle Vanlerberghe
Unit of General Epidemiology and
Disease Control
Public Health Department
Institute of Tropical Medicine
Antwerp, Belgium
E-mail: vvanlerberghe@itg.be
31. Vijay K. Saxena
Independent Consultant
(Vector Borne Diseases)
New Delhi, India
E-mail: saxena101@yahoo.com

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Dengue in South-East Asia: an appraisal of case management and vector control

AP Dash,^{a#} Rajesh Bhatia^b and NL Kalra^c

^a*Vector-Borne and Neglected Tropical Diseases, Department of Communicable Diseases, World Health Organization, Regional Office for South-East Asia, New Delhi, India*

^b*Department of Communicable Diseases, World Health Organization, Regional Office for South-East Asia, New Delhi, India*

^c*Former Deputy Director, National Malaria Eradication Programme, Delhi, India*

Dengue disease severity

Dengue is regarded as one of the most important arboviral infections in the world. Dengue fever (DF), including its variants, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), is caused by four antigenically distinct but related dengue viruses (DENV-1, DENV-2, DENV-3 and DENV-4), also known as serotypes, belonging to genus *Flavivirus*, family *Flaviviridae*.¹

There exists a considerable variation within each serotype in the form of phylogenetically distinct subtypes or genotypes. Currently, each serotype has subtype/genotype as follows:¹

- DENV-1: Three
- DENV-2: Two (one is found in non-human primates)
- DENV-3: Four
- DENV-4: Four (one is found in non-human primates).

Infection with any one serotype confers lifelong immunity to that serotype but only two- to three-months' immunity to other serotypes. Infection with another serotype or multiple serotypes leads to severe forms of dengue (DHF/DSS).¹

Today, all serotypes/genotypes are circulating globally and all areas, which used to report epidemics of dengue earlier, are now hyperendemic areas including the WHO South-East Asia Region.

[#]E-mail: dasha@who.int



Dengue is transmitted primarily by *Aedes (Stegomyia) aegypti*, and *Aedes (Stegomyia) albopictus* is the secondary vector. *Ae. aegypti* is a native of Africa which spread to other continents through slave trade and, subsequently, by globalization of trade and commerce. Today, it is regarded as a cosmopolitan species breeding in urban areas between latitude 45°N and 35°S.²

Disease burden

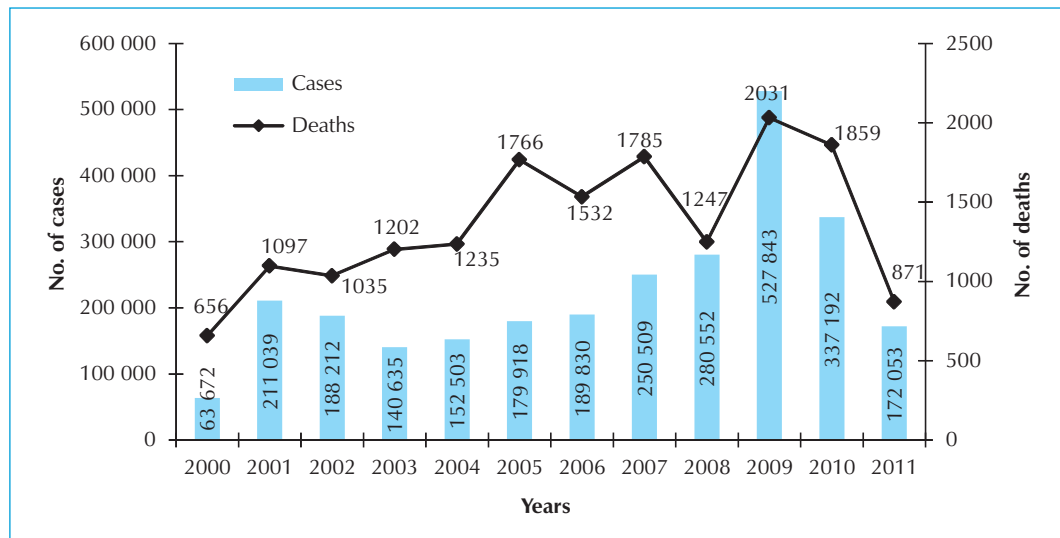
Global

As per the World Health Organization, dengue has shown a 30-fold increase globally over the past five decades.³ Some 50 to 100 million new infections are estimated to occur annually in more than 100 endemic countries. Every year, hundreds of thousands of severe cases arise resulting in 20 000 deaths.³

WHO South-East Asia Region

The WHO South-East Asia Region comprises 11 countries with a population of 1.3 billion. Bhutan and Nepal reported epidemics in 2004 only, whereas Democratic People’s Republic of Korea has not yet reported any dengue outbreak. Figure 1 shows dengue cases and deaths for the years 2000 to 2011 in SEAR.

Figure 1: Dengue cases and deaths in the WHO South-East Asia Region



Source: WHO Regional Office for South-East Asia, New Delhi



From the figure, it is apparent that the South-East Asia (SEA) Region has become hyperendemic with regular reporting of dengue cases since the year 2000. The maximum number of cases (527 843) and deaths (2031) were recorded during 2009. Since then, a declining trend is being reported. Maybe it is a cyclic trend and/or non-induction of virulent serotypes/genotypes (source: WHO Regional Office for South-East Asia, New Delhi).

Case definition/Revised classification

Dengue is now most prevalent in tropical countries in Asia, Latin America and the Caribbean. Early detection of dengue cases and their treatment is essential to prevent deaths. The first-ever attempt for classification and management of DF/DHF was developed on the basis of 123 Thai children admitted to the Children's Hospital in Bangkok. Subsequently, researchers' recommendations evolved into WHO guidelines in 1974, which were updated in 1986, 1994 and in 1997.⁴ These guidelines were adopted by the WHO South-East Asia Region⁵ and the Region of the Americas.⁶ However, these guidelines were not validated. During 2009, Dr Alex Kroger, Scientist, Tropical Diseases Research, WHO headquarters⁷ presented a paper entitled "Reclassifying dengue" before a meeting of the WHO Advisory Committee on Dengue and Other Flavivirus Vaccines in 2009.⁷ He gave details of the difficulties faced by clinicians in some situations in classifying their patients as per existing WHO guidelines.⁴ These conditions included the following:

- dengue with haemorrhagic manifestations but without vascular leakage;
- dengue with shock syndrome (up to 18%) but without fulfilling the four criteria;
- organ failure reported in severe disease that does not meet WHO criteria.

He further presented the details of DENCO (dengue control), a multicentre prospective clinical study conducted during 2004–2008.⁷ On the basis of this study, a revised classification system on 'severity score' on the basis of interventions was presented. This was proposed in order to make it possible to distinguish between 'severe' and 'non-severe (mild/moderate)' dengue with a sensitivity and specificity of around 95%. To determine which case might progress from mild/moderate dengue to severe dengue, the following 'warning signs' were identified as predictors of progression:

- abdominal pain/tenderness
- mucosal bleeding
- rash
- lethargy
- low albumin
- low platelets
- increased haematocrit.



Dr Kroger added: “It is planned to validate the study of revised classification in clinical practice and surveillance involving 18 countries, further analyse the predictive value of ‘warning signs’ and the signs and symptoms of probable dengue.”

With this background, and on the recommendation of an expert group, WHO adopted the revised classification in 2009 based on the level of severity, i.e. dengue with warning signs and severe dengue.⁸

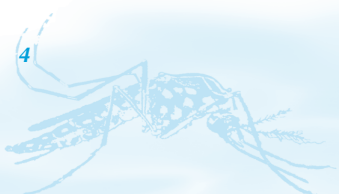
The validation studies carried out in 18 countries concluded that the applicability of the DF/DHF/DSS classification was limited even when strict DHF criteria were not applied (13.7% dengue cases could not be classified using the DF/DHF/DSS classification by experienced reviewers, compared with only 1.6% with the revised classification). Therefore, it was concluded that the revised dengue classification carried a high potential of facilitating dengue case management and surveillance. However, the need for the validation of “warning signs” and “probable dengue” required further research.⁹

Subsequently, WHO-TDR took the further initiative of reviewing the development, evidence base and application of the revised dengue case classification.¹⁰ This document established that the revision meant a major change not only for the health care professionals, but also for surveillance officers and researchers. An analysis has shown that the revised dengue case classification is better able to standardize clinical management, raise awareness about unnecessary interventions, and match patients’ categories with specific treatment.

In yet another initiative, WHO-TDR has brought out a *Handbook for clinical management of dengue*¹¹ for reorientation of clinicians/health care professionals for the clinical management of dengue cases as per the revised classification.

The subject of WHO revised classification 2009 is being hotly debated. Kalayanarooj in Thailand¹² carried out a prospective study of suspected dengue patients admitted in the Dengue Unit of the Queen Sirikit National Institute of Child Health (also the WHO Collaborating Centre for Case Management of Dengue/DHF/DSS) between June and August 2009. The final diagnosis was based on the current WHO classification together with laboratory confirmation. The TDR classification was applied later by the author using the data from the study case-report form of each patient. A statistical analysis comparing the clinical and laboratory data between each group of patients was done using software SPSS version 14. As a conclusion, the author recommended continued use of the current WHO classification because, in her opinion, the newly suggested TDR classification creates about twice the workload on health-care workers. In addition, the TDR classification needs dengue confirmatory tests. More than 90% of the DHF defined by the WHO case definition is dengue-confirmed.

However, the current WHO classification needs to be modified to be simple and more user friendly. The suggested modification is to address plasma leakage as a major criterion.



Positive tourniquet test or bleeding symptoms can be combined as minor criteria. Unusual dengue is proposed to be added to the current WHO classification to cover those patients who do not fit in the current WHO classification.

Hadinegoro¹³ found difficulties in the application of revised classification in Indonesia and suggested that elements from the revised classification (2009) should be incorporated into the 1997 guidelines,⁴ which remain relevant for use. He further suggested multicentre, i.e. prospective studies using standardized protocols, in Asia and Latin America in a full range of patients' age groups.¹³

The WHO-TDR Expert Meeting on Effective, Affordable and Evidence-based Dengue Early Warning and Response System held on 28–30 June 2012 Freiburg, Germany, recommended separate guidelines for dengue case management of children and adults.¹⁴ Sri Lanka has already developed national guidelines for management of DF and DHF separately for children and adults (personal communication: Dr S.L. Hoti, Vector Control Research Centre, WHO Collaborating Centre for Research and Training in Lymphatic Filariasis and Integrated Methods of Vector Control, Puducherry, India).

In view of the aforesaid, WHO's Regional Office for South-East Asia adopted an attitude of 'wait and see' until validation of the revised classification. Furthermore, in view of the experience gained over a decade, the expert group of WHO-SEAR did not follow the results of the DENCO clinical study,⁷ but further expanded the existing guidelines by incorporating the range and frequencies of constitutional symptoms under each category of DF, DHF and DSS. The existing DHF grades 1–4 have been re-categorized as DHF without shock and DHF with shock.¹⁵

A comparative case definition of dengue guidelines adopted by WHO headquarters⁸ and the WHO South-East Asia Regional Office¹⁵ are detailed in Table 1.

Validation of warning signs/classification – case-studies

Abdominal pain/tenderness has been identified as an important predictor of progression of DF to DHF (alarm signal). Khanna et al.,¹⁶ in a Delhi (India) hospital, analysed 100 patients presenting acute fever with abdominal pain. A probable diagnosis of DF was made, based on the presence of acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations and leucopaenia. The diagnosis was confirmed by enzyme immunoassay-based serology. DHF was diagnosed based on WHO criteria.⁴

Out of the 100 DF patients with abdominal pain, 55 patients' diagnosis was attributed to DF. The remaining 45 cases had other causes of fever with abdominal pain related to other causes.

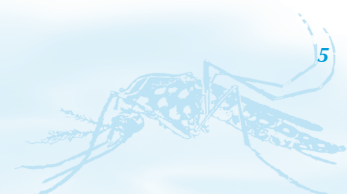
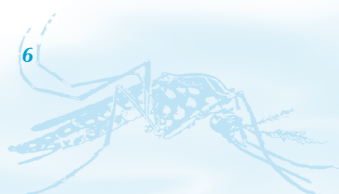


Table 1: Comparative case definition of DF/DHF/DSS by WHO Regional Office for South-East Asia and WHO-headquarters

WHO – regional guidelines, SEARO, New Delhi (2011) ¹⁵	WHO headquarters guidelines, Geneva (2009) ⁶
Scheme includes the following categories	
<p>I Undifferentiated fever DENV primary infection. Self-limiting, recovery Mixed with other arboviral, bacterial and parasitic infections</p>	<p>I Undifferentiated fever Not taken into account</p>
<p>IIA Dengue fever without haemorrhage Age group – Older children <15 years – Adolescents – Adults Constitutional symptoms – Fever – acute, febrile – Sometimes biphasic fever – Severe headache – Myalgia/arthralgia – Rash – Leucopaenia – Bone/joint pains especially in adults – Retro-orbital pain – Anorexia – Colicky pain/abdominal tenderness</p>	<p>IIA Non-severe dengue fever (Probable) Age group – not taken into account Constitutional symptoms – Living in DF-endemic areas or travellers – Acute fever (with two of the following) • Nausea/vomiting • Rash • Aches/pain • Positive tourniquet test • Leucopaenia • Any warning sign Laboratory-confirmed dengue – Important when sign of plasma leakage</p>
<p>IIB DF with unusual haemorrhage – All signs as under DF (IIA) – Skin haemorrhage as positive tourniquet test and/or petechiae – Massive epitaxis – Thrombocytopenia – Occasionally gastrointestinal bleeding, hyper menorrhoea</p>	<p>IIB Dengue with warning signs* – Abdominal pain/tenderness – Persistent vomiting – Clinical fluid accumulation – Mucosal bleed – Lethargy/restlessness – Liver enlargement >2 cm – Laboratory increase in HCT concurrent with decrease in platelet count.</p>

*Requires strict observation and medical intervention

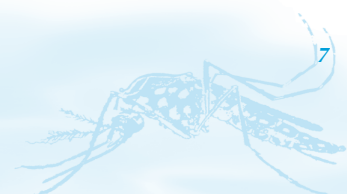


WHO – regional guidelines, SEARO, New Delhi (2011) ¹⁵	WHO headquarters guidelines, Geneva (2009) ⁶
<p>III DHF (with plasma leakage)</p> <p>IIIA DHF – Non-shock</p> <p>Age group</p> <p>More common in children <15 years</p> <p>However, incidence in adults is increasing</p> <ul style="list-style-type: none"> – Acute febrile illness with all signs of DF in early febrile phase – Positive tourniquet test – Petechiae, easy bruising and/or – GL haemorrhage – Marked thrombocytopenia – Generalized abdominal pain – A rising haematocrit, e.g. 10% to 15% above baseline 	<p>III Severe disease</p> <p>Severe plasma leakage leading to</p> <ul style="list-style-type: none"> – Shock (DSS) – Fluid accommodation with respiratory distress <p>Severe bleeding</p> <ul style="list-style-type: none"> – As evaluated by clinician <p>Severe organ involvement</p> <ul style="list-style-type: none"> – Liver AST or ALT ≥ 1000 – CNS impaired consciousness – Heart and other organs
<p>IIIB DHF (with shock)</p> <ul style="list-style-type: none"> – Towards end of febrile phase – Hypovolemic shock – Pleural effusion – Gall bladder oedema – Acute abdominal pain 	
<p>IV DF with expanded dengue syndrome</p> <ul style="list-style-type: none"> – Unusual manifestations – Involvement of liver, kidney, brain or heart are increasingly being reported in DHF – Occurred in DF cases also with no evidence of plasma leakage – May be due to co-morbidities 	

SEARO – WHO Regional Office for South-East Asia.

In another study from Sri Lanka by Weerakoon et al.,¹⁷ 14 cases of acute fever with abdominal pain of DHF/DSS were analysed during the dengue epidemic in 2009. All patients had secondary infection, probably DENV-1 (isolated elsewhere during the same period). The findings included:

- severe thrombocytopenia, mean platelet count $18 \times 10^9/l$, range 12–48;
- high liver enzyme mean ALT 374 U/l, range 82–2692;
- asities in all cases (100%);



- acute hepatitis in 11 cases (79%);
- acalculitis cholecystitis in 5 cases (42%);
- renal involvement in 3 cases (25%);
- normal serum amylase level (100%);
- normal-looking pancreas (100%).

Abdominal pain in dengue infection warrants investigation to find a specific cause.

Epidemiological change: Affected age group of DHF cases

Sedhain et al.¹⁸ reported the first-ever large outbreak in central Nepal (virgin soil) during 2010. A total of 414 dengue-confirmed cases following application of WHO guidelines⁵ were included in the study. Out of 414 cases, 329 were of DF and 85 of DHF including 2 cases of DSS. DHF was more common in the elderly population than DF (mean age of 31.59 vs 35.42). These findings are contrary to the known fact that DHF is basically the disease of children >15 years.

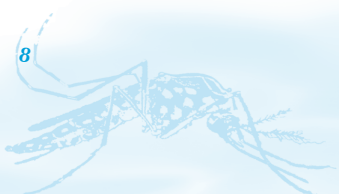
Validation of WHO probable case definition⁸

Nujum et al.¹⁹ conducted a study to check the performance of “probable dengue” case definition as per WHO headquarters guidelines.⁸ The study was carried out in Thiruvananthapuram district of Kerala, India. It included 254 patients with acute febrile illness of 2–7 days without a definite diagnosis for the community and primary and secondary case settings. The performance was assessed using RT-PCR as gold standard in the case of fever <5 days and IgM antibody detection for fever >5 days. WHO case definition had a very high negative predictive value of 97.4% (90.2, 99.6). The sensitivity and specificity were 71.4% (35.9, 91.8) and 30.7% (25.3, 36.7), respectively. The diagnostic odds ratio of the WHO classification was 1:1 which could be increased to 13.6 **if any five items listed in the case definition were used.**

Host genetics and DHF

Guha-Sapir and Schimmer²⁰ have discussed various factors involved in the pathogenesis of DF. High viremia, virulence of virus, age and racial and ethnic resistance to DHF are some of the important factors.

Host genetics in dengue viral infection is considered to be an important area of research to understand the end-stage complications of DHF. Aggressive studies are required in different ethnic groups in different countries on a large number of patients during the acute phase of DHF.^{21,22}



It is noteworthy that human populations have undergone migrations for millennia globally. For example, in India, Kondrashin and Rashid²³ identified 19 ethnic tribes (about 54 million population as per 1981 census) speaking over 100 languages/dialects representing ethnic diversity with different gene pools. Dudley Stamp²⁴ gave historical accounts of earlier migrations into India. According to him, Dravidians came from the south, Ahom from the east, Mongols from the north, Aryans from Central Asia and invaders and Moghuls from the west during the twelfth to fifteenth centuries, and finally, the British, the French and the Portuguese from Europe. The mixing of these diverse groups of populations is bound to generate 'gene pools' of great diversity. A study of the genetic make-up of these gene pools and their interaction with viral genome may provide useful information on the clinical manifestations and severity of DHF.

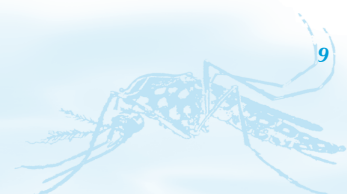
Vector control

In the absence of antiviral drugs and vaccines, vector control is the only option against dengue. *Ae. aegypti*, the primary vector of dengue, is a 'hygrophilic species' i.e. humidity-loving. Therefore, it has adapted to breeding in water-storage containers in domestic habitation and is governed by 'microclimatic conditions' and, to a lesser extent, by macrolevel climatic environment. Thus, to a large extent, this species can breed and transmit the virus comfortably under extreme macrolevel environmental conditions. During the rainy season, when temperatures come down and humidity increases, the species invades peridomestic areas and breeds profusely in any natural or manmade container holding rainwater, building up a very high density.

A study by Sharma et al.²⁵ in the National Capital Territory of Delhi (India), which experiences extreme weather conditions, recorded round-the-year breeding of *Ae. aegypti* in 1998.²⁵ The container indices varied from 83 in the winter, 1147 in the rainy season and 51 in the summer. It also recorded the corresponding incidence of DF cases which were hospital-based and serologically confirmed. The monthly distribution of 332 cases was 2, 6, 19, 79, 203 and 33 corresponding to the months from July to December, respectively. The transmission continued even in the severe winter month of December.

In contrast to this, Bohra and Andriansolo²⁶ reported the occurrence of outbreaks of DF/DHF in 1985 and 1990 during the months of March–April (spring season) in an arid zone of Jalore, Rajasthan (India).²⁶

This highlights the need for the study of the co-relation of microclimatic conditions to determine the threshold levels of *Ae. aegypti* breeding and transmission of DF/DHF.



Major breeding sites in urban areas

- Indoors
 - Overhead tanks, underground cement tanks, large earthen jars, flower vases, pitchers, desert coolers (evaporation coolers) and other water-storage receptacles. All these sites act as mother foci for breeding round the year.
- Outdoors (seasonal breeding sites)
 - solid waste materials with collection of rainwater
 - non-biodegradable containers
 - improper management of used tyres.
- Lack of building bye-laws resulting in:
 - construction of curing tanks
 - collection of water in basements
 - absence of a control strategy at construction sites.
- Housing
 - non-maintenance of roof gutters
 - extended exteriors of buildings holding rainwater
 - storm water drains with concrete floors – preventing percolation.
- Execution of small-to-large development projects, particularly non-eco-friendly designs, in both urban and rural areas, without **health impact assessment**.

Globally, vector control has been executed using chemicals, bio-control agents and personal protection measures including insecticide-treated nets (ITNs) but without much success. A successful vector control programme requires intersectoral coordination, and active individual and community participation.

A recent meta-analysis study²⁷ concluded that dengue vector control is effective in reducing vector population when interventions use a community-based integrated approach tailored to local eco-epidemiological and sociocultural settings and combined with educational programmes to increase the knowledge and understanding of best practices.

Recently, a WHO-sponsored research project entitled “Eco Bio-social Research on Dengue in Asia”²⁸ concluded that variable influence on vector breeding is complex and public health response should go beyond larviciding/spraying of insecticides. The study emphasized the need to develop close interaction between political leaders, religious leaders, all sectors of economics and municipal authorities, which is critical for the success of dengue vector control.



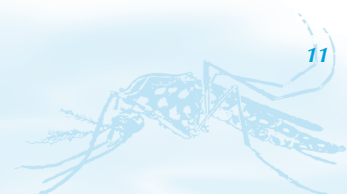
New avenues for build-up of high breeding potential of *Ae. aegypti*: a future challenge

Globally, old cities and towns were established along river banks for easy availability of potable water. With rapid expansion of urban areas, availability of potable water has become acute. Even groundwater resources are dwindling due to overuse. The existing cities and towns have already expanded to their full capacity.

To meet the growing demand for residential accommodation, governments are depending on public-private partnership (PPP). Under this arrangement, private infrastructure companies are building expressways (e-ways) to connect existing cities. The companies are given concessions to collect toll fees and build residential-cum-commercial complexes on e-ways. These complexes have to depend heavily on rainwater harvesting to meet their requirement of potable water. Rainwater harvesting will involve collection of rainwater from rooftops, paved surfaces, outlets and storm-water drains and will involve a number of ground-water charging prototypes. In the absence of efficient and effective health impact assessments, different types of prototypes will not only provide a high potential for breeding of *Ae. aegypti* but also for *Anopheles stephensi*, the vector of urban malaria. Adoption of eco-friendly designs and technologies for varied prototypes will be essential. **Any omission at this stage will prove a perilous oversight.** This problem was highlighted at a WHO workshop on “Adverse effects of development projects on mosquito-borne diseases” held in Bangkok in 1999.²⁹

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Identifying requirements for targeted risk communication in prevention of dengue transmission in vulnerable areas, Mawlamyaing, Myanmar

Pe Thet Zaw[#], Khin Thet Wai, Tin Oo, Zaw Win, Hlaing Myat Thu, Moe Thida, Phyu Sin Aye and Tin Tin Wai

Department of Medical Research (Lower Myanmar), No. 5, Ziwaka Road, Dagon Township, 11191 Yangon Region, Republic of the Union of Myanmar

Abstract

This cross-sectional study focused on the largest periurban ward of Mawlamyaing Township in Myanmar to identify the requirements for targeted risk communication in the prevention of dengue transmission in vulnerable areas. During May 2011, 200 structured interviews of householders and six in-depth interviews of health personnel and ward administrators were conducted. Most of the respondents lived in wooden houses (145/200, 73%). Only 18% of the respondents had high school and higher education. Two thirds of the households had children under 15 years of age, and 8% reported hospitalization for dengue infection in the past one year. Only 30% (60/200) knew that dengue could occur all the year round. The majority acknowledged that dengue was preventable. However, their mean score for five specific preventive measures was 1.8 ± 0.8 . The mean risk perception scores in chances of reinfection, and in severity, were 5.7 ± 2.2 and 6.0 ± 2.5 , respectively. They stored rainwater mostly outdoors in cement tanks and in ceramic jars (52/102, 51%). They were unable to clean these containers within seven days, and they did not change water regularly. Only 42% of cement tanks and 67% of ceramic jars had complete covering. Nearly 81% of the households had a few to abundant water-retainable discarded materials in their compounds. In-depth interviews revealed the need to strengthen advocacy for more community engagement in the removal of potential dengue vector breeding sites. In conclusion, extensive rainwater storage in key containers without adequate management and improper environmental management may lead to increased dengue vector breeding sites. The householders' low knowledge scores in preventive measures, and their low-risk perceptions towards reinfection may aggravate the situation. Greater emphasis should be on multisectoral collaboration and coordination to use advocacy as the best tool for risk communication in order to motivate community engagement. This will complement effective and sustained integrated vector management measures in vulnerable areas.

Keywords: Dengue transmission; Integrated vector management; Prevention; Risk perception; Water storage; Waste disposal; Risk communication; Vulnerable areas; Mawlamyaing; Myanmar.

[#]E-mail: pethetzaw@gmail.com



Introduction

In the South-East Asia and the Western Pacific regions of WHO, around 1.8 billion people are at risk for dengue.¹ In Myanmar, Mon State, Yangon Region, Ayeyarwaddy Region and Kayin State reported the maximum number of dengue cases in 2009 and 2010.² Dengue transmission is likely to occur under favourable climatic conditions, during population mobility and when there is inadequate water supply. Water scarcity leads to storage of water in various types of containers for domestic use and for drinking purpose. These containers may act as potential breeding sites for dengue vectors, especially when rainwater is stored.³ Preventing or reducing dengue virus transmission depends entirely on the control of mosquito vectors, and control measures have been carried out in the most affected townships of Mon State. However, the participation of householders in the management of key containers as potential breeding sites of *Aedes aegypti* requires exploration. In addition, their practices of the disposal of discarded materials that can hold water increase the chance of the potential for dengue vector breeding. Apart from their knowledge and attitudes, their risk perceptions are important for taking precautionary actions in effective risk management of epidemic infectious diseases, including dengue, which does not have any specific treatment or vaccination.^{4,5}

Risk communication is an interactive process of exchange of information and opinions among individuals, groups and institutions.⁴ This issue is consistently understood as an effective strategy for infectious diseases prevention and control. Advocacy at various levels is an integral component of mobilizing the community and should be linked to the risk communication framework.⁶ From this study, evidence-based requirements to improve knowledge and risk perceptions can be identified for effective risk communication, especially in times of an outbreak. This may lead to an enhancement in adoption and adaptation of locally specific actions for adequate, timely and sustainable container management apart from chemical larviciding carried out by vector control services. Therefore, the objectives of this study were to find out the vulnerability of the study site to dengue infection, to outline the knowledge, attitudes and risk perceptions of householders of dengue infection, and to find out their water-storage patterns, container management practices specific to rain-filled water storage containers, and management of water-retainable discarded materials in a peri-urban ward of the district affected by dengue.

Materials and methods

Study design

A cross-sectional and community-based descriptive study was conducted in May 2011.



Study area and study population

The study area was the largest peri-urban ward of Mawlamyaing Township in Mon State. It covered an area of 1049 acres with 3498 households and a population of 25 755 in 2010. The average annual rainfall was 190 inches (482.6 cm).² The study population included householders and their water-storage containers in and around the households.

Sample size determination and sampling procedure

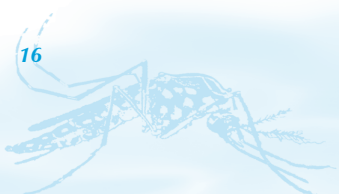
The sample size of 200 households met the assumptions of correct container management practices in peri-urban areas, being 15% at 95% confidence level, and 5% precision. The study site was purposively selected due to the presence of the reported high number of dengue cases compared to others during the past three years.⁷ The streets were selected at random followed by visiting all households in each street till the required sample size was reached. In each selected household, one adult respondent, either head of the household or the assigned person, was recruited to participate in the structured interview. For in-depth interviews, two health personnel from the State Vector Borne Diseases Control (VBDC) team, two midwives responsible for dengue vector control in the study site and two ward authorities were recruited.

Data collection methods

Six trained interviewers used pretested and modified structured questionnaire for eligible householders. They used the observation sheet to note down the water-storage containers and their characteristics and the presence of water-retainable discarded materials in the household compounds. Water-storage containers mainly focused upon were metal and plastic drums, cement drums, ceramic jars and cement tanks. These large and medium-sized containers were specifically chosen because these were likely to be the most productive containers (key containers) for larvae and pupae of *Aedes aegypti* already proved in other studies from Yangon Region and Kayin State.^{8,9} In-depth interviews of personnel from VBDC, basic health staff and ward administrators were conducted using the pretested guideline to underscore their ideas towards prevention of dengue transmission in risk areas and opportunities and challenges.

Data processing and analysis

The SPSS version 19.0 software was used for data entry and analysed after thorough form checks, range and consistency checks. Frequency distributions and cross tabulations of variables of interest were carried out. The questionnaire covered 37 knowledge items: general knowledge (4 items), mode of transmission (5 items), symptoms (8 items), dengue vectors (8 items), prevention (6 items) and container management (6 items). The knowledge score



was computed by assigning 1 for correct response. Risk perceptions related to dengue in the questionnaire included perceived susceptibility, perceived severity, perceived response efficacy and self-efficacy with reference to the “Health Belief Model”.¹⁰ The questionnaire also covered 10 items for risk perceptions and 2 attitudinal statements. These items were measured by the visual analogue scale (0–10). Mean scores and standard deviations were computed. The reliability coefficient was computed for knowledge scores and the value of ≥ 0.7 was considered as reliable. Qualitative data from in-depth interviews were triangulated with quantitative findings as appropriate for meaningful interpretations.

Ethical considerations

Privacy and confidentiality issues were observed during the interviews, following verbal informed consent for every form of data collection.

Findings

Vulnerability to dengue transmission

The study site was the community vulnerable for dengue transmission in which 75% (150/200) of the households had children below 15 years of age. Some 70% (140/200) of respondents were either heads of the household or their spouses. Only 18% of respondents had attained an educational level of high school and above. A vast majority of the people were living in wooden houses (145/200, 73%). Of the 200 households surveyed, 8% reported hospitalization for dengue infection in the past one year.

Knowledge of dengue infection, dengue vectors and prevention

Over half of the respondents did not know that adults could also contract dengue infection. Only 30% (60/200) knew that dengue could occur all the year round. This finding indicated the likelihood of less attention being given to water-storage containers to prevent dengue vector breeding, especially in the dry season. Symptoms of dengue shock were not widely known (<30%). Moreover, 83.5% (167/200) of respondents thought that there was medication available to cure dengue. The majority of them knew that dengue infection was transmitted via mosquitoes (186/200, 93%). However, only a few knew that dengue could be transmitted and spread due to unclean environment (Table 1).

Approximately 61% (113/200) could name the mosquitoes causing dengue in local terms, and 58.6% (109/200) correctly stated that dengue was caused by daytime biting of mosquitoes. Only 21.5% (40/186) cited clean water as dengue vector breeding sites which required attention. A vast majority (184/186, 92%) knew that dengue was preventable. Nearly 90% (165/184) of respondents knew it was necessary to avoid being bitten by mosquitoes



Table 1: Knowledge of dengue infection, dengue vectors and prevention

Characteristic*	No.	%
Knowledge of dengue shock	(n = 200)	
• Sudden decrease in fever and got cold extremities	39	19.5
• Frequent vomiting	59	26.5
• Blood streaks in the vomitus	55	27.5
Medication that can cure dengue	167	83.5
Transmission of dengue infection		
• Via mosquitoes	186	93.0
• Via unclean environment	14	7.0
Knowledge of dengue vector		
• Can name dengue-causing mosquitoes	113	60.8
• Daytime biting of dengue-causing mosquitoes	109	58.6
Where the dengue causing mosquitoes lay their eggs	(n = 186)	
• Clean water	40	21.5
Knowledge of prevention		
• Dengue is preventable	184	92.0
Methods of prevention	(n = 184)	
• Prevent from being bitten by mosquitoes during daytime	165	89.7
• Prevent water-storage containers as breeding sites	80	43.5
• Keeping the household utensils neat and tidy	54	29.3
• Proper disposal of water-retaining discarded materials	35	19.0

*Columns do not add up to 100 per cent due to single item, yes responses only

during daytime. Conversely, knowledge of preventive measures against vector breeding was not high. Nearly 44% (80/184) knew about protecting water storage containers as breeding sites, and only 19% (35/184) knew how to carry out proper disposal of water-retainable discarded materials in the neighbourhood. A majority attained the maximum score of '5' in the knowledge sub-score of five items related to dengue transmission (170/200, 85%). However, their mean knowledge sub-score on dengue vector breeding sites was not high, and their mean knowledge sub-score on five specific preventive measures focused on



container management was only 2.2 ± 0.69 . The mean knowledge score of 20.5 ± 2.3 for all knowledge items was low in the study households. The overall reliability coefficient for 37 knowledge items was 0.8, which indicated that the responses were reliable.

The respondents mentioned the channels that they had received messages from on dengue as follows: health personnel (114/200, 57%) and audiovisual materials (61/200, 30.5%). They mostly preferred health personnel as risk communicators (189/200, 94.5%). The findings indicated the confidence and trust of the householders towards health personnel as risk communicators in conveying comprehensive dengue-related information. Among those who knew how to prevent breeding of mosquitoes in water storage containers, 65% (52/80) said that they should discard the water at least once a week. Only 35% (28/80) knew that proper covering of the water containers was necessary to prevent dengue vector breeding. Nearly 78% could cite the requirement to discard the water stored for more than seven days. The least-known practices were the application of ABATE (Temephos) for larviciding and the requirement of thorough cleaning/scrubbing of the inside of the containers (<15%). Nearly 81% of households had few to abundant water-retainable discarded materials in their compounds, according to observations. Nearly 30% of respondents preferred special garbage bags to store the water-retainable discarded materials before systematic disposal, followed by turning them upside down and burial (Table 2).

Attitudes and risk perceptions

Around 75% (150/200) of the respondents acknowledged that all household members were responsible for the reduction of dengue vector breeding sites. The respondents' attitudes towards the seriousness of dengue attained high scores (Table 3) such as *"Dengue is life-threatening"* (8.84 ± 1.53), and *"Consequences of dengue as a serious concern"* (9.64 ± 6.62). The respondents scored high for perceived efficacy towards *"Ability to carry out effective vector control at home"* (7.25 ± 2.13). In the area of prevention of dengue, they scored high for *"Perceived effectiveness to prevent dengue by controlling vector breeding sites"* (7.61 ± 2.02), and *"Perceived effectiveness to prevent dengue at places where children gather during daytime"* (7.75 ± 2.19). However, they gave low scores especially for *"Own chance of being infected by dengue"* (4.19 ± 2.35), *"Possibility of reinfection"* (5.39 ± 2.52), and *"Possibility of severity when reinfected"* (6.08 ± 2.52) (Table 3).

Water storage patterns and container management practices

Nearly 96% of the study households stored water for domestic use and drinking purpose in 459 containers, an average of 2.4 ± 1.2 containers per household (range 1 to 8). They were filled by river water, either piped in or carried manually from the small reservoir in the ward (314/459, 68.4%), rain water (126/459, 27.5%), and water from tubewells (19/459, 4.1%). Of these, we elaborated on the storage of rainwater, which mostly favoured dengue vector



Table 2: Knowledge of management of water storage containers, presence of water-retainable discarded materials in premises during the survey and preferences

Characteristic*	No.	%
Container management to prevent from becoming the breeding site	(n = 80)	
• Discarding water at least once a week	52	65.0
• Proper covering of the water container	28	35.0
• Discarding water stored for more than 7 days	62	77.5
• Application of chemical larvicide (ABATE)	10	12.5
• Thorough cleaning of inner part of the container	2	2.5
• Placing larvae-eating fish	24	30.0
Water-retainable discarded materials (observations)	(n = 200)	
• Abundant	29	14.5
• Few	132	66.0
• Absent	39	19.5
Preferred methods of disposal for water-retaining discarded containers	(n = 35)	
• Special garbage bag	10	28.6
• Turning upside down	9	25.7
• Burial	9	25.7
• Burning and throwing out into the river	4	11.4
• Discarded at the public dumping site	3	8.6

*Columns do not add up to 100 per cent due to single item, yes responses only

breeding. Among the 126 rain-filled containers, metal and plastic drums were common (39/126, 30.9%) and 102/126 (81%) of containers were kept outdoors (Table 4). The householders stored rainwater outdoors in 51% (52/102) of the cement tanks and ceramic jars. Only 42% (14/33) of the cement tanks and 67% (22/33) of the ceramic jars had complete covering. The rain-filled large cement tanks were mainly located outside (29/33, 87.9%). The householders reported that they changed water only in 26% (24/33) of these tanks within seven days. Moreover, 75.8% (25/33) of the inner surfaces of the cement tanks were not scrubbed when changing the water. Therefore, the likelihood of these tanks becoming



Table 3: Attitudes and risk perceptions related to dengue

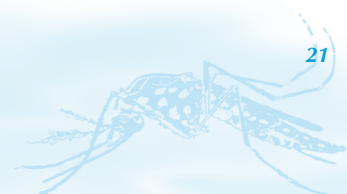
Attitudes and risk perceptions	Mean ± SD	Range
Attitudes		
Dengue infection is life-threatening	8.84 ± 1.526	1–10
Consequences of dengue as a serious concern	9.64 ± 6.619	1–10
Risk perceptions		
Own chance of being infected by dengue	4.19 ± 2.354	1–10
Possibility of dengue infections in other children if there is one infected child at home	5.39 ± 2.524	1–10
Possibility of dengue infection at home if in the vicinity of 10 houses that had dengue	5.20 ± 2.159	1–10
Possibility of reinfection	5.66 ± 2.225	1–10
Possibility of severity when reinfected	6.08 ± 2.520	1–10
Perceived effectiveness to prevent dengue by controlling vector breeding sites	7.61 ± 2.020	1–10
Ability to carry out effective vector control at home	7.25 ± 2.128	2–10
Perceived effectiveness to prevent dengue at places where children gather during daytime	7.75 ± 2.199	1–10
Perceived willingness to participate regularly in ward cleanup activities	6.42 ± 2.671	1–10

Table 4: Characteristics of rain-filled water storage containers

Type of rain-filled containers	Total (n = 126)		Location*				Best practices**					
			Indoor		Outdoor		Complete cover		Change within 7 days		Scrubbing the interior	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ceramic jars	33	26.2	10	30.3	23	69.7	22	66.7	4	12.1	24	72.7
Cement tanks	33	26.2	4	12.1	29	87.9	14	42.4	24	26.0	8	22.4
Metal and plastic drums	39	30.9	9	23.1	30	76.9	14	35.9	15	38.5	19	58.0
Cement drums	21	16.7	1	4.8	20	95.2	6	28.6	13	61.9	9	27.2

*row percentages

**single items computed based on row totals of different types of rain filled containers



key breeding sites for *Ae. aegypti* increased (Table 4). The findings supported application of specific mechanical, chemical and biological control measures to prevent dengue vector breeding in key containers.

On the other hand, challenges faced by the ward administrative authorities and basic health staff while mobilizing dengue vector control efforts were described in the following words:

“Whenever we asked householders to discard the water if we found larvae and pupae in it, they were reluctant to do so. They had to buy water out of their own pockets”. (Ward authority)

“People are very poor. They can’t spare sufficient time for cleaning the water containers, as they try hard to make their ends meet”. (Midwife)

“It’s more convenient for us to put ABATE in the water containers rather than persuading householders for effective container management”. (VBDC personnel)

Discussion

In this study, 8% of the surveyed households with reported hospitalization reflected the severity of the dengue infection. Others might have had sub-clinical infection which was uneventful, hence increasing the risk of having a more serious infection later.³ This study focused on behavioural determinants and potential dengue vector breeding sites that may increase the risk of transmission in the locality, accentuated by inadequate water supply and water storage practices. After the commencement of the river water distribution project which included the construction of a small reservoir to collect water from the Attaran river, since 2009 the problem of water scarcity in the study site was overcome. The regional administrative authorities and municipal services provided technical and financial assistance, with partial cash contributions made by the householders. However, people continued to store rainwater which was preferred over the river water due to its cleanliness. Thus, dengue transmission continued in the locality due to the presence of potential breeding sites.

A majority (92%) of the respondents acknowledged that dengue infection was preventable. Yet, their mean knowledge score on five specific preventive measures was only 1.84 ± 0.77 . However, the householders expressed a positive attitude toward accepting the life-threatening nature of dengue and the seriousness of its consequences. On the other hand, their misconceptions about contracting dengue could lead to an increase in more severe forms of dengue infection. Moreover, heavy rainfall in the area multiplied the favourable conditions for vector breeding in and around premises.³ Low level of knowledge among householders related to dengue vectors and infection highlighted the need for more comprehensive risk communication through innovative approaches. Their positive



attitudes may act as a trigger when arranging for community advocacy to strengthen risk communication, targeting householders in vulnerable areas.

Risk perception is an individual's perception of the magnitude of the risk.⁵ In this study, the score for perceived efficacy in controlling dengue vector breeding sites was high, but not consistent with adequate container management practices, especially regular changing of the water. Though several KAP studies on dengue in Asia and Latin America have disproved this linkage,¹¹⁻¹⁴ in this study, the householders' knowledge and risk perceptions towards dengue infection are of great importance, offering the potential for improved container management and reduction in pupal and larval productivity, which is supported by a recently completed multi-country study on eco-bio-social research for dengue vectors.¹⁵

The knowledge-to-action gap was found especially in the regular changing of water within seven days. Around 65% of the respondents knew of this specific requirement and 44% put it into actual practice. The programme personnel and ward administrators noticed that the householders were reluctant to change water regularly due to economic reasons. Besides, poor knowledge (2.5%) about the need for cleaning the inner part of the water storage containers may have a link with the low percentage (22.4%) of householders scrubbing the interior of rain-filled cement tanks (Table 4). The presence of larvae and pupae of *Aedes* mosquitoes required inspection, together with the KAP study. But owing to financial and operational constraints, this activity could not be included in the present study.

Rainwater storage in both indoor and outdoor containers increased the chances of becoming potential vector-breeding sites. Large cement tanks were found to be the most productive type of water-holding containers, which is already documented in Viet Nam.¹¹ Therefore, rain-filled large cement tanks in this study, which were without appropriate container management, were subjected to biological control or regular chemical control.¹⁶ In southern Viet Nam of the Mekong Delta, in response to water infrastructure projects, the storage of rainwater was based upon their perceptions of cost, quality and security of supply.¹⁷ Likewise, apart from the piped water available from the Attaran river water distribution project, rainwater is still being used. Using both rainwater and river water without adequate household water treatment and safe storage facilities might result in a double burden of diarrhoea and dengue. This issue calls for putting in place integrated services as outlined in integrated vector management principles.¹⁸

In addition, unsatisfactory disposal of water-retaining discarded materials may lead to more dengue vector breeding. Thus, we also need to put emphasis on improvement in household waste disposal and ward waste collection system apart from improved water storage practices. In this context, efforts to prevent dengue transmission by enlisting householders' involvement require intensification. For example, fully empowered, environment-friendly, ward-based multistakeholder partner groups and trained volunteers successfully carried out inspection and removal of dengue vector breeding sites together with environmental management in periurban households of Yangon Region.¹⁶ Such actions may encourage less reliance on chemical control, which is costly and likely to face householders' resistance.¹



Conclusions and recommendations

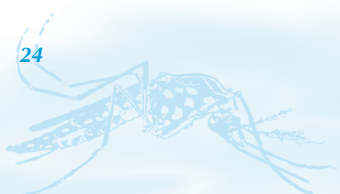
Despite the river water distribution project, the stored rainwater being preferred for its cleanliness may become a potential dengue vector breeding source, particularly when stored in outdoor containers under the shade. The misconceptions of householders about the risk of dengue infection still existed despite being exposed to IEC messages. However, householders in the study site accepted dengue infection as a matter of serious concern. They could be motivated through an improved understanding of the risk of dengue infection and active engagement in the management of water storage containers as well as water-retainable discarded materials. Barriers in the way of prevention and control of dengue vector breeding and the overriding concerns of the community should be addressed through advocacy at various levels. Greater emphasis should be placed on multisectoral collaboration and coordination in the use of advocacy as a communication tool for community engagement. This will complement effective and sustained integrated vector management efforts in vulnerable areas.

Acknowledgments

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The benefits and challenges of scaling up dengue surveillance in Saudi Arabia from a GIS perspective

Hassan M Khormi,^{a,b,#} Ramze A Elzahrany^b and Lalit Kumar^a

^aEcosystem Management, School of Environmental and Rural Sciences, Faculty of Arts and Sciences, University of New England, Armidale, NSW 2351, Australia

^bDepartment of Geography, Umm Al-Qura University, PO Box 6928, Makkah 21955, Saudi Arabia

Abstract

The most common mosquito-borne virus in Jeddah, Saudi Arabia, is dengue and it is predominantly transmitted by *Aedes aegypti* mosquitoes. This study was aimed to assess the status of dengue surveillance in Jeddah, develop recommendations to improve surveillance data quality, investigate prospective uses of geographic information systems (GIS) to monitor dengue incidence, vector prevalence and patterns, and introduce ways for improving the current dengue surveillance system in Jeddah. Continuance of the current system is recommended, but its effectiveness can be improved by increasing its accuracy and collection and use of data.

Keywords: GIS; Surveillance system; Dengue; *Aedes aegypti*; Jeddah; Saudi Arabia.

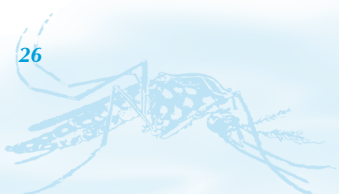
Introduction

It is estimated that around 3.6 billion people in more than 124 countries are at the risk of dengue infection. An estimated 34 million cases of clinical dengue fever (DF), 2 million cases of dengue haemorrhagic fever (DHF) and over 20 000 deaths occur each year.¹ Disease prevention is limited to vector control and case management. Treatments are limited to supportive care;² to date, no drug can cure the disease and no vaccine has been found which can prevent it. Thus, dengue control and prevention have mainly relied on vector control in collaboration with community action.

During outbreaks, vector control programme activities focus on reducing mosquito vector populations to levels where dengue virus transmission is no longer sustainable and the role of the mosquito is reduced to that of a nuisance.^{3,4}

This study aims to assess the status of dengue surveillance in Jeddah, Saudi Arabia, where the most common mosquito-borne virus is the dengue virus, mainly transmitted by

[#]E-mail: hkhormi@une.edu.au



the *Aedes aegypti* mosquito⁴ and to develop recommendations to improve surveillance data quality, investigate prospective uses of geographic information systems (GIS) to monitor dengue incidence, vector, prevalence, and patterns, and introduce ways to improve the current dengue surveillance system.

Discussion

Previous studies using geographic information systems in Jeddah

The results of previous studies conducted in Jeddah using GIS show that the incidence of DF and its vector patterns were significantly clustered.⁵ Khormi and Kumar's⁵ study provides evidence that mosquito density is significantly associated with DF disease in Jeddah. The variation in both spatial and temporal distribution of the disease and its vector indicates a complex ecology that is likely to be attributable to different environmental and socioeconomic variables.

The results of the analysis on climatic determinants of the dengue fever vector indicate that the distribution of the vector varied remarkably with temperature, relative humidity and rainfall.⁶ The disease vector in Jeddah was negatively associated with the weekly average minimum temperature.⁷ Also, Khormi et al.⁷ found that rainfall and average minimum temperature play determining roles in the abundance of the vector. These variables may be used to assist in forecasting outbreaks of DF disease in Jeddah.

Khormi and Kumar⁸ endeavoured to develop a risk model based on socioeconomic factors to forecast the risk of DF epidemics. To our knowledge, this was the first attempt to develop an epidemic risk model for predicting areas at risk in Jeddah. The model developed in the study had a high degree of accuracy, and therefore, may have implications for the planning of DF control and risk management programmes. The result of this model suggests that socioeconomic factors, such as neighbourhood quality and population density, directly affect mosquito and DF distributions.⁸ Areas with a low neighbourhood quality, high population density and a high percentage of non-Saudi residents were more likely to have a higher prevalence of DF.⁸

Spatiotemporal risk modelling of dengue based on a weekly frequency index is a useful tool for interpreting and applying surveillance data. It has great potential to be used as a decision support tool for DF particularly, and mosquito-borne diseases generally.⁹ Such models often require daily data collection. The rationale for the use of such techniques is that it increases the precision of the estimates. The spatiotemporal risk model, based on case notification data, is a simple and inexpensive technique. It was proposed for early warning, categorization and identification of areas at risk that can be incorporated into routine monitoring by health authorities.¹⁰

The overall findings of the previous DF studies in Jeddah using GIS suggest that changes in environment and socioeconomic factors may have direct and indirect effects on the



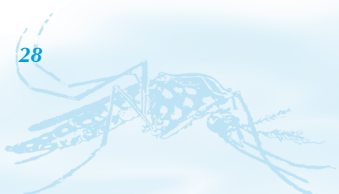
transmission of DF. For instance, in the early years of Khormi and Kumar's⁸ study period (2006–2010), which was designed to model dengue risk based on socioeconomic parameters, high numbers of recorded cases were noted among Saudis, especially in 2006. However, increases in the per capita income of Saudi citizens (by 15%) around mid-2006 had a direct impact on the standard of living.^{11,12} This gave Saudi nationals greater opportunity to move to more affluent areas that were less dengue-prone and to purchase equipment (e.g. window screens) that could protect them from *Aedes aegypti* vector bites. Environmental and socioeconomic factors can influence the length and efficiency of extrinsic incubation of DF, as well as the breeding, survival, longevity, dispersal and many other aspects of the biology of the vector and the host. These factors also influence human behaviour and demographics and may determine the likelihood of human exposure to DF.

It is important to understand, assess and use an extensive range of variables (such as clinically confirmed cases of DF, mosquito counts, population density based on inhabited areas, total population per area, neighbourhood quality, subsurface water and monthly spatiotemporal risk of DF, based on average weekly frequency) in order to model DF risk. The resulting model can identify areas whose inhabitants have a higher likelihood of contracting DF in the future due to the prevalence of relevant environmental and socioeconomic conditions as described above.¹³

This model can be used by decision-makers in the Jeddah Municipality to prioritize major infrastructure projects being planned in the city. They can initiate redevelopment in low-quality neighbourhoods with a high population density, high-risk subsurface water, high or medium numbers of DF cases recorded in the previous years, and high or medium numbers of trapped mosquitoes, to minimize the prevalence of the disease.

The models developed in previous studies can be used for different control management purposes in Jeddah. The model of hotspots,^{5,14} based on yearly data, can be used to give an overview of high-impact areas (in terms of cases of DF and populations of *Aedes* mosquitoes) during a year. The disadvantage is that this model does not indicate appropriate weekly or monthly spatial shifts in DF incidences and *Aedes* mosquito population densities. As a result, it is difficult to assess whether or not dengue epidemics have broken out or have been kept under control. Therefore, modelling spatiotemporal risk based on a frequency index¹⁰ is a better way to demonstrate changing temporal- and spatial-based DF risk, based on weekly or monthly data that can later be used to improve DF surveillance systems. Risk models of DF based on a combination of environmental and socioeconomic variables help to define the causes behind the prevalence of the disease.¹³

It is important to have a study that shows different models for surveillance systems of DF, with appropriate temporal and spatial scales. Models can be categorized in different ways. One may focus on modelling the risk of DF based on yearly data, or modelling the risk based on monthly data, but the question here is whether these two models are optimally scaled and sufficiently timely for effective management programmes.



In light of these considerations, Khormi and Kumar's¹⁵ study was designed to illustrate the impact of different temporal and spatial scales on DF management decisions. The resulting models show that understanding (and ultimately being able to predict) the spatial and temporal dynamics of mosquito-borne diseases and their vector prevalence at scales ranging from sub-district to district and sub-municipality, and at time scales ranging from weekly to monthly and yearly, is critical to our ability to manage and monitor such diseases. The same applies to dengue fever and its vector, *Aedes aegypti*. The resulting models show how hotspots and risk levels vary, depending on what scales are used, underscoring the importance of understanding this variability. It also shows that selecting the wrong temporal and spatial scales can lead to sub-optimal decisions concerning monitoring and management of the disease and its vector.

Current dengue surveillance system and management in Jeddah

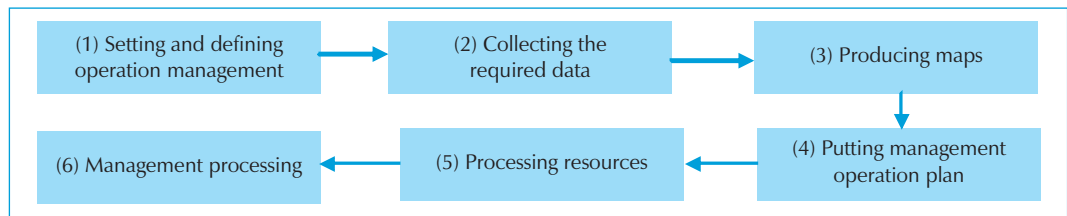
In Jeddah, there are two main governing authorities that are responsible for dengue monitoring programmes; the Jeddah Municipality and Jeddah Health Affairs. The Jeddah Municipality acquires daily mosquito samples using black hole traps, and these samples are returned to the mosquito laboratory for filtering and sorting according to species, sex, date of collection, coordinates, and number of mosquitoes for each location. According to Aburas,³ black hole traps are considered the most efficient traps for the study area. For the capture of mosquitoes, 504 black hole traps have been in operation since 2006. These traps are distributed geographically based on population density and different environmental factors and capture mosquitoes by producing carbon dioxide. The clinically confirmed case registries of dengue fever have been collected continuously and systematically since 2006 by the Dengue Fever Operation Room of Jeddah Health Affairs.

The map of dengue management, monitoring and management operations in Jeddah is based on six consecutive steps (Figure 1). The first step is setting and defining operation management. The second step is collecting mosquito data, and receiving reports on dengue cases, mosquito density and public input. In this step, there are some sub-sections that include receiving: (1) information about confirmed dengue cases by fax; (2) reports of mosquito density from the mosquito laboratory; (3) dengue case reports; (4) a report on the density of mosquitoes on a daily basis from the geographic information system (GIS) unit; and (5) resident reports, delivering the civil report to dengue programme control and, lastly, archiving all of these reports.

The third step involves producing a detailed map of dengue cases and mosquitoes for the prevention and management operation. To produce these maps, there are some sub-steps that are used by GIS unit staff, including receiving coordinates of dengue cases and mosquitoes that are recorded by Jeddah Health Affairs and the management of the mosquito laboratory, building a spatial database including all the required details such as date, street name and district name, adding the coordinates, showing the coordinates on maps and printing them.



Figure 1: Six consecutive steps of dengue management, monitoring, and management operations in Jeddah, Saudi Arabia



The fourth step is putting a management plan in place, including: receiving detailed maps of mosquito and dengue case locations from the GIS unit; gathering citizen reports from the General Directorate of Operations and Research; classifying the maps based on sub-municipality name; determining control type depending on the number of dengue cases and mosquito density that were mapped previously; distributing a statement that includes locations that will be targeted and maps of those locations to dengue control supervisors in each sub-municipality; classifying priorities for the locations that will be targeted; and, finally, having every dengue management supervisor distribute a list of those locations to his/her team.

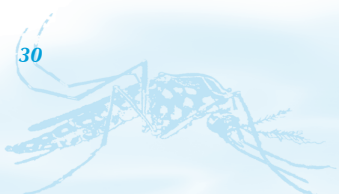
The fifth step is processing sources, including pesticides, control teams and guidance. This step includes estimating the reliance and disbursement of the amount of pesticides; receiving the pesticides; automatic mixing of the pesticides inside reservoirs; writing a report about pesticide mixing; filling spraying devices; delivering the devices; and providing maps of locations that will be targeted to the control teams. The last step is management processing, i.e. processing and delivering achievement reports to the management commission, as well as analyzing the achievement reports and distributing the results, including maps that show locations targeted by pesticides.

Ways to improve current surveillance system and management in Jeddah

Indoor application of insecticide and collecting detailed data on a house-by-house basis

Different studies have noted the presence of dengue vectors in the homes of patients, which confirms the value of indoor application of insecticide.¹⁶ This is an effective method to destroy infected mosquitoes and thus prevent patients' visitors from being bitten by them, which could later start new transmission foci in other areas.

In Jeddah, similar situations have been found. Khormi and Kumar⁵ found in their study that, from 2006 to 2008, most of the DF cases originated in areas that were mosquito hotspots. However, from 2009 to 2010, some areas that had originally been identified as at

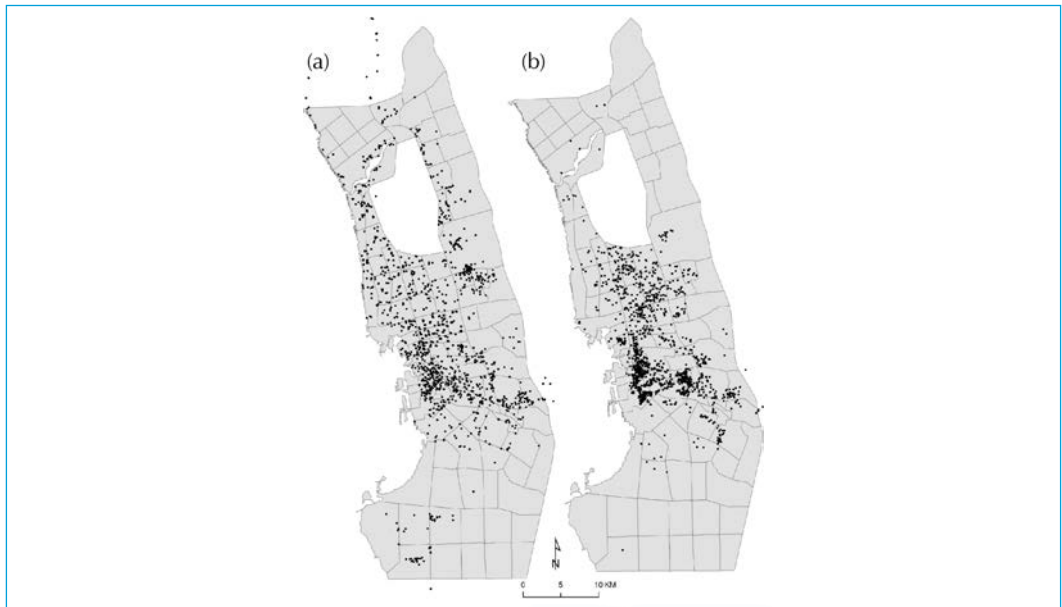


high or highest risk of dengue infection had been subsequently determined to have low and moderate mosquito risk.⁵ One of the reasons for this was that most infected people were adults who are highly mobile, working and travelling outside of their districts and visiting friends within districts that have high densities of female *Aedes* mosquitoes. These facts confirmed the value of indoor application of insecticide.¹⁶ Regarding indoor activities that could be used to carry out source reduction, detailed environmental and socioeconomic data (e.g. temperature, humidity, income, house design and house conditions) must be collected on a house-by-house basis. The collected data can be later used to precisely determine the best environmental and socioeconomic conditions for mosquitoes to breed, and this information can also be used to model DF risk accurately.

Geographic information systems: implications and suggestions to improve current surveillance system

Since 2006, GIS has been used to map DF cases and vector locations by some Jeddah-related authorities (Figure 2). It is also used as an inquiry tool to identify the locations of buildings under construction, general information about dengue patients, weekly mosquito population densities and number of notified cases.¹⁷ However, it has not been used widely to analyse and identify DF incidence and its vector patterns and trends, i.e. to find the association between the disease and its vector, other environmental and socioeconomic variables, and the distribution of the disease and its vector based on the impact of these variables.

Figure 2: (a) Locations of *Aedes aegypti*; (b) locations of dengue cases, Jeddah, Saudi Arabia



Recently, several published studies^{5,7-9,15} conducted in Jeddah show a variety of GIS implications in the planning of health interventions that can be used to enhance the surveillance system. Spatial analytic methods developed through these studies may be used to augment the current surveillance system by better identifying and monitoring high-risk areas for the disease, and to find the association between dengue distribution and environmental and socioeconomic factors, among others. The spatial approaches used in these studies allow changes in the environmental conditions, which are one of the key determinants of DF incidence, to be taken into account.

In Jeddah, the DF surveillance system can be integrated into a GIS environment with social, biological, economic and environmental factors. This integration may contribute to greater accuracy in the development of epidemic forecasting models. Also, it may have significant implications for DF monitoring, management, decision-making and practices, which may help the authorities prioritize more wisely and use resources more effectively and efficiently.

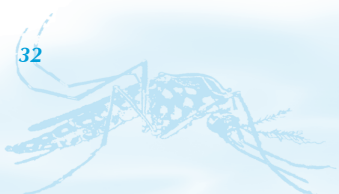
There are some points that must be considered at the time of collecting data. We observed that different spatial data are collected using different coordinate systems and map projections. Some coordinates are also recorded incorrectly due to misunderstanding of the differences between the longitude and latitude (or X and Y) for the required data (e.g. in DF cases). Therefore, more training is required for the local programme staff and health workers who are responsible for recording the locations of breeding sites, cases and transmission sources according to house lot, block and neighbourhoods in the city.

Currently, there are no temperature, relative humidity and rainfall spatial data for Jeddah, which is one of the weaknesses of the risk model. To improve monitoring and forecasting, we suggest that every trap that is used to capture adult mosquitoes must have devices for measuring the temperature and relative humidity to give a better understanding of the climatic conditions in the area. This information can be used later to create temperature, relative humidity and rainfall GIS data layers for all Jeddah districts, to be used as parameters for modelling dengue risk areas.

Other weaknesses and strengths, and some suggestions to improve current surveillance system

There are effective links between various stakeholders, especially health care providers, laboratory staff and public health and vector management authorities. However, many of these relationships are dependent on personal contacts, which are affected by staff turnover.

Although the dengue surveillance system in Jeddah is generally operating well, information bias is still possible in the process of notification. Failure to report dengue cases is an important source of information bias. For example, the case register in one of the Jeddah districts fails to include residents in Jeddah who develop the disease in Makkah city during their visits there.



To avoid this information bias, patients must report their travel history to their doctor when travelling in endemic areas, in order to increase the quality of the surveillance system. Patients' travel history can then be used in the development of the dengue forecasting model.

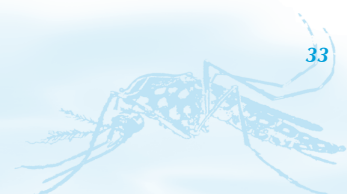
Data collected from different staff members during different observation periods and in different locations might lead to variations in notification results. According to Rothman and Greenland,¹⁸ disease interventions, changes in the requirements for disease reporting and modifications to the surveillance system might all affect the quality of data, and thus the validity of the findings.

