

Think of a mosquito and malaria invariably comes to mind; however, in South-East Asia it is just as likely to trigger thoughts of Japanese encephalitis. In the second in his series of articles on infectious disease in the Far East, Richard Collins contemplates a condition that affects mainly young children.

Japanese encephalitis – a growing threat?

In June 2004, Hong Kong recorded its first fatality from locally acquired Japanese encephalitis since 1996. Within weeks, two other locally acquired infections were confirmed. Following so quickly after the triple threats of avian influenza H5N1, severe acute respiratory syndrome (SARS) and dengue fever, the Hong Kong media was quick to speculate on the possibility that a new viral epidemic was about to be born. With travel to South-East Asia on the increase, the potential for exposure to new pathogens is increasingly likely. So, what progress has been made with the detection and prevention of Japanese encephalitis?

Cause and symptoms

Japanese encephalitis is caused by a mosquito-borne flavivirus. The viral genome comprises a single-stranded, non-segmented, positive-sense RNA molecule of about 11,000 nucleotides in length. The virus particle is enveloped, spherical and 40–50 nm in diameter. Japanese encephalitis virus (JEV) is closely related to several other pathogenic viruses, including those that cause dengue fever, West Nile fever, St Louis encephalitis and yellow fever.

Japanese encephalitis virus infects animals and humans, and is transmitted by the bite of an infected *Culex tritaeniorhynchus* mosquito (Fig 1). Humans and other mammals (eg horses) are dead-end hosts as infection produces only low-grade, short-term viraemia, making infection of subsequent biting mosquitoes highly inefficient. In contrast, pigs and aquatic birds (eg egrets, herons etc) are termed amplifying hosts because infection produces persistent high-grade viraemia, which increases the probability that subsequent biting mosquitoes will become infected and able to spread the disease.

In humans the incubation period is between four and 16 days, and JEV infection causes inflammation of the membranes around the brain. Most cases are mild and may be either asymptomatic or cause only fever and headache. However, infection in one case in 25–1000 results in severe disease characterised by rapid onset of high fever followed gradually by disturbances in speech, gait or other motor dysfunctions, headache, neck stiffness, disorientation, coma, seizures, spastic paralysis and ultimately death.^{1,2}

In children the disease usually begins with

gastrointestinal symptoms of anorexia, nausea, or abdominal pain. Irritability, vomiting and diarrhoea, or an acute convulsion, may be the earliest objective signs of illness in an infant or child. The case fatality rate can be as high as 60% among those with disease symptoms. Of those who survive, up to 50% suffer lasting damage to the central nervous system (CNS).¹ In areas where JEV is common, encephalitis occurs mainly in young children. Older children and adults tend to have been infected previously and thus are immune.

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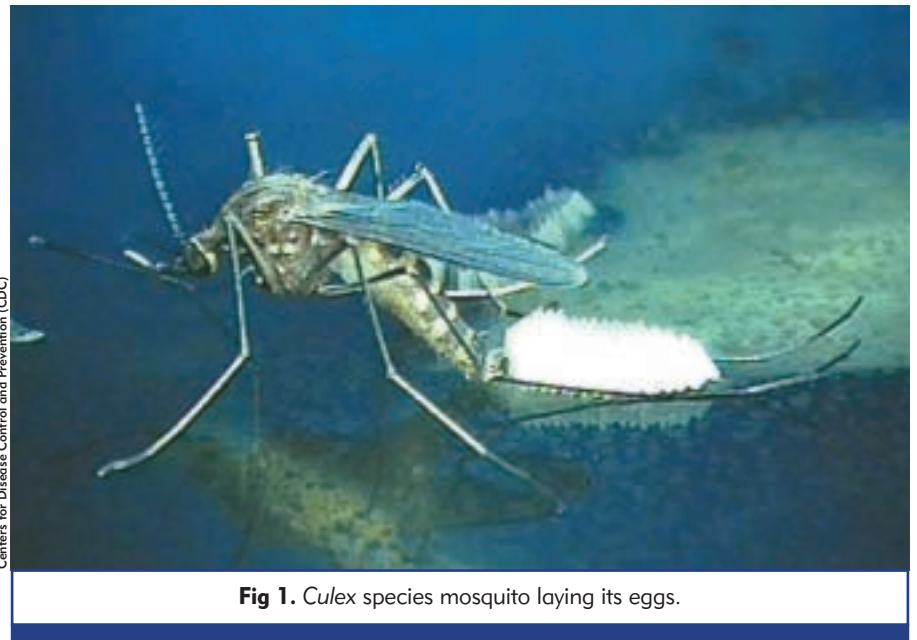


Fig 1. *Culex* species mosquito laying its eggs.



