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Flavivirus Infections: An Emerging and Spreading Encephalitis and Neurological Manifestations

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ABSTRACT

The flaviviruses are spread by the bite from a diseased mosquito or tick and, classified as arboviruses. Individuals become infected fortuitously but they are hosts because they do not have adequately protracted echelons of virus in blood to spread the virus more. Flavivirus encephalitis (inflammation of the brain) includes West Nile Virus (WNV) Japanese encephalitis virus (JEV), St. Louis encephalitis (SLV), and Murray Valley encephalitis (MVE). These diseases typically develop after an incubation period of 5 to 15 days. The neurologic appearances hinge on which part of the nervous system is infected. The meanings (to cause meningitis), the body of the brain (encephalitis), or the spinal cord (myelitis). The more important features include consciousness, seizures, a flaccid (floppy) paralysis. Seizures are particularly common in children with flavivirus encephalitis. Other movement disorders include generalized rigidity, jaw dystonia, opisthotonos, choreoathetosis, and myoclonus. In this chapter discuss transmission, diagnosis and clinical complications of flavivirus infections.

INTRODUCTION

Flaviviruses are significant global human pathogens that primarily cause encephalitis and hemorrhagic disease [1]. Flaviviruses are enveloped, positive-stranded RNA viruses, furthermost human flavivirus infections are asymptomatic, primarily transmitted to man by the bite of an infected mosquito or tick and are maintained in nature in animal reservoirs also be transmitted between humans by transfusion or transplantation of contaminated tissue as shown in Figure 1 [2]. All flaviviruses are exactly connected so diagnosis of human disease can be problematic. Most flavivirus diseases are restrained emerging infections [3]. There are no active drugs or treatments for flavivirus infections. Accepted human flaviviruses vaccines are existing for tick-borne encephalitis, and yellow fever (YFV) [4]. Regulator of flavivirus epidemics mainly depend on vector-control measures [3]. JEV is statistically the most important cause of encephalitis with up to 70,000 cases annually across Asia [5]. Clinical features include a non-specific febrile illness, aseptic meningitis, febrile seizures, encephalitis, with Parkinsonian movement disorders, and myelitis, causing a poliomyelitis-like flaccid paralysis [6]. There is no specific treatment, but good supportive care is essential [7]. Recognition and control of JE has been improved in recent years through better surveillance, improved diagnostics, on disability and disease burden and greater use of vaccines. Being a mosquito-borne zoonotic Flavivirus, WNV is approximately comparable to JEV [8]. Humans tend to become exposed to infected ticks in forested extents finished travel or work. The neurological manifestations including encephalitis have been recognised increasingly over the last twenty years [9]. The diversity of arthropod vectors, disease characteristics and the wide geographic distribution of the flaviviruses makes these viruses especially interesting, particularly if one considers that most people throughout the world live in a flavivirus endemic region [10]. The relative ease with which some of these viruses can be introduced into new environments should also raise concerns and highlight the need for extensive additional research on these viruses, both in the lab and in the field [11].

TRANSMISSION

Many flaviviruses are transmitted via arthropod vectors to humans. DV, WNV, ZV, JE, MVE are mosquito-borne, and tick-borne encephalitis and Kyansanur Forest disease are tick-borne [12]. Arbovirus transmission permits flavivirus to cross species barriers since the same arthropod may bite birds, reptiles and mammals that rarely come in contact with one another naturally [13]. The virus perseveres in insects through transovarian spreads and in vertebrates such as birds, pigs and monkeys through host amplification [14]. In these animals, except in the case of dengue and yellow fever, humans are not involved in the primary transmission cycles. Humans only get involved when they come into close contact with infected arthropods or drink milk from infected animals [15]. Arboviruses are enzootic and disease is endemic, but in most areas arthropod transmission and flavivirus infection only reaches epidemic proportions at the end of the wet season or after a sudden population movement which disturbs the arthropod habitat. Irrigation, deforestation and long-distance air travel also increase flavivirus transmission.