Since 2006, we have observed that, in some respects, disease prevention is less important than disease management.^{5,7-9,13,15} Observations have confirmed that outbreak response through spraying insecticide is considered more important than outbreak prevention because of the public demand for action. According to Beatty et al.,¹⁹ this action often leads to insecticide spraying that is unlikely to be effective, since insecticide released in streets is unlikely to reach the adult *Ae. aegypti* resting and feeding inside homes. Lower reporting rates for the disease by health care providers leads to more diagnostic tests, which complicate the situation, especially since the results are difficult to interpret by health-care providers and public health practitioners unfamiliar with the limitations of the tests.²⁰

Data are rarely used locally even when adequate infrastructure exists; rather, they are forwarded to the central health affairs offices or other authorities for official evaluation, missing the opportunity for an immediate local response. Having laboratory confirmation of dengue cases available only at the central level, and a lack of funding for these services were reported as further weaknesses.¹⁹ Additionally, full coordination of entomological surveillance and data sharing by human disease surveillance and vector control units conducted by epidemiologists are needed for detection of increased transmission sufficiently early to prevent or control disease prevalence and its vector abundance.

To avoid the above-mentioned weaknesses, some guiding principles can be suggested. The responsible people in all the related departments/authorities in Jeddah need to make additional efforts to improve and maintain a high level of quality reporting. All the suspected cases and the number of trapped mosquitoes and other valuable information must be reported to a central dengue unit as soon as possible and in a systematic way. Information providers must be reminded that timely reporting can increase the effectiveness of response.²¹ Data must be collected and analysed as soon as possible. Public and private health-care facilities should be encouraged to report the required data (e.g. DF case incidence).

Reporting can be reduced to every two weeks during periods of low transmission to conserve reporting resources. Reporting would be necessary and more frequent (perhaps on a daily basis) during an outbreak or in peak seasons. At such frequencies, reporting would be affected by the operating hours of the reporting facilities, which must be noted. For instance, these facilities are usually closed on weekends and holidays, which could artificially reduce the number of reported cases and create surveillance artifacts. Within 24–48 hours of form



completion, reports should reach the surveillance units; therefore, a better way is setting up an electronic system or web-based reporting system. Periodic additional studies (e.g. using capture–recapture methods) should be conducted and incorporated into the system when possible in order to confirm and understand the burden of dengue, which will also determine the representativeness of the surveillance data.¹⁹

Conclusion

The current dengue surveillance regime in Jeddah must continue to improve its effectiveness in delivering accurate and detailed surveillance data. To examine the likelihood of disease under-reporting and over-reporting, rigorous assessment is required. This will provide forewarning of dengue outbreaks. In addition, valuable information needs to be made available for public health decision-making, vector surveillance and monitoring programmes.

Using GIS and spatial analysis identifies and monitors hotspots of dengue, its vector, and the threshold of dengue disease. Also, special education campaigns and mosquito control activities in Jeddah, providing systematic and integrated training for public health professionals and general practitioners and, finally, increasing collaboration between microbiologists, environmental health practitioners, epidemiologists, public health physicians, ecologists and GIS and spatial information technology specialists in order to assess major determinants of dengue transmission can improve the effectiveness of public health interventions.

Two important approaches for reducing disease transmission are community participation and health education. Conducting regular health promotion campaigns is important for managing mosquito breeding sites throughout communities. This includes advising people to keep swimming pools full and well maintained, covering water containers and changing the water regularly, screening living areas, using mosquito bednets to keep mosquitoes out, using insect repellent in areas where mosquitoes are active and wearing loose light-coloured long-sleeved clothes and covered footwear to prevent mosquito bites.

Vector control is an effective and economical approach to reducing dengue transmission. In Jeddah, *Ae. aegypti* is the major mosquito species that is the disease vector. More attention is needed from Jeddah public health authorities to the monitoring and managing of this mosquito species. Assessment of various vector control strategies is essential to examine the feasibility and effectiveness of vector control measures. Improved district planning, development and vector control strategies in Jeddah should be coordinated; this is of growing importance, as people seek lifestyle changes and urban spread impinges on districts at risk.

More geographical epidemiological research is needed in Jeddah particularly, and in other Saudi cities that have recorded dengue incidences recently, such as Makkah and Jazan, for a better understanding of the natural transmission of DF infection. These studies can include research on the spatial distribution of vectors and their movement, the virus carrier rate of vectors and sero-epidemiological investigation. There is also a need for further studies into



the complex ecology of the virus, the epidemiology of the disease and the uncertainties associated with the impact of predicted socioeconomic and environmental factors. All this will improve understanding the determinants of DF, which is important for the development of an accurate DF forecasting system.

It is effective economically to increase research into vector control strategies and improved urban development planning due to limited spatial and geographical distribution of DF in Saudi Arabia generally. Additionally, more research on the environmental and behavioural determinants of the infection is needed. For public health measures, some work can yield evidence of DF incidence reduction by informing the public about how to reduce or eliminate mosquito breeding sites, and also through behavioural changes to reduce the chance of being bitten by *Aedes* mosquitoes.

This study shows how various spatial models can be incorporated into the DF management and monitoring system in Saudi Arabia in order to improve the current system. The same methodology can be used in other parts of the world as well.

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Trends of dengue infections (1997–2008) in Cebu Province, Philippines

Frances Edillo^{a#} and Susana Madarieta^b

^aDepartment of Biology, University of San Carlos, Cebu City 6000, Philippines

^bDepartment of Health, Region 7, Cebu City 6000, Philippines

Abstract

This study analysed the epidemiological trends of dengue cases and deaths in Cebu province, Philippines, recorded at sentinel hospitals of the Department of Health, Regional and Epidemiological Surveillance Unit in Central Visayas during 1997–2008. A total of 34 326 dengue cases [dengue fever (DF) and dengue haemorrhagic fever (DHF)] and 831 deaths occurred in the 12-year period. The average case–fatality rate (CFR) was 2.46%; the average incidence rate of hospitalized dengue cases was 0.854/1000 population. The annual incidence rates of hospitalized DHF cases (average = 0.55 cases/1000 population; 17 540 total cases) and DF cases (average = 0.323 cases/1000 population; 9977 total cases) differed ($P < 0.05$) in 2000–2008. The incidence rates of hospitalized DHF cases were significant ($P < 0.05$) from year-to-year in 2000–2008, whereas those of DF were mostly significant, except between 2001 and 2003 ($P > 0.05$). Dengue cases, deaths and CFR increased annually in the rainy season. Cebu city was the highest (average = 0.356 cases/1000 population) in its yearly incidence rate of hospitalized dengue cases and differed ($P < 0.05$) among the top 10 municipalities and cities, followed by Mandaue city (average = 0.076 cases/1000 population). Dengue cases differed ($P < 0.05$) across age groups. One- to five-year-old (average = 31.82%) and 6 to 10-year-old children (30.66%) were significantly higher compared with other age groups. Urbanization, increased population, inadequate public health infrastructure, poor solid waste management and lack of effective mosquito surveillance system were the reasons.

Keywords: Dengue infection; Epidemiological trends of dengue; Cebu province; Philippines.

Introduction

Mosquito vector-associated human diseases directly cause much misery while creating massive obstacles to economic development.¹ Dengue fever (DF) is an infectious disease naturally transmitted between humans by the primary vector, *Aedes aegypti*, and by the secondary vector, *Ae. albopictus*. It is caused by any of the four serotypic single-stranded RNA dengue viruses (DENV-1-4; family Flaviviridae, genus *Flavivirus*). Infection with one serotype provides a life-long immunity against that serotype but not against the others.² Although dengue has been known as a human disease for over two centuries, in the last

[#]E-mail: aegyptifra@hotmail.com



two decades, DF, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) have emerged as the most important arthropod-borne viral diseases of international public health concern.^{2,3} DHF is characterized by fever; spontaneous bruising and bleeding from mucosa, gingival, injection sites; thrombocytopenia (<100 000 platelets per cu mm); and plasma leakage with haematocrit more than 20% higher than expected.⁴ DSS is defined as DHF with weak rapid pulse and narrow pulse pressure (<20 mmHg). It is estimated that 50–100 million cases of DF, including 250 000–500 000 cases of DHF and DSS, and 30 000 deaths occur each year worldwide.^{2,5} More than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific are now endemic of dengue infection, of which South-East Asia and the Western Pacific are the most seriously affected.⁶ Before 1970, only nine countries had experienced DHF epidemics, which number had increased more than fourfold by 1995.

In the Philippines, major dengue epidemics occurred in 1966, 1983, 1998 and 2001; of which the 1998 epidemic was the country's worst.² In 1998, a total of 31 829 dengue cases and 497 deaths (case–fatality rate, CFR=1.6%) were reported nationwide, of which Central Visayas where Cebu belongs had 10.11% dengue cases and 19.12% deaths with 3% CFR.⁷ The Philippine Department of Health (DOH)⁸ initiated the National Dengue Prevention and Control Programme in 1993, and its pilot sites were Central Visayas and the National Capital Region. This programme has only been implemented nationwide in 1998.⁹ The thrust of the DOH⁸ is directed towards community-based prevention and control in endemic areas. Their programme includes: integrated vector control, early case detection and management, surveillance, health education to multiple sectors, and implementation of the 4S *Kontra* dengue (a *Bisayan* dialectal term which means “against”) and the 4 *o'clock habit*. The 4S *Kontra* dengue involves: (i) search and destroy mosquito breeding sites; (ii) self-protection measures; (iii) seek early treatment; and (iv) say “no” to indiscriminate fogging. The 4 *o'clock habit* programme requests householders to clean up their surroundings and to drain water containers to prevent the spread of mosquitoes.

To date, there has been no scientific publication on dengue infections in Cebu. This study aimed to investigate the epidemiological trend of dengue cases and deaths in Cebu province by analysing the 12-year (1997–2008) dengue data recorded at sentinel hospitals of the Department of Health – Regional and Epidemiological Surveillance Unit Region 7 (DOH-RESU 7). The results may serve as a valuable guide for health personnel who develop effective measures for the improvement of public health.

Materials and methods

Description of Cebu province

Cebu province (9°25'N–11°15'N latitude; 123°13'E–124°15'E longitude) stretches 225 km north to south of Cebu Island, located in the centre of the Philippine archipelago (Figure 1). Its capital is Cebu city, the oldest city in the country. The other major cities are: Carcar,

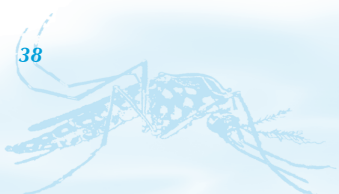


Figure 1: Map of Cebu province indicating the top 10 municipalities and cities with highest dengue cases in 1997–2008.
Inset: Philippines map showing Cebu province



Danao, Lapulapu, Mandaue, Naga, Bogot and Talisay. Cebu province has 48 municipalities and 8 cities. The province is one of the most developed in the country and is the main centre of commerce, trade, education and industry in central and southern Philippines.

Collection of dengue data

DF admissions at all sentinel hospitals of DOH-RESU 7 from their earliest record in 1997 until 2008 were gathered from DOH-RESU Central Visayas Office, because all sentinel hospitals in Cebu province are required to submit their dengue surveillance reports there.¹⁰ Sentinel hospitals are tertiary hospitals that have speciality departments that may vary in services and may include paediatrics, general medicine, and various branches of surgery and/or psychiatry. Suspected dengue cases were diagnosed on the basis of haematological tests (haematocrit level and platelet counts), and acute febrile illness for 2–7 days with two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations and leucopaenia. This is according to the Philippine DOH guidelines which are adapted from the 1997 World Health Organization (WHO) guidelines.¹¹ Resource limitations of the DOH 7 and sentinel hospitals hindered confirmatory tests in the laboratory such as isolation of the dengue virus from blood serum, plasma or leukocytes, and test for immunoglobulin M (IgM) antibody, hence, data are suspected to be of dengue



cases only. Separate annual data of DF and DHF in the province, but not across age groups, were available from the year 2000; prior to that, dengue cases were combined DF and DHF cases according to Central DOH, Manila. The incidence rate of hospitalized dengue cases per 1000 population each year was estimated based on the number of dengue cases obtained from sentinel hospitals of DOH-RESU 7 and the population data from the Philippine National Statistics Office web site.¹²

Statistical analysis

All analyses were performed in SPSS statistics 17 software.¹³ A univariate analysis of variance (ANOVA) was used in most statistical tests. Post hoc ANOVA using Duncan's Multiple Range test for multiple comparisons between means was used to determine differences in dengue cases across age groups. Two-proportion Z-test or Kruskal Wallis test was used to determine significant differences in year-to-year incidence rates of hospitalized dengue cases and CFR. Two-sample *t*-test was used to know the difference in the incidence rates of hospitalized DF and DHF cases in the province.

Results

The data on dengue cases and deaths from the 56 municipalities and cities in Cebu province were taken from 10 to 18 sentinel hospitals of DOH-RESU 7 between 1997 and 2008.¹⁰ Not all municipalities and cities had dengue cases every year; hence, these cases occurred in 51–56 municipalities and cities in the past 12-year period of the study. There were ten sentinel hospitals of DOH-RESU 7 in 1997–2004, and later, their number increased to 18 until at present. Two sentinel hospitals are located in Lapu-lapu and Naga cities, namely, Camp Lapu-lapu Hospital and South General Hospital, respectively. The rest are located in Cebu city such as Cebu City Medical Center, Cebu Doctors' University Hospital, Cebu Velez General Hospital, Chong Hua Hospital, North General Hospital (formerly *Kauswagan* Hospital prior to 2004), Miller Sanitarium Hospital, Perpetual Succor Hospital, Sacred Heart Hospital, Saint Vincent General Hospital, Vicente Gullas Memorial Hospital, Saint Anthony Mother and Child Hospital, Visayas Community Medical Center, Central Command Army Station Hospital, Cebu Puericulture Center and Maternity House, and Vicente Sotto Memorial Medical Center. Guba Emergency Hospital in Guba, Talamban, Cebu city existed as a sentinel hospital during 1997–2007 and resumed in the later part of 2008. Thus, not all municipalities and cities in Cebu province have sentinel hospitals. Most dengue patients go to Cebu city sentinel hospitals for admission.

Trends of annual incidence rate of hospitalized dengue and CFR

A total of 34 326 dengue cases (DF and DHF) and 831 deaths occurred in Cebu province in 12 years (1997–2008). The highest annual incidence rate of dengue cases among hospitalized patients occurred in 1998 (1.29 cases/1000 patients), 2007 (1.17 cases/1000 patients) and

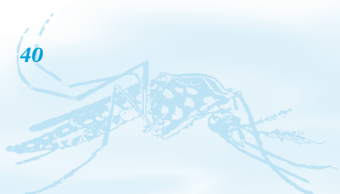
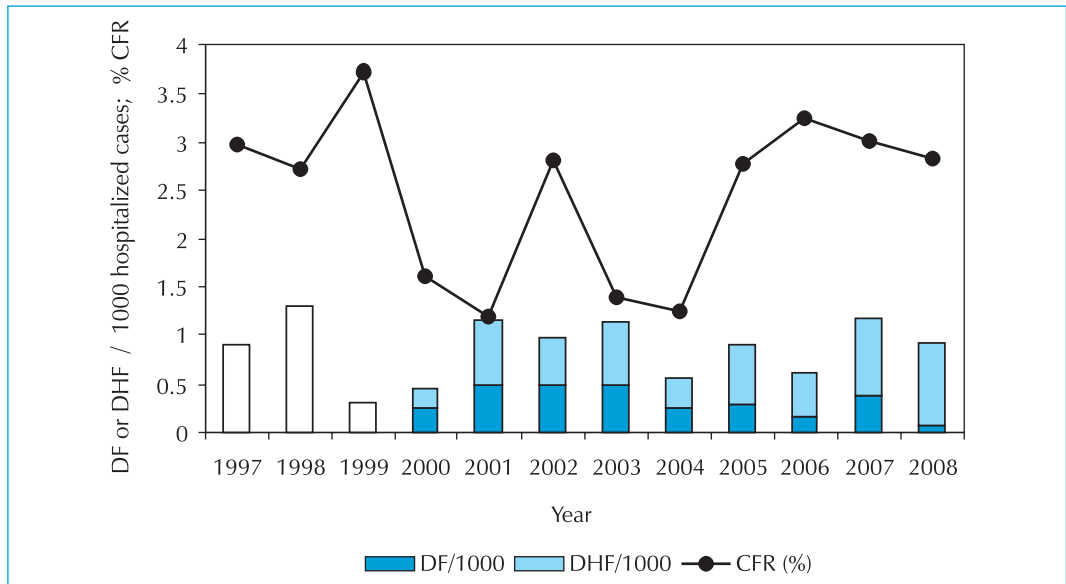


Figure 2: Yearly incidence rates (cases/1000 hospitalized patients) of dengue cases (white bar), DF (solid dark blue bar), DHF (light blue bar) and CFR (%; trend line on top) in Cebu province (1997–2008)

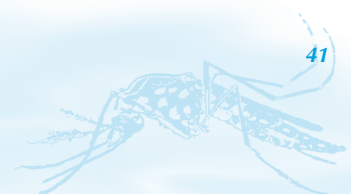


2001 (1.15 cases/1000 patients) (Figure 2), indicating that at least one case of dengue for every 1000 hospitalized patients occurred in each year in 12 years. The lowest happened in 1999 (0.0312 cases/1000 patients). The average CFR in Cebu province during this 12-year period was 2.46% (range = 1.19–3.73%), implying three deaths for every 100 cases that occurred each year (Figure 2). A CFR greater than 3% occurred in 1999, 2006 and 2007.

Total DF cases in 2000–2008 were 9977. The highest incidence of hospitalized DF cases occurred in 2001 and 2002 (0.496 cases/1000 hospitalized patients), in 2003 (0.485 cases/1000) and in 2007 (0.388 cases/1000) (Figure 2). Total DHF cases in the same period were 17 540. The highest incidence of hospitalized DHF cases occurred in 2008 (0.838 cases/1000 hospitalized patients), next was in 2007 (0.779 cases/1000 patients), and in 2003 (0.656 cases/1000 patients). The incidence rates of hospitalized DF and DHF cases in 2000–2008 differed ($t=2.68$, $df=16$, $P=0.016$) at 0.05 level of significance. The incidence rates of hospitalized DHF cases were significant ($P<0.0001$) from year-to-year in 2000–2008, whereas those of DF were mostly significant ($P<0.001$), except in 2002 (z-value = 0; $P>0.99$) and 2003 (z-value = 0.63; $P=0.53$).

Monthly trends of dengue cases and deaths

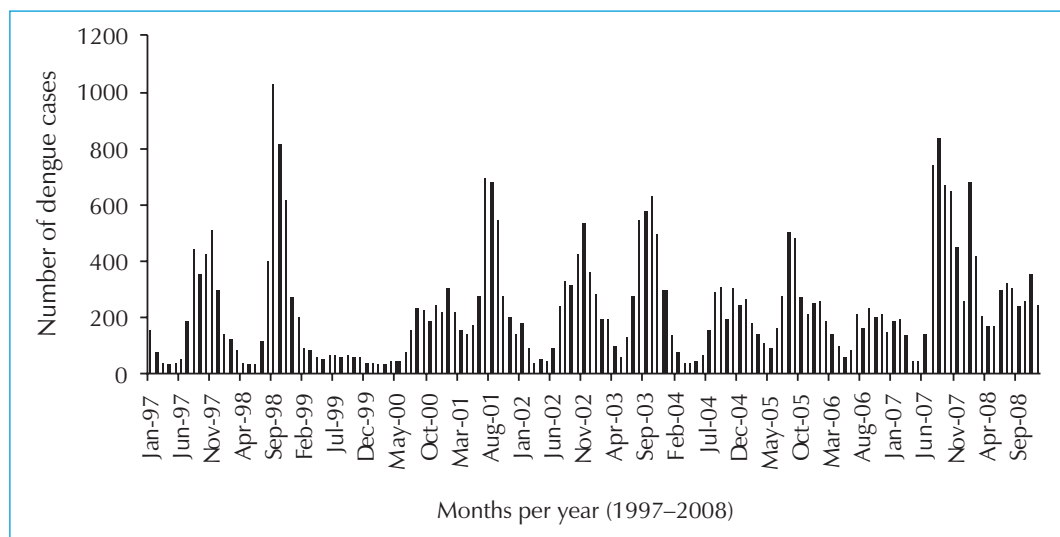
The annual trend of the monthly dengue cases (Figure 3A) increased in the rainy season (June–February) and decreased in summer (March–May). Dengue cases increased dramatically



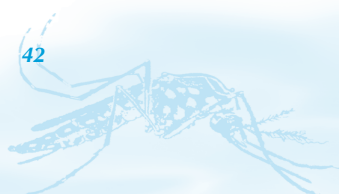
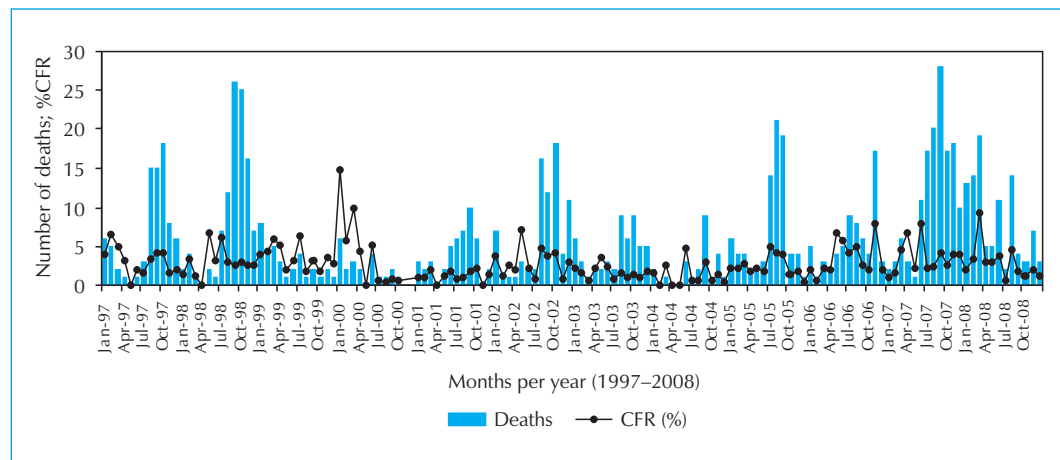
in July, reached their highest level in September, after which they gradually decreased in October through February. A similar trend was observed for the monthly deaths and CFR during 1997–2008 (Figure 3B). Obviously, summer months had lower dengue cases, deaths and CFR.

Figure 3: (A) The monthly number of dengue cases; (B) dengue deaths and CFR, in Cebu province in 1997–2008

A



B



Top 10 municipalities or cities in Cebu province with dengue infections

Based on the average yearly hospitalization rates in the 12-year period (1997–2008), Cebu city recorded the highest number of cases (0.356 cases/1000 population), followed by Mandaue city (0.076 cases/1000 population) consistently each year (Table 1). The rest of the top ten municipalities and cities were Talisay (0.055 cases/1000 population), Lapulapu (0.041 cases/1000 population), Minglanilla (0.038 cases/1000 population), Toledo (0.033 cases/1000 population), Danao (0.027 cases/1000 population), Consolacion (0.025 cases/1000 population), Liloan (0.022 cases/1000 population), and Naga (0.018 cases/1000 population). Cebu city differed in its yearly incidence rates of hospitalized dengue cases among the rest of the top 10 municipalities and cities ($\chi^2=58.16$, $df=9$, $P=0.00$). Its highest incidence occurred in 2001 (0.635 cases/1000 population) and the lowest (0.177 cases/1000 population) in 1999.

The yearly CFR among the top ten municipalities and cities did not vary significantly ($\chi^2=15.316$, $df=9$, $P=0.083$) in the 12-year period (Table 1). Lapulapu had the highest average CFR (4.69%), followed by Consolacion (2.94%), and next by Liloan (2.63%). The average CFR of the top 10 cities and municipalities ranged from 1.47% to 4.69%, implying that 2–5 deaths for every 100 cases occurred in each year in each of these municipalities and cities.

Trends of age distribution

The ages of dengue patients admitted in different sentinel hospitals of DOH-RESU 7 between 1998 and 2008 were categorized into the following age groups: 0–11 months, 1–5 years, 6–10 years, 11–15 years, 16–20 years, 21–40 years, 41–60 years, 61–80 years, and 81–100 years. Separate data for DF and DHF across age groups, and for patients across age groups in 1997 were not available from DOH-RESU 7; hence, excluded in the analysis. One- to five-year-old children had the highest average rate of hospitalized dengue cases (31.82%), followed by 6- to 10-year old children (31.82%) throughout the 11-year period (Table 2). These age groups were homogeneous and had a significantly (P -value <0.001) higher incidence rate of hospitalized dengue cases compared with all other age groups. These were followed by other homogeneous age groups composed of 11–15 years old (average=15.85%), 16–20 years old (average=8.59%) and 21–40 years old (average=8.33%). Infants aged less than one year were the least affected (average = 3.38%) among children. Overall, the least affected were the 81-100-year olds (average = 0.01%). There was a significant difference in the incidence of dengue cases across age groups ($F=527.49$, $df = 8$, $P<0.05$).



Table 1: Yearly incidence rates of hospitalized dengue (cases/1000 population) and CFR (%) of the top 10 municipalities and cities in Cebu province in 1997–2008

Year	Incidence rate (IR) of hospitalized dengue (cases/1000 population) and CFR (%; enclosed in parentheses) per year in top 10 municipalities and cities in Cebu Province									
	Cebu city	Mandaue	Talisay	Lapulapu	Minglanilla	Toledo	Danao	Consolacion	Liloan	Naga
1997	0.391 (2.28)	0.088 (2.34)	0.063 (4.37)	0.035 (3.96)	0.017 (0.02)	0.042 (2.46)	0 (0)	0.04 (4.27)	0.024 (1.43)	0.017 (2.08)
1998	0.528 (1.88)	0.132 (1.82)	0.094 (1.82)	0.023 (10.61)	0.036 (0.94)	0.048 (1.42)	0.033 (1.05)	0.033 (2.11)	0.038 (4.55)	0.045 (2.29)
1999	0.177 (3.86)	0.035 (2.94)	0.011 (0)	0.007 (21.05)	0.008 (0)	0.006 (11.77)	0.003 (0)	0.009 (3.7)	0.008 (8.33)	0.007 (0)
2000	0.246 (1.33)	0.061 (0.98)	0.019 (4.48)	0.019 (0)	0.009 (3.23)	0.005 (0)	0.007 (8.33)	0.025 (0)	0.018 (1.64)	0.005 (0)
2001	0.635 (0.85)	0.078 (1.15)	0.048 (1.85)	0.047 (1.28)	0.022 (1.37)	0.036 (1.65)	0.055 (2.16)	0.024 (1.22)	0.022 (1.39)	0.019 (3.13)
2002	0.397 (2.48)	0.067 (0.44)	0.054 (1.67)	0.031 (2.89)	0.025 (2.38)	0.052 (2.86)	0.024 (2.53)	0.016 (3.85)	0.012 (7.32)	0.007 (8.33)
2003	0.495 (1.08)	0.107 (1.38)	0.071 (1.68)	0.035 (2.54)	0.027 (2.17)	0.05 (1.79)	0.062 (0.97)	0.018 (3.33)	0.019 (0)	0.019 (1.54)
2004	0.203 (1.17)	0.073 (1.22)	0.03 (0.99)	0.035 (2.59)	0.138 (2.04)	0.029 (2.06)	0.013 (0)	0.034 (2.66)	0.05 (1.19)	0.012 (0)
2005	0.255 (3.39)	0.057 (2.62)	0.051 (2.34)	0.046 (1.95)	0.039 (1.53)	0.039 (1.52)	0.066 (0.45)	0.018 (3.28)	0.018 (3.39)	0.014 (2.22)
2006	0.18 (4.13)	0.049 (4.24)	0.032 (1.87)	0.055 (2.19)	0.026 (1.15)	0.012 (2.56)	0.014 (0)	0.029 (4.08)	0.024 (1.22)	0.019 (0)
2007	0.433 (3.41)	0.108 (3.36)	0.072 (3.96)	0.03 (4.31)	0.084 (2.79)	0.038 (0.69)	0.027 (2.91)	0.028 (3.74)	0.023 (1.12)	0.037 (2.82)
2008	0.335 (3.65)	0.058 (4.04)	0.058 (1.79)	0.129 (3.02)	0.028 (0)	0.039 (2.03)	0.025 (2.06)	0.025 (3.09)	0.001 (0)	0.02 (1.32)
Ave. IR	0.356	0.076	0.050	0.041	0.038	0.033	0.027	0.025	0.022	0.018
Ave. CFR	2.460	2.211	2.240	4.690	1.470	2.570	1.710	2.940	2.630	1.980



Table 2: Yearly number (N) and percentage (%) of dengue cases across age groups in Cebu province in 1998–2008

Year	Number (N) and percentage (%; enclosed in parentheses) of dengue cases per year by age groups									
	0–11 months	1–5 years	6–10 years	11–15 years	16–20 years	21–40 years	41–60 years	61–80 years	81–100 years	Total (N)
1998	180 (4.75)	1356 (35.81)	1092 (28.84)	607 (16.03)	285 (7.53)	224 (5.92)	29 (0.77)	13 (0.34)	1 (0.03)	3787
1999	0 (0)	358 (40.96)	291 (33.30)	110 (12.59)	59 (6.75)	49 (5.61)	6 (0.69)	1 (0.12)	0 (0)	874
2000	66 (4.24)	496 (31.84)	522 (33.51)	231 (14.83)	121 (7.77)	109 (7.00)	13 (0.83)	0 (0)	0 (0)	1558
2001	126 (3.26)	1129 (29.22)	1314 (34.01)	614 (15.89)	332 (8.59)	310 (8.03)	36 (0.93)	3 (0.08)	0 (0)	3864
2002	83 (2.99)	919 (33.08)	834 (30.02)	428 (15.41)	252 (9.07)	217 (7.81)	35 (1.26)	9 (0.32)	1 (0.04)	2778
2003	144 (3.76)	1240 (32.38)	1119 (29.22)	597 (15.59)	337 (8.80)	338 (8.83)	44 (1.15)	10 (0.26)	0 (0)	3829
2004	70 (3.65)	621 (32.38)	534 (27.84)	301 (15.69)	175 (9.12)	189 (9.85)	21 (1.10)	7 (0.37)	0 (0)	1918
2005	115 (3.80)	915 (30.21)	910 (30.04)	538 (17.76)	225 (7.43)	285 (9.41)	35 (1.16)	6 (0.20)	0 (0)	3029
2006	71 (3.44)	643 (31.14)	631 (30.56)	306 (14.82)	189 (9.15)	192 (9.30)	31 (1.50)	2 (0.10)	0 (0)	2065
2007	133 (2.95)	1115 (24.77)	1426 (31.68)	843 (18.73)	457 (10.15)	445 (9.89)	67 (1.49)	15 (0.33)	1 (0.02)	4502
2008	159 (4.34)	1035 (28.25)	1036 (28.28)	622 (16.98)	372 (10.15)	365 (9.96)	56 (1.53)	19 (0.52)	0 (0)	3664
Total (N)	1147	9827	9709	5197	2804	2723	373	85	3	31 868
Ave. %	(3.38)	(31.82)	(30.66)	(15.85)	(8.59)	(8.33)	(1.13)	(0.24)	(0.01)	

Discussion

This retrospective study of a total of 34 326 patients describes the epidemiological profile of dengue cases admitted to all sentinel hospitals of DOH-RESU 7¹⁰ in all municipalities and cities in Cebu province, Philippines, between 1997 and 2008. The results showed a surging hospitalization of suspected dengue cases in these 12 years. The highest incidence rate of hospitalized suspected dengue cases occurred in 1998, 2007 and 2001, indicating one case for every 1000 hospitalized patients each year. DHF patients were significantly higher than DF patients in the province based on the 2000–2008 records.

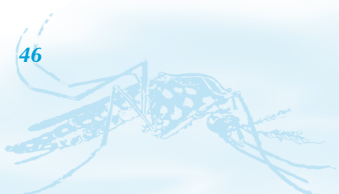


Cebu city had consistently recorded the highest annual incidence rates of hospitalized dengue cases and CFR among all municipalities and cities in the province, which is consistent with the previous report.¹⁴ The rest of the top 10 municipalities and cities included Mandaue, Talisay, Lapulapu, Minglanilla, Toledo, Danao, Consolacion, Liloan and Naga, in that order. These 10 municipalities and cities accounted for 73.89% of the total dengue deaths in Cebu province. Factors that contribute to this growing public health problem, besides the fact that Cebu city has been developed as the centre for commerce, trade, education and industry outside of Manila, include growing population, migration, rapid industrial growth and development, inadequate health-care systems and lack of an effective mosquito surveillance programme. The lack of an adequate solid waste management system creates numerous small artificial breeding sites such as discarded cans, bottles, used vehicle tyres, and the like that are suitable for dengue mosquitoes to breed. The Philippine Ecological Solid Waste Management Act of 2000 (Republic Act 9003) is not fully implemented, which contributed to the increase of dengue cases in Metro Manila.¹⁵ This scenario is similar to many South-East Asian countries.^{16–18} Limited water supply in lower-income populations and water storage in larger tanks in better socioeconomic areas were identified as the causes that might have potentially contributed to dengue transmission in Brazil.¹⁹

Furthermore, increased travel to Cebu Island as a vacation spot for foreigners has been observed, and Cebu city has become popular with many foreign students from Kenya, Republic of Korea, Thailand and middle eastern countries. This potentially increases the number of dengue-infected travellers into the city.^{20,21}

Dengue cases and deaths in Cebu province start to rise in June, reach their highest peak in September, and decrease gradually after that until February, coinciding with the rainy season. This implies that dengue infections exhibit a distinct seasonal pattern and are weather-sensitive.^{22–25} Rainfall played an important role in the occurrence of dengue infections in Metro Manila from 1996 to 2005.²⁵ The El Niño Southern Oscillation (ENSO) phenomenon, a large-scale ocean-atmosphere climate event linked to a periodic warming of the sea surface temperatures across the central and east-central equatorial Pacific,²⁶ has been incriminated in the increase of vector-borne diseases, including dengue infection.^{27,28} Recently, global warming has also contributed to potential disease risks^{26,29,30} because of increased geographical range of mosquitoes and shortened viral incubation inside them, although caution is needed in interpreting these predictions.^{23,31,32}

Although the present study lacks information on primary or secondary infections, the CFR of all dengue patients in Cebu province ranged from 1.19% to 3.73% (mean=2.46%) in 12 years, implying that there were three persons who died of dengue infection per 100 hospitalized patients. This finding is relatively similar to other Asian countries (0.5–3.5%).¹⁵ The bulk of dengue cases occurred in paediatric dengue patients, which can be explained by the fact that because of their endemicity, adults have developed some immunity against the infection. The top three age groups with the highest age-specific cases in Cebu province include: (i) 1- to 5-year-old children; (ii) 6- to 10-year-old children; and (iii) 11–15-year-



old group, in that order. This is consistent with previous reports in the Philippines^{29–34} and countries in Asia^{34,35} where dengue infection was a leading cause of childhood hospitalizations. Infants under one year of age are the least affected (3.38%) among children, which is similar to previous work.³⁶ In Viet Nam, 6- to 10-year old children had the highest risk of DSS.³⁵ Moreover, dengue infections equally affect males (51.1%) or females (48.1%) of all ages in Cebu province. In Thailand, the male to female ratio of infants affected with DHF was 1.3:1,³⁷ however, in Hong Kong Special Administrative Region,³⁸ males were in a majority among dengue cases. A substantial and unexpected bias to males was also observed among dengue cases in Viet Nam in 2003–2006,³⁵ but the relative contribution of innate susceptibility and health-care-seeking behaviour remains unclear.

Thus, the surging public health problem of dengue cases and deaths in Cebu province during the 12-year period (1997–2008) could be attributed to growing urbanization and population, inadequate public health infrastructure, poor solid waste management and lack of an effective mosquito surveillance system.

Acknowledgments

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Dengue in the northernmost part of Brazil from 1999 to 2011: characterization of circulating DENV strains

Pablo Oscar Amézaga Acosta,^{a#} Joel da Silva Cordeiro,^a Fabiana Granja,^a Thalita Caroline da Silva Siqueira,^a Francisco Eduardo Gomes Brito,^a Aline Gondim de Freitas,^a Débora Dinelly de Sousa,^a Joel de Melo Lima,^b George Allan Villarouco da Silva,^c Raphaela Honorato Barletta-Naveca,^d Victor Costa de Souza,^c Vera Margarete Scarpassa^d and Felipe Gomes Naveca^c

^aMolecular Biology Laboratory, Biodiversity Studies Center, Federal University of Roraima (UFRR), 2413 Ene Garcez Avenue, Campus de Paricarana, Bairro Aeroporto. Boa Vista, Roraima, Brazil

^bCoordination of Health Surveillance in Roraima, Boa Vista-Roraima, Brazil

^cLeônidas and Maria Deane Institute, Fiocruz, Manaus-Amazonas, Brazil

^dNational Institute for Amazonian Research (INPA), Av. André Araújo, 2936, Aleixo, Manaus-Amazonas, Brazil

Abstract

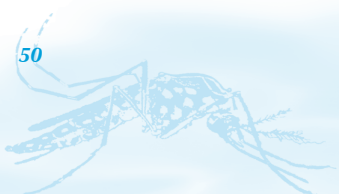
Roraima is the northernmost state of Brazil and its western and southern portion is covered by the Amazon rainforest. This region is one of the most affected by dengue viruses in Brazil. Reports from 1999 to 2011 pointed out a high incidence of dengue epidemics, and the state evolved from hypoendemic to hyperendemic. This led to the isolation and identification of the four dengue virus (DENV) serotypes with a substitution pattern of one serotype for another until 2010, when simultaneous circulation of the four serotypes was detected. In line with the increased risk of secondary infection and entry of new serotypes/genotypes, the number of severe cases increased in recent years. Roraima borders Venezuela and Guyana, where all four dengue serotypes are in circulation, and so it is considered an important port of entry for new genotypes into Brazil.

Keywords: Dengue; Serotypes; Genotypes; Roraima; Brazil.

Introduction

Classic dengue fever (DF) and the severe forms of dengue such as dengue haemorrhagic fever (DHF), dengue shock syndrome (DSS) and other cases which do not fulfil the strict criteria of DHF, being named dengue with complications, are caused by the dengue virus (DENV), which is primarily transmitted by *Aedes aegypti* mosquitoes. Dengue belongs to the

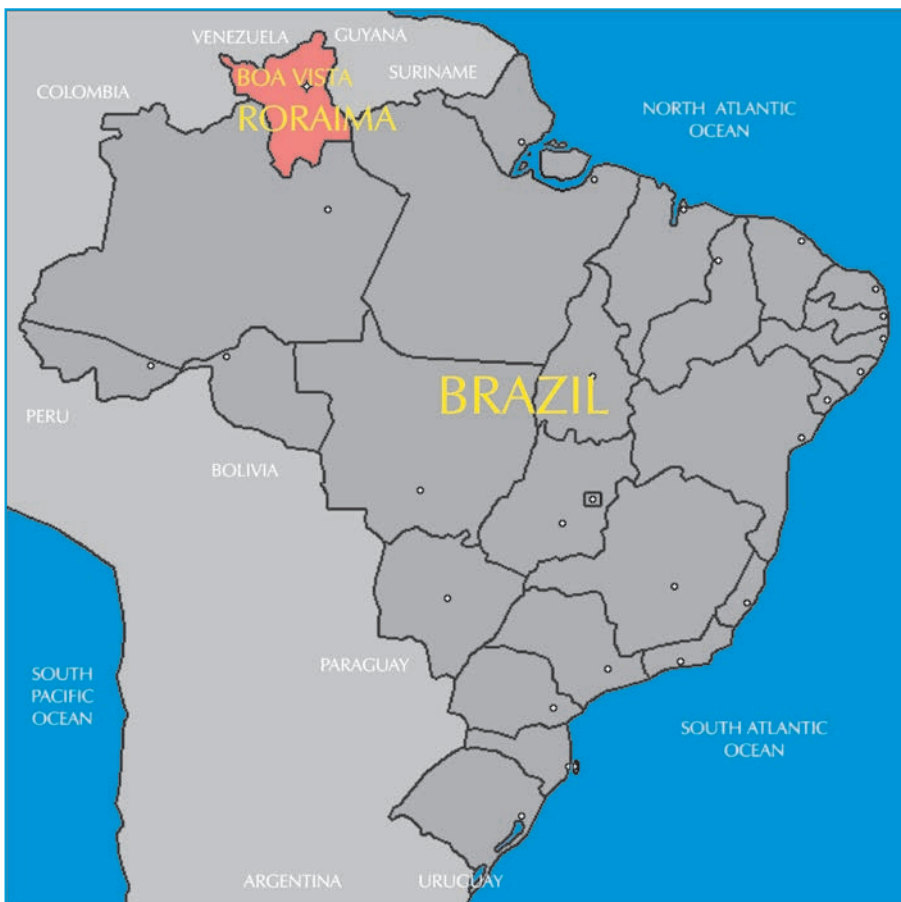
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Flavivirus genus, family *Flaviviridae*, with four antigenically distinctive serotypes: DENV1–4.¹ Within each DENV serotype there are genetic variants known as genotypes, and some of these have been related to the more severe or milder forms of the disease.²⁻⁴

Roraima was the first Brazilian state where a dengue fever epidemic was confirmed by laboratorial tests.⁵ Although dengue is regarded as the most important arboviral disease affecting humans in terms of morbidity and mortality, until recently there was not even a single method for isolation and identification of the virus in the state and little research was conducted on dengue fever. With the establishment of the Molecular Biology Laboratory at the Federal University of Roraima in 2005, virological and molecular techniques have been introduced to isolate and identify the possible different dengue serotypes, as well as to detect the entry of new serotypes/genotypes in the state.⁶ This paper highlights the epidemiological profile of the disease in Roraima, Brazil, from 1999 to 2011 (Figure 1).

Figure 1: Localization of Roraima state, Brazil



Regional characteristics of dengue in Roraima

The first known cases of dengue in the state of Roraima occurred by 1981 when its capital, Boa Vista, was struck by an epidemic of classic dengue fever which lasted until August 1982. Serotypes DENV-1 and DENV-4 were isolated from both patients and *Ae. aegypti* mosquitoes. There were approximately 11 000 cases of DF for an estimated population of 60 000 and no death reports.⁵ It was apparently a local and limited occurrence; local because there was no further case report in other Brazilian states, and limited because after 1982, there was no dengue case reported in Roraima. This epidemic probably stemmed from an outbreak of dengue that occurred in the Caribbean.⁷ After this event, an intensive campaign to eliminate potential mosquito breeding sites, improving water management system and use of insecticide-treated mosquito bednets led to the eradication of the vector. Thus, both entomological and epidemiological surveillance phases were implemented in order to prevent a new entry and outbreak of dengue in Brazil through the state of Roraima. After preventing the occurrence of the dengue epidemic for 14 years, failures in the surveillance system management led to the dispersal of *Ae. aegypti*, facilitating dengue circulation which was already present in other Brazilian states and neighbouring countries.

This provided the entry of dengue virus to the Roraima state, with reports of 116, 54 and 26 cases of DF in the years 1996, 1997 and 1998, respectively,⁸ without serotype identification. With the number of cases increasing, in 1999, the Secretariat of Health recorded the second dengue epidemic in Roraima, with a total of 1920 cases reported, and the first isolation of dengue serotype 2 (Table 1). Even though the pre-existing heterologous immunity in part of the population (with antibodies to DENV-1 and/or DENV-4 acquired in the epidemic of the years 1981–1982) and the possibility of secondary infection, no DHF/DSS cases were reported.

Since 1999 when DENV re-emerged in Roraima until 2011, successive epidemics have occurred and the four serotypes of DENV (1–4) have been identified (Figure 2). The Brazilian Ministry of Health has ranked the state in terms of high incidence rate of dengue fever (>300 cases per 100 000 inhabitants) in 10 of the 13 years examined in this study (Table 1). The circulation of multiple serotypes simultaneously contributed to an elevation of the incidence culminating in 2010 with more than 1600 cases per 100 000 inhabitants and the simultaneous identification of all four DENV serotypes in the same year (Table 1).

Despite the high incidence rate, these numbers are probably underestimated. Studies by Vasconcelos and colleagues concerning epidemiological surveys in Brazil point out that underreporting may vary over 20 times the number of reported cases.⁹

The records of severe dengue occurred since 2003 (Table 1), which is probably due to the hyperendemic situation with co-circulation of multiple serotypes/genotypes with enhanced virulence, as well as the enhanced skills of health professionals in identifying severe forms of dengue.

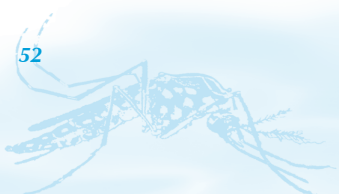


Table 1: Type infection of dengue and serotypes isolated in Roraima, Brazil, 1999–2011

Year	Total cases of dengue	IR	Severe dengue	Deaths	% Severe dengue	DENV serotypes isolated
1999	1920	451.3	0	0	0.00	2
2000	4107	965.4	0	0	0.00	1, 2
2001	3345	786.3	0	1	0.00	1, 3
2002	1268	298.1	0	0	0.00	1, 2, 3
2003	4982	1171.1	7	2	0.14	2, 3
2004	564	132.6	7	0	1.24	3
2005	2317	544.7	54	0	2.33	2, 3
2006	817	192.1	11	1	1.35	1, 2, 3
2007	672	158.0	5	0	0.74	1, 2, 3
2008	5537	1301.6	389	2	7.03	1, 2, 3
2009	3134	736.7	172	2	5.49	1, 2
2010	7037	1654.2	285	6	4.05	1, 2, 3, 4
2011	1325	311.5	21	0	1.58	1, 2, 4

Source: Data from the health department of the state of Roraima-Brazil, Laboratory of Molecular Biology of Federal University of Roraima-Brazil and IBGE

Note: Severe dengue = dengue hemorrhagic fever + dengue shock syndrome + dengue with complications

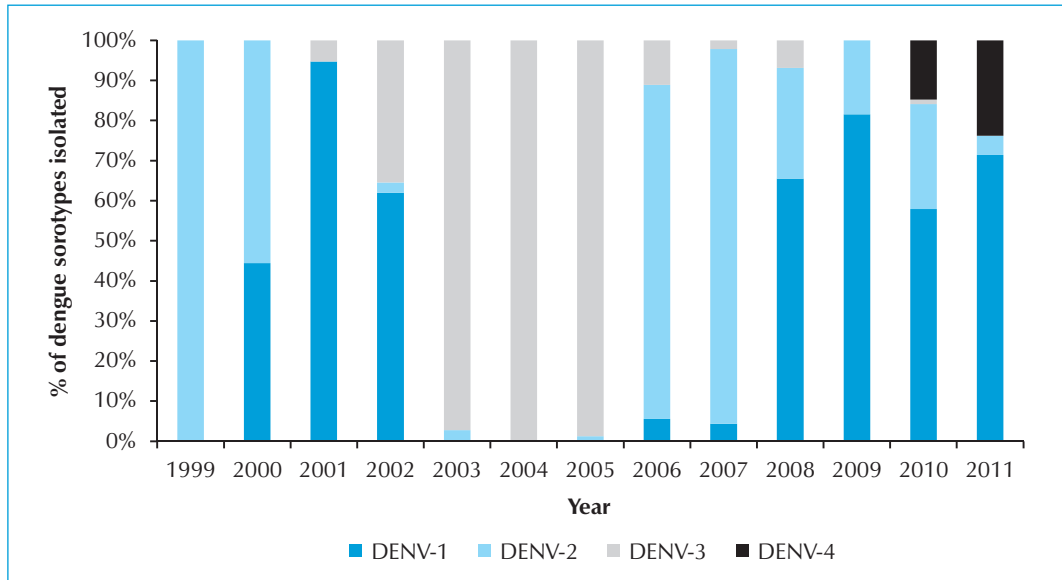
% Severe dengue = (Severe dengue/Total cases dengue) X 100

IR – Incidence rate per 100 000 inhabitants

In order to study the relationship of the incidence of dengue cases and the pluviometric index in the rainy season (April–September) and the dry season (October–March), a study was carried out in the state capital, Boa Vista, during 1999–2009 when the dengue incidence rates between the wet season and the dry season did not differ significantly (Student's *t*-test, $p=0.5034$, $\alpha=0.05$) (Table 2). These data differ from most Brazilian states where dengue has a seasonal pattern with an increase in the number of cases in the wet season.¹⁰



Figure 2: Variations in prevalence of dengue serotypes by years, Roraima, Brazil, 1999–2011



Source: Data from the Health Department of the State of Roraima-Brazil and Laboratory of Molecular Biology of Federal University of Roraima, Brazil.

Table 2: Incidence rate of dengue and rainfall (RF) in Boa Vista, Roraima, during the dry and rainy seasons from 1999 to 2009

		1999–2000	2000–2001	2001–2002	2002–2003	2003–2004	2004–2005	2005–2006	2006–2007	2007–2008	2008–2009
Wet season	% RF	78.6	86.6	83.0	94.8	89.2	87.9	83.4	91.8	82.2	60.0
	% DEN	48.8	52.7	28.0	40.2	78.8	22.9	98.9	27.3	47.0	59.3
Dry season	% RF	21.4	13.4	17.0	5.2	10.8	12.1	16.6	8.2	17.8	40.0
	% DEN	51.2	47.3	72.0	59.8	21.2	77.1	1.1	72.7	53.0	40.7

Source: Data from the health department of the state of Roraima, Brazil, 1st District of Meteorology/AM/AC/RR, IBGE/RR

Note: IR - No statistically significant differences t -test at $p=0.5034$ and $\alpha=0.05$. The t -test was performed in SAEG statistical software Sistema para Análises Estatísticas, Versão 9.1: Fundação Arthur Bernardes - UFV - Viçosa, 2007¹¹



Characterization of circulating DENV strains

Is there an incursion of dengue strains from neighbouring countries into Brazil through Roraima?

The state of Roraima has been considered an important port of entry of new serotypes and genotypes of dengue in Brazil, mainly for its geographical location and features linking the Brazilian Amazon and the Caribbean through international borders with the Bolivarian Republic of Venezuela and the Cooperative Republic of Guyana, countries that have circulation of four dengue serotypes (Figure 1). Here we present data from our research studies of the genotypes of DENV that have been circulating in the state.

DENV-1

DENV-1 serotype was introduced in Brazil through Roraima during the 1981 epidemics.⁵ Afterwards that serotype was reintroduced in the country through the state of Rio de Janeiro in 1986, and then it spread over the rest of the country.^{12,13} Studies on molecular epidemiology of DENV-1 have revealed that such strains belonged to the genotype V, the same genotype circulating in other countries in the Americas.^{14,15}

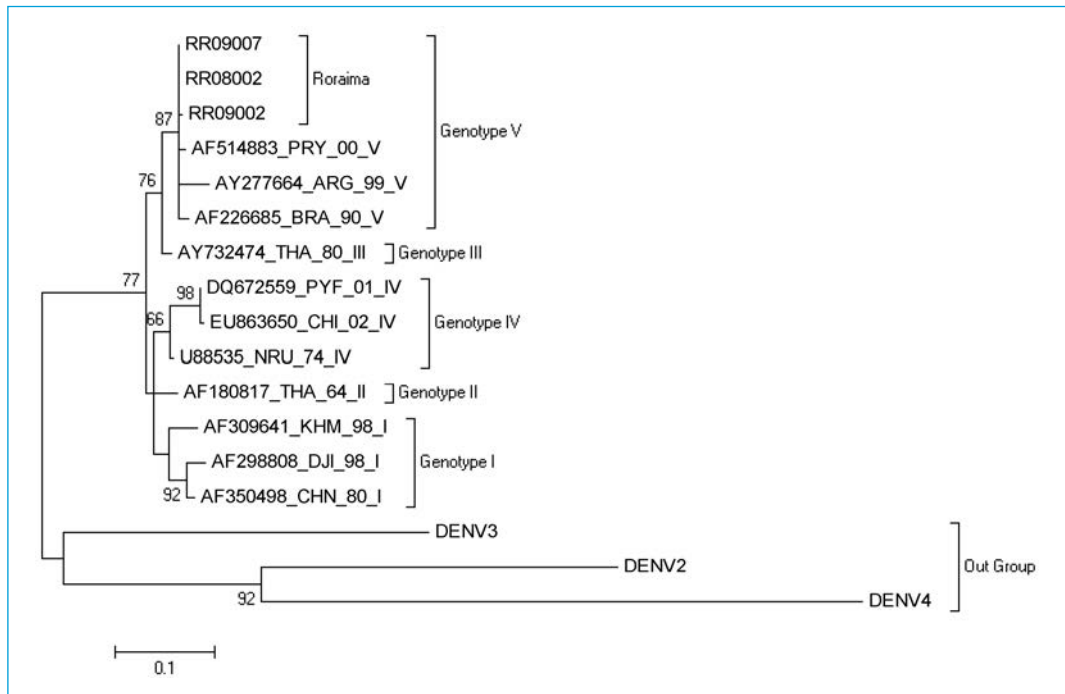
After a brief stint in the state of Roraima in 1981 DENV-1 was reintroduced in 2000 and has been isolated in nine of the 13 years analysed (Table 1 and Figure 2). Phylogenetic studies based on sequence analysis isolates of 2008 and 2009 from Roraima have revealed that these strains belong to the genotype V (Figure 3),¹⁶ the same genotype of DENV-1 that was circulating in the state during 1981 and has been present since it re-emerged in the country in 1986.^{14,17} Cordeiro, using envelope gene to analyze the phylogenetic relationship of DENV-1 isolated in Roraima 2009, described that this genotype was clustered with isolates from Venezuela in 1997 and 2007, but not with a strain isolated in Brazil in 1997.¹⁶ These results might point out that DENV-1 had at least two independent routes of introduction into Brazil: throughout the state of Roraima by means of strains from Venezuela, and from other Latin American and Caribbean countries.

DENV-2

DENV-2 entered Brazil during 1990–1991 via the state of Rio de Janeiro (RJ), and thus came the reports of the first severe forms of dengue mostly associated with secondary infections since DENV-1 was already circulating in the country.²⁰ Genetic studies with such strains revealed that they belonged to the Jamaican genotype. This Asian variant was isolated in the Americas during a severe outbreak that harshly affected many areas of the Caribbean in 1981 and which is now known as the Asian/American genotype.^{21,22} This confirms that the



Figure 3: DENV-1 genotype
Maximum likelihood phylogenetic tree reconstructed using a 317nt fragment corresponding to junction region of Capsid and Membrane precursor (C/prM) genes



Note: [Molecular evolution model used was TrN+I+G selected by jModel Test software and phylogeny was made using MEGA software Version 5.0. The reliability of tree was evaluated using Bootstrap method (only values above 60% are shown). Three Roraima strains were used and 14 strains identified in previous studies, besides the three other serotypes of DENV used as outgroup. Roraima strains clustered with genotype V]^{18,19}

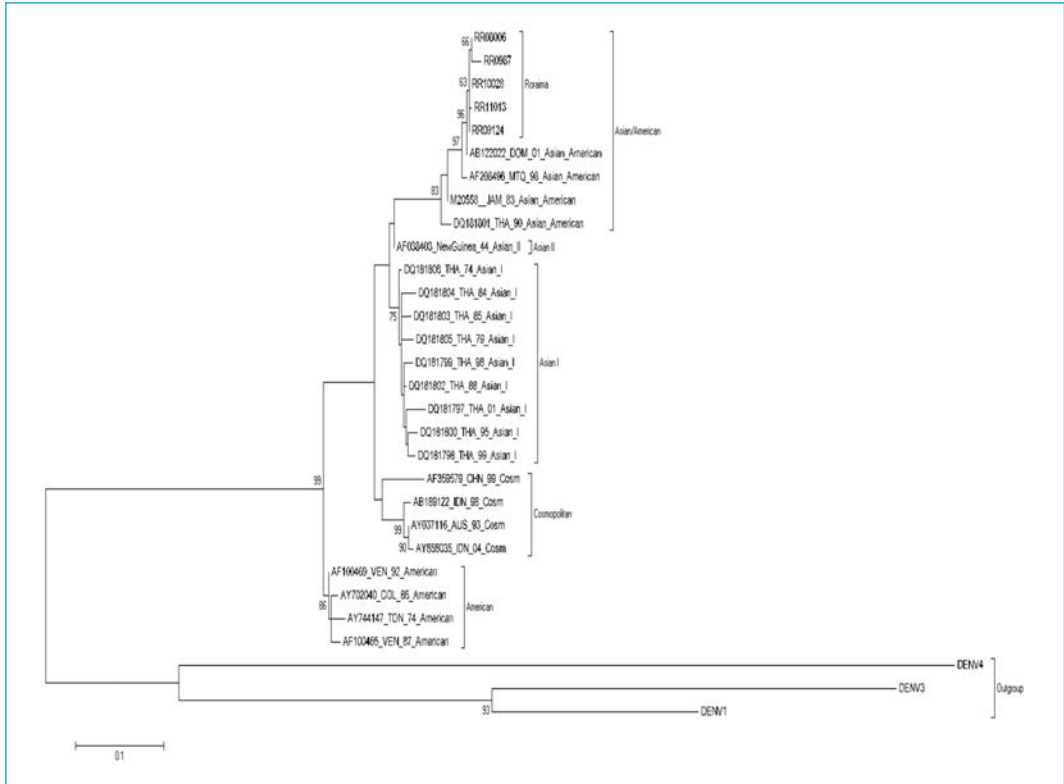
DENV-2 strains circulating in Brazil originated in the Caribbean and hit the country through infected people and vectors.

Serotype DENV-2 was first isolated in Roraima in 1999 (Table 1), and probably has been circulating during the entire analysed period once it was isolated in 11 of the 13 study years. The genetic analysis of the isolates from 2008 to 2011 within this serotype indicated that these samples belong to the Asian/American genotype (Figure 4). The phylogenetic analysis of 2009 strains formed a clade with samples circulating in Brazil since 1990 and isolated in Cuba, the Dominican Republic, Martinique, and Puerto Rico, places with several DHF/DSS epidemics.¹⁶



Figure 4: DENV-2 genotype

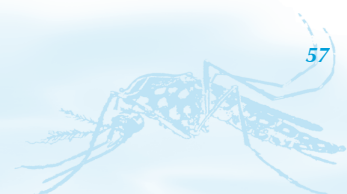
Maximum likelihood phylogenetic tree reconstructed using a 392nt fragment corresponding to junction region of Capsid and Membrane precursor (C/prM) genes



Note: [Molecular evolution model used was GTR+G selected by jModel Test software and phylogeny was made using MEGA software Version 5.0. The reliability of tree was evaluated using Bootstrap method (only values above 60% are shown). Five Roraima strains were used and 22 strains identified in previous studies, besides the three other serotypes of DENV used as outgroup. Roraima strains clustered with “Asian/American” genotype]^{18,19}

DENV-3

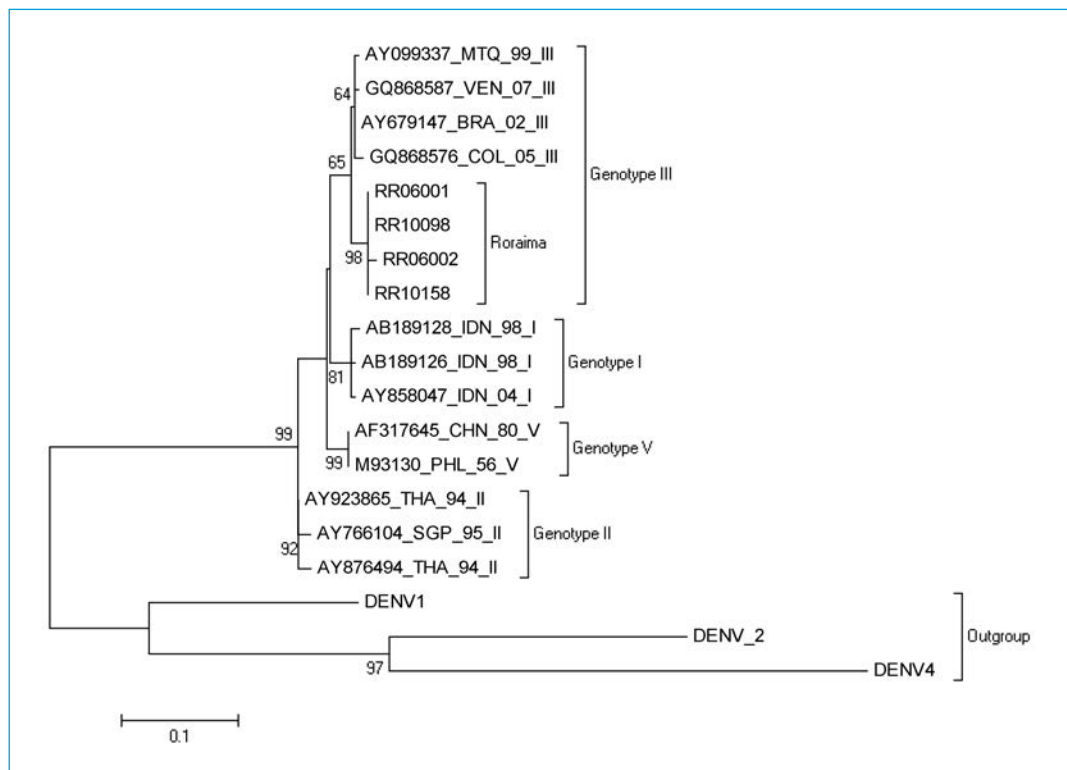
In Brazil, DENV-3 was first isolated in 1999 from a non-autochthonous case. Its entry into the country is considered from January 2001 when it was identified in an epidemic that hit the state of Rio de Janeiro and swept across 25 out of the 26 states in the country during three years.^{13,23} There was a significant increase in both incidence rates and severe forms of the disease. DENV-3 genotype III (Sri Lanka/India) was identified in almost all states involved in those epidemics.²⁴



Unlike what happened to DENV-2, sooner identified in other states, DENV-3 was detected in Roraima earlier. In 2001, ten months after its entry into Brazil, it was responsible for an epidemic with a pattern similar to the rest of the country.²⁵ Since its entry, DENV-3 has replaced DENV-1 and DENV-2 serotypes that were circulating in the state, and it was virtually the only serotype isolated from 2003–2005 associated with a rise in the number of severe forms of dengue (Figure 2 and Table 1). The molecular analysis of the strain isolated in the state indicated that it belongs to the genotype III (Sri Lanka/India) (Figure 5), the one with the widest circulation in Brazil and the Americas. Studies on the chronology of virus isolation show that the introduction of DENV-3 genotype III into Brazil occurred across the south-north direction, more specifically from the state of Rio de Janeiro to the northern states.

Figure 5: DENV-3 genotype

Maximum likelihood phylogenetic tree reconstructed using a 295nt fragment corresponding to junction region of Capsid and Membrane precursor (C/prM) genes



Note: [Molecular evolution model used was TrN+I+C selected by jModel Test software and phylogeny was made using MEGA software Version 5.0. The reliability of tree was evaluated using Bootstrap method (only values above 60% are shown). Four Roraima strains were used and 12 strains identified in previous studies, besides the three others serotypes of DENV used as outgroup. Roraima strains clustered with genotype III]^{18,19}



However, phylogenetic studies performed with strains in northern states (Roraima and Pará) showed they are more related with circulating viruses isolated in the Caribbean islands and in northern South America (Guyana, Venezuela, Peru and Ecuador). This suggests that the entry of DENV-3 into the northern region would have directly occurred through the Caribbean and not only via Rio de Janeiro,^{26,27} i.e. the entry of DENV-3 into Brazil through Roraima via Venezuela and Guyana was a distinct event, not related to the Rio de Janeiro cases.

