Dengue

Major John G Aaskov, BSc, PhD, FASM, FRCPath, RAAMC

Abstract

- An estimated 50–100 million cases of dengue occur annually in more than 100 tropical and sub-tropical countries.
- Dengue has been a source of unquantifiable morbidity in many of Australian Defence Force campaigns in the Asia-Pacific region. There were more confirmed dengue cases in ADF personnel in Timor in the first six months of operations (215) than were reported in Vietnam in seven years (4).
- A new generation of assays allows point-of-care diagnosis of dengue infection by semi-skilled operators.
- There are opportunities for the ADF to become involved in the evaluation of the first dengue vaccines likely to be effective.
- Training of ADF medical practitioners might include attachments to hospitals which handle significant numbers of tropical infections, including dengue.
- Communicable disease surveillance, including that for dengue, should include an interaction with the civilian community, particularly during peacekeeping deployments.

ADF Health 2003; 4: 66-71

DENGUE is a disease caused by four serotypes of a virus of the same name (dengue 1, 2, 3 and 4). The severity of dengue infections is influenced by the age and genetic background of the host, the strain and serotype of the infecting virus and the prior history of dengue infections of the host.[1-6]

History

Dengue fever was accepted as an occupational hazard of living and working in the tropics. The 1905 edition of Manson's Tropical Diseases[7] states: "In Europeans, an attack of dengue very often leads to a condition of debility necessitating temporary change of climate, or even return to Europe". Perhaps the first reports of dengue haemorrhagic fever and an associated mortality were made by Hare in Charters Towers in northern Queensland in 1896–97 (Box 1).[8] He wrote: "as epidemic succeeds epidemic, the disease appears to be more severe and fatal cases more frequent. Second attacks are as severe or more severe than the first".[8] However, it took 60 years and outbreaks of a "new" haemorrhagic fever in Thailand and the Philippines[9] before the significance of these observations was appreciated. There is now compelling evidence that severe dengue occurs most commonly following infection with a second or subsequent dengue virus serotype.[5,10]

Several other seminal observations relating to dengue and its pathogenesis also were made in Australia. Following a dengue outbreak in Brisbane in 1905, in which it was estimated that one third of the workforce was incapacitated, Bancroft, who was a general practitioner in the then rural Brisbane suburb of Alderly, demonstrated that *Aedes aegypti* mosquitoes which had fed on a dengue patient were able to transmit virus to previously healthy members of the Alderly community.[11] These observations subsequently were confirmed by Cleland, Bradley and MacDonald in Sydney[12] and were extended to determine the interval from infection to onset of symptoms, the duration of viraemia in patients and to show that the virus was present in both serum and blood cells. In some notes on failed experiments, these authors mentioned "the unexpected difficulty in obtaining volunteers even with a considerable monetary inducement" and " in two experiments the finding of a positive Wasserman test (syphilis?) in a volunteer prevented further utilisation of the virus in the blood". Outbreaks of dengue continued in northern Australia on an annual basis until the mid-1920s. Cases of dengue have been reported in Australia since the 1980s, due to the arrival or return of human hosts who have been infected with dengue virus in another country. Since World War II, there also have been numerous examples of local transmission of dengue viruses introduced into Australia (1954–5, dengue 3; 1981–2, 1990–1, dengue 1; 1992–3, 1995, 1996–7, dengue 2; 1997–9, dengue 3; 2001, dengue 2).[13,14]

The primary vector of dengue is the mosquito *Aedes aegypti*, a peridomestic mosquito (ie, found in and around homes) with a short flight range. It breeds in a variety of containers, usually associated with human refuse or water storage. In Australia, it is found in northern Queensland. A secondary vector, *Aedes albopictus*, has similar habits to *Ae. aegypti* and has recently invaded the south-west Pacific region. *Ae. albopictus* is common in Papua New Guinea and poses a constant risk to Australia.

