Break-bone fever

The dengue virus exacts a devastating and growing toll on public health in the tropics, yet remains little studied. Tom Clarke talks to the scientists who are intent on raising dengue's profile.

JOSE GOITIA/AP

Smoking out the enemy: a pest controller in Havana, Cuba, fumigates a house against *Aedes aegypti* (left), the mosquito that transmits the dengue virus to humans.

WHO/TDR/STAMMERS

"You don't die from it, but you wish you could," says Duane Gubler. He should know — he's been stricken by dengue fever three times. Gubler's first infection consigned him to bed for more than a week with a raging temperature and the agonizing limb pains that have earned the disease its sobriquet 'break-bone fever'. In addition, some sufferers lose hair and develop a measles-like rash, bleeding gums and depression that can last for weeks.

They're the lucky ones. In a small percentage of cases, mostly in infants, the infection causes dengue haemorrhagic fever (DHF) — which is a killer. Its victims vomit and pass blood in their faeces and urine as their capillaries leak fluid. If spotted early, DHF responds to intensive hospital treatment. But if it isn't caught, mortality can reach 15%.

The dengue virus is spread by the mosquito *Aedes aegypti*. For Gubler, the disease is an occupational hazard — he has spent his career studying dengue in the field, and now directs the vector-borne diseases division of the US Centers for Disease Control and Prevention, based at the National Center for Infectious Diseases in Fort Collins, Colorado.

GERALD ANKER/CORBIS

Huge troop deployments in the Second World War sowed the seeds of Asia's dengue problem.

Growing concern

Once a sporadic illness, dengue is on the rise. Epidemics are now regular in southeast Asia, India, the western Pacific and much of South America (see map, opposite). Outbreaks are also getting bigger and more serious, with a higher proportion of DHF cases. The World Health Organization (WHO) receives reports of about 500,000 dengue fever cases each year, but estimates that as many as 50 million people are infected annually.

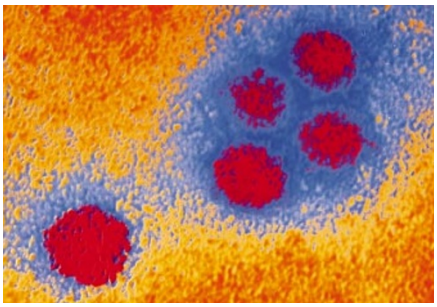
Yet the disease is neglected. Almost entirely absent in the developed world and difficult to study, dengue has received little attention — and a fraction of the research funding devoted to better-known tropical diseases. "It's malaria's poor cousin," says Gubler.

Although dengue doesn't rival malaria as a killer, the sheer number of people infected and the fever's debilitating nature mean that it has an enormous economic impact. Measured in units devised by the World Bank called disability-adjusted life years, which quantify disruption to quality of life and economic productivity, dengue's burden on some societies is comparable to that of HIV, tuberculosis or hepatitis. In South America, its impact rivals that of malaria, and in southeast Asia, it is public-health enemy number one[1].

"It's an orphan disease, but one that's increasingly screaming at us," says Mike Nathan, a dengue specialist at WHO's headquarters in Geneva. He, Gubler and other experts are now trying to push dengue up the public-health agenda — with some success. In January, WHO's executive board drafted a resolution urging international agencies and national governments to spend more on studying and tackling dengue. This will be considered by the World Health Assembly, WHO's governing body, next month.

This effort to raise dengue's profile is partly due to renewed hope that it will be possible to produce a vaccine, which would bolster efforts in mosquito control and disease surveillance. But this will only happen, say dengue experts, if scientists rise to the challenge and are supported by adequate funds.

How did the situation get so bad? Demographic changes — particularly urbanization — are largely to blame. *Ae. aegypti* prefers to feed on human blood, and can spread the dengue virus most effectively when people are living cheek-by-jowl[2]. Mas-



LONDON SCH. HYG. TROP. MED./SPL

DADO GALDIERI/AP

Virus and vector: left, dengue virus particles; right, Brazilian children examine *Aedes aegypti* larvae.

sive troop deployments and refugee movements in the Second World War established southeast Asia as the world's dengue hotbed. But in the years after the war, efforts to combat yellow fever and malaria held the disease in check by ridding the region of *Ae. aegypti*.

Dengue's comeback was triggered by the arrival in South America of widespread vaccination for yellow fever in the 1950s and the scaling back of a worldwide anti-malaria initiative in the 1970s. Mosquito-control efforts slackened, as urbanization continued to gather pace[3]. Over the past five decades, the disease's incidence has grown 30-fold[4]. But its tendency to lie dormant in any given region before exploding into a severe outbreak has contributed to dengue's neglect by public-health experts. Cuba and Brazil have this year been hit by severe epidemics. But in countries experiencing a lull, it is easy for health authorities to give dengue a low priority. "With tight budgets, the disease gets pushed into a corner," says Nathan.