DENV-4

The first entry of DENV-4 into Brazil was via Roraima during the 1981 dengue epidemics.⁵ Phylogenetic analysis of these samples showed that these belonged to the American genotype.²⁸ And it was not until 25 years later that this serotype was detected in another Brazilian state (2008), when researchers at the Tropical Medicine Foundation of Amazonas in Manaus isolated and identified samples from three DENV-4 patients. It was, however, an example of the genotype I (Asian) which was not isolated in other states of the country yet. It was likely to be an independent event and the virus entry may have occurred, as Melo et al. proposes, through a direct route to Asia due to close economic relations between Manaus and Japan.^{29,30}

The next entry of DENV-4 into Brazil occurred in late July 2010 through Roraima again, but this time it swept through several Brazilian states.³¹ Naveca et al. were the first ones to complete the Brazilian DENV-4 genome sequence which phylogenetic analysis has shown that it belongs to the American genotype (Figure 6), i.e. the same genotype circulating in the first entry into the country.³² It seems that this genotype has never ceased to move around since its appearance in Roraima-Brazil and other American countries in 1981. Instead, it has spread and has been circulating in the Americas with isolation and identification in several countries of the West Indies, Central and South America, but not in Brazil.^{33,34} These results allow us to suggest that DENV-4 may have entered Roraima in 2010 via Venezuela and Guyana, the countries that have reported such serotype/genotype circulation, and that border Roraima and have close economic relations with.

The nucleotide sequences used in this paper can be accessed at GenBank under the accession number: JX502768(RR08002); JX502769(RR09007); JX502770(RR09002); JX502771(RR08006); JX502772(RR09124); JX502773(RR0987); JX502774(RR10028); JX502775(RR11013); JX502776(RR06001); JX502777(RR10158); JX502778(RR10098); HQ822125(RR10_II); HQ822126(RR10_II); JN983813(RR10_II).

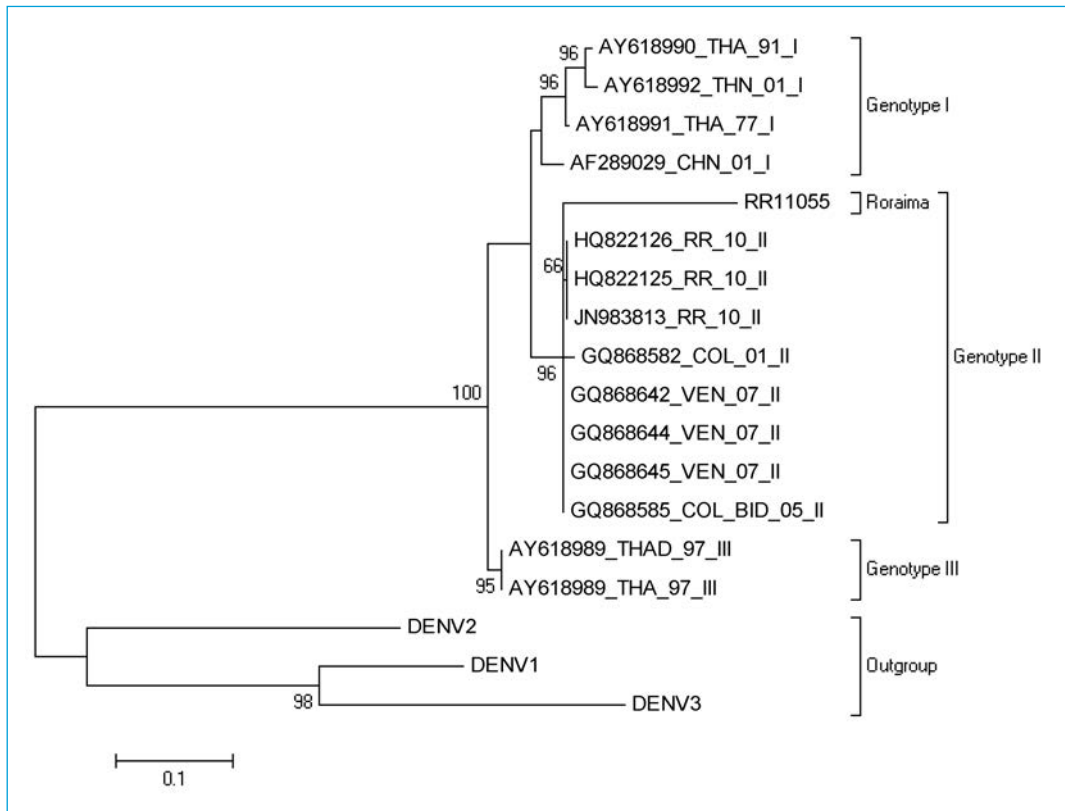
Conclusion

Roraima is a hyperendemic and high dengue-incidence state in Brazil, so the increase of secondary infections and virulence of circulating strains is a real possibility that could lead to a rise in the number of severe forms of the disease. Besides, its public health importance to the



Figure 6: DENV-4 genotype

Maximum likelihood phylogenetic tree reconstructed using a 330nt fragment corresponding to junction region of Capsid and Membrane precursor (C/prM) genes



Note: [Molecular evolution mode lused was TrN+G selected by jModel Test software and phylogeny was made using MEGA software Version 5.0. The reliability of tree was evaluated using Bootstrap method (only values above 60% are shown). One Roraima strains were used and 14 strains identified in previous studies, besides the three others serotypes of DENV used as outgroup. Roraima strains clustered with genotype II]^{18,19}

population of Roraima, data presented in this study reveal that the state has a crucial role to play in the introduction/reintroduction of new variants of DENV in the country. This reinforces the importance of monitoring the virus in a state key to Brazilian dengue epidemiology for providing expeditiously reliable information to the national health authorities so that they can respond appropriately to a health problem that affects the entire population. These data could also contribute to the development of mathematical models and other tools to evaluate dengue dispersion in Brazil.



Acknowledgments

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Climatic factors affecting dengue fever incidence in Lahore, Pakistan

Hassan Z Baig and Anem J Nawaz[#]

Shifa College of Medicine, Pitrus Bukhari Road, H-8/4 Islamabad, 44000, Pakistan

Abstract

The study aimed to investigate the relationship of climatic parameters such as temperature, rainfall and humidity with cases of dengue fever during the 2011 outbreak in Lahore, and their possible role in making the epidemic so devastating.

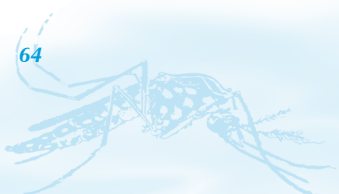
Line lists of more than 25 000 serologically confirmed dengue fever patients during the past five-year period (2007–2011) were obtained from the WHO Disease Surveillance Unit at Islamabad and Lahore. Daily data of mean, maximum and minimum temperature (°C), amount of precipitation (mm) and humidity (%) for this period were obtained from the web site www.tutiempo.net/en/ and arranged epidemiologically week-wise. To determine the type of relationship between the meteorological variables and dengue incidence, Spearman correlation was conducted. The number of dengue cases was the dependent variable, while the climate variables were the independent variables. Cross-correlation between the dengue cases and climate variables was also carried out with lag times of 2, 4, 6, and 8 weeks. The data were entered into Free Statistics Software (2012).

Total rainfall seemed to be more associated with dengue cases than the other variables graphically. Positive correlations which were statistically significant were observed between dengue cases and mean temperature with lag times of 4, 6, and 8 weeks. Minimum temperature and relative humidity at all lag times also had statistically significant correlations with dengue case incidence. With lag times of 4 weeks and especially 6 and 8 weeks, statistically significant positive correlations were observed for precipitation and dengue cases. Precipitation was found to have the strongest correlation with dengue cases with a lag time of eight weeks (correlation coefficient 0.363, p -value <0.05). Maximum temperature was the only variable which had no statistically significant correlation with dengue cases at all lag times.

Our study suggests that optimal temperature with abundant stocks of fresh water reservoirs (generated due to rain) provide conditions conducive for mass breeding and propagation of the vector and transmission of the virus. Greater insight into dengue vector ecology and disease transmission patterns, together with more targeted use of environmental management strategies, may offer improved potential for combating dengue fever, the world's fastest growing vector-borne disease.

Keywords: Dengue fever; Temperature; Rainfall; Humidity; Lahore; Pakistan.

[#]E-mail: anemjaved@gmail.com



Introduction

Dengue fever (DF) has been recognized as one of the world's emerging tropical infectious diseases. WHO estimates that dengue is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, and the South-East Asia and Western Pacific regions¹ and that around 50–100 million people are affected with DF every year across the globe.

Pakistan first reported an epidemic of dengue fever in 1994.² Since then, the country has experienced numerous outbreaks. In 2011, Lahore experienced an outbreak of dengue in proportions that outnumbered past records. Not only were many people affected by dengue, that outbreak caught the attention of all due to the high number of lives it claimed.

Studies show that the vector, *Aedes aegypti*, has adapted to domestic human habitation.^{3,4} In tropical and subtropical regions, it has been seen that optimum temperature and rainfall levels enable adult vectors to remain active throughout the year.⁵ This contributes to a continuous transmission cycle that makes the disease endemic in a particular region.

Previous studies have revealed that climatic changes influence transmission of the dengue virus and *Ae. aegypti* reproduces rapidly and bites more frequently at higher temperatures.^{6,7} Temperature, precipitation and humidity are critical to mosquito survival, reproduction and development and can influence mosquito existence and abundance.⁸

Materials and methods

Study area

The study was centered around the city of Lahore, Pakistan. The summer season is from May till September. The monsoon season begins in July and ends in September. The average annual rainfall is 471.1 mm.⁹

Study design

A retrospective study was performed to determine the correlation between various meteorological variables and the incidence of dengue cases in the city of Lahore from the years 2007 to 2011.

Data collection

Daily climate data were obtained from an online source (www.tutiempo.net/en/).¹⁰ The data from this source was obtained directly from the local weather station in Lahore. Daily mean,



minimum and maximum temperatures, relative humidity and precipitation for the years 2007 to 2011 were obtained. The daily values were used to obtain weekly averages. Line lists of more than 25 000 patients affected with dengue fever in the past five years who were serologically confirmed, were obtained from the Disease Surveillance Unit, WHO Lahore.

Data analysis

In order to determine the type of relationship between the meteorological variables and dengue incidence, Spearman correlation was conducted. The number of dengue cases was the dependent variable while the climate variables were the independent variables. Cross-correlation between the dengue cases and climate variables was also carried out with lag times of 2, 4, 6, and 8 weeks. The data were entered into Free Statistics Software (2012).¹¹

Results

Trends of meteorological factors and dengue cases

The mean, minimum and maximum temperatures rose according to a linear regression analysis. The mean temperature rose approximately 2.0°C and the minimum temperature rose approximately 2.0°C. The maximum temperature rose approximately 1.5°C and the mean relative humidity rose approximately by 2%. Total annual rainfall kept decreasing from 2007 until 2010. However, in 2011, there was 2.5 times more rainfall than in 2010. From 2007 to 2008, the number of dengue cases decreased from 1673 to 1420. The year 2009 saw a major decline in the cases with a total of only 87. In 2010, the dengue cases rose significantly reaching almost 4500; in 2011, these quadrupled to approximately 16 800.

Correlation between meteorological factors and dengue cases

An association between the climate variables and dengue cases was observed; the amount of rainfall seemed to be more associated than the other variables (Figure 1). In order to determine the presence and type of association, Spearman correlation was conducted. The correlation was not statistically significant for any variables (Table 1). Furthermore, cross-correlation was carried out with a lag time of 2, 4, 6, and 8 weeks and it revealed some significant correlations (Table 2).

Positive correlations which were statistically significant were observed between dengue cases and mean temperature with lag times of 4, 6, and 8 weeks. Minimum temperature and relative humidity at all lag times also had statistically significant correlations with dengue case incidence. A positive correlation between precipitation and dengue cases was observed but was not statistically significant with a lag time of two weeks. However, with lag times of 4 and especially 6 and 8 weeks, statistically significant positive correlations were observed

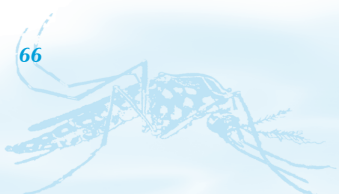


Figure 1: Rainfall and cases in Lahore, Pakistan, 2007–2011

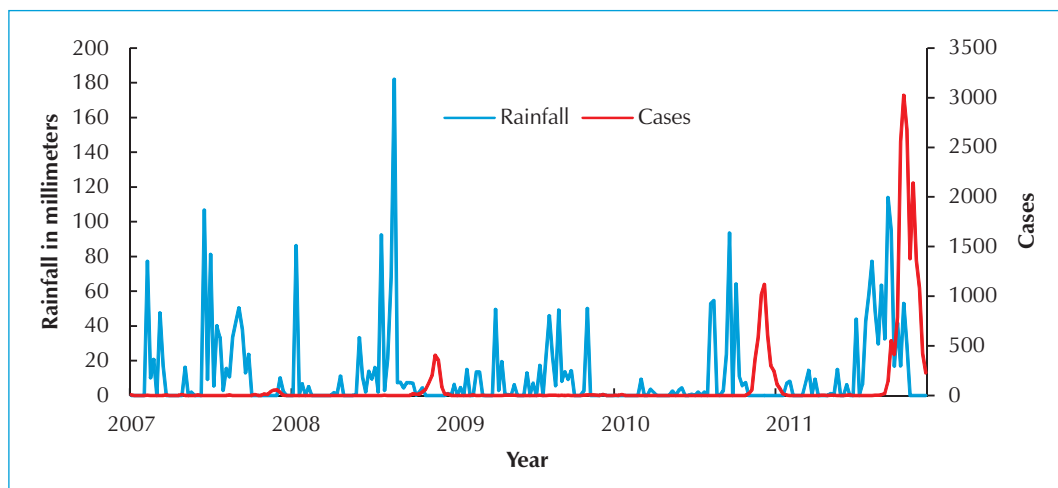


Table 1: Correlation coefficients of climate variables and dengue incidence, Lahore, Pakistan, 2007–2011

Meteorological variable	Spearman correlation coefficient	p-value
Mean temperature	-0.07499	0.235576
Maximum temperature	-0.06976	0.269896
Minimum temperature	-0.03475	0.582997
Precipitation	-0.11014	0.080985
Relative humidity	0.055127	0.38352

Table 2: Correlation coefficient at various lag times of climate variables and dengue incidence, Lahore, Pakistan, 2007–2011

Meteorological variable	Correlation coefficient at various lag times			
	2 weeks	4 weeks	6 weeks	8 weeks
Mean temperature	0.100239	0.125767*	0.151752*	0.162535*
Maximum temperature	0.065805	0.079132	0.102537	0.110343
Minimum temperature	0.146203*	0.172545*	0.194927*	0.204657*
Precipitation	0.104082	0.285056*	0.317675*	0.363426*
Relative humidity	0.206623*	0.244996*	0.24742*	0.258841*

*p-value < 0.05



for precipitation and dengue cases. Precipitation was found to have the strongest correlation with dengue cases with a lag time of eight weeks (correlation coefficient 0.363, p -value <0.05). Maximum temperature was the only variable which had no statistically significant correlation with dengue cases at all lag times.

Discussion

Studies show that most of the vector-borne diseases exhibit a distinctive seasonal pattern and association to climatic parameters such as rainfall, temperature and other weather variables.¹²

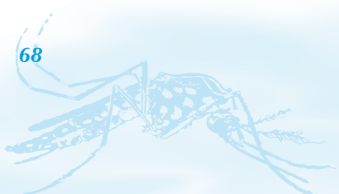
Temperature

The greatest effect of climate change on transmission is likely to be observed at the extremes of the range of temperatures at which transmission occurs. According to a WHO study, laboratory experiments have demonstrated that the incubation period of dengue virus could be reduced from 12 days at 30°C to 7 days at 32–35°C in *Ae. aegypti*.^{13,14} Various other studies estimate that temperature rise of 1.0–3.5°C increases the likelihood of accelerated transmission of vector-borne diseases in endemic and new areas.¹³ Temperature trends in Lahore from 2007 to 2011 show that mean, minimum and maximum temperatures are on the rise according to a linear regression analysis. Mean temperature has risen approximately 2.0°C, minimum temperature has risen approximately 2.0°C, while maximum temperature has risen by approximately 1.5°C. This trend of rising temperature may help explain the rise in dengue incidence over the last few years.

Another important observation about temperature is that the increasing minimum temperature has a significant and non-linear impact on the extrinsic incubation period,⁶ and consequently disease transmission, while, at the upper end, transmission could cease. However, at around 30–32°C, vectorial capacity can increase.¹³ The results of our study also indicate a positive correlation between minimum temperature and dengue incidence with lag times of 2, 4, 6, and 8 weeks. A negative correlation was observed between maximum temperature and dengue incidence, but it was not statistically significant.

Precipitation and humidity

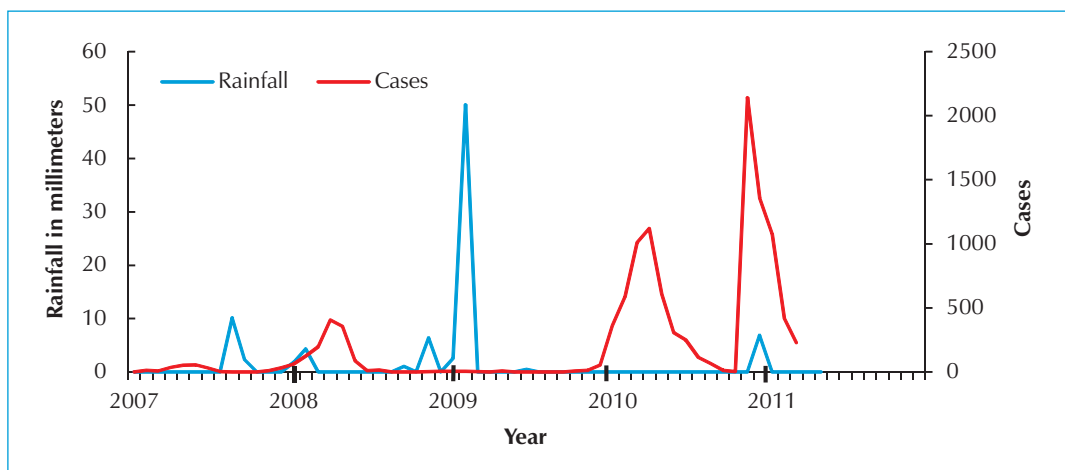
Various studies have consistently shown that precipitation and humidity affect the incidence of dengue.^{3,15-17} As humidity increases, the metabolism of the vector increases as well, thus improving survival and the probability of disease transmission. However, as humidity decreases, the vector becomes more susceptible to dehydration and results in decreased survival and disease transmission.¹⁸ From 2007 to 2011, mean relative humidity has risen



approximately 2% in Lahore which may help explain the rise in dengue cases especially over the past two years. Furthermore, observations from our study also revealed significant positive correlations between mean relative humidity and dengue cases with lag times of 2, 4, 6, and 8 weeks.

Rainfall is an important climate variable for vector development. Several studies have shown the role precipitation plays in dengue incidence.¹⁹⁻²¹ From 2007 to 2010, total rainfall per year kept decreasing. In 2011, the rainfall was 2.5 times more than in 2010. In Pakistan, the monsoon period is taken from July to September. According to the Pakistan Meteorological Department, the rainfall during the 2011 monsoon months across the country was in excess of 87 per cent and was the highest since 1994 and ranked the second highest in the last 50 years.⁹ In our study, the largest proportion of serologically positive cases was recorded in the post-monsoon period (Figure 2), which is in agreement with studies from this geographical region.²²⁻²⁴ A study of the surge of dengue cases has shown that the post-monsoon period is the most favourable period for mosquito breeding in Bangladesh as well.²⁵ Our study also confirms the importance of rainfall as the most significant meteorological variable. This can be seen by the significant positive correlation between precipitation and dengue cases with lag times of 4, 6, and 8 weeks. The association is lagged, which may indicate the requirement for adequate precipitation for dengue transmission. However, precipitation must be moderate;¹⁹⁻²¹ excessive rainfall may disturb the peridomestic breeding environment. This may explain why even though the year 2009 had the most rainfall in the post-monsoon season, it had the least number of cases over the five years (2007–2011). Furthermore, the year 2011 had the most total rainfall as compared to the other years, but a modest amount of rain fell during the post-monsoon season as compared with 2009. The year 2011 also had the highest number of dengue cases on record. These observations may explain the large increase in dengue cases in 2011 by pointing towards a permissive effect of rainfall.

Figure 2: Post-monsoon rainfall and dengue cases in Lahore, Pakistan, 2007–2011



Rising minimum and mean temperatures, humidity and especially total precipitation, seem to be important climatic factors for dengue incidence. However, disease transmission and incidence depend not only on climate, but also on non-climatic variables which have not been addressed in this study. For example, the effect of various construction projects in urban and suburban areas of Lahore could have created many new breeding sites for the mosquito vector. As dengue incidence has been on the rise, government interventions have also increased, which could have had an effect on disease transmission as well.

Conclusions

There is a need for the strengthening of the dengue surveillance programme. Its main objective should be to “identify risk factors of human infection and then ways and means aimed at reducing them”.²⁶ Currently, the initiative for identification of risk factors is lacking. Only when certain risk factors are brought into focus will a surveillance programme be able to effectively predict an impending epidemic and the onset of an outbreak. For this, cohort studies need to be undertaken. Ambient temperature “should also be considered an operationally viable component of large-scale surveillance programmes”.²⁶ The study of temperature in Lahore over the past five years has pointed towards several trends. Furthermore, the study of spatial (not political) boundaries will allow for a better understanding of vector distribution.²⁶

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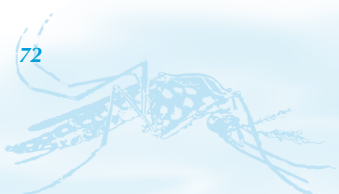
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Risk assessment and risk maps using a simple dengue fever model

José Eduardo Marques Pessanha[#]

Belo Horizonte Observatory for Urban Health (OSUBH) and Municipal Health Secretariat of Belo Horizonte City, MG, Brazil, Rua Curitiba, 1588/1101, 30170-122, Belo Horizonte, MG, Brazil

Abstract

Since 1986 through 2012, the incidence of dengue in Brazil has shown an upward trend with over 1 million cases per year reported during 2010 and 2011. Despite efforts to control *Aedes aegypti*, an uncomfortable situation has remained with large epidemics involving all the four dengue serotypes (DENV-1 to 4). Brazil has been subject to more extreme peaks. This paper discusses the use of simple tools to predict dengue incidence through a risk map based on entomological and human cases parameters. We apply a mathematical model for dengue infection that takes into account vector indicators and historical incidence, in the years 2005–2010 in Belo Horizonte city, Minas Gerais, Brazil. We created a risk map of the intra-urban areas and compared it with the incidence coefficient in the next year. We compared the impact in 2011 when it was adopted for control strategies based on the priorities defined in the risk map. It is demonstrated that the measures were effective in reducing the number of cases in the year after the start of control measures.

Keywords: Arbovirus; Dengue outbreaks; DF/DHF; Burden of dengue; Brazil.

Introduction

Dengue fever (DF) is a viral mosquito-borne infection caused by four antigenically distinct viruses, designated as dengue serotypes (DENV1–4).¹ The main vectors of dengue are *Aedes aegypti* and *Aedes albopictus*.¹

The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people are now at risk from dengue. WHO currently estimates there may be 50–100 million dengue infections worldwide every year.¹ Cases across the Americas, South-East Asia and the Western Pacific exceeded 1.2 million in 2008 and over 2.2 million in 2010. Lately, the number of reported cases has continued to increase. In 2010, 1.6 million cases of dengue were reported in the Americas alone, of which 49 000 cases were of severe dengue. Not only is the number of cases increasing as the disease spreads to new areas, but

[#]E-mail: edumpessanha@hotmail.com



also explosive outbreaks are occurring. The threat of a possible outbreak of dengue fever now exists in Europe and local transmission of dengue was reported for the first time in France and Croatia in 2010, and imported cases were detected in three other European countries.¹

In Brazil, DF reappeared in the north-western Amazon region, in the state of Roraima, caused by DENV-1 and DENV-4, in 1982.² By 1986, DENV-1 had caused a sharp outbreak of DF in Rio de Janeiro, then spread towards the north-east and mid-west regions.³ In 1990, DENV-2 was reported in Rio de Janeiro, from whence it spread across the country, becoming endemic in some areas.⁴ Since 2000, all 27 Brazil Federations Unity had reported DF cases. In the period 1990–2007, DF caused 6 642 936 officially reported cases (MS 2012a)⁵ with 1454 deaths (MS 2012b).⁶

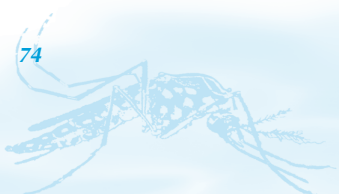
The increase in the incidence of dengue cases in 2002 and the emergence of DENV-3 led to a prediction of an increased risk of dengue epidemics and an increase in the dengue haemorrhagic fever (DHF) cases.⁷ To face the expected risks for 2002, the Brazil Ministry of Health, in collaboration with the Pan-American Health Organization, organized an international seminar in June 2000 to evaluate the dengue epidemic and to prepare a National Dengue Control Programme (PNCD).⁷ Currently, DF is the most important arthropod-borne viral disease in Brazil and all the four serotypes (DENV-1 to 4) are circulating in the country.⁸

Successive infections in Asia resulted in DHF epidemics. In Brazil, epidemics of dengue, DENV-1, DENV-2 and DENV-3 caused mainly DF, with surprisingly few cases of DHF.⁹

In 1996, the first dengue epidemic in Brazil occurred in Belo Horizonte (BH), the capital of Minas Gerais state, the third most densely populated in the country's metropolitan area, and since then, several epidemics have occurred every year. The serotype initially identified was DENV-1 in 1998, and DENV-2 and DENV-3 were isolated in 2002 for the first time.¹⁰ From 2007 to 2010, the most prevalent serotypes were: DENV-3 in 2007, DENV-2 and DENV-3 in 2008, DENV-2 in 2009 and DENV-1 in 2010.¹¹ However, after the introduction of DENV-4 in 2012, the four serotypes now coexist.¹¹

In recent years, mathematical modelling has become an interesting tool for the understanding of infectious diseases epidemiology and dynamics. Newton and Reiter¹² were the first to initiate research on dengue modelling. They introduced the minimal model for dengue for the human population disaggregated in Susceptible (S), Exposed (E), Infected (I) and Recovered (R) compartments, but the mosquito population was not modelled in this work. Focks et al. described mosquito populations in a Dynamic Table Model,^{13,14} where later the human population (as well as the disease) was introduced to make a local dengue epidemic model.¹⁵

Dengue infection by one serotype confers life-long immunity to only that serotype, and temporary cross-immunity to other related serotypes. The susceptible become infected and



infectious, are cured and become recovered. After a waning immunity period, the recovered individual can become susceptible again to re-infection by other serotypes.

Multi-strain dengue model introduces the idea of competition of multiple strains in dengue epidemics; however, it cannot be easily performed. Dengue epidemiology may be modelled with SEIR-type models, and the basic model motivated by modelling dengue fever epidemiology is able to predict future outbreaks of dengue in the absence of human interventions, a major goal if one wants to understand the effects of control measures. At the moment, such simple models have a chance to be understood well and have tested existing data. The simplicity of the model offers a promising perspective on parameter values inference from the DF case notifications.¹⁶⁻²⁰

The Risk Assessment and the Risk Map are tools used to identify and evaluate illnesses and outbreak risks. The map allows visualizing risks in relation to each other, gauging their extent, and planning what type of controls should be implemented to mitigate them.²¹

In Brazil, an alternative approach, to estimate the larval index (LI), that is, the proportion of houses with breeding places harbouring vector larvae and/or pupae, was proposed in order to optimize the logistics of its operation. It consists of a random sampling technique in which the sample unit corresponds to 9000 to 12 000 buildings. The surveillance of foci positive for breeding places is carried out simultaneously in several cities around the country. This strategy has been called Larval Index Rapid Assay (LIRA).²²

In September 2010, the Brazil Ministry of Health suggested identification of areas of increased vulnerability to occurrence of dengue transmission with a view to support the intensification of control, including epidemiological and entomological indicators.²³

In this manuscript, we present the properties of the basic SEIR epidemic model applied to dengue fever epidemiology by the use of a Risk Map. We focused on the effects on the host population and the effect of the vector dynamics.

The aim of this work was the identification of risk areas of transmission of dengue fever through a mapping based on a composite indicator of risk factors. Discussed are the determinants of the distribution of cases and the vector in urban space and an evaluation of potential scenarios of transmission of the virus, through an examination of epidemiological, entomological and environmental indicators of areas with the presence of cases and the vector. These scenarios represent clues for the improvement of dengue surveillance actions. We did a dengue fever “risk map” with a measure of risk with a causal approach. It will give public health services up to ten weeks of early warning for the next outbreak – enough time to reduce impact. We propose modelling the daily evolution of dengue prevalence at a town-scale. These models have a chance to be understood well and eventually tested against existing data.



Materials and methods

Study area

This ecological study was conducted in Belo Horizonte (BH), the capital of the state of Minas Gerais, in the south-east region of Brazil (19°55'S 43°57'W).

Occupying an area of 330.23 sq km²⁴ with 2 375 151 inhabitants in approximately 600 000 households,²⁵ BH is Brazil's sixth most populous city. Situated at altitudes ranging from 700 to 1200 metres (mean 858 metres), BH has a tropical wet and dry climate with an average annual temperature of approximately 21°C.²⁶

The model

In the present model, mosquito and human populations is the urban unit (UU) in a spatial unity, with 9000 to 12 000 buildings (LIRA's sample unit), tuned to Belo Horizonte city. The model then assumes that mosquitoes belong to the urban unity and not to the houses, and they blood-feed with equal probability on any human resident in the UU. *Aedes aegypti* is assumed to disperse, seeking places to lay eggs. The number of bites per day, dispersal flights and adult mortality information per UU is estimated from the mosquito model.

To the mosquito indicators we used as proxy the eggs average (vector density) in ovitraps, which cover a radius of 200 metres—installed every two weeks,²⁷ and the building larval index (LI) as proposed by Connor and Monroe²⁸ to measure the dispersion of *Aedes aegypti* in urban areas and represent the proportion of houses with *Aedes aegypti* larvae.

The application of these methods to estimation of dengue was based on the R_0 expression for dengue fever and the SEIR model:

$$R_0 = \frac{N_m}{N_h} \times \frac{a^2 b c^{-\mu T}}{\gamma \mu}$$

where,

N_h is the human population;

N_m is the mosquito population;

a is the average daily bite rate;

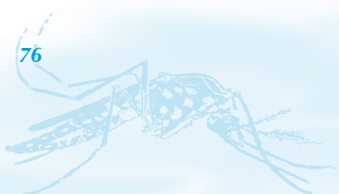
$1/\gamma$ is the average duration of viremia (infectious period in humans);

μ is the mortality rate for female mosquitoes;

T is the average duration of the extrinsic incubation period (in days);

b is the transmission coefficient from mosquitoes to humans; and

c is the transmission coefficient from humans to mosquitoes.



SEIR models

“S” is represented by human population without “R”.

“E” is represented by human population adjusted by the horizontal building proportion and the mosquito presence.

“I” was estimated by the dengue cases that occurred in the July to October period of the current year.

“R” was estimated equal of the cumulated cases in the last five years (considering the low human dengue lethality in Brazil).

The 2010 and 2011 risk map

Risk indicators associated with the dengue models

- S proportion = $(N_h - R) / N_h$ or $1 - \text{Cumulated Incidence Coefficient last five years (2005 to 2009) and (2006-2010)}$
- R proportion = $1 - \text{Cumulated Incidence Coefficient last five years (2005 to 2009) and (2006-2010)}$
- I = dengue cases occurred from July to October of the current year (2009 to 2010) and (2010 to 2011)
- E = human population in relation to the proportion of horizontal buildings (2009-2010), N_m density and N_m dispersion.
- N_m density = Eggs average (2009 July–October to 2010) and (2010/July–October to 2011)
- N_m dispersion = Building larval index (2009 October to 2010) and (2010 October to 2011)

Risk indicators are adjusted by the division by the greatest value.

Weight of risk indicators

A given risk factor may matter more than others.

Where risk factors overlap, risk heightens.

Risk indicators were carefully analysed (linear regression) and weighted:

- Eggs average (v_1), (weight=2)
- Building larval index (v_2), (weight=1)



- S proportion represented by $\frac{(N-R)}{N} = 1 - \frac{R}{N}$ or $1 - \text{Cumulated Incidence Rate } (p)$ last five years, (weight=2)
- R proportion or $(1-p)$, (weight=2)
- I represented by dengue cases (c) occurred from July to October of the current year, (weight=2)
- E represented by human population in relation to the proportion of horizontal buildings (d), (weight=1).

The risk-factor score

- Risk-factor score = $2*v_1 + v_2 + 2*p - 2*p^2 + 2*c + d$

Risk-factor score label = 0 to 10

Data analysis

We estimate R_0 from epidemic data as the growth rate of the epidemic measure by the Incidence Coefficient (number of infected / exposed population), since higher R_0 imply a higher epidemic. The dengue Incidences Coefficients (IC) per 1000 inhabitants, in the 71 UU, were correlated with their respective risk-factor score. To estimate the correlations between the variables, and define the weight by risk indicator, we carried out a linear regression analysis that correlated the risk indicator versus IC. The strength of the above correlations was estimated by the calculation of the Pearson's correlation coefficients. All the statistical analyses were carried out with the software R (R Development Core Team; <http://www.r-project.org>).

Risk maps, by combining the risk-factor layers, taking into account their relative importance, show areas at lower to higher risk, for both introduction and spread for the analysed urban unities. TabWin 3.5 (<http://www.datasus.gov.br>) was used to make BH maps.

Results

Table 1 presents the multiple linear regression of the values of the variables that compose the risk indicators, from 2005 to 2009, and IC of the subsequent year. All variables were statistically significant with p -value <0.05 .

Table 2 shows the 71 UUs analysed with the respective values of the risk-factor scores to 2010 and 2011, and IC per 1000 inhabitants to 2010 and 2011. The IC for 2011 was lower than in 2010 in all UUs.

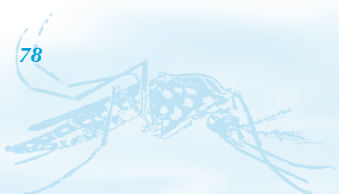


Table 1: Multiple linear regression model for Urban Units (UU) Incidences Coefficients (IC), Belo Horizonte, Brazil

lm (formula = IC_year_subsequent ~ p + p ² + v ₂ + v ₁ + c + d)					
Residuals:					
Min	1Q	Median	3Q	Max	
-0.305856	-0.033258	-0.009319	0.007877	1.055429	
Coefficients:					
	Estimate	Std error	t value	Pr(> t)	
(Intercept)	-0.29838	0.12485	-2.390	0.0173	*
p	1.05758	0.13025	8.119	5.28e-15	***
p ²	-101358	0.16518	-6.136	1.96e-09	***
v ₂	0.14710	0.03577	4.112	4.72e-05	***
v ₁	0.31926	0.07269	4.392	1.42e-05	***
c	0.47385	0.09815	4.828	1.94e-06	***
d	0.28401	0.12796	2.219	0.0270	*
Residual standard error: 0.1046 on 419 degrees of freedom					
Multiple R-squared: 0.4455, adjusted R-squared: 0.4375					
F-statistic: 56.1 on 6 and 419 DF, p-value: <2.2e-16					

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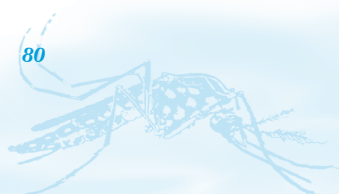
The empirical data available for dengue cases consist of the cumulative incidence of reported cases in 2010. For this type of data, the risk map indicator presents a qualitative good agreement between empirical data and model output, as Figure 1 shows by the graphic of linear regression to risk-factor score for 2010 and dengue IC per 1000 inhabitants in 2010. In the result of the model's simulation for 2010, we can see that the model tallies with the actual data with reasonable accuracy, with *R* equal a 0.6 and *p*-value <0.05.

Finally, we presented the relation between the Map Risk for 2010 and the Map of the IC by UU in 2010 (Figure 2), and Figure 3 shows the Map Risk for 2011 and the Map of the IC by UU in 2011.



Table 2: Risk-factor score and Incidence Coefficient (IC) per 1000 inhabitants to 2010 and 2011 of the 71 Urban Units (UU), Belo Horizonte, Brazil

Urban Unit	2011 Risk-factor score	IC 2011	2010 Risk-factor score	IC 2010
Barreiro1	1.10	0.30	1.76	4.26
Barreiro10	1.15	0.50	1.52	13.46
Barreiro2	1.36	0.50	1.47	4.98
Barreiro3	1.17	0.40	1.89	6.57
Barreiro4	1.17	0.50	1.30	6.20
Barreiro5	1.42	0.30	1.62	6.63
Barreiro6	1.34	0.70	1.53	12.09
Barreiro7	1.35	1.90	1.47	5.71
Barreiro8	1.16	0.30	1.22	6.83
Barreiro9	1.17	1.10	1.59	3.87
Centro-Sul1	1.13	0.20	1.44	2.87
Centro-Sul2	1.22	0.40	1.29	2.20
Centro-Sul3	1.21	0.30	1.22	2.20
Centro-Sul4	1.13	0.30	1.12	2.68
Centro-Sul5	1.27	0.50	1.63	4.73
Leste1	1.92	0.80	1.44	32.09
Leste2	1.90	0.60	1.82	19.79
Leste3	2.07	1.40	2.27	26.33
Leste4	1.85	0.50	1.46	17.78
Leste5	2.10	0.60	1.68	12.65
Leste6	2.07	0.60	2.06	13.98
Leste7	1.92	0.90	1.88	15.84
Leste8	1.97	0.60	1.78	16.16
Nordeste1	2.64	0.50	1.95	21.88
Nordeste10	1.12	0.60	1.72	36.67
Nordeste2	2.11	0.80	2.57	25.61
Nordeste3	2.10	0.40	2.07	18.89
Nordeste4	2.20	0.80	1.89	8.77
Nordeste5	1.75	1.00	2.18	21.23
Nordeste6	1.94	0.50	1.57	5.77
Nordeste7	2.18	0.10	1.86	30.06
Nordeste8	1.62	0.50	2.25	14.09
Nordeste9	1.68	0.90	1.96	12.98
Noroeste1	2.32	1.40	2.22	37.20
Noroeste2	2.03	0.50	2.08	38.66



Urban Unit	2011 Risk-factor score	IC 2011	2010 Risk-factor score	IC 2010
Noroeste3	1.97	0.80	2.21	19.29
Noroeste4	1.83	1.20	1.90	17.22
Noroeste5	2.20	0.40	1.88	15.66
Noroeste6	1.74	0.50	1.58	26.20
Noroeste7	1.81	2.00	1.73	26.70
Noroeste8	1.93	0.60	1.87	17.06
Noroeste9	2.08	0.30	1.71	25.95
Norte1	1.88	1.00	2.86	22.46
Norte2	1.83	3.20	2.11	67.93
Norte3	1.86	0.90	2.30	48.77
Norte4	1.61	1.40	2.78	43.98
Norte5	1.75	1.20	2.01	39.94
Norte6	1.72	0.50	2.33	28.35
Norte7	1.62	0.60	2.14	49.33
Oeste1	2.51	0.30	2.33	11.00
Oeste2	1.88	0.70	2.04	36.12
Oeste3	1.57	0.30	1.50	49.44
Oeste4	1.87	0.60	1.71	13.72
Oeste5	2.03	0.30	1.60	30.75
Oeste6	1.54	0.50	1.50	25.24
Oeste7	1.72	0.50	1.81	13.26
Oeste8	1.81	0.30	2.38	18.31
Pampulha1	2.42	0.60	2.36	46.15
Pampulha2	2.07	0.40	3.29	56.52
Pampulha3	2.39	0.40	1.86	38.68
Pampulha4	2.26	0.60	2.00	15.60
Pampulha5	2.07	0.50	2.04	26.59
Venda Nova1	1.64	0.10	4.10	40.81
Venda Nova2	1.59	0.30	2.15	36.67
Venda Nova3	1.62	0.50	2.13	23.61
Venda Nova4	1.86	0.10	2.09	50.05
Venda Nova5	2.17	0.60	4.64	48.53
Venda Nova6	1.61	1.30	2.53	91.04
Venda Nova7	1.74	0.60	2.58	58.01
Venda Nova8	1.56	1.50	1.89	41.04
Venda Nova9	1.63	0.40	4.32	78.77

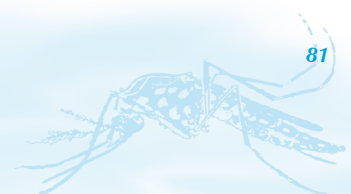
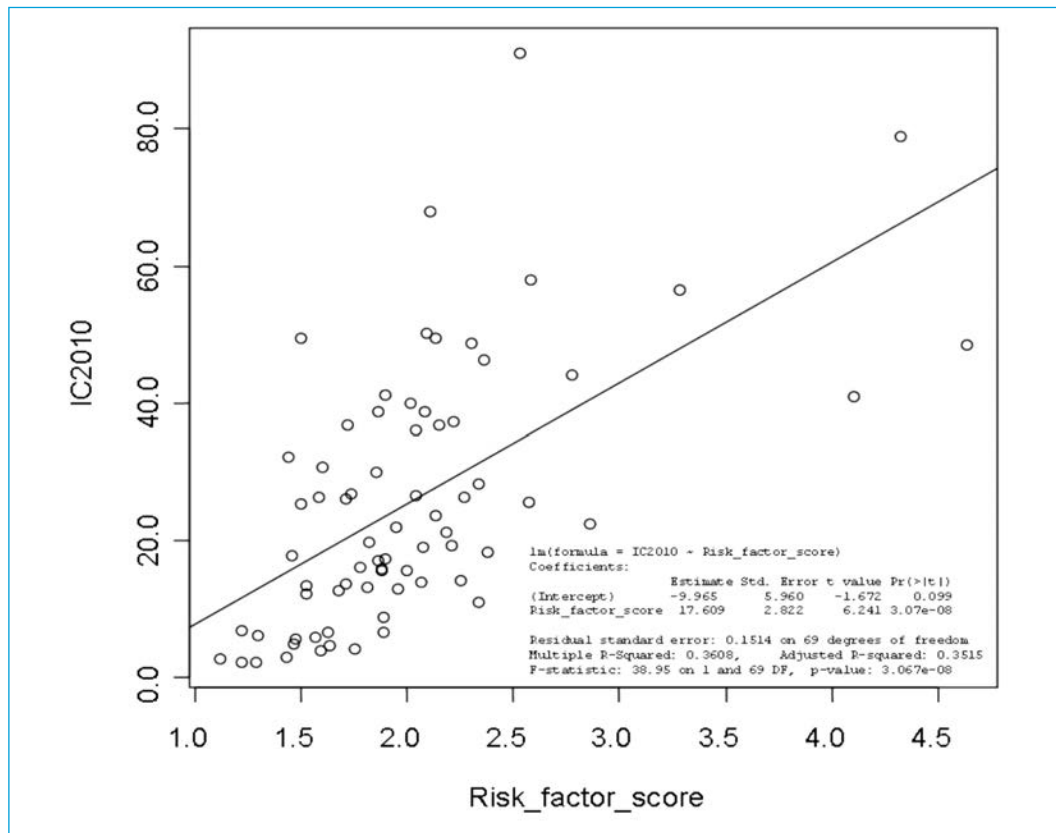


Figure 1: Linear regression graphic to risk-factor score for 2010 and dengue IC per 1000 inhabitants in 2010, urban unit, Belo Horizonte, Brazil



Discussion

We should stress here that the estimated indicator used in this analysis refers to urban unit averages. It was demonstrated that dengue, like many of the other vector-borne infections, has heterogeneous spatial distribution over the affected areas. This theoretically can predict the dengue incidence. The model is based on known cases and the vector monitoring. Moreover, dengue control measures, as proposed by the Brazilian National Programme of Dengue Control, should prioritize the foci where transmission is likely to occur.

The regression analysis indicates that, despite the values obtained for the correlation coefficients, they are significant for the association between the risk-factor score and the dengue incidence coefficient in the next season. The use of the LIRA indicators and the average eggs in the traps is a proxy entomologically-derived variable and the dengue incidence coefficient were estimated from human cases. Here, it is noteworthy that there are significant correlations between them.



Figure 2: Map risk for 2010 and the Map of the incidences coefficients (IC) by urban unit (UU) in 2010, Belo Horizonte, Brazil

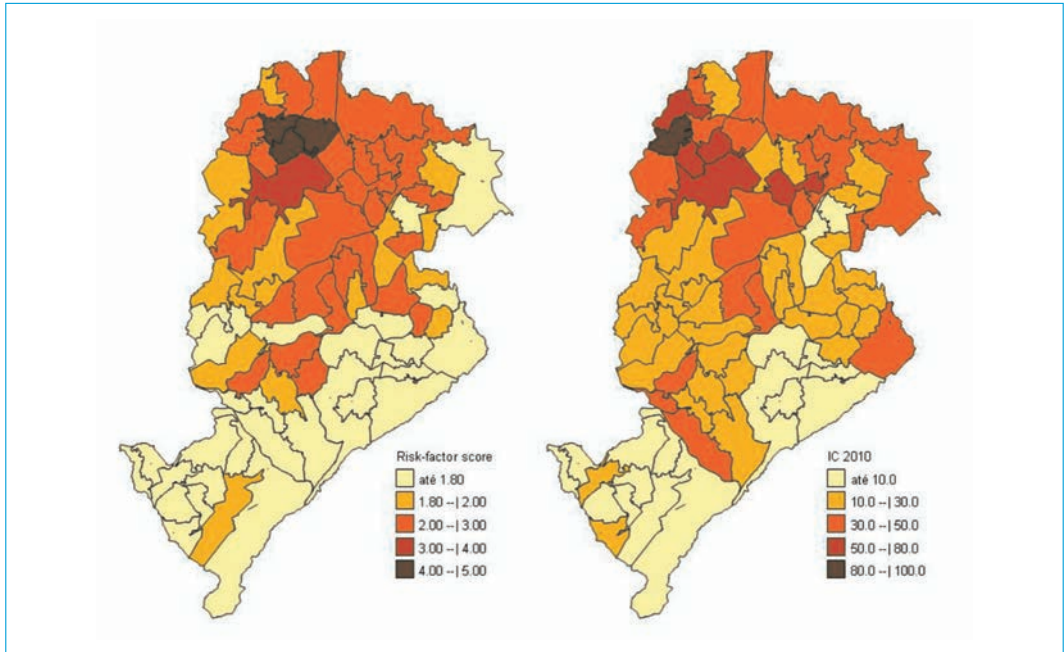
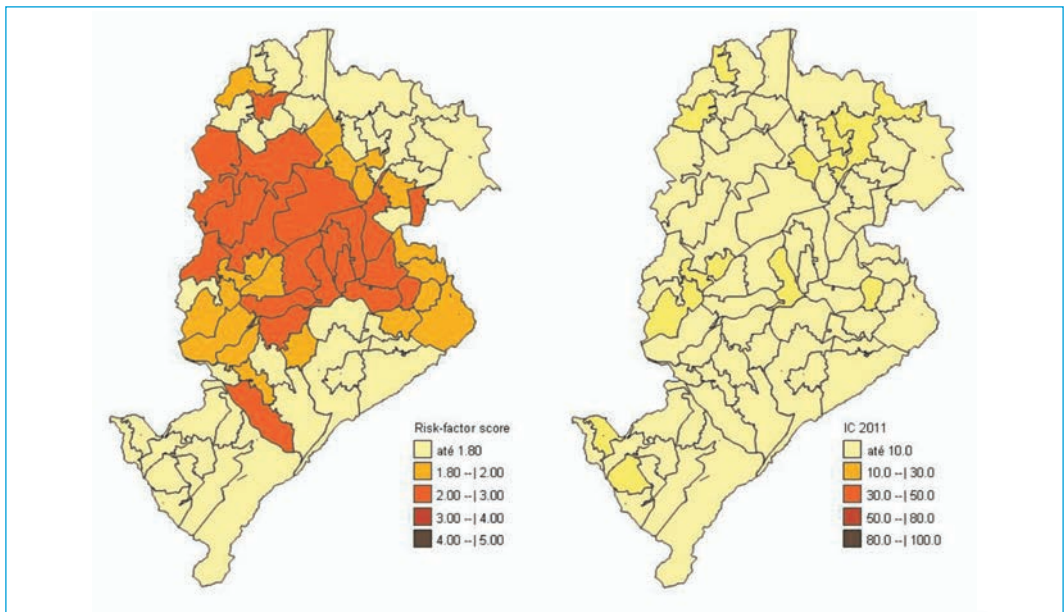


Figure 3: Map risk for 2011 and the Map of the incidences coefficients (IC) by urban unit (UU) in 2011, Belo Horizonte, Brazil



However, in view of the p-values found, we may conclude that the risk-factor score is a good predictor of the dengue incidence, probably so because it is also related to human dengue epidemiological factors, such as previous dengue outbreaks and herd immunity effects, in addition to entomological parameters.

The risk map performed in October has some predictive power on the velocity of epidemic growth, as demonstrated by the association found with dengue incidence. Nevertheless, a prediction of dengue epidemic obtained almost two months before the beginning of a dengue outbreak could serve as a guide for early intervention.

The simplicity of the model (low number of parameters and state variables) offer a promising perspective on parameter values inference from the DHF case notifications.

A vaccine against dengue is not yet available, since it would have to simulate a protective immune response to all four serotypes, although several candidates of tetravalent vaccines are at various stages of development. So far, prevention of exposure and vector control remain the only alternatives to prevent dengue transmission.

In October 2010, the government of Brazil implemented intensive control measures in the priority areas. The impact of these measures, theoretically, would associate with the low dengue incidence in 2011.

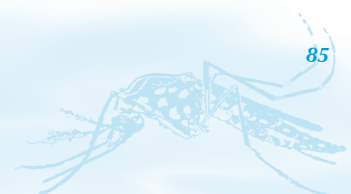
The model is based on short-term predictability to be applied to proposed targets for intervention and control design according to the expected impact of the disease. Our special interest would be to get the model fully parameterized on data referring with a framework so that we would be able to gain an insight into the predictability of upcoming dengue outbreaks. This epidemiological tool would help to prioritize control measures and therefore, to guide policies of prevention and control of the dengue virus transmission.

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Aedes survey following a dengue outbreak in Lahore, Pakistan, 2011

Muhammad Saeed Akhtar,^{a#} Ayesha Aihetasham,^a Mehwish Saeed^b
and Ghazanfer Abbass^a

^aDepartment of Zoology, University of the Punjab, P.O. Box No. 54590, Lahore, Pakistan

^bDepartment of Biotechnology and Microbiology, Lahore College University for Women, Lahore, Pakistan

Abstract

During the study period (October–December 2011), a total of 793 houses in 13 localities of Lahore, Pakistan, were surveyed for the presence of larvae of the dengue vectors, *Aedes aegypti* and *Ae. albopictus*, in different types of water containers. The House Index (HI) determined was 2.77. Species structure based on mosquito larvae collected from clean water receptacles showed that *Ae. aegypti* larvae were more abundant (65.3%), followed by *Ae. albopictus* (22.2%) and *Culex species* (12.5%).

The relationship between temperature and humidity with the number of larvae in a 200 ml sample taken from water containers showed a positive correlation ($r=0.357$) between the number of larvae and the temperature recorded during the study period. The number of larvae declined with increase in humidity ($r=-0.177$). However, during this period, the temperature also decreased.

Keywords: *Aedes aegypti*; *Aedes albopictus*; Survey; Lahore; Pakistan.

Introduction

Dengue is an acute febrile viral disease transmitted from person to person by the bite of infective mosquitoes. *Aedes aegypti* is the major vector for the virus (DENV) in many parts of the world; *Ae. albopictus* is considered a secondary vector.¹ *Ae. aegypti* has wider greater geographical distribution at present than at any time in the past and is established in virtually all tropical and subtropical countries.²

Entomological surveillance is used to determine changes in the geographical distribution and density of the vector and in evaluation of control programmes. Dengue vector breeding

[#]E-mail: msakhtarch@yahoo.com



can be influenced by human practices, with *Ae. aegypti* preferring to breed in domestic water storage receptacles and peridomestic artificial containers like buckets, evaporation coolers (also known as desert coolers), plant saucers, traps, fountains, used tyres, and other water-holding containers around houses. Pakistan experienced a dengue fever epidemic in some cities during May–October 2011 when Lahore suffered the most. As a result, entomological studies were initiated on dengue vector surveillance to control the epidemic. The present paper describes the results from the collections made of immature mosquitoes from water-holding containers in different localities of Lahore.

Materials and methods

For dengue vector surveillance, a total of 793 houses (selected at random) in the suburbs of Lahore were visited during the dengue fever outbreak from October to December 2011. The numbers and types of containers, viz. desert coolers, water tubs, pots, fridge trays, water tanks, pitchers, etc., infested with mosquito larvae and pupae were recorded and the species infesting the containers were identified using the WHO guidelines.³ If a container was positive for the presence of larvae, a sample of water of 200 ml jar was taken.

Data were analysed based on House Index (HI) and relative species abundance. The relation of the occurrence of larvae with temperature and humidity conditions of the area was analysed by Pearson Correlation using SPSS Version 2012. The daily maximum temperature and humidity record (at 8:00 a.m.) was taken from the Meteorological Office, Lahore.

Results

The relative abundance of species based on mosquito larvae collected from clean water habitats is given in Table 1. *Ae. aegypti* was the most commonly found species (65.3%). *Ae. albopictus* accounted for 22.2% and *Culex* spp. 12.5%. *Culex* species, although very common in sewer/dirty water, is rarely found in clean water habitats but was found breeding during November–December in desert coolers and fridge trays, maybe under ecological compulsions.

House Indices for the months of October, November and December are given in Table 2. The HI in October 2011 was 4.6%. Later, when the temperature decreased, the breeding of *Aedes* larvae also went down. Consequently, in November 2011, the HI declined to 2.76%. In December, it further declined to 2.32%. The overall HI for the entire period (October–December) was 2.77.

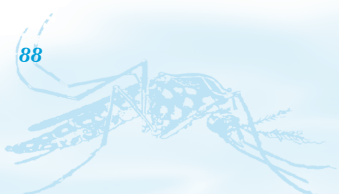


Table 1: *Aedes* mosquito larvae recovered from different localities of Lahore, Pakistan, during October 2011

Date	Locality	No. of houses visited	No. of houses infested	Type of container	No. of larvae	No. of pupae	Adults emerged
20 Oct 11	Wahdat Colony	20	1	water tub	20 <i>Ae. aegypti</i>	–	–
21 Oct 11	Chuburji	30	1	desert cooler	35 <i>Ae. aegypti</i>	–	4 <i>Ae. aegypti</i>
26 Oct 11	Gulshan-i-Ravi	15	1	desert cooler	35 <i>Ae. albopictus</i>	–	6 <i>Ae. albopictus</i>
Total for Oct 11		65	3 (4.6)		90		
2 Nov 11	Khyber Block	80	1	desert cooler	4 <i>Ae. aegypti</i> , 2 <i>Culex</i> spp.	–	1 <i>Culex</i> spp.
15 Nov 11	Baker Mandi	45	1	water tub	2 <i>Ae. aegypti</i>	6	6 <i>Ae. aegypti</i> , 2 <i>Culex</i> sp.
16 Nov 11	Islamia Park	120	2	desert cooler	1 <i>Ae. aegypti</i>	10	–
17 Nov 11	Ichhra Baba Azam Chowk	60	1	fridge tray	6 <i>Ae. aegypti</i> , 4 <i>Culex</i> spp.	3	1 <i>Ae. aegypti</i>
22 Nov 11	Band Road	30	1	water tank on roof	8 <i>Ae. aegypti</i>	37	3 <i>Ae. aegypti</i>
22 Nov 11	Awan Town	2	1	desert cooler	12 <i>Ae. aegypti</i>	2	–
23 Nov 11	Mansoorā	10	1	desert cooler	40 <i>Ae. aegypti</i> , 3 <i>Culex</i> spp.	15	1 <i>Ae. aegypti</i>
24 Nov 11	Gulshan-i-Ravi	50	1	water tub	5 <i>Ae. aegypti</i>	4	–
24 Nov 11	Sherakot Bund Road	30	1	water tank on roof	6 <i>Ae. albopictus</i>	14	–
25 Nov 11	Band Road	20	1	fridge tray	2 <i>Ae. albopictus</i>	8	–
29 Nov 11	Mughalpura	3	1	pitcher	5 <i>Ae. aegypti</i>	2	4 <i>Ae. aegypti</i>
Total for Nov 11		470	13 (2.76)		100		



Date	Locality	No. of houses visited	No. of houses infested	Type of container	No. of larvae	No. of pupae	Adults emerged
1 Dec 11	Garden Town	75	1	water tank on roof	4 <i>Ae. albopictus</i>	–	–
1 Dec 11	Itefaq Colony	85	1	water tub	1 <i>Ae. aegypti</i>	1	–
1 Dec 11	Clifton Colony	30	1	water hose	1 <i>Ae. aegypti</i>	1	–
2 Dec 11	Sabzazar	30	1	water tub	3 <i>Ae. albopictus</i>	–	–
12 Dec 11	Muslim Town	35	1	fridge tray	7 <i>Culex</i> spp.	4	5 <i>Culex</i> spp.
15 Dec 11	Old Campus	3	1	desert cooler	12 <i>Culex</i> spp.	2	–
Total for Dec 11		258	6 (2.32)		28		

Figures in brackets = House Index

Table 2: House index (HI)* of *Aedes* mosquitoes in Lahore, Pakistan (October–December 2011)

Months	No. of houses inspected	No. of houses infested	% infested
October	65	3	4.61
November	470	13	2.76
December	258	6	2.32
Total	793	22	2.77

*Percentage of houses infested with dengue vector mosquitoes

The container types of *Ae. aegypti* breeding and the infestation rates are given in Table 3. Desert coolers were the most preferred site for breeding (38.09%), followed by water tubs/pots (28.5%), fridge tray (14.28%), rooftop water tanks (14.28%) and pitchers (4.46%).

Table 4 gives the details of water temperature and humidity in relation to abundance of larvae.



Table 3: Number (*n*) and type of containers (%) that were *Aedes* breeding sites in Lahore, Pakistan, October–December 2011

Desert coolers	Water tubs/pots	Fridge trays	Water tanks	Pitchers
38.09 (<i>n</i> =8)	28.59 (<i>n</i> =6)	14.28 (<i>n</i> =3)	14.28 (<i>n</i> =3)	4.46 (<i>n</i> =1)

Table 4: Abundance of larval mosquito species from clean water habitats/containers (data based on 21 positive samples of 200 ml each from October–December 2011), Lahore, Pakistan

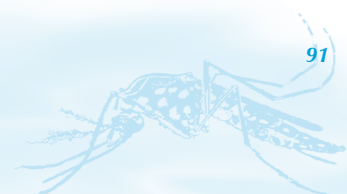
Species	No. of specimens	Percentage of total
<i>Ae. aegypti</i>	147	65.3
<i>Ae. albopictus</i>	50	22.2
<i>Culex</i> spp.	28	12.5

The relationship between the number of larvae collected from containers and the temperature and humidity levels was also investigated. There was a slight but insignificant negative correlation between the number of larvae and humidity ($r=-0.177$). However, during this period, the temperature also decreased. The correlation between the number of larvae collected and the temperature was, however, positive ($r=0.359$).

Discussion

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are emerging as major public health problems in many tropical and subtropical countries. Effective prevention and control by providing early warnings of dengue epidemics will depend on improved surveillance.⁴ Control of the vectors of dengue can be best achieved by community participation and effective entomological surveillance. The dengue epidemic in 2011 in Punjab, and particularly in Lahore, affected 28 394 people. In Lahore alone, 274 deaths occurred due to dengue. Among the affected population, 70% were males and 30% were females (source: Daily Times, 19 October 2011).

Dengue fever is caused by any one of the four antigenically-related but distinct dengue virus serotypes DENV-1–4. Infection with one serotype does not protect against the others, and sequential infections put people at greater risk for dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Teixeira et al⁵ have pointed out that as long as a vaccine is



not available, further dengue control depends on potential results on basic interdisciplinary results and intervention evaluation studies, integrating environmental changes, community participation and education, epidemiological and virological surveillance and strategic technological innovations aimed to stop transmission.

The present paper reports the findings from 793 houses which were examined for the presence of mosquito larvae. Out of these, 9.69% houses were infested and larvae of *Ae. aegypti*, *Ae. albopictus* and *Culex* spp. were collected from a variety of peridomestic water containers, viz. desert coolers, fridge trays, rooftop water tanks, water tubs in bathrooms and pitchers. The overall HI for the area was 2.77. The threshold values of *Ae. aegypti* indices that permit transmission of dengue viruses has been a topic of debate.⁶ An HI of 5 was selected as the target for control of yellow fever in Quito, Ecuador⁷. However, in Singapore where the HI has been less than 1 for many years, dengue transmission continues to occur.⁸

In the present study, *Ae. aegypti*, *Ae. albopictus* and *Culex* spp. were found breeding at the household level. *Ae. aegypti* was the predominant species, accounting for 65.3% of all larvae collected.

The epidemic behaviour of dengue viruses seemingly correlates closely with fluctuations in temperature and rainfall. For example, dengue outbreaks in the Indian subcontinent frequently occur during the hot, dry and wet seasons because *Ae. aegypti*, breeds abundantly in the water trays of desert coolers (38.09% of all water containers studied) and multiplies the vector population.⁹

During the study period (October–December 2011), a negative relationship between the number of larvae and humidity was observed. During the last week of November 2011, humidity increased, but at the same time, there was a decrease in the temperature. Consequently, mosquito population and larval counts in clean water habitats declined. With the onset of winter, the dengue vector immature population decreased considerably and larvae were not collected again until March 2012, and at that point only *Culex* were detected. The subsequent hot weather further adversely affected the mosquito populations, with temperatures reaching 46°C in June 2012. As a result of these environmental factors, i.e. extreme winter and summer temperatures, larvae of dengue vector mosquitoes were not reported again until June 2012 from the localities which were positive during 2011.

As the study was confined only to houses, small water sites that developed in open grounds as a result of rains, water ponds (without sewer water) and larval collections from tyres are not included in this study. However, it is suspected that the main cause of the dengue epidemic during 2011 could be the mosquito breeding in the large number of tyres that were imported from Thailand around that time. The government took severe action against tyre sellers and all old tyres were confiscated, destroyed or recycled by the time (28 October 2011) sampling was carried out from Lahore. It is hoped that in future import of tyres, especially old tyres, is done strictly under quarantine supervision, and mosquito breeding sites reduction is continued.



Conclusion

It is essential that mosquito surveillance in Lahore is continued for 3–4 years to better understand the population dynamics of the local dengue vector mosquitoes.