Origin of dengue viruses

The origin of dengue viruses is uncertain. Some have speculated that they originated in Africa and moved out of that continent along with its mosquito vector *Ae. aegypti* as a result

Major John Aaskov is an Arbovirologist with the Army Malaria Institute and Senior Lecturer in Immunology and Virology in the School of Life Sciences at the Queensland University of Technology. He has dengue research programs in Vietnam and Myanmar and is undertaking a dengue genotyping project with collaborators throughout Asia.

Australian Army Malaria Institute, Gallipoli Barracks, Enoggera, QLD.

John G Aaskov, BSc, PhD, FASM, FRCPath, RAAMC, Arbovirologist.
Correspondence: Major John G Aaskov, Australian Army Malaria Institute, Gallipoli Barracks, Enoggera, QLD 4052. j.aaskov@qut.edu.au

of the slave trade.[15] Others have proposed that the viruses may have evolved from a jungle cycle involving lower primates and canopy dwelling mosquitoes in the Malay peninsula.[16] Whatever their origin, there has been an exponential diversification in dengue virus genotypes which has paralleled the increase in the human population over the last 200–300 years.[17] While there is clear evidence of the introduction of dengue viruses into non-endemic countries like Australia and Cuba and the introduction of an "Asian" strain of dengue 2 into South America,[6] most changes in virus genotypes in countries where the virus is endemic appear to be due to local evolution.[18,19]

1: Dengue in northern Queensland, 1897

Dr Hare, of Charters Towers, made perhaps the first report of dengue haemorrhagic fever in the medical literature,[8] as part of his report of an epidemic of dengue fever that swept northern Queensland in 1897.

Above: The medical staff of the Charters Towers Hospital in 1896. Dr Hare, who recognised dengue haemorrhagic fever cases, is seated on the extreme left in the front row. Below: the hospital.

"I have collected some account of 60 fatal cases occurring in North Queensland during the epidemic of 1897. Half the number were adults. In many, pre-existing conditions appeared to determine the fatal issue. Among these were old age, diabetes, chronic bronchitis, opium smoking, pregnancy and especially alcoholism. There is a widespread popular idea that alcohol has prophylactic influence against the disease, and this, I am sure, acted disastrously at times." — F E Hare [8]

Variation in the competence of mosquito vectors *Ae. aegypti* and *Ae. albopictus* to transmit different strains of dengue virus also suggest local co-evolution of virus and vector.[20] Nonetheless, in areas where there is extensive movement of human hosts it may be possible to identify the introduction of new strains of virus even if they do not become established.[19]

From phylogenetic studies carried out by the Australian Army Malaria Institute and the Queensland University of Technology, it appears that all four serotypes of dengue virus are circulating in East Timor (Box 2). The Timorese dengue 1 strains recovered between 1999 and 2001 were related to older "Pacific" strains, but appear to be evolving locally. In contrast, there were four genotypes of dengue 2 circulating in Timor in 1999, only one of which (Timor 2001 D2-79) appeared to have continued in circulation. These data suggested that some or all of the dengue 2 virus genotypes were introduced, possibly with UN personnel from dengue endemic areas such as India and Singapore. Too few dengue 3 isolates were recovered to draw any conclusions about this virus serotype. There appeared to be two distinct genotypes of dengue 4 in Timor in 1999, which were quite distinct from dengue 4 viruses from other countries. One lineage may have disappeared, while the Timor 99/00 D4-252 lineage appeared to have continued to evolve locally until 2002. In an illustrative example of the peridomestic nature of the mosquito vector of dengue, four of these dengue 4 virus isolates were recovered sequentially, at about weekly intervals, from four defence personnel who were sharing accommodation.

Clinical features

Most dengue infections are inapparent, but symptoms, when they occur, vary in severity from a mild "flu-like" illness to a haemorrhagic fever and hypovolaemic shock which, if untreated, may be fatal.[21] The mildest form of clinical dengue infection is dengue fever, but because of the broad spectrum of signs and symptoms, the World Health Organization (WHO) has suggested there should not be a detailed clinical definition for dengue fever. Clinical features of dengue fever include abrupt onset high fever, headache, retro-orbital pain, muscle and bone or joint pain, nausea, rash and, occasionally, petechiae.

