ARBOVIRUSES OF VETERINARY SIGNIFICANCE IN THE ASIA-WESTERN PACIFIC REGION, SUCH AS JAPANESE ENCEPHALITIS VIRUS

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Summary: Arboviral diseases receive serious consideration as the emerging infectious diseases globally. In veterinary medicine there are both zoonotic agents and primarily animal pathogens warranting concern. Japanese encephalitis virus and West Nile virus are two zoonotic arboviral agents that have had a major impact globally in recent years, Japanese encephalitis in the Asia-Western Pacific area and West Nile in Europe, the Middle east and North America. OIE Member Countries of the Regional Commission for Asia, the Far East and Oceania were surveyed by questionnaire to assess levels of concern and preparedness for these diseases within the region, as well as seeking information on other arboviral infections of domestic animals that may be recognized. Responses indicated varying levels of diagnostic capability and hence of specific information regarding the nominated viruses. There was widespread recognition of the public health implications of Japanese encephalitis. Bovine ephemeral fever was the most widely reported arboviral animal pathogen. Where Member Countries rate it a priority, there is particular scope for collaboration in development of common approaches to laboratory diagnosis of arboviral diseases and the systems of laboratory quality assurance to manage the diagnostic capability. More generally, there is scope to discuss common approaches to emergency animal disease preparedness, including preparedness for zoonotic arboviral disease.

1. INTRODUCTION

Arboviruses are prominent among the emerging infectious disease agents globally. They are viruses with a life cycle that includes replication in an arthropod vector and that are transmitted by that vector. Their range can increase quite dramatically either through movement of infected vertebrate hosts to regions that already have competent vector species, or independently of the vertebrate host by movement of infected vectors. Such vector movement may be by gradual extension of range during climatically suitable periods, more long distance dispersal of insects by winds, or by the human-assisted short or long distance transport of infected insects on transport such as shipping and aircraft. Recent examples of extensions to the range of such agents include outbreaks of infection and disease associated with serotypes of the bluetongue viruses (BTV) in southern and eastern Europe (for example Stylas, 2001; Marabelli, 2001; Danevski, 2001), Rift Valley fever in the Middle East (Nasher et al., 2000), West Nile (WN) virus infections in North America (Nash et al., 1999; Petersen and Roehrig, 2001) and Japanese encephalitis (JE) infections moving from East Asia westwards into South Asia and eastwards into Oceania (reviewed by Tsai, 1997; Mackenzie et al., 2001; Daniels et al., 2002). This report summarises the current distribution, clinical significance, laboratory diagnostic capability and control measures for arboviruses of veterinary and zoonotic significance in Asia, the Far East and Oceania as advised by Member Countries and as reported in the scientific literature.

Five of the 15 disease agents in the OIE List A may technically be classified as arboviruses: African horse sickness, African swine fever, BTV, Rift Valley fever and vesicular stomatitis viruses, although African swine fever and the latter two agents are also contagious and may be spread by means other than infected arthropods. Arboviruses in List B include equine encephalomyelitis (Eastern, Western and Venezuelan), JE and Nairobi sheep disease. Of these List A and B agents, only BTV and JE are known to be widespread in the Asian and Oceanic regions covered by the Commission. African horse sickness has made one incursion into South Asia some 40 years ago (Uppal, 1992).

Since the BTV serogroup in the genus Orbivirius of the family Reoviridae comprises 24 serotypes of virus with variation in virulence of strains even of the same serotype, and BTV infections occur on all continents with susceptible ruminants, BTV could properly be the subject of a major, dedicated review. This report will concentrate mainly on the List B agent JE which has been identified as a serious emerging disease threat in the region, the closely related virus WN that has shown the capacity for dramatic emergence as a disease causing agent in new areas in the USA, and other viruses reported from time to time in the Asia Pacific region but not currently included in the OIE Lists A or B.
2. OVERALL LEVELS OF CONCERN IN THE REGION REGARDING ARBOVIRAL DISEASE

There does not seem to be a high level of monitoring for arboviruses by veterinary agencies in the Asia-Western Pacific region. Of 13 countries that responded to a recent questionnaire (Daniels 2001), most did not report the presence of arboviruses or arboviral disease (Table 1). (The response from the USA was not included in the analysis since that country is in a different geographic region.) The most widely recognized arboviruses in the region were JE and bovine ephemeral fever (BEF).

Table 1: Summary of responses from veterinary authorities in 13 countries in the Asia/Western Pacific region regarding the presence of certain arboviral infections and associated disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Presence of infection recognized</th>
<th>Animal or human disease recognized</th>
<th>Virus isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akabane (AKA)</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Bovine ephemeral fever (BEF)</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Bluetongue (BTV)</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Epizootic haemorrhagic disease (EHD)</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Getah</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Japanese encephalitis (JE)</td>
<td>9</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Murray Valley Encephalitis (MVE)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>West Nile (WN)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3. AN OVERVIEW OF ARBOVIRUSES IN THE REGION