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Performance of WHO probable case definition of dengue in Kerala, India, and its implications for surveillance and referral

Zinia T Nujum,^{a#} Vijayakumar K,^a Pradeep Kumar AS,^b Anoop M,^c Sreekumar E,^c Ramani Bai JT,^a Dalus D,^a Lalitha Kailas,^a Saritha N,^a Anitha Abraham,^a Anto Varghese,^a Raji RT^a and Sudheesh Kumar TK^a

^aMedical College, Thiruvananthapuram, Kerala, India

^bDepartment of Health Services, Kerala, India

^cRajiv Gandhi Centre for Biotechnology, Kerala, India

Abstract

Case definitions are inevitable for public health surveillance. The WHO case definition for probable dengue (2009) is being used in some areas. Validating this case definition in different regions is essential because of differences in disease epidemiology and clinical presentations. This study attempted to find the performance of the WHO case definition in the Thiruvananthapuram district of Kerala, India, and to make predictions on the hidden disease burden. The study recruited 254 persons with acute febrile illness of 2–7 days' duration without a definitive diagnosis from the field, which included community, primary and secondary care settings of the district. The performance of the case definition was assessed, using RT-PCR as gold standard in case of fever less than five days and IgM antibody detection in case of fever more than five days. The WHO case definition had a very high negative predictive value of 97.4% (90.2, 99.6). The sensitivity and specificity obtained were 71.4% (35.9, 91.8) and 30.7% (25.3, 36.7), respectively. The diagnostic odds ratio of the WHO case definition was 1.1, which could be increased to 13.6 if any of the five items listed in the case definition were used. The use of the five-item criteria also improved the specificity of the case definition for dengue. A two-stage categorization of dengue is proposed based on the results of the study, using the five-item criteria, to supplement the WHO case definition for surveillance and referral. The limitations of using a case definition for dengue and the estimations of disease burden from the study point towards the need for active sentinel surveillance and universal fever surveillance to find the hidden magnitude of the problem more accurately, especially in resource-poor settings.

Keywords: Dengue; Case definition; Diagnostic odds ratio; Surveillance; Kerala; India.

#E-mail: drzinia@gmail.com



Introduction

Case definitions have been recognized to be important elements of public health surveillance systems.¹ They assure comparability and consistency of surveillance data. It has a crucial impact on the sensitivity and positive predictive value of a surveillance system. The World Health Organization (WHO) has been encouraging the use of case definitions to make surveillance data comparable between countries.²

Recommendations for the classification and management of dengue and dengue haemorrhagic fever (DHF) were developed by WHO following a study of Thai children in Bangkok in 1964. Over the succeeding three decades, better case definitions have evolved.^{3,4} Several authors have reported that these case definitions have some drawbacks and are not always reproducible in different countries. This reproducibility is crucial to effective surveillance and reporting as well as global disease comparisons. The prevailing difficulties lead to creation of new case definitions to represent the observed patterns of disease more accurately.^{5,6,7} In 2009, WHO revised the guidelines and classified dengue into probable dengue, dengue with warning signs, and severe dengue.⁸ A probable case of dengue is defined as fever of 2–7 days' duration in an individual in an endemic area with any two clinical manifestations, namely, nausea/vomiting, rash, aches and pains, tourniquet test positive, leucopaenia and any warning sign. The warning signs enlisted include abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy/restlessness, liver enlargement of more than 2 cm and an increase in haematocrit concurrent with a decrease in platelet count. Although the guidelines are being widely used in clinical practice and surveillance, they have rarely been formally validated in the community setting.^{9,10}

In 2003, Kerala state reported the maximum number of deaths due to dengue in India. Over the years, the reported cases of dengue have been increasing. The district of Thiruvananthapuram reports the maximum number of cases in the state.¹¹ A case definition for surveillance is most relevant in this part of the region. In this context, an attempt was made to evaluate the performance of the WHO probable case definition (2009)⁸ of dengue in Thiruvananthapuram district during an inter-epidemic period. An effort was also made at estimating the hidden disease burden using the study findings and the data from the existing surveillance system in the state.

Methods

A cross-sectional study (diagnostic test evaluation) was done in the primary and secondary care settings of Thiruvananthapuram district. Using the existing surveillance system, laboratory-confirmed dengue cases were identified. Subjects meeting the inclusion criteria were recruited from household surveys conducted in 25 houses around each confirmed case of dengue. Recruitment was also done from corresponding primary and secondary care settings. The inclusion criteria was acute febrile illness of 2–7 days' duration. The fevers with a definite



diagnosis and those who did not give informed written consent were excluded. The study period was from February 2011 to July 2011 and included pre-monsoon and monsoon seasons. A total of 254 fever cases were recruited. The sample size was calculated using the formula given below:¹²

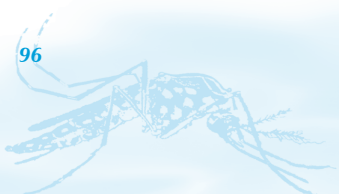
$$N = \frac{4Z_{1-\alpha/2}^2 p(1-p)}{[I(S+C-1)]^2}$$

where p is prevalence, I is precision, S is sensitivity and C is specificity

After obtaining consent from the health authorities and the necessary ethical approval from the institutional ethical committee of the Medical College, Thiruvananthapuram (IEC No. 3/44b/2011/MCT), the study was started. Informed written consent was obtained from all the study participants. Then training of the personnel involved in data collection was done. Questionnaires were filled for all cases meeting the inclusion criteria. Study variables were baseline socio-demographic variables, clinical symptoms and signs included in the WHO case definition. Aseptically 3 ml each of venous blood was collected using vacutainers for haematological tests, namely, total count, differential count, platelet count, packed cell volume and serology/RT-PCR. The procedure of blood collection, storage and transportation was according to the guidelines of the National Institute of Communicable Diseases (NICD), Delhi.¹³ The gold standard used for confirmation of dengue was RT-PCR in those patients with fever less than or equal to five days and IgM antibody detection in case of fever for more than five days. The name of the patient, identification number and date of collection were indicated on the label of the vacutainer. The specimens were transported to the corresponding laboratory on the same day.

Blood samples for RT-PCR analysis were allowed to clot at room temperature, and serum was separated immediately. Serum samples were made into aliquots in RNase-free vials and stored at -80°C till processing. The samples were processed in the laboratory strictly as per the procedures approved by the institutional bio-safety committee. Viral RNA (ribonucleic acid) was isolated from 150 μl of serum samples using Nucleospin RNA Virus kit (Macherey Nagel) as per the manufacturer's instructions. Dengue viral detection and typing was carried out by RT-PCR as previously reported.¹⁴ Extreme care was taken to avoid PCR contamination by products of previous amplifications in the laboratory by spatially separating the pre- and post-amplification procedures. Absence of contamination was also ensured by including a non-template, negative control in the PCR reactions and detecting no amplification in them. The positive samples were detected by checking the PCR products in agarose gel electrophoresis.

The samples for serology were tested for dengue virus IgM antibodies by capture ELISA (SD Dengue IgM ELISA). This is a qualitative detection of IgM antibodies against dengue virus antigen (DENV-1–4) in human serum. The test kit contains a micro plate pre-coated with mouse-monoclonal antihuman IgM antibodies on well. During the first incubation, IgM antibody in the patient's serum binds to the mouse monoclonal antihuman IgM antibody in the well and then this IgM-anti IgM complex binds to a mixture of dengue antigen and



mouse monoclonal anti-dengue HRP (Horse Radish Peroxidase) conjugate. All the unbound materials are removed by washing. The residual enzyme activity in the well is proportional to dengue IgM antibody in the serum and is evidenced by incubating with a substrate solution. Colorimetric reading was performed using a spectrophotometer at 450 nm.

The sensitivity, specificity, predictive values and likelihood ratios of the probable case definitions of WHO for dengue were calculated. Clinical manifestations of these patients were also scored and a receiver operator characteristic (ROC) curve was drawn to find the optimum discriminating score. Unconditional logistic regression was done for multivariable analysis following a bivariable analysis to find the symptoms and signs associated with a positive dengue and the significant items were used for framing a new case definition. The performance of this new definition was tested. The likelihood of disease for a positive result, was calculated as $[\text{Sensitivity}/(1-\text{Specificity})]$ and likelihood of no disease for a negative result was calculated as $[(1-\text{Sensitivity})/\text{Specificity}]$.¹⁵ The diagnostic odds ratio (DOR) was also calculated as a single indicator of performance of the case definition.¹⁶ It is the ratio of odds of disease in test-positives relative to the odds of disease in test-negatives – $(\text{TP}/\text{FP})/(\text{FN}/\text{TN})$ or the ratio of odds of positivity in disease relative to the odds of positivity in the non-diseased – $(\text{TP}/\text{FN})/(\text{FP}/\text{TN})$. The value of a DOR ranges from zero to infinity and higher values indicate a better discriminatory test performance. DOR does not depend on the prevalence of the disease.

Results and discussion

Nearly 15% of the 254 fever cases were from urban areas and the rest were from various rural areas of Thiruvananthapuram district. The bulk of fever cases studied were in the productive age group of 19–40 years. They constituted 52.3% of the fever studied. In the study group there were more males (54.7%) than females. Among the 254 fever cases, 79% (198/254) had fever of 5 days or less. In the study, 176 (69.2%) fever cases satisfied the WHO probable case definition and 73.2% (186/254) had at least one warning sign (Figure 1).

Seven of the fever cases (7/254) were dengue-positive, of which four were confirmed as dengue by RT-PCR and three by serology. The RT-PCR analysis of 198 serum samples, of persons who had fever of five days' duration, yielded two cases of DENV-3 and two of DENV-2. The WHO case definition had a sensitivity of 71.4% (35.9, 91.8) and a specificity of 30.8% (25.3, 36.7). The ROC showed that the performance was best at a score of 1.5, suggesting that the WHO criteria of two manifestations in addition to fever would be ideal for initial work up (Table 1 and Figure 2).

Symptoms/signs significantly associated with dengue, considering a level of significance of 0.05 were abdominal pain, diarrhoea, icterus, thrombocytopenia and raised haematocrit. A new set of criteria with those items having a significance less than 0.5 was tried that included symptoms such as headache, myalgia, arthritis/arthralgia, altered taste, abdominal pain, diarrhoea, icterus, raised haematocrit and thrombocytopenia. This new definition also did



Figure 1: Manifestations and warning signs as mentioned in the WHO case definition⁸ among the fever cases, Kerala, India

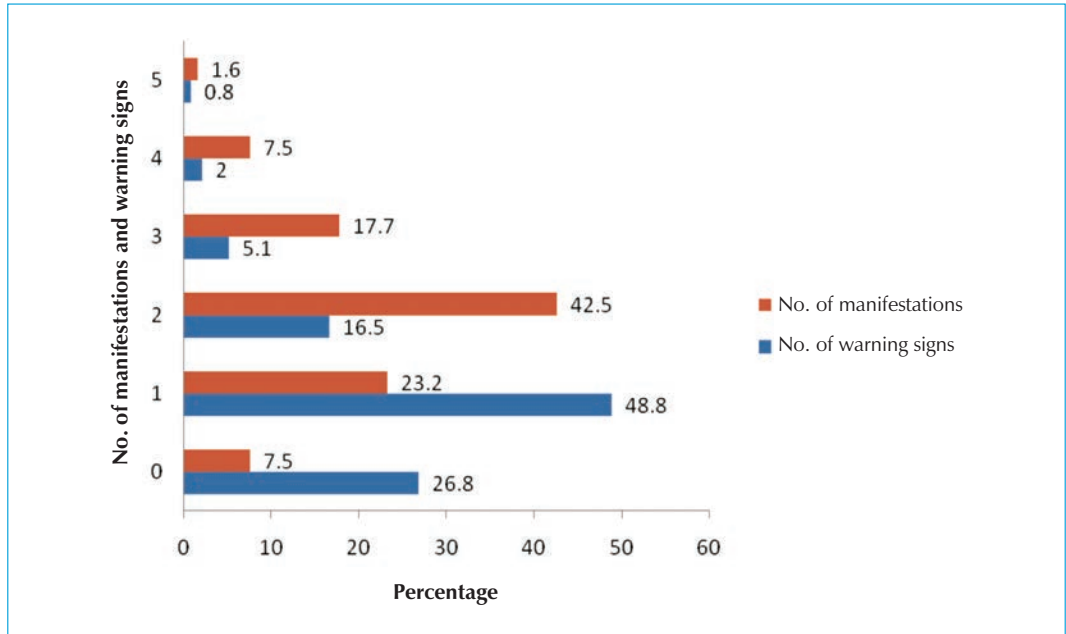
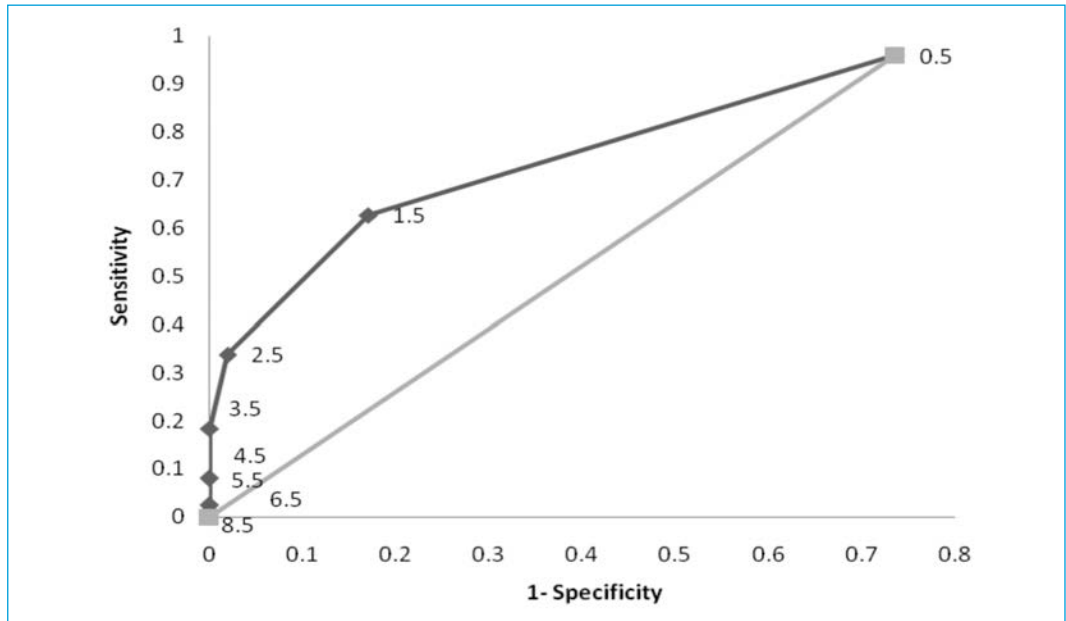


Figure 2: ROC curve drawn using a scoring system for manifestations described in the WHO case definition, Kerala, India



not improve the performance of the case definition. The results are given in Tables 1 and 2. All analyses yielded a consistently high negative predictive value (NPV) of above 97%.

Table 1: Classification of the fever cases as per various case definitions for dengue used in the study, Kerala, India

		WHO case definition ⁸ (N = 254)		New case definition (N = 254)		Using five items in the WHO case definition ⁸ , instead of two (N = 254)	
		Probable dengue		Probable dengue		Probable dengue	
		Yes	No	Yes	No	Yes	No
Lab test	Confirmed	5	2	5	2	1	6
	Not confirmed	171	76	196	51	3	244

Table 2: Performance of the different case definitions compared, Kerala, India

	WHO probable case definition ⁸ – two-item criteria	New definition	WHO probable case definition ⁸ – five-item criteria
Sensitivity % (95%CI)	71.4 (35.9, 91.8)	71.4 (30.3, 94.9)	14.3 (0.8, 58.0)
Specificity % (95%CI)	30.8 (25.3, 36.7)	20.6 (15.9, 26.3)	98.8 (96.2, 99.7)
PPV % (95%CI)	2.8 (1.1, 6.9)	2.5 (0.9, 6.0)	25 (1.3, 78.1)
NPV % (95%CI)	97.4 (90.2, 99.6)	96.2 (85.9, 99.3)	97.6 (94.6, 99.0)
LR+ (95%CI)	1.0 (0.6–1.7)	3.5 (2.0–5.9)	11.9 (1.4–99.5)
LR–(95%CI)	0.9 (0.2–3.0)	0.7 (0.1–0.2)	0.9 (0.6–1.2)

Among the dengue-positive cases (4/7), 57.1% had high fever compared with 28.6% having mild fever and 14.3% moderate fever. The other symptoms of the fever cases were analysed and presented in order of decreasing frequency (Table 3). Rash was found in eight cases of fever. These were found in different parts of the body, namely, the arms (5/8), legs (4/8), chest (3/8), face (3/8), back (2/8), abdomen (1/8) and over the body (1/8) in that order of frequency. The duration of rash varied from 1 to 3 days. The rash was mostly erythematous, but also in the form of petechiae and maculopapular. The symptoms found in more than 50% of fever cases identified were headache, myalgia, chills and lethargy. Among the warning signs mentioned in the WHO case definition, lethargy was most common, followed by abdominal pain.



Table 3: Distribution of manifestations among fever cases, Kerala, India

Symptoms	Number (N = 254)	Percentage	Symptoms	Number (N = 254)	Percentage
Headache	193	75.98	Dehydration	6	2.36
Myalgia	185	72.83	Constipation	3	1.18
Chills	153	60.24	Mucosal bleed	2	0.79
Lethargy	148	58.27	Skin bleed	2	0.79
Arthralgia	112	44.09	Epistaxis	1	0.39
Orbital pain	85	33.46	Gum bleed	1	0.39
Nausea	83	32.68	Menorrhagia	1	0.39
Anorexia	74	29.13	Conjunctival congestion	41	16.14
Flushed face	68	26.77	Lymphadenopathy	11	4.33
Abdominal pain	60	23.62	Hepatomegaly	4	1.57
Vomiting	56	22.05	Hypertension	3	1.18
Altered taste	51	20.08	Altered sensorium	2	0.79
Asthenia	47	18.50	Pleural effusion	2	0.79
Restlessness	22	8.66	Orbital oedema	1	0.39
Diarrhoea	15	5.91	Splenomegaly	1	0.39
Pallor	13	5.12	Torniquet test	0	0.00
Pruritis	12	4.72	Leucopaenia	23	9.1
Persistent vomiting	12	4.72	Thrombocytopenia	8	3.1
Abdominal tenderness	12	4.72	Raised haematocrit	8	3.1
Rash	8	3.15			



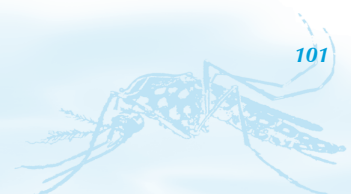
The WHO case definition⁸ is most useful to rule out the disease because of the high negative predictive value. Although the sensitivity obtained was low, it could be because of the less number of confirmed dengue cases. The specificity and positive predictive values were also low. Passive surveillance using the WHO case definitions⁸ alone lack specificity, since many other infectious diseases, such as influenza, chikungunya, enterovirus infections, leptospirosis, malaria and typhoid fever all present with similar symptoms and signs as dengue in the acute phase of illness.^{9,10} The use of the existing WHO case definition⁸ of dengue may overload tertiary care centres in resource-poor countries like India. This over-estimation may be particularly problematic during dengue outbreaks or periods of high incidence. Hence, primary care centres need to be strengthened to handle uncomplicated cases.

Those cases which are probable case-definition positive need to be further evaluated before referral since the specificity and positive predictive values are low. In order to improve specificity, more than two manifestations were included for fulfilling the criteria of case definition for dengue and the analysis was repeated. It was found that a five-item criterion raises the specificity to 98.8% (96.2, 99.7). Using the existing case definition, the odds of diagnosing dengue (DOR) in acute non-specific febrile illness are 1.1% (0.2, 5.85), but by increasing the number of symptoms/signs to five instead of two, the odds of diagnosing dengue are increased to 13.6% (1.2, 149.9), almost 12 times more. If WHO case definition of fever, with any two symptoms/signs, is used for initial work up and before labelling as probable case/referral, if the five-items criterion is applied, the likelihood of disease may be increased. When this criterion is used, the specificity and positive predictive value (PPV) also improves. However, since the sensitivity of this criterion is very low if used alone, there is a high chance of missing out probable cases. Therefore, we suggest a two-stage screening and categorization of acute febrile illness using the five-items criterion to supplement the WHO case definition for surveillance and referral as shown in Table 4.

Table 4: Suggested modifications in the application of WHO case definition⁸ for surveillance and referral of dengue suspected cases based on study results, Kerala, India

	Surveillance	Referral
Not satisfying the WHO case definition	Not dengue	No
Satisfying the WHO case definition less than or equal to five symptoms/signs	Suspect dengue	No – evaluate further with laboratory criteria and monitor closely in the primary or secondary setting
Satisfying the WHO case definition more than or equal to five symptoms/signs	Probable dengue	Yes

From this study, the incidence of dengue in acute febrile illness of 2–7 days' durations can be projected as 2755/100 000 fevers. For the year 2010 (January to December), 2 760 720 fevers of unidentified etiology were reported in Kerala state. The expected number of



symptomatic dengue cases is therefore 76 057. This is much less than the reported figures.¹¹ The population of Kerala is 33 387 677 as per the 2011 census. If we ignore repeat infections, the annual dengue fever incidence would be 227/100 000 population. The incidence of non-apparent and symptomatic dengue virus infection ranges from 0.96 to 1.75.¹⁷ The annual incidence of dengue infection should therefore be at least 454/100 000 to 681/100 000 population. This is higher than the estimated worldwide incidence of 250–500/100 000 population.¹⁸ Some of the reasons for the gaps in the estimation of dengue burden are lack of an uniform application of the WHO case definition, limited capabilities and standards of dengue laboratories, limited accuracy of rapid tests, misdiagnosis and misclassification in the reporting of dengue.¹⁹

The limitations of the study include the use of a single value of IgM as gold standard instead of a paired titre and the limited area of the study. The fact that the study was carried out during an inter-epidemic period, which resulted in the relatively few number of confirmed cases, is also a limitation since the performance of the case definition can vary based on this. This study was planned and done prior to the release of the revised and expanded edition of the WHO guidelines.²⁰ The probable case definition the way it would be useful in settings where confirmatory facilities are not available makes the study still relevant. The findings of the study also reiterate the need for active surveillance for dengue, including sentinel clinician, hospital and fever alert, as mentioned in the revised guidelines.²⁰ The predictions of disease burden is a worst-case analysis since Thiruvananthapuram district reports the maximum number of cases and thus may be limited in application to other endemic and epidemic-prone areas of Kerala. Although the applicability of the classification system for dengue has been studied in a clinical setting,^{21–25} its relevance for dengue surveillance and case identification in the community setting prospectively has not been studied much. This is one of the strengths of the study. This study can be replicated in all areas with good fever surveillance and sentinel laboratory surveillance mechanism for dengue (if the necessary information on clinical manifestations is also available) to produce more generalizable results. Besides, if the sensitivity and specificity of the case definition is known, the case definition can be used for surveillance and the true positives can be calculated and fed into the surveillance system. This will provide a better application of the use of case definition in surveillance for estimating the disease burden even if the performance of the case definition is low.

Conclusions

The high negative predictive values and sensitivity of the WHO case definition are in favour of ruling out dengue in acute febrile illness. The WHO case definition, although lacks specificity, is the most acceptable basis for surveillance and referral, since dengue is a disease for which the clinical manifestations are not very specific. Health professionals who use this definition have to be better informed on its usefulness and interpretation. The use of the five-item criteria to supplement the WHO case definition for categorization of acute febrile illness may help to improve specificity for surveillance and referral in settings where confirmatory tests



cannot be done. Estimations of the disease burden from the study point towards the need for sentinel laboratory/hospital/clinical surveillance and stronger fever surveillance along with the use of passive surveillance using case definitions for more accurate information to base health planning in resource-poor settings for dengue prevention and control.

Acknowledgements

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Early predictors of dengue infection in adults (EPOD) – a Malaysian outpatient experience

Emer Breen,^{a#} Indrani Pemmulu,^a Daisy SH Ong,^a Shamala Devi Sekaran,^a Praba Appana,^a Thien Hoong Tew,^b EM Khoo,^a Lesley Pillans,^a Noor Azina Ismail,^b Sharifah FS Omar,^a Sasheela Ponnampalavanar,^a R Vhimalleshwari^a and Lucy CS Lum^a

^aFaculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

^bDepartment of Applied Statistics, Faculty of Economics and Administration, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Dengue infection is a significant public health problem in Malaysia. While there is no specific treatment for dengue, we may, if the disease is recognized early, through close monitoring and judicious use of fluids, be able to prevent deterioration and ultimately demise. To understand the early predictors of dengue infection in adults, a prospective cohort study was undertaken at the primary care clinic of a teaching school in Kuala Lumpur, Malaysia.

Out of 238 patients originally recruited for the study, 214 (90%) patients were ultimately enrolled in it. Of them, 121 (57%) had dengue fever (DF), 16 (7%) had dengue haemorrhagic fever (DHF), 3 (1%) had dengue unclassifiable (DU) and 74 (35%) had a diagnosis of other febrile illness (OFI). 52 (37%) patients with dengue were admitted to hospital. There were no cases of dengue shock syndrome (DSS) or fatalities.

In the first 72 hrs of illness, logistic regression showed that having recent dengue in the family (odds ratio [OR] = 6.0, 95% confidence interval [CI] = 1.1–31.7, $P < 0.037$) or neighbourhood (OR = 6.2, 95% CI = 2.0–18.5, $P < 0.001$), postural dizziness (OR = 2.8, 95% CI = 1.2–6.6, $P < 0.019$), nausea or vomiting (OR = 2.8, 95% CI = 1.1–6.9, $P < 0.028$), low white cell count (OR = 0.8, 95% CI = 0.7–0.9, $P < 0.011$) were independent predictors for dengue. The area under the receiver operating curve (ROC) was 0.83. In dengue patients we were unable to identify early predictors for dengue haemorrhagic fever/dengue shock syndrome.

We concluded that in the early stages of fever, it is possible to determine markers that are predictors for dengue in adults. This allows for early identification and close monitoring of the disease.

Keywords: Dengue fever; Dengue haemorrhagic fever; Dengue shock syndrome; Early febrile; Predictors; Malaysia.

#E-mail: emerbreen@yahoo.com



Introduction

The dengue virus is a flavivirus transmitted mainly by the *Aedes aegypti* mosquito. It is the most rapidly spreading vector-borne disease in the world.¹ In Malaysia, dengue infection is endemic, and, it is an important public health problem. There has been a gradual rise in the incidence of reported dengue cases here in the last decade.² In 2010, there were over 45 000 cases of dengue reported, with 134 deaths, and 35% of these cases were from the state of Selangor.³ The highest incidence of the disease is in the age groups of 15 years and above.² The WHO classification of 1997 for dengue infection was revised in 2009.⁴ However, as this study commenced before the revision, the 1997 classification has been followed.

Symptomatic dengue infection is characterized by fever, headache, body ache, nausea, vomiting and a low platelet count. Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) tend to be more severe forms of the disease. Severe disease is characterized by plasma leakage and bleeding tendencies, and can be fatal.

Populations that do not have access to sophisticated laboratory tests need early clinical and basic laboratory indicators that can help in dengue diagnosis.⁵ This early identification allows appropriate management to be instituted to prevent consequences of DHF/DSS and fatality. However, in the early stages of fever, it can be difficult to distinguish dengue from other viral fevers as the presentation is non-specific and undifferentiated.

When this study commenced there were only two reported studies that had attempted to identify predictors of dengue in the first 72 hours of illness, in patients with undifferentiated fever.^{6,7} The first, from Thailand, included children only.⁶ It was able to identify laboratory markers as predictors of early dengue; however, multivariate analysis was not performed to assess the independence of variables. Also, as children have a higher risk of developing DHF compared to adults, the study findings may not be similar in adults.⁷ The second, the Early Dengue Infection and Outcome (EDEN) Study, looked at Singaporean adults.⁷ Cases were selected based on finding a positive early PCR result. It is unclear whether this result was available prior to collecting patient information and thus there is a potential for bias. It seems that significant results tabulated were for univariate and not multivariate analysis and thus were unable to find independent clinical or laboratory predictors for dengue in the early febrile period.

The objectives of our study were to determine early independent predictors for dengue and DHF/DSS in the early stages (72 h or less) of fever, in adults, in a Malaysian primary care setting, using a combination of clinical and laboratory findings.

Materials and methods

The early predictors of dengue (EPOD) study is a prospective cohort study. The patients were recruited from the primary care outpatient clinic at a university hospital situated on the border



between Kuala Lumpur and Selangor (Malaysia) from 2007 to 2010. Ethical approval was obtained from the University Malaya Medical Centre (UMMC) Ethics Committee. Informed consent was obtained from the patients.

Criteria for selection into the EPOD study

Patients were selected if aged ≥ 16 years with undifferentiated fever for ≤ 72 hours and had a temperature of ≥ 38 °C (or ≥ 37.5 °C with recent use of paracetamol). Those patients were excluded if there was an obvious cause for the fever, e.g. tonsillitis, sinusitis, and they did not have complex medical conditions such as malignancy or HIV infection.

At the time of enrolment, the subjects were interviewed by a study nurse to collect demographic, social and medical history, using a standardized collection form. They were then examined by a doctor and the data obtained was added to the form. All patients were reviewed daily, until they were clinically better, and then at convalescence (defined as 10–14 days from the onset of fever). Day 1 was taken as the first day the patient enrolled in the study. The patients were managed in the outpatient clinic unless their condition warranted admission.

Postural hypotension was defined as the experience of dizziness when standing up from the sitting or lying position. A person was said to have a positive recent history of family exposure to dengue, if he/she had a case of confirmed or suspected dengue in someone living in their household, in the previous two weeks. A positive recent history of dengue in the neighbourhood was one where the patient had heard of a confirmed or suspected case of dengue within their neighbourhood, in the previous two weeks.

Laboratory tests

At recruitment, 4 ml of blood was sampled for full blood count, renal and liver function tests, and serum cholesterol. Changes in these blood markers have been associated with dengue infection.^{8–10} Urinalysis was performed to check for haematuria, which could indicate internal bleeding from the genitourinary tract.

Serology and virology

At recruitment, at defervescence (defined as the time when oral temperature fell below 37.5 °C) and convalescence, an additional 4 ml of blood was taken. The serum obtained was subjected to serological and molecular tests to determine the presence of dengue antibodies and dengue virus. These tests included an IgM capture ELISA¹¹ for detection of dengue IgM antibodies, real-time polymerase chain reaction (RT-PCR)¹², dengue early ELISA (Panbio, Australia) and standard diagnostics (SD, Republic of Korea) to confirm the presence of



dengue NS1 antigen, and haemagglutination inhibition (HI).¹³ A case was said to be positive for dengue if the PCR was positive, NS1 was positive or there was a fourfold rise in IgM or HI. Due to the cross-reactivity of the HI with other flaviviruses and an increasing incidence of travel-related exposure in Malaysia, if there was a fourfold rise of HI without any other positive laboratory test, the case was excluded from the study.

Radiological tests

At defervescence and one day post-defervescence, all patients had an ultrasound taken of the thorax and abdomen to check for pleural effusions, ascites or gall bladder thickening. These are recognized markers of plasma leakage.¹⁰

Diagnosis of dengue

All cases confirmed by laboratory tests were categorized into DF, DHF or DSS as per WHO classification.⁴ Those cases that had some but not all features of DHF were termed as ‘dengue unclassifiable (DU)’.¹⁴ Those whose laboratory results were negative for dengue were termed as ‘other febrile illness (OFI)’.

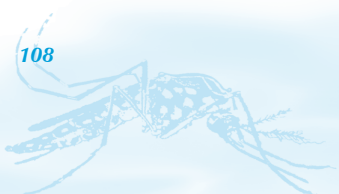
Analysis

Data were recorded on a standardized data collection form by trained staff and analysed using SPSS Version 13. Background epidemiological characteristics, symptoms, signs and laboratory indicators found on Day 1 of recruitment for dengue and non-dengue patients were subjected to univariate and multivariate logistic regression analysis. A variable with a *P* value less than 0.15 in the univariate analysis was added to the multivariate model.¹⁵ Independent predictors were those with a *P* value less than 0.05 in the multivariate logistic regression.

The Hosmer Lemeshow test was used to see if the multivariate model fitted the data well.¹⁶ The area under the ROC curve was calculated to indicate how well the final model performed.¹⁷ This analysis was then repeated comparing DHF/DSS with other dengue cases.

Results

We recruited 238 patients. 15 patients failed to return for follow-up, 1 withdrew from the study, 8 patients had a fourfold rise in HI but no other laboratory markers of dengue. The results are based on the remaining 214 patients of whom we were able to obtain a confirmatory diagnosis of either dengue or OFI.



A total of 140/214 (65%) patients had a discharge diagnosis of dengue while 74/214 (35%) had OFI. Of the dengue patients, 121/140 (86.4%) had DF, 16/140 (11.4%) had DHF, none had DSS and 3/140 (2.1%) had DU. 52/140 (37.1%) of the dengue patients required hospitalization. There were no fatalities.

The results from univariate analysis are presented in Table 1. Multivariate analysis showed that having a recent history of family exposure to dengue, a recent history of neighbour exposure to dengue, nausea or vomiting, postural dizziness and lower white cell count were predictive of dengue infection (Table 2). The Hosmer-Lemeshow test concluded that the five independent predictors fitted the data well (P value = 0.70). The area under the ROC was 0.83, showing a very good discriminative power (Figure).

Table 1: Univariate analysis results for dengue compared with other febrile illness on Day 1 of presentation, Malaysia

	Dengue (n=140)	Controls (n=74)	Odds ratio	95% Confidence interval	P value
Background					
Being a foreigner	27 (19.4)	6 (8.1)	2.7	1.1–6.0	0.035 ^
Family exposure	22 (15.9)	3 (4.1)	4.4	1.3–15.3	0.019 ^
Neighbour's exposure	56 (40.9)	8 (11.1)	5.5	2.5–12.4	0.000 ^
Age	30.7 (12.3)	32.2 (11.3)	1.0	1.0–1.0	0.385
Sex = Male	98 (70.0)	48 (64.9)	0.8	0.4–1.4	0.443
Chronic disease	15 (10.7)	8 (10.8)	1.0	0.4–2.4	0.983
Clinical symptoms and signs					
Oral temperature (°C)	38.4 (0.7)	38.3 (0.7)	1.5	1.0–2.2	0.061 ^
Headache	127 (91.4)	66 (90.4)	1.0	1.0–1.0	0.847
Retrobulbar pain	65 (46.8)	26 (35.6)	1.4	0.9–2.4	0.172
Nausea or vomiting	112 (80.0)	39 (52.7)	3.6	1.9–6.6	0.000 ^
Flushing	40 (28.8)	8 (11.4)	3.1	1.4–7.1	0.007 ^
Postural dizziness	86 (62.3)	28 (38.4)	2.7	1.5–2.8	0.001 ^
Aches and pains	128 (91.4)	65 (87.8)	1.5	0.6–3.7	0.403
Clinical dehydration	62 (45.3)	22 (30.1)	1.9	1.0–3.5	0.034 ^
Positive TT	23 (18.)	12 (17.6)	1.0	0.5–2.2	0.955
Laboratory results					
WBC (×10 ⁹ /l)	5.3 (3.1)	8.3 (4.6)	0.8	0.7–0.9	0.000 ^
Cholesterol (mmol/l)	4.2 (1.0)	4.7 (1.1)	0.6	0.5–0.9	0.004 ^
Platelets (×10 ⁹ /l)	168 (62)	195 (57)	1.0	1.0–1.0	0.017 ^
ALT (IU/l)	62.1 (40.0)	57.8 (44.7)	1.0	1.0–1.0	0.237 ^
AST (IU/l)	53.7 (39.5)	42.2 (39.7)	1.0	1.0–1.0	0.063 ^
Urea (mmol/l)	3.7 (1.1)	3.8 (1.9)	1.0	0.8–1.2	0.478
Microhaematuria	67 (58.8)	34 (55.7)	1.0	1.0–1.9	0.452

Data are presented as number (%) or mean (standard deviation)

TT = tourniquet test, WBC = white cell count, ALT = alanine aminotransferase, AST = aspartate aminotransferase

^ Indicates significant variables at the 0.15 level which are selected for multivariate mode



Table 2: Independent predictors for having dengue on Day 1 as shown by multivariate analysis, Malaysia

Independent predictors for dengue	Odds ratio	95% Confidence interval	P value
History of family exposure*	6.0	1.1–31.7	0.037
History of neighbour exposure*	6.2	2.0–18.5	0.001
Nausea or vomiting	2.8	1.1–6.9	0.028
History of postural dizziness	2.8	1.2–6.6	0.019
White cell count ($\times 10^9/l$)	0.8	0.7–0.9	0.011

Colinearity tests, VIF and tolerance statistic are <10 and 0.1 , respectively, indicating that colinearity is unlikely. This table shows only those variables that are significant at the $P < 0.05$ level from those variables chosen for the multivariate analysis

Figure: ROC curve displaying how the model using the five independent predictors for early dengue in adults fits the data, Malaysia

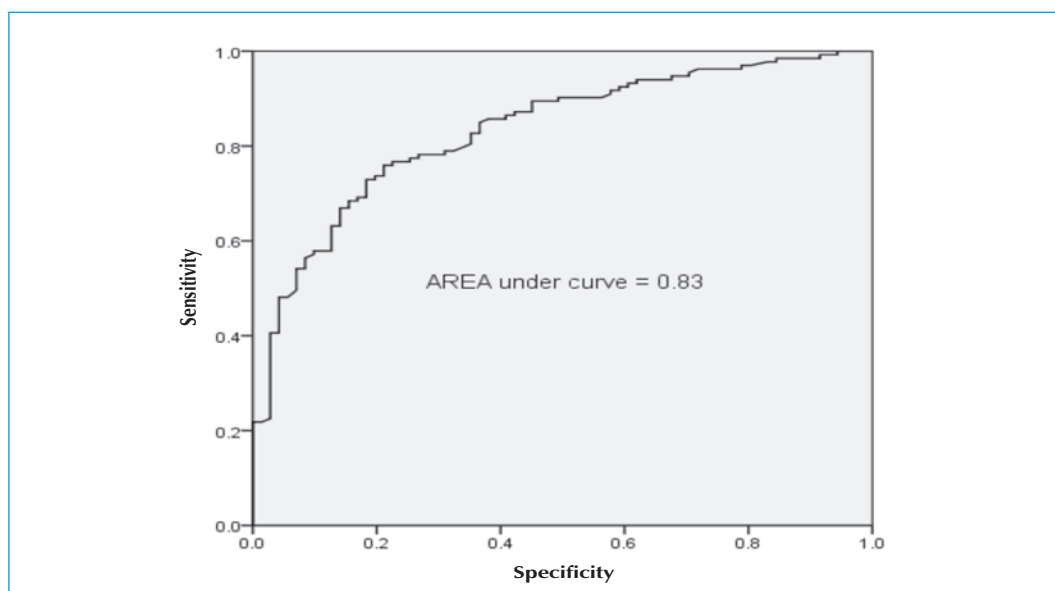


Table 3 shows the results for the univariate analysis comparing DHF with other dengue on Day 1 of presentation of the patient. The significant variables were subject to multivariate analysis. However, this was unable to show any independent variables associated with an increased risk of DHF compared with other dengue.



Table 3: Univariate analysis results for dengue haemorrhagic fever compared with other dengue on Day 1 of presentation

	DHF N=16	Other dengue N=124	Odds ratio	95% Confidence interval	P value
Background					
Being a foreigner	3 (18.7)	24 (19.5)	0.9	0.3–3.6	0.942
Family exposure	4 (25.0)	18 (14.8)	1.9	0.6–6.6	0.299
Neighbour's exposure	4 (25.0)	52 (42.6)	0.5	0.1–1.6	0.243
Age (years)	39.8 (14.4)	29.6 (11.6)	1.0	1.0–1.1	0.004 ^
Sex = Male	1 (6.3)	14 (11.3)	2.0	0.7–5.7	0.208
Duration of fever at D1 (days)	2.1 (0.72)	2.2 (0.89)	0.9	0.5–1.7	0.815
Chronic disease	1(6.3)	15 (12.1)	0.5	0.1–4.2	0.546
Clinical symptoms and signs					
Oral temperature (°C)	38.6 (0.85)	38.4 (0.69)	1.6	0.3–3.4	0.192
Headache	16 (1.0)	111 (90.2)	2.3	0.0–	0.999
Retrobulbar pain	8 (50.0)	57 (47.1)	1.0	0.8–1.1	0.889
Nausea or vomiting	14 (87.5)	98 (79.0)	0.8	0.2–3.7	0.724
Flushing	5 (31.3)	35 (28.5)	1.1	0.4–3.5	0.816
Postural dizziness	13 (81.3)	73 (59.8)	2.9	0.8–10.7	0.109 ^
Aches and pains	16 (1.0)	112 (91.1)	1.0	1.0–1.0	0.844
Clinical dehydration	10 (62.5)	52 (43.0)	2.2	0.8–6.5	0.148 ^
Positive TT	3 (20.0)	20(17.7)	1.2	0.3–4.6	0.79
Laboratory results					
WBC (x10 ⁹ /l)	4.2 (1.65)	5.4 (3.20)	0.8	0.7–1.1	0.154
Cholesterol (mmol/l)	4.1 (0.96)	4.2 (1.03)	1.0	0.6–1.6	0.871
Platelets (x10 ⁹ /l)	122 (50.6)	174 (61.1)	1.0	1.0–1.0	0.002 ^
ALT (IU/l)	81.3 (48.2)	59.7 (38.3)	1.0	1.0–1.0	0.062 ^
(AST) (IU/l)	77.7 (51.9)	50.6 (36.9)	1.0	1.0–1.0	0.021 ^
Urea (mmol/l)	3.4 (1.31)	3.8 (4.17)	1.9	1.2–3.0	0.007 ^
Microhaematuria	8 (72.7)	59 (57.3)	1.0	1.0–1.0	0.15 ^

Data are presented as number (%) or mean (standard deviation)

TT = Tourniquet test, WBC = White cell count, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

^ Indicates significant variables at the 0.15 level which are selected for multivariate mode



Discussion

We were able to show early, independent, predictors of dengue infection in Malaysian adults with undifferentiated fever. We believe that because dengue in Malaysia is more common among adults, and since our hospital is located in an area with a large number of dengue patients, our results could be generally applicable to other Malaysian settings and indeed to other resource-poor, endemic settings with similar disease epidemiology and where instant confirmation with PCR is not available. Two studies have subsequently used algorithm trees to predict dengue early. However, they use different cut-off values which is confusing and we feel that they may not be applicable in real-life settings as dengue is an evolving disease.^{18,19}

We have used multiple laboratory tests for dengue confirmation, which has been shown to increase the sensitivity for detection of dengue infection, especially in areas with a high prevalence of secondary dengue, as in Malaysia.²⁰ Because serological and virological results were not available on Day 1 of recruitment, the staff were blinded to the diagnosis, which helped avoid bias.

It is not surprising that we were unable to find any variables to predict DHF/DSS due to the small numbers present and because we applied the WHO criteria for definition of DHF/DSS very strictly. A 2009 Vietnamese paper subsequently was able to confirm one early clinical indicator, frequent vomiting, and several laboratory indicators, lymphopenia, thrombocytopenia, elevated liver enzymes in dengue patients with severe disease, i.e. had plasma leakage, gall bladder thickening or internal bleeding.¹⁰ This study was able to show independent predictors for severe dengue but did not report any predictors for dengue (both severe and not severe) to distinguish it from OFI. In a clinical situation, one would want to detect dengue early so as to follow it up for development of severe disease.

We did find, however, that a recent family or neighbourhood history of dengue, nausea or vomiting, postural dizziness and white cell count, were independently predictive of dengue infection in the first 72 h of undifferentiated fever in adults.

The lowering of the WCC is the first haematological marker to alter due to suppression of the bone marrow by the dengue virus.⁸ This is a consistent and well-recognized feature in early dengue infection. Nausea and vomiting as early dengue predictors have been reported in Thai children.¹⁹ We have been able to show that having either one of these is an independent predictor in adults. WHO, recognizing the importance of these symptoms, has incorporated them into its new guidelines for dengue diagnosis.¹

This is the first study that has reported postural dizziness as an independent early predictor of dengue. However, anecdotal evidence from Mumbai, India, reports dizziness as an early symptom of dengue and that admission of patients with suspected dengue and dizziness reduces mortality.²¹ Clinically, it is not unexpected to find patients who have nausea and vomiting to present with postural dizziness as they may have subclinical fluid depletion.



Having a history of recent exposure, either by the family or a neighbour, to dengue infection, which we found are predictors of dengue infection, again reaffirm the importance of the clinician notifying the disease to the public health authorities, so that immediate public health measures can be undertaken.

Limitations of the study

We had a 10% non-completion rate. The most common reason for non-completion was a failure to return for convalescent blood sampling. This group was excluded, as we could not be certain if the patients were or were not dengue cases. Dengue patients tend to feel sicker for a longer period than OFI patients⁷ and therefore, might be more likely to return for sampling. If we excluded more OFI than dengue patients then the results could be biased. However, the differences between OFI and dengue would be stronger than those we have described.

Our real-time RT-PCR positive rate for dengue was 27%. This is very low, given that it has a reported sensitivity as high as 99% in the early stages of fever.¹² This is probably due to sample damage during transportation, or, the delay in analysing the results.

Conclusion

Knowing dengue predictors in adults, it is possible to highlight those with probable dengue sooner in the illness and help ensure that these patients have closer follow-up, with clear advice about the warning signs and severe manifestations of the disease. This earlier diagnosis has the potential to reduce morbidity and mortality as well as to ensure the implementation of timely public health measures. Ultimately, education of frontline health professionals to be aware of the possibility of dengue in a patient with suggestive symptoms is crucial in making an early diagnosis.

Acknowledgements

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Levels of serum transaminases in patients of dengue fever during the 2011 outbreak in Lahore, Pakistan

Sabrina Rashid[#] and Farhana Shahzad

Department of Immunology, Children Hospital and Institute of Child Health, Lahore, Pakistan.

Abstract

To assess the levels of serum transaminases in patients with dengue virus infection, 175 serologically confirmed cases were analysed. The mean age of males and females was 8.90 ± 3.52 years and 8.74 ± 3.67 years, respectively. The male to female ratio was 2:1. Signs and symptoms that included fever, abdominal pain, body aches, nausea, anorexia, headache, vomiting, rash, bleeding were present in 175/175 (100%), 122/175 (69.7%), 109/175 (62.3%), 106/175 (60.6%), 77/175 (44%), 69/175 (39.4%), 54/175 (30.9%), 2/175 (1.1%), 1/175 (0.6%) patients, respectively. Deranged serum transaminases, aspartate transaminase (AST) and alanine transaminase (ALT) was present in 130/175 (74.3%) and 108/175 (61.7%) patients, respectively. The mean value of AST (111.26 ± 151.77) was higher than the mean value of ALT (77.07 ± 132.86). Similarly, the serum levels of AST were significantly higher than the serum levels of ALT ($p=0.025$). Thus, preferentially high AST may serve as an early indicator of dengue virus infection.

Keywords: Dengue virus infection; Aspartate transaminase (AST); Alanine transaminase (ALT); Lahore; Pakistan.

Introduction

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are arboviral diseases caused by viruses belonging to genus *Flavivirus*, family *Flaviviridae*. There are four antigenetically-related but distinct serotypes — DENV-1, DENV-2, DENV-3 and DENV-4. In humans, one serotype produces lifelong immunity against re-infection, but only temporary and partial immunity against the other serotypes. *Aedes aegypti* is the principal vector while *Aedes albopictus* is known to be the secondary vector. These mosquitoes mostly breed in man-made water storage containers or natural containers holding rainwater in and around houses.¹

Dengue is a major cause of morbidity and mortality in tropical and subtropical regions. It is estimated that 100 million cases of dengue fever and 500 000 cases of dengue haemorrhagic fever, with 25 000 deaths, occur annually.²

[#]E-mail: sabrinarasheed@gmail.com



The first case of dengue fever in Pakistan was reported in 1994 in Karachi. Now, nearly two decades later, the disease is rapidly assuming epidemic proportions, specifically in the central province of Punjab and its capital, Lahore, where, in September 2011, according to the Punjab Health Department, 131 people were reported dead and over 12 000 people were reported infected. Sindh province, too, was hit by the dengue virus. According to the Sindh Provincial Dengue Surveillance Cell, nearly 400 people were infected with the virus, with over 300 people in Karachi alone, and the death toll stood at five at the end of September 2011.³

Dengue virus replicates within mononuclear cells, including skin dendritic cells, tissue macrophages, peripheral blood monocytes and hepatocytes.⁴

When the liver is damaged, the hepatocyte cell membrane becomes more permeable and some of the enzymes leak out into the blood stream. The two transaminases, which are commonly measured, are alanine transaminase (ALT) and aspartate transaminase (AST). These levels were previously called the serum glutamate-pyruvate transaminase (SGPT) and the serum glutamate-oxaloacetate transaminase (SGOT). Transaminases levels are higher in dengue haemorrhagic fever and dengue shock syndrome (DSS) than in dengue fever and tend to return to normal 14–21 days after infection. Liver damage may be potentiated by the intake of drugs during the early phase of the illness. In dengue virus infection, elevation in serum AST appears to be greater than ALT level. It is due to excess release of AST from damaged myocytes during dengue virus infection. The elevated AST levels tend to return to normal more rapidly than ALT levels because AST has shorter life (12.5–22 hours) than ALT (32–43 hours).

Most people infected with dengue virus are commonly asymptomatic or only have mild symptoms such as an uncomplicated fever; others have a much more severe illness, and in a small proportion of patients, it can be life-threatening. Children often experience symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhoea) but are more susceptible to the severe complications.

Materials and methods

An outbreak of dengue virus infection occurred in Lahore during the summer of 2011 when several children presented to the Children Hospital and Institute of Child Health, Lahore, with acute febrile illness. The suspected clinical diagnosis was made on the basis of acute fever (less than 10 days) and constitutional symptoms. These patients were tested for dengue IgM ELISA. Among them, 175 patients were found to be serologically positive. They were analysed for hepatic involvement by evaluation of serum transaminases. On the basis of the levels of serum transaminases, two groups were made. Group A, with normal values and Group B, with elevated values. The data was entered and analysed using statistical package



for social sciences (Spss) 16.0. Mean \pm SD was given for quantitative variables. Frequencies and percentages were given for qualitative variables. Two independent sample *t* tests were applied to observe mean differences between serum transaminases.

Results

Gender and age

A total of 175 serologically confirmed dengue patients were included in this study. The data of gender and age are summarized in Table 1. 114/175 (65.1%) patients were males while 61/175 (34.9%) were females and the male to female ratio was 2:1. The mean age of male and female patients was 8.90 ± 3.52 and 8.74 ± 3.67 , respectively.

Table 1: Gender and age distribution of dengue patients, Lahore, Pakistan, 2011

Gender	Number of patients (<i>n</i>)	Percentage (%)	Age (mean \pm SD)	Male to female ratio
Male	114	65.1	8.90 ± 3.52	2:1
Female	61	34.9	8.74 ± 3.67	

Historical data

This data is summarized in Table 2.

Most of the patients in this study presented with typical signs and symptoms of dengue viral infection. Fever was the most common presenting symptom in 175/175 (100%) patients, followed by abdominal pain in 122/175 (69.7%), body ache in 109/175 (62.3%), nausea in 106/175 (60.6%), anorexia in 77/175 (44%), headache in 69/175 (39.4%), vomiting in 54/175 (30.9%), rash in 2/175 (1.1%) and bleeding in 1/175 (0.6%) patients.

Serum transaminases (AST and ALT)

The data is summarized in Tables 3 and 4.

In the study population, the serum transaminases (AST and ALT) levels were significantly elevated ($p=0.00$ for AST and $p=0.00$ for ALT). Although both serum transaminases levels were high in dengue patients, the level of serum AST was significantly higher than the serum ALT ($p=0.025$) levels. The mean \pm SD of AST and ALT were 111.26 ± 151.77 and 77.07 ± 132.86 , respectively.



Table 2: Clinical signs and symptoms of dengue patients (n=175), Lahore, Pakistan, 2011

Signs and symptoms	No. of patients	Percentage (%)
Fever	175	100
Abdominal pain	122	69.7
Body aches	109	62.3
Nausea	106	60.6
Anorexia	77	44
Headache	69	39.4
Vomiting	54	30.9
Rash	2	1.1
Bleeding	1	0.6

Table 3: Comparison of optical densities of dengue IgM antibodies and serum transaminases (AST and ALT), Lahore, Pakistan, 2011

Parameter	Mean ±SD	p-value	Parameter	Mean ± SD	p-value
Dengue	1.98±0.62	0.00	Dengue	1.98±0.62	0.00
AST	111.26±151.7		ALT	77.07±132.86	

AST = aspartate transaminase

ALT = alanine transaminase

p-value was determined by independent sample t test

Table 4: Comparison of serum transaminases (AST and ALT) in dengue patients, Lahore, Pakistan, 2011

Serum transaminases	Mean ±SD	p value
AST	111.26±151.77	0.025
ALT	77.07±132.86	

AST = aspartate transaminase

ALT = alanine transaminase

p-value was determined by independent sample t test.



Classification of serum transaminases

This data is summarized in Table 5.

On the basis of the levels of transaminases, the study subjects were divided into two groups. For AST, Group A (1–47 U/L) had 45/175 (25.7%) subjects and Group B (48–>470 U/L) had 130/175 (74.3%) subjects. For ALT, Group A (1–39 U/L) had 67/175 (38.3%) subjects and Group B (40–>390U/L) had 108/175 (61.7%) subjects.

Table 5: Classification, number and percentage of transaminases (AST and ALT) in dengue patients, Lahore, Pakistan, 2011

AST			ALT		
Group	<i>n</i>	Percentage (%)	Group	<i>n</i>	Percentage (%)
Group A (1–47 U/L)	45	25.7	Group A (1–39 U/L)	67	38.3
Group B (48–>470 U/L)	130	74.3	Group B (40–>390 U/L)	108	61.7

n = number of patients

AST = aspartate transaminase

ALT = alanine transaminase

Sub-classification of serum transaminases

These data are given in Table 6. On the basis of elevation in transaminases levels the Group B subjects were, both for AST and ALT, further divided into three subgroups. For AST, subgroup BI (48–141 U/L) had 98/175 (56%) patients, subgroup BII (142–470 U/L) had 28/175 (16%) patients and subgroup BIII (>470 U/L) had 4/175 (2.3%) patients. For ALT, subgroup BI (40–117 U/L) had 86/175 (49.1%) patients, subgroup BII (118–390 U/L) had 18/175 (10.3%) patients and subgroup BIII (>390 U/L) had 4/175 (2.3%) patients. On the basis of this data a larger number of patients was present in subgroup BI and BII of AST as compared with that of ALT. Similarly, the serum AST levels were significantly higher in subgroup BI and BII as compared to ALT levels in these groups ($p=0.00$ for subgroup BI and $p=0.01$ for subgroup BII) (Figure 1).



Table 6: Sub-classification, number and percentage of elevated serum transaminases in dengue patients, Lahore, Pakistan, 2011

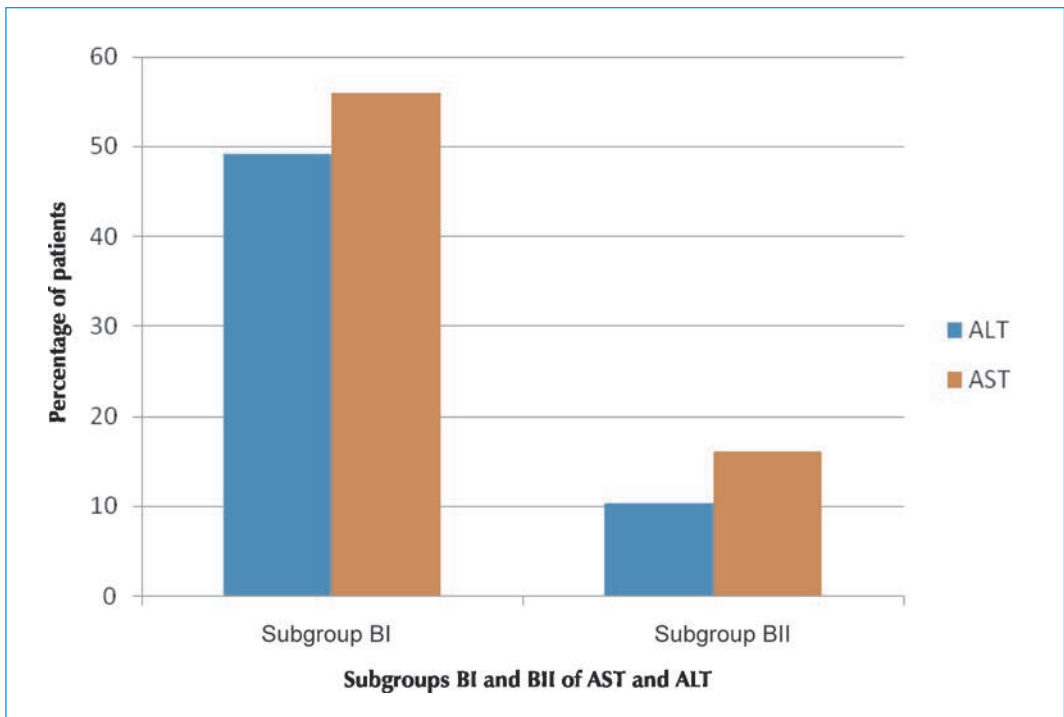
AST			ALT		
Group	n	Percentage (%)	Group	n	Percentage (%)
Group BI	98	56	Group BI	86	49.1
Group BII	28	16	Group BII	18	10.3
Group BIII	4	2.3	Group BIII	4	2.3

AST = aspartate transaminase

ALT = alanine transaminase

n = number of patients

Figure 1: Comparison of ALT and AST elevations in patients with dengue virus infection, Lahore, Pakistan, 2011



ALT = Alanine transaminase

AST = Aspartate transaminase

Group BI (p value = 0.00)

Group BII (p value = 0.01)

p value was determined by independent sample t test



Discussion

Dengue fever is an important cause of morbidity in most South Asian countries, including Pakistan. In Pakistan, the first documented case of DF was reported in 1985⁵. The first major DF outbreak was reported in Karachi in 1994–95. Dengue fever was more commonly seen in the age group 20–40 years; however, some unpublished data also shows children as its major victims.

During this study the maximum cases were males, which may be due to the fact that males spend more time outside their homes and thus are more likely to be exposed to mosquito bites. Some other studies have also reported similarly.^{5,6,7}

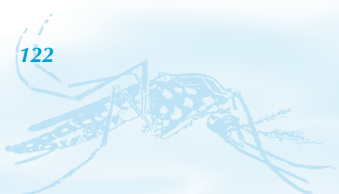
According to our study, the peak levels of cases were among children aged 5–10 years, though some other studies have suggested that dengue is more prevalent among adults. According to a study, dengue is found in all age groups, with a significant number among young adults (20–29 years) and less common among children below 9 years. In one more study, the maximum number of patients belonged to the age group 21–30 years.⁸ However, in a study of south-east Asia, DHF is reported to be primarily a children's disease and showed similar clinical features in all age groups, though the incidence is reported to be higher in children below 15 years of age. Thus, this study correlates with the findings of our study about the incidence of dengue in children.⁹

The AST levels in dengue virus infection tend to be greater than ALT levels.¹⁰ The exact cause of this is uncertain, but it has been suggested that it may be due to excess release of AST from damaged myocytes during the infection.¹¹

Biochemical liver dysfunction, in the form of increased transaminases, was found in most of patients in this study. Similarly, in another study serum transaminases were significantly elevated.¹² However, in another study, AST and ALT were deranged only to a lesser extent as compared to the present study.¹⁰

Although in this study, AST levels were found to be raised in dengue patients but to a lesser extent. According to other studies, AST and ALT levels were highly elevated, up to 90% and 84% respectively, which may be due to the fact that these studies were done in adults.^{12,13} In another study, AST levels were significantly higher than ALT levels.⁸ It may be due to the same reason that the quoted study was done in adults and included a number of DF, DHF and DSS patients. (The degree of rise of AST is significantly higher in DHF and DSS patients as compared to DF, but in our study, a mixed population of dengue virus infection was selected without separate specification of DF, DHF and DSS cases.)

In this study, more patients were in subgroup BI (48–114 U/L) and subgroup BII (142–470 U/L) of AST. However, in another study from India, more population was in the group of AST which had values more than three to ten times the original value of AST like subgroup BII of present study.⁸



In conclusion, it can be reported that liver involvement occurs during dengue virus infection with increased serum liver enzymes (AST and ALT), but AST, rises significantly more than ALT. Therefore, preferentially high AST levels may serve as an early indicator of dengue virus infection, but cannot be a definitive confirmatory parameter of dengue virus infection; only serology and viral confirmation can provide a definitive confirmation of dengue virus infection.

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Dengue-specific IgA in different body fluids: a prospective alternative for dengue diagnosis in resource-poor settings

Rabeya Sharmin,^{a#} Shahina Tabassum,^b KZ Mamun,^a Afzalun Nessa^b and Munira Jahan^b

^aDepartment of Virology, Dhaka Medical College, Dhaka, Bangladesh

^bDepartment of Virology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Abstract

Early diagnosis of recent dengue virus infection is very important as morbidity and mortality associated with untreated cases are exceedingly high. The present study attempted to detect anti-dengue IgA by immunochromatographic test (ICT) from serum, whole blood, saliva and urine in order to establish a reliable alternative for early diagnosis of dengue infection in resource-limited settings. To differentiate between primary and secondary infections, all serum samples were also tested for IgM and IgG antibodies by capture ELISA. Out of 201 clinically-suspected dengue patients, 137 (68.2%) tested positive for anti-dengue antibodies by ELISA, of which 80 (58.4%) were primary and 57 (41.6%) were secondary dengue cases. Among primary cases, the rate of anti-dengue IgA detection was 65% from serum, 53.3% from whole blood, 12.5% from saliva, and 3.75% from urine; in secondary cases, these were 80.7%, 85.2%, 42.1% and 16.25% respectively. The overall sensitivity for anti-dengue IgA from serum, whole blood, saliva and urine were 71.5%, 64.8%, 24.8% and 11.7% (95% CI: 67.0–74.7, 58.5–70, 21.9–24.8, 8.6–12.9), while their specificities were 85.9%, 77.4%, 100% and 96.9% (95% CI: 76.3–92.6, 66.1–86.4, 93.7–100, 90.2–99.4) respectively.

Anti-dengue IgA production elucidated from serum and whole blood in relation to duration of fever showed a statistically significant difference ($P=0.001$) among primary cases. A statistically significant difference was also observed between the appearances of IgA in saliva and duration of fever in primary cases ($P=0.043$). However, urinary anti-dengue IgA positivity was observed more before five days of fever, decreasing gradually after five days of fever in both primary and secondary cases. Thus, serum anti-dengue IgA may be a potential diagnostic tool if used after five days of fever. The sensitivity of anti-dengue IgA from saliva and urine was low in our study and thus needs further evaluation. If the sensitivity can be increased, it may be useful for large-scale epidemiological studies and during outbreaks, especially for dengue-endemic, resource-poor countries such as Bangladesh.

Keywords: Anti-dengue antibodies; IgA; ICT; Whole blood; Serum; Saliva; Urine; Bangladesh.

#E-mail: sharmin.rabeya@gmail.com



Introduction

Dengue is a mosquito-borne viral illness caused by four serotypes (DENV1–4) of dengue viruses. Dengue fever (DF) and dengue haemorrhagic fever (DHF) are major public health problems causing millions of DF cases and thousands of deaths in tropical countries every year, including Bangladesh.¹⁻⁴ Symptoms of dengue virus infections vary from relatively mild DF to more severe life-threatening forms of DHF and dengue shock syndrome (DSS).^{2,3,5,6} Hence, accurate diagnosis of dengue, both in the laboratory and in field settings, is important for understanding the disease spectrum and tracing the source of infection.