Dengue haemorrhagic fever is characterised by four major clinical manifestations: high fever, haemorrhage, hepatomegaly and circulatory failure. An abbreviated WHO case definition for dengue haemorrhagic fever is:

- Fever, lasting 2–7 days and perhaps biphasic
- Haemorrhage (bleeding from the mucosa or gut, positive tourniquet test, petechiae, ecchymoses or purpura, haematemesis or melaena)
- Thrombocytopenia (< 100 000 cells/mL)
- Plasma leakage (> 20% rise in age and sex adjusted haemocrit, pleural effusion, ascites)

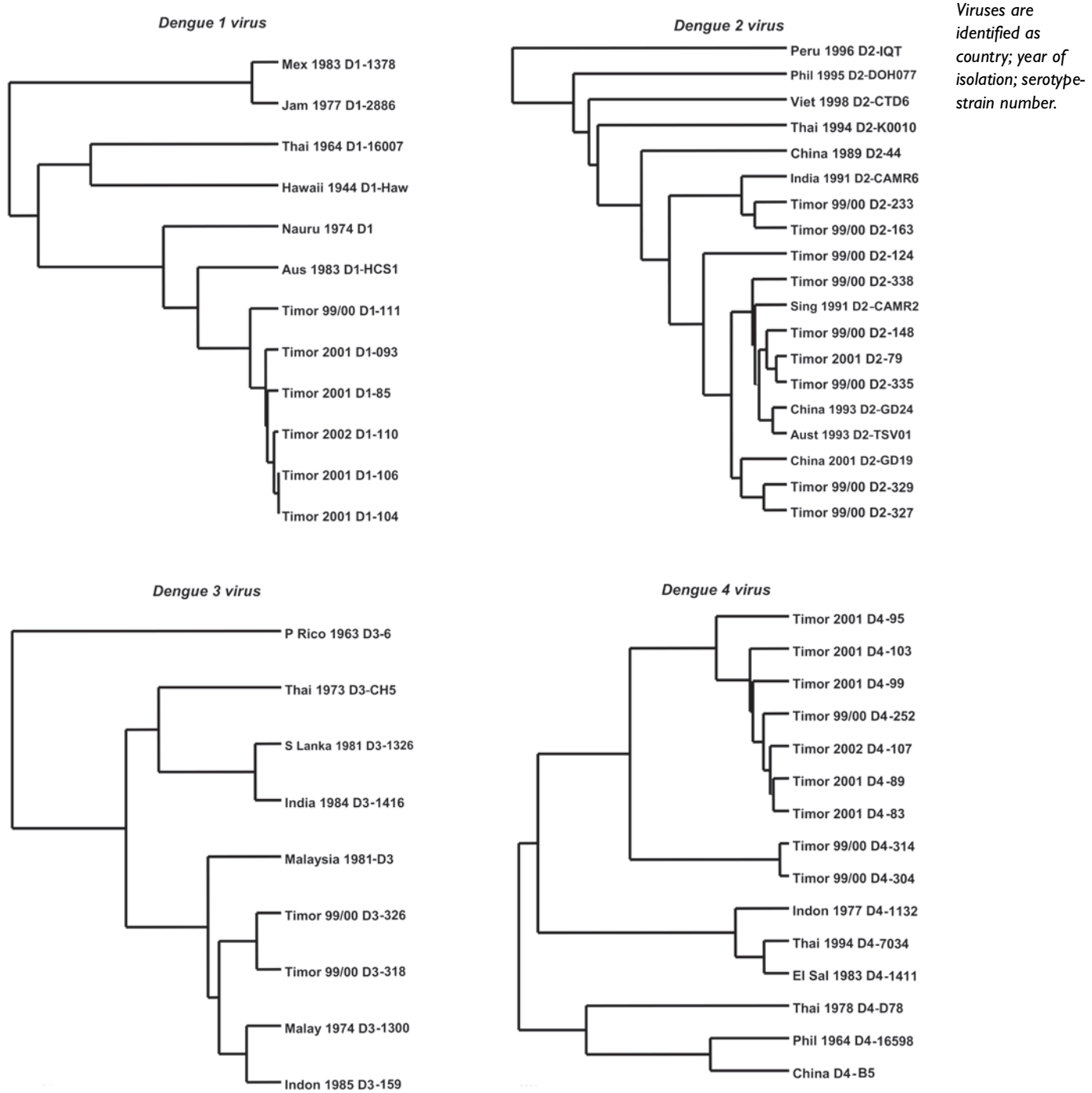
Dengue shock syndrome usually occurs in patients with dengue haemorrhagic fever after 2–7 days of fever. Patients