BEF, in the family Rhabdoviridae, is associated with a recognized clinical syndrome, three day sickness, of a valuable livestock species, cattle, and hence may have higher prominence than other arboviral infections. In Indonesia clinical BEF has been recognized since 1918, with major epidemics in 1928 and 1931 (reviewed by Daniels et al., 1993). Recent serological studies have confirmed field reports that BEF is widespread in that country, and BEF or related viruses have been isolated (Daniels et al., 1993; Soleha et al., 1993). The disease has been recognised in China since the mid 1930s, with epidemic activity being well documented (Bai Wenbin, 1993). BEF virus was isolated in China in 1976 (Liu Shanggao, 1993). In Japan epidemics have also been recognized for more than 50 years, and BEF virus isolation from infected cattle was reported in 1968. BEF is considered economically important in that country, and vaccination is practiced (reviewed by Tanaka and Imaba, 1986). Epidemics of BEF have occurred in Australia since at least 1936, and possibly prior to that (St George, 1986b). Clinical BEF is also reported in Africa and the virus isolated there (reviewed by Davies, 1993), and BEF is considered endemic in India (Patel et al., 1993). Hence BEF may be considered endemic throughout tropical and subtropical Africa, Asia and Australasia wherever climatic conditions favour the insect vectors. Species of Culicoides are involved, and there is also evidence for mosquito vectors (Muller and Standfast, 1986).

In the country responses received for this report only Japan indicated a high level of concern for BEF, with the disease being notifiable in that country. Taipei China reported epidemics at 6 to 7 year intervals, with vaccination being practiced. Australia, Indonesia and Nepal reported seasonal activity and Malaysia reported serological evidence of infection. For the region there seems to be no evidence that BEF is extending its range, but continuing epidemic activity can be expected depending on seasonal influences and the immune status of the susceptible cattle populations.

BTV is widespread in the region, being recognized in India (Prasad et al., 1992), Malaysia (Sharifah et al., 1995), Indonesia (Sendow et al., 1991, 1993; Daniels et al., 1995), Australia (St George et al., 1978; ) and China (Zhang et al., 1996). In India, BTV is clearly virulent, being associated with disease in Asian breeds of sheep as well as imported breeds from Europe (Prasad et al., 1992; Sreenivasulu et al., 1996). Further to the east, in countries such as Malaysia and Indonesia, bluetongue disease is not seen in Asian breeds of sheep, with cases having been reported only when European breeds were imported (Sudana and Malole, 1982; Sharifah et al., 1995). Hence, in the absence of bluetongue disease, there has not been the incentive to develop a diagnostic capability for this virus in many countries, with the consequent possibility that infections remain unidentified. In terms of disease preparedness, it may be prudent to investigate the basis of this apparent difference in virulence within the region, and to assess under what conditions BTV strains of greater
virulence may extend their range (Daniels et al., 1997). Such an event would see BTV become an emerging infectious disease agent of great concern to the Regional Commission.

In Australia where arboviral infections have been extensively studied, other arboviruses such as Akabane (AKA) and epizootic haemorrhagic disease (EHD) viruses tend to have a similar distribution to some BTV serotypes, having the same or similar insect vectors (St George, 1986a). Hence it might be expected that infections with AKA (family Bunyaviridae) and EHD (family Reoviridae, genus Orbivirus) may also be under reported throughout the area covered by the Regional Commission, again associated with lack of clinical disease to stimulate a need for development of a laboratory capability. As an example, in Australia monitoring for BTV has also resulted in isolation of 6 of the 8 EHD serotypes recognized internationally (St George et al., 1983, Weir et al., 1997). These viruses of ruminants could not have occurred naturally in Australia, but more likely have arrived from areas further to the north in Southeast Asia after ruminants were introduced (St George 1986a).

In Australia EHD has not been associated with animal disease, while disease due to AKA, in the family Bunyaviridae, is diagnosed only at the extremities of the range of its distribution in occasional epidemics associated with local expansion of the range of the insect vector due to favourable climatic conditions (Kirkland et al., 1989). It is likely throughout much of Asia that EHD viruses, although possibly widespread, are not serious pathogens. Similarly in might be expected that AKA is endemic throughout much of the tropics and subtropics of Asia and infecting animals before they reach breeding age and hence susceptibility to reproductive disease. A serological survey in Indonesia found a seroprevalence of 80% to AKA and 99% to the closely related Aino virus (Miura et al., 1982). The seroprevalence of EHD reactors was lower, but again the evidence was for a wide distribution of these viruses (Sendow et al., 1991; Daniels et al., 1995).

In responses to the current questionnaire, apart from Australia and Indonesia, only Japan and Taipei China reported information regarding AKA. In Taipei China AKA was isolated in association with foetal deformities in cattle in 1992, while in Japan widespread outbreaks were reported. In Japan an outbreak associated with Aino virus was reported in 1999, but Aino virus has not been reported from Taipei China. Interestingly, New Caledonia in the Pacific reported serological evidence of Aino virus infection, with but with evidence of disease. Only Australia and Japan reported the current presence of EHD viruses.

A special case of EHD is that of Ibaraki virus, classified as serotype 2 in the EHD serogroup, which comprises 8 serotypes (van Regenmortel et al., 2000). This strain was isolated from cattle suffering a disease characterised by stomatitis, swelling of the conjunctivae, ulceration of the nasal pad and difficulty in swallowing (Omori, 1961). Although EHD serotype 2 has been isolated in the USA (reviewed by Karabatsos, 1985) and the EHD serotype 2 isolated in Australia is antigenically very similar to the Ibaraki strain (Della-Porta et al., 1986), Ibaraki disease does not appear to have been identified as a problem outside of East Asia. In responses to the current questionnaire, Japan recorded that Ibaraki is a notifiable disease in that country, and that a small outbreak was diagnosed in Okinawa in the year 2000. Taipei China recorded isolation of Ibaraki virus in 1990 from a bovine showing nonsuppurative encephalitis, a syndrome noted sporadically since 1985.

