

## Review Article

Indian J Med Res 118, September 2003, pp 101-108

# West Nile virus: the Indian scenario

R. Paramasivan\*, A.C. Mishra & D.T. Mourya

*Microbial Containment Complex, National Institute of Virology (ICMR), Pune, India*

Received November 20, 2002

West Nile virus (WNV) is an important arthropod borne flavivirus; usually causes a mild infection called West Nile fever (WNF) in human and horses. Mosquitoes are the principal vectors of WNV. Various *Culex* species are found to act as vectors in different geographical regions. The virus is maintained in a bird-mosquito cycle in nature. In India, *Culex* mosquitoes are tentatively incriminated as vectors of WNV. Experimental studies have shown that *Culex tritaeniorhynchus*, *Cx. vishnui*, *Cx. bitaeniorhynchus* and *Cx. univittatus*, *Culex pipiens fatigans* and *Aedes albopictus* could act as potential vectors of WNV. Transovarial transmission of WNV has been experimentally demonstrated in *Culex* mosquitoes. Apart from mosquitoes, the role of other arthropods is also considered in the maintenance of WNV during inter-zoonotic periods. The possible role of ardeid birds in the maintenance of WNV has been described in India. Though very few clinically overt cases of human encephalitis due to WNV are observed, Japanese encephalitis virus (JEV) is found to dominate in southern India. WNF in horses has not been documented in India. JEV immunized monkeys were protected from WNV challenge and the WNV immunization was found to reduce the disease severity due to JEV. Based on the limited genome sequence analysis, the Indian isolates are grouped together under the genetic lineage-I. WNV infection is diagnosed by IgM antibody capture enzyme linked immunosorbent assay, haemagglutination inhibition test, neutralization test and reverse transcriptase-polymerase chain reaction (RT-PCR). For the effective control of *Culex* mosquitoes, integrated vector control strategies are recommended. Specific methods are not available for the treatment of WNV infection. However, in patients with encephalitis supportive therapy is recommended. Though a few candidate vaccines are under laboratory trial, no vaccine has been available commercially for the control of WNV infection in human and animals. In view of the global interest on WNV, this paper describes the present status of WNV in India.

**Key words** Arthropod borne virus - Indian scenario - West Nile fever - West Nile virus

West Nile virus (WNV) is an arthropod borne virus of public health importance. WNV is a member of the genus flavivirus and belongs to the Japanese encephalitis virus (JEV) antigenic complex under family flaviviridae. The other members of the serocomplex are Japanese encephalitis, Murray Valley encephalitis, Alfuy, Kokobera, Koutango, St. Louis encephalitis, Stratford and Usutu. Kunjin Virus which was considered closely related to WNV<sup>1</sup>, has recently been classified as a sub type of WNV<sup>2</sup>.

WNV infection is a self-limited, non-fatal mild febrile illness but occasionally reported to cause encephalitis<sup>3</sup>.

It causes mortality in horses<sup>4</sup>, domestic and wild birds<sup>3</sup>. The emergence of WNV in America<sup>5</sup> and its impact on the health of humans, horses and birds, have caused global concern about this virus. In India, this virus is known to be active in mosquitoes, birds and pigs. It has also been associated with human encephalitis cases<sup>6</sup>. Therefore, it is felt necessary to review the present status of WNV in context with Indian scenario.