may have a rapid weak pulse, complain of abdominal pain and become restless, and the skin may become cool and blotchy. Blood pressure and pulse become imperceptible. The WHO case definition for dengue shock syndrome is:

- Rapid and weak pulse
- Narrow pulse pressure (<20 mmHg[2.7kPa])
- Hypotension for age
- Cold, clammy skin and restlessness[21]

None of the clinical signs and symptoms listed above are specific for dengue and so the disease is frequently misdiagnosed, even by paediatricians and physicians who have worked with these patients all their careers. Laboratory tests are essential if a definitive diagnosis is to be made. For these reasons, there are no reliable figures for the number of cases of dengue the ADF experienced during the Pacific campaign of World War II. In the first two years of the ADF deployment in

2: Phylogenetic relationships between dengue viruses recovered in East Timor and representative examples of clades (ie, commonly descended branches) of each dengue serotype



Timor, more *laboratory confirmed* cases of dengue were reported (234) than there were unconfirmed dengue cases (4) reported for the duration of the commitment to Vietnam.[22]

Diagnosis

There are three criteria for a definitive diagnosis of a dengue infection.

1. Detection of dengue virus or dengue virus RNA in an acute phase serum/tissue sample *or*
2. Detection of anti-dengue virus IgM antibody or detection of anti-dengue IgG antibody at a titre equivalent to a haemagglutination-inhibiting antibody titre of 1280, in serum collected within 10–14 days of onset of symptoms compatible with dengue *or*
3. Detection of a four-fold or greater rise in anti-dengue virus antibody titre in paired sera collected 7–14 days apart and tested in parallel.

There remain a number of problems associated with the laboratory diagnosis of dengue. Serological tests are easier and often faster to perform than virological ones, and there are a range of dengue serological tests available commercially. However, it may be 5–6 days after onset of fever before diagnostic levels of anti-dengue virus antibodies are produced. Conversely, dengue viruses may not be detected in all seronegative, acute-phase, serum samples. In a small percentage of cases, seroconversion may not occur.

If doctors or paramedical personnel suspect a case of dengue among military personnel they can perform a "rapid" immunochromatographic dengue assay[23] on serum as soon as the patient presents. This test can be performed in a Regimental Aid Post or similar facility, provided attention is paid to the manufacturer's recommended procedures for performing and interpreting the test. If the test is performed on serum collected before the fifth day of fever, and is negative, a second test should be performed 6–7 days later. Suspected patients should not be returned to their unit before this time because they may still be viraemic and so act as a source of further infection. While in medical care, these patients should be confined under a mosquito net at all times that they are not moving about. There is strong evidence from the number and type of dengue viruses recovered from patients in the Dili Hospital, early in the deployment of the ADF, that dengue virus transmission occurred inside the hospital because these precautions were not taken/enforced.

If a large dengue outbreak is suspected, it is far more efficient to use enzyme-linked immunosorbent assays (ELISA),[24,25] which require basic laboratory facilities. These assays should be read spectrophotometrically in an ELISA Plate Reader, but in an emergency they can be read by eye (ELISAs were read by eye in the early stages of the dengue outbreak in Australian troops in Timor with almost 100% sensitivity and specificity. Major Scott Kitchener, personal communication).