The immune response is different in primary and secondary dengue infections. In primary infection, specific immunoglobulin M (IgM) levels are detectable 3-5 days after the onset of symptoms, rise subsequently for the next 1-3 weeks and persist in blood for more than 2-3 months.⁷⁻⁹ Immunoglobulin G (IgG) is detectable approximately 14 days after the onset of symptoms and may be maintained at low level for life.^{7,9-10} In secondary infection, IgM titre rises slowly and may not be detectable in approximately 5% of patients. However, IgG appears approximately two days after the onset of symptoms and is detectable at higher titres, persisting for 10 months to the rest of life.^{7,10,11} Dengue-specific IgA is detected at the same time as IgM, persisting in blood for 40 days only and in saliva for less than one month, in contrast to IgM and IgG antibodies in serum which may persist as long as six months. A higher amount of anti-dengue IgA is obtained in secondary dengue, DHF or DSS than in primary cases.¹²⁻¹⁴ The usefulness of saliva for dengue diagnosis has been infrequently evaluated.^{12,15} However, reports on the use of urine in dengue diagnosis is very limited,¹⁶ and needs further investigation.

The increasing incidence of DF and DHF in Bangladesh underlines the importance of early detection in controlling the spread of the disease. Presently, dengue-specific IgM detection in serum by ELISA is one of the most important and useful methods of dengue diagnosis.^{10,17} Although serum is the preferred sample for most serological assays, its collection is difficult in infants and small children and in field conditions.^{1,16} Saliva and urine are non-invasive specimens, and thus present an interesting alternative for dengue diagnosis. Therefore, in addition to anti-dengue IgA in serum and whole blood, we also investigated the use of anti-dengue IgA in saliva and urine to identify a simple and cheap diagnostic approach and compare its sensitivity and specificity to IgM and IgG from serum for the early diagnosis of dengue in resource-poor settings.

Materials and methods

During January to December 2008, clinically-suspected dengue fever patients were selected from different hospitals of Dhaka city, Bangladesh. Dengue fever cases were selected on the basis of “WHO criteria for case definition of dengue fever, dengue haemorrhagic fever and dengue shock syndrome”.² Serum, saliva and urine samples from a total of 201



patients and whole blood from 147 patients were tested at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. All samples were tested for anti-dengue IgA antibody by ICT. Moreover, to differentiate between primary and secondary dengue infections, anti-dengue IgM and IgG antibodies were detected by IgM and IgG antibody capture ELISA from serum samples only. The cut-off value of the IgG ELISA is used to differentiate between the high levels of IgG characteristic of secondary dengue virus infections and the lower IgG levels characteristic of primary or past dengue infections. A majority of secondary dengue virus infections are detected on the basis of IgG, and most of them also show an elevation of IgM. In contrast, the majority of primary dengue virus infections show an elevation of IgM but not of IgG.^{18,19} In our study done according to the manufacturer's instructions, the index value of IgG >2.2 was suggestive of active secondary dengue infection and index value of IgM >1.1 was suggestive of active primary or secondary dengue infection.

Immunochematographic Test (IgA)

IgA antibody from human serum, whole blood and saliva was detected by ASSURE® Den IgA point-of-care test (POCT) (MP Biomedical Asia Pacific Pte Ltd., Singapore)²⁰ according to the manufacturer's instructions. Interpretations were made as positive, negative and invalid.

Capture ELISA

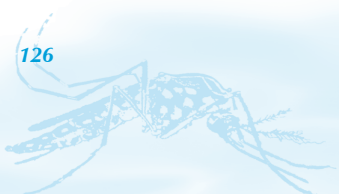
Dengue IgM and IgG capture ELISA (Panbio Diagnostics, Australia, Catalog No. E-DEN01M and E-DEN02G) were used according to the manufacturer's instructions.

Data analysis

The numerical data obtained from the study were analysed and the significance of difference was estimated by using computer-aided statistical package (SPSS) version 15. Data were expressed in frequency and percentage. Sensitivities and specificities were determined. 95% confidence intervals (CI) around the point estimates of sensitivity and specificities were evaluated. Comparison between groups was done by chi-square test. Probability less than 0.05 was considered as significant.

Results

Among the 201 clinically-suspected dengue fever patients, 137 (68.2%) tested positive for anti-dengue antibodies by ELISA. Of these, 80 (58.4%) were suggestive of primary and 57 (41.6%) cases showing very high levels of anti-dengue IgG during the acute phase of infection were considered as suggestive of secondary cases. Out of the 80 primary dengue cases, anti-



dengue IgA was detected in 65% cases from serum, 12.5% cases from saliva, 3.75% cases from urine and 53.3% cases from whole blood. Among the 57 secondary dengue cases, anti-dengue IgA was detected in serum of 80.7% cases, in saliva of 42.1% cases, in urine of 16.25% and in 85.2% cases from whole blood (Table 1). Out of the 64 ELISA-negative cases, anti-dengue IgA was detected from serum in 9 (14.1%) and from urine in 2 (3.1%) cases. Overall, anti-dengue IgA was detected from a significantly higher number of secondary cases than primary cases ($P=0.045$ for serum; $P=0.001$ for whole blood; $P=0.001$ for saliva; $P=0.001$ for urine).

Table 1: Comparison between appearances of IgA in serum, whole blood, saliva and urine among primary and secondary dengue cases, Dhaka, Bangladesh

ICT IgA	Dengue		P value*
	Primary (n=80)	Secondary (n=57)	
Serum	52 (65.0)	46 (80.7)	0.045
Saliva	10 (12.5)	24 (42.1)	0.001
Urine	3 (3.75)	13 (16.25)	0.001
	Primary (n=60)	Secondary (n=34)	
Whole blood	32 (53.3)	29 (85.2)	0.001

Figure within parenthesis indicates percentage.

*Chi-square test was done to measure the level of significance.

Based on the duration of fever, among primary cases, serum anti-dengue IgA was present in 25.0% cases before five days of fever, 72.0% cases at day 5 of fever and 90.3% cases after five days of fever. In 35% cases no IgA could be detected. However, among secondary cases, serum anti-dengue IgA was present in 80.0% cases before 5 days of fever, 75.0% cases at day 5 of fever and 83.9% cases after five days of fever (Table 2). No IgA was detected in 19.3% secondary cases.

Among primary cases, the rate of salivary anti-dengue IgA detection increased from 0% before five days of fever to 12.0% at day 5 of fever, increasing further to 22.6% cases after five days of fever. Salivary IgA was not detectable in 87.5% of primary cases. Among secondary cases, salivary IgA was detected in 10.0% cases before five days of fever, rising to 60.0% cases at day 5 of fever but fell again to 45.2% cases after five days of fever. In 32 (56.1%) secondary cases no salivary IgA was detected. Salivary IgA was not detectable from any of the 64 serologically (ELISA)-negative cases. A statistically significant difference was observed between the appearances of anti-dengue IgA in saliva and duration of fever in primary cases but not in secondary cases ($P=0.043$ for primary dengue; $P=0.059$ for secondary dengue).

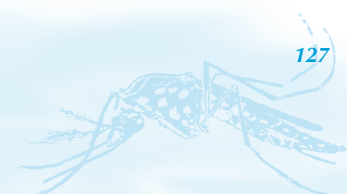


Table 2: Comparison between appearances of IgA in serum, saliva and urine among primary and secondary dengue cases, Dhaka, Bangladesh

Dengue	Duration of fever	Number of cases	IgA		
			Serum	Saliva	Urine
Primary (n=80)	<5 days	24	6 (25.0)	0 (0.0)	2 (8.3)
	5th day	25	18 (72.0)	3 (12.0)	1 (4.0)
	>5 days	31	28 (90.3)	7 (22.6)	0
Secondary (n=57)	<5 days	10	8 (80.0)	1 (10.0)	5 (50.0)
	5th day	16	12 (75.0)	9 (60.0)	5 (31.25)
	>5 days	31	26 (83.9)	14 (45.2)	3 (9.7)
Negative (n= 64)			9* (14.1)	0	2* (3.1)

Figure within parenthesis indicates percentage.

Chi-square test was done to measure the level of significance.

P value of serum IgA for primary cases 0.001

P value of serum IgA for secondary cases 0.764

P value of saliva IgA for primary cases 0.043

P value of saliva IgA for secondary cases 0.059

P value of urine IgA for primary cases 0.271

P value of urine IgA for secondary cases 0.019

* Anti-dengue IgA positive

The detection rate of anti-dengue IgA was comparatively low in urine. Among the primary cases, it was detectable in 8.3% cases before five days of fever, which fell to 4% at day 5, but after five days no IgA could be detected in any of the cases. In 77 (96.3%) primary cases no IgA was detected. Among the secondary cases, detection of anti-dengue IgA in urine decreased from 50% cases before five days of fever to 31.25% cases at day 5, decreasing further to 9.7% cases after five days of fever. No IgA was detected from urine in 44 (77%) secondary cases.

In whole blood, a steady rise in anti-dengue IgA was observed among primary cases from 9.1% before five days of fever to 61.1% cases at day 5, which increased to 95.0% cases after five days of fever. Among secondary cases, IgA was detected in 88.9% cases before five days of fever, 66.7% cases at day 5 but increased to 93.8% cases after five days of fever (Table 3). Although there was a statistically significant difference between appearances of anti-dengue IgA in whole blood and duration of fever in primary dengue cases ($P=0.001$), no significant difference was observed among the secondary dengue cases ($P=0.174$). Among the 53 serologically (ELISA)-negative cases, IgA was detected in 22.6% cases from whole blood.



Table 3: Comparison between appearances of IgA in whole blood among primary and secondary dengue cases, Dhaka, Bangladesh

Dengue	Duration of fever	IgA whole blood		P value*
		Positive	Negative	
Primary (n=60)	<5 days	2 (9.1)	20 (90.9)	0.001
	5th day	11 (61.1)	7 (38.9)	
	> 5 days	19 (95.0)	1 (5.0)	
Secondary (n=34)	<5 days	8 (88.9)	1 (11.1)	0.174
	5th day	6 (66.7)	3 (33.3)	
	> 5 days	15 (93.8)	1 (6.3)	
Negative (n=53)		12 (22.6)	41 (77.4)	

Figures within parenthesis indicate percentage.

*Chi-square test was done to measure the level of significance.

The results of anti-dengue IgA in serum, whole blood, saliva and urine were compared with the results of serum IgM and/or IgG. The highest sensitivity was observed as reference standards for serum (71.5%), and the lowest for urine (11.7%). In contrast, the highest specificity was observed for saliva (100%), and the lowest for whole blood (77.4%). Overall, the sensitivity and 95% confidence intervals around the point estimates of sensitivity obtained for serum, whole blood, saliva and urine were 71.5% (CI: 67.0–74.7), 64.8% (CI: 58.5–70.0), 24.8% (CI: 21.9–24.8), and 11.7% (CI: 8.6–12.9) while their specificity and 95% confidence intervals around the point estimates of specificity were 85.9% (76.3–92.6), 77.4% (66.1–86.4), 100% (93.7–100) and 96.9% (90.2–99.4) (Table 4).

Discussion

In this study, we evaluated the response of anti-dengue IgA from serum, whole blood, saliva and urine from both primary and secondary cases to determine which sample yielded the best results for early diagnosis of dengue infection. We found this serological marker to be a promising alternative diagnostic tool to serum IgM and IgG. A higher percentage of anti-dengue IgA was obtained from secondary cases than from primary cases in all four types of samples, and this difference was statistically significant. In our study, the sensitivity of anti-dengue IgA for serum was 71.5% and 95% confidence intervals around the point estimates of sensitivity was (67.0–74.7), while the specificity was 85.9% and 95% confidence intervals around the point estimates of specificity was (76.3–92.6). A similar specificity (86.05%) but higher sensitivity (86.7%) for serum anti-dengue IgA was reported by Tan et al.²⁰ Their study detected anti-dengue IgA in 77.4% primary and more than 90% secondary dengue cases, while our study detected anti-dengue IgA from serum in 65% primary and 80.7% secondary dengue cases. A higher anti-dengue IgA response in secondary cases has also been reported

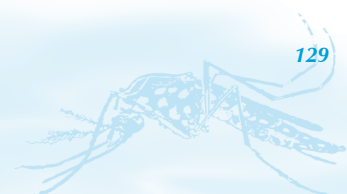


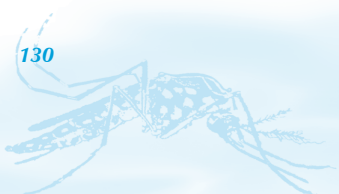
Table 4: Comparison of results of serum, whole blood, saliva and urine for detection of IgA antibody among the dengue positive cases, Dhaka, Bangladesh

	Dengue		Sensitivity (95% CI)	Specificity (95% CI)
	Positive	Negative		
Serum IgA				
Positive	98 (48.7)	9 (4.4)	71.5% (67.0-74.7)	85.9% (76.3-92.6)
Negative	39 (19.4)	55 (27.3)		
Total (n=201)	137 (68.1)	64 (31.9)		
Whole blood IgA				
Positive	61 (41.4)	12 (8.2)	64.9% (58.5-70.0)	77.4% (66.1-86.4)
Negative	33 (22.4)	41 (27.8)		
Total (n=147)	94 (63.9)	53 (36.1)		
Saliva IgA				
Positive	34 (16.9)	0 (0)	24.8% (21.9-24.8)	100.0% (93.7-100.0)
Negative	103 (51.2)	64 (31.9)		
Total (n=201)	137 (68.1)	64(31.9)		
Urine IgA				
Positive	16 (7.9)	2 (1.0)	11.7% (8.6-12.9)	96.9% (90.2-99.4)
Negative	121 (60.2)	62 (30.8)		
Total (n=201)	137 (68.1)	64 (31.9)		

Figures within parenthesis indicate percentage.
CI = Confidence Interval

by other investigators.^{12,13,21-22} Significantly higher levels of anti-dengue IgA in acute sera have been reported from patients with DSS rather than DF or DHF, thus associating the level of IgA with disease severity.⁷ However, further studies using sequential samples in different geographical locations is required to define the kinetics of anti-dengue IgA production among primary and secondary dengue cases.

In our study, the appearance of anti-dengue IgA in serum and whole blood related to the duration of fever showed a statistically significant difference among primary dengue cases only ($P=0.001$) but not among secondary cases. Among primary cases, serum anti-dengue IgA was detected in 25.0% cases before five days of the onset of fever, 72.0% cases at day 5 of fever and 90.3% cases after five days of fever. Investigators have reported that serum IgA was detected in more than 50% cases on the third day of fever which increased to 83.3% between five and seven days of fever.²⁰ A 100% positive serum IgA response in both primary and secondary cases has been reported after 7-8 days of illness, decreasing more rapidly than IgM.²³ Thus, anti-dengue IgA may be a good diagnostic tool if used after five days of fever. Our study found that 14% of serum samples and 22.6% of whole blood samples that were IgA-positive, were ELISA-negative. It should be helpful to confirm these results with



supplementary tests. However, as no confirmatory tests such as viral culture or PCR were performed on these samples due to financial and logistical limitations, no conclusive remarks regarding this can be made.

Both saliva and urine specimens present interesting alternatives for dengue diagnosis, particularly for large-scale epidemiological studies, as their collection is done by non-invasive method. It is also helpful when blood collection is difficult due to cultural factors or difficult venous access, especially in very young children.¹⁶ In our study, a higher percentage of salivary anti-dengue IgA-positive cases was detected among secondary than primary cases ($P=0.001$). Other studies have suggested that salivary IgA may be a good marker, especially in secondary cases.^{12,16} A study from Cuba observed that although serum IgM and IgA were first detected on average at days 4.1 and 4.8 respectively, in saliva, these appeared at 4.4 and 5.7 days after onset of fever.²³ Our study detected salivary IgA before five days of fever in only one case of secondary infection, but after five days, this was present in 22.6% primary and 45.2% secondary cases. A statistically significant difference was observed between the appearance of IgA in saliva and duration of fever in primary cases ($P=0.043$), but not in secondary cases ($P=0.059$). The extent of the role of salivary anti-dengue IgA as a diagnostic marker for dengue infection could not be fully ascertained in our study, as it showed only 24.8% sensitivity but excellent (100%) specificity. The 95% confidence intervals around the point estimates of sensitivity and specificity was (21.9–24.8) and (93.7–100). The low sensitivity of this marker may probably be due to the high concentration of non-specific IgA present in saliva which competes with dengue-specific IgA.²⁴ Therefore, prior to dismissing salivary IgA as a diagnostic marker, an ELISA antigen capture dengue-specific IgA (indirect method) is worth investigating.

Urine, another non-invasive specimen, has low yet determinable concentrations of immunoglobulins.²⁵⁻²⁶ In our study, urinary anti-dengue IgA positivity was observed more before five days of fever, but the positivity decreased gradually thereafter in both primary and secondary cases, unlike the pattern observed for serum and saliva. A similar pattern of anti-dengue IgA positivity in urine, serum and saliva samples from both primary and secondary cases has been reported earlier.¹⁶ Although anti-dengue IgA was detected in a less significant number of cases from urine than serum, urine showed a high specificity but the lowest sensitivity in our study, thereby making this a less reliable marker for diagnosis of recent dengue infection. However, as the use of urine in dengue diagnosis is as yet very limited, further studies addressing the early phase of the disease with this marker are warranted before reaching any conclusive decision.

As anti-dengue IgA gives the indication of early dengue infection, its detection from serum and whole blood may be a useful alternative to IgM, especially in secondary dengue infection when IgM is not detected. Although serum IgM and IgG remain the preferred markers for early serological diagnosis of dengue infection, serum anti-dengue IgA along with IgM and/or IgG antibody may be a valuable tool in the diagnosis of recent dengue infection. To evaluate the role of anti-dengue IgA in saliva and urine, large-scale epidemiological studies need to



be undertaken. If the results are promising, then these tests may be of great importance, especially for dengue-endemic, resource-poor countries such as Bangladesh, as these clinical specimens are more cost-effective than serum, being cheaper and easier to collect and process, requiring no special materials or technical expertise. Therefore, estimation of these specific antibodies may provide valuable guidance in order to determine the need for observation and treatment of dengue, thereby lowering disease fatality.

Acknowledgments

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A clinicoradiological and laboratory analysis of dengue cases during an outbreak in central Nepal in 2010

**Arun Sedhain,^{a#} Shital Adhikari,^a Gandhi R Bhattarai,^b Sudhir Regmi,^a
Lok R Subedee,^a Sunil K Chaudhary,^a Manoj Shah^a and Barun Shrestha^a**

^aChitwan Medical College, Bharatpur Chitwan, Nepal

^bOptumHealth Care Solutions, Golden Valley, MN 55427, United States of America

Abstract

Dengue fever (DF), which was considered a rare disease entity in Nepal till the recent past, showed a major outbreak in the central part of the country in 2010. This study was aimed at finding the clinical and laboratory profiles of DF patients during the outbreak in Chitwan and adjacent districts. Among the 1456 patients with acute febrile illness, 426 (29%) tested positive for DF, out of which 414 patients were included in the study. 85% of the patients were in the 16–60 years age group and males (58%) outnumbered females (42%). 56% of the patients came from the urban area while 34% and 10% came from sub-urban and rural areas, respectively. 69% of the patients tested positive for IgM, 10% tested positive for IgG only and 21% tested positive for both IgG and IgM. The most common clinical presentations were fever (100%), headache (97%), bodyache (93%), nausea (85%) and vomiting (63%). Other symptoms included retro-orbital pain (49%), itching (43%), abdominal pain (42%), skin rashes (27%) and loose motion (26%). Pneumonia was seen in 10% of patients. 90% of patients were admitted in hospital and 3% required ICU admission. Around 10% of patients were managed as outpatients. Dengue fever (DF) and dengue haemorrhagic fever (DHF) were present in 79% and 21% of the patients, respectively. Only 2 out of 414 patients had dengue shock syndrome (DSS). Thrombocytopenia (platelet count less than 100 000 mm³) was present in 70% of patients and 18% of patients had platelet count less than 50 000 mm³. Leucopaenia was seen in 54% of patients. The most common ultrasound finding was hepatomegaly (31%) followed by thickened gall-bladder wall and ascites (12% each), splenomegaly (9%) and pleural effusion (8%). Only one patient with DHF who had co-morbidity with bronchiectasis and alcoholic liver disease died in the hospital during the course of treatment.

Keywords: Dengue fever; Dengue haemorrhagic fever; Dengue outbreak; Chitwan; Nepal.

#E-mail: purkang@gmail.com



Introduction

Dengue is considered as one of the most important of the arboviral infections of humans.¹ Dengue fever (DF) and dengue haemorrhagic fever (DHF) are caused by four antigenically distinct but related dengue viruses (DENV1–4). DENV serotypes are transmitted primarily by *Aedes aegypti*. The global incidence of DF and DHF has increased dramatically in recent decades.^{1,2} Dengue virus is now the most common cause of arboviral disease in the world, with an estimated annual occurrence of 100 million cases of DF and 250 000 cases of DHF with a mortality rate of 25 000 per year.³

Dengue virus infection has been recorded in Nepal since the 1990s, and the first case of dengue was reported in 2004.⁴ A confirmed dengue outbreak was observed in nine districts of the Terai region in Nepal in 2006.⁵ However, no national surveillance programme exists in the country to establish the incidence of dengue or its disease burden.

An unusual outbreak of DF and DHF was observed in Chitwan district and adjacent areas of central Nepal during 2010. There was a rapid surge in the number of patients with atypical clinical and biochemical symptoms in early to mid-August. Though dengue had not been considered a common disease in the Nepalese context, we still thought of it as one of the differential diagnoses. As the testing facilities were not available locally, blood samples of a few patients were sent to the national reference laboratory in Kathmandu for testing for dengue and leptospirosis. Out of 15 test samples, 4 were positive for dengue and seven were positive for leptospirosis. This established the presence and possible threat of dengue in Nepal. Later, reliable testing facilities were established at local level and a large number of dengue cases were recorded.

Materials and methods

Objectives

This study was carried out by the Department of Medicine, Chitwan Medical College, with the broad objective to analyse the trend of dengue during a disease outbreak, along with its clinical features, complications and disease outcome in association with laboratory data as predictive factors of dengue diagnosis. We also aimed to analyse the radiological findings in dengue patients and compare them as per clinical and laboratory parameters.

Geographical setting

Chitwan, one of the 75 districts of Nepal, lies in the central part between 27°21'45" to 27°52'30" N and 83°54'45" to 84°48'15" E. The altitude of the district ranges from 141 to 1947 metres above the sea level. Its total population is 468 699 with a density of 354 persons/km².



Of the district, 38.75% is in the plains, while 20.65% is in the hilly area. The rest (40.6%) of the land area is occupied by the renowned Chitwan National Park.

Bharatpur, the district headquarters, is emerging as a health city of Nepal with the establishment of new and well-equipped hospitals and other health care facilities. The Chitwan Medical College is a newly established 500-bed teaching hospital located in the center of the district, which gets referrals from adjacent districts such as Nawalparasi, Gorkha, Lamjung, Tanahun, Makawanpur and Rupandehi. Around 30 000 patients visit the outpatient department of the hospital annually.

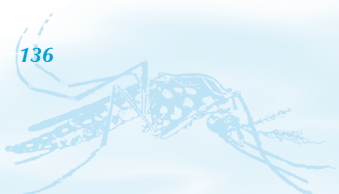
Study population and dengue case definition

From September to December 2010, during the dengue outbreak in Chitwan and adjacent areas of central Nepal, patients with a history of fever in the 10 preceding days (acute febrile illness) and no clinical evidence of bacterial infection anywhere in the body were examined and had at least one blood sample collected for specific dengue and other laboratory tests. See Table 1 for district-wise distribution of dengue patients in 2010 in and around Chitwan district. A total of 1456 patients were tested for dengue serology, of which 426 tested positive for the disease and 414 cases were included in the study. The definition of DHF included fever, thrombocytopenia (platelet count less than 100 000 per mm³), haemorrhagic manifestations, and objective evidence of plasma leakage revealed by more than 20% rise in haematocrit over baseline, or more than 20% drop in haematocrit after treatment, or presence of plasma leakage signs, such as pleural effusion, ascites and hypoproteinemia.¹ For the diagnosis of dengue shock syndrome (DSS), in addition to the previously mentioned criteria, the presence of hypotension or narrow pulse pressure was also required. Any subject with serological evidence of acute dengue infection who did not meet the criteria for DHF/DSS was considered as having DF.

Study design

After written informed consent, demographic and personal details of the patients were recorded. Clinical data were collected through interviews with patients or their attendants. Detailed clinical information with special focus on the duration and highest intensity of fever, and associated symptoms such as headache, nausea, vomiting, anorexia, body ache and bleeding diathesis, were recorded. A meticulous physical examination of the patients was conducted. Hepatomegaly and ascites were ascertained by physical examination and on reports of ultrasonography and X-rays.

All blood samples were tested for dengue virus IgM or IgG antibodies. SD Dengue IgM/IgG capture ELISA kit and SD Dengue IgM/IgG rapid test kit, both produced by Standard Diagnostics Inc., Kyonggi-do, Republic of Korea, was used for testing in the laboratory. In the initial days of the study, when there were limited resources and testing mechanisms, rapid test



for dengue was applied.⁶ Subsequently, rapid IgM-IgG capture ELISA test, which has become the standard for serological diagnosis of dengue fever,⁷ was initiated. All laboratory tests were done at the National Reference Laboratory (NRL) located within the Chitwan Medical College Hospital. Additional laboratory investigations carried out in these patients included haemoglobin, total and differential leucocyte count, platelet count and liver function tests. Chest X-ray and ultrasound of the abdomen were done whenever clinically indicated.

Treatment

The patients were closely monitored. Intravenous fluids, platelet-rich plasma (PRP), fresh whole blood and fresh frozen plasma were infused as needed. In patients with isotonic dehydration, 5% dextrose in normal saline (dextrose normal saline solution) was used. Platelet-rich plasma was administered to the patients with a platelet count of less than 50 000/mm³. Fresh whole-blood transfusion was given when patients had either a massive haemorrhage or a falling haematocrit. Paracetamol was the only antipyretic agent given when body temperature was higher than 39 °C. Combinations of paracetamol, ibuprofen, salicylates and intramuscular injections were avoided.

Statistical analysis

SPSS version 16 was used for the statistical analysis of data. Graphical maps were prepared using open source GIS software DIVA-GIS version 7.

Results

Geographical distribution

More than two thirds of the patients were from Chitwan district, followed by Nawalparasi and Rupandehi districts in the distant second and third places respectively (Figure 1). Table 1 shows the district-wise distribution of dengue patients by their place of residence.

Most of the patients from Chitwan district were residents of Bharatpur municipality, followed by Ratnanagar municipality and adjacent village development committees (VDCs). Figure 2 shows the geographical distribution of dengue patients in Chitwan district.

Age- and sex-wise distribution of patients

A majority of the patients (84.57%) were in the age group 16–60 years, which is considered to be the most productive part of one's life (Figure 3).



Table 1: District-wise distribution of dengue patients during 2010 in and around Chitwan district, Nepal

District	No. of cases	Percentage
Chitwan	291	70.36%
Nawalparasi	78	18.79%
Rupandehi	25	6.02%
Tanahun	11	2.65%
Makwanpur	6	1.44%
Parsa	2	0.48%
Lamjung	1	0.24%

Figure 1: Map of Nepal showing the districts affected by dengue outbreak in 2010

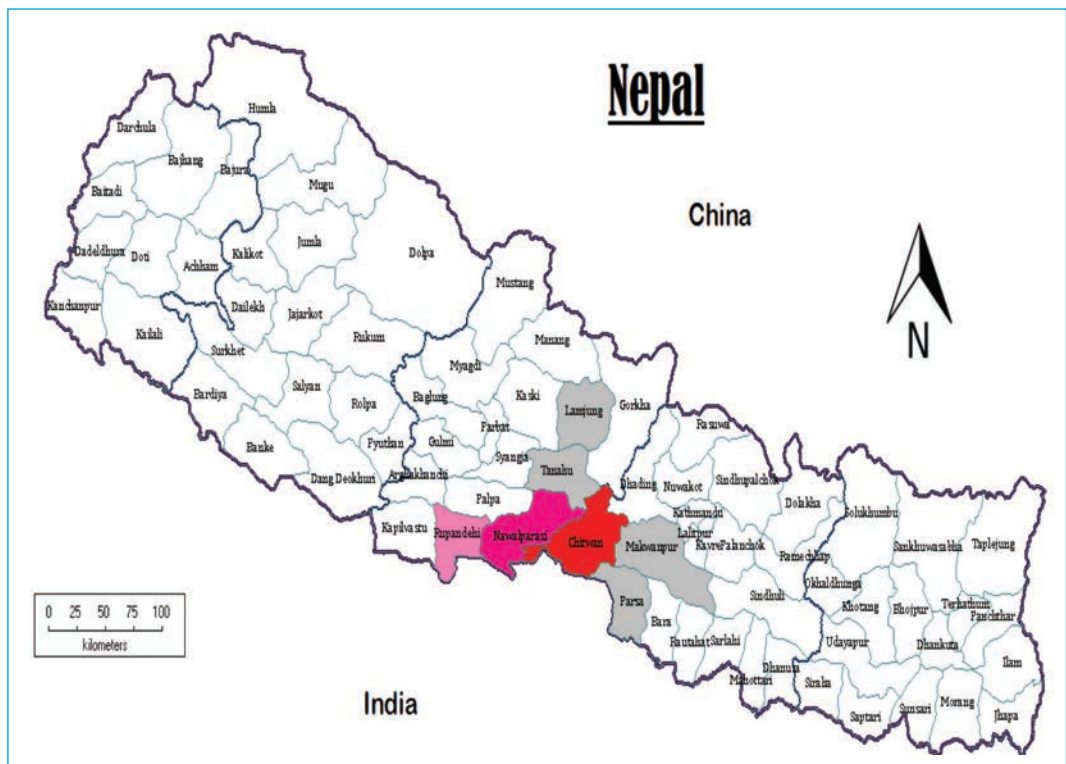


Figure 2: Map of Chitwan district showing the areas affected by dengue outbreak, 2010

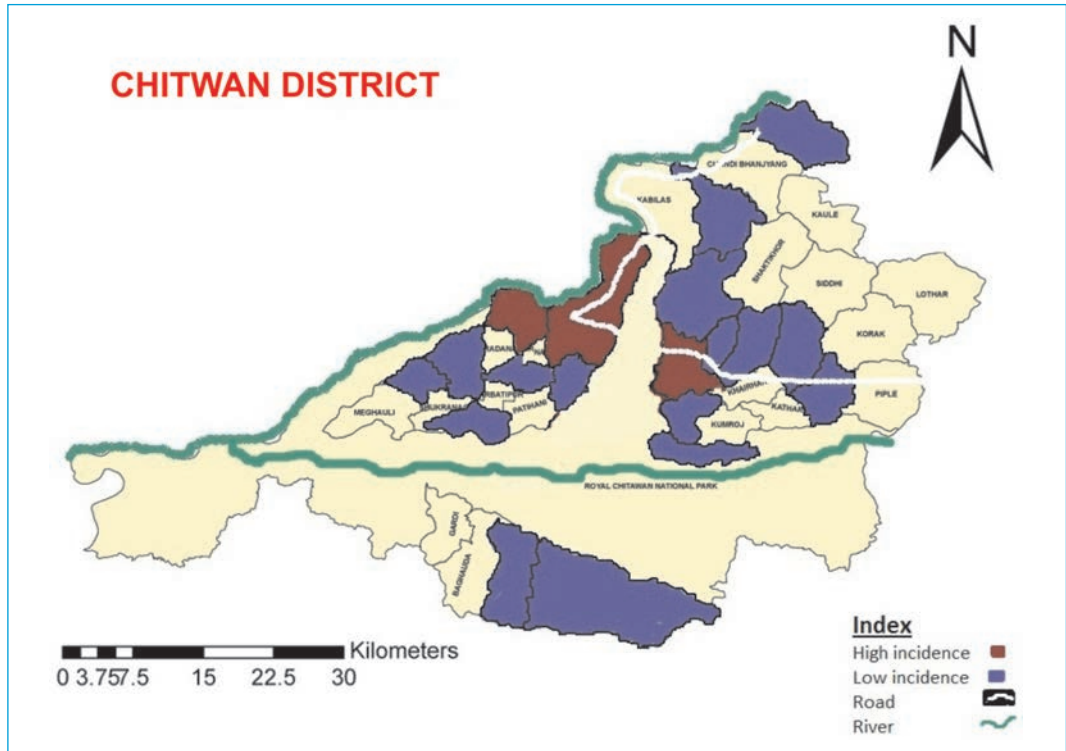
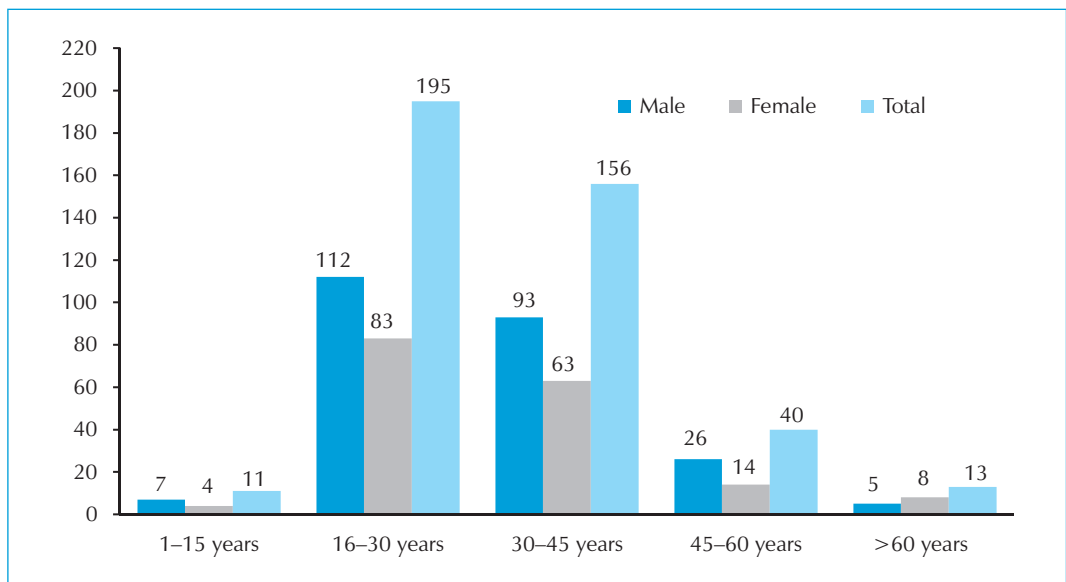


Figure 3: Age- and sex-wise distribution of dengue patients, Chitwan, Nepal, 2010



Seasonal variation of dengue cases:

Since the first case of dengue was detected in the last week of August, the disease incidence showed an increasing trend, which peaked towards the last week of October. The following two graphs highlight the month-wise (Figure 4) and week-wise (Figure 5) distribution of dengue cases.

Figure 4: Month-wise distribution of dengue cases, Chitwan, Nepal, 2010

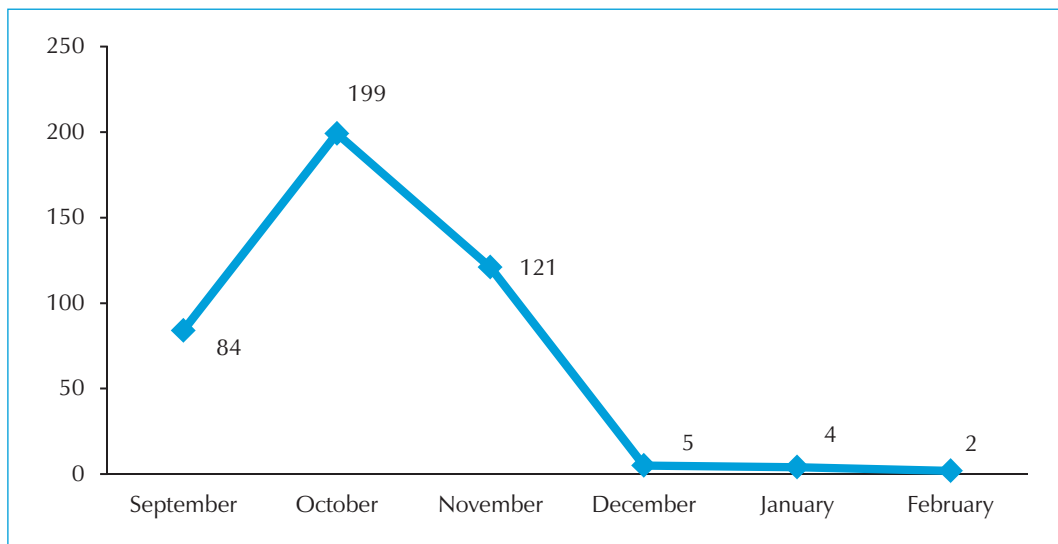
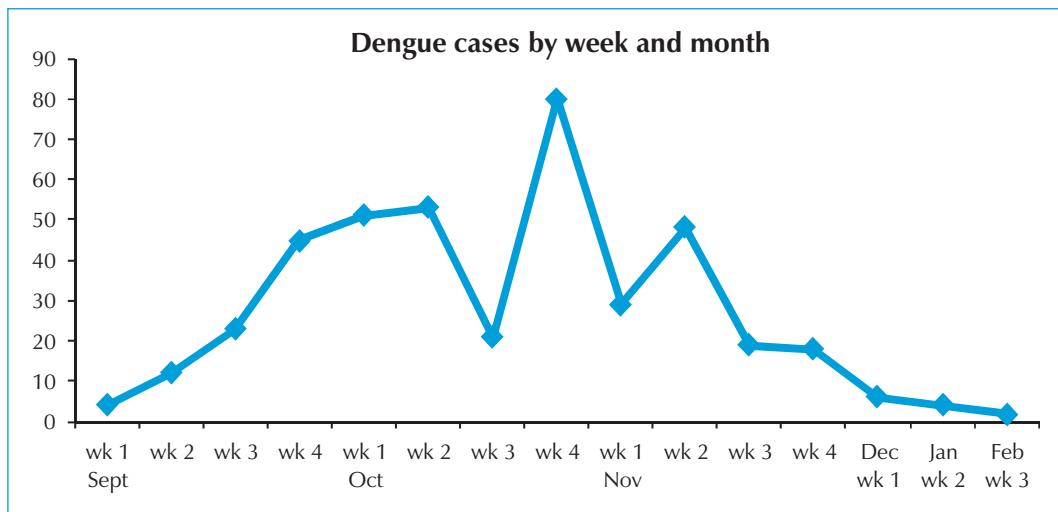


Figure 5: Week-wise distribution of the dengue cases, Chitwan, Nepal, 2010



Symptoms of dengue fever

Table 2 indicates the signs and symptoms of all patients who presented with fever and were diagnosed with DF. Headache, body ache and nausea were present in majority of the patients. Other clinical manifestations are also given in the table.

Table 2: Symptomatic presentations of dengue patients, Chitwan, Nepal, 2010

Clinical manifestation	Number of patients (%)
Fever	415 (100)
Myalgia	401 (96.62)
Body ache	386 (93.06)
Nausea	351 (84.57)
Vomiting	260 (62.79)
Retro-orbital pain	204 (49.15)
Itching	177 (42.65)
Abdominal pain	174 (41.93)
Hepatomegaly	162 (39.06)
Skin rash	111 (26.74)
Loose motion	103 (24.81)
Conjunctival suffusion	99 (23.85)
Splenomegaly	67 (16.14)
Lymphadenopathy	57 (13.73)

Comparative analysis of symptoms in DF and DHF

Table 3 gives the comparative analysis of DF and DHF cases.

Following the existing WHO criteria, 79.27% ($n=329$) of the patients were diagnosed with classic DF and 20.48% ($n=85$) with DHF. Three of them had clinically significant bleeding. One of the patients, who was diagnosed with DSS, had co-morbidities such as post-TB bronchiectasis and alcoholic liver diseases. He died during the course of the treatment in the ICU. One of the patients was admitted with features of fulminant hepatic failure with altered sensorium, deep jaundice and deranged transaminase level (AST of 14 100 IU and ALT of 5760 IU). However, this patient survived and AST and ALT values returned to 105 IU and 120 IU, respectively, on the 11th day of illness.



Table 3: Comparative analysis of clinical manifestations among patients with dengue fever and dengue haemorrhagic fever, Chitwan, Nepal, 2010

Symptoms	DF (n=329)		DHF (n=85)		P value
	Count	Percentage	Count	Percentage	
Headache	316	96.05	85	100.00	0.063
Bodyache	303	92.10	83	97.65	0.069
Nausea	277	84.19	76	89.41	0.226
Retro-orbital pain	161	48.94	43	50.59	0.786
Itching	137	41.64	40	47.06	0.368
Vomiting	112	34.04	48	56.57	<0.001
Hepatomegaly	100	30.40	62	72.94	<0.001
Loose motion	74	22.49	33	38.82	0.002
Conj. suffusion	63	19.15	36	42.35	<0.001
Skin rash	49	14.89	62	72.94	<0.001
Splenomegaly	37	11.25	30	35.29	<0.001
Lymphadenopathy	34	10.33	23	27.06	<0.001
Drowsiness	1	0.30	3	3.53	0.007
Bleeding	0	0.00	3	3.53	<0.001

Laboratory investigations

Tables 4 and 5 indicate the laboratory parameters and ultrasonologic manifestations of DF cases, respectively.

Thrombocytopenia with values less than 100 000/mm³ was seen in 72% of patients. The lowest platelet count was 13 000 and the highest was 280 000/mm³. Around 19% (n=76) of patients had platelet count less than 50 000/mm³. Leucopenia was seen in 53.49% (n=222) of patients; 31% (n=129) had lymphocytes more than neutrophils in differential leucocyte count.



Table 4: Laboratory parameters of DF cases, Chitwan, Nepal, 2010

Variables	DF (n=329)		DHF (n=85)		P value
	Count	Percentage	Count	Percentage	
Platelet <50 000	32	9.73	43	51.19	<0.001
Requirement of PRP	41	12.46	44	52.38	<0.001
Lymphocytes > Neutrophils	100	30.40	34	40.48	0.078
ALT more than 50	258	78.42	81	96.43	<0.001
AST more than 50	177	53.80	62	73.81	<0.001

Table 5: Ultrasonologic manifestations of DF cases, Chitwan, Nepal, 2010

Variables	DF (n=329)		DHF (n=85)		P value
	Count	Percentage	Count	Percentage	
Splenomegaly	37	11.25	30	35.29	<0.001
Thickened gall bladder	20	6.08	22	25.88	<0.001
Third space loss	18	5.47	84	98.82	<0.001
Hepatomegaly	100	30.40	62	72.94	<0.001

Discussion

The dengue outbreak started between end-August and early September and peaked by late October. Pandey et al. have reported 11 cases of DF during September – December from different geographical areas of Nepal.⁵ September is the beginning of the post-rainy season in Chitwan region. Despite the fact that October, November and December are colder months, there was a continuous rise in the pattern of the disease until the end of December and some sporadic cases were seen until mid-February 2011.

Dengue-specific IgM was positive in 69% of patients, which is comparable to other studies.^{8,9} According to WHO criteria for diagnosis, 329 (79.27%) of the patients were diagnosed as classic DF, 83 (20.48%) as DHF, and 2 of them had DSS, which is comparable to a similar study done in Chennai, India (DHF in 25%),¹⁰ but much lesser than the previously-cited Lucknow study,⁹ where as many as 56% among the total dengue cases were DHF cases.



In our study, the majority of patients were in the age group 16–45 years (84.78%), with the mean age of 32.37 years and a similar trend was observed in studies from Singapore^{11,12} and Malaysia.¹³ DHF was more common than DF in the elderly population (mean age of 31.59 years vs 35.42 years), which is statistically significant ($P=0.010$). Our finding was contrary to the fact that in most dengue endemic areas, DHF cases are reported to occur mainly in children below 15 years of age,¹ which has been supported by a few other studies as well.^{9,14–16}

90.16% of the patients were managed as in-patients and the rest were managed on out-patient basis. 3.38% ($n=14$) of the patients required ICU admission. DHF patients were admitted longer (mean hospital stay of 5.33 days) than DF patients (mean hospital stay of 3.61 days), which was statistically significant.

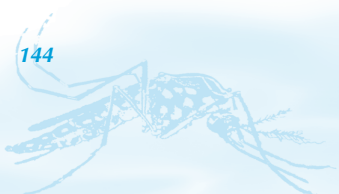
Dengue is considered to be common in most tropical and subtropical regions worldwide. Despite having large populations of mosquitoes and presence of vector-borne diseases, there were no detectable significant numbers of dengue cases in the past in Nepal. However, it is not surprising to have the outbreak of dengue in the plain areas of Chitwan and adjacent Terai districts of Nepal, which lie in the tropical climatic zone. Though there were a few reports of detection of *Aedes aegypti* in six different districts of Nepal,⁵ Chitwan, where the current outbreak occurred, had never been reported as having this species of mosquito. But the alarming part of the current outbreak was that there were significant numbers of patients from the hilly regions of Nepal like Tanahun, Lamjung and hilly areas of Chitwan, which were supposed to be free from vector-borne diseases (Source: Health Department, Nepal).

Regarding the geographical distribution of the cases, 55.9% of these were from urban area followed by 33.73% from semi-urban and 10.12% cases from rural areas.

High grade fever (100%), headache (96.86%), myalgia (93%) and nausea (85.3%) were the most common symptoms, followed by vomiting (63%), retro-orbital pain (48.67%), itching (42.75%), abdominal pain (41.93%), skin rashes (26.74%) and diarrhoea (25.78%). Similar clinical manifestations are reported in different studies.^{6,17–19} Bleeding from various sites was noted in 3.53% of DHF patients. Bleeding could be the result of thrombocytopaenia, consumption coagulopathy, capillary fragility or platelet dysfunction.²⁰

The typical clinical symptom we noticed in our study was body itching which was complained by 42.75% of the patients. However, there was no statistically significant difference of this symptom in DF or DHF patients with values of 41.64% and 47.06% respectively ($P=0.368$). Patients with dengue fever developed itching when the body temperature started falling back to normal.

The platelet count was below 100 000/mm³ in 70% of patients, which is slightly higher than in a few other studies.¹¹ The platelet count was below 50 000 in more than 18% of patients. Thrombocytopenia below 50 000 counts was significantly lesser in DHF patients in comparison to DF (51.76% vs 9.73%) with P value of <0.001 . Severe thrombocytopenia (less



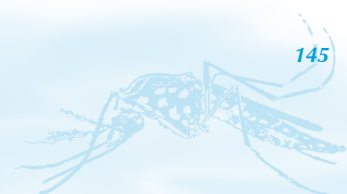
than 20 000/mm³) was observed only in 1.69% ($n=7$) of the patients. Rapid fluctuation in daily platelet count was seen in some of our patients and the possible explanation of this could be that platelets align themselves onto the leaking endothelial cells, which are lining the blood vessels damaged by the viruses, and with recovery, these cells return to circulation.²¹

The average number of white blood cell count (TLC) was 4701/mm³, with 4806/mm³ in DF patients and 4289/mm³ in DHF patients, though the difference in TLC was not statistically significant ($P=0.403$). Leucopaenia with TLC less than 4000/mm³ was seen in 53.38% of patients, which is similar to a few other studies.^{17,18} Leucopaenia is a common laboratory finding in DF and DHF.²² The leucocyte count may be normal or slightly increased with the predominance of neutrophils initially. Towards the end of the febrile phase, there is a reduction in the number of TLC and neutrophils. The leucopaenia usually reaches its nadir shortly before or at the time of a drop in temperature and returns to normal 2–3 days after defervescence.

Liver enzyme AST was increased with the value more than 50 IU/mL in 78.42% and 96.47% of cases of DF and DHF respectively, whereas the ALT value was raised in 53.8% of DF and 74.12% of DHF patients. The difference in the rise of liver enzymes in DHF and DF was statistically significant ($P<0.001$). These findings are slightly higher than the north Indian study¹¹ but the rise in liver transaminases are slightly lower than a few of the other studies.^{23–28} Similar to our findings, Wahid et al. found liver dysfunction to be more common in DHF than in DF patients.²⁹

The AST levels in dengue infection tend to be greater than ALT levels.^{30,31} This differs from the pattern in viral hepatitis, but is similar to that seen in alcoholic hepatitis. The exact cause of this is uncertain, but it has been suggested that it may be due to excess release of AST from damaged monocytes during dengue infection.²⁷ The elevated AST level tends to return to normal more rapidly than the ALT levels. This is possibly due to the fact that AST has a shorter life of 12.5–22 hours in comparison to 32–43 hours of ALT.³² Our study has also shown the significant rise in AST than in ALT, and this abnormality may be the early indicator of dengue infection. Though there are many studies signifying liver abnormality in dengue infection and alterations in liver functions are well known, these abnormalities are not included in the dengue case definition.¹

In our study, the most common ultrasound finding was hepatomegaly seen in 39.13% ($n=162$) of patients, followed by third space loss in the form of ascites and pleural effusion in 24.64% ($n=102$) of patients. Hepatomegaly has been described in 20.4% of patients in a New Delhi study²⁰ and in 22.2% of patients in a Kolkata study.³³ Splenomegaly was observed in 16.18% ($n=42$) of patients and thickened gall-bladder wall in 10.14% ($n=42$) patients. The occurrence of splenomegaly in our study has been higher than in the study from Delhi (8.2%)²² and Kolkata (9.3%).³³ The gall-bladder wall thickness of more than 3 mm is considered abnormal.³⁴ In a study of 96 children with mild or severe DHF, Setiawan et al³⁵ found a gall-bladder wall thickness of more than 3 mm in 33% of patients with mild DHF and 94% of patients with severe DHF.

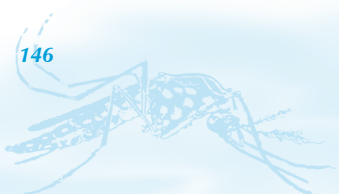


Conclusion

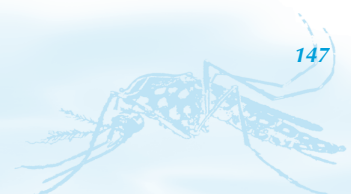
Limited technical and economic resources in developing countries, such as Nepal, are stimulating the investigation of predictive validity of clinical data and non-specific tests for dengue diagnosis. A combination of symptoms that are commonly seen in dengue could improve surveillance of the disease and thus help in timely decision-making in simple and economically-deprived clinical settings.

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Evidence for the use of intravenous rehydration for treating severe dengue with plasma leakage in children and adults: a systematic review

Usman HB,^a Safitri I,^b Lum L,^c Martinez E,^d Kroeger A,^{e,f} and Horstick O^{#g}

^aDepartment of Public Health and Epidemiology, University of Birmingham, United Kingdom

^bDepartment of Child Health, Faculty of Medicine, Gajah Mada University/ Dr. Sarjito Hospital, Yogyakarta, Indonesia

^cDepartment of Child Health, University of Malaya, Kuala Lumpur, Malaysia

^dInstituto Medicina Tropical Pedro Kuori, La Habana, Cuba

^eSpecial Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland

^fLiverpool School of Tropical Medicine, United Kingdom

^gInstitute of Public Health, University of Heidelberg, Germany

Abstract

Dengue, in its severe form, is mostly related to plasma leakage, requiring intravenous rehydration. This study reviews the evidence available for intravenous rehydration to prevent and reduce dengue deaths due to severe dengue in both children and adults.

The comprehensive systematic literature review followed AMSTAR criteria: search terms were: (i) dengue and severe dengue (dengue deaths); (ii) therapy (rehydration therapy, intravenous fluid); and (iii) types of intravenous fluids (isotonic, hypotonic and hypertonic).

Nine studies met the inclusion criteria; study designs were heterogeneous and were recorded differently. All included studies looked at children as a study population. There are two systematic reviews for treatment options for children, concluding that aggressive fluid resuscitation is an effective treatment for dengue with plasma leakage. Two literature reviews suggest that Ringer's lactate, or isotonic crystalloids and hypotonic solution, are appropriate for rehydration and treatment of severe dengue. Three randomized controlled trials (RCTs) demonstrated the efficacy of intravenous rehydration in preventing or reducing death due to severe dengue. One before-and-after study demonstrated that administration of intravenous fluids reduced the duration of hospitalizations and ICU admissions. One prospective study comparing different types of fluids showed that patients with severe dengue get a favourable outcome with effective fluid replacement. Also, blood transfusion as well as dextran may be required for patients with severe dengue.

[#]E-mail: Olaf.Horstick@uni-heidelberg.de



In conclusion, studies show that intravenous rehydration with isotonic fluids is the appropriate treatment in cases of children with plasma leakage. Training on how to use intravenous rehydration may be the key to good outcomes, far more important than the type of fluid used. However, no specific evidence has been found for the treatment of adults. These shortcomings need to be highlighted in existing algorithms. Studies specifically addressing best practice of treatment for severe dengue in adults are urgently needed, especially in pregnancy and with co-morbidities.

Keywords: Dengue; Intravenous rehydration; Systematic review.

Introduction

Dengue is the most common mosquito-borne viral infection worldwide and every year, almost 50 million people are estimated to suffer from the disease.^{1,2} Around 2.5 billion people all over the world are living in countries at risk of dengue.³

Dengue is classified into dengue and severe dengue.³ Children and cases with secondary infections are at a greater risk of suffering from severe dengue.⁴ Most of the cases of severe disease are related to plasma leakage and shock. The pathogenesis of plasma leakage is not clear, but the antibody-dependent enhancement (ADE) of infection has been hypothesized as a mechanism to explain severe dengue in the course of a secondary infection and in infants with primary infections.^{5,6}

Early detection of the disease along with its appropriate management can reduce the number of hospitalizations resulting from the severity of the disease. The critical phase of the ailment starts at defervescence.^{7,8} Cases with plasma leakage are at risk of shock, usually when the amount of plasma lost through leakage is severe. Prolonged shock may lead to disseminated intravascular coagulation and metabolic acidosis along with severe damage to the organs causing hepatitis, myocarditis or encephalitis.⁹ This clinical picture can often be fatal. Severe bleeding may initiate a reduction in haematocrit levels. With further deterioration, warning signs may become more apparent and early intervention with intravenous rehydration may prevent shock.

The life-saving role of rapid replacement of fluids lost from the vascular compartment in severe dengue began to be appreciated in the 1960s.¹⁰ With growing understanding of the pathophysiology of severe dengue, intravenous rehydration has been shown to be life-saving and to reduce the case-fatality rate of severe dengue from more than 20% to less than 1%.³ Although this intervention is commonly accepted as being the only effective intervention reducing case fatalities of severe dengue, the evidence for this has not been systematically evaluated and summarized, especially not for children and adults. Also, treatment algorithms exist for both children and adults and the level of evidence for these has never been evaluated.



In countries with high case fatalities of severe dengue, clinicians are often reluctant to accept this intervention and a systematic review on this issue would be of high importance to underline that the evidence is not only derived from expert opinion.

The main objective of this study, therefore, is to establish the level of evidence available for intravenous rehydration to prevent and reduce dengue deaths due to severe dengue in both children and adults.

Specifically, this systematic review defines: (i) the impact of intravenous rehydration in severe dengue cases in both children and adults; (ii) the fluids that are recommended; (iii) the fluid regimens used; and (iv) discusses the major findings in various studies found in association to the use of intravenous fluids in severe dengue.

Methods

An initial survey of the studies and literature revealed that extensive work on this issue is not available. Therefore, this review applied a broad search strategy, searching for all studies directly relevant to the use of intravenous fluids to treat cases with severe dengue with plasma leakage, in both adults and children.

Search terms included free text terms and Medical Subject Heading (MeSH) terms/subjects, taking into consideration the population, intervention, comparator and outcome measure (PICO) related to the research question, using the words and combinations of:

- Illness: dengue and severe dengue, including dengue deaths
- Relevant therapy: rehydration therapy, intravenous fluid
- Types of intravenous fluids: isotonic, hypotonic and hypertonic

Included databases were searched from inception (no lower time limit) to 28.02.2012: EMBASE, Cochrane Database of Systematic Reviews, WHOLIS, PubMed and Lilacs. References from identified papers and grey literature were searched from sources like WHO, unpublished evaluations, reports, conference papers and theses.

All available studies dealing with dengue, intravenous rehydration and different intravenous fluids/regimens, regardless of study design, were included. Articles were assessed by the abstract and titles, when meeting the inclusion criteria, and full studies were assessed against the exclusion criteria: studies not dealing with severe dengue (or related terms for severity) were excluded (Table 1). The quality of studies was assessed according to study type, using the Jadad checklist for single studies¹¹ and AMSTAR criteria¹² for systematic reviews. Only studies with a very poor methodology or grossly incorrectly conducted studies were excluded from review. The search was restricted to English language only.



Table 1: Inclusion and exclusion criteria for systematic literature review

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none">• All available studies were included, if dealing with<ul style="list-style-type: none">– dengue– intravenous rehydration– different intravenous fluids/regimens• All study types with clinical cases were included	<ul style="list-style-type: none">• Studies not dealing with severe dengue (or related terms for severity) were excluded• Only studies with<ul style="list-style-type: none">– very poor methodology, or– grossly incorrectly conducted studies were excluded from review• Studies reported in languages other than English

All included papers for the systematic review were assessed and grouped according to the ‘hierarchy of study designs’.¹³

Data were extracted to a pilot checklist; a data matrix containing different information on each study was documented in a table; data synthesis was carried out by comparing and grouping the studies; data extraction (Annex 1) comprised studies which conformed to the inclusion and exclusion criteria; and data were separated into different sections which included study design, types of intravenous fluids used, dosages, patient characteristics, comorbid conditions, and other medicines used and predefined outcomes.

All steps of the systematic review were independently followed by two reviewers. Discrepancies were solved through discussion and with the help of a third reviewer.

Results

A total of 41 potentially eligible publications were identified, excluding duplicates. Applying the inclusion and exclusion criteria resulted in a total of nine articles (initially 11 articles; however, two articles were excluded because they were not specifically on dengue shock but rather on shock generally (Figure 1)).^{14,15}

The remaining nine articles were: (i) five single studies; and (ii) two literature reviews and two systematic literature reviews (Table 1). For the single studies, three were randomized controlled trials (RCTs), one before-and-after study, and one comparative prospective study. Furthermore, two systematic reviews and two literature reviews were found, mostly relying for the research question of this systematic review on the five studies identified as well in this systematic literature review. Most studies were in Asia, one review included a dataset from Africa, and one study had been done in Latin America. All the included clinical studies presented data related to the paediatric population.

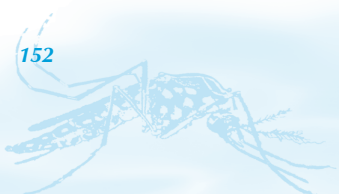
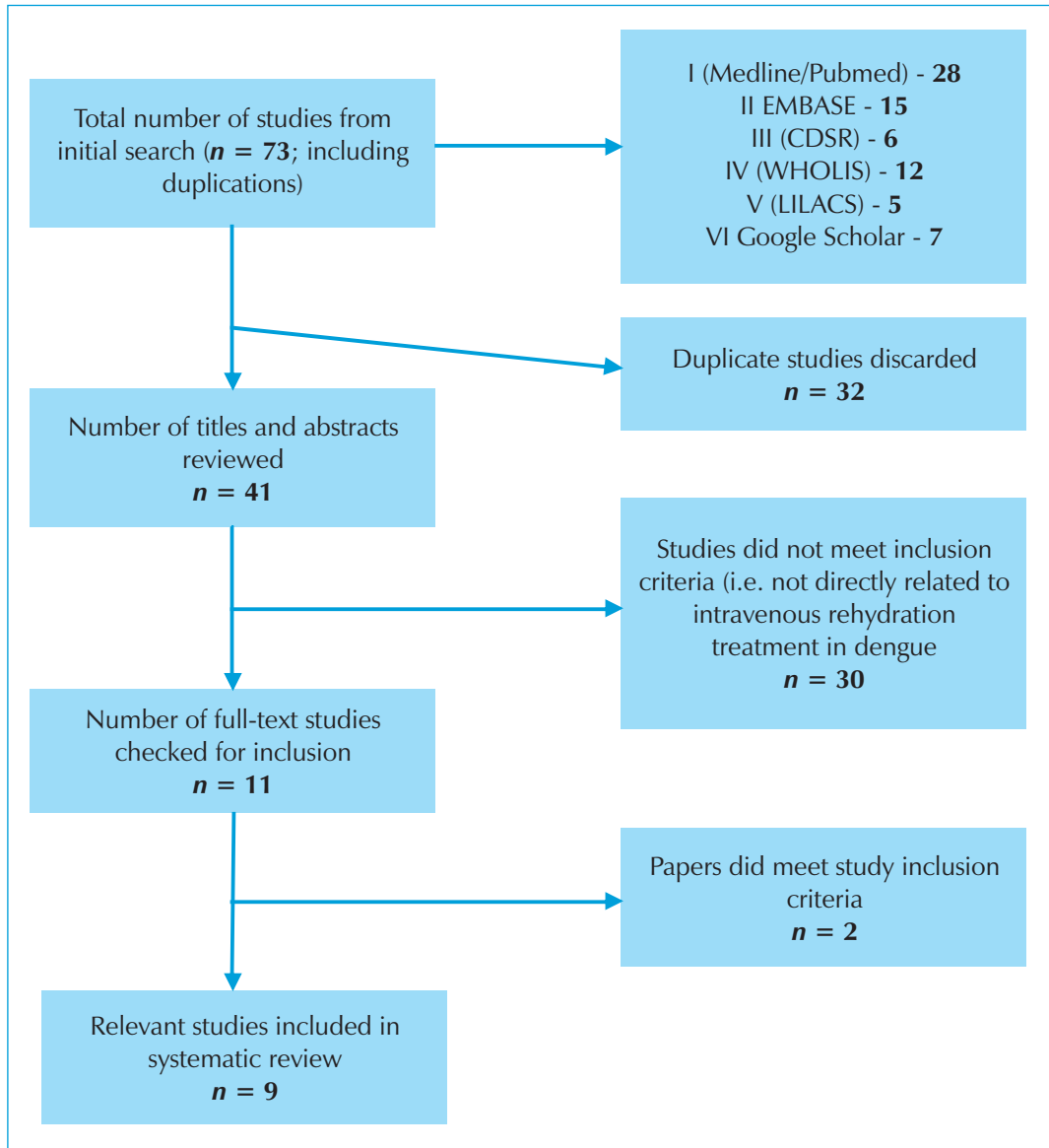
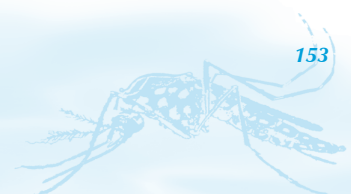


Figure 1: Flow diagram of literature search identifying relevant studies for systematic review



Because of the different study designs included in this systematic review, an overall quality score is difficult to achieve. However, when applying the Jadad Score,¹¹ the included RCTs scored high. The included reviews could not be assessed for quality; however, the systematic reviews were assessed by AMSTAR¹² and found to be of high quality.



Single studies

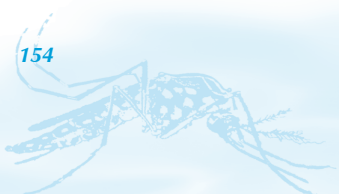
- (1) Nhan et al. (2001)¹⁶ conducted a randomized, double-blinded comparative study of children in the age group of 1-15 years with four intravenous fluid regimens which included dextran, gelatin, lactated Ringer's solution and normal saline. These were given in the first hour of hospital admission for 230 Vietnamese children with dengue shock syndrome (DSS). This study did not show any clear advantage for any of the four fluids used, but demonstrated that the longest recovery time occurred with the lactated Ringer's group. The study graded the cases according to severity into mild dengue haemorrhagic fever (DHF) and Grade III and Grade IV DHF and DSS. The results in the differences between the fluid treatment groups showed a small but significant difference ($p=0.03$) between the four fluid-recipient groups in the median pulse pressure recovery times. Overall, they proposed that there was no statistically significant difference between the use of crystalloids and colloids in the management of severe dengue infection. However, in this study, a small subset of severely affected children fared better with colloids.