## Natural cycle

WNV is reported to be maintained in the nature in a cycle involving certain birds and mosquitoes<sup>7</sup>. Mosquitoes

\*Present address : Centre for Research in Medical Entomology (ICMR), No-4, Sarojini Street, Chinna Chokkikulam, Madurai 625 002.

are principle vectors of WNV. Outbreaks of WNV infection coincided with the increased population of the *Culex* mosquitoes during summer in temperate regions and during rainy seasons in tropics<sup>8</sup>. This virus has been mainly isolated from *Cx. univittatus* in Egypt<sup>9</sup> and South Africa<sup>10</sup>; *Cx. pipiens* and *Cx. perexiguus* in Israel<sup>11</sup>; and from *Cx. molestus* in France<sup>12</sup>; *Cx. vishnui* complex in Pakistan<sup>13</sup>; *Cx. restuans*, *Cx. pipiens*, *Culiseta melanura* and *Cx. salinarius* in Connecticut, USA<sup>14</sup>. The role of non Culicine arthropods has also been considered in the maintenance of WNV during inter-enzootic periods. WNV has also been isolated from both hard and soft ticks (*Hyalomma marginatum*, *Omithodoros maritimus*, *Argas hermanni*,) and swallow bugs (*Oeciacus hirundinis*)<sup>3</sup>. Experimental transmission of WNV has been observed in *Ornithodoros savignyi*, *O. maritimus*, *O. moubata*, *O. erraticus*, *Rhipicephalus sanguines*, *R. rossicus*, *Dermacentor reticulatus* and *Haemophysalis leachii*<sup>15</sup>.

In India, this virus has been isolated from human beings<sup>6,16,17</sup>, frugivorous bat<sup>18</sup> (*Rousettus leschenaulti*), domestic pigs (NIV, unpublished data) and mosquitoes<sup>19-21</sup>. The WNV isolate (724268) was recovered from a pool of 100 *Cx. vishnui* mosquitoes, collected resting outdoors in bushes at Mudikonda village, Khammam taluk, Khammam district, Andhra Pradesh<sup>19</sup>. In another study, two strains of WNV have been isolated from two pools (pool size 48 and 50 females respectively) of *Cx. fatigans* (*Cx. quinquefasciatus*) at Manjri, Pune<sup>20</sup>. Of 5553 *Cx. vishnui* mosquito pools (containing 738, 291 mosquitoes), Dandawate *et al*<sup>21</sup> isolated five strains of WNV from five pools with a pool sizes ranging from 74 to 150 mosquitoes collected from North Arcot districts of Tamil Nadu and Chittoor district of Andhra Pradesh. Experimental studies have shown that mosquitoes *viz.*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. bitaeniorhynchus* and *Cx. univittatus*<sup>22</sup>, *Cx. pipiens fatigans* (*Cx. quinquefasciatus*) and *Aedes albopictus*<sup>23</sup> could act as potential vectors of WNV. Transovarial transmission (TOT) of WNV has been experimentally demonstrated in *Cx. vishnui* mosquito<sup>24</sup>.

Several birds in different geographical regions have been implicated as vertebrate reservoirs *viz.*, passerine birds in North America, house sparrow in Europe, hooded crow and other birds in Africa, turtle dove in Middle

east and herons in Oceania<sup>3</sup>. In southern India, JEV/WNV neutralizing antibodies were detected in ardeid birds mainly from the pond herons (*Ardeola grayii*) and cattle egrets (*Bubulcus ibis*). This indicates the possible involvement of ardeid birds in the natural cycle of WNV in India<sup>25</sup>. Experimental infection of equines with WNV has been reported previously<sup>26-29</sup>. A recent study<sup>30</sup> has shown that horses cannot act as amplifying host of WNV in nature. In southern India, domestic pigs were shown to develop antibodies to WNV<sup>31</sup>, but are considered poor hosts<sup>32</sup>.