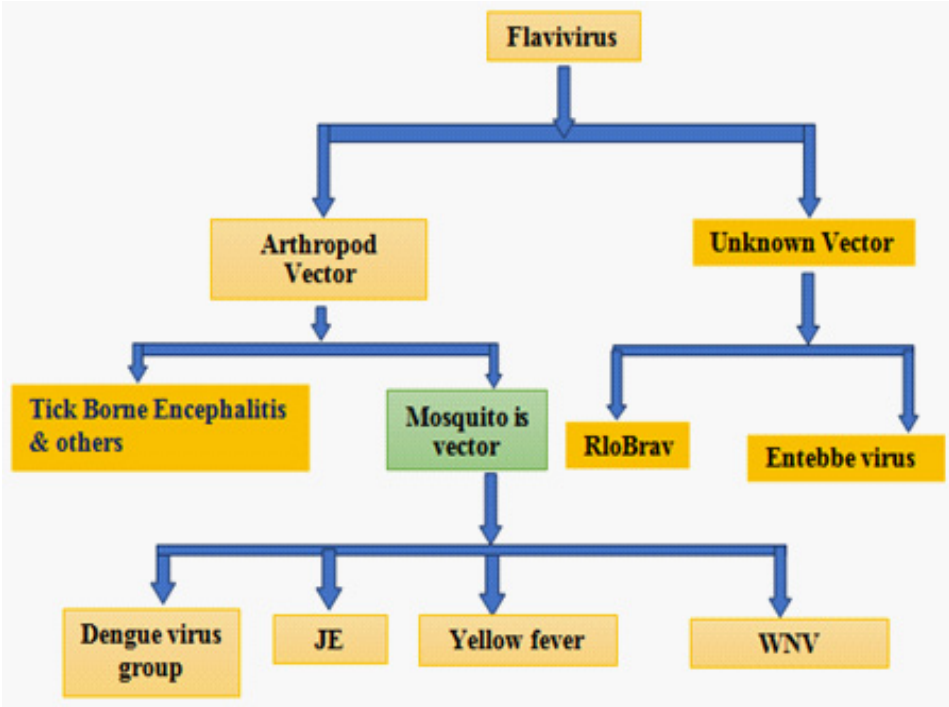

Figure 1: Representations of Flavivirus Vector.

CHARACTERISTICS OF VIRAL PROPERTIES

Flaviviruses are ssRNA (Single Stranded -RNA) enveloped viruses, 40-50 nm in diameter positive RNA genome of around 10,000 nucleotides 3 structural proteins an envelope glycoprotein and a number of non-structural proteins. One single reading frame, no sub genomic mRNA classified in terms of cross-reactivity and the host [16]. The flavivirus family is divided into seven subgroups of viruses [16]. The antigenic determinants are carried by the envelope glycoprotein which is recognized by both hemagglutination inhibition assay and neutralization tests. The envelope glycoprotein contains mainly type-specific determinants and to a lesser extent complex-reactive and group-reactive determinant. [17] The nucleocapsid proteins had only been shown to contain group-reactive determinants.

CLINICAL FEATURES

General symptoms of flavivirus infection include fever, body aches, headache, joint pain, vomiting and diarrhoea as shown in table. The incubation period varies from 3 to 6 days, Generalized myalgias and GI complaints (N+V) follows and signs may include facial flushing, red tongue and conjunctival injection as shown in Figure 2[18]. Some patients may experience an asymptomatic infection or a mild undifferentiated febrile illness [19]. After a period of 3 to 4 days,

improvement should be seen in most patients [20]. The moderately ill should begin to recover, however, the more severely ill patients with a classical YF course will see a return of fever, bradycardia (Faget's sign), jaundice, and haemorrhagic manifestations [21]. The haemorrhagic manifestations may vary from petechial lesions to epitaxis, bleeding gums, GI haemorrhage (black vomit of YF). 50% of patients with frank YF will develop fatal disease characterized by severe haemorrhagic manifestations, oliguria and hypotension [22]. Frank renal failure is rare. Rarely, other clinical findings such as meningo encephalitis in the absence of other findings have been described as shown in Figure 3 [23]. Below is a table showing some of the important human pathogens that belong to the Flaviviridae family, the natural hosts, and the symptoms for human infections:

Table 1: Clinical features of flavivirus.