Another Orbivirus associated with disease only in Japan to the present time is Chuzan virus, which was isolated initially from healthy cattle and Culicoides oxystoma midges (Miura et al., 1988). It was implicated serologically in an epidemic of congenital abnormalities in calves at that time (Goto et al., 1988). Subsequently Chuzan virus has been reclassified (van Regenmortel et al., 2000), being considered identical with Kasba virus isolated in Vellore, India, in 1957 (reviewed by Karabatsos, 1985). The virus is in the Palyam serogroup of the genus Orbivirus. In Australia there has been serological evidence for involvement of a Palyam virus in an outbreak of congenital abnormalities (Kirkland et al., 1989). Chuzan disease is also a notifiable disease in Japan, with cases reported in 1999 and 2000 in the Kyushu district. No other respondent countries reported infections with this virus.

Getah virus, in the Alphavirus genus in the family Togaviridae (van Regenmortel et al., 2000), was first isolated in Malaysia from Culex gelidus mosquitoes feeding on cattle. It was subsequently isolated in Japan, Cambodia, the Philippines and Russia, with one report from Australia. Although infecting a range of species, it has mainly been associated with a febrile disease in horses accompanied by nasal discharge, rash and oedema. A number of epidemics have been reported in racing establishments in Japan. Getah virus has been isolated from aborted foetuses in pigs, and serological evidence presented to indicate a role in reproductive failure in that species. However Getah virus is considered mainly an equine pathogen. A range of mosquito species may act as vectors (Karabatsos, 1985; Kono, 1988; Geering et al., 1995). With the exception of Japan, respondents to the current survey do not recognize its presence in their countries at the present time. In Japan vaccines are reported to be available for use in both horses and pigs.

Murray Valley encephalitis (MVE) virus and Kunjin (KUN) viruses are in the JE serogroup of the genus Flavivirus of the family Flaviviridae, as are JE and WN (van Regenmortel et al., 2000). Both MVE and KUN occur predominantly in Australia, although MVE has been isolated in Papua New Guinea and KUN from Sarawak in East Malaysia. The more westerly limit to the distribution of the two viruses has not been accurately determined, but they are believed to be
primarily associated with the Australian zoo-geographic region (Mackenzie et al., 1997). Neither virus is regarded as a pathogen of domestic animals, although MVE has been suspected of causing rare cases of encephalitis in horses.

4. JAPANESE ENCEPHALITIS VIRUS

Distribution

Responses to the current questionnaire confirmed that JE is currently recognized by veterinary authorities in Indonesia, Japan, Malaysia, Myanmar, Nepal, the Philippines, Singapore, Taipei China and in the islands of the Torres Strait to the north of Australia.

The distribution of JE has been well studied from a public health perspective. It has been reported that the range of JE extends from Japan, Korea, and maritime Siberia southwards through China to the Philippines, and throughout Southeast Asia, from Indonesia to Thailand, Vietnam, Laos, and Myanmar (Burke and Leake, 1988). More recently the known range has extended westwards into India, Bangladesh, Nepal and Sri Lanka (Umenai et al., 1985; Vaughn and Hoke, 1992; Tsai, 1997). The virus was detected in south-eastern Pakistan in 1993 (Igarashi et al., 1994). Even more recently, outbreaks occurred in people in far northern Australia, in the Torres Strait Islands (Hanna et al., 1996, 1999), with eastern Indonesia and Papua New Guinea now known to be infected also (Daniels and Ginting, 1992; Johansen et al., 2000; Mackenzie et al., 2001). Hence much of the area covered by the Regional Commission may be exposed to endemic or epidemic JE activity.

Epidemiology

The natural history of JE virus has been described in early studies in Japan (Buescher and Scherer, 1959; Scherer et al., 1959) followed by others in Thailand (Grossman et al., 1974; Johnsen et al., 1974) and Indonesia (van Peenan et al., 1974, 1975). The main mosquito vector throughout Asia is Culex tritaeniorhynchus. The major sylvatic hosts are ardeid birds, especially the black-crowned night heron, Nycticorax nycticorax, which is considered to be the most important avian maintenance host. Egrets and other herons may also be involved. This water bird-mosquito life cycle may be considered the natural ecosystem for JE (Hammon et al., 1951). However the major mosquito species are rice field breeding, which brings the natural system into close contact with man and domestic animals. The domestic pig is susceptible to JE infection, showing high levels of viraemia, and is considered an amplifier host. It can function as a maintenance host in the absence of water birds. The above studies emphasized the importance of the pig in the epidemiological chain of events that result in human disease, a factor confirmed in studies of more recent extensions to the geographic distribution of JE (Peiris et al., 1992; Hanna et al., 1996).

JE activity tends to be epidemic in more temperate climates, with discrete and obvious epidemics (Takashima et al., 1988; Tsai, 1997) and a relatively low seroprevalence in human and animal populations. This pattern of activity probably reflects susceptibility of the vectors to unfavorable climatic fluctuations. In tropical areas with monsoonal rainfall, the virus tends to be endemic, showing a high seroprevalence in susceptible populations, including pigs, and with only occasional cases of disease, in young children not protected by antibodies.