### Geographical distribution

WNV is recognized as the most widespread virus among flaviviruses. It was first isolated during 1937 in the West Nile district of Uganda from a patient suffering from mild illness<sup>33</sup>. WNV has been reported from Algeria, Russia, Azerbaijan, Botswana, Central African Republic, Cote d'Ivoire, Cyprus, Democratic Republic of Congo, Egypt, Ethiopia, Israel, Kazakhstan, Madagascar, Morocco, Mozambique, Nigeria, Pakistan, Senegal, South Africa, Tajikistan, Turkmenia, Uganda and Uzbekistan<sup>15</sup>. Several epidemics have been reported from middle East, Africa and Israel and the WNV is endemic in Middle East, Africa and Southwest Asia<sup>34,35</sup>. WNV specific neutralizing antibodies have been detected in Armenia, Borneo, China, Georgia, Iraq, Uganda, Kenya, Lebanon, Malaysia, Philippines, Sri Lanka, Syria, Thailand, Tunisia, Turkey Belgian Congo and Sudan<sup>15,34</sup>. Recently, the virus has been recognized in New York, America<sup>5</sup>.

In India, presence of West Nile antibodies in humans was first reported from Bombay (now Mumbai) by Banker in 1952<sup>36</sup>. Smithburn *et al*<sup>37</sup> confirmed the report by detecting the WNV neutralizing antibodies. During a post sero-epidemiological study, Risbud *et al*<sup>38</sup>, detected WNV neutralizing antibodies among humans at South Arcot district of Tamil Nadu. WNV has been isolated from sporadic cases of encephalitis and mosquitoes. Work<sup>39</sup> postulated a hypothesis of a zoogeographical interface of Japanese encephalitis and West Nile virus. The hypothesis proposed the intermingling distribution of JEV and WNV at the south Indian peninsular region. The relative prevalence of JEV and WNV needs to be studied in India. From the available data, it is evident that different viruses may predominate in different years since in South Arcot District of Tamil Nadu,

Risbud *et al*<sup>38</sup> observed a higher prevalence of neutralizing antibodies to WNV than JEV during a post encephalitis outbreak survey in 1982, whereas in the same area, during 1989 and 1990, Gajanana *et al*<sup>40</sup> found a low prevalence of WNV.

### Biological characterization

Strain analysis studies have been reported earlier<sup>41-45</sup>. The prevalence of similar antigenic strains and a single heterogeneous domain based on epitope mapping of envelope protein among Indian strains were reported<sup>46</sup>. The study on understanding the nature of passive immunity in WNV infected mice elucidated the transfer of maternal antibodies through placenta and colostrum<sup>47</sup>. Two-way cross protection studies between JEV and WNV revealed that the JEV immunized macaque was protected from WNV challenge. On the other hand, WNV immunization only reduces the disease severity due to JEV<sup>48</sup>. Recently, Tesh *et al*<sup>49</sup> demonstrated that immunization with heterologous flaviviruses (JEV and St. Louis encephalitis virus) protects hamsters against WNV challenge by reducing the severity from fatal encephalitis.

The comparative analysis of the degree of pathogenicity among Indian isolates of WNV in mice, and their plaque size in cell culture were studied<sup>50</sup>. A varying degree of pathogenicity was observed in adult mice by the peripheral route. Among the six strains studied, bat (68856) and human strains (672698) were found to produce smallest and largest plaques respectively. A serological heterogeneous group has been observed among Indian WNV isolates. The poor immunogenicity of the human isolate and poor antigenicity in serological testing of a mosquito isolate (643009) were also observed<sup>51</sup>. The study also reported the non-identical nature of the two mosquito isolates G2266 and G22886 though appear similar.

### Genomic characteristics

WNV are small spherical, enveloped particles containing single-stranded positive-sense RNA genome. The viral genome is approximately 11,000 nucleotides in length. The viral genome encodes for three structural (C, M and E) and seven non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) proteins. It has a

single large open reading frame (ORF), flanked by non-coding regions (NCR) at both the ends<sup>52</sup>. The mosquito borne flaviviruses lack poly (A) tail at its 3' end<sup>53</sup>.

WNV is mainly divided into two genetic lineages, namely lineage-1 and lineage-2. The strains grouped under lineage-1 are associated with human clinical encephalitis. The members of the lineage-2 have not been found to be associated with human encephalitis and are maintained in enzootic foci in Africa<sup>34</sup>. Genome characterization of WNV has been previously described<sup>54-59</sup>. Phylogenetic study conducted among the isolates recovered from Europe and Africa suggested the introduction of WNV into Europe by birds migrating out of sub-Sahara Africa<sup>55</sup>.