GENUS	NATURAL HOST	VIRUS	DISEASE	SYMPTOMS
Flavivirus	Mosquitoes	DENV	Dengue fever	haemorrhagic fever
Flavivirus	Ticks	YFV	Yellow fever	haemorrhagic fever, jaundice
Flavivirus	Mosquitoes	TBEV	Tick-borne encephalitis	headache, vomiting, Fever, respiratory distress, fatigue
Flavivirus	Mosquitoes	WWNV	West Nile fever West Nile encephalitis/ meningitis	joint pain, Fever, headache, chills, fatigue,excessive sweating, swollen lymph nodes
Flavivirus	Mosquitoes	JEV	Japanese encephalitis	Neck rigidity, convulsions Fever, headache, malaise, excessive sweating,

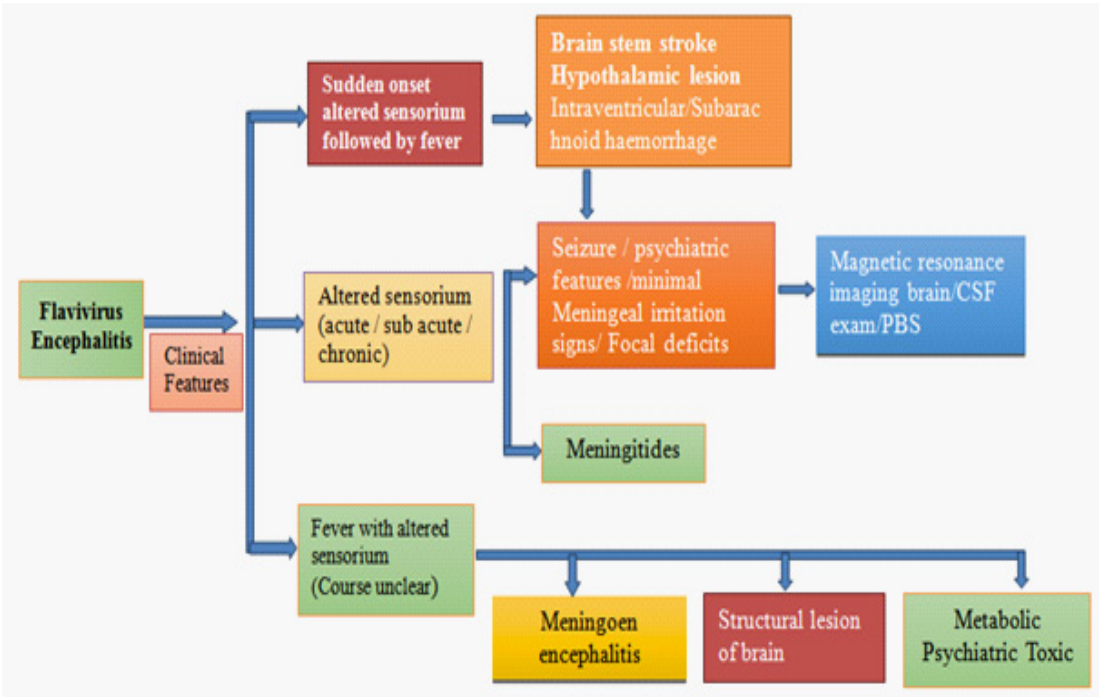

Figure 2: Clinical features of flavivirus encephalitis.