JE as an emerging disease

As noted above, the geographic distribution of JE is increasing, resulting in its classification as an emerging disease (Tsai, 1997; Mackenzie et al., 2001). Hence identification of the factors favoring dispersal is necessary to account for its emerging importance. Human factors are likely to be involved, both directly and indirectly. Indirect influences include the increase in area of land under irrigation for rice in much of Asia, increasing the habitat for the mosquito vectors. There has also been a major expansion in the pig industry in many countries. The figures for pig populations and the structure of the pig industry as supplied by respondents confirmed the trend to intensification of this industry in recent times. Only 3 of 9 countries reporting on pig industry structure had a village pig component of 30% or more of the porcine population. Together these changes in agriculture have increased the size of the ecological niche available for JE and its insect vectors. Natural pressure to fill this expanded niche would be expected to result in the spread of JE into new areas, and an increase in virus activity in those areas where it was already established. From a public health perspective, increasing human populations and the resulting spread of urbanization have resulted in more people living in closer proximity to large pig farming areas in some countries.

Increases in the frequency and speed of aircraft and shipping movements may have created more direct opportunities for the distribution of JE, via infected people, animals or mosquitoes. However some preliminary interpretations of molecular studies of JE viral isolates from throughout its global range tend to indicate local encroachments of range rather than long distance dispersal as the predominant pattern (Williams et al., 2000; Daniels et al., 2002). Such a pattern of spread could still be facilitated by factors such as the increase in ferry and aircraft traffic, as has occurred between the
islands of central and eastern Indonesia in recent decades, for instance, that could have contributed to the easterly dispersion of JE in that part of the region.

Nature undoubtedly also has played a role in what may be expected to be a complex and multifaceted process. Wind may be a factor in driving encroachments of virus, through the dispersal of infected mosquitoes, as has been suggested to explain the southward movement of JE from West Papua province of Indonesia and from PNG into northern Australia (Ritchie and Rochester, 2001). The migration of water birds has been identified from the early years of the study of JE as a possible mechanism for movement of virus from endemic areas to areas of epidemic activity (Buescher and Scherer, 1959), but as noted above, molecular analyses of isolates do not indicate that such long distance movements are a regular feature of JE ecology (Chen et al., 1990; Williams et al., 2000), although they remain a possibility.

Early studies of JE identified that epidemics were correlated with the abundance of mosquito vectors (Buescher and Scherer, 1959). Peiris et al. (1992) observed that the major difference between epidemic and non-epidemic years was the lower vector biomass in the latter case, and an accompanying lower infection rate in the vectors. Hence it may be expected that climatic factors will influence JE dispersal through creating or denying favourable environments for the mosquito populations. Factors particularly likely to influence the range and activity of JE in this way have been expected: in the usually wet area of western PNG it has been hypothesized that relative drought conditions allowed breeding of large mosquito populations, usually washed away by torrential rain, that were subsequently blown south into northern Australia with a resultant JE outbreak (Hanna et al., 1999; Ritchie and Rochester, 2001).

**Disease in horses**

Since pigs routinely show a high seroprevalence in serological surveys but reports of disease are rare, it is clear that most JE infections of pigs are asymptomatic. However JE virus is regarded as a porcine pathogen, causing fetal encephalitis, abortion, and stillbirth in pigs, and hypospermia and aspermia in boars (Joo and Chu, 1999). Burns (1950) described reproductive failure with mummified fetuses and birth of piglets too weak to survive. Encephalomalacia and hydrocephalus were described in a high proportion of affected young. The condition was widespread in Japan during years when there were epidemics of JE disease in people. JE virus was isolated from affected litters.

Of the responding countries in the current survey, only Japan reported that JE caused disease in pigs, although a number of countries reported laboratory confirmation of infection in pigs. Nepal reported undiagnosed reproductive failure in pigs. The publication of Takashima et al. (1988) confirmed that reproductive disease in pigs still occurs where JE infections are seasonally epidemic, and are diagnosed where there is a laboratory capability to support investigations of cases.

**Disease in horses**

In spite of sizeable equine populations being reported by most respondent countries, none reported JE disease in horses. This may be because in countries with an active diagnostic capability the horses that are under closer supervision are usually vaccinated.

Clinical JE has been reported in horses for over a century (Nakamura, 1972). The literature regarding the equine disease in Japan and elsewhere has recently been reviewed (Ellis et al., 2000). In Japan epidemics of equine disease occurred concurrently with epidemics in the human population. The clinically signs were variable, with both mild and severe disturbances of nervous function being reported. The case fatality rate in endemic areas has been reported as low as 5%, but as high as 40% during epidemics. Mild JE has been reported to interfere with racing performance. A group of breeding mares imported into northern Thailand suffered 33% mortality from encephalitis. JE was diagnosed on the basis of serology. Some recovered animals suffered permanently from ataxia and urinary incontinence.