The available data on the genomic sequences of Indian WNV isolates are limited. Porter *et al*<sup>54</sup> analyzed the NS3 region of the Indian bat isolate (68856) and compared with six other WNVs recovered from different geographical regions. In the reverse transcriptase-polymerase chain reaction (RT-PCR) assay, the expected PCR product of the bat isolate (258-bp) was found to be less distinct compared to the other strains studied. The analysis showed that the twelve bases of the 5' primer-binding site of the Indian bat isolate showed a 25 per cent mismatches than the AME (Asian Middle East group) group strains (0-16%). In the PCR product nucleotide sequence analysis the Indian isolate-shared 92-98 per cent sequence homology with other isolates *i.e.*, E-101, AN4767, AN4766 and Dak B310. The interesting point is that all the five other strains (including 68856) showed only 77-82 per cent homology with the WNV Nigerian strain (WN-Wengler). Based on the E and NS5/3' UTR gene region analysis, the Indian isolates (G-2266 and G-22886 mosquito isolates, and 804994 a human isolate) were grouped in the lineage-I<sup>58</sup>. These strains were further grouped with one of the three clusters of lineage-I. The isolates grouped under lineage-I shared an average sequence identity of 80 per cent (E gene) and 77 per cent (NS5/3'UTR). The Indian isolates are clustered together. They shared an average sequence identity of 97 per cent and 98 per cent in the E region and NS5/3'UTR regions respectively<sup>58</sup>. However, the analysis of very limited number of isolates, may not represent the complete picture of the molecular epidemiology of WNV in India.

## Epidemiology

WNV is an emerging virus infection of the globe. Several outbreaks in different countries with various range of severity has been reported earlier<sup>5,11,15,35,60</sup>. The first known outbreak of WNV in the northern United States was observed during late August 1999<sup>5</sup> and due to the widespread virus activity, Northeastern USA is becoming endemic to WNV<sup>61</sup>. In human, clinically WNV appears as a mild, self limited, non-fatal, febrile illness rarely leading to encephalitis. However, myocarditis, a rare non-neurological complication<sup>62</sup> and pancreatitis associated WNV infection<sup>63</sup> have also been reported. In monkey model, WNV has been reported to cause persistent infection and a sub acute inflammatory degenerative processes in the nervous system<sup>64</sup>.

WNV is highly prevalent in India<sup>65</sup> and usually causes a mild, non-fatal dengue like illness in humans. However, febrile illness in epidemic form and clinically overt encephalitis cases were observed in Udaipur area of Rajasthan, Buldhana, Marathwada and Khandesh districts of Maharashtra<sup>66</sup>. WNV neutralizing antibodies (about 20-30%) have been detected in human sera collected from Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa and Rajasthan<sup>67</sup>. Serologically confirmed cases of WNV infections were reported from Vellore<sup>68</sup> and Kolar districts during 1977, 1978 and 1981<sup>6</sup>. The incubation period is 1 to 6 days. The WNV strain P-4230 has been isolated from a laboratory worker who got lab infected while handling the Indian mosquito strain G-2266 and the Egyptian human strain E-101 on consecutive two days (Virus Research Centre, Annual Report-1956). However, as WNV was prevalent in Pune area, the source of infection could not be ascertained<sup>69</sup>.

Horses encounter the WNV infection like humans and experience encephalitis. Though the WNV encephalitis in horses is rare, a considerable mortality rate is reported in the case of encephalitic horses. WNV fever in horses has been reported in Egypt<sup>26</sup>, France<sup>4</sup>, Morocco<sup>70</sup>, Italy<sup>71</sup> and in USA<sup>72</sup>. WNV infection in horses has not been documented in India. However in a recent survey, a significant rate of serological evidence against WNV has been noticed among horses in and around Pune City (NIV; unpublished data). Extensive studies of WNV infection in horses in India need to be carried out.