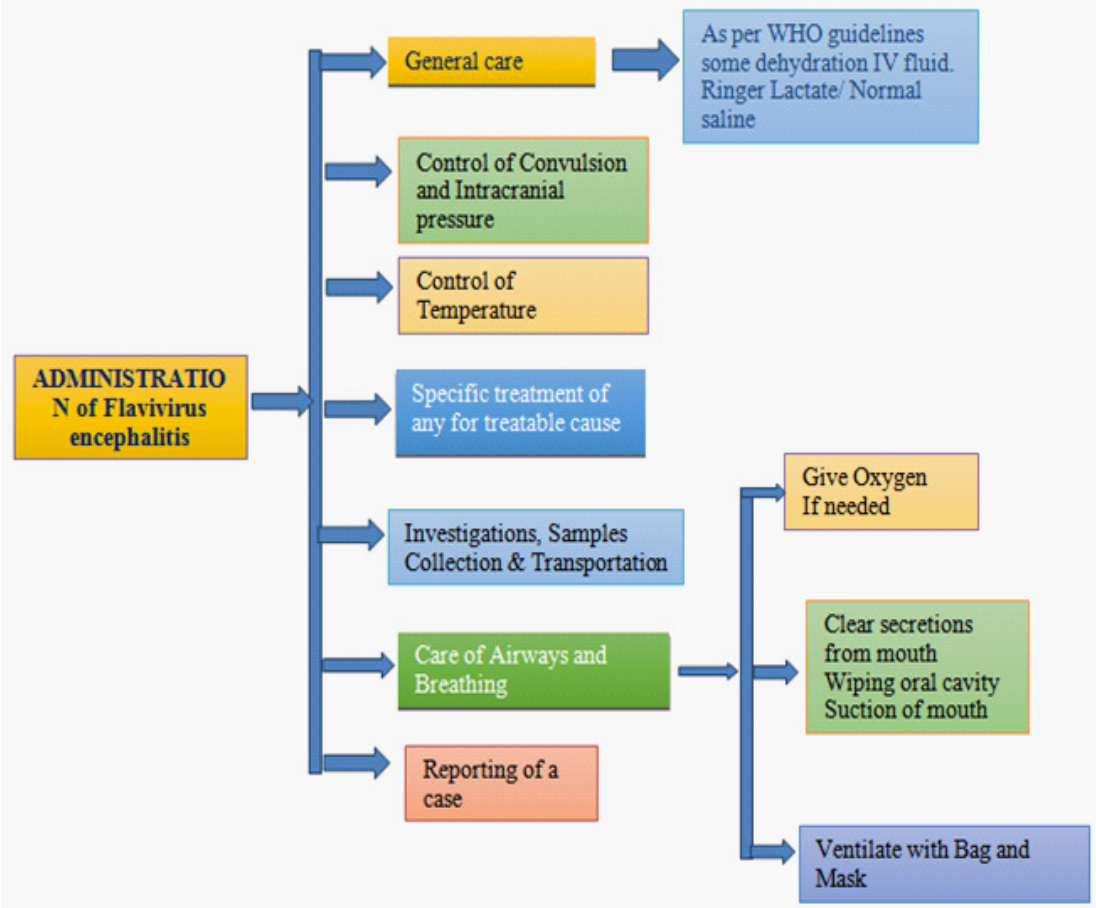

Figure 3: Representation of Administration of cases of flavivirus encephalitis.

DIAGNOSIS OF FLAVIVIRUSES

Cell Culture

The viral sample is initially inoculated on to a mosquito cell line C6/36 and incubated for 3-4 days at 28°C. In directive to get a cytopathic effect (CPE) this must be blind pass aged to mammalian cell lines such as Vero or BHK cells and incubated at 37°C for a few days [24]. Virus is then identified by the binding of specific monoclonal antibodies, by neutralisation with specific antisera, or by specific nucleic acid detection tests.

SEROLOGICAL INVESTIGATIONS

Serological assay is the leading techniques for finding of flavivirus infections in human. Since antibodies caused during flavivirus infections cross-react with other flavivirus members, plaque reduction neutralization test (PRNT) is apply to prevent the contaminating of types of

flaviviruses [25]. Since PRNT need culturing raw viruses, it must be used in bio safety level-3 or level-4 suppression for many flaviviruses and takes more than ten days to complete Novel assay involves such as VLPs (viral-like particles) from a panel of flaviviruses are incubated with flavivirus-infected sera at 37°C for 1 h; the neutralized VLPs are used to infect Vero cells; and the infected cells are measured for luciferase activities at 22 h post-infection [26]. The virus types whose VLP is most efficiently neutralized by the serum specimen (as quantified by the luciferase activities) is the etiologic agent.