Most assays for the rapid detection of dengue viruses are still research tools or are "in house" assays performed in large specialised laboratories.[26] The one commercial assay for the detection of dengue viruses in serum lacks sensitivity. A number of polymerase chain reaction (PCR) based assays are approaching commercialisation and these have the potential to be rapid, sensitive and specific and to provide laboratory confirmation of dengue virus infection in acute phase serum. The Australian Army Malaria Institute and the Combatant Protection and Nutrition Branch of the Defence Science and Technology Organisation have adapted "real time" PCR protocols for the detection of dengue viruses using the Ruggedised Advanced Pathogen Identification Device (RAPID) and have deployed this to Timor. The system worked well in the laboratory and the field, but this technology is not at a stage where it could be employed by paramedical staff at a Regimental Aid Post.

Clinical management

Managing patients with dengue haemorrhagic fever, and even dengue shock syndrome, does not require sophisticated medical facilities. Perhaps the greatest risk to such a patient is overcompensation for plasma leakage when giving intravenous fluids. Early oral rehydration may be adequate for mild cases of dengue haemorrhagic fever and is a useful initial treatment in patients who may go on to severe disease requiring more comprehensive management.

Dr Suchitra Nimmannitya from the former Bangkok Childrens Hospital has developed a simple, effective, step-by-step, treatment protocol based on decades of experience, which has the endorsement of WHO, and is used extensively in hospitals in dengue endemic areas.[21] This protocol is based on regular monitoring of platelet counts and haematocrit to guide treatment. Antipyretics may be given during the febrile phase of dengue haemorrhagic fever but these will not reduce the duration of fever. Salicylates should not be used because they affect platelet function and they may precipitate Reye syndrome in children. A rise in haematocrit of 20% or more is the trigger to begin fluid replacement. Colloids (dextran 70 or gelafundin 35 000) have been found to restore cardiac index and blood pressure and to normalise haematocrit more rapidly than crystalloids (Ringers lactate).[27]

In severe cases of dengue shock syndrome, hyponatraemia and metabolic acidosis may occur. Prompt fluid replacement and correction of the acidosis with sodium bicarbonate usually overcomes these complications. In patients experiencing significant bleeding, fresh whole blood may be given to restore a normal red blood cell volume. There appears to be a window of about six months to five years after a dengue infection in which a person is at greater risk of severe disease if infected with a second dengue virus serotype. This may be due to "enhancement" of the subsequent infection by dengue virus cross-reactive antibodies produced following the first infection. The magnitude of this risk for re-deployment of ADF personnel who have experienced prior dengue infection(s) depends on the interval between the most recent infection and deployment, whether there are multiple dengue virus serotypes circulating in the area of operations, the rate of infection in the area of operations and the number of people with prior dengue infections deployed. It is unlikely that there would be

significant numbers of cases of severe dengue in ADF personnel re-deployed to Timor. People who have been infected with three or four dengue virus serotypes are probably totally immune to re-infection.

Vaccine development

The watershed in the study of dengue and its causative agent was the isolation and culture of dengue viruses; first by Hotta in Japan[28] and then by Sabin (of subsequent polio vaccine fame) in the United States of America.[29] Hotta succeeded in growing the virus in mice but had to inject infected mouse tissue into his mother to confirm he had isolated dengue virus; whereas Sabin relied on human volunteers to grow his virus isolates until he too managed to adapt his viruses to grow in mice.[30] These experiments took place during World War II, so each group was unaware of the work of the other. Once the viruses could be cultured, diagnostic tests were developed and the first candidate vaccines were produced. Sixty years later there are still only "candidate" dengue vaccines. Dengue poses some particular challenges for vaccine development. There are four serologically distinct viruses and long-term immunity is specific for the infecting serotype. Sequential infections with different serotypes may result in severe disease.[5,10] Since the vaccine is to be used in endemic areas, there must be no risk that pre-existing anti-dengue virus antibody in a vaccine will "enhance"[31] the vaccine infection and cause severe disease, and the vaccine must induce simultaneous, life-long immunity to all four virus serotypes if it is not to "sensitise" vaccinees to severe disease following a natural dengue virus infection. Added to these difficulties is the absence of an animal model of dengue haemorrhagic fever in which to test a vaccine and the lack of definitive markers of virus attenuation.