## Laboratory diagnosis

WNV infection is diagnosed by serological methods. Demonstration of four-fold rise or drop of antibody titer in paired serum samples by hemagglutination inhibition test is still widely used<sup>73</sup>. The interference of cross-reactions of the co-existing other closely related virus especially JEV has been reported. In a single human serum sample MAC (IgM-antibody capture) ELISA is routinely used for the diagnosis of acute infection in human<sup>74</sup>. A commercially available arboviral immunofluorescence assay has been used for the screening of West Nile virus infection in human<sup>75</sup>. The commercially available West Nile virus specific monoclonal antibody (MicroBix Bio system INC, Canada) could be exploited in the detection of the virus. Quick complement fixation test<sup>76</sup>, kinetic complement fixation test<sup>77</sup>, single radial haemolysis test<sup>78</sup> and neutralization test<sup>79</sup> are some of the tests which are routinely used. Plaque reduction neutralization test is very useful in survey and confirmation of virus isolates<sup>73</sup>. Recently, the RT-PCR method for the detection of virus specific genome has been extensively used by several workers<sup>80-82</sup>. To increase the sensitivity and specificity certain modified assays viz., TaqMan assay<sup>80</sup> and nucleic acid sequence based amplification method (NASBA)<sup>83</sup> have been reported. The TaqMan assay has been successfully used for the detection of WNV in human (cerebrospinal fluid) CSF that was found negative in conventional cell culture technique<sup>80</sup>. The other NASBA method involves the use of three enzymes and a detection system of electrochemiluminescence (ECL). Alternately, molecular beacon probes have also been used as detection system<sup>83</sup>. However, the test requires real time PCR machine.

## Prevention, treatment and control

The guidelines for detection, prevention and control of WNV in the United States of America have been published<sup>84</sup> and are available in the internet (<http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm>). In India, since no studies have been carried out for the control of WNV, the strategies recommended for control of *Culex* mosquitoes, the known vectors of JEV could be applicable. The integrated vector control strategies includes the use of personal protection measures like protective clothing, bed nets, both chemical and nem

based repellants, insecticides, insecticide impregnated curtains, and biological control methods by larvivorous fish, introducing natural parasites and predators and bacterial agents<sup>85</sup>. In clinically overt cases, specific methods are not available for the treatment of WNV infection. However, in encephalitis cases supportive therapy is recommended<sup>3</sup>. Several drugs have been tried for the treatment of encephalitis in animal models<sup>86-90</sup>. The encouraging result of an appetite stimulant<sup>91</sup> and intravenous administration of donor immune serum against WNV in severe human cases has been described<sup>92</sup>. Though a few candidate vaccines are under laboratory trial<sup>93</sup>, no vaccine is available commercially for the control of WNV infection in human and animals.

### Conclusion

WNV is not a new disease to India. The recent emergence of WNV in America has clearly indicated the spread of WNV to newer areas. The spread of US strain of WNV to India may not create an impact as the circulation of WNV among Indian population has been well documented. Along with WNV, the coexisting closely related JEV could also be expected to provide a limited cross protection to the community. The possible threat of spread of US WNV strain to Australia has been addressed<sup>94</sup>, where the circulation of Kunjin virus (a sub type of WNV) and the closely related JEV and Murray Valley encephalitis virus (MVE) is known. Inclusion of molecular diagnostic methods for the accurate diagnosis of WNV infection could probably improve the surveillance of WNV in India, where other closely related flaviviruses coexist.

### Acknowledgment

The authors acknowledge Dr M.A. Ilkal, former Deputy Director, NIV, Pune for providing important references.