These are the only trials used regularly separate reference laboratories for the diagnosis of flavivirus infections other than dengue. The flaviviruses evoke antibodies that are widely cross-reactive within the genus. This causes substantial trouble in characterising antibody to the equal obligatory to classify the infecting virus. Furthermore, due to the antigenic relatedness, infection with a second flavivirus may reason a deceptive increase of antibody to the beforemet flavivirus [27]. Kits are available for the detection of antibody to certain flaviviruses by EIA, and some laboratories may have in-house EIAs. Detection of flavivirus IgG may indicate recent or past infection with any flavivirus or may be due to flavivirus vaccination (against YFV or JEV). It is of little value in diagnosis of recent infection unless paired sera are tested [28]. Changes in optical density between acute and convalescent samples should not be used to measure rises in antibody level unless the test has been specifically validated for the purpose. If an EIA is to be used for measuring antibody, then replication dilutions should be used with loss of reactivity as an endpoint [29]. For IgM tests that do not use an IgM capture format, it is essential that rheumatoid factor and IgG be removed from the sample before testing to avoid false positives and false negatives [30]. Kit controls as stated for profitable kits. A low optimistic patient serum is also suggested as a peripheral control for batch-to-batch difference. For in-house tests, known positive (preferably high and low) and negative samples should be included. For IgM tests the control serum should come from a case of infection due to that virus [31].

The epitope-blocking and competitive EIAs detect total antibody (IgG and IgM) and are not, in themselves useful for proving presence of IgM. Also, specific blocking may not be demonstrable early after onset due to the lower specificity of IgM and early IgG responses. Testing of convalescent serum is recommended for determining species specificity of flavivirus IgG.

IMMUNO FLUORESCENT ANTIBODY (IFA)

IFA tests can be developed to detect IgG, IgM or IgA antibody to any of the flaviviruses. Commercial indirect IFA slides are now available for detection of IgM directed against a number of flaviviruses, including DENV, JEV, WNV, YFV and ZIKV [32]. Known positive and negative viral samples should be used. For IgM tests the control serum should come from a case of infection due to that virus. However, it is recognised that suitable samples may not be available for the less common infections. In those situations, a serum with IgM to another flavivirus may be used provided it shows reliable and consistent cross reactivity. Negative control and positive controls

show appropriate reactivity. IgG-EIA seems to be precise for flavivirus antibody, but it will not distinguish between IgG to dissimilar flaviviruses. The IgM analysis have usually had limited evaluation for a range of flaviviruses and should not be assumed to be specific.

HEMAGGLUTINATION INHIBITION (HI)

The flaviviruses possess a hemagglutinin antigen that will agglutinate goose red cells within a narrow pH range. Antibody to the HA will inhibit its activity, HI measures total antibody but is less sensitive than EIA for detecting IgM [33]. Serum control serum plus goose RBC to check for nonspecific hemagglutinins, RBC control, antigen control for each antigen, positive control serum of known titre, negative control serum.No evidence of nonspecific agglutination or auto-agglutination, antigen controls within expected range, positive control serum within expected range, negative control serum negative. HI titres are relatively insensitive for both IgG and IgM compared with the EIA and IFA tests, but are valuable for determining comparative antibody titres between acute and convalescent sera. HI antibody responses are usually highest to the antigen of the infecting virus and may give some clues as to the likely cause. Due to the cross-reactivity, it is generally accepted that any flavivirus antigen can be used to test for antibody to the range of flaviviruses, though the sensitivity will be highest for antibody to the virus from which the antigen was derived [34].