**Public health significance of Japanese encephalitis**

The main importance of JE is as a threat to public health. Figures usually given to indicate the scope of disease in people in the region are over 50,000 cases of encephalitis annually with about 15,000 deaths (reviewed by Umenai et al. 1985; Burke and Leake, 1988; Vaughn and Hoke, 1992; Tsai, 1997; Solomon and Vaughn, 2002). Fortunately most JE infections are asymptomatic, with estimates indicating that as few as 1 in 300 infections results in clinical disease (Vaughn and Hoke, 1992; Tsai, 1997). However the consequences of developing clinical disease are severe, with approximately 25% of cases of JE being fatal, and a further 50% resulting in neurological sequelae such as motor impairment or mental retardation. WHO recognizes JE as an important emerging pathogen.

Six of the responding countries reported that JE disease still occurs in people in their country. Reported patterns of disease varied, with the elderly being more at risk in Japan and children in Nepal. Japan, Nepal and Taipei China reported vaccination of the human population, but 6 countries reported public education campaigns. Most countries
reported the pig as the important reservoir host. Water birds were also mentioned by Nepal. In Singapore JE has been eliminated by removal of the pig population, and neither vaccination nor public education were reported by that country.

**Diagnostic Capability**

The responding countries reported an uneven range of diagnostic capability within the region, reflecting the varying importance given to JE by veterinary and public health agencies. The information is summarised in Table 2. Ideally veterinary laboratories should have the capability to investigate encephalitic disease in horses and reproductive failure in pigs for involvement of JE. Since JE is such a major public health issue, it would be ideal for labs to be able to monitor pigs or other animals serologically for public health surveillance where this was requested by health agencies.

### Table 2: Summary of responses from veterinary authorities in 13 countries in the Asia/Western Pacific region regarding laboratory capability for Japanese encephalitis virus diagnosis

<table>
<thead>
<tr>
<th>Test capability by country</th>
<th>JE diagnostic laboratories</th>
<th>Tests to detect virus</th>
<th>Serological tests</th>
<th>Type strain</th>
<th>Source of reagents</th>
<th>Inter-laboratory comparison of tests?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>6</td>
<td>Isolation, FAT, PCR, IHC</td>
<td>C-ELISA, HI, CFT, PRNT</td>
<td>Nakayama</td>
<td>Made in country</td>
<td>Yes, formal and informal</td>
</tr>
<tr>
<td>Indonesia</td>
<td>8</td>
<td>Isolation, IHC</td>
<td>HI, ELISA, others</td>
<td>Not advised</td>
<td>CDC Fort Collins; AAHL and UQ, Australia</td>
<td>Yes, informal</td>
</tr>
<tr>
<td>Japan</td>
<td>6+</td>
<td>Isolation, PCR, IHC</td>
<td>HI, CFT, ELISA</td>
<td>Nakayama</td>
<td>Made in country</td>
<td>Yes, NIAH acts as a reference lab</td>
</tr>
<tr>
<td>Malaysia</td>
<td>3</td>
<td>PCR, FAT, IHC</td>
<td>HI, ELISA</td>
<td>Nakayama</td>
<td>Japan, and made in country</td>
<td>Yes, among the 3 laboratories</td>
</tr>
<tr>
<td>Myanmar</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nepal</td>
<td>3</td>
<td>None</td>
<td>HI, ELISA</td>
<td>Not advised</td>
<td>AFRIMS, Bangkok</td>
<td>Yes, with AFRIMS acts as a reference lab</td>
</tr>
<tr>
<td>New Caledonia</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>1</td>
<td>Isolation, PCR</td>
<td>Not available</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Philippines</td>
<td>2</td>
<td>Isolation</td>
<td>HI, ELISA</td>
<td>Not advised</td>
<td>Made in country</td>
<td>Not yet established</td>
</tr>
<tr>
<td>Russia</td>
<td>None advised</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td>3</td>
<td>Isolation, PCR</td>
<td>HI, CFT, PRNT</td>
<td>Nakayama</td>
<td>Japan and in country</td>
<td>Natl University of Singapore acts as a reference lab</td>
</tr>
<tr>
<td>Taipei China</td>
<td>2</td>
<td>Isolation, PCR</td>
<td>HI, ELISA, PRNT</td>
<td>Nakayama, Beijing</td>
<td>Made in country</td>
<td>Informally between labs</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Definitive serological diagnosis of JE infections in regions where other flaviviruses are circulating is difficult due to cross reactions that are the result of close antigenic similarities among the viruses (van Peenan et al., 1974; reviewed by Mackenzie et al., 1998; Williams et al., 2001; Daniels et al., 2002). Sequential infections with two flaviviruses, or multiple flaviviral infections, result in anamnestic and heterotypic responses that make identification of the infecting viruses on the basis of the serological responses almost impossible. These problems of differentiation of antibody responses with respect to JE occur with infections with other flaviviruses such as dengue viruses as well as to viruses in the actual JE serogroup.
Diagnosis of animal infections by detection of the virus is also a little difficult due to the short period of viraemia, usually 2 to 4 days. However, in animals suffering encephalitis, detection of viral nucleic acid has been possible even in the absence of viable virus (Williams, Lunt and Daniels, unpublished data). A useful panel of tests for JE diagnosis has been tabulated by Ellis et al. (2000), but there is an urgent need for a serological test that will specifically detect antibodies produced in response to JE infection and not produced in response to single or multiple infections with other viruses in the JE serogroup (Williams et al., 2001). Even the plaque reduction neutralization test (Takashima, 2001) does not satisfy this need.