The Jadad checklist score is 5, indicating a high quality. However, the study was not powered to detect an effect.

- (2) Wills et al. (2005)¹⁷ conducted a double-blinded, randomized controlled trial by comparing three fluids — Ringer's lactate, dextran and starch — as a resuscitation treatment for 383 children of age group between 2-15 years, admitted for the treatment of DSS. In the study, there was no demonstrable benefit of colloids over crystalloids (Ringer's lactate). Comparing Ringer's lactate and any of the colloid solution, the main result for children with moderate shock showed that Ringer's has a relative risk ratio (at the 95% confidence interval) of 1.08 (0.78 to 1.74; $p=0.65$) and 1.13 (0.74 to 1.74; $p=0.59$). Among the children with severe shock who received dextran as compared to starch, the relative risk (95% confidence interval) was 0.88 (0.66 to 1.17; $p=0.38$). Only one patient died, resulting in a mortality rate of less than 0.2% for the study. The authors concluded that all three treatment regimens were equally effective in the treatment of DSS. While Ringer's lactate initiated a longer recovery time, it was definitely safer than the two colloid solutions.

This study, too, scored 5 on the Jadad questionnaire. The study did not address the issue of the use of crystalloids for patients with profound or recurrent shock for ethical reasons.

- (3) Dung et al. (1999)¹⁸ conducted a double-blinded, randomized controlled trial study in 50 children with DSS between the age group 5-15 years in Viet Nam using four intravenous fluid regimens: dextran 70, 3% gelatin, normal saline and Ringer's lactate. The study mentioned a faster recovery rate for colloids (dextran 70) as compared to crystalloids (Ringer's lactate and normal saline). This study concluded that while fluid resuscitation was the mainstay of treatment in DSS, dextran 70 and 3% gelatin



restored blood pressure and other parameters more rapidly than Ringer's lactate or normal saline.

According to the Jadad checklist, this study too scored 5. However, with four treatment arms in the study and a sample size of 50, the study was not powered to detect an effect, but to determine if such studies are feasible in the study environment.

- (4) Rocha et al. (2009)¹⁹ carried out a before-and-after study in Nicaragua of two periods before 2003 and after 2005. 182 hospitalized, laboratory-confirmed cases of dengue (children 0–14 years of age) and another group of 46 children were recruited in 2005. In the study, changes in hospital morbidity and mortality indicators are described after changes were made in the management of dengue in Nicaragua. The results indicated significant reductions in patients receiving IV fluids ($p < 0.0001$), days of IV fluid administration ($p = 0.0001$) and the duration of hospitalization ($p < 0.0001$), after using aggressive oral rehydration during the acute febrile phase. Also, there was a reduction in the number of admissions to the intensive care unit to zero in 2005 from eight in 2003 ($p = 0.36$). The authors concluded that the prompt use of IV fluids during the critical phase, continuous monitoring of clinical and laboratory indicators and the aggressive use of oral rehydration wherever possible had a significant positive impact on the number of admissions to intensive care and the duration of hospitalization. The importance of training to achieve optimum outcomes has been highlighted.
- (5) Hung et al. (2006)²⁰ studied the amount of volume replacement required in the treatment of 208 infants with DHF and DSS, aged less than 12 months. The authors accepted that effective fluid replacement resulted in a favourable outcome in most patients with severe dengue disease. Their prospective study concluded that an average of 110.4 mL/kg was required as intravenous fluid replacement over a mean period of 25.8 hours. They used Ringer's lactate as primary fluid replacement and used dextran only when patients failed to improve with Ringer's lactate.

Since the study aims were different from a comparative efficacy trial, this study was neither double-blinded nor randomized. However, all patients were followed-up completely until the end of treatment and details of treatment were given and the therapeutic response obtained were recorded and presented accurately and thoroughly.

Reviews

- (6) The literature review by Molyneux and Maitland (2005)²¹ includes different studies to review intravenous rehydration in different diseases, but also in dengue. For dengue, the article relies on Wills et al. (2005)¹⁷ which is one of the included studies in this systematic review. The review described in detail the different types of intravenous fluids such as hypotonic solutions, normal saline and Ringer's lactate which could



be used in the emergency care of children, especially those suffering from DSS, mentioning that the use of hypotonic solutions could be more appropriate when the primary problem was depletion of intracellular volume due to dehydration. While these may not cause a sodium overload, they are not usually advocated for the correction of shock until the circulatory volume has been restored to normal. This can be done more easily with solutions like normal saline and Ringer's lactate, which are cheap and widely available. However, large volumes of normal saline may cause acidosis and electrolyte imbalance. Being a physiological solution, Ringer's lactate is stated to be more suitable in the treatment of DSS since large volumes of this solution do not cause metabolic acidosis.

The quality of this article could not be assessed, since it is a review without specifying the methods chosen.

- (7) Singhi et al. (2007)²² conducted a literature review describing epidemiology, clinical features and treatment of dengue fever and DSS, including intravenous rehydration. They carried out a literature search on Pubmed and WHO's and Pan American Health Organization's (PAHO's) electronic libraries using the terms 'dengue' and 'dengue shock syndrome'. In relation to the study question, they concluded that while no single fluid was superior to others in the form of intravenous infusion, Ringer's lactate solution proved to be a safer and more practical treatment as compared to other intravenous fluids.

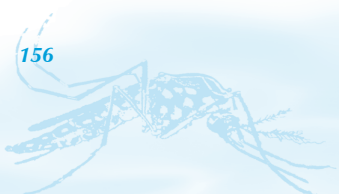
No quality criteria could be established, since the search was very broad and the methodology is only described very briefly.

Systematic reviews

- (8) A systematic review by Smart and Safitri²³ reviews different treatments in the management of shock in severe dengue in children, also including intravenous rehydration.²³ This review screened nine articles which were all RCTs. It excluded two studies conducted on monkeys and one which was unblinded. The remaining seven articles included for review were declared to be of types Ib and IIb in the hierarchy of evidence.

Three studies compared the use of crystalloids to colloids in the treatment of DSS. All three studies have also been found by this systematic review and are described in detail as single studies.¹⁶⁻¹⁸ The review also included four further randomized controlled trials (RCTs) with other treatments for DSS in children, not intravenous rehydration.

The systematic review concluded that intravenous fluids formed the mainstay of treatment in DSS, that there was no difference in the clinical efficacy of crystalloids and colloids, and that other concurrent treatments like steroids were not very



beneficial. However, with the appropriate use of intravenous fluids, the mortality rates in DSS were even less than 0.2%.²³

The quality of the study is high as a systematic review only considering RCTs and fulfilling the AMSTAR criteria.¹²

- (9) In a further systematic review (Alejandria, 2009),²⁴ 13 RCTs were assessed in relation to the effectiveness of treating children with DHF and DSS. The same three RCTs as in the systematic review above have been found in relation to intravenous rehydration and the treatment of DHF and DSS using colloids, crystalloids and intravenous fluids.

Alejandria concluded that crystalloids and colloids were equally effective treating children with DSS. The conclusion in Alejandria's study²⁴ is that – despite its better response in decreasing pulse rates and hematocrit – no significant advantage of colloid was found over crystalloid for all outcomes: (i) need for rescue colloids; (ii) reducing recurrence of shock; (iii) need for diuretics; (iv) total volume of fluids given; and (v) mortality.

The quality of the systematic review is high and the article has been assessed in relation to its quality using Grading of Recommendations, Assessment, Development and Evaluations (GRADE).²⁵

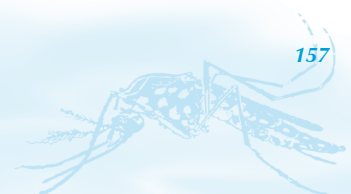
In summary, the main findings from a full text review of the above included studies are:

- There is good evidence that intravenous isotonic fluids form the mainstay of treatment in severe dengue with plasma leakage in children.
- This evidence is based on three RCTs assessing children.
- Almost all the studies have been conducted in Asia, thus limiting generalizability.
- Throughout all studies, there is a general consensus on the therapeutic utility and efficacy of intravenous rehydration in severe dengue disease; however, there is no strong evidence for the superiority of one fluid over another, though trends seem to favour initiation of therapy with isotonic crystalloids, and, if necessary, by colloids.

Discussion

Discussion of the findings

As for the results, the literature reviewed suggests that intravenous rehydration therapy is a very efficacious treatment for severe dengue due to plasma leakage and can definitely have an impact on preventing or reducing deaths due to severe dengue in children. Thus, while there is some difference of opinion about the individual use of different intravenous fluids, the overall consensus is in favour of instituting intravenous fluid therapy at the earliest signs of shock.¹⁷



This finding coincides with the fact that countries applying intravenous rehydration have a lower mortality. A good example is a study from Martinique,²⁶ the authors concluding that seriously-ill cases of dengue respond well to intravenous rehydration. Furthermore, there is evidence emerging that service reorganization in cases of dengue outbreaks may be helpful, as in the case of “dengue tents”-like fast-track facilities during the 2008 outbreak in Sao Paulo.²⁷

However, a study done by Srikiatkhachorn et al.⁷ reported that even though plasma leakage is the major cause of mortality and morbidity in patients with DHF, there is a lack of a detailed assessment of the natural cause. This study underlines the need for pathophysiological studies to establish the processes underlying plasma leakage.

Factors that can contribute to a good clinical outcome of dengue with plasma leakage can be: (i) when to use fluids; (ii) what fluids to use; (iii) how much to use; (iv) how fast the fluids should be given; and (v) when to stop intravenous fluids. This review addresses especially question (ii).

Throughout all reviewed studies, there is a general consensus about the therapeutic utility and efficacy of intravenous rehydration in severe dengue disease; however, there is no strong evidence for the superiority of one fluid over another though trends seem to favour initiation of therapy with isotonic crystalloids, followed later, if necessary, by colloids, this also being specified by guidelines of intravenous fluid management of dengue patients with severe shock. However, one study investigating dextran fractional clearance in acute dengue suggested that dextran might have a direct therapeutic effect on capillary permeability in dengue.²⁸ These findings clearly need to be studied in clinical trials and in different settings. In addition, the adverse effects of different intravenous fluids need to be studied in more detail, especially for adults.

It is possible that appropriate training how to use fluids is the key for good outcomes — with studies conducted in hospitals seeing dengue cases on a regular basis leading to better outcomes. Once the medical staff is trained on how to use intravenous fluids, the type of fluids may not be such an important determinant of the outcome.

A complete awareness of the medical personnel about the impact of prompt intravenous rehydration and training for the reduction of mortality in severe dengue infection is necessary. This requires alertness and discipline on the part of public health authorities, along with the capacity and willingness to make use of adequate funds for this purpose. Algorithms need to be locally adapted; public health authorities need to respond with countrywide training of clinicians at primary, secondary and tertiary levels although training material is a further necessity.

Furthermore, studies conducted in this field have only assessed the paediatric population mostly within the age range between 1 and 15 years. Hence it is difficult to generalize the results and to use similar dosages in adults that were used in children. No research has been



found on obese patients and people suffering from co-morbid conditions like asthma, heart disease, renal disease and diabetes, or other high-risk populations where fluid regimen may have to follow different algorithms. Clearly, research is needed, but also field-testing of algorithms is required. While it is established that the institution of intravenous rehydration can drastically reduce the mortality rate in severe dengue, a much larger number of rigorous and scientific studies need to be conducted and published in order to provide convincing scientific evidence for data which can be accepted easily by health care delivery systems of affected countries. This will have strong, positive implications for public health at a time when nearly two fifths of the world's population is at risk from dengue infection.

The sample size of the studies is a further issue. Of the three RCTs included in this review, the study by Dung et al.¹⁸ had a sample size of only 50 cases for a four-arm study and therefore appears to be highly under-powered. The authors of the remaining two RCTs accept that better evidence can be obtained from larger trials with similar aims and objectives. Overall, much larger clinical trials are needed in the future in order to produce statistically significant and convincing results.

Further technical recommendations for studies should be developed, including recommendations for laboratory diagnosis of dengue and clinical case definitions.

In summary, all the nine main studies included in the systematic review are consistent and coherent with the overall therapeutic recommendations in severe dengue infections to institute intravenous rehydration therapy, initially with isotonic fluids like Ringer's lactate and normal saline, to be complemented if necessary with colloidal solutions like dextran or starch. The principles of treatment and intravenous fluid regimens concur on the whole with treatment recommendations from WHO: isotonic intravenous fluids like Ringer's lactate and normal saline can be given as a preferred choice for initial intravenous therapy in the resuscitation of patients suffering from severe dengue. Colloidal solutions like dextran 70 may be added if the response is not satisfactory. The easy availability and much lesser costs of isotonic solutions like Ringer's lactate and normal saline make these fluids more acceptable for initial use in intravenous rehydration therapy. Overall, intravenous isotonic fluid rehydration can significantly reduce the case-fatality rate in severe dengue disease of children, but evidence for adults is not available.

Limitations of the study

The key limitation of this study is the paucity of studies, especially for adults; this is a limitation but it also is a result of the systematic review. Most studies have been conducted in Asia, with one review using data from Africa. While countries in Latin America and the Caribbean are major dengue areas in the world, only one study could be identified from these regions. Again, this is a serious limitation, but as well a result of the review.



There are methodological limitations associated with systematic reviews, only being as good and as far as the search strategy goes. But this has been limited by searching reference lists of included studies and grey literature.

Dengue occurs predominantly in the non-English speaking world, and the search was limited to English. However, searches have been via abstracts that are often translated into English; no article has been found with an English abstract and was later excluded because the full article was written in other languages.

Furthermore, the quality of the included studies has been assessed and found to be very good (especially for the RCTs). The reviews could not be analysed for quality; however, the systematic reviews were of good quality.

To further assess the quality of this systematic review in the form of a self-assessment, the whole systematic review was assessed for quality with AMSTAR and it scored well, considering the few available studies.

Conclusion

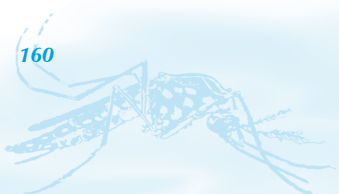
From the results and discussion detailed in previous chapters, the following overall conclusions can be made:

- Intravenous rehydration forms the mainstay of treatment in severe dengue infection due to plasma leakage.
- Isotonic intravenous fluids should be the first choice of treatment and colloid fluids should be reserved for cases not responding to isotonic fluid rehydration.
- Research is urgently needed for intravenous rehydration in adults, dengue in pregnancy, co-morbidities and other risk populations.
- Research is urgently needed in dengue-affected areas with few available studies, notably in Latin America and the Caribbean.

However, it has to be considered that training on how to use intravenous rehydration may be the key to good outcomes, far more important than the type of fluids used.

Statement of contributions

HU is the principal author of the article, IS second reviewer. OH supervised the research and advised on all stages of the article, including solving differences in the data search between the first and second reviewer. All other authors contributed to the research and the final version of the article.

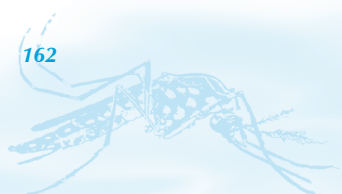


Annex 1: Studies included in search for evidence for intravenous rehydration for dengue

	Reference/ location	Study design	Study objectives/ age group of study population	Intervention	Main results from relevant outcomes	Conclusion of study authors
Single studies						
1	Nhan et al., 2001 Dong Nai, Viet Nam	RCT	To compare the efficacy of four different fluid regimes in the initial management of DSS in children (1-15 years)	Dextran, gelatin, lactated Ringer's and normal saline	No clear difference for any of the four fluids, other than lactated Ringer's solution performed the least well	Choice for resuscitation of the majority of patients with DSS is 0.9% saline (a crystalloid)
2	Wills et al., 2005 Viet Nam	RCT	To compare the effectiveness of three different fluid solutions used for resuscitation in DSS (2-15 years)	Ringer's lactate, 6% dextran (a colloid) and 6% hydroxyethyl starch (a colloid)	The primary outcome measure is the use of colloid	For initial resuscitation in children with moderately severe DSS, Ringer's lactate is preferred. All three fluids are effective also in the treatment of DSS
3	Dung et al., 1999 Ho Chi Minh City, Viet Nam	RCT	The effect of fluid replacement in dengue shock syndrome: randomized, double-blind comparing four intravenous – fluid regimens. Age 5-15 years	Saline & chloride, Ringers lactate solution, calcium and chloride	Fluid resuscitation is the mainstay of treatment in DSS. Colloids restored cardiac index and blood pressure and normalized haematocrit more rapidly than crystalloids	Dextran 70 provided the most rapid normalization for acute resuscitation in DSS



	Reference/ location	Study design	Study objectives/ age group of study population	Intervention	Main results from relevant outcomes	Conclusion of study authors
4	Rocha et al., 2009 Nicaragua	Before and after study	The impact on hospital indicators after changes were made in the management of dengue in Nicaragua before 2003 and after 2005 within 0–14 year old children	Oral rehydration and/or intravenous fluids	A positive reduction in the duration of hospitalization, days of IV fluids administration and number of patients receiving IV fluids ($p \leq 0.0001$). Also a reduction in the number of admissions to the intensive care unit to zero in 2005 from eight in 2003 ($p = 0.36$)	The immediate use of IV fluids during critical phase and the use of oral rehydration had a significant positive impact on hospitalization in intensive care
5	Hung et al., 2006 Ho Chi Minh City, Viet Nam	Prospective study	The impact of volume replacement in infants with dengue haemorrhagic fever/ dengue shock syndrome	Intravenous fluids	Effective fluid replacement resulted in a favourable outcome in patients with severe dengue disease. Patients with DSS required dextran and blood transfusions significantly more than non- shock infants. An average of 110.4mL/kg was required as intravenous fluid replacement over a mean period of 25.8 hours	Intravenous fluids must be administered to infants with DHF/ DSS.



	Reference/ location	Study design	Study objectives/ age group of study population	Intervention	Main results from relevant outcomes	Conclusion of study authors
Reviews and systematic reviews						
6	Molyneux and Maitland, 2005	Literature review	To review the different types of intravenous fluids used in emergency care of children in cases such as DSS		In the presence of dehydration, hypotonic solution is more appropriate to use. For the circulatory volume to be restored to normal, solutions like normal saline and Ringer's lactate should be administered also	Ringer's lactate is the most suitable in the treatment of DSS
7	Singhi et al., 2007	Literature review	To review the different types of DSS treatment		No single fluid was superior to others	Ringer's lactate is the best and safest intravenous fluid for this use
8	Smart and Safitri, 2009	Systematic literature review	Evidence behind the WHO Guidelines: Hospital Care for Children: What treatment is effective for the management of shock in severe dengue?		No difference in the clinical efficacy of crystalloids and colloids	Aggressive fluid resuscitation is the only known effective treatment in DSS
9	Alejandro, 2009	Systematic literature review	Effectiveness of treatment of DHF and DSS in children		Ringer's lactate or isotonic crystalloids are effective as well as safe in DSS treatment and in reducing shock occurrence	Crystalloids and colloids are equally effective in DSS treatment

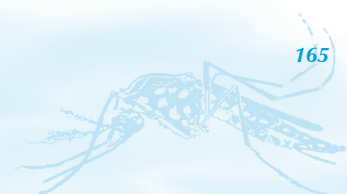


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Relationships between *Aedes* indices and dengue outbreaks in Selangor, Malaysia

Shamsul Azhar Shah,[#] Jamiatul Aida Mohd Sani, Mohd Rohaizat Hassan, Nazarudin Safian, Azimatun Noor Aizuddin and Rozita Hod

Department of Community Health, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

Abstract

Dengue is the most important vector-borne disease globally. Malaysia has been witnessing its increasing incidence over the years. Despite continuous surveillance using methods such as *Aedes* indices (*Aedes* Index and Breteau Index), dengue outbreaks continue to persist. This study aimed to identify the relationship between *Aedes* indices and dengue outbreak and its effectiveness.

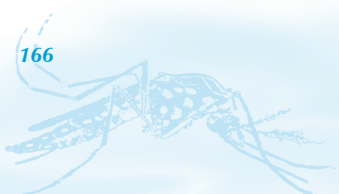
The study found an increasing trend of dengue incidence over the years in the Sepang district. Most of the cases occurred among Malay males, aged between 21 and 30 years, and with an upward trend among non-citizens. Almost all dengue notifications came from a hospital, with 68% of cases laboratory confirmed. The majority of dengue outbreaks occurred in localities with low *Aedes* indices. Although significant relationships existed between *Aedes* indices and dengue outbreaks, these were inconsistent throughout the four-year study period (2004–2007). *Aedes* indices were shown not to be related with dengue outbreak areas. More accurate and sensitive indices to monitor and act as an early warning system should be evolved for dengue surveillance.

Keywords: Dengue outbreak; *Aedes* index; Breteau index; Selangor; Malaysia.

Introduction

Despite being a century-old infectious disease in Malaysia, dengue fever continues to be an important public health problem. In 2004, there were 33 895 cases of dengue fever reported with 102 fatalities and an incidence rate of 132.5 cases for 100 000 population.¹ Selangor in that year recorded the highest number of dengue cases in Malaysia, with 9189 reported cases, 2806 confirmed cases and 37 fatalities. In 2008 however, the problem worsened with 49 335 dengue cases reported with 113 fatalities; 63% of the cases occurred in the Klang Valley, which forms part of Selangor and the Federal Territory of Kuala Lumpur. In Malaysia, the dengue vectors commonly found are *Aedes aegypti* and *Ae. albopictus*.

[#]E-mail: drsham@ppukm.ukm.edu.my



Among the strategies used in the Dengue Fever Control Programme are disease surveillance for early detection and control of dengue fever and dengue haemorrhagic fever. These include detection of cases, notification of cases, case and outbreak investigation, virological surveillance, vector surveillance and vector control. Three indices were used to monitor the presence of *Aedes* larvae, namely, *Aedes* Index, Breteau Index and Ovitrap Index.² For dengue control, the Ministry of Health, Malaysia, has identified localities with the level of *Aedes* Index of more or equal to 5% and Breteau Index of more or equal to 20 as high-risk localities for dengue outbreaks.³ These indices are also used as a measure of the effectiveness of dengue control and prevention measures. The Pan American Health Organization (PAHO) has used three levels of risk for dengue transmission, i.e. low risk (*Aedes* Index <0.1%), medium risk (*Aedes* Index 0.1–5%) and high risk (*Aedes* Index >5%).⁴ In Singapore, dengue outbreaks still occurred even at a national *Aedes* Index of less than 1%.⁵ At *Aedes* Index level of 0.05% to 0.91%, dengue outbreak involving 138 cases still occurred in Havana, Cuba, from September to October 2000.⁶ In Brazil however, a study found that dengue outbreaks never occurred when *Aedes* index was less than 1%.⁷ Nevertheless, no study has been conducted to determine the critical level of those indices with accuracy.

Although various strategies are utilized to control dengue, its cases continue to increase with outbreaks occurring every year. The use of larval survey indices to detect 'sensitive' areas to warn against the occurrence of a dengue outbreak has been in place for more than 30 years, but despite that, dengue outbreaks remain a reality. Hence, it is important to study the effectiveness of using the *Aedes* indices as predictors to warn against the occurrence of dengue outbreaks so that early control measures can be taken in order to reduce morbidity and mortality caused by the disease.

The general objective of this study was to identify the relationship between *Aedes* Index and Breteau Index with the occurrence of dengue outbreaks.

Materials and methods

A cross-sectional study was conducted by collecting secondary data on notified dengue cases and *Aedes* and Breteau indices from Sepang District Health Office. Sepang is one of the nine districts in the State of Selangor, Malaysia. All clinically diagnosed dengue fever and dengue haemorrhagic fever cases notified to Sepang District Health Office from 2004 to 2007 were taken as a study sample. This study was approved by the Ethical Committee of the Ministry of Health and Universiti Kebangsaan Malaysia.

The dependent variables were dengue cases and the presence or absence of dengue outbreaks. Dengue cases were then categorized into 'outbreak' or 'non-outbreak'. Dengue outbreaks were defined to occur when two or more cases were notified in the same area within 400-metre radius from the case, whereby the date of onset between one case and the



following case was 14 days or less.³ The independent variables included socio-demographic profile of the dengue cases (age, gender, ethnicity, notification source(s), status of dengue based on serology and *Aedes* larval indices (*Aedes* Index and Breteau Index) for each of the dengue outbreak area. *Aedes* Index was defined as the percentage of premises positive for *Aedes* larval breeding in a locality, while Breteau Index was defined as the number of containers positive for *Aedes* larval breeding in 100 premises inspected in a locality.

Data analysis was divided into two parts. The first part of the analysis was a descriptive analysis of dengue cases. The second part of the analysis was to determine the relationship between dengue outbreaks with *Aedes* indices using Pearson's Chi square. Receiver operating curve (ROC) analysis was used to evaluate the ability of the indices to discriminate between the outbreak and non-outbreak area. All analyses were performed using software SPSS version 16.0.

Results

A total of 1378 dengue cases were notified to the Sepang District Health Office from 2004 to 2007. A majority of cases (68.1%) were serologically confirmed and only 9% of the cases were classified as dengue haemorrhagic fever (DHF). There was an increasing trend of dengue cases throughout the four-year period (Table 1). The incidence of dengue cases increased from 24.32 per 10 000 population in 2004 to 30.45 per 10 000 population in 2007.

Table 1: Total number and incidence of dengue from 2004 to 2007 in Sepang, Malaysia

Year	2004	2005	2006	2007	Total
Dengue cases	281	329	338	430	1378
Incidence (per 10 000 population)					
DF	24.32	25.11	25.76	30.45	
DHF	1.47	3.57	2.24	3.38	
Classification					
DF	265 (94.3%)	288 (87.5%)	311 (92.0%)	387 (90.0%)	1251 (90.8%)
DHF	16 (5.7%)	41 (12.5%)	27 (8.0%)	43 (10.0%)	127 (9.2%)
Status					
Confirmed	182 (64.8%)	213 (64.7%)	238 (70.4%)	306 (71.2%)	939 (68.1%)
Suspected	99 (35.2%)	116 (35.3%)	100 (29.6%)	124 (28.8%)	439 (31.9%)

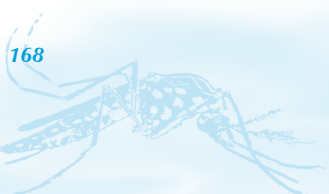


Table 2 shows that more than half of the dengue cases were male (62%), and among Malays (62%). The number of cases among non-citizens are increasing over the years. A majority of cases occurred among the 21–30-year age group (34%), followed by 31–40-year age group (20%), 11–20-year age group (19%), 0-10-year age group (12%), 41–50-year age group (9%) and 51-year-and-above age group (6%). Most of the cases (74%) were from urban areas. There was an increasing trend of dengue cases reported from the suburbs during the four-year study period. Most of the cases were notified by hospitals, especially the government hospital, while only 0.7% of the cases were notified by government health clinics.

Table 2: Sociodemographic distribution of dengue cases, 2004–2007, Sepang, Malaysia

Year	2004	2005	2006	2007	Total
Gender					
Male	168(59.8%)	220(66.9%)	194(57.4%)	276(64.2%)	858(62.3%)
Female	113(40.2%)	109(33.1%)	144(42.6%)	154(35.8%)	765(59.9%)
Ethnicity					
Malay	182(64.8%)	214(65.1%)	183(54.1%)	272(63.3%)	851(61.7%)
Chinese	54(19.2%)	40(12.2%)	53(15.7%)	24(5.6%)	171(12.4%)
Indian	31(11.0%)	39(11.8%)	64(18.9%)	53(12.3%)	187(13.6%)
Bumiputra	3(1.1%)	6(1.8%)	3(0.9%)	3(0.7%)	15(0.1%)
Others (Non-citizen)	11(3.9%)	30(9.1%)	35(10.4%)	78(18.1%)	154(11.2%)
Area category					
Urban	228(81.1%)	262(79.6%)	263(77.8%)	265(61.6%)	1018(73.9%)
Rural	52(18.5%)	66(20.1%)	51(15.1%)	47(10.9%)	216(15.7%)
Suburban	1(0.35%)	1(0.3%)	24(7.1%)	118(27.4%)	144(10.4%)

Data on *Aedes* Index (AI) and Breteau Index (BI) were available for every dengue outbreak reported in Sepang. The analysis was performed in two parts. In the first part, AI and BI was classified respectively into $AI \geq 5\%$ or less and $BI \geq 20$ or less in reference to localities sensitive for dengue outbreaks as defined by Ministry of Health, Malaysia.³ In the second part, AI and BI was categorized, respectively, into $AI \geq 1\%$ or less and $BI \geq 5$ or less in reference to one of the objectives of the National Dengue Control Programme (NDCP), which was to reduce the *Aedes* breeding to $AI < 1\%$ and $BI < 5$.⁸

The relationship between the number of dengue outbreaks and *Aedes* indices was tabulated at a different level of cut-off point (Table 3). Only 22.3% of dengue outbreaks in Sepang occurred in localities with $AI \geq 5\%$, while only 8.2% of dengue outbreaks occurred in localities with $BI \geq 20$. However, there were significant relationships between $AI \geq 5\%$ and the occurrence of dengue outbreaks in Sepang in 2007 ($p < 0.001$) and overall ($p < 0.001$) and between $BI \geq 20$ and dengue outbreaks in 2007 ($p < 0.001$) and overall ($p < 0.001$).

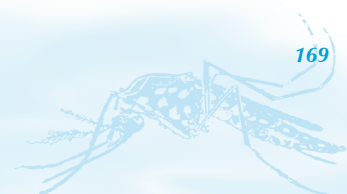


Table 3: Relationship between dengue outbreak and *Aedes* indices at different levels of cut-off point, Sepang, Malaysia

Aedes indices	Year	Dengue outbreak N (%)	Chi square value	p value
Aedes Index ≥5%	2004	22 (16.1)	0.260	0.610
	2005	43 (23.8)	3.428	0.064
	2006	37 (19.8)	0.363	0.547
	2007	67 (26.4)	32.307	<0.001**
	Overall	169 (22.3)	21.649	<0.001**
Breteau Index ≥20	2004	1 (0.7)	0.717	0.371#
	2005	7 (3.9)	0.443	0.506
	2006	5 (2.7)	0.939	0.333
	2007	49 (19.3)	38.319	<0.001**
	Overall	62 (8.2)	16.023	<0.001**
Aedes Index ≥1%	2004	63 (46.0)	1.448	0.229
	2005	78 (43.1)	2.158	0.142
	2006	85 (45.5)	4.739	0.029*
	2007	104 (40.9)	22.312	<0.001**
	Overall	330 (43.5)	21.900	<0.001**
Breteau Index ≥5	2004	25 (18.2)	0.012	0.914
	2005	44 (24.3)	1.770	0.183
	2006	37 (19.8)	0.198	0.657
	2007	67 (26.4)	32.307	<0.001**
	Overall	173 (22.8)	15.040	<0.001**

**Significant at 1% level

*Significant at 5% level

Fisher exact test

When the NDCP’s cut-off points were used, 43.5% of dengue outbreaks occurred in localities with AI ≥1%, while only 22.8% of dengue outbreaks occurred in localities with BI ≥5. However, significant relationships were found between AI ≥1% and occurrence of dengue outbreaks in 2006 ($p=0.029$), in 2007 ($p<0.001$) and overall ($p<0.001$). Significant relationships were also found between BI ≥5 and the occurrence of dengue outbreaks in 2007 ($p<0.001$) and overall ($p<0.001$).

The ROC curve for Aedes Index was only 0.572 (95%CI: 0.542-0.602) (Figure 1). The optimal sensitivity was 37% with specificity of 77% at AI 2.5%. At AI 1%, the sensitivity was only 44% with specificity of 69%, the positive predictive value was at 63% while the negative predictive value was 50%. At AI 5%, sensitivity reduced to 23%, with specificity of 87%, positive predictive value of 68% while the negative predictive value was 48%.



Similarly, the Breteau Index ROC curve was only 0.571 (95%CI: 0.541-0.601) (Figure 2). The optimal sensitivity was 42% with specificity of 70% at BI value of 2. At BI 5, the sensitivity was only 23% with specificity of 85%, positive predictive value of 66% while the negative predictive value was 47%. At BI 20, the sensitivity reduced to only 8% but with good specificity at 97%, positive predictive value of 77% while the negative predictive value was 46%.

Figure 1: Receiver operating characteristics (ROC) curve for *Aedes* Index in Sepang, Malaysia

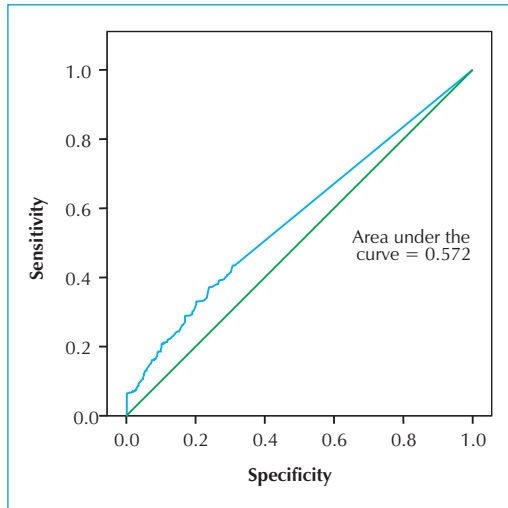
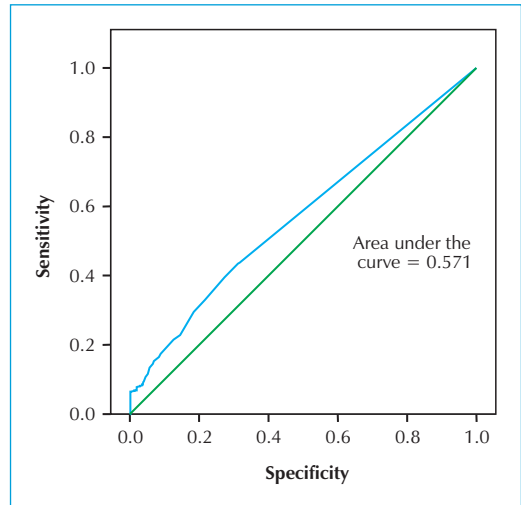


Figure 2: Receiver operating characteristics (ROC) curve for Breteau Index in Sepang, Malaysia



Discussion

Our study found that most dengue outbreaks in Sepang occurred in localities with low *Aedes* indices and were inconsistent throughout the four-year period. *Aedes* indices were shown not to be related with dengue outbreak areas. One of the limitations of this study is that all dengue cases were included regardless of their confirmation status. The location where the patients contracted dengue was sometimes doubtful, as it was not necessarily from their housing area. Hence, selection bias might have occurred during the analysis. However, the strength of this study is the large number of cases and most of them were investigated thoroughly. In this study, the majority of dengue cases throughout the four-year period were males, similar to the situation in Sri Lanka where 60% of the dengue cases nationally were males.⁹ Throughout the four years, most of the cases occurred among the Malay ethnic group. However, the incidence of dengue cases among non-citizens showed an increasing trend. More than half of the cases occurred among people below 30 years and the ones between 21-30 years were the most affected. 11.8% of dengue patients were less than 10 years old.



The elderly were the least affected, comprising only 1.8% of total cases in Sepang. This trend was also seen in Singapore where most dengue cases occurred among those aged 16 to 25 years.¹⁰ The success in controlling mosquitoes might have contributed to the low immunity of young adults for dengue infection; hence they became the group most infected.¹⁰

A majority of dengue cases in Sepang occurred in urban areas which were densely populated, in line with the State of Selangor where 85% of the cases in 2005 were from urban areas.¹¹ Singapore reported the incidence of dengue was higher in densely populated residential areas compared to industrial areas.¹⁰ This study also found the increasing trend of dengue cases in the suburbs where new developments and residential areas were on the rise. Most notifications of dengue cases in this study were done by hospitals, similar to Selangor, where 97% of cases in 2005 were notified by hospitals, 70% being from government hospitals.¹¹ The Ministry of Health also reported a similar scenario throughout Malaysia.¹² This was probably because most doctors required a confirmation of dengue infection before the cases were notified to the Ministry of Health.

WHO has recommended the use of AI and BI to determine the high risk area of dengue transmission for the purpose of its control.¹³ If control measures are not taken in these areas, it is most likely that dengue outbreaks will occur. Our study showed the inconsistent relationship between the *Aedes* indices and dengue outbreak occurrence. In contrast, a study in Brazil found that dengue outbreaks never occur when *Aedes* Index was less than 1%.⁷ Our results could affect our national target which uses similar level with Thailand, using *Aedes* Index $\leq 1\%$ and Breteau Index ≤ 5 as a low dengue transmission risk.^{8,14} The transmission of dengue infection depends on the infected *Aedes* mosquito density and population density. Another possible explanation is that in the area with high *Aedes* breeding, not all cases would be reported because most dengue infections are asymptomatic.¹⁵ Furthermore, the laboratory confirmation of dengue is rather intricate due to the differences in the immunological response time to dengue infection.¹⁶ The presence of these undiagnosed patients in the area with high *Aedes* breeding provides a suitable environment for dengue outbreaks.¹¹

The occurrence of a dengue outbreak with low *Aedes* indices could be rationalized in several ways. It may be due to the fact that clusters of *Aedes* larvae were not related to the clusters of adult *Aedes* population. The reason for this was that as larval mortality was high, not all larvae in a container would survive into adults and transmit the dengue infection. Studies have shown that these indices were inadequate to measure the risk of transmission.^{17,18} Secondly, adult mosquitoes could fly to areas of no spatial relationship from their original breeding places. Thirdly, inspections of premises for *Aedes* larvae were usually done by taking only a few houses as sample.¹⁹

The adult mosquito population size is more important compared to the larval index as the number of people infected by dengue has a more direct relationship with adult vector density.¹⁴ This could also explain why most dengue outbreaks occurred in localities with low *Aedes* indices. In contrast, studies of dengue outbreaks in Havana, Cuba, reported that



the maximum Breteau Index recorded every two months in a locality was more effective in predicting the transmission of dengue with area under ROC curve of 0.71. At Breteau Index value of 4, its sensitivity was 78% with specificity of 63%.⁶

Another possible reason why most dengue cases occurred in localities with low *Aedes* indices is the improper technique of larval survey. This incorrect technique of larval survey might be performed by public health staff with different backgrounds and experience, either with or without the supervision of health inspectors or entomologists. There could be a possibility that the *Aedes* Index recorded was less than the actual level.

In conclusion, our study showed that *Aedes* and Breteau indices were inconsistent and not accurate in relation to dengue outbreaks in Sepang. Its usage in dengue surveillance and control is arguable. We need to look for other methods, such as adult or pupae survey, that could be more effective as early warning systems of a possible dengue outbreak.

Acknowledgements

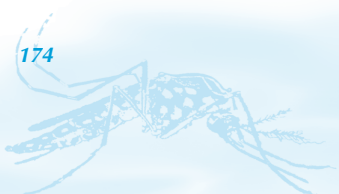
The authors express their sincere gratitude to the health personnel of the District Health Office of Sepang, Selangor. Universiti Kebangsaan Malaysia Research Grant (FF-046-2007) funded this study.

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Water quality and *Aedes* larval mosquito abundance in Caloocan city, Philippines*

Glenn Sia Su,^{a#} Abigail Beronilla^b and Kathleen Beatrice Yao^b

^aBiology Department, College of Arts and Sciences, University of the Philippines, Manila, Philippines

^bBiology Department, College of Science, De La Salle University, Manila, Philippines

Abstract

The aim of our study was to examine the water quality of the breeding habitats of *Aedes* mosquitoes from randomly selected communities of Caloocan city, Philippines, and determine the relationship of water quality parameters in the breeding habitats to the abundance of the *Aedes* larval mosquitoes. Water samples obtained from the breeding habitats were assessed for dissolved oxygen, pH, conductance and salinity. Mosquito larvae surveys were conducted in all breeding habitats. The relationship between the abiotic variables (dissolved oxygen, pH, conductance and salinity) and the abundance of mosquito larvae was investigated. Results showed that barrels had the most abundant *Aedes* mosquito larvae occurrence and the *Anopheles* sp. and *Culex* spp. were incidentally detected in selected breeding habitats of the *Aedes* sp. All water samples obtained from the breeding habitats were within water quality standards. Results of the multiple regression analysis suggest that dissolved oxygen is the best predictor variable associated with the abundance of the *Aedes* mosquito larvae ($r^2=0.144$, $P<0.05$). The dissolved oxygen in the water in breeding habitats plays an important role in the abundance of *Aedes* larval mosquitoes.

Keywords: *Aedes* larvae; Water quality; Abundance; Dissolved oxygen; Caloocan city; Philippines.

Introduction

Aedes mosquitoes are important vectors of viral diseases. They are recognized as the greatest menace as they are responsible for the transmission of dengue worldwide. These mosquitoes continue to proliferate in our environment and affect the quality of life of each individual as they contribute to the morbidities and mortalities reported in both rural and urban areas. Previous studies^{1,2,3} have shown that the spread of mosquito-borne diseases may have been

*Generally, in urban areas in countries of the WHO South-East Asia and Western Pacific regions, *Aedes aegypti* comprises >90% of mosquito population and the rest comprises of *Ae. albopictus*, particularly in peripheral areas. Breeding of *Anopheles* and *Culex* species in *Aedes* type of breeding waters is incidental. – Editor.

#E-mail: glss76@yahoo.com



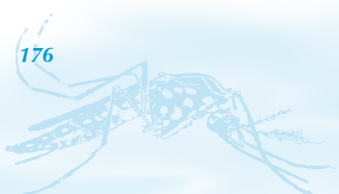
associated with the availability of breeding habitats and the changes in the climate and the environment.¹⁻³ In a study conducted by Takken et al,³ it was found that the abundance and distribution of mosquitoes are primarily dependent on their breeding habitats and the environmental conditions predominating in these habitats. It is likely that water chemistry, geographical location, size of water body, vegetations present and abundance of predators may likewise affect mosquito density and distribution in the area.⁴⁻⁶ Kengluetcha et al⁷ further indicated that the existence of mosquitoes may vary from one place to another and is primarily dependent on biological and physicochemical conditions emanating from their breeding habitats.

The present increase of mosquito-borne diseases has been attributed to numerous factors influencing the behavioural pattern and the biology of the vector. Although these factors are not fully understood, it is believed that the environment plays a vital role in the development of mosquitoes. To date, limited research has been conducted on how environmental factors, particularly water quality of breeding habitats, influence the dynamics and distribution of mosquitoes, particularly in urban areas of the Philippines. There is a need to look into the relationship of environmental factors, such as water quality, to the abundance of mosquitoes to understand and predict the occurrences of mosquitoes in our environment, particularly the causes of heterogeneity and abundance of mosquito distribution in the environment. This study aimed to assess the water quality of the breeding habitats of *Aedes* mosquitoes and relate the water quality factors to the abundance of the *Aedes* larval mosquitoes in the breeding habitats. Results pertaining to this study are significant, as they enable us to better understand the vector's dynamics and help local communities in predicting the occurrences of *Aedes* larval mosquitoes in relation to environmental factors in order to better prevent and control *Aedes* mosquitoes.

Methodology

The study was conducted in Caloocan city, Philippines. A sampling frame was used to randomly draw out the three study sites identified in the area. The sampling frame was a listing of all the communities in the study area. The selection was done through the use of random numbers generated by the computer. On the basis of the given random numbers, the number corresponding to the community in the listing was selected as the study site. *Aedes* larval surveys and water quality assessments of the breeding habitats for Barangays (communities) 13, 62, and 104 were conducted. Caloocan city is situated at 14.653° latitude and 120.983° longitude in the Philippines. Community 13 is a populated residential area, Community 104 is a mixed residential and commercial area, and Community 62 is an industrial area. All the communities included in the study were urban areas with low vegetation coverage.

All the households, schools, commercial centres and factories in the randomly selected study sites were included in the study. All the possible breeding sites of the *Aedes* mosquitoes were surveyed. Collection of mosquito larvae was done using the dipping method. *Aedes*



larvae were collected using a ladle wherein one dip was calibrated to collect 120 ml of water. The dipper was gently immersed until the edge was just under the water surface. After filling, the dipper was quickly lifted out and inspected for the presence of *Aedes* larvae. Depending on habitat and quantity of water present in each habitat, a range of one to five dips were taken per site. The field-collected fourth instar larvae were examined morphologically up to genus level by using standard keys.

Water quality testing

Water samples were obtained from all breeding habitats within the water quality guidelines and general effluent standards set by the government. These samples were evaluated for four abiotic factors: salinity, conductance, pH, and dissolved oxygen. Salinity and conductance of the water samples were determined using an SCT meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). pH was determined colorimetrically using a pH paper (Merck, Germany), and dissolved oxygen was determined using a DO meter (Jenway Ltd., Gransmore Green, Felsted Dunmow, Essex, United Kingdom).

Data analysis

The mosquito larvae per study site was recorded and compared. The skewness and kurtosis tests were performed to determine whether the distribution of the data deviated from the distribution of the normal curve. A multiple regression analysis by stepwise elimination method was employed to obtain the best predictor variables contributing to the abundance of the *Aedes* mosquito larvae for the study area. The model equation that was developed followed a regression relationship: $Y = \alpha + \beta X$, wherein Y is the abundance of the *Aedes* mosquito larvae, α is the intercept variable, β is the slope parameter, and X is the measurement of the abiotic factors (salinity, conductance, pH and dissolved oxygen). The criterion used a fixed preset α (for entry set, $\alpha = 0.05$; for removal set, $\alpha = 0.10$). The linear model was developed, and all statistical analyses were performed using SPSS software.

Results

A total of 30 outdoor breeding habitats of the *Aedes* mosquito were identified in all the three randomly selected communities. No indoor breeding habitats were obtained since most of the water storage containers were found outdoors. The breeding habitats of the mosquitoes collected were tyres, barrels, ornamental ponds, constructed water receptacles and wet floors.

A total of 319 of fourth instar larvae were collected from all breeding sites and identified up to genus level. Using standard keys the three genera of mosquito larvae obtained were



Aedes (88%), *Culex* (11%) and *Anopheles* (1%). Table 1 shows the abundance of mosquito larvae obtained in the three different communities along with aggregate percentage.

Table 1: Distribution of larval mosquito genera obtained in the study habitats, Caloocan city, Philippines

Study sites	Larval mosquito genera			Total
	<i>Aedes</i>	<i>Anopheles</i>	<i>Culex</i>	
Community 13	179	2	17	198
Community 104	83	1	16	100
Community 62	19	1	1	21
Total	281 (88%)	4 (1%)	34 (11%)	319

Figures in brackets denote percentage

Table 2 shows the breeding habitats and larval mosquito abundance. *Aedes* was found in all the habitat types situated in all the three communities, namely, barrels, grotto, ornamental ponds, tyres, wet floor and constructed water receptacles, whereas *Anopheles* and *Culex* were found incidentally in selected breeding habitats of the *Aedes* mosquito. The most abundant *Aedes* mosquito larvae were found in the barrels in Community 13.

Table 2: Number of breeding habitat types and abundance by genera in Caloocan city, Philippines

Study sites	Breeding habitats	Number of breeding habitats (N)	Larval mosquito genus abundance			Total
			<i>Aedes</i>	<i>Anopheles</i>	<i>Culex</i>	
Community 13	Barrel	15	141	1	15	157
	Grotto	1	38	1	2	41
Community 104	Barrel	1	2	0	0	2
	Ornamental ponds	4	59	0	9	68
	Tyres	5	21	1	5	27
	Wet floor	2	1	0	2	3
Community 62	Constructed water receptacle	1	1	0	0	1
	Tyres	2	18	1	1	20
Total		31	281	4	34	319



Table 3 shows the physicochemical quality of water in all breeding habitats.

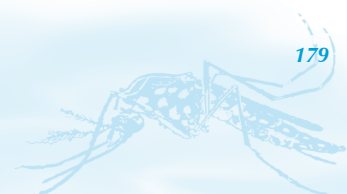
Table 3: Physicochemical quality of water in all breeding habitats in Caloocan city, Philippines, 2011

Study area	Breeding habitats	Physico-chemical quality of the water			
		pH Mean \pm SD	DO (ppm) Mean \pm SD	Conductance ($\mu\Omega$) Mean \pm SD	Salinity (%) Mean \pm SD
Community 13	Barrel (n=15)	6.67 \pm 0.82	5.92 \pm 0.40	152.00 \pm 46.63	0
	Grotto (n=1)	8.00 \pm 0.00	6.52 \pm 0.00	5.00 \pm 0.00	0
Community 104	Barrel (n=1)	7.00 \pm 0.00	5.58 \pm 0.00	280.00 \pm 0.00	0
	Ornamental Pond (n=4)	8.50 \pm 0.58	5.87 \pm 0.38	256.17 \pm 253.75	0
	Tyres (n=5)	7.29 \pm 0.76	6.18 \pm 0.57	157.86 \pm 105.59	0
	Wet floor (n=2)	9.00 \pm 0.00	5.61 \pm 0.08	630.74 \pm 454.00	0
Community 62	Constructed water receptacle (n=1)	8.00 \pm 0.00	5.57 \pm 0.00	70.00 \pm 0.00	0
	Tyres (n=2)	6.50 \pm 2.12	5.82 \pm 0.42	187.5 \pm 81.32	0

The results of the multiple linear regression analysis demonstrated that, among the different water quality parameters examined, dissolved oxygen was an important factor in the abundance of the *Aedes* mosquito larvae ($Y = -37.90 + 8.00 [\text{DO}]$, $r^2 = 0.144$, $P < 0.05$). No significant correlation was established between pH, salinity and conductivity and the abundance of the *Aedes* larval mosquitoes ($P > 0.05$).

Discussion

This was a cross-sectional study and its scope was limited to determining the water quality of the *Aedes* breeding habitats. This study was also limited in relation to the abiotic variables (pH, conductance, salinity and dissolved oxygen) to the abundance of the *Aedes* larval mosquitoes obtained from the breeding habitats in the randomly-selected communities in Caloocan city, Philippines. We detected breeding habitat heterogeneity and incidentally found the *Anopheline* and the *Culicinae* mosquitoes in selected breeding habitats of the *Aedes* mosquito. We likewise identified that the dissolved oxygen was a key environmental variable that determined the occurrence and relative abundance of the *Aedes* larval mosquitoes. Community 13 had the most number of *Aedes* mosquito larvae collected among the study sites examined. *Aedes* larvae were present in a wide range of breeding habitats. Since we did not identify the *Aedes* mosquitoes up to its species level, a previous study⁸ has shown that the *Ae. aegypti* mosquitoes are commonly found in urban areas and inhabit water storage containers.

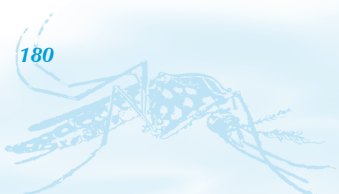


The water quality assessed in the breeding habitats was all within government standards. The dissolved oxygen of water in all the breeding habitats was above 5 ppm, indicating that the water quality was good. The variability in the abundance of the *Aedes* larvae in the breeding habitats indicated that the *Aedes* larvae might have certain oxygen requirements for them to be able to thrive. A study by Opoku et al.⁹ supports the results of this study, wherein they have presented that the oxygen requirement of different genera of mosquito larvae varies. They have presented that the *Aedes* mosquitoes can thrive on breeding habitats that have variable dissolved oxygen concentrations. Results of the study have indicated that the *Aedes* mosquito larvae are capable of surviving in a wide range of pH, although the study showed breeding habitat pH ranges of 4 to 9. Studies showed that pH is an important parameter, as it indicates favourable habitats for mosquito larval development. According to Clark et al.,¹⁰ mosquitoes prefer to breed in habitats that have pH ranging from 4 to 11, whereas in a separate study by Umar and Don-Pedro,¹¹ it has been indicated that mosquito larvae prefer breeding habitats with pH ranging from 6.5 to 8. The pH variable in this study did not indicate a significant relationship with the abundance of the *Aedes* larval mosquitoes ($P>0.05$).

This study has brought out that the *Aedes* mosquitoes can thrive in a wide array of breeding habitats which can be acidic, normal (pH) or basic. The abundance of the *Aedes* mosquitoes may likewise be dependent on conductance¹² and salinity of the waters in the breeding habitats. Results of this study have shown a wide range of conductance readings on the waters of the breeding habitats, indicating that the waters contained inorganic dissolved solutes, which may provide nourishment to the *Aedes* larval mosquitoes. All the waters obtained in the breeding habitats contained 0% salinity, indicating that all were freshwater sources. Both the conductance and salinity were likewise observed not to significantly correlate to the abundance of the *Aedes* larval mosquitoes ($P>0.05$). Although studies point out that salinity and conductance play an important role generally in the larval mosquito development, the results of this study do not show that kind of relationship. Previous studies^{13,14} indicate that although conductance and salinity are important in mosquito development, different mosquitoes would have specific tolerances that enable them to osmoregulate and adapt to the varying water conductance and salinity concentrations in the water.

Conclusion

This study assessed the water quality of the breeding habitats and related the water quality parameters to the abundance of the *Aedes* larval mosquitoes in the breeding habitats. Among the water quality parameters tested with the abundance of the *Aedes* larval mosquitoes, dissolved oxygen was an important factor in their abundance. Further research should examine other abiotic and biotic factors with a more detailed analysis of water chemistry and investigate the effects of water quality on the *Aedes* mosquito biology, occurrence, abundance and competence of transmitting infectious diseases.



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Baseline and key container survey for *Aedes aegypti* and *Aedes albopictus* in Albay Province, Philippines

A Schapira,^{a#} C Garcia,^b M Asor,^b I Riquentino,^b L Bahillo,^c M Palattao,^b P Morano,^d T Mirandilla,^d K Discaya,^d M Banzuela,^b R Berango,^b F Buban,^b R Caldino,^b M Cedeno,^b D Dayandante,^b R Delfin,^b L Grageda,^b S Oguan,^b E Olitan,^b A Quitasol,^b S Rabano,^b S Sabareza,^b MR Sy,^b B Tapit,^b L Vasquez,^b L Vicera,^b J Aguilar,^b Coper,^b S Candila,^b G Flores,^b J Garcia,^b L General,^b A Gutierrez,^b L Lazona,^b JA Balangat,^d K Rodriguez,^d K Almiñe,^d MM Camata,^d C Aquino^c and L Mendoza^b

^aIndependent Consultant, Legazpi City, Albay Province, Philippines

^bAlbay Provincial Health Office, Legazpi City, and City/Municipal Health Units, Albay Province, Philippines

^cRegion V Centre for Health Development (CHD), Daraga, Albay Province, Philippines

^dDepartment of Biology, Faculty of Science, Bicol University, Daraga, Albay Province, Philippines

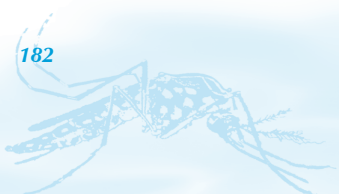
Abstract

Albay Province in the Philippines has a population of 1.3 million. On the background of a dengue epidemic in 2010, a survey was carried out in late 2011 to provide entomological baseline data on *Aedes aegypti* and *Ae. albopictus* and information on key containers. A two-stage cluster sampling was used to select 1800 households. Classical entomological indices, viz. House Index (HI), Container Index (CI), Breteau Index (BI) and Pupae per Person Index (PPI) were determined in four ecological strata.

Across the province, the indices of *Ae. albopictus* were about three times as high as those of *Ae. aegypti*. There was a significant correlation of dengue surveillance data with PPI summed for both vector species, and, based on these findings, it was concluded that both vectors were present in the province. The mean (and standard deviation) values of the main indices for the province were for *Ae. aegypti* and *Ae. albopictus* together: HI 31.9% (15.5%); BI 46.4 (28.3); PPI: 0.33 (0.37), compatible with low to moderate risk.

Tyres accounted for 21% of *Aedes* vector pupae, drums for 13%, and 'other' containers for 39%. 'Others' included mainly coconut shells, bromeliads and various man-made containers. For nearly all the container types examined, the initial field finding of mosquito larvae or pupae of any genus was associated with the presence of *Aedes* vectors in more than 90% of cases. Thus, the presence

#E-mail: allan.schapira@gmail.com



of mosquito pupae in a container belonging to these types is sufficient grounds for incriminating it as a dengue vector breeding site with a high degree of probability.

A dengue vector control strategy for the province is outlined based on attention to the container types, which account for most pupal production, inspection by lay community health volunteers, communication and enforcement, possible specific measures for the disposal of tyres, and chemical larviciding or netting of about 6000 large drums (estimated from survey data). The effectiveness of this strategy will be monitored by a re-survey 2–3 years after the baseline.

Keywords: Dengue; *Aedes aegypti*; *Aedes albopictus*; Entomological; Survey; Vector control; Pupae per Person Index; Key container; Albay; Philippines

Introduction

Albay province is located in the southern part of Luzon, the largest of the Philippine islands; it has a population of 1.32 million^a living in three cities, Legazpi City, Tabaco and Ligao, and 15 municipalities, which are subdivided in 721 *barangays*.^b The climate is tropical and humid with no dry season and the heaviest rainfall is from December to January. Typhoons are frequent, especially from July to November. From 2005 to 2009 the average annual rainfall was 4187 mm, the maximum monthly temperature, in May, 32 °C and the minimum, in January, 22 °C. In 2010, the annual precipitation was only 3019 mm, and in 2011, as high as 5145 mm.^c The province comprises of a central riverine plain with highland areas and narrow coastal plains to the east and west, and a chain of smaller islands off the Pacific coast. In most years, the reported incidence rate of dengue in Albay has been much lower than in the Philippines as a whole. However, in 2010, the dengue incidence reached an all-time high of 1.41 per 1000 population (Figure 1). The seasonal distribution suggests that the transmission usually picks up in the middle of the year, when rains increase (Figure 2). The epidemic in 2010 could be related to prolonged low precipitation during the first half of the year, leading to increased water storage.

After the epidemic in 2010 it was decided to generate systematic information on dengue vectors in the province, and the survey reported here was planned with the following objectives: (i) to characterize breeding sites and identify key containers by examining a sample of households representative of the province; (ii) to obtain entomological baseline data for evaluation by repeat surveys in the same sites, for example, every two years. It was decided to collect data for calculation of House Index (HI), Container Index (CI) and Breteau

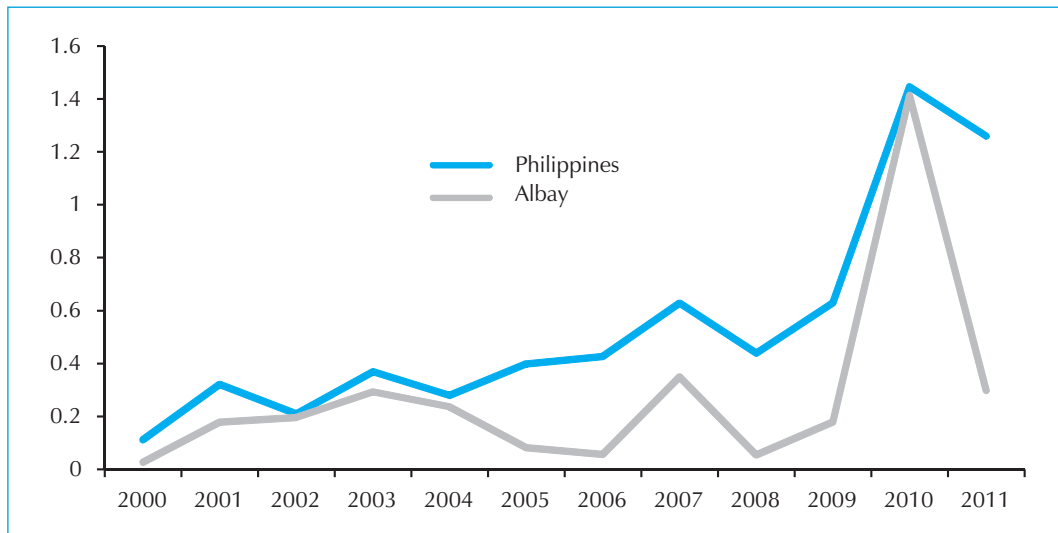
^a2011 unofficial figure, Region V Centre for Health Development

^bA *barangay* is the smallest administrative division in the Philippines.

^cPAGASA Legazpi Complex (Meteorological) Station

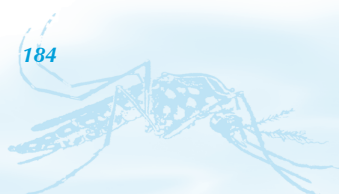
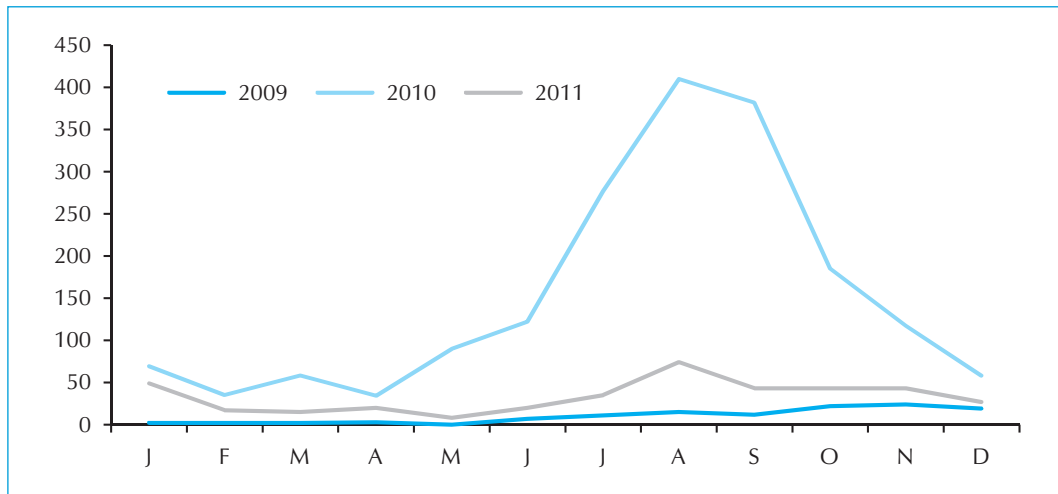


Figure 1: Reported annual incidence rate per 1000 of dengue in Albay province and the Philippines, 2000-2011



Source: WHO Western Pacific Regional Office and Albay Provincial Health Office

Figure 2: Reported number of dengue cases by month, Albay province, Philippines, 2009-2011



Index (BI), as well as the Pupae per Person Index (PPI), which is nowadays considered the most important, as it reflects the number of adult mosquitoes available to bite humans.¹ The identification of key containers is meant to pinpoint the types of containers, which in a given area are responsible for most of the production of adult mosquito vectors. Targeting the containers, which hold at least 55–70% of vector pupae, can provide the same entomological effect as targeting all productive containers, and at lower cost.^{1,2}

Methods

A two-stage cluster randomized survey was conducted. The sample size was decided as follows: for an assessment of key containers sufficient to inform control strategy, 100 positive households are adequate even if the dispersion index is high.¹ In a dengue vector survey in Cebu in the Philippines, the lowest HI found in one *barangay* was 33%,³ so a sample size of 300 households should be sufficient. We wished to have an assessment in each of four ecological strata, so we opted for a total sample of 1800 households. The assumption of a high dispersion index was conservative, but balanced the need for a larger sample size due to the design effect of a cluster survey. It was decided to select clusters of only 30 households per *barangay* as this would allow more *barangays* to be examined and therefore, lead to a more precise estimation being made at province and stratum levels. Thus, 60 *barangays* were selected by systematic random sampling so that the chance of inclusion was proportional to population.⁴ In each *barangay*, 30 households were selected as a systematic random sample based on household lists. *Barangays* were classified ecologically based on maps and knowledge of provincial health staff, as belonging to one of four strata: (i) urban, without agricultural fields adjacent to habitation; (ii) rural plain (rice field areas); (iii) highland (more than 100 m above sea level); and (iv) coastal fishing communities. In addition to households, 30 institutions, companies and other public spaces were selected randomly from tourist maps (EZ[®]) of the three cities.