All of the tetravalent dengue vaccines in trial or about to enter clinical trials[32,33] are derived from a single genome of each dengue virus serotype or a plaque-purified population (ie, a very homogeneous population of virus). In some cases, the viruses used in the vaccines are those which were circulating 20–30 years ago. Dengue viruses have RNA genomes and because of the error-prone nature of RNA polymerases, populations of virus might be expected to be diverse. Recent experiments have confirmed this.[34] If a virus population is diverse, it has subpopulations that may be ideally suited to occupy new ecological niches or to escape the immunological pressures of a host immune response (ie, the immune response to a single dengue genotype in a vaccine might not protect against all the viruses in a diverse, natural, virus population of the same serotype).

Two other influences may be acting to force change on dengue virus populations. There is extensive evidence of intra-serotypic recombination occurring in dengue viruses.[34,35] This occurs when a host is infected with two different dengue virus populations and part of the genome of one replaces a corresponding region of the second to give rise to a new virus. Although recombinant dengue viruses have been identified for some time, it was only in 2002 that we identified a single *Ae. aegypti* mosquito which contained two different dengue virus populations, along with a third which was a recombinant of these two.[34] *Ae. aegypti* is easily disturbed when feeding and we postulate that this insect fed on two patients in order to complete a blood meal and acquired a virus population from each. Viruses from each population then recombined.

3: Measures to control dengue among ADF personnel deployed in dengue-endemic areas

- Appropriate wearing of permethrin-treated uniform.
- Applying DEET-based repellant to exposed skin.
- Sleeping under bed-nets or in screened enclosures wherever possible.

Rapid and dramatic changes in dengue virus genotype have been detected in Thailand[19,36] and Myanmar (unpublished observations) due to what is believed to be genetic bottlenecks. These occur at times of low virus transmission when there is a possibility that a rare virus variant may be the only one to be transferred to a susceptible host.

These observations do not indicate that dengue vaccines will not be effective, but they do suggest that they may be aiming at moving targets.

It has been only in the past few years that a dengue vaccine has become a possibility. Both Hotta and Sabin failed in their attempts to produce dengue vaccines.[30,37] Subsequent efforts by the US Army met with little more success, with only a dengue 2 vaccine progressing to phase I clinical trials.[38] A tetravalent vaccine developed at Mahidol University in Thailand using viruses attenuated by in-vitro passage [32] and commercialised by Aventis Pasteur showed initial promise, but is now being reformulated. The US Army also has a classically attenuated tetravalent vaccine which is entering trials. A tetravalent dengue vaccine containing chimeric yellow fever–dengue viruses also has entered trials.[33] The viruses in this vaccine are composed of a backbone of the core and non-structural protein genes of the 17D yellow fever virus vaccine, into which the pre-membrane and envelope protein genes of each of the dengue virus serotypes has been inserted. This results in a virus particle with dengue virus proteins on its surface enclosing a chimeric yellow fever–dengue virus genome. This approach has the potential to overcome the difficulties encountered with earlier tetravalent dengue vaccines in which the four virus serotypes appeared to replicate at different rates.

Dengue vaccine trials face some additional hurdles. The virus record is incomplete because many of the countries in which dengue occurs lack the facilities for systematic collection and identification of dengue viruses, and some of those that do have collections lack the resources to analyse the viruses in a timely or systematic manner. Without information on the serotypes and genotypes circulating in a region and the infection rates in those areas, it will be extremely difficult to plan vaccine efficacy trials.

The US Armed Forces Research Institute of Medical Sciences study site at Kampong Phet, Thailand, is perhaps the only site anywhere in the world for which sufficient data are available to undertake a dengue vaccine efficacy study. The US Army is attempting to overcome this problem by

developing dengue virus preparations that will cause mild disease in all who are infected with them. Such a virus preparation could then be used safely in challenge tests of dengue vaccinees. This would be much simpler than undertaking large scale vaccine efficacy studies in populations in which only a few per cent will develop clinical symptoms of dengue each year.