Fig 2. Global distribution of Japanese encephalitis (1970–1998).

Distribution

The global distribution of Japanese encephalitis is shown in Figure 2.³ In South-East Asia, India, China, Japan and Korea over 50,000 infections and 15,000 deaths, mostly of children, are recorded annually.¹ The disease occurs mainly in rural and agricultural areas of Asia and the western Pacific. In temperate regions such as China, Japan and Korea, transmission reaches a peak between April and September; in northern India and Nepal, peak transmission occurs between June and November; while in the tropical regions of Asia and Oceania, Japanese encephalitis occurs all year around.

Diagnostic tests

A complete blood count often shows a non-specific, modest leucocytosis in the first week of illness. A mild anaemia also may be present. In addition, serum sodium may be depressed because of inappropriate antidiuretic hormone secretion.¹

Immunoglobulin M capture enzyme-linked immunosorbent assay (ELISA) of serum or cerebrospinal fluid (CSF) is the standard diagnostic test for Japanese encephalitis.^{4–7} Specific IgM can be detected in serum or CSF in approximately 75% of patients within the first four days after onset of illness and nearly all patients are positive within seven days. In general, IgM is detected in CSF earlier than in serum but sensitivity approaches 100% when both serum and CSF are tested.

A specific diagnosis can be confirmed by demonstrating at least a four-fold increase in antibody titre by other serological procedures (eg haemagglutination inhibition,

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complement fixation, immunofluorescence or neutralisation).

Heterologous flaviviral antibodies (eg to dengue fever and West Nile viruses and from Japanese encephalitis and yellow fever vaccinations) are a potential source of false-positive reactions. False-negative results may occur if the samples are tested too early (eg within seven days of the onset of illness). For field diagnosis, IgM dot ELISAs for CSF and serum are portable, simple tests that compare favourably with the capture ELISA (sensitivity 98.3%, specificity 99.2% compared with capture ELISA as standard).⁸

In common with most of the pathogenic flaviviruses, nucleic acid tests have now been developed for JEV, with the first report of a reverse-transcriptase polymerase chain reaction (RT-PCR) assay for the organism appearing in 1991.⁹ The assay detected JEV successfully in cell culture, frozen brain tissue and in formalin-fixed paraffin-embedded brain tissue. An alternative RT-PCR used to analyse infected culture fluid was described in 1993.¹⁰

Owing to the importance of JEV, assay sophistication has kept pace with advances in

technological development. For example, a highly sensitive fluorogenic TaqMan RT-PCR has detected viral RNA from single infected mosquitoes in pools of 1000 mosquitoes, indicating its suitability for use in mosquito surveillance programmes;¹¹ an *in situ* RT-PCR has been used to detect JEV genome segments in mouse peripheral blood monocytes without the need for RNA extraction and later was used to demonstrate the transient nature of viraemia in the circulating blood and migration of the virus into the CNS;¹² and a LightCycler SYBR Green RT-PCR assay for JEV has been described for use in insect cell culture.¹³ However, extension of such tests to clinical use may be limited because the period of viraemia is very short and an effective neutralising antibody response means that cheaper and less technologically demanding serological methods of detection can be used. This is especially relevant to South-East Asian countries where access to expensive diagnostic tools is limited.

Prevention

Physical

The simplest way to prevent Japanese encephalitis is to avoid being bitten. Mosquitoes breed in pools of stagnant water and such pools should be drained or filled in. Mosquito nets over windows and beds are efficient and cost-effective, and exposed skin should be covered when venturing outdoors. In addition, one should avoid going out when mosquitoes are active (eg *Culex* species are most active from dusk until dawn). Mosquito repellents such as DEET (N, N-diethyl-m-toluamide) or other deterrents such as citronella oil, incense sticks and the newer electronic repellents are readily available.