Messy business

Any effort to control dengue will involve spraying with insecticides, and outlawing of open water containers and litter such as discarded tyres — which can collect water and serve as perfect nurseries for aquatic mosquito larvae. But although mosquito control works reasonably well, it is "a little bit messy", says Nathan. As such, it can be hard to sell to public-health authorities. A vaccine, however, would be a different matter — more likely to gain a high profile and financial backing. "To many people's eyes it's a tool that's neat and clean," says Nathan.

Work began on dengue vaccines in the 1970s, but progress has been slow. Part of the problem is that — unlike yellow fever — dengue is caused not by a single virus but by four distinct viral 'serotypes'. Each is equally infectious and pathogenic. And the catch is that once a person's immune system has fought off and memorized an infection by one dengue serotype, a secondary infection with a different serotype is thought to be the biggest single risk factor for developing DHF.

The reasons for this are not well understood. Different dengue serotypes are seen as similar by the immune system — when a new serotype infects someone who has had a previous bout of dengue, it is recognized. But although antibodies bind to the new virus, they do not do so as effectively as they would to the serotype they encountered previously. So when immune cells called macrophages arrive on the scene to ingest the virus–antibody complexes, the virus remains infectious. And unfortunately, macrophages are the very cells that dengue prefers to infect.

As a result, exposure to a second dengue serotype can cause higher levels of infection than occurred the first time around[5,6]. Researchers led by Francis Ennis, an immunopathologist at the University of Massachusetts Medical School in Boston, have found that this has knock-on effects on another arm of the immune system: the 'killer' T cells that attack virus-infected cells. When exposed to a burgeoning infection with a second dengue serotype, the T cells can overreact. "It's like an immunological explosion," says Ennis. The killer cells start producing excessive quantities of cytokines[7], molecules that at normal concentrations help to coordinate immune responses. In excess, they can cause other cells, and capillaries, to leak fluid. This, Ennis suspects, leads to DHF[8].

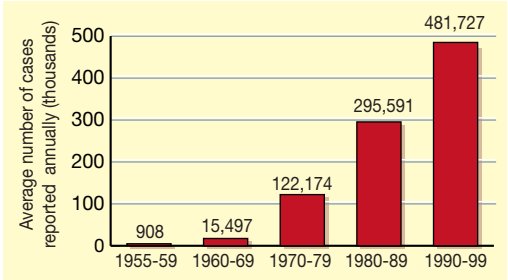
The phenomenon is worst in very young children with naive immune systems. They may receive antibodies against one serotype in their mother's milk and then become infected by a mosquito carrying a different one. So any reliable dengue vaccine must provide complete protection against not just one serotype but all four, and be safe for babies. Otherwise, vaccination in an endemic area could cause a rise in DHF incidence, even if it reduces the total number of dengue cases.

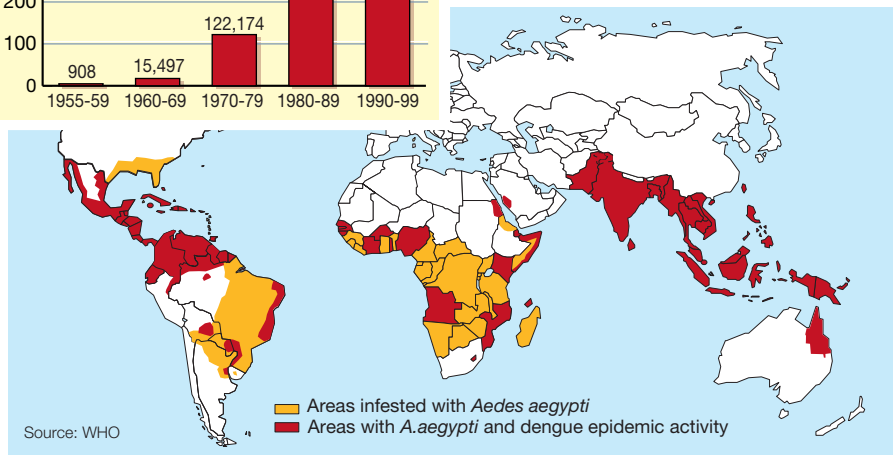
To make matters worse, says Alan Rothman, who works in Ennis's team, there is no good animal model with which to study dengue and DHF. "Mice get infected, but they don't get sick in any way," he says. Although monkeys produce antibodies against the virus and sometimes develop a fever, they never get the haemorrhagic form. So research into dengue vaccines involves a largely blind leap from the test tube to clinical trials.

Promising prospects

Despite these obstacles, dengue vaccines are now edging towards clinical use. Two of them, one developed at Mahidol University in Bangkok, Thailand[9], and another at the Walter Reed Army Institute of Research in Silver Spring, Maryland[10], are based on living, weakened dengue viruses, selected by growing all four serotypes through multiple generations in cultured cells. The Bangkok vaccine is now licensed to Aventis Pasteur of Lyon, France. Safety and efficacy trials are ongoing and show that the vaccine is safe and protects up to 90% of recipients from all four serotypes. The Walter Reed vaccine, licensed to GlaxoSmithKline Biologicals in Rixensart, Belgium, will begin safety and efficacy trials in infants later this year.