**Surveillance**

The regular, sequential sampling of sentinel animals is currently the most appropriate surveillance method for detecting JE activity. Sentinel pigs have been used successfully in Thailand (Johnsen et al., 1974; Burke et al., 1985b; Gingrich et al., 1987), Japan (Maeda et al., 1978), India (Geevarghese et al., 1987a,b, 1991), Indonesia (Van Peenen et al., 1974b; Daniels et al., 1995) and more recently in the Torres Strait Islands and northern Australia (Hanna et al., 1999). However, as pigs are an amplifying host there is an increased public health risk to people in the immediate vicinity if the sentinels become infected. Cattle are being investigated as an alternative sentinel animal. They are not believed to participate in natural JE transmission cycles, but show a high prevalence of antibody positive animals in endemic areas. Mosquito vector species frequently show a feeding preference for cattle.

**Vaccination**

Japan, Nepal and Taipei China have some vaccination programs in pigs, but the numbers reported as vaccinated represent only a small proportion of the pig population in each case. Nepal reported annual usage of 56,000 doses of live attenuated vaccine of tissue culture origin produced in Korea, and Taipei China reported vaccination of 92,000 head annually, with a number of products manufactured from Nisseiken, M, SA and AT strains being available. Japan reported only 25,000 head vaccinated by government agencies, but a large range of vaccines available on the market (Table 3). Both inactivated vaccines and live attenuated products are available for use in pigs, and of the latter there are a number of polyvalent products incorporating porcine parvovirus and Getah virus vaccination.

<table>
<thead>
<tr>
<th>Type</th>
<th>Manufacturer</th>
<th>JE Virus Strain</th>
<th>Culture Method</th>
<th>For use in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated</td>
<td>CTSI*</td>
<td>Nakayama</td>
<td>Mouse brain</td>
<td>Horses, pigs</td>
</tr>
<tr>
<td>Inactivated</td>
<td>Nisseiken Co Ltd</td>
<td>BM</td>
<td>Mouse brain</td>
<td>Horses, pigs</td>
</tr>
<tr>
<td>Inactivated</td>
<td>KBL#</td>
<td>Nakayama, Yakken line</td>
<td>Chicken embryo fibroblast cells</td>
<td>Horses, pigs</td>
</tr>
<tr>
<td>Live</td>
<td>Nisseiken Co Ltd</td>
<td>AT</td>
<td>Hamster kidney cells</td>
<td>Pigs</td>
</tr>
<tr>
<td>Live</td>
<td>KBL</td>
<td>M</td>
<td>Hamster lung cells (HmLu-1)</td>
<td>Pigs</td>
</tr>
<tr>
<td>JE/swine parvovirus, live</td>
<td>CSTI</td>
<td>M</td>
<td>Swine kidney cells (SK)</td>
<td>Pigs</td>
</tr>
<tr>
<td>JE/swine parvovirus, live</td>
<td>KBL (joint venture with above)</td>
<td>M</td>
<td>Swine kidney cells (SK)</td>
<td>Pigs</td>
</tr>
<tr>
<td>JE/swine parvovirus, live</td>
<td>CBI**</td>
<td>S</td>
<td>Koushi kidney cells</td>
<td>Pigs</td>
</tr>
<tr>
<td>JE/swine parvovirus/Getah virus, live</td>
<td>KBL</td>
<td>M</td>
<td>HmLu-1 cells</td>
<td>Pigs</td>
</tr>
<tr>
<td>JE/Getah virus, inactivated</td>
<td>Nisseiken Co Ltd</td>
<td>BM</td>
<td>MPK- aCI cells</td>
<td>Horses</td>
</tr>
<tr>
<td>JE/equine influenza/tetanus, inactivated</td>
<td>CSTI</td>
<td>Peking</td>
<td>Mouse brain</td>
<td>Horses</td>
</tr>
<tr>
<td>JE/equine influenza/tetanus, inactivated</td>
<td>Nisseiken Co Ltd</td>
<td>BM</td>
<td>MPK- aCI cells</td>
<td>Horses</td>
</tr>
<tr>
<td>JE/equine influenza/tetanus, inactivated</td>
<td>CSI (joint venture with above)</td>
<td>BM</td>
<td>MPK- aCI cells</td>
<td>Horses</td>
</tr>
</tbody>
</table>

* CTSI Chemo-Sero-Therapeutic Research Institute
The vaccines available for horses in Japan are also listed in Table 3. Japan, Malaysia, Singapore and Taipei China all reported vaccination of horses. Singapore and Malaysia reported the use of the BM III strain inactivated vaccine prepared in tissue culture by the Nisseiken Company in Japan. This product is also used in Macau and Hong Kong, and vaccination of horses using products prepared in-country has been reported in China and Korea (Ellis et al., 2000). Australia vaccinates performance horses being exported to countries in the region where this is required by the importing country.

Horse racing organizations in Singapore, Malaysia, Hong Kong, Macau and Japan all have this requirement, and the introduction of vaccination has greatly reduced the incidence of JE in horses. The last reported case of equine JE in Hong Kong was in 1981 (Ellis et al., 2000).

Other control measures

Other approaches to JE control are vector control and vector avoidance. Insecticides to kill either adult or larval mosquitoes are not considered an effective long term approach, requiring frequent use and often resulting in insect resistance (reviewed in Burke and Leake, 1988; Vaughn and Hoke, 1992). Wide scale insecticide use raises environmental concerns and potential human health (toxicologic) problems, and usage may be best targeted to local objectives during outbreaks.