Immunological

Vaccination is the most effective means of preventing Japanese encephalitis. A vaccine has been available in Japan since 1954. It was licensed in the USA in 1992 and, although not licensed for use, it is available in the UK on a named-patient basis. The vaccine is a formalin-inactivated, mouse brain-derived vaccine that is approximately 100% immunogenic after three doses (two doses are used in native populations in endemic areas). The vaccine is recommended for persons living in endemic areas and for at-risk travellers planning extended trips (ie more than 30 days) to rural areas.

The current dosing schedule for patients aged three years or over is 1.0 mL subcutaneously on days zero, seven and 30 (0.5 mL in patients aged 1–2 years). The last dose of vaccine should be administered at least 10 days prior to travel to an endemic area. A booster may be given after two years.

Mild adverse reactions have been reported in up to 20% of people. Adverse reactions include local pain and redness, fever, gastrointestinal symptoms, headache and myalgia. The incidence of reactions usually decreases with each subsequent dose.

Hypersensitivity, including angioedema or urticaria, occurs in 0.6% of patients, with 2.6 per 100,000 of those vaccinated requiring hospitalisation. The hypersensitivity reaction may be delayed by several days and patients should have access to medical care for 10 days after the last dose. Patients with a history of allergies or urticaria may be at higher risk of adverse reactions.

An inactivated cell-culture vaccine and a live attenuated vaccine have been used in China for more than 30 years. The live vaccine has proved safe and highly effective in several epidemiological studies but it is not readily available outside China.^{14,15}

Advances in genetic sequencing ushered in a new era of vaccine development and second-generation recombinant JEV vaccines were being evaluated as early as 1988.¹⁶ In 1998, the potential of DNA vaccines against JEV was demonstrated when 70–90% of mice injected intramuscularly with plasmid DNA encoding JEV glycoproteins were protected from a lethal JEV challenge.¹⁷ DNA vaccines have also been shown to provide cross-protection against heterologous JEV strains.¹⁸

A live attenuated vaccine derived from recombinant chimaeric JEV was demonstrated in 1999.¹⁹ A chimaeric yellow fever/Japanese encephalitis virus was constructed by inserting the pre-membrane and envelope genes of an attenuated human JEV vaccine strain between the core and non-structural genes of an infectious yellow fever clone.¹⁹

The vaccine produced using the chimaeric virus is now in advanced clinical trials.²⁰

The safety and effectiveness of a Vero cell-derived inactivated JEV vaccine has been tested in phase I clinical trials and found to be equivalent to the currently available conventional vaccine.²¹ Recombinant subunit vaccines (non-replicating protein vaccines) and formalin-inactivated whole virus vaccines are also in development.²²

A growing threat?

Japanese encephalitis is a major cause of morbidity and mortality in South-East Asia. However, it is the native population and long-term residents who are at increased risk of contracting the disease. The risk to the majority of travellers is extremely low.

Existing vaccines have a relatively high risk of hypersensitivity reactions and require a protracted vaccination schedule, which may limit acceptability and uptake rates by the general population. Newer recombinant vaccines in development offer the prospect of a cheaper, purer and more reproducible source of antigen with a high degree of immunogenicity.

Although the sensitivity and specificity of rapid nucleic acid-based diagnostic tests continue to be improved, access to such technology by developing countries remains limited. Thus, serological methods continue to play an extremely valuable role in the clinical laboratory.

As deforestation increases across Asia and the rural population encroaches on previously undisturbed habitats, the risk of exposure to Japanese encephalitis and other vector-borne disease increases significantly. Consequently, the number of cases of Japanese encephalitis and new vector-borne pathogens may increase in the future.

In Hong Kong, Japanese encephalitis has recently been made a notifiable disease, emphasising its growing importance as a public health threat in this region. In addition, mosquito surveillance has been enhanced to include the JEV vector *Culex* spp. and the dengue fever vector *Aedes* spp.

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Dr Richard A Collins AIBMS is the Scientific Review Director in the Research Office of the Health, Welfare and Food Bureau of the Hong Kong Government.