NEUTRALISATION TITRES

Traditional neutralisation titres (NT) can be performed in reference laboratories and measure the ability of the patient's serum to stop replication of the virus by binding to surface antigens and preventing virus uptake [35]. Other methods use a monoclonal antibody with a dye-attached to detect infected cell (focus-reduction neutralisation tire or FRNT) and PCR-based methods have been developed for some. Any of these can be used provided that the appropriative range of viruses is tested, and proper criteria are used for interpretation. Known samples negative for flaviviruses [36]. Positive control samples for all the flaviviruses being considered. It is recognised that positive control material may not be available for some of the less common viruses. Neutralisation titres are highly sensitive for low level flavivirus antibody detection. They do not distinguish between IgG and IgM. The exception is second infections where the early antibody response may be specific for the previously infecting flavivirus, though convalescent samples will usually show a dominance of antibody to the recent virus [37].

RNA Detection by Nucleic Acid Testing

A number of studies have reported at the application of NAT for detection of flaviviruses, either using flavivirus universal primers, or primers targeted at sequences specific for individual viruses. Several studies have been published for detection of viral RNA in clinical specimens and in mosquitoes [37]. The 3'-UTR, NS5 and C/PrM gene targets have been used. Many of the studied RT-PCR assays are developed and a number of commercial kits are available. A study evaluated

four commercially available RT-PCR assays compared to an in-house hemi-nested RT-PCR. MVE-RNA has been detected in serum and CSF of cases. NAT assays for detection of other flaviviruses rarely found in Australia such as JEV, ZIKV and YFV have also been developed. ZIKV PCR has proven useful in the confirmation of cases in returned travelers. The virus can be detected in a number of body fluids, including blood, urine, semen, genital tract secretions and saliva, as well as amniotic fluid, placenta and feral tissues in congenital infection. ZIKV-RNA load in the urine has been found to be higher than blood levels in some studies, and to persist for longer [38].

ROLE OF NEURO-IMAGING IN ENCEPHALITIS

The important neurological complications are encephalopathy and encephalitis, the former being more common. Along with serological and CSF examinations, imaging with CT or MRI is important to look for structural changes in brain and if present, to define the pattern and extent of involvement of brain parenchyma [38]. Patients with suspected encephalitis with Detailed clinical information are collected from each patient underwent extensive laboratory investigation. The case definition for encephalitis included any person of any age admitted to hospital with encephalopathy (altered level of consciousness persisting >24 hours, including lethargy, irritability, or a change in personality and behaviour) and two or more of the following: fever or history of fever (38C) during the presenting illness; seizures and/or focal neurological findings (with evidence of brain parenchyma involvement); CSF pleocytosis (>4 white blood cells/ml); electroencephalogram (EEG) findings compatible with encephalitis; abnormal results of neuroimaging (computed tomography CT/MRI) in keeping with encephalitis [39]. Wherever possible, CT and MRI images are collected from each patient. These are only possible in a subset of recruiting centres due to practical complexities, including computer and software compatibility issues; however, all regions are represented. Available CT and MRI images were independently rated by three consultant neuroradiologists with expertise in reporting adult and pediatric neuroimaging. The ratters are blinded to patient and clinical details and used a pre-defined proforma. Age, gender, and aetiology of patients with available CT and MRI images are analysed [40].

NEUROLOGICAL COMPLICATIONS

Neurologic dysfunction usually follows the systemic symptoms by several days. The most common symptoms include headache, altered level of consciousness, and focal weakness, observed in various combinations [41]. Flavivirus meningitis manifests as headache and fever following back pain, myalgias, and rash in 20% to 50% of patients. Meningeal signs are often absent on physical examination, with neck stiffness and photophobia observed in only 19% to 27%. Aseptic meningitis tends to occur more in younger patients, and it usually resolves without major sequelae. Meningo encephalitis is the most common diagnosis in hospitalized flavivirus infected patients, affecting 50% to 84% of patients; it manifests with behavioural or personality changes such as irritability, confusion, or disorientation that can evolve into stupor