Modification of the local environment to reduce or contain mosquito populations at low numbers can be useful. Approaches include reducing breeding sites for vectors around pig farms and removing pig farms and pig pens from near human habitation.

Protection of susceptible animals against insect bite has been recommended as a control measure against JE in horses. A variety of approaches can be used, such as screened housing, the use of residual insecticides on housing and equipment, and even the use of systemic insecticides to reduce insect numbers in the vicinity (Ellis et al., 2000).

5. WEST NILE VIRUS

The natural history of WN has been comprehensively reviewed (Hayes, 1989). It was discovered in Africa, firstly in Uganda and subsequently in Egypt, where much of its life cycle was studied. It can infect a wide range of vertebrates, from rodents and birds to dogs, horses, camels and humans. Similarly, numerous mosquito species have been identified as potential vectors, particularly *Culex* sp but also *Aedes* sp and *Anopheles* sp, and also some species of ticks normally associated with birds. *Cx univittatus* may be the main vector in Africa. Wild birds were identified as the likely natural host of the virus, particularly but not exclusively the hooded crow. The geographical distribution of the virus to that time was from South Africa throughout Africa to the Middle East, southern Europe, the USSR and eastwards into Pakistan and India. Movement is believed to be by facilitated by migratory birds, which land in places such as Egypt and southern France while en route between southern Africa and the USSR and eastern Europe. Epidemics of WN disease were reported in the 1950s in Israel, southern France in the 1960s and South Africa in the 1970s and 1980s. Humans were the main species reported as susceptible to disease, with occasional cases of encephalitis in horses. Crows were shown to be susceptible in experimental studies.

More recently, in 1996, an epidemic of WN encephalitis occurred in Romania (Tsai, 1997). The outbreak was attributed to a spill over of WN from a sylvatic bird-mosquito cycle in contact with migratory birds to an urban cycle based on *Cx pipiens*, which infests housing throughout the affected area. Domestic chickens and urban passerine birds were also numerous in the cities and towns of the region. Serological studies have indicated that the sylvatic cycle was not a new phenomenon in the region, whereas the serological pattern of exposure in the human population indicated no prior endemic urban cycle if infection.

The occurrence of a WN epidemic in the USA, commencing in 1999, was a major expansion to the range of the virus (Nash et al., 1999). It occurred against a background of increasing WN associated disease globally. Since the epidemic in Romania there have been outbreaks in Morocco, Tunisia, Italy, Russia, Israel and France. There has been an increase in severe human disease associated with recent outbreaks, and high avian mortalities have been observed where human disease has been more severe (Petersen and Roehig, 2001). Molecular analyses show a relationship between the isolates from outbreaks in Europe and Asia and the strain involved in Romania. The US outbreak strain is most closely related to a strain circulating in Israel from 1997 to 2000, which has not been identified elsewhere to the present time. This strain, IS98/NY99, showed genetic stability into the following year and across all species affected in the US (reviewed by Petersen and Roehig, 2001). As well as severe human disease, the US outbreak has been associated with a high avian death rate and disease in horses. Horses were particularly involved in the outbreaks in Morocco and Italy (Deubel et al., 2001).
In the area covered by the Regional Commission, WN has been known for many years in Pakistan and India, with virus isolations in India from as early as 1955 (reviewed by Hayes, 1989). Now that JE has extended its range westwards, as reviewed above, JE and WN co-circulate in South Asia. In an outbreak of human encephalitis in Karachi in 1993 both viruses were identified (Igarashi et al., 1994). There do not yet appear to be reports from public health authorities in the region of the more recent WN strains to emerge in Europe, the Middle East and the US. The introduction of these strains would be of concern, and as a minimum level of preparedness it is recommended that undiagnosed cases of encephalitis in humans or horses and undiagnosed incidents of mortalities in free living bird populations be specifically investigated for possible WN involvement.

Since WN has not been identified in most countries in the region, most respondents to the questionnaire had little to contribute. Only Australia, Japan and Singapore reported a diagnostic capability, although countries with virus isolation capability could attempt virus isolation in the event of an outbreak. Given the close relationship between WN and KUN (van Regenmortel et al., 2000), serological tests for KUN could be expected to be useful for WN diagnosis and surveillance if a need arose. In 1999-2000 Singapore conducted a serological study of 55 crows for evidence of WN infection by plaque reduction neutralization test, with no reactors detected.

6. PREPAREDNESS FOR EMERGING AND EMERGENCY ARBOVIRAL DISEASES

A comprehensive platform of preparedness for emerging and emergency diseases, including arboviral disease, will most appropriately be based on 4 pillars.

A capability for surveillance. Surveillance in the first instance is a strategy to detect new or unusual events, such as the incursion of a specific virus or vector into a previously free area, or the occurrence of a new or unusual disease syndrome. Surveillance is supported by monitoring, a strategy to measure the incidence or changes in prevalence of an event of concern. For both activities there is an absolute requirement to have the ability to make and record observations of the target animal or human population, and preferably the additional ability to sample the population or aspects of its environment that may allow timely detection of changes in risk of exposure to an infectious agent or its vector. Hence surveillance may be directed against specific viruses such as BTV, JE or WN; may be designed to detect occurrence or changes in abundance of vector species; or may have the objective of ensuring that changes in the expected pattern of human or animal disease are detected early. A mature surveillance strategy will have elements of all these approaches.