Surveillance and control

In Asia, epidemics of dengue occur in cycles of 3–5 years, probably due to the phenomena of enhancement of infection by cross-reactive antibody produced in earlier infections,[39] so it remains to be seen whether the remedial actions taken to reduce exposure of ADF personnel to *Aedes* mosquito vectors following the dengue outbreak in Timor in 1999–2000 (Box 3) have been responsible for the subsequent reduction in the number of cases.

Disease surveillance is a key component in disease prevention: know your enemy. The ADF may have good qualitative data about communicable diseases in areas where it may be called on to operate, but it lacks quantitative data that would help to prioritise disease risks. It may be impossible to obtain these data before a deployment, but one of the best disease surveillance systems available to peacekeeping forces, once deployed, is the local civilian population. A case might be made to develop the interfaces needed to be able to obtain reliable, timely, public health data — including that for dengue — from civilian populations in areas where the ADF operates.

Competing interests

The author participated in the development of commercial assays for the diagnosis of dengue and receives a financial benefit from the sale of these assays.

References

1. Guzman MG, Kouri G, Bravo J, et al. Effect of age on outcome of secondary dengue 2 infections. *Int J Infect Dis* 2002; 6: 118-124.
2. Loke H, Bethell DB, Phuong CXT, et al. Strong HLA class I restricted T cell responses in dengue haemorrhagic fever: a two edged sword? *J Infect Dis* 2001; 184: 1369-1373.
3. Stephens HAF, Klaythong R, Sirikong M, et al. HLA-A and –B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. *Tissue Antigens* 2002; 60: 309-318.
4. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viraemia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000; 181: 2-9.
5. Sangkawibha N, Rojanasuphot S, Ahandrik S, et al. Risk factors in dengue shock syndrome: a prospective epidemiological study in Rayong, Thailand. *Am J Epidemiol* 1984; 120: 653-669.
6. Leitmeyer KC, Vaughn DW, Watts DM, et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999; 73: 4738-4747.
7. Manson P. Tropical diseases. London: Cassell and Company, 1905: 195-204.
8. Hare FE. The 1897 epidemic of dengue in North Queensland. *Australas Med Gaz* 1898; 17: 98-107.
9. Hammon WMcD, Rudnick A, Sather G, et al. New haemorrhagic fevers in children in the Philippines and Thailand. *Trans Assoc Am Physicians* 1960; 73: 140-155.
10. Thein S, Aung MM, Shwe TN, et al. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* 1997; 56: 566-572.
11. Bancroft TL. On the aetiology of dengue fever. *Australas Med Gaz* 1906; 25: 17-18.
12. Cleland JB, Bradley B, McDonald W. Dengue fever in Australia. *J Hyg (Camb)* 1918; 16: 317-418.
13. Hanna JN, Ritchie SA, Merritt AD, et al. Two contiguous outbreaks of dengue type 2 in north Queensland. *Med J Aust* 1998; 168: 221-225.
14. Hanna JN, Ritchie SA, Phillips DA, et al. An epidemic of dengue 3 in far north Queensland, 1997-1999. *Med J Aust* 2001; 174: 178-182.
15. Ehrenkranz NJ, Ventura AK, Cuadrado RR, et al. Pandemic dengue in Caribbean countries and the southern United States — past present and potential problems. *N Engl J Med* 1971; 285: 1460-1469.
16. Smith CEG. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *J Trop Med Hyg* 1956; 59: 243-251.
17. Zanotto PMdeA, Gould EA, Gao GF, et al. Population dynamics of flaviviruses revealed by molecular phylogenies. *Proc Nat Acad Sci USA* 1999; 93: 548-553.