Other candidates are on the way. The National Institute for Allergy and Infectious Diseases in Bethesda, Maryland, is working on a dengue serotype in which virulence genes are deleted[11] — early indications are that this is safe and produces an antibody response. The next step is to insert genes that encode the





Global trend: the wide distribution of *Aedes aegypti* — largely due to lapses in efforts to control mosquito populations — means that dengue fever can strike throughout the tropics.

protein coat of the other three serotypes. Similarly, Gubler's team has genetically modified one of the weakened Bangkok viruses by adding coat-protein genes from the other three serotypes[12]. This vaccine has stimulated antibody production in rhesus monkeys.

One-shot deal

Another promising candidate is based on a modified form of the successful yellow fever vaccine — a live, weakened yellow fever virus carrying genes for the dengue protein coat, developed by Acambis of Cambridge, UK. Unlike the Bangkok and Walter Reed vaccines, which require two rounds of vaccination to provide immunity, the Acambis vaccine should require just one shot.

Encouragingly, Acambis has used the same approach to develop a vaccine against Japanese encephalitis, which is caused by a virus related to dengue, but with only one serotype. This vaccine is performing well in clinical trials[13]. Although the company has so far added genes from only one serotype to its dengue vaccine, it intends to add those from the other three. "We still need to show that immunity is lasting and persists for all four serotypes," says Tom Monath, head of vaccine development at Acambis, based at its US branch in Cambridge, Massachusetts.

Other approaches are at earlier stages of development. The US Naval Medical Research Center in Silver Spring and the biotech firm Maxygen of Redwood City, California, for instance, are both developing a vaccine consisting of genes for dengue coat proteins, packaged with other DNA that promotes the recognition of these proteins by the immune system. Each has been tested in animals, and human safety trials are planned for this year. Such 'naked' DNA vaccines[14] have the advantage of being completely non-infectious — whereas live, weakened vaccines can potentially revert to a disease-causing form.

Trail of blood: Thomas Scott is using DNA analysis to shed light on mosquitoes' activites.

MOHAMMED ARSHAD/AP

Breeding ground: open water containers serve as nurseries for the dengue virus's mosquito vector.

Given that dengue mostly afflicts poor countries, a vaccine is unlikely to be a major moneyspinner. So experts argue that public funds may be needed to bring vaccines to the people who need them. To focus fundraising and vaccine-development efforts, and to provide a forum to discuss the merits of the various candidates, the New York-based Rockefeller Foundation intends to set up a dengue vaccine programme at the International Vaccine Institute in Seoul, South Korea — which was established to promote the development of vaccines for diseases of the developing world. "This is a critical effort," claims Scott Halstead, a dengue researcher and the Rockefeller's associate director of health sciences.

Know your enemy

But even if Halstead and others successfully invigorate vaccine development, it will be years before vaccination against dengue is routine. In the meantime, a few researchers are getting intimate with dengue and its mosquito vector. They hope to understand exactly how the disease spreads, why it waxes and wanes — and perhaps to discover new ways to avoid or prepare for epidemics.

Working near Mae Sot in western Thailand, Thomas Scott of the University of California, Davis, has found that a female *Ae. aegypti* may need five blood meals to reproduce successfully, compared with the single blood meal required by most other mosquito vectors[15]. This finding, confirmed by individually marking female mosquitoes and recapturing them around people's homes, helps to explain why dengue can persist even when very few mosquitoes are present. It also shows that just a few infected mosquitoes or people are needed to kickstart an outbreak. "It's not good news for those trying to eradicate dengue by killing mosquitoes," says Scott.

Right now, Scott is developing techniques to evaluate the risk of dengue outbreaks in specific communities. Using DNA fingerprinting, he has been able to match the blood in captured mosquitoes' stomachs to DNA samples from villagers. He is now using this technique to discover which segment of the population mosquitoes bite, how frequently, and how far they roam in search of a meal[16].

In another project in Iquitos, Peru, Scott's team has begun to relate the dynamics of dengue transmission to the density of mosquito populations. They are building on previous research in which Halstead's team took blood samples from 1,300 schoolchildren and screened them for antibodies against dengue. They then monitored the arrival and subsequent spread a new serotype of dengue in this isolated Amazonian city, which previously has been host to only one serotype[17].

Basic research such as this, Scott hopes, may provide clues that will allow public-health officials to predict an imminent outbreak. "Our hope is that we can identify some fundamental principles that can be tested elsewhere," he says. Predicting an epidemic before it happens might not help to stop it, but it could help health departments prepare to treat the youngest and most susceptible victims, and so prevent unnecessary deaths.

After decades of neglect, Gubler is confident that dengue research and efforts to develop an effective vaccine will soon start getting the attention that he and others believe they deserve. "The other option is to sit around and watch thousands die every year," he says. ■

Tom Clarke works in *Nature*'s science writing team.

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WHO dengue factsheet

www.who.int/inf-fs/en/fact117.html

Draft resolution for World Health Assembly

www.who.int/gb/EB_WHA/PDF/EB109/eeb109r4.pdf