The survey was carried out by medical technologists from the municipalities and cities, in some cases together with biology students. The work was supervised by senior technologists from the provincial and regional levels, who accompanied the city/municipality staff on the first day of field and laboratory work. All surveyors and supervisors were trained by entomologists from the Research Institute of Tropical Medicine, Alabang, and the WHO country office, Manila, Philippines at a workshop held in the provincial capital, Legazpi city, in July 2011. The households and most public spaces were examined from August to November 2011. Because of operational problems, some work in public spaces in Legazpi city took place in March 2012.

A team comprising a medical technologist and a *barangay* health worker covered 10–20 households per day. After obtaining permission from the household head, the team carried out a systematic search for any water-holding container on the premises of the selected

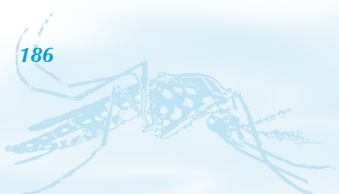


households. Indoors (sheltered by a roof), all spaces and rooms were surveyed. Containers were categorized as follows:

- **Drum:** A cylindrical container made of plastic or metal with >50 litre capacity.
- **Water storage jar:** A jerry-can made of plastic or metal, or an earthenware water container >10 litre capacity.
- **Concrete tank:** Any container made of concrete irrespective of size.
- **Small pot:** A container <10 litre capacity, made of plastic, metal or earthenware, which does not fit in any other category.
- **Flower vase, pot or tray:** Anything used for holding flowers or plants, including the bases.
- **Tyre:** Self-explanatory.
- **Bottle/tin/can:** Self-explanatory.
- **Others:** Include trays under refrigerators and air-conditioners, roof gutters, drains, wells, septic tanks, coconut shells, cacao pods, leaf axils, tree holes, garden ponds, etc.

The teams checked all wet containers for larvae and pupae. All live pupae and instar III/IV larvae were collected from smaller containers by pouring or with dippers or pipettes. From large containers (which were all cylindrical), immatures were collected using a ten-sweep net method, and the numbers were corrected by multiplying with 3.2, intermediate between WHO-recommended values of 3.0 for two thirds full containers and 3.5 for full containers.^{5,6} The number of pupae and instars III/IV of *Ae. aegypti* and *Ae. albopictus* was determined by microscopy. Larvae were considered instar III/IV, if more than 3 mm long. Larvae of *Culex spp.* and *Anopheles spp.* were likewise recorded; pupae of the two main vector species were differentiated by chaetotaxy;⁷ due to difficulties of taxonomy, pupae, which could not be identified as *Ae. aegypti* or *Ae. albopictus*, were not counted. Quality assurance was carried out by placing all biological material from days 1, 3 and 8 of work in each municipality/city in glass vials with 60% alcohol, which were cross-checked at the Bicol University Biology Department with the initial results concealed from those checking.

We used 2010 and 2011 data from the dengue disease surveillance in the province for correlation by linear regression to assess the predictive utility of different entomological indices and the local importance of the two vector species. The disease surveillance is based on reporting of inpatient cases and deaths from 11 hospitals located in the three cities of the province. The cases are usually clinically (not serologically) diagnosed and not differentiated according to severity. Data from household forms were double-entered on an *Epidata* format, validated, rechecked in case of discrepancy between the two encodings; random records were checked for data consistency. Data analysis was carried out in *Epi-Info* and *Excel*. All indices were calculated by *barangay* and stratum for *Ae. aegypti* alone and for *Ae. aegypti* plus *Ae. albopictus*. PPI and Immatures per Person Index (instar III/IV larvae plus pupae as numerator)⁶ were also calculated separately for *Ae. albopictus*. The identification of key containers was done for *Ae. aegypti* alone and for the two species together.



Results

The survey covered 1797 of 1800 planned households and 23 of 30 planned public spaces. The quality assurance showed that the field staff could identify the two vector species of *Aedes* correctly. However, in about 10% of samples, the counts of larvae or pupae, respectively, by the field staff were significantly higher than that found at double-checking. In some cases, for larvae, this could be ascribed to the field staff having erroneously included instars I/II in their counts; in other instances, the field staff had misunderstood instructions and included only a fraction of each positive sample for quality assurance. As no species misclassification was detected, it was decided to use the field results without correction, recognizing that larval counts could, in a few instances, be too high because of inclusion of instars I/II.

The data summarizing the entomological indices by stratum are presented in Table 1. It may seem surprising that most of the selected *barangays* belonged to the highland stratum. The explanation is that peri-urban residential areas are often concentrated in hills to avoid risk of flooding in this region. The values for *Ae. aegypti* alone were generally low; the PPI for that species was above 0.5 only in two *barangays* (data not shown). For the two vectors together, the values for all indices were about four times as high, and *Ae. aegypti* accounted for 24% of all *Aedes* pupae recorded. For *Ae. aegypti* alone, indices were higher (though not high) in the urban stratum than in the others.

The number of reported dengue cases with a residential address in each surveyed *barangay* was summed for the years 2010–2011 and divided by the mean of the estimated *barangay* population for those years to calculate a dengue incidence rate for each surveyed *barangay* for the two-year period. A total of 159 reported cases were available for this analysis. Entomological indices for the surveyed *barangays* were correlated with this incidence rate by linear regression. The correlations with BI, HI and CI, whether for *Ae. aegypti* or for both vector species, were weak and far from statistical significance (not shown). In contrast, we found a correlation between PPIs and Immatures per person indices, which were statistically significant, when pupae of both species were summed (Table 2, Figure 3). The pupal indices gave a better correlation (higher slope and lower *P*-values) than the immature indices. The reported dengue attack rate was statistically significantly higher in urban areas. While the densities of *Ae. aegypti* were relatively higher in urban areas, *Ae. albopictus* was the commonest, even there (Table 3). There was only a very weak correlation (slope=0.317; *P*=0.28; *r*²=0.02) between PPIs for *Ae. aegypti* and for *Ae. albopictus*. A multivariate linear regression of dengue incidence rate with PPI for each vector species yielded correlations, which are not statistically significant, but with *P*-values low enough to suggest that they are real (Table 4). The slopes suggest that *Ae. aegypti* is a more efficient vector than *Ae. albopictus*.

The distribution of *Aedes* pupae by container type across the province and in each stratum is shown in Figure 4 (a and b). At the provincial level, the categories 'Drum', 'Tyre' and 'Others' together accounted for 73% of pupal productivity. Figure 5 shows the sub-types of 'Others', which were most commonly associated with *Aedes* pupae: in urban areas,

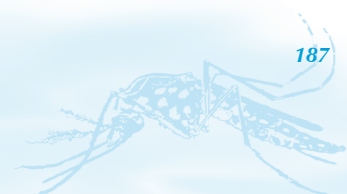


Table 1: Entomological indices for *Ae. aegypti* and *Ae. albopictus* by stratum from a sample of 60 *barangays* in Albay province, Philippines

		Stratum								All	
		Urban		Rural		Highland		Coastal			
No. households		540		329		688		240		1797	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Ae. aegypti</i>	HI	14.8	10.9	2.8	4.3	6.7	8.8	7.9	11.1	8.6	9.9
	BI	20.0	17.7	2.8	4.3	8.0	12.1	10.8	15.2	11.0	14.4
	CI	6.5	5.4	1.5	3.5	3.4	6.7	2.3	3.6	3.8	5.6
	PPI	0.13	0.19	0.08	0.18	0.02	0.05	0.10	0.14	0.08	0.14
<i>Ae. aegypti</i> and <i>Ae. albopictus</i>	HI	25.0	13.0	33.7	16.0	36.1	17.4	32.9	14.4	31.9	15.5
	BI	36.7	22.1	51.3	32.2	50.1	30.7	51.3	33.5	46.4	28.3
	CI	12.0	7.4	13.7	8.4	16.1	16.9	10.6	8.2	13.6	11.8
	PPI	0.29	0.43	0.40	0.44	0.30	0.31	0.40	0.43	0.33	0.37

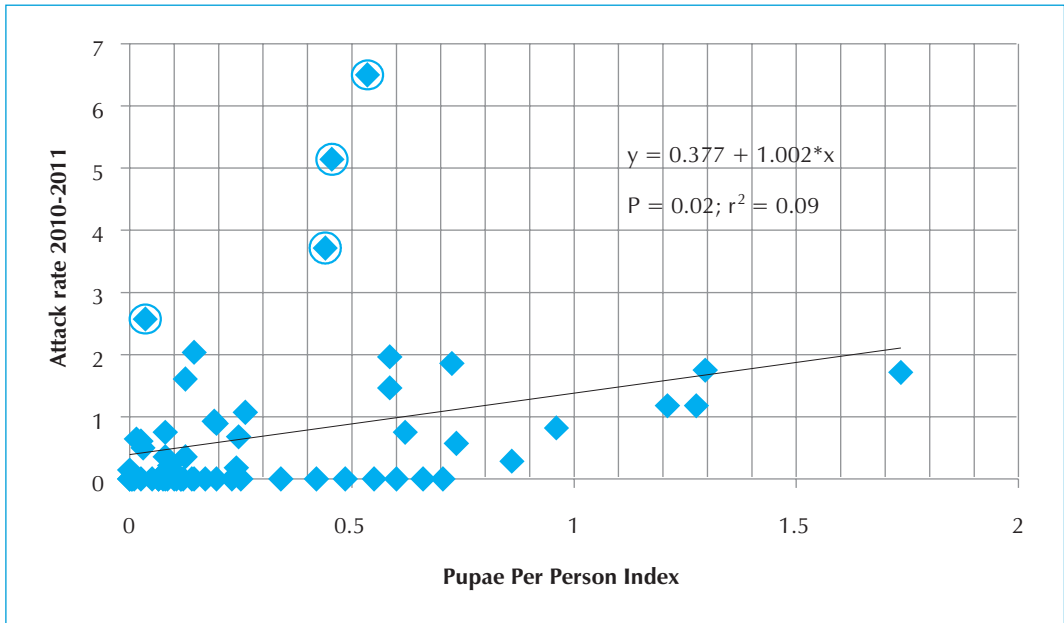
Note: [The figures were derived for each *barangay* surveyed and the summary statistics computed from the *barangay* values. HI=House Index=Percentage of positive households; BI=Breteau Index=No. positive containers per 100 households; CI=Container Index=Percentage of positive containers; PPI=Pupae per Person Index=No. of pupae/no. persons inhabiting the examined households]

Table 2: Simple linear regressions between reported dengue incidence rate 2010-11 (dependent variable) and selected *Aedes* indices by survey *barangay*, Albay, Philippines

Independent variable	Constant	Slope	F-statistic	P-value	Correlation coefficient r^2
Immatures per person (<i>Ae. aegypti</i>)	0.587	0.329	1.568	0.22	0.03
Immatures per person (<i>Ae. albopictus</i>)	0.499	0.165	3.401	0.07	0.06
Immatures per person (all)	0.41	0.182	4.744	0.03	0.08
Pupae per person (<i>Ae. aegypti</i>)	0.555	1.973	3.389	0.07	0.06
Pupae per person (<i>Ae. albopictus</i>)	0.472	0.934	3.779	0.06	0.06
Pupae per person (all)	0.377	1.002	5.993	0.02	0.09



Figure 3: Linear regression of dengue attack rate 2010-2011 against Pupae per Person Index (sum of *Ae. aegypti* and *Ae. albopictus*), Albay, Philippines



Note: [While the P-value is below 0.05 indicating statistical significance, the correlation coefficient, r^2 is low; this may be due to a large extent to the four circled outliers, where the attack rate values are much higher than “expected”. Three of these correspond to urban areas in Legazpi city; the fourth to a highland area in Daraga with good health service access]

Table 3: Correlations between strata and dengue incidence rate 2010–2011, and PPI for *Ae. aegypti* and *Ae. albopictus* in 60 surveyed *barangays*, Albay, Philippines

Stratum	N	Incidence rate		PPI (<i>Ae. aegypti</i>)		PPI (<i>Ae. albopictus</i>)	
		Mean	SD	Mean	SD	Mean	SD
Urban	18	1.39	1.96	2.42	0.04	2.86	0.09
Rural	11	0.46	0.47	0.91	0.03	3.48	0.15
Highland	23	0.35	0.59	0.54	0.00	6.41	0.09
Coastal	8	0.56	0.72	0.79	0.02	2.42	0.18
ANOVA P-value		0.042		0.12		0.54	

N=number of *barangays*



Table 4: Multivariate linear regression of reported dengue incidence rate 2010-2011 on PPI for *Ae. aegypti* and *Ae. albopictus* in surveyed *barangays*, Albay, Philippines

Independent variable	Constant	Slope	F-statistic	P-value	Correlation coefficient r^2
Pupae per person (<i>Ae. aegypti</i>)	0.37	1.71	2.58	0.11	0.1
Pupae per person (<i>Ae. albopictus</i>)		0.82	2.96	0.09	

Figure 4: (a) Distribution of *Ae. aegypti* and *Ae. albopictus* pupae by container category in Albay province as a whole; and (b) in the four strata, Philippines

Figure 4(a)

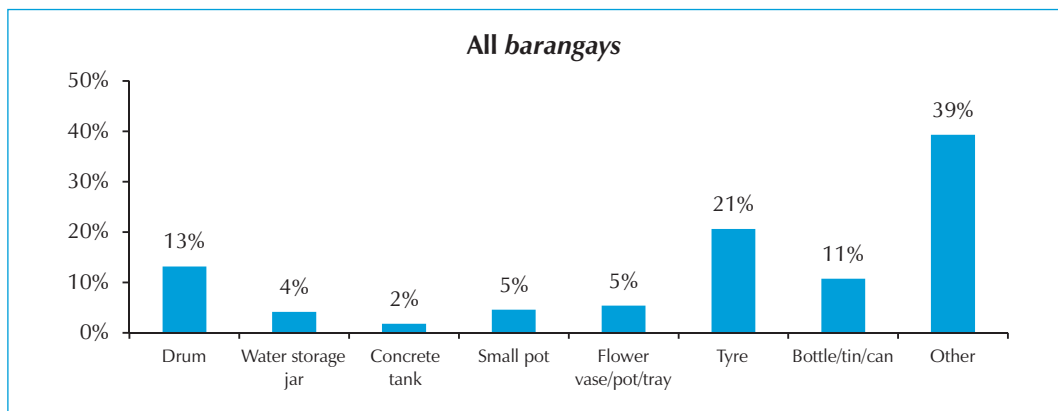


Figure 4(b)

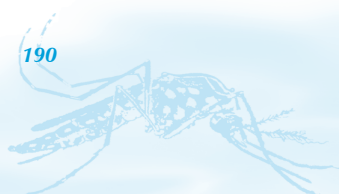
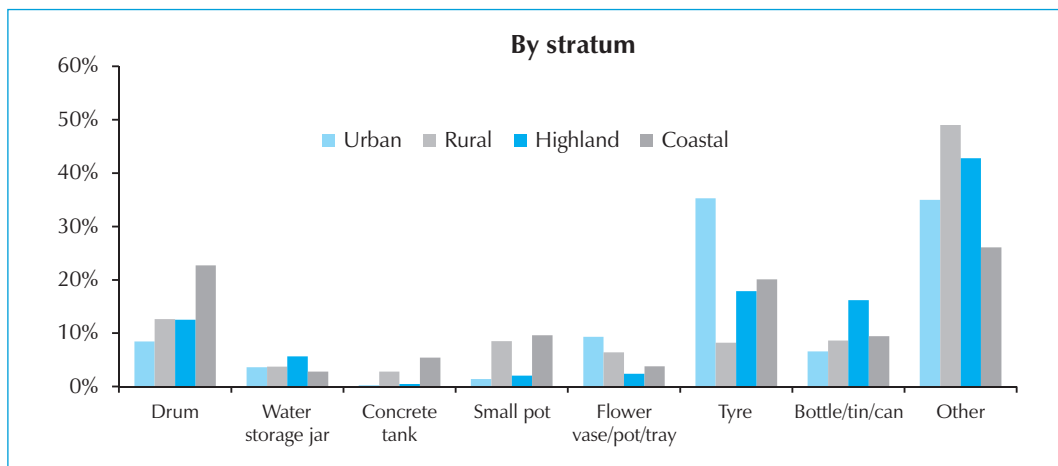
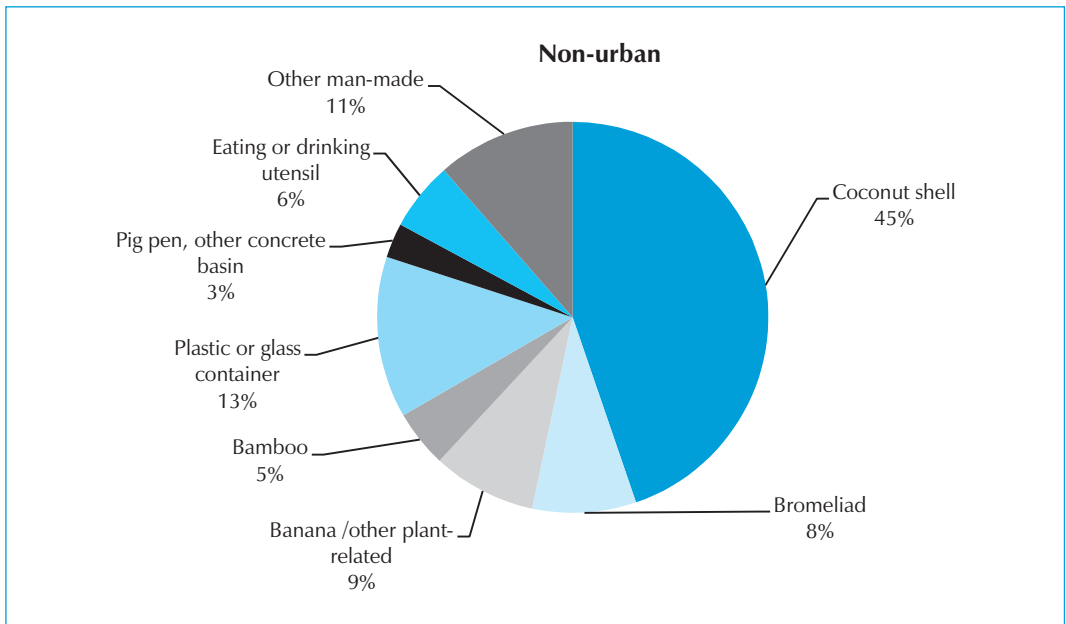
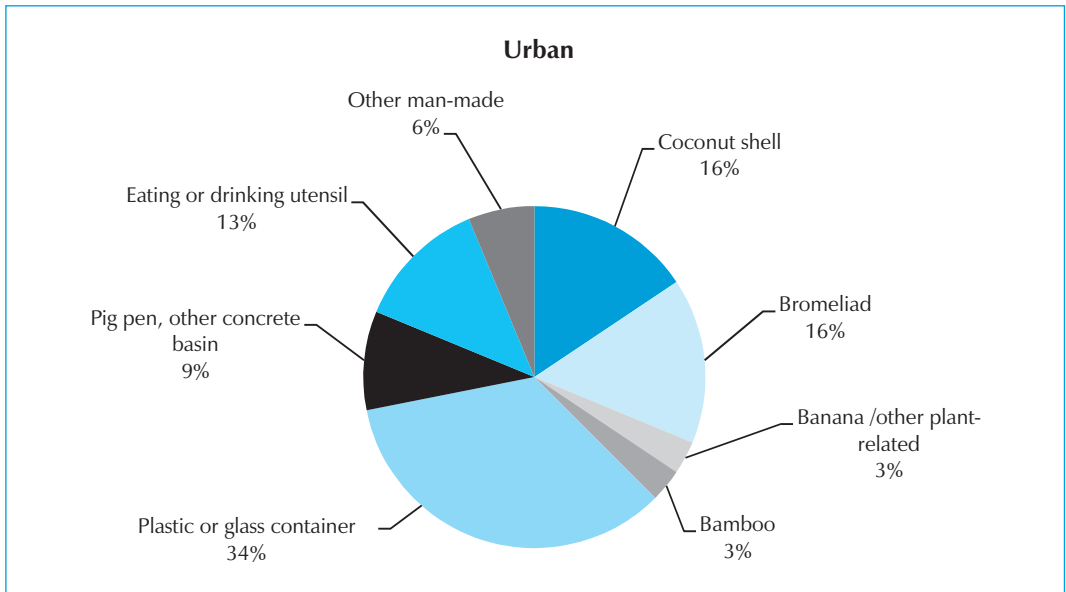


Figure 5: Distribution of households with >1 *Aedes* pupae according to presence of 'Other' container(s) of each sub-type. Separately for the urban stratum and the other three strata (non-urban), Albay, Philippines



bromeliads, coconut shells and an array of discarded man-made containers, in other strata, coconut shells. Unfortunately, the data recordings within the category ‘Others’ did not make it possible to quantify the exact contribution to pupal production of the container types included. The three categories of ‘Concrete tank’, ‘Small pot’ and ‘Flower vase/pot/tray’ played a negligible role. An analysis by municipality indicated that drums and water-storage jars are important in some of the more populous municipalities; tyres were important in Legazpi city, and the adjacent highly urbanized municipality of Daraga, but not in the other cities (data not shown). Across the sample, pupae were found mainly outdoors: only 8.9% of *Ae. aegypti* and 7.0% of *Ae. albopictus* pupae were found in indoor containers.

According to interviews with owners of the productive drums, these were filled only with rainwater, and it was used only for cleaning and gardening. The total number of *Aedes*-positive drums in the sample was 43, of which 41 were placed outside. The most common type is a blue plastic drum, which has a surface area of 0.92 m² and typically holds around 500 litres. The total number of persons in the surveyed households was 9123, which is 0.69% of the estimated total population. The maximum likelihood estimate of the total number of productive drums in the province is therefore $43/0.69\% = 6236$ with a total surface area of 5713 m² and water volume of 3 118 000 litres.

We examined the relationship between the field observation of “any mosquito” and the laboratory identification of one or both of the *Aedes* vector species (Table 5). The results of the survey were recorded with household as the basic unit, so there may be instances where several containers in one household would be recorded as having “any mosquito”, but only one of them contained *Aedes* dengue vectors. The positive predictive values may, therefore, be a little higher than if they had been calculated from a container database. Nonetheless, it is noteworthy that for all container categories the presence of “any mosquito” was associated with *Aedes* in more than or nearly 90% of households. The positive predictive value for *Culex sp.* was much lower, 3% to 20%. In three cases, in Daraga and Legazpi city,

Table 5: The probability of finding *Ae. aegypti* and/or *Ae. albopictus*, or, respectively, *Culex sp.* in each container category, when “any mosquito”, larvae and/or pupae, had been identified in a container of that category in the household, Albay, Philippines

	Container type	Drum	Water storage jar	Concrete tank	Small pot	Flower vase/pot/tray	Tyre	Bottle/tin/can	Other
No. with “any mosquito”		70	59	17	35	48	83	104	273
“Positive predictive value” of “any mosquito”	<i>Ae. aegypti</i> and/or <i>albopictus</i>	93%	88%	94%	100%	92%	98%	98%	91%
	<i>Culex sp.</i>	11%	20%	5%	3%	8%	6%	6%	4%



the technicians identified *Aedes* species, which were neither *aegypti* nor *albopictus*. Re-examination by senior technical staff led to the identification of *Ae. pseudoscutellaris*, which is common in the Philippines. In the data, this species was disregarded. No anophelines were detected.

The 23 public spaces surveyed included 10 schools and other educational institutions, three stores, three hotels, two hospitals, a restaurant, a bank, a fire station, a *barangay* hall and a waste disposal facility. Calculating as if these units were households, the following mean indicator values for both vector species were derived: HI: 43%; BI: 96 per 100 and CI: 7%, which is not far from the mean values for the households (Table 1). The mean number of *Aedes* pupae per premise was 1.5 for these units against 1.8 for the households. However, 14 of the 35 pupae were found in tyres on a single premises.

Discussion

A level of PPI for *Ae. aegypti* above 0.5 is considered compatible with transmission, when ambient temperatures are around 28 °C.⁸ We found this indicator to be below the threshold in nearly all *barangays*. As the densities of *Ae. albopictus* were much higher, we explored the vector status of *Ae. albopictus*, although it is usually considered to be of minor importance in tropical areas.⁹ Linear regressions between recorded dengue incidence rates and PPI values for each or both species in the survey *barangays* indicated that both species are important vectors. Could the association between *Ae. albopictus* and the incidence rate be a result of confounding? As the correlation between PPIs for *Ae. albopictus* and *Ae. aegypti* was very weak, the correlation of the former with dengue incidence cannot be due to shared habitats. The reported dengue incidence rate is higher in urban areas, which would be explained in part by better health service access, but *Ae. albopictus* is relatively more dominant in other areas (Table 3), so any geographical confounding would tend to weaken the association between *Ae. albopictus* and dengue incidence. It is possible that the situation in 2011 had changed from that in 2010, a drought year, when people could have stored water to a larger extent, causing extensive breeding of *Ae. aegypti* in many localities where it could not be found in 2011. However, this could also not explain the correlation between dengue incidence and *Ae. albopictus*.

It, therefore, appears that because of its high density, *Ae. albopictus* is an important vector in Albay province. The high density matches the high precipitation, making the entire province, including urban areas, highly suitable for a mosquito, which tends to rest in foliage. The slopes of the multivariate correlation suggest that the efficiency of *Ae. aegypti* is higher than that of *Ae. albopictus* (as expected), but the numbers are not sufficient to support quantitative conclusions. Correlations of recorded dengue attack rates 2010–2011 and entomological indicators for either *Ae. aegypti* or *Ae. aegypti* and *Ae. albopictus* across the 60 *barangays* surveyed showed poor correlation with House, Breteau and Container indices. This suggests that PPI should be the preferred indicator for assessing dengue risk



and evaluating control measures; in Albay province, this PPI should be based on counts of both vectors or possibly, pending further investigation, with a correction factor to account for the lower efficiency of *Ae. albopictus*. It is questionable whether the measurement of HI, BI and CI have any value at all.

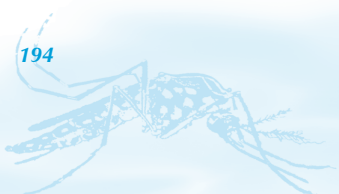
The PPI for the two vector species together was the highest in highland areas, followed by urban, rural and coastal *barangays* (Table 1). This suggests that dengue may be transmitted anywhere in the province. The higher attack rates reported from urban areas probably result more from better access to health services and the higher risk of various dengue virus serotypes being introduced there by travelling people rather than from higher vectorial capacity.

The generally low level of entomological risk which fits well with the marked inter-annual fluctuations exhibited by the surveillance data suggest that the disease burden could be significantly reduced if the vector abundance of both species as reflected by PPI can be reduced by at least 50%.^{6,8}

The identification of key containers showed that drums, water-storage jars, tyres, coconut shells, bromeliads and a variety of discarded artificial containers are important. In an adapted *Search and Destroy* strategy, it is important to pay attention to these containers, while other small containers, such as flower-pots, cans and bottles can normally be disregarded. All the important container types except drums can be dealt with by measures of environmental hygiene, which can be carried out by communities, if properly mobilized. Splitting of tyres can, however, be difficult; it is hoped that the survey results can be used to motivate decision-makers to establish a tyre facility at a newly constructed waste disposal facility in Legazpi city. For the drums, the most realistic options are chemical or biological larviciding or covering with a shower-cap type of net. As the survey was carried out in a probability sample of the population of the province, it provided an estimate of the number of drums that need to be covered – an essential element for planning.

For nearly all the container types examined in this survey, the initial field finding of mosquito immatures of any genus was associated with the presence of *Ae. aegypti* or *Ae. albopictus* in more than 90% of cases and with *Culex* in only about 3%–20% of cases. This means that in practical control operations, the presence of mosquito pupae in a container belonging to these types is sufficient grounds for incriminating it as a dengue vector breeding site, which must be controlled.

It was observed during the survey that the lay volunteer *barangay* health workers could quickly learn to detect mosquito larvae and pupae. In Albay province, there is more than one *barangay* health worker per 100 households. Following a one-day training, it will, therefore, be possible for them to check all outside areas of all households twice a year with minimal supervision. This will be combined with person-to-person communication, enforcement supported by collaboration between *barangay* captains and city/municipal health units, and, as mentioned above, limited specific measures for drums and water-storage jars. The



objective of this strategy will be to prevent a future epidemic like the one in 2010, and its effectiveness will be monitored by a repeat survey in 2013 or 2014. Such a strategy will depend on a considerable degree of sustained motivation on the part of unpaid volunteers and the financing of a supply chain of insecticides, predators or nets to cover drums. Not only must the communities be mobilized, other sectors need to be involved as well (our survey did not include, for example, junk shops, which often store many tyres horizontally!).

Retrospectively, some elements could have improved the quality of the study: A small pilot study could have generated a locally-adapted classification of containers, so that it could have been avoided to end up with 'Others' as one of the most important categories, without precise quantification of the contribution of the container types included in it. The incrimination of *Ae. albopictus* in the present study implies that more attention should have been paid to ground-level water collections, where this species in particular might be found, and which are not covered by the category 'Others' (Methods). A local calibration of the sweep-netting method applied would have improved the precision of the estimation of the contribution of drums, as there is a wide variation in correction factors in different studies (S. To et al., manuscript under preparation).

Acknowledgements

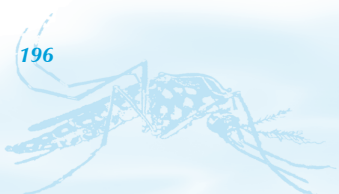
Barangay health workers from all *barangays* surveyed were extremely helpful and diligent; without their strong involvement, this survey would not have been possible. Many *barangay* captains went out of their way to solve problems and provide logistical support. Thanks are due to the trainers, Ms Estrella Cruz, Mr Fred Aure, Mr Paul Malijian and Dr Jeffrey Hii. Drs Hii and Chang Moh Seng from WHO provided many useful comments on the protocol and the manuscript. Staff of the Provincial Health Office assiduously entered the data from the paper survey forms. The training in preparation for the survey was supported financially by WHO and Region V CHD. Financial support for implementation of the survey amounted to Philippine pesos (PHP) 20 000 from WHO. We are grateful to the Provincial Governor, Mr Jose Salceda, for his kind encouragement and interest.

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Knowledge of dengue and related preventive attitude and practices among urban slum dwellers of Jaipur city, Rajasthan, India

Vivek Lal,[#] Dinesh Chandra and Shiv Dutt Gupta

*Institute of Health Management Research, Jaipur (IHMR)
1, Prabhu Dayal Marg, Sanganer Airport, Jaipur - 302011, India.*

Abstract

We interviewed 411 residents in several slums in Jaipur city, Rajasthan, India, to assess their knowledge of dengue fever and compare use of preventive practices between people with and without any knowledge of the disease. More than 80% of the respondents were found to have knowledge of dengue. Fever, headache and rash were the most frequently mentioned disease symptoms by the respondents (85%, 81% and 79%, respectively). Dirty water (67%) and garbage (53%) were identified as the most common breeding sites of the mosquitoes that transmit dengue. However, only 10% respondents with knowledge of dengue were able to identify a possible breeding place for the mosquitoes inside their houses. Age, level of education and family structure were found to be significantly related to knowledge of the disease. In terms of preventive practices, using mosquito-repellent coils and keeping water containers covered were found to be significantly associated with knowledge of the disease. Effective control of dengue requires collaboration of various public and private agencies and community participation. Communication should aim to inform the public that a majority of the dengue cases may originate from within the home and support positive mosquito-control actions within households.

Keywords: Dengue fever; Knowledge, attitudes and practices; Community participation; Jaipur; India.

Introduction

Dengue has been identified as one of the 17 neglected tropical diseases by the World Health Organization.¹ Current estimates show that there may be 50 million dengue infections worldwide every year. India reported a total of 28 292 cases and 110 deaths in 2010, which was the highest in the country in the last two decades.²

Demographic and societal changes leading to unplanned and uncontrolled urbanization have resulted in creation of urban slums. Concurrent population growth has put severe constraints on civic amenities, particularly water supply and solid waste disposal, thereby increasing the breeding potential of disease vectors and the resulting dengue outbreaks.

[#]E-mail: doc.lal@gmail.com



Disease control activities require a sustained high-level government commitment and strengthening of public health infrastructure, intersectoral collaboration and community participation.³ Timely control of dengue epidemics requires preparedness and capacity to undertake suitable and effective control activities during the inter-epidemic period. As dengue disease transmission occurs at home, the ultimate success of the programme would depend on community participation and cooperation.⁴

As part of the National Vector-Borne Disease Control Programme (NVBDCP) strategy, health education is conducted through different channels of interpersonal communication, group educational activities and mass media. Although education campaigns may lead to people's increased awareness of dengue, it remains unclear as to what extent this knowledge is put into practice. We interviewed residents in several slums in Jaipur city of Rajasthan, India, to assess their knowledge of dengue fever and compare the preventive practices employed by people with and without any knowledge of the disease.

Materials and methods

The study was conducted among residents of various urban slums of Jaipur, is situated at 26°55'N and 75°52'E and is the capital of Rajasthan state in India. The hot semi-arid climate of Jaipur presents challenges for water management in general and water storage habits in particular. Jaipur is endemic for dengue fever and accounted for almost half of the reported cases in the state in 2010.⁵

The study was conducted during September – November 2011. This is the usual period when the number of dengue fever cases are on the increase.

A knowledge, attitudes and practices (KAP) questionnaire was designed to collect data on socio-demographic characteristics, knowledge of dengue fever, people's attitude towards the disease and practices in preventing it. The number of questions related to each of these points was 11, 9, 6 and 5, respectively. The KAP questionnaire was pretested in a slum locality in Jaipur which was not included in the study and appropriate modifications were made in the final questionnaire.

We assumed a 50% prevalence of knowledge regarding dengue fever in the community. For a confidence level of 95% and an absolute precision of 5%, the required sample size was calculated to be 384. We increased the sample size by 10% to account for non-response, resulting in a total sample size of 422.

Using a probability proportional to size (PPS) cluster sampling method, 30 clusters from among 46 slums under Jaipur Development Authority were identified. Within each of these clusters, 14 households were systematically selected for interviews. The respondents were an adult member of the household, aged more than 18 years who was present at the time of visit by the study team. Only those families who had been residing in the house for at



least one year were included in the study. Informed consent was obtained from each study participant prior to administering the questionnaire.

Knowledge of dengue was defined as the respondent mentioning at least one of the following symptoms: fever, headache, rash or muscular pain.

The data was entered in Microsoft Excel 2007 and analysed using SPSS version 16.0. Chi-square test was applied to determine associations between sociodemographic variables and knowledge of dengue fever. The variables found to be statistically significant (associated p -value ≤ 0.05) on bivariate analyses were entered into a logistic regression model, with knowledge of dengue fever being the dependent variable.

Results

Eleven families refused to participate in the study. Of the remaining 411 respondents, 229 (56%) were aged 30-44 years. Almost an equal proportion of males and females participated in the study. More than 60% of the respondents were literate, with some formal schooling (less than primary). One third of the respondents were housewives. The total monthly income was less than Indian Rupees 5000 (US\$ 100) for more than 60% of the respondent families. More than half (51.6%) of the families lived as joint families comprising head of household and spouse, with married son(s) and/or daughter(s) and their spouses and/or parents, with or without other not currently married relation(s).

The major sources of potable water were hand pumps and public taps. Drinking-water was usually stored in earthen pitchers, water drums and cemented water tanks kept within the premises of the house. More than 70% of the households did not have provision for a separate toilet and used open fields to relieve themselves. The majority of the households (66%) did not have drains.

All the respondents reported to have heard of dengue fever. However, of the 411 study participants, only 67 could not mention any disease symptom, resulting in 344 (84%) being classified as having knowledge of dengue fever. Among the persons with knowledge of dengue, fever, headache and rash were the disease symptoms mentioned most frequently by 85%, 81% and 79% of the respondents respectively. The least frequently mentioned symptom was muscular pain (63%). Of those with some knowledge of the disease, 10% knew of only one symptom, while 75% had knowledge of three or more symptoms.

Among those who had knowledge of dengue fever, dirty water (67%) and garbage (53%) were identified as the most important breeding places for mosquitoes transmitting dengue (Table 1). However, only 10% of them were able to identify a possible breeding place for mosquitoes inside their houses; of these, the most frequently mentioned sites were water coolers and water tanks. Only 13% were aware that these mosquitoes bite during daytime. However, an additional 34% mentioned that such mosquitoes could bite at any time during



the day and night. For them the main source of information on dengue fever was from television (62%) and radio (44%).

Table 1: Knowledge of dengue fever symptoms and mosquito breeding sites and practice of prevention measures, Jaipur, India

What are the symptoms of dengue fever* (n=344)	Total (%)
Fever	291 (84.6)
Headache	279 (81.1)
Rash	272 (79.1)
Muscular pain	217 (63.1)
What are the breeding places for mosquitoes causing dengue fever* (n=344)	Total (%)
Dirty water	231 (67.1)
Garbage	182 (52.9)
Tyres and flower pots	158 (45.9)
Drinking containers for animals	155 (45.0)
House drains	119 (34.6)
Water jars	94 (27.3)
Water cooler	84 (24.4)
Clean stagnant water	40 (11.6)
What preventive measures are practised by you to prevent dengue fever* (n=344)	Total (%)
Keeping water containers covered	283 (82.3)
Mosquito-repellent coils	274 (79.7)
Eliminate standing water in containers	178 (51.7)
Bednet	158 (45.9)

*Multiple responses

Dengue fever was considered to be a serious illness by 83% of those with knowledge of the disease, and that they were at risk of getting the disease. A majority of those with knowledge of dengue fever (90%) felt that the nearest hospital should be approached if a person suffered from symptoms of dengue.

Keeping water containers covered (82%) and use of mosquito-repellent coils (80%) were the most commonly reported preventive measures practised by those having knowledge of dengue fever. Use of bed nets was reported by less than half of the respondents. Twenty-seven (7.8%) respondents did not practise any preventive measure. Among those who had knowledge of the disease, 133 had water coolers. More than 60% of the respondents with water coolers reported that these were cleaned once a week. Water-filled containers in the house were reported to be cleaned once a week by half of the respondents.



Furthermore, we compared the differences in the use of preventive measures (mosquito-repellent coils, bednets, keeping water containers covered and eliminating standing water in containers) between persons with and without knowledge of dengue fever. Overall, persons with knowledge of dengue fever reported the use of preventive measures more frequently than those without any knowledge. This difference was found to be statistically significant for all four preventive measures (Table 2). On applying logistic regression, use of mosquito-repellent coils and keeping water containers covered were found to be significantly associated with knowledge of the disease.

Table 2: Difference in use of preventive practices between persons with and without knowledge of dengue fever, Jaipur, India

	n	Per cent with knowledge	Unadjusted		Adjusted	
			OR	95% CI	OR	95% CI
Keeping water containers covered	304	93.1	10.16*	5.65 – 18.25	4.36*	2.08 – 9.11
Mosquito-repellent coils	292	93.8	10.65*	5.84 – 19.42	5.36*	2.35 – 12.21
Eliminate standing water in containers	197	90.4	2.70*	1.52 – 4.79	0.93	0.44 – 1.92
Bednets	179	88.3	1.86*	1.06 – 3.25	0.68	0.33 – 1.36

*Statistically significant
OR = Odds ratio

In the opinion of the respondents, both households and the government had a shared responsibility for controlling mosquito breeding and preventing dengue fever. However, 78% of the respondents reported that dengue fever awareness activities by the government or NGOs had not been undertaken in their localities in the past one year. Nonetheless, willingness to cooperate with health authorities in activities to prevent dengue fever were expressed by 73% of the respondents and 63% were of the opinion that dwellers of houses where mosquitoes were found to be breeding should be penalized.

In bivariate analyses (Table 3), sex, age, level of education and family structure were significantly related to knowledge of dengue fever. However, in multivariable analysis, after adjustment for other factors, age, level of education and family structure were found to be significantly related to the knowledge of the disease. Compared to persons aged 60 years and above, those in the age groups of 30–44 years and 45–59 years had a better knowledge of dengue fever [adjusted odds ratio 3.31 (95% confidence interval (CI) 1.09–10.06) and adjusted odds ratio 4.29 (95% CI 1.14–16.10), respectively]. The knowledge of dengue fever was significantly lower among those with a primary school education in comparison

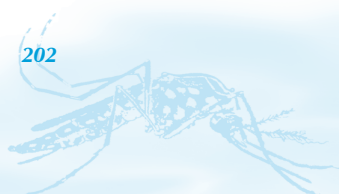


Table 3: Determinants of knowledge of dengue fever, Jaipur, India

	n=411	Percent with knowledge	Unadjusted		Adjusted	
			OR	95% CI	OR	95% CI
Sex						
Male	212	79.2	1.0			
Female	199	88.4	2.0*	1.16–3.46	1.67	0.78–3.56
Age (in completed years)						
More than 59	18	50.0	1.0			
45–59	64	89.1	8.1*	2.42–27.37	4.29*	1.14–16.10
30–44	229	85.6	5.9*	2.19–16.06	3.31*	1.09–10.06
15–29	100	82.0	4.5*	1.58–13.08	2.96	0.92–9.51
Level of education						
Secondary and above	48	91.7	1.0			
Primary	153	71.2	0.22*	0.07–0.66	0.26*	0.08–0.82
Literate, without formal education	49	83.7	0.46	0.13–1.66	0.57	0.15–2.18
Illiterate	161	93.2	1.2	0.37–4.08	1.28	0.36–4.54
Occupation						
Skilled worker	90	88.9	1.0			
Unskilled worker	152	80.3	0.50	0.23–1.09	0.52	0.23–1.19
Housewife and unemployed	169	84.0	0.65	0.30–1.42	0.41	0.15–1.08
Family structure						
Nuclear family	199	88.4	1.0			
Joint family	212	79.2	0.49*	0.28–0.86	0.53*	0.29–0.95

*Statistically significant

OR = Odds ratio



with persons having a secondary education and above [adjusted odds ratio 0.26 (95% CI 0.08–0.82)]. Respondents living in joint families had significantly less knowledge of dengue fever as compared to those living in a nuclear family [adjusted odds ratio 0.53 (95% CI 0.29–0.95)] (Table 3).

Discussion

In this study, 84% of the respondents had some knowledge of dengue fever measured by an awareness of symptoms related to dengue fever; 75% of those with knowledge of the disease cited three or more symptoms. The most commonly reported symptom was fever. The high level of awareness could be due to a greater exposure to mass media. Among those classified as having knowledge of the disease, 86% cited radio and/or television as the major sources of information on dengue fever. The importance of television in disseminating awareness of dengue has been reported by other studies as well.^{6,7}

Individuals over the age of 59 years had significantly less knowledge of the disease than younger persons. Despite being at risk due to lesser mobility and confinement at homes,⁸ lower levels of knowledge among the elderly clearly indicate a gap in prevention programmes. The study participants with primary school education were found to have significantly lower levels of knowledge of the disease than those with higher education. Interestingly, those individuals who were part of and resided in joint families had a significantly lower knowledge of dengue. This could be indicative of poorer socio-economic conditions among joint family members in comparison to those residing as nuclear families.

As most transmission occurs at home,⁴ the ultimate success of the dengue prevention and control programme will depend on active community participation and cooperation. The majority of respondents in our study identified dirty water (67%) and garbage (53%) as the breeding sites for the vector causing dengue fever; only 10% could identify breeding places within their homes. Confusion about the breeding sites for the mosquito transmitting dengue fever has been reported in earlier studies.⁹ The lack of awareness in this regard has implications for its prevention and control and clearly needs to be emphasized. Communication should aim to inform the public that the disease may originate from within the home and should support positive mosquito-control behaviours and practices among individuals and the community.¹⁰ They should be advised to observe a weekly dry day when all water containers should be cleaned and dried. Unfortunately, over the years, the community has transferred the responsibility for vector control to the government health sector. People assume that the mosquito vector is ubiquitous and therefore, beyond their control.¹¹

Overall, the respondents showed positive attitudes toward prevention and control of the disease, with 73% expressing their willingness to cooperate with the health authorities in activities to prevent the dengue disease. When asked whether penalties should be levied on those who allow mosquito breeding in their houses, a slightly lower proportion of respondents (63%) agreed. In a study conducted in Malaysia, more than 95% of respondents showed a



willingness to cooperate with the health authorities; however, a much lower 72% agreed that households should be penalized if mosquito breeding was found in their vicinity.¹²

Among those who had knowledge of dengue fever, the most prevalent preventive measures adopted were keeping water containers covered (82.3%) and using mosquito-repellent coils (79.7%); use of mosquito nets was the least practised preventive method by the community (45.9%). The use of such preventive measures was found to be significantly associated with knowledge of dengue fever. Our results are similar to those reported from Thailand and Malaysia where persons with knowledge of dengue reported a significantly higher use of prevention measures than persons without knowledge of dengue.^{8,12,13} However, since a majority of the respondents did not know of the daytime biting habit of the dengue vector, the reported preventive practices may not have been used during the day and therefore, not specific for prevention against dengue fever. Indeed, some studies have shown no relationship between knowledge of dengue and mosquito-reduction practices, and have found that measures against mosquitoes were used only when people experienced a mosquito nuisance.¹⁴⁻¹⁶

The high incidence of vector-borne diseases is an indicator of deficient health and well-being of people, resulting in individual and national economic loss. Emphasis should be given to preventive measures rather than containment of epidemics. WHO issued the integrated vector management (IVM) strategy on the premise that effective control is not the sole responsibility of the government health sector, but requires the collaboration of various public and private agencies as well as the community. IVM is described as “a rational decision-making process for the optimal use of resources for vector control”.¹⁷ This can be achieved through sustained social mobilization for behavioural change communication and community involvement in source reduction activities using the ‘communication for behavioural impact’ (COMBI) approach. This is an innovative approach which entails purposive and tailor-made strategic communication solutions intended to engage a specific target audience to translate information into responsive action and integrate it with advocacy and social mobilization initiatives to create an enabling environment. Such an environment will result in desired behavioural outcomes and impact.¹⁸

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Community-based dengue source reduction interventions in two townships of Yangon region that significantly reduced entomological indices

Zaw Lin,^a Tun Lin W,^{b#} Tin Oo,^c Khin Thet Wai,^c Hla Myint^d and Monti F^e

^aVector Borne Disease Control (VBDC), Department of Health, Mandalay Region, Myanmar

^bDirector (Research) (Retd.), Department of Medical Research (Lower Myanmar) cum Project Consultant

^cDepartment of Medical Research (Lower Myanmar)

^dRegional Health Department, Yangon Region, Myanmar

^eThe Mentor Initiative, Myanmar

Abstract

A community-based dengue source reduction Project was implemented during a six-month period, from March to August 2009, in two townships of Yangon Region — Insein and Hlaingthayar. The two main interventions were implementation of community-based environmental management activities and a larviciding campaign with two rounds of temephos application. Two entomological and two knowledge-attitude-practice (KAP) surveys were undertaken before and after the interventions to assess the impact of the control activities on key indicators. The baseline survey showed a significant difference ($P < 0.01$) on the entomological indices between the two townships, with higher values in Hlaingthayar, but after the interventions the reduction was effective ($P < 0.01$) and similar in both the townships. In Insein, the Breteau Index (BI) decreased from a pre-intervention index of 86 ± 23.58 to a post-intervention index of 45 ± 24.03 ($P < 0.02$), and the Pupae per Person Index (PPI) from a pre-intervention 0.39 ± 0.12 to a post-intervention 0.06 ± 0.05 ($P < 0.02$). In Hlaingthayar, the BI was reduced from 138 ± 19.17 to 63 ± 28.43 ($P < 0.02$), and the PPI from 3.1 ± 1.48 to 0.31 ± 0.28 ($P < 0.02$). Productive containers were mainly drums, spirit-worship/religious flower bowls/vases, cement tanks and ceramic jars. The behavioural assessment provided controversial results: while household members reported a pre-post improvement on disposal of discarded containers and cleaning of larval sites (from 38.6% to 81.6% in Insein and 26.8% to 64.1% in Hlaingthayar), the actual pre-post assessment conducted by the surveyors showed that the proportion of households with discarded containers lying around the premises did not change in Insein (25.6% to 29%) whereas it improved in Hlaingthayar (73.5% to 53.2%). The results of the study indicated that a significant reduction of entomological indices was achieved within a short period of three months mainly due to larviciding by temephos, whereas the contribution of the community-based environmental management efforts was heterogeneous and of controversial evaluation.

Keywords: Dengue; Survey; Key containers; Epidemic; Source reduction; Temephos; Myanmar.

#E-mail: wtunlin@mptmail.net.mm; dr.wtunlin@gmail.com



Introduction

In Myanmar, dengue is one of the leading causes of morbidity among children under the age of 10 years, with approximately 85% of cases occurring in this age group. Some adult cases have also been reported, especially from rural areas.¹

An annual average of 7000–10 000 cases of DF/DHF are reported nationwide. However, in 2001, 2002 and 2007, this number rose to over 15 000 cases. National figures indicate that the largest number of cases occur in Yangon Region. In the 2007 epidemic, a total of 4759 cases with 54 deaths were reported from Yangon Region alone (comprising 45 townships). These data should be considered as the proverbial tip of the iceberg as only those patients who attend public health services, mainly hospitals, get reported, whereas a large majority who use private health care providers do not get reported in the official statistics, not to mention the mild or asymptomatic cases which remain undiagnosed. (Source: Vector Borne Disease Control (VBDC), Department of Health, Myanmar).

On 2–3 May 2008, the cyclone Nargis devastated Myanmar; and Ayeyarwady and Yangon Regions were the most affected. The destruction caused by the cyclone and the consequent disruption of all sectors and services became a risk factor for a possible increase in several infectious diseases, including dengue.

WHO, in collaboration with national and international partners, drew up an Action Plan for scaling up dengue prevention and control for the cyclone Nargis-affected populations. This Action Plan gave first priority to townships in the Ayeyarwady Region and second priority to dengue-prevalent townships in the Yangon Region. The Health Department of Yangon Region, in collaboration with “The Mentor Initiative”, an international nongovernmental organization which was part of this coordinated effort, implemented a community-based dengue source reduction project.

Materials and methods

Study area and study period

Two townships, Insein and Hlaingthayar, belonging to the northern districts of Yangon Region, which were among the top 10 townships with the highest dengue incidence in the previous five years, were selected for project implementation by the Health Department of Yangon Region.

Yangon city has a population of 5 million and is 60 m above sea level. The average daytime temperature is 31.4°C, the relative humidity is 67–91.9% and the average annual rainfall is 2833 mm. There are three seasons: wet season (June–October), dry-cool (November–February) and dry-hot (March–May). The study period was of six months from March to August 2009.



Study design

The objective of the study was to assess the effectiveness of a community-based source reduction project with pre- and post-intervention surveys including two components: a larval survey, collecting the entomological indices, and a knowledge–attitude–practice (KAP) survey investigating a few key behavioural indicators. The findings are intended for reproducibility and coverage of this particular intervention model rather than generalization/representativeness for the whole township.

Sample size

The sample size estimation for this study is based upon the estimation of one multi-country study supported by WHO/TDR, namely: “Eco-bio-social (EBS) research on dengue vectors in Asia” (2007–2011). Phase 1 of the multi-country study in Yangon had been completed in early 2008. Baseline data (already published in *WHO Bulletin*, March 2010), based on negative binomial distribution, revealed different levels of Pupae per Person Index (PPI) with lower dispersion than anticipated.² Therefore, the sample size calculation for Phase 2 (intervention phase) of EBS research study starting April 2009 was based on the assumption of normal distribution of PPI and also for Breteau Index (BI) for analysis on sample cluster instead of household level.

In this study, there were no “control clusters” to implement cluster randomization of the intervention. The formula of Lwanga and Lemeshow (1991) was used rather than the formula from Hayes and Bennett (1999).^{3,4} The assumptions of mean level of PPI being 0.3 in baseline clusters and 0.1 in post-intervention clusters with SD of 0.1; mean level of 40 for BI in pre-intervention clusters and 10 in post-intervention clusters with SD of 20 for this study. This formula was used after thorough discussion among public health specialists, entomologists, and statisticians at Community of Practice Workshop for EBS dengue vector research in Chennai, India (February 2008) sponsored by WHO and IDRC. For a significance level of 5% and a power of 80% to detect the difference between changes in Pupae per Person Index (PPI) as mentioned above, the needed number of clusters per township was 4. In actual implementation, six clusters were selected in each township. The formula used for sample size calculation was:

$$n = 2 \sigma^2 (Z_{1-\alpha} + Z_{1-\beta})^2 / (\mu_1 - \mu_2)^2$$

where,

$1 - \alpha$ = level of significance

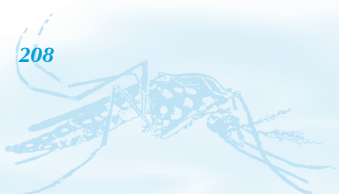
$1 - \beta$ = power of the test

σ = population standard deviation

σ^2 = population variance

μ_1 = test value of population mean

μ_2 = anticipated population mean



For the KAP study, two additional clusters were selected in each township, which means 800 KAP questionnaires per township, to achieve a statistical significance of 95% and a statistical power of 90% in detecting at least a 10% behavioural change, assuming a baseline level of 50% and a design effect of two. In total, 1600 KAP questionnaires were filled in both pre-intervention and post-intervention surveys.

Sampling procedure and entomological surveys

Sampling for houses in each cluster were carried out according to Tun-Lin et al. (2009) with some modification and based on the concept of Tun-Lin et al. (1995a).^{5,6} This was to ensure that the sampling will contain maximum number of highly productive containers. This is a modification of the WHO/TDR method to include key containers, especially rare but extremely productive containers (REPC), as much as possible, as these containers are not normally distributed.

For residential areas, the method of a household cluster survey was adopted and each cluster included 100 households. After the number of clusters necessary to achieve the requested statistical significance was calculated, (six for the entomological and eight for the KAP survey), they were randomly selected among the wards covered by the project — 14 wards in Insein and 12 wards in Hlaingthayar — with no more than one cluster per ward. A cluster comprises 100 households within a 'U' shape area of a ward.

Interventions

The activities aimed at reducing dengue vector breeding sites were largely inspired by the social mobilization and communication methods of the Communication for Behavioural Impact (COMBI) approach and were influenced by similar COMBI experiences carried out in other South-East Asian countries.^{7,8}

In collaboration with the Vector Borne Disease Control Department (VBDC) and the Dengue Prevention Assistant (DPA) volunteers, a two-round campaign was implemented, eight weeks apart, for the application of the larvicide, temephos (Abate, 1% SG) in water containers that could not be appropriately covered or cleaned weekly. Family members comprising a total beneficiary population of about 363 106 living in 63 187 households were prompted to regularly carry out every Saturday, with the support of 2000 DPA volunteers, two specific actions: clean and scrub water containers, and inspect the home, both inside and outside, for potential mosquito larvae sites/containers and discard them properly.

Routine dengue control measures in Yangon Region

These are: sweep method by supervised local people based on the method by Tun-Lin et al. (1994, 1995b), dragon-fly nymphs based on the method by Sebastian et al. (1990), fish plus



overall routine interventions (source reduction) and temephos in some wards by community volunteers and MOH staff.^{9–11} However, the level of implementation varied from township to township.

Public places

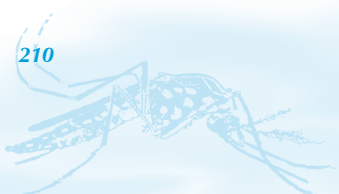
As regards public places, those within 100 metres of the nearest cluster houses such as religious places, police station, bazaar, schools, administrative buildings and hospitals were inspected in both the townships (Insein: $n=28$; Hlaingthayar: $n=47$) during the pre-intervention survey. The same public places were inspected during the post-intervention survey. In Insein, an additional two places were inspected ($n=30$). In Hlaingthayar, due to limited time for the surveyors, only 34 public places were inspected.

Data entry and statistical analysis

Data was entered in EpiData and an analysis was undertaken using SPSS software. After entering and cleaning the data in each township, they were then merged into a single database and analysed with SPSS 15. Repeated measures were not taken into account, as there was only one post-intervention survey measurement. Statistical significance using non-parametric tests (test of difference between paired samples and comparison of two groups (Wilcoxon signed rank test and Mann-Whitney U test) were applied as there was non-normality of the data sets which could not be corrected by a suitable transformation.^{12,13}

Key containers and highly pupal-productive containers

A key container is a container which contributes significantly to the total *Ae. aegypti* immatures in a ward/suburb or a region. It is difficult to determine the criteria (the cut-off point of the high numbers of *Ae. aegypti* immatures) for defining a key container. Based on a previous study (Tun-Lin et al. 1995), it was determined that the mean larvae-pupae per positive drum ($n=28$) obtained by absolute count was 450 and, so, arbitrarily, a key container was defined as ≥ 500 immatures.¹⁰ Of the immatures, 10% are usually the numbers of pupae in most of the moderate to highly positive containers and, so, 50 pupae was arbitrarily taken as the minimum number for a container having high numbers of pupae. This is the nearest assumption, but may not be applicable for all moderate to highly positive containers. Temephos is commonly used in dengue vector control in the study sites. Therefore, ethical approval is not necessary for the conduct of these implementation activities for dengue control by the Health Department. However, verbal informed consent was obtained from every household before application of temephos in water containers and the conduct of structured interviews. Confidentiality was maintained regarding the household data supplied.



Results

Entomological findings

A summary of pre-intervention and post-intervention findings and the details of entomological indices are shown in Table 1.

Table 1: Comparison between results of pre-intervention and post-intervention entomological surveys in Insein and Hlaingthayar townships, Yangon Region, May and August 2009

Indicator	Insein		Hlaingthayar	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Premises visited	600	600	600	600
Number of residents	3048	3019	3811	3696
Premises positive for larvae and/or pupae	249	133	350	181
House Index Premises positive for pupae	42±6.7 101	22±9.33 22	58±5.66 233	30±11.7 79
House Pupae Index Number of containers with water Containers positive for larvae and/or pupae	17±5.01 5147 514	3.6±2.35 4939 272	39±7.84 4929 827	13.1±5.55 4777 380
Container Index Containers positive for pupae	10±3.28 154	5.5±2.6 30	17±1.37 372	7.9±3.09 103
Container Pupae Index Total number of pupae	3±1 1216	0.6±0.33 180	8±1.37 11 857	2.1±0.91 1138
Pupae per Person Index (Number of pupae per person)	0.39±0.12	0.06	3.1±1.48	0.31±0.28
Breteau Index (Positive containers/100 houses)	86±23.58	45±24.03	138±19.17	63±28.43

A comparison between the percentage reduction and significant levels of pre-intervention and post-intervention PPI and BI entomological indices (test of difference between the same six clusters, pre-post intervention) in Insein and Hlaingthayar townships respectively are shown in Table 2. The results showed significant levels in the percentage reduction of the entomological indices in each township.



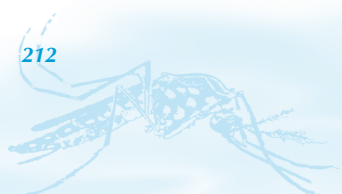
Table 2: Test of difference between paired observations on PPI and BI in Insein and Hlaingthayar townships, Yangon Region

Indicators	Insein		Percentage reduction	Significant level
	Pre-intervention	Post-intervention		
Pupae per Person Index (PPI) (number of pupae per person)	0.39 Mean = 0.398 SD = 0.13	0.06 Mean = 0.058 SD = 0.066	84.60	$P < 0.02$ Wilcoxon signed rank test
Breteau Index (BI) (positive containers/100 houses)	86 Mean = 85.67 SD = 28.03	45 Mean = 45.33 SD = 26.32	48.00	$P < 0.02$ Wilcoxon signed rank test
Indicators	Hlaingthayar		Percentage reduction	Significant level
	Pre-intervention	Post-intervention		
Pupae per Person Index (PPI) (number of pupae per person)	3.1 Mean = 3.12 SD = 1.63	0.31 Mean = 0.31 SD = 0.32	90.20	$P < 0.02$ Wilcoxon signed rank test
Breteau Index (BI) (positive containers/100 houses)	138 Mean = 137.8 SD = 21.01	63 Mean = 63.33 SD = 31.2	54.30	$P < 0.02$ Wilcoxon signed rank test

A comparison between pre-intervention PPI and BI entomological indices in Insein and Hlaingthayar showed that there was a significant difference (Mann Whitney U test, $P < 0.01$), but post-intervention, PPI and BI entomological indices showed that there was no significant difference (Mann Whitney U test, $P > 0.05$) between the two. It showed that regardless of the significant differences in the entomological indices in the pre-intervention period (as in the two study townships), the indices in the post-intervention period were about the same (i.e. gave the same results) ($P > 0.05$).

Some clusters belonged to moderate socioeconomic status (more concrete cement buildings) and the majority were in the low socioeconomic status (more bamboo/wood buildings). The entomological indices per socioeconomic level could not be produced, since it was not designed to cover this aspect.

In Insein township, during the pre-intervention survey, out of 5147 assorted positive containers detected, 1216 pupae were counted/estimated and 420 pupae (34.5%) were from 869 positive drums. In Hlaingthayar, during the pre-intervention survey, out of 4929



assorted positive containers detected, 11 857 pupae were counted/estimated and 7765 pupae (68.5%) were from 984 positive drums. The percentage distribution of productive containers during the two surveys in the two studied townships are shown in Tables 3A & 3B.

Table 3A: Productive containers, Insein township, Myanmar, May 2009

Type of container	No. of containers	Pupae count
Drum	869	420 (34.5%)
Cement tank (any shape)	141	129 (10.6%)
Ceramic jar	182	40
Overhead tank	117	0
Spirit worship flower (vase/bowl)	933	292 (24.0%)
Religious flower vase	2028	184 (15.1%)
Car tyre	13	15
Bucket (metal, plastic)	144	6
Coconut shell	0	0
Plastic bag for fire prevention	63	0
Tin	8	25
Flower pot	16	0
Miscellaneous discarded broken items	26	7
Toilet container	193	8
Bamboo stumps	0	0
Small drum	24	0
Other	390	90
Total	5147	1216



Table 3B: Productive containers, Hlaingthayar township, Myanmar, May 2009

Type of container	No. of containers	Pupae count
Drum	984	7765 (65.5%)
Cement tank (any shape)	118	689 (5.8%)
Ceramic jar	191	1085 (9.1%)
Over-head tank	38	0
Spirit worship flower (vase/bowl)	778	1457 (12.3%)
Religious flower vase	1857	164
Car tyre	54	121
Bucket (metal, plastic)	217	42
Coconut shell	41	0
Plastic bag for fire prevention	15	5
Tin	20	5
Flower pot	69	45
Miscellaneous discarded broken items	116	38
Toilet container	239	89
Bamboo stumps	1	5
Small drum	73	204
Other	118	143
Total	4929	11 857

Key containers

In Insein, the pre-intervention survey results showed that there were three key containers (with larvae and/or pupae ≥ 500 immatures) but during the post-intervention survey, not a single key container could be detected. In Hlaingthayar, there were 29 key containers during the pre-intervention survey, but during the post-intervention survey, only four key containers were detected. In comparing positive containers with pupae ≥ 50 between pre-intervention and post-intervention surveys, it was found that there were no positive containers with pupae ≥ 50 in Insein township; but in Hlaingthayar township, the % reduction was 90.6% (from 43 positive containers with pupae ≥ 50 to only four positive containers with pupae ≥ 50 (Table 4).