18. Twiddy SS, Farrar JJ, Chau NV, et al. Phylogenetic relationships and differential selection pressures among genotypes of dengue 2 virus. *Virol* 2002; 298: 63-72.
19. Wittke V, Robb TE, Thu HM, et al. Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. *Virol* 2002; 301: 148-156.
20. Gubler DJ, Nalim S, Tan R, et al. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*. *Am J Trop Med Hyg* 1979; 28: 1045-1052.
21. Dengue haemorrhagic fever. Diagnosis, treatment, prevention and control. 2nd ed. Geneva: World Health Organization, 1997. Available at: www.who.int/emc/diseases/ebola/Denguepublication/index.html (accessed 16 Jun 2003).
22. O'Keefe BG, Smith FB. Medicine at war. Medical aspects of Australia's involvement in Southeast Asia 1950–1972. Sydney: Allen and Unwin, 1994.
23. Cuzzubbo AJ, Endy TP, Nisalak A, et al. Use of recombinant envelope proteins for serological diagnosis of dengue virus infection in an immunochromatographic assay. *Clin Diagnostic Lab Immunol* 2001; 8: 1150-1155.
24. Innis BL. Nisalak A, Nimmannitya S, et al. An enzyme-linked immunosorbent assay to characterise dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989; 40: 418-427.
25. Cuzzubbo AJ, Vaughn DW, Nisalak A, et al. Comparison of PanBio Dengue Dou enzyme linked immunosorbent assay (ELISA) and MRL Dengue Fever Virus Immunoflogulin M Capture ELISA for diagnosis of dengue virus infections in southeast Asia. *Clin Diagnostic Lab Immunol* 1999; 6: 705-712.
26. Raengsakulrach B, Nisalak A, Maneekarn N, et al. Comparison of four reverse transcription-polymerase chain reaction procedures for the detection of dengue virus in clinical specimens. *J Virol Meth* 2002; 105: 219-232.
27. Dung NM, Day NPJ, Tam DTH, et al. Fluid replacement in dengue shock syndrome: a randomised, double blind comparison of four intravenous fluid regimes. *Clin Infect Dis* 1999; 29: 787-794.
28. Hotta S, Kimura R. Experimental studies on dengue. I. Isolation, identification and modification of the virus. *J Infect Dis* 1952; 90: 1-9.
29. Sabin AB. Research on dengue during World War II. *Am J Trop Med Hyg* 1952; 1: 30-50.
30. Sabin AB, Schlesinger MC. Production of immunity to dengue with virus modified by propagation in mice. *Science* 1945; 101: 640-642.
31. Kliks SC, Nisalak A, Brandt WE, et al. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue haemorrhagic fever. *Am J Trop Med Hyg* 1989; 40: 444-451.
32. Bhamarapravati N, Yoksan S. Live attenuated tetravalent dengue vaccine. In: Gubler DJ , Kuno G, editors. Dengue and dengue haemorrhagic fever. Oxford: CAB International, 1997: 367-377.
33. Guirakhoo F, Arroyo J, Pugachev KV, et al. Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. *J Virol* 2001; 75: 7290-7304.
34. Craig S, Thu HL, Lowry K, et al. Diverse dengue type 2 virus populations contain recombinant and both parental viruses in a single mosquito host. *J Virol* 2003; 77: 4463-4467.
35. Holmes EC, Worobey M, Rambaut A. Phylogenetic evidence for recombination in dengue virus. *Mol Biol Evol* 1999; 16: 405-409.
36. Sittisombut N, Sistayanarain A, Cardosa MJ, et al. Possible occurrence of a genetic bottleneck in dengue serotype 2 viruses between 1980 and 1987 epidemic seasons in Bangkok. *Am J Trop Med Hyg* 1997; 57: 100-108.
37. Hotta S. Dengue and dengue related haemorrhagic diseases. St Louis, MO: Warren H Green, 1969.
38. Bancroft WH, Scott RM, Eckels KH, et al. Dengue virus type 2 vaccine: reactogenicity and immunogenicity in soldiers. *J Infect Dis* 1984; 149: 1005-1010.
39. Ferguson N, Anderson R, Gupta S. The effect of antibody-dependent enhancement on the transmission dynamics and persistence of multistrain pathogens. *Proc Nat Acad Sci* 1999; 96: 790-794.

(Received 20 Jan 2003, accepted 5 Mar 2003) ❑