and even coma, with mental status changes persisting for up to several weeks. Reduced level of consciousness, a general symptom of encephalitis, is frequently related with other, more restricting symbols such as tremor, bulbar dysfunction, ataxia, or principal faintness, shiny more specific areas of CNS involvement. Physical examination usually reveals hyper reflexia, as would be expected with upper motor neuron injury, unless there is associated myelitis, where are flexia becomes the rule. Earlier studies suggested that older age and medical comorbid conditions could predispose to weakness [42]. The limb weakness is of a lower–motor neuron pattern, with flaccid tone, areflexia, or hyporeflexia. It is typically asymmetrical and rapidly progressive, reaching nadir weakness within 2 to 8 days of symptom onset [40]. The clinical pattern consists of flaccid quadriparesis, asymmetrical paraparesis, or monopoiesis. The weakness typically involves proximal musculature, and the upper lumbar segments can be affected in isolation, mimicking an upper lumbar radiculopathy or plexopathy. Other neurologic manifestations include movement disorders, rhomb encephalitis, and cerebellar dysfunction [41]. Rhomb encephalitis with associated bulbar dysfunction and swallowing difficulties can contribute to morbidity and prolonged hospitalization. Cerebellar involvement with gait or truncal ataxia was described and even appeared to correlate with overall morbidity and mortality. Most of the salient neurologic manifestations become obvious several days or even weeks into the illness, as the patient is recovering from the meningo encephalitis and beginning rehabilitation. Although the tremors can be mistaken for seizure activity in severely affected patients, focal motor seizures have rarely also been described [42].

TREATMENT

The treatment of flavivirus is currently supportive, with particular attention to the risk of respiratory compromise secondary to muscle weakness and aspiration secondary to bulbar dysfunction [43]. Trials of several medications, including intravenous immunoglobulins, ribavirin, interferon, and steroids, have shown no effect, although none has been assessed in large clinical trials. In the absence of specific therapy, prevention becomes crucial. Approaches to prevention include reduction of the mosquito population with draining of water from mosquito breeding sites and use of mosquito larvicides and maturation inhibitors to reduce the numbers of mosquitoes [44]. Lifestyle modifications include avoiding outdoor activities during the hours around dawn and dusk, when mosquitoes are most active and wearing protective, light-colored clothing to limit insect bites. Insect repellents containing 10% to 50% N, N-diethyl-3-methylbenzamide (DEET) have also been recommended as an alternative to the organophosphate insecticides, which have significant side effects [45]. A vaccine has been developed for veterinary use in horses but is not approved for use in humans.

INFECTION PREVENTION AND CONTROL

Infection prevention and control is required to prevent the transmission of communicable diseases in all health care settings. Infection prevention and control strains a basic considerate

of the epidemiology of diseases risk factors that increase patient susceptibility to infections [46]. The risk of acquiring a healthcare-associated infection is related to the mode of transmission of the infectious agent, the type of patient-care activity or procedure being performed and the underlying patient's host defenses [47]. Personnel at risk for exposure to tuberculosis should be screened per recommendations. The spread of mosquito-borne flaviviruses can be prevented by demanding vaccination of all individuals traveling into or out of flavivirus endemic regions [48]. Insect quarantines and strict airport controls should also be implemented to prevent the introduction of infectious arthropods into new areas. In endemic regions, pesticides and insect repellents (DEET) should be utilized and stagnant pools of water drained to reduce the population of mosquitoes in the vicinity of towns [49,50]. Since the development of pesticide resistance, it has become even more difficult to kill the mosquitoes. Ticks persist throughout the year and live through more than a single breeding cycle of their host. Thus, they are more difficult to control than mosquitoes [1-5].

CONCLUSIONS

Flavivirus infection can be a significant cause of CNS morbidity and mortality. The virus can cause salient neurologic manifestations ranging from aseptic meningitis to flaccid quadriplegia. Heightened awareness is essential for early diagnosis, and prevention remains crucial in the absence of effective targeted therapy.

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