The technical approaches to arboviral surveillance may include serological surveys or monitoring of sentinel animals, vector trapping and identification, virus isolation or other molecular or antigenic detection of virus in either sentinel animals or trapped vector species, and structured observation and reporting of disease in susceptible populations (Daniels et al., 1996).

A laboratory capability. The ability to accurately detect and diagnose infection with specific arboviruses is a crucial value-adding step in arboviral preparedness. A virus specific diagnostic capability based on laboratory tests allows targeted surveillance for agents of concern, and correct diagnosis of disease syndromes. The more sensitive and specific the diagnostic testing capability, and the more confidently it is managed and used, the more focused can be the responses.

Some of the issues regarding arboviral diagnosis in the region have been identified above. Diagnosis is laboratory based, and hence depends on the general level of sophistication of laboratory capability from place to place. For example, some labs support HI but not ELISA, some have cell culture capability and some not, similarly some have embarked on development of molecular diagnosis. Some labs are now starting to manage their activities under an accredited quality system. Thinking slightly more broadly, entomology skills are usually rare. Important samples are frequently collected great distances from the lab, and hence the logistics of getting the sample to the test are important. Laboratory tests can be expensive to maintain, and other uses of funds may have priority. Collaboration among countries could help to raise overall levels of capability.

One area that should not prove too expensive or difficult would be exchange of reagents for tests that can be supported on common platforms, such as ELISA. Where a number of countries are using a common test approach there is a role for external proficiency testing to support the quality assurance of the test capability. A coordinated approach to developing such collaboration has not yet been addressed in the region.

Where laboratory based diagnosis has been established, the resulting capability can be used to address other issues relating to preparedness. There is an ongoing need for diagnostic test R&D to continuously improve the capability. In Australia laboratory studies have provided information on a range of issues important for JE preparedness in that country, such as whether native fauna can support replication of the virus, whether exposure to other flaviviruses already
established will confer herd immunity to JE in feral animal populations, and which vaccines could be used in pigs to suppress JE viraemia in the face of an outbreak, as a public health measure (Daniels, Middleton, Lunt and Williams, unpublished results).

A response capability. The investment to detect, diagnose and quantify a disease problem is made to facilitate a response. Timely detection through an effective surveillance mechanism may allow prevention of disease, or some other effective management strategy.

In the case of arboviral infections the appropriate response may be vector control to protect susceptible animal or human populations from infection. Where the technology is available, protection of these populations by vaccination may be appropriate. Factors governing a decision to vaccinate will include product related factors such as the cost, the effectiveness and the safety of the vaccine; disease related factors such as the risk of becoming infected and the consequences of the infection; and population related factors such as the logistics of applying the vaccine, the acceptance of vaccination as a procedure, and the competing uses for the resources needed to carry out a vaccination campaign. Where countries have supported accurate monitoring of the distribution of infections, the management response may be zoning to facilitate trade from zones that can be recognized as uninfected.

The application of surveillance and diagnostic capability over time leads to the development of informed local knowledge, a most valuable asset. It is against a background of such local knowledge that effective responses can be initiated, based on an understanding of the likely course that events may take and the likely outcomes in each case. Refinements in the management planning for future events may be undertaken, such as the writing of emergency response plans supported by appropriate staff training.

A predictive capability. Just as a stool or table can function quite usefully with 3 legs, so can an arboviral preparedness platform. Addition of a fourth leg makes for a more robust product. Our predictive capability is usually based on human experience, interpretation of available information, and judgment. It is the assumption of such a predictive capability that leads to response management.

Modern computing science offers the possibility for another level of refinement, the development of the predictive model as an additional tool. Perhaps not every country will have this as a priority at present, and certainly not for every infectious agent. However the capability exists and is being developed towards greater usefulness with time. Mathematical approaches to the predictive modeling of vector borne diseases have been described, for example Anderson and May (1992) or Standfast and Maywald (1991). The increasing availability and analytical power of geographical information systems (GIS) in recent years allows integration of previous modeling concepts with spacial information, the ability to relate information and events to specific geographical locations and to analyse the relationships among locations. These tools are currently under utilized in disease management and control. Already there have been investigations of how this technology may assist veterinary authorities in the region, in Thailand (Cameron and Sharma, 1999). A plan to assist countries develop competency with these new tools has been prepared (Sharma and Cameron, 1999). Of expected particular benefit in the development of management strategies for vector borne disease is the escalating capability globally in remote sensing, the making of observations of the earth’s surface from satellite based sensors, that can be analysed for information on changes in climate or habitat. The satellite images can be analysed in GIS models that allow prediction of the probability of transmission of targeted diseases (Beck et al., 2000). Satellite images were used in the response to the Rift valley fever outbreak in the Yemen (Nasher et al., 2000).

It seems probable that future developments will include GIS-based platforms loaded with basic information regarding countries or regions and their animal and human populations, that also support modules that model the transmission dynamics of specific disease agents. Into these platforms could be loaded the results of surveillance and the changes in information regarding the climatic factors which influence vector populations, collected by remote sensing. The outputs would include prediction of the changes in the risk of transmission of the targeted arboviruses.

REFERENCES