Table 4: Comparison of key containers and positive containers with pupae ≥ 50 , Myanmar

Positive container type	Insein		Hlaingthayar	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Positive but not key containers	511	272	798	376
Key containers (≥ 500 immatures)	3	0	29	4
Positive containers with pupae ≥ 50	0	0	43	4

Temephos (Abate) coverage

Temephos coverages of the major water-storage containers (≥ 30 gallons) were 71.2% ($n=2878$) and 78.3% ($n=2908$) in Insein and Hlaingthayar, respectively, according to the survey results. For spirit-worship flower bowls/vases, temephos treatment was encouraged if changing of the water was not regular, and so the percentages of treated spirit-worship flower bowls/vases were only 31.7% and 16.8% in Insein and Hlaingthayar, respectively.

KAP

With regard to four main indicators assessing the project impact on dengue prevention among the target population, the findings are given in Table 5.

In Insein, the percentage of households which reported having assured appropriate and regular (once a week) care of their major water-storage containers (≥ 30 gallons) in the past two weeks was 84.5% during the pre-intervention survey, which decreased to 58.5% during the post-intervention survey. Similarly, in Hlaingthayar, it was 88.7% that decreased to 60.5% respectively. This is not surprising and is in line with the recommendation of not emptying the containers treated with temephos.

In Insein, households which reported having disposed of discarded containers or cleaned potential larvae sites at least once during the past two weeks was 38.6% during the pre-intervention survey, which increased to 81.6% during the post-intervention survey. Likewise, in Hlaingthayar, it was 26.8% which increased to 64.1% respectively.

In Insein, it was observed that households with discarded containers (tyres, tins, cans, coconut shells, etc.) lying around their premises/compounds was 25.6% during



Table 5: Four main behavioral indicators assessing the project impact on dengue prevention among the target population in Insein and Hlaingthayar townships, Yangon Region, 2009

Indicator	Insein		Hlaingthayar	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Households reported appropriate and regular (once a week) care of their major water-storage containers (≥ 30 gallons) in the past two weeks	650 84.50% (n = 769)	451 58.50% (n = 771)	668 88.70% (n = 753)	462 60.50% (n = 763)
Households reported having disposed of discarded containers or cleaned potential larvae sites at least once during the past two weeks	309 38.60% (n = 800)	653 81.60% (n = 800)	215 26.80% (n = 800)	513 64.10% (n = 800)
Households with discarded containers (tyres, tins, cans, coconut shells, etc.) laying around the premise/compound*	205 25.60% (n = 800)	232 29.00% (n = 800)	588 73.50% (n = 800)	426 53.20% (n = 800)
Households with major water-storage containers (≥ 30 gallons) not well covered*	615 79.90% (n = 769)	689 89.40% (n = 771)	691 91.70% (n = 753)	729 95.20% (n = 763)

*Observations

pre-intervention and increased slightly only to 29.0% during post-intervention period. In Hlaingthayar, it was 73.5% which decreased to 53.2% during post-intervention.

In Insein, it was observed that the percentage of households with major water-storage containers (≥ 30 gallons) not well covered was 79.9% during the pre-intervention survey which increased to 89.4% during the post-intervention survey. In the same way, in Hlaingthayar, it was 91.7% initially and increased only minimally to 95.2%, respectively.



The slight worsening of this indicator indicates that household members, confident in the larviciding activity of temephos, reduced their attention in covering the water-storage containers. Additionally, the discrepancies found between reported behaviour and actual observation in the field of covering water-storage containers and disposing of discarded containers suggests an incomplete reliability of the answers provided.

Regarding public places, during the pre-intervention survey, Insein had only four pupae/hectare and during the post-intervention survey, pupae were not detected. In Hlaingthayar, during the pre-intervention survey, there were 57 pupae/hectare and during the post-intervention survey, only two pupae/hectare were detected. It is worth noticing that in Hlaingthayar, during the pre-intervention survey, out of 2072 pupae counted, 1211 pupae (58%) were found in a police station where the staff and their family members were living. The market was second, with 276 pupae (13.3%), and the schools third, with 256 pupae (12.4%).

Discussion

The present study reported effective reductions of at least 80% for PPI and approximately 50% for BI as a result of the community-based implementation of an integrated approach tailored to local eco-epidemiological and sociocultural settings in the two selected townships.

With regard to the four results describing people's behaviour: two results were worse after the interventions than before. The explanation could be that the household members were aware that Abate insecticide would prevent the breeding of mosquito aquatic stages in their major water-storage containers even if the big containers were not well covered. Some households may not be giving the correct answers and observations made on the peri-domestic areas will provide the right answers.

On the other hand, one cannot expect an effect of a community-based intervention only three months after its start, but community-based activities may have contributed, even if in small proportion, to the reduction in indices, which was caused by mostly the application of temephos (Abate) but not by "temephos (Abate) alone". To prove both opinions is difficult, because the two interventions were simultaneously implemented in the same areas. The entomological survey was conducted after the first round of Abate was completed, so its impact cannot be separated from the community's own actions. In Myanmar, *Ae. aegypti* is the main target of dengue prevention and control measures. No temephos (Abate) resistance to *Ae. aegypti* has been reported in Myanmar so far.

It seems that when temephos (Abate) is distributed and used, people don't clean/discard or cover their containers any more. This is potentially harmful, as people only rely on Abate and do not realize the other necessary preventive actions. More information should be provided to the community and stakeholders for the long-term dengue control measures to be successful.



In Myanmar, the majority of the population is Buddhist and there are religious flower vases kept for worshipping. In addition, there are also people who worship “Nats” or spirits and keep spirit-worship flower vases (SWFV) either inside or just outside their houses. These containers are big bowls containing 2–3 litres of water and when they become positive for larvae and/or pupae, the volunteers and health staff are reluctant to touch the containers to tip the water. Thus, many SWFVs become highly productive containers. In our study, the volunteers and health staff were instructed to put Abate in these SWFVs after asking prior permission from the house owners.

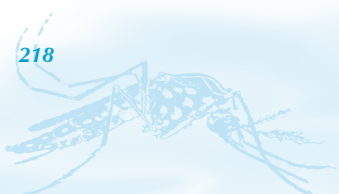
Pupae serve as a proxy measure for adult mosquitoes. Unlike PPI, other entomological indices BI, HI and Container Index (CI) do not offer a measure of its relative importance as a breeding site because it does not provide information on how many adult mosquitoes develop and are ultimately produced in it. Thus, in Hlaingthayar, five key containers which are drums (with estimated pupae counts varying from 500 to 2000) were responsible for half the value of PPI. However, in a flower vase factory premises on the outskirts of Insein, hundreds of small flower vases were producing high numbers of larvae and adult *Ae. aegypti*. This will be a high-risk area for the community in Insein.

In Hlaingthayar township, during the inspection of public places, it was found that a police station compound, where the staff and their families were living, was home to approximately 50% of the total pupae population discovered. This inspection was done without prior warning to the police station staff and thus the true situation was determined. It is important that prior warning should not be given to the householders at the sites to be inspected since the breeding sites could be cleaned/removed by them. Biased results will then occur. This is what happened to this public place during the post-intervention survey, or it could also be due to the application of temephos that there were only a few containers found positive at the time.

Tyres lying along the roads in public spaces and beside or underneath houses are breeding sites that are difficult to treat with temephos. Key containers are also high-risk sites for human-vector contact and should be removed or treated regularly.

These entomological and behavioural indicators allowed us to make a statistical comparison of the situations occurring before and after the interventions, and, in particular to: assess the impact on the density of *Aedes* immatures (larvae and pupae); identify the most productive water containers to prioritize interventions on key containers; and assess the impact on dengue prevention behaviour among the target population. Hence, a simple calculation on the percentage reduction was preferred for dissemination of the salient findings to target beneficiaries.

The larvae and/or pupae in the highly productive drums are routinely removed by volunteers or health staff using locally available cotton net sweepers. The container-variety dragon-fly nymphs, *Bradynopyga geminata* (Rambur) (about 2–5 cm in length), are usually present and found clinging to the inner-side of the drums. In such drums, there are no larvae



and/or pupae found and thus the use of cotton net sweepers is not needed. Sometimes, house owners introduce local larvivorous fish, such as *Aplocheilus panchax*, into the drums. Thus, locally available mechanical and bio-control measures are already routinely used in some townships in Yangon Region, but the above activities were minimal in the two study townships.

The use of locally available cotton net sweepers (which have been approved by the Ministry of Health, Myanmar) and the dragon-fly nymphs and larvivorous fish should be encouraged for the long-term sustainability of dengue prevention and control measures. It was encouraging to note that Mentor Initiative was supporting the Yangon Health Division and the two study townships with extra cotton net sweepers. Seed money was also provided to the Ward 18 Administrative Council in their endeavour to successfully breed local larvivorous fish in their office compounds. This is a good feature for the sustainability of the dengue control project in Yangon Region, as community-based dengue source-reduction measures using locally available mechanical and biological control methods should be a viable alternative to the long-term use of temephos for dengue control activities.

Conclusion

The results of the study indicated that significant reductions in entomological indices were possible within a short period of three months mainly due to larviciding by temephos, whereas the contribution of the community-based environmental management efforts was heterogeneous and subject to controversial evaluation.

Acknowledgements

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Comparative field efficacy of different formulations of trypsin modulating oostatic factor-*Bacillus thuringiensis israeliensis* (TMOF-Bti) against *Aedes aegypti* Linnaeus by manual application and ultra-low volume (ULV) spray at Setapak high-rise flats in Kuala Lumpur, Malaysia

Saiful Azlan Nordin,^{a#} Lau Ming Seong,^a Sallehudin Sulaiman,^a
Paramessarvathy Ramanathan,^b Zainol Ariffin Pawanchee,^c
Sallehudin Abu Bakar^b and Hidayatulfathi Othman^a

^aDepartment of Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia

^bDepartment of Health, Kuala Lumpur City Hall, 4, Jalan Cheras, 51600, Kuala Lumpur, Malaysia

^cDepartment of Health (Public Health) of Kuala Lumpur and Putrajaya, Jalan Cenderasari, Kuala Lumpur, Malaysia

Abstract

Bacillus thuringiensis israeliensis (Bti), an entomopathogenic bacteria, is a naturally occurring soil bacterium registered for mosquito larvae control. Trypsin modulating oostatic factor (TMOF), a peptide hormone originally isolated from the ovaries of adults *Aedes aegypti*, is currently under commercial development as a new insecticide with a novel mode of action for the control of mosquito larvae. This hormone stops mosquito larvae from producing trypsin, a crucial digestive enzyme ultimately leading to starvation, and death. The objective of this study was to evaluate the effectiveness of TMOF-Bti formulations against *Ae. aegypti* under field situation. Three blocks of 17-storey flats at Ayer Panas, Setapak, Malaysia, were chosen for this study. Each block received a total of 36 containers, each one containing 20 first instar *Ae. aegypti* larvae in 4 l of water. Crushed dry leaves were provided as food. The containers were placed at alternate levels of the 17-storey flats. Two units of flats in each selected level were provided with two containers for each unit. Two formulations of TMOF-Bti were tested: 20% TMOF + 20% Bti wettable powder formulation, 4% TMOF + 4% Bti rice husk formulation. The first block was selected as control, the second block was treated with a concentration of 50 mg/4 l of 4% TMOF + 4% Bti rice husk which was applied manually into the containers. The third block was sprayed using ULV with 30 g/3 l formulation. The field trial was repeated by using 25 and 10 mg/4 l of 4% TMOF + 4% Bti rice husk, 60 and 90 g/3 l of TMOF-Bti wettable powder respectively. New batches of first instar *Ae. aegypti* larvae were introduced into each container for every two weeks with addition of TMOF-Bti formulations. Larval mortality was recorded every 24 hours and weekly after spraying and manual application of TMOF-Bti. The results showed that 4% TMOF + 4% Bti rice husk formulation resulted in 100% larvae mortality on the first instar compared to wettable powder which presented a lower mortality percentage of 60% to 80% in both trials. Both TMOF-Bti in rice husk and wettable powder formulations were proven to be effective against first instar larvae of *Ae. aegypti* in the field.

Keywords: TMOF-Bti; *Aedes aegypti*; 4% TMOF + 4% Bti rice husk; Wettable powder; Mousticide; Malaysia.

[#]E-mail: saifulazlan87@gmail.com



Introduction

Bacillus thuringiensis israelensis (*Bti*) is a gram positive bacterium used as a larvicide that causes death to *Aedes aegypti* larvae. Delta endotoxin from *Bti* are ingested by the larvae which bind to the cells of the mid gut's epithelium, causing disruption to the osmotic balance within the mid gut's epithelium cells by forming pores in the cell membrane and lysing the cells.¹

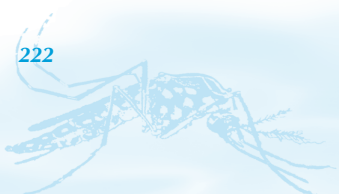
Trypsin Modulating Oostatic Factor (TMOF) is a decapeptide hormone developed from the ovaries of the mosquito itself. It halts trypsin biosynthesis in the mosquito gut. The hormone, when orally administered to different species of mosquito larvae, stops food, digestion, resulting in larval mortality.² When combined with *Bti*, it exhibits a synergistic effect. Initially, there is a low level of endogenous trypsin present in the gut which assists in the activation of *Bti* Cry toxin. Ingestion of food stimulates additional trypsin synthesis. The release of the induced trypsin is prevented by TMOF. *Bti* binds on a receptor within the mid gut cells of mosquito larvae in order to kill the larvae.³ When there is extra food available for the larvae, the receptor gets blocked by the food. TMOF causes starvation and prevents the exogenous proteins from blocking the access of the *Bti* Cry toxins to the mid gut cells, thereby allowing cell lysis to occur, which then increases the access of TMOF to the larvae resulting in more starvation and then more cell lysis by *Bti* Cry toxins.⁴ This self-amplifying mechanism is what is termed as the "mutual synergistic effect" of TMOF and *Bti*. It showed an increase in efficacy in killing mosquito larvae compared with TMOF or *Bti* alone. We evaluated the efficacy of TMOF-*Bti* (Mousticide) in rice husk and wettable powder formulations against *Ae. aegypti* in high-rise flats under field conditions.

Materials and methods

Field trial at high-rise flats in Kampung Baru Air Panas, Setapak

Two different formulations of TMOF-*Bti* were evaluated against first instar larvae of *Ae. aegypti* in high-rise flats in Kuala Lumpur, viz. wettable powder and rice husk formulations.* Three blocks of 17-storey high-rise flats consisting of 136 units of flats in Setapak (3°11'N 101°42' E), namely, Block A, H and F, in Kampung Baru Air Panas were selected in order to test the efficiency of TMOF-*Bti* against larvae of *Ae. aegypti* in field conditions. Each block was located at least 500 m apart. The three blocks were chosen randomly. Kampung Baru Air Panas was chosen because this area has a very high potential for dengue infection being located in Setapak, which is one of the blacklisted areas with a high incidence of dengue cases, according to Kuala Lumpur City Hall Council. Furthermore, the baseline study of the mosquito population in this area, which was done before the trial was started, showed that the population of *Ae. aegypti* mosquitoes in this area was quite high.

* Both formulations were supplied by EntoGenex Industries Sdn Bhd, Malaysia.



These three blocks were chosen as the study sites based on a comparison of the ecology of natural mosquito breeding habitat to the area around the study sites. *Ae. aegypti* tends to breed in locations with human habitations. It usually stays indoors and rests on the wall, ceiling and in dark spaces in the house. It breeds in clean, clear water, which is stored in any water-holding container and prefers to feed on human blood. The surrounding environment around the study blocks had high-potential breeding sites.

A survey of the potential mosquito breeding sites around the blocks was undertaken before the trials were started. The potential breeding sites found included flower pots and buckets in front of most of the units. During the rainy season, damaged floors, corridors and solid waste around the units support heavy breeding of *Ae. aegypti*.

TMOF-Bti in wettable powder formulation sprayed by ULV was treated to Block H. Block F was treated with TMOF-Bti in rice husk formulation distributed manually, while Block A was kept as control.

Six weeks before the field trial, an ovitrap survey was conducted to obtain the baseline information of *Ae. aegypti* ovipositions. Thirty-six ovitraps per high-rise block were distributed on the 1, 3, 5, 7, 9, 11, 13, 15, 17 levels, with four ovitraps on each floor chosen from two units. The units were randomly chosen on each floor. One ovitrap each was put outside and inside the chosen unit; the ovitraps were replaced weekly. Paddles of the ovitraps were taken to the laboratory for egg counting. Crushed leaf litters were supplied as food source. The larvae that hatched from eggs were identified and counted.

The study consisted of two formulations, each with three different weights for each trial. TMOF-Bti in rice husk formulation was manually applied at 50 mg/4 l in the first trial, 25 mg/4 l in the second trial and 10 mg/4 l in the third trial, while wettable powder was sprayed using ULV at 30 g/3000 m², 60 g/3000 m² and 90 g/3000 m² in the first, second and third trials respectively. The wettable powder formulation in three litres of water for each concentration was sprayed using LECO/Model 1600 cold aerosol generator mounted on a vehicle. The head nozzle of the sprayer was adjusted at an angle of 45° to the ground. The flow rate of ULV application was estimated at 104 ml/min and the velocity of the vehicle was approximately 6 km/h.

In each trial, 20 first instar *Ae. aegypti* larvae were distributed into each bucket early in the morning (0900 hrs) for each block. TMOF-Bti in rice husk formulation was manually treated at F block. Wettable powder was sprayed using ULV at Block H later in the evening at 1700 hrs, as this time is the active period for *Ae. aegypti* biting. A block was sprayed by ULV with three litres of water only as the control. In all cases, all windows and doors remained open during ULV spraying. Larval mortality was assayed in all buckets 24 hours after spraying of compound application. The buckets were covered by mosquito netting. (In case of any dengue cases occurring which required fogging in that area, buckets were covered by lids provided during fogging operation.) Larval mortality was assayed in all buckets 24 hours after spraying of compound application. After one week, all buckets were re-assayed for



larval mortality. All dead and surviving larvae were taken out from the buckets. Treatment was repeated in the following week with a new batch of 20 first instar *Ae. aegypti* larvae and application of TMOF-Bti in rice husk and wettable powder formulation. In each trial, it consisted of eight treatments. Between each trial, there was one-month interval to help build up the *Aedes* population.

Results

Table 1 includes the baseline population of *Ae. aegypti* in Kampung Baru Air Panas, Setapak, which was conducted for seven weeks before the first trial. In Block A the eggs ranged from 83 to 1232, in block F their number ranged from 32 to 223, and in Block H these ranged from 41 to 368 eggs.

Table 1: Baseline of natural population of *Ae. aegypti* mosquito's eggs in PPR Kampung Baru Air Panas, Setapak, Malaysia

Block/week	1	2	3	4	5	6	7
A	83	184	198	964	588	1232	441
F	188	32	93	175	223	183	221
H	368	181	251	173	41	192	198

Tables 2, 3 and 4 show the natural population of the eggs of *Ae. aegypti* during the first, second and third trials. Table 2 shows a statistically significant difference in the number of eggs between the three blocks where the significance level is $p < 0.05$, $p = 0.01$. Table 3 shows the number of eggs during the third trial where there was no significant difference between the three blocks $p < 0.05$, $p = 0.955$, with a mean rank of 28.39 for Block A, 27.25 for Block F and 26.86 for Block H when tested with Kruskal Wallis test. Table 4 indicates the number of eggs between the three blocks in the third trial. There was a significant difference in the number of eggs between the three blocks where $p < 0.05$, $p = 0.01$ with a mean rank of 40.31 for A block, 21.78 for F block and 20.42 for H block when tested with Kruskal Wallis test.

Table 2: Natural population of *Ae. aegypti* mosquito's eggs in PPR Air Panas, Setapak, Malaysia (1st trial)

Block/week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A (control)	68	175	195	966	563	1221	492	751	200	572	452	425	366	1426	440	408	272	823
F (50 mg/4 l)	194	39	101	159	239	172	243	212	129	238	195	301	293	235	347	261	254	105
H (30 g/4 l)	326	160	278	141	50	185	187	252	279	413	284	285	263	518	376	367	295	381

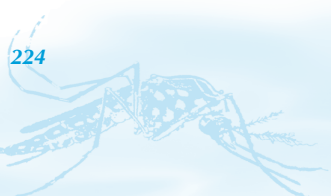


Table 3: Natural population of *Ae. aegypti* mosquito's eggs in PPR Air Panas, Setapak, Malaysia (2nd trial)

Block/week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A (control)	356	289	259	382	47	227	125	146	118	49	21	35	75	90	84	95	87	81
F (25 mg/4 L)	571	452	299	213	134	1117	95	254	34	68	45	60	118	87	49	63	71	56
H (60 g/4 L)	351	431	342	365	148	163	76	92	75	105	35	50	85	65	70	65	61	63

Table 4: Natural population of *Ae. aegypti* mosquito's eggs in PPR Air Panas, Setapak, Malaysia (3rd trial)

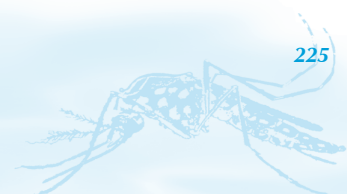
Block/week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A (control)	461	601	551	641	501	489	402	513	385	274	394	352	461	435	382	408	301	305
F (10 mg/4 l)	571	417	511	402	376	321	201	164	101	100	142	81	87	76	66	41	91	58
H (90 g/4 l)	511	491	450	366	239	115	187	211	125	97	82	86	85	72	75	69	84	82

Table 5 shows the result of larval mortality at A block which was the control. The percentage of larval mortality was much lower in the second and the third trials compared to the first trial. The average of weekly larval mortality ranged between 15% and 40% respectively.

Table 5: Percentage of *Ae. aegypti* mosquito's larval mortality (Block A – control), Setapak, Malaysia

Trial/ Treatment	1st treatment		2nd treatment		3rd treatment		4th treatment		5th treatment		6th treatment		7th treatment		8th treatment	
	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h
Block A (4 l water)	56.94	87.22	24.72	33.33	40.56	49.44	50.55	70.14	47.92	57.36	38.89	42.64	31.25	45.56	34.31	47.08
Block A (4 l water)	24.52	33.61	21.25	27.63	40.69	47.5	26.39	40.69	10.2	36.11	11.38	42.36	6.67	34.31	5.51	24.72
Block A (4 l water)	1.8	18.47	24.72	33.33	16.94	24.17	22.22	41.25	14.72	47.64	12.36	34.58	14.17	26.11	6.67	36.11

Table 6 shows the result after spraying with TMOF-Bti in wettable powder formulation using ULV. In the first trial, the percentage of larval mortality was around 30% in the first 24 hours and increased up to 50% after one week. In treatment 5, the percentage of larval mortality decreased to 30% in the first 24 hours, but then increased to 80% after one week. In treatment 6 to 8, there were increases in the percentage of larval mortality to 40–50%, and one week later, the larval mortality increased to 70–90%, respectively. However, in the second and third trials, the percentages of larval mortality increased compared to the first



trial. The rate of larval mortality in 24 hours was 50–70% for the second trial and 40–60% in the third trial. After one week, the percentage of larval mortality increased to between 60%–80% for the second trial and to 70–80% in the third trial.

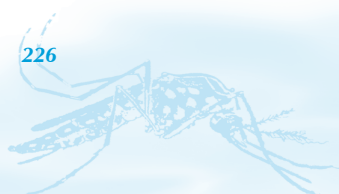
Table 6: Percentage of *Ae. aegypti* mosquito’s larval mortality against TMOF-Bti (wetable powder) at H block, Setapak, Malaysia

Trial/ Treatment	1st treatment		2nd treatment		3rd treatment		4th treatment		5th treatment		6th treatment		7th treatment		8th treatment	
	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h
Block H (30 gm/4 l)	64.77	75.83	31.67	55	33.66	57.08	31.67	52.22	31.67	78.47	44.44	74.03	45.42	90.69	54.72	80.69
Block H (60 gm/4 l)	50.83	78.1	73.89	83.08	56.57	81.94	54.08	65.4	50	73.61	54.36	75.42	49.43	72.28	50	73.5
Block H3 (90 gm/4 l)	57.80	76.97	41.08	72.81	59.87	80.42	38.61	72.08	40.84	76.04	49.40	74.73	47.43	81.49	52.36	77.10

Table 7 indicates the percentages of larval mortality in F block when TMOF-Bti in rice husk formulation was applied directly to all the buckets containing *Ae. aegypti* larvae. In the first trial, TMOF-Bti in rice husk formulation was able to provide 100% larval mortality after 24 hours, except in the third and sixth treatments when only 98.25% and 99.75% *Ae. aegypti* larvae were killed. However, after one week, eventually all the larvae died. The second trial was much better than the first trial, whereby the percentage of larval mortality was 100% in all treatments throughout the study period. Although the concentration of rice husk used decreased from 50 mg to 25 mg for four litres of water, the formulation still killed all larvae effectively. On the third trial, the amount of TMOF-Bti in rice husk formulation was decreased to 10 mg. Even with the lower dose the mortality percentage recorded was still 100% in every application.

Table 7: Percentage of *Ae. aegypti* mosquito’s larval mortality against TMOF-Bti (wetable powder) at F block, Setapak, Malaysia

Trial/ Treatment	1st treatment		2nd treatment		3rd treatment		4th treatment		5th treatment		6th treatment		7th treatment		8th treatment	
	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h	24 h	168 h
Block F (50 mg/4 l)	100	100	100	100	98.19	100	100	100	100	100	99.72	100	100	100	100	100
Block F (25 mg/4 l)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Block F (10 mg/4 l)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

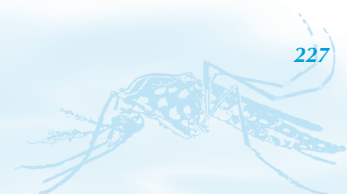


To find out whether there was a significant difference in the first, second and third evaluations of Mousticide in rice husk and wettable powder formulations, the data were tested with Anova using SPSS version 17.0. In the first trial, there was a significant difference in the larval mortality between the three blocks where $p < 0.05$, $p = 0.01$. The second and third trials also recorded the same result as the first where $p < 0.05$, $p = 0.01$ respectively. Thus, Mousticide™ in rice husk and wettable powder formulations were able to control the mosquito population in the field. To determine the best concentration to be used for wettable powder application using ULV machine, Anova test was used. The result indicated that $p < 0.05$, $p = 0.146$, stating that there was no significant difference between different weights of wettable powder used in this trial.

Discussion

TMOF is a peptide hormone which comes from the ovary of the mosquito itself, which is rich with oostatic hormone.⁵ In the mosquito, it serves as a hormone that binds at a specific binding site in the mid gut epithelial cells. In a condition where trypsin biosynthesis is at its highest level, the follicular epithelium of the ovary begins to synthesize and release TMOF into the hemolymph. Once in the hemolymph, TMOF binds to the gut epithelial cells and signals them to cease trypsin biosynthesis, which rapidly declines and stops.⁶ To transform TMOF into a new potential larvicide, TMOF was incorporated into yeast and fed to the larvae. Because of the feeding of TMOF, there will be some amount of TMOF in the mosquito gut even though there is still low level of trypsin in the gut.⁷ This will cause the mosquito not being able to synthesize trypsin in order to digest the food it eats and thus lead it to death by starvation.² In order to increase the efficacy of TMOF, it is mixed with *Bti* and *Pichia pastoris*, thus leading to a synergistic effect between them.⁴ This shows that TMOF has a great potential as a larvicide to be used in controlling dengue vector along with other larvicides such as temephos and *Bti*. In this study, it showed that TMOF-Bti in wettable powder and rice husk formulations were very effective as a larvicide in the field.

TMOF expressed in *P. pastoris* combined with *Bti* will cause synergistic effects and increase their efficacy on the larvae.⁴ TMOF with *Bti* rice husk (4% TMOF plus 4% *Bti*, 3% TMOF plus 1% *Bti*, 1% TMOF plus 3% *Bti*, 2% TMOF plus 2% *Bti* and 1% TMOF plus 1% *Bti*) caused high mortality (95%–97%) within one hour of application and caused complete mortality (100%) until 96 hours after treatment when tested in the laboratory. The mortality effect of TMOF with *Bti* was influenced by the rapid killing of *Bti* and prolonged killing of TMOF due to the residual effect.⁸ When TMOF in *P. pastoris* was combined with *Bti* toxin and exposed to 20 first instar larvae for six days, the mean of larval survival was 0.7 ± 0.7 (surviving larvae \pm S.E.M),⁴ indicating a very low larval survival after exposed to TMOF-Bti. In the present field study, TMOF-Bti in rice husk formulation was placed manually in buckets and it caused rapid mortality within 24 hours in every evaluation using different weights.

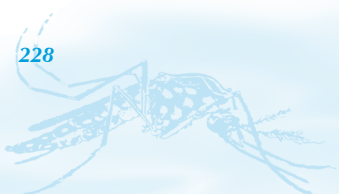


When *Ae. aegypti* and *Culex quinquefasciatus* larvae were fed TMOF that was adsorbed onto yeast cells, the larvae stopped synthesizing trypsin and stopped growing.² Based on a study conducted in the laboratory, 400 ppm *Pichia*-TMOF and 300 ppm *Pichia*-TMOF were able to cause 100% and 67% cumulative mortality respectively on *Ae. aegypti* larvae on the eighth day. At 200 ppm, 100 ppm and 50 ppm concentration *Pichia*-TMOF showed obvious stunted effect on *Ae. aegypti* larvae.⁹ Another study using TMOF, which was combined with yeast (*Pichia* 11 and *Pichia* 12), showed that by one hour of treatment and at 24 hours, there was 70% and 97.5% larval mortality, respectively. However, *Pichia* 11 and 12 caused complete mortality at 96 and 120 hours, respectively.⁸ In the present field study, the application of the TMOF-Bti in the wettable powder formulation using ULV indicated that there was low larval mortality in the first 24 hours after the application. However, after a week, there was an increased larval mortality up to 80%. This result could support the usage of TMOF-Bti in wettable powder formulation for field use as larvicide. It had been reported that TMOF-Bti in wettable powder formulation was able to cause high mortality even within one hour of exposure and resulted in complete mortality in 24 hours.⁸

Our study showed a significant difference in the number of eggs between the control block and the treatment block, where the number of eggs in the treatment block were much lower as compared to the control block.

A study in the laboratory using different ratios of TMOF with *Bti* rice husk (4% TMOF plus 4% *Bti*, 3% TMOF plus 1% *Bti*, 1% TMOF plus 3% *Bti*, 2% TMOF plus 2% *Bti* and 1% TMOF plus 1% *Bti*) caused high mortality (95–97%) within one hour of treatment and caused complete mortality (100%) 96 hours after treatment. From this data, we can see that even at lower ratios of TMOF and *Bti* (1%:1%), the effectiveness was as good as at higher concentrations.⁸ In the present field study, even by using a very low amount of 20% TMOF-20% *Bti* wettable powder and 4% TMOF-4% *Bti* rice husk (10 mg) formulation, these were still effective in killing *Ae. aegypti* larvae and in controlling the mosquito population.

TMOF-Bti is a combination of TMOF that is incorporated in yeast and mixed with *Bti*. This combination has been proven to have a synergistic effect where it increased the efficacy up to 100-folds.⁴ This combination is said to be able to overcome the problem posed by *Bti*. *Bti* effectiveness in controlling dengue vector has been demonstrated widely in the world.¹⁰ However, some concern has arisen as there are reports that resistance has developed against it as demonstrated by *B. sphaericus* and its decreased efficacy in the polluted water environment. A report has stated that when high toxicity of recombinant *E. coli* expressing *cry4Aa*, *cry11Aa* and *cyt1Aa* to 1st instar *Ae. aegypti* larvae was dramatically reduced in the presence of *P. pastoris* as a nutritious food source. However, the mortality was restored upon replacing it with recombinant *P. pastoris* producing TMOF. TMOF and *Bti* are synergistic against larvae of *Ae. aegypti* in organic-rich environment. TMOF enhances the toxicity of *d*-endotoxins by inhibiting trypsin biosynthesis, causing anorexia, starvation and death.^{4,11} In a condition without TMOF acting together with *Bti*, ingested nutritious particles in the larvae are able to reduce the susceptibility of insects against *d*-endotoxins.¹² Another



study has stated that sediment acted to decrease the efficacy of *Bti* by increasing settling of the toxic particles, but did not decrease the persistence of the *Bti* cells.¹³ In our study, we provided food to the larvae in the buckets to mimic a natural breeding condition. Even with the additional food supply to the larvae, the efficacy of TMOF-*Bti* was still the same where it killed all the larvae in 24 hours.

We noted that in this study, there was a difference in efficacy between TMOF-*Bti* in rice husk formulation and in wettable powder due to the different ways of application. By applying TMOF-*Bti* in rice husk formulation directly to the breeding site, the efficacy was much better compared to ULV spraying. The researchers observed that during spraying, some residents were not present at home, thus the windows and doors were not open. Furthermore, some residents did not comply with the agreement to put the buckets behind the doors and in the kitchen. They also closed the lid of the bucket so that the larvicide did not come into contact with the larvae when spraying was done.

Conclusion

Both TMOF-*Bti* in rice husk and wettable powder formulation were proven to be effective against first instar larvae of *Ae. aegypti* in the field.

Acknowledgements

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Guillain-Barré syndrome as a rare complication of dengue fever: a report of two cases

**Dinesh Srivastava, Kailash Kumar Goyal,[#] Sudeep Vaniyath and
Dhananjaya Melkunte Santhaiah**

*Department of Medicine, Postgraduate Institute of Medical Education and Research,
Dr Ram Manohar Lohia Hospital, New Delhi – 110001, India*

Dengue fever is the most rapidly spreading vector-borne disease in the world and more so, in the Asia subcontinent. The diagnosis of dengue is made by clinical and serological evidence. Available literature suggests the possibility of dengue virus as an etiological agent causing Guillain-Barré syndrome (GBS). The following case report describes two patients who presented at our tertiary care centre with paraparesis and quadriparesis, respectively, with areflexia which was preceded by fever and rash.

Case reports

Case 1

A 30-year-old male patient, from New Delhi, was admitted on 25 September 2012 with a history of acute high-grade fever for five days, accompanied by aches and pains, and was not associated with chills or rigors. The patient also gave history of noticing rashes all over the body on the third day of fever. The patient however became afebrile on the sixth day of the onset of the disease. During the stay in hospital, after being afebrile for two days, the patient noticed weakness in both the lower limbs. There was no history of bowel, bladder or sensory involvement. No recent history of diarrhoea, immunization or dog bite was given by the patient.

On examination, the patient was found to be conscious and oriented, with a blood pressure of 110/80 mm of mercury, pulse rate of 70/min and a respiratory rate of 15/min. The patient was afebrile to touch. A diffuse macular rash was observed all over the body (Figures 1 and 2). The neurological examination revealed decreased power in the four limbs with hypotonia. Deep tendon reflexes were absent in all the limbs. No sensory or bowel and bladder involvement was observed. An initial clinical diagnosis of viral fever with ascending type of motor polyneuropathy was made.

[#]E-mail: goyal31st@gmail.com

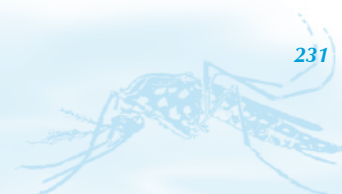


Figure 1: Rash classical of dengue fever seen on the upper limb of the patient (Case 1)

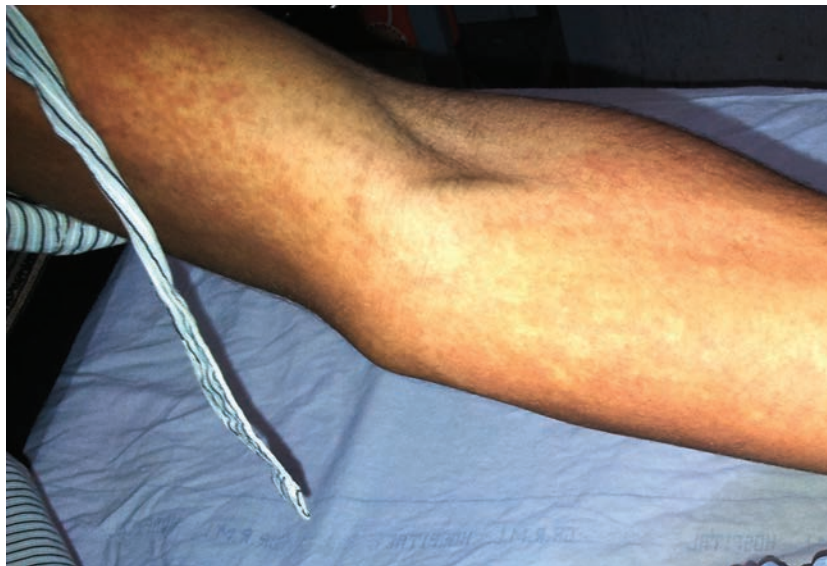
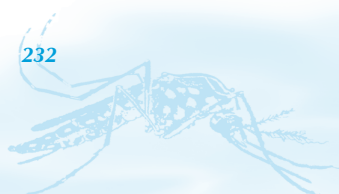


Figure 2: Purpuric spots observed on both legs of the patient (Case 1)



Routine investigations showed decreased platelet count (89 000/cu mm), with slightly elevated packed cell volume (46%), normal haemoglobin (14.8%) and leucopaenia (TLC-3000 cells/cu mm). His liver function test, renal function test and serum electrolytes were normal. Serum for HIV ELISA, HbsAg antigen, ANF were negative. Serological examination for IgM antibodies against dengue virus was positive, which was reconfirmed with a second test.

The electrophysiological study of bilateral lower limbs showed pure motor axonal radiculoneuropathy involving both the lower limbs with absent F waves in right peroneal nerve and reduced distal latency. CSF analysis done one week after the development of weakness showed raised protein (150 mg %) and 2 cells/cu mm, which were all mononuclear cells.

Considering the diagnosis of both dengue fever and GBS, the patient was treated with intravenous immunoglobulins and fluids. Our patient started recovering from the third day of starting immunoglobulins with recovery of power and reflexes over one week. Platelet counts reached normal levels after four days of admission. The patient was discharged on Day 9 of admission and, on subsequent follow-up after two weeks, had no further complaints.

Case 2

A 29-year-old male, from New Delhi, was admitted on 6 November 2012 with complaints of sudden onset of weakness in bilateral lower limbs, which progressed to involve bilateral upper limbs over a period of one week. The patient also gave a history of high-grade fever with generalized rash one week prior to the development of weakness, for which he was admitted in a private hospital. There was no history suggestive of cranial nerve, bladder or bowel involvement. No recent history of diarrhoea, immunization or dog bite was given by him.

On examination, the patient was found to be conscious and oriented with a blood pressure of 120/80 mm of Hg in right upper limb in supine position and his pulse rate was 80/min. the patient was afebrile to touch. The neurological examination revealed decreased power in all the four limbs with absent tendon reflexes and without any sensory involvement. Other system examinations were normal.

Routine investigations including complete haemogram, serum electrolytes were within normal limits. Other serological investigations including ELISA for HIV, ANF titres, HbsAg antigen, Anti-HCV antibodies were negative. IgM ELISA for dengue antibodies was positive which was reconfirmed with a second test. Electro diagnostic studies were done for all four limbs which revealed pure motor axonal neuropathy (Table 1). CSF analysis showed total protein of 189 mg/dl and 3 cells which were all lymphocytes. Previous records of the private hospital were procured. The records revealed thrombocytopenia and positive NS1 antigen test during the patient's febrile illness. The patient was treated with intravenous immunoglobulins and fluids and he started improving on the second day of treatment with complete recovery by Day 7 of hospitalization. The patient did not have any complaints on routine follow-up.

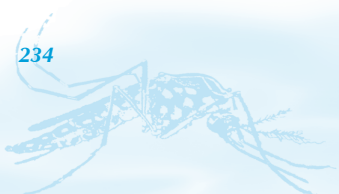


Table 1: Nerve conduction studies of patient (Case 2) showing reduced amplitudes of action potential and normal conduction velocity indicating motor axonal neuropathy

Site	Onset (ms)	Norm Onset (ms)	O-P Amp (mV)	P-T Amp (mV)	Norm P-T Amp	Site1	Site2	Delta-0 (ms)	Dist (cm)	Vel (m/s)	Norm vel (m/s)
Right median motor (Abd Poll Brev)											
Wrist	3.0	<4.2	3.0	5.6	>5	Elbow	Wrist	3.6	23.0	64	>50
Elbow	6.6		2.9	4.3							
Left peroneal motor (Ext Dig Brev)											
Ankle	4.1	<6.1	0.2	0.2	>2.5	B Fib	Ankle	5.3	30.0	57	>38
B Fib	9.4		0.2	0.2							
Right peroneal motor (Ext Dig Brev)											
Ankle	3.3	<6.1	0.4	0.7	>2.5	B Fib	Ankle	6.2	30.0	48	>38
B Fib	9.5		0.3	0.5							
Left tibial motor (Abd Hall Brev)											
Ankle	5.6	<6.1	0.2	0.3	>3.0	Knee	Ankle	8.2	41.0	50	>35
Knee	13.8		0.0	0.0							
Right tibial motor (Abd Hall Brev)											
Ankle	5.4	<6.1	0.4	0.6	>3.0	Knee	Ankle	8.4	41.0	49	>35
Knee	13.8		0.0	0.0							
Right ulnar motor (Abd Dig Minimi)											
Wrist	2.0	<4.2	1.1	2.6	>6	Elbow	Wrist	4.0	25.0	63	>53
Elbow	6.0		1.6	2.5							

Discussion

Dengue fever, also called break bone fever, is caused by an arbovirus and is a common cause of fever in north India during the months of August–November. The virus is transmitted by *Aedes aegypti* mosquito. Dengue fever is characterized by clinical and laboratory features of fever, retro-orbital pain, rash, myalgia, thrombocytopenia, leucopaenia, deranged liver function tests and haemoconcentration. It is a self-limiting disease. Common complications of dengue fever include mucosal bleeding, pleural effusion, ascites. Neurological complications in dengue fever are quite rare and include encephalitis, meningitis myelitis and rarely Guillain-Barré syndrome. Most dengue-associated complications arise once the patient becomes afebrile and develops antibodies against the virus. Some authors have suggested the development of cross-reacting antibodies against myelin and axon in patients suffering from dengue fever.^{1,2}



Guillain-Barré syndrome is a neurological condition associated with acute onset of symmetrical weakness of limbs with areflexia and subjective sensory loss. Immune-mediated destruction of myelin and axon is thought to be pathogenic mechanism behind GBS. Antecedent infections with campylobacter jejuni, mycoplasma, cytomegalovirus and swine flu influenza vaccination are thought to have causal association with GBS. Cases of Guillain-Barré syndrome following dengue fever have been reported from various parts of the world.³⁻⁵

In our patients, dengue fever was diagnosed clinically with supportive serological evidence and it preceded the development of GBS, the diagnosis of which was supported by classical findings on electrophysiological study. Similar to the previous case report from Mumbai,⁵ our patients recovered from GBS after the starting of immunoglobulins, suggesting autoimmune process initiated by dengue virus infection. The temporal association between the two conditions suggests the possibility of dengue virus as the etiological agent of Guillain-Barré syndrome which developed in our patients.

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Dengue and severe dengue factsheet (Revised January 2012)[#]

Weekly Epidemiological Record (<http://www.who.int/wer>)

24 February 2012, No. 8, 2012, 87, 65–72

Overview

Dengue is a mosquito-borne infection occurring in tropical and subtropical regions around the world. In recent years, transmission has increased predominantly in urban and semi-urban areas and has become a major international public health concern.

Severe dengue (previously known as dengue haemorrhagic fever) was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today, severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children in these regions.

There are four distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue.

Global burden of dengue

The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world's population – are now at risk from dengue. WHO currently estimates there may be 50–100 million dengue infections worldwide every year.

Before 1970, only nine countries had experienced severe dengue epidemics. The disease is now endemic in >100 countries in Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. South-East Asia and the Western Pacific regions are the most seriously affected.

[#]<http://www.who.int/wer/2012/wer8708.pdf>



Cases across the Americas, South-East Asia and Western Pacific have exceeded 1.2 million cases in 2008 and over 2.2 million in 2010 (based on official data submitted by Member States). Recently the number of reported cases has continued to increase. In 2010, 1.6 million cases of dengue were reported in the Americas alone, of which 49 000 cases were severe dengue.

Not only is the number of cases increasing as the disease spreads to new areas, but explosive outbreaks are occurring. The threat of a possible outbreak of dengue fever now exists in Europe and local transmission of dengue was reported for the first time in France and Croatia in 2010 and imported cases were detected in three other European countries.

An estimated 500 000 people with severe dengue require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die.

Transmission

The *Aedes aegypti* (*Ae. aegypti*) mosquito is the primary vector of dengue. The virus is transmitted to humans through the bites of infected female mosquitoes. After virus incubation for 4–10 days, an infected mosquito is capable of transmitting the virus for the rest of its life.

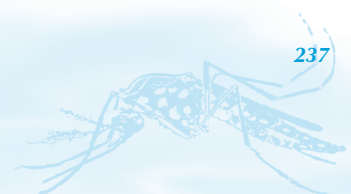
Infected humans are the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. Patients who are already infected with the dengue virus can transmit the infection (for 4–5 days; maximum 12) via *Aedes* mosquitoes after their first symptoms appear.

The *Ae. aegypti* mosquito lives in urban habitats and breeds mostly in man-made containers. Unlike other mosquitoes *Ae. aegypti* is a daytime feeder; its peak biting periods are early in the morning and in the evening before dusk. Female *Ae. aegypti* bites multiple people during each feeding period.

Aedes albopictus, a secondary dengue vector in Asia, has spread to North America and Europe largely due to the international trade in used tyres (a breeding habitat) and other goods (e.g. lucky bamboo). *Ae. albopictus* is highly adaptive and therefore can survive in cooler temperate regions of Europe. Its spread is due to its tolerance to temperatures below freezing, hibernation, and ability to shelter in microhabitats.

Characteristics

Dengue fever is a severe, influenza-like illness that affects infants, young children and adults, but seldom causes death.



Dengue should be suspected when a high fever (40°C/104°F) is accompanied by two of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito.

Severe dengue is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38°C/100°F) and include: severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness, blood in vomit. The next 24–48 hours of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death.

Treatment

There is no specific treatment for dengue fever. For severe dengue, medical care by physicians and nurses experienced with the effects and progression of the disease can save lives – decreasing mortality rates from >20% to <1%. Maintenance of the patient's body fluid volume is critical to severe dengue care.

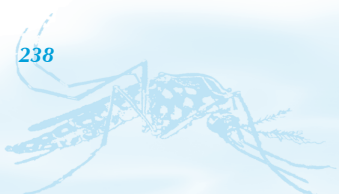
Immunization

There is no vaccine to protect against dengue. Developing a vaccine against dengue/ severe dengue has been challenging although there has been recent progress in vaccine development. WHO provides technical advice and guidance to countries and private partners to support vaccine research and evaluation. Several candidate vaccines are in various phases of trials.

Prevention and control

At present, the only method to control or prevent the transmission of dengue virus is to combat vector mosquitoes through:

- preventing mosquitoes from accessing egg-laying habitats by environmental management and modification;
- disposing of solid waste properly and removing artificial man-made habitats;
- covering, emptying and cleaning of domestic water storage containers on a weekly basis;
- applying appropriate insecticides to water storage outdoor containers;
- using of personal household protection such as window screens, long-sleeved clothes, insecticide treated materials, coils and vaporizers;



- improving community participation and mobilization for sustained vector control;
- applying insecticides as space spraying during outbreaks as one of the emergency vector control measures;
- active monitoring and surveillance of vectors should be carried out to determine effectiveness of control interventions.

WHO response

WHO responds to dengue in the following ways:

- supports countries in the confirmation of outbreaks through its collaborating network of laboratories;
- provides technical support and guidance to countries for the effective management of dengue outbreaks;
- provides training on clinical management, diagnosis and vector control at the regional level with some of its collaborating centres;
- formulates evidence-based strategies and policies;
- develops new tools, including insecticide products and application technologies;
- gathers official records of dengue and severe dengue from >100 Member States;
- publishes guidelines and handbooks for dengue prevention and control for Member States.



Global strategy for dengue prevention and control 2012–2020*

World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

Dengue is a major public health concern throughout tropical and subtropical regions of the world. It is the most rapidly spreading mosquito-borne viral disease, with a 30-fold increase in global incidence over the past 50 years. The World Health Organization (WHO) estimates that 50–100 million dengue infections occur each year and that almost half the world's population lives in countries where dengue is endemic. While dengue is a global concern, with a steady increase in the number of countries reporting the disease, currently close to 75% of the global population exposed to dengue are in the Asia–Pacific region.

Epidemics of dengue result in human suffering, strained health services and massive economic losses. In some countries, the burden of dengue is comparable to that of tuberculosis and other communicable diseases with high disease burdens; unexpected surges in cases and the challenge to health systems of triaging thousands of cases without knowing which severe cases will require hospital care are additional challenges. There has not, however, been concerted action against dengue, and the 1995 WHO strategy** warrants revision in the light of new advances. This *Global strategy for dengue prevention and control, 2012–2020* aims to address this need.

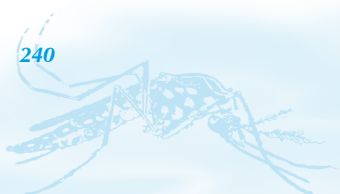
The goal of the global strategy is to reduce the burden of dengue. The specific objectives are to reduce mortality and morbidity from dengue by 2020 by at least 50% and 25% respectively (using 2010 as the baseline). These objectives can be achieved by applying existing knowledge.

Dengue mortality can be reduced by implementing early case detection and appropriate management of severe cases; reorienting health services to identify early cases and manage dengue outbreaks effectively; referral systems, at primary health care levels.

Dengue morbidity can be reduced by implementing improved outbreak prediction and detection through coordinated epidemiological and entomological surveillance; promoting the principles of integrated vector management and deploying locally adapted vector

*<http://www.who.int/denguecontrol/9789241504034/en/index.html>

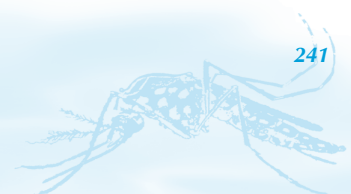
**WHO (1996). *Report of the consultation on key issues in dengue vector control towards the operationalization of a global strategy*, WHO, Geneva, 6–10 June 1995. Geneva, World Health Organization (CTD/FIL(DEN)/IC/96.1)



control measures including effective urban and household water management. Effective communication can achieve behavioural outcomes that augment prevention programmes.

Research will continue to play an important role in reversing the trend in dengue, a neglected tropical disease, by improving methods and systems for surveillance, prevention and control.

Reversing the trend requires commitments and obligations from partners, organizations and countries, as well as leadership by WHO and increased funding. Fund-raising is probably best addressed by a combined effort, with consideration for dengue as a public health problem in countries with substantial local and national funding resources that must be effectively channelled through sound technical support. Dengue prevention and management can now exploit opportunities presented by promising advances in vector control technology interventions, diagnostics, prognostic systems for triage, evidence-based clinical interventions and candidate vaccine developments. In order to realize these opportunities, we need to ensure they are implemented, coordinated and adequately resourced.



Handbook for clinical management of dengue^a

TDR/World Health Organization, 20, Avenue Appia, 1211 Geneva 27, Switzerland

This handbook has been produced to help health-care practitioners at all levels manage dengue. Aspects of managing severe cases of dengue are also described for practitioners at higher levels of health care. Additional and more specific guidance on the various areas related to clinical management of dengue (from other sources in WHO and elsewhere) are cited in the reference sections. This handbook is not intended to replace national treatment training materials and guidelines, but it aims to assist in the development of such materials produced at a local, national or regional levels.

This publication complements the 2009 edition of *Dengue: Guidelines for diagnosis, treatment, prevention and control*.

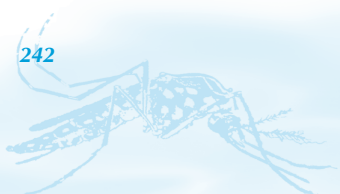
Since publication of the new edition of *Dengue: Guidelines for diagnosis, treatment, prevention and control* by the World Health Organization (WHO) in 2009^b, the need to provide more training to health-care workers in this area has become increasingly evident. Existing training materials need to include more detail to help clinicians recognize the evolution of the course of dengue disease in its various forms of severity, and to enable them to apply the knowledge and principles of management accordingly.

With this aim in mind and following previous successful collaborations, the WHO Department of Control of Neglected Tropical Diseases (WHO/NTD) and the Special Programme for Research and Training in Tropical Diseases (WHO/TDR), set out to develop new training materials.

This handbook has been produced to be made widely available to health-care practitioners at all levels. Aspects of managing severe cases of dengue are also described for practitioners at higher levels of health care. Additional and more specific guidance on the various areas related to clinical management of dengue (from other sources in WHO and elsewhere) are cited in the reference sections.

^ahttp://www.who.int/tdr/publications/handbook_dengue/en/index.html

^bAvailable from: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf, last accessed July 2012.



Contributions and reviews, by many experts both within and outside WHO, have facilitated the preparation of this publication through consultative and peer review processes. We are most grateful to all contributors who are listed in the acknowledgements section. All information is up-to-date at the time of writing, to the best knowledge of the authors.



Handbook for integrated vector management*

TDR/World Health Organization, 20, Avenue Appia, 1211 Geneva 27, Switzerland

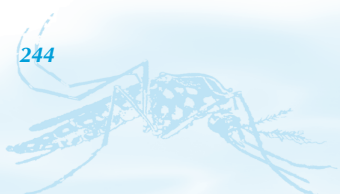
Integrated vector management (IVM) is a rational decision-making process to optimize the use of resources for vector control. The aim of the IVM approach is to contribute to achievement of the global targets set for vector-borne disease control, by making vector control more efficient, cost effective, ecologically sound and sustainable. Use of IVM helps vector control programmes to find and use more local evidence, to integrate interventions where appropriate and to collaborate within the health sector and with other sectors, as well as with households and communities. By reorientating to IVM, vector control programmes will be better able to meet the growing challenges in the control of malaria, dengue and other vector-borne diseases in the face of dwindling public sector human and financial resources.

This handbook presents an operational framework to guide managers and those implementing vector-borne disease control programmes in designing more efficient, cost-effective systems. As a national IVM policy and an intersectoral steering committee are essential for establishing IVM as a national strategy, the handbook begins with the policy and institutional framework for IVM. Policy analysis is a means for identifying options for policy reform and suggesting instruments for implementing policy.

IVM transforms the conventional system of vector control by making it more evidence-based, integrated and participative. This may require changes in roles, responsibilities and organizational links. The transition to IVM involves both reorientation of vector-borne disease control programmes and embedding IVM within local health systems. Intersectoral partnerships and collaboration at both national and local levels will result in cost savings and benefits to other health services. Other relevant sectors, such as agriculture, environment, mining, industry, public works, local government and housing, should incorporate IVM and vector control into their own activities to prevent vector proliferation and disease transmission.

Planning and implementing IVM involve assessment of the epidemiological and vector situation at country level, analysis of the local determinants of disease, identification and selection of vector control methods, assessment of requirements and resources, and design of locally appropriate implementation strategies. Solid evidence on the cost effectiveness of interventions, their underlying parameters and a comprehensive vector surveillance system are essential for locally appropriate decision-making.

*http://whqlibdoc.who.int/publications/2012/9789241502801_eng.pdf



Capacity-building, in particular human resource development, is a major challenge, because the IVM strategy requires skilled staff and adequate infrastructure at central and local levels. The handbook outlines the core functions and essential competence required for IVM at central and local levels, complementing a separate set of documents containing the *Core structure for training curricula on integrated vector management* and associated training materials.

Like any new approach, IVM must be actively advocated and communicated in order to become established. The handbook lays out the elements and processes of IVM to enable policy-makers, donors and implementing partners to use it for vector-borne disease control. During the period of transition and consolidation of an IVM strategy, regular feedback is required on performance and impact in order to ensure continued support. The general public must also be made aware of the strategy and participate in its implementation. The communication tools for reaching the public are the media and various types of educational interventions to increase their knowledge and skills, which should lead to behavioural change and empowerment.

The final section presents a comprehensive framework for monitoring and evaluation of IVM, covering aspects discussed in the previous sections. Indicators and methods for measuring process, outcomes and impact are proposed.

In conclusion, IVM is the preferred approach to improving vector control in countries. The means for establishing IVM are indicated in the operational framework of this handbook. IVM offers an opportunity and a method, as described in this handbook, for setting up partnerships and developing the capacity to find solutions and implement programmes in an efficient, cost effective, ecologically sound and sustainable manner.



In memory of the late Professor Susumu Hotta

Eiji Konishi[#]

*BIKEN Endowed Department of Dengue Vaccine Development, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand**



Dr Susumu Hotta, Emeritus Professor of Kobe University, Japan, who succeeded in isolating the dengue virus for the first time, passed away on 17 November 2011. On behalf of his students who had been involved in research on dengue and Japanese encephalitis viruses under his guidance for a long period of time, I would like to send a farewell message to him.

Born in Osaka on 26 September 1918, Professor Hotta spent his childhood there. After graduating from the Faculty of Medicine, Kyoto Imperial University (the former Kyoto University) in 1942, he joined the Department of Microbiology in the Faculty of Medicine of the school, led by Professor Ren Kimura, to conduct research on the dengue virus. The following year, he succeeded in isolating the dengue virus from dengue fever patients, which was published in a Japanese journal in 1944¹ and later in an English journal in 1952.² There was an outbreak of dengue fever in Nagasaki after Japanese soldiers returning from south-east Asia during World War II brought it into the country. The virus was named “Mochizuki strain” after the name of a patient, and was recognized as the world’s first isolate of dengue virus; later this strain was classified into dengue virus type 1. From 1953 to 1955, he conducted a study of the dengue virus under the guidance of Professor Charles A Evans of the Department of Microbiology, Faculty of Medicine of the University of Washington, Seattle, USA, and was awarded a PhD by the university in 1958. Immediately after returning to Japan in 1957, he assumed the post of Professor at the former Hyogo Prefectural Kobe Medical University (integrated into the School of Medicine of Kobe University, a national university, in 1964). He devoted himself to research on dengue and Japanese encephalitis viruses until he retired from Kobe University in 1982. After his retirement from the university, he served as the Director of the Institute for Tropical Medicine, Kanazawa Medical University, from 1985 to 1989, providing research guidance on the dengue and Japanese encephalitis viruses.

[#]E-mail: ekon@biken.osaka-u.ac.jp

^{*}Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan, to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.



Immediately after World War II, Professor Hotta, along with up-and-coming virus researchers at that time, established the Society for Virus Research (the predecessor of the Japanese Society for Virology) in 1941, and served as a member on the editorial board of *VIRUS*, a journal published since 1951. When the Japanese Society for Virology created the digital archive of the journal in PDF form a few years ago, the first and some of the subsequent issues were not stored in most of the universities and research institutions around Japan. However, they were discovered later in the Department of Microbiology, Kobe University School of Medicine, which had long been a research base for Professor Hotta.

Professor Hotta actively promoted academic exchange with researchers in countries in south-east Asia who were involved in research on the dengue virus. As early as in 1964, he joined a Kobe Medical University medical surveillance team in Indonesia, and, in 1965, organized the second surveillance team to conduct field research in various regions of the country.³ These international activities for medical and academic exchange were highly acclaimed by the Ministry of Education, which led to the establishment of the International Center for Medical Research, Kobe University School of Medicine, in 1979. Professor Hotta greatly contributed to the work of the center as the director. He maintained close relations with Professor Sujudi in particular, who was the Dean of the Faculty of Medicine, the Rector of the University of Indonesia, and the Indonesian Minister of Health, and trained a large number of young Indonesian medical educators and researchers. Academic exchange with Indonesia was further promoted through the “Academic Exchange Project in Asia/Core University Program between Two Countries” and “Core University Program between Multiple Countries based on the Large-scale Collaborative Research System” developed by the Japan Society for the Promotion of Science (JSPS).

The above-mentioned projects for overseas academic exchange are currently being facilitated and implemented by the Center for Infectious Diseases, Kobe University Graduate School of Medicine, as part of the “Program for Founding Research Centers for Emerging and Re-emerging Infectious Diseases” and “Japan Initiative for Global Research Network on Infectious Diseases (J-GRID)”, organized by the Ministry of Education, Culture, Sports, Science, and Technology, and “Science and Technology Research Partnership for Sustainable Development (SATREPS)”, organized by Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA).

In recent years, Kobe University Professor Hak Hotta, his second son, has played an important role in J-GRID and SATREPS activities. This is an indication of the continuation of long-term academic exchange across generations, which, I believe, is one of Kobe University’s proud traditions. I am also very honoured to have been able to conduct research on dengue and Japanese encephalitis viruses in Kobe University, including collaborative studies with Indonesian researchers, for a long period of time.



Professor Hotta received numerous awards for his achievements in dengue virus research and his contribution to the promotion of international academic exchange; these include the Hyogo Prefectural Award of Science in 1960 and The Order of the Rising Sun, Gold Rays with Neck Ribbon in 1992.

Although he was confined to a wheelchair later in his life, he continued to write research reports and articles with his ever-increasing intellectual curiosity of dengue fever. He even published a book entitled “Dengue and Dengue Virus” written in English a few months before he passed away.⁴ In other words, he conducted dengue research for approximately 70 years. I feel I should learn from his life and cultivate my own desire to learn as much as possible.

I would like to express my sincere respect and gratitude for the outstanding achievements of Professor Hotta and the advice and guidance he gave us during the long years of his career, and extend my heartfelt condolences to his family.

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Instructions for contributors

Dengue Bulletin welcomes all original research papers, short notes, review articles, letters to the Editor and book reviews which have a direct or indirect bearing on dengue fever/dengue haemorrhagic fever prevention and control, including case management. Papers should not contain any political statement or reference.

Manuscripts should be typewritten in English in double space on one side of white A4-size paper, with a margin of at least one inch on either side of the text and should not exceed 15 pages. The title should be as short as possible. The name of the author(s) should appear after the title, followed by the name of the institution and complete address. The e-mail address of the corresponding author should also be included and indicated accordingly.

References to published works should be listed on a separate page at the end of the paper. References to periodicals should include the following elements: name and initials of author(s); title of paper or book in its original language; complete name of the journal, publishing house or institution concerned; and volume and issue number, relevant pages and date of publication, and place of publication (city and country). References should appear in the text in the same numerical order (Arabic numbers in parenthesis) as at the end of the article. For example:

- (1) Kroeger A, Lenhart A, Ochoa M, Villegas E, Levy M, Alexander N, et al. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. *BMJ* 2006;332:1247-52.
- (2) *Dengue: guidelines for diagnosis, treatment, prevention and control*. Geneva: World Health Organization; 2009.
- (3) Nathan MB, Dayal-Drager R. *Recent epidemiological trends, the global strategy and public health advances in dengue: report of the Scientific Working Group on Dengue*. Geneva: World Health Organization; 2006 (TDR/SWG/08).
- (4) *DengueNet database and geographic information system*. Geneva: World Health Organization; 2010. Available from: www.who.int/dengueNet [accessed 11 March 2010].
- (5) Gubler DJ. Dengue and dengue haemorrhagic fever: Its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G (ed.), *Dengue and dengue haemorrhagic fever*. CAB International, New York, NY, 1997, 1-22.

Figures and tables (Arabic numerals), with appropriate captions and titles, should be included on separate pages, numbered consecutively, and included at the end of the text with instructions as to where they belong. Abbreviations should be avoided or explained at the first mention. Graphs or figures should be clearly drawn and properly labelled, preferably using MS Excel, and all data clearly identified.

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