### References

- Zanotto PMA, Gould EA, Gao GF, Harvey PH, Holmes EC. Population dynamics of flaviviruses revealed by molecular phylogenies. *Proc Natl Acad Sci USA* 1996; 93 : 548-53.
- Heinz FX, Collett MS, Purcell RH, Gould EA, Howard CR, Houghton M, *et al*. Family: Flaviviridae In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, *et al*, editors. *Virus taxonomy: classification and nomenclature of viruses*. San Diego: Academic Press 2000; p. 859-78.
- Komar N. West Nile viral encephalitis. *Rev Sci Tech* 2000; 19 : 166-76.
- Murgue B, Murri S, Zientara S, Durand B, Durand J, Zeller H. West Nile outbreak in horses in southern France, 2000: The return after 35 years. *Emerg Infect Dis* 2001; 7 : 692-6.
- Asnis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis* 2000; 30 : 413-8.
- George S, Gourie-Devi M, Rao J A, Prasad SR, Pavri KM. Isolation of West Nile virus from the brains of children who had died of encephalitis. *Bull World Health Organ* 1984; 62 : 879-82.
- Hayes CG. West Nile fever. In : Monath, TP, editor. *Arboviruses: epidemiology and ecology*, Vol. V. Boca Raton FL: CRC Press; 1989 p. 59-88.
- Baqar S, Hayes CG, Murphy JR, Watts DM. Vertical transmission of West Nile virus by *Culex* and *Aedes* species mosquitoes. *Am J Trop Med Hyg* 1993; 48 : 757-62.
- Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of West Nile virus in Egypt. *Am J Trop Med Hyg* 1956; 5 : 579-620.
- McIntosh BM, Jupp PG, Dickinson DB, Mc Gillivray GM, Sweetnam J. Ecological studies on Sindbis and West Nile viruses in South Africa. I. Viral activity as revealed by infection of mosquitoes and sentinel fowls. *S Afr J Med Sci* 1967; 32 : 1-14.
- Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, David DB, *et al*. West Nile fever outbreak, Israel. 2000: Epidemiologic Aspects. *Emerg Infect Dis* 2001; 7 : 686-91.
- Hannoun C, Panthier R, Mouchet J, Eouzon JP. Isolement en France du virus West Nile à partir de malades et du vecteur *Culex molestus* Ficalbi. *C R Acad Sci* 1964; 259 : 4170-2.
- Igarashi A. Virus isolation from mosquitoes collected in Karachi, Pakistan. In: Takasu T, editor. *Encephalitides, mosquitoes and a virus in Karachi - A neuro-viro-patho-epidemiological survey*, 2nd Official Report. 1987 p. 233-41.
- Andreadis TG, Anderson JF, Vossbrinck CR. Mosquito surveillance for West Nile virus in Connecticut, 2000: Isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius* and *Culiseta melanura*. *Emerg Infect Dis* 2001; 7 : 670-4.
- Hubalek Z, Halouzka J. West Nile fever- a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis* 1999; 5 : 643-50.
- Paul SD, Narasimha Murthy DP, Das M. Isolation of West Nile virus from a human case of febrile illness. *Indian J Med Res* 1970; 58 : 1177-9.
- Kedarnath N, Prasad SR, Dandawate CN, Koshy AA, George S, Ghosh SN. Isolation of Japanese encephalitis & West Nile viruses from peripheral blood of encephalitis patients. *Indian J Med Res* 1984; 79 : 1-7.
- Paul SD, Rajagopalan PK, Sreenivasan MA. Isolation of the West Nile virus from the frugivorous bat, *Rousettus leschenaulti*. *Indian J Med Res* 1970; 58 : 1169-71.

19. Rodrigues FM, Bright Singh P, Dandawate CN, Soman RS, Guttikar SN, Kaul HN. Isolation of Japanese encephalitis and West Nile viruses from mosquitoes collected in Andhra Pradesh. *Indian J Parasitol* 1980; 4 : 149-53.
20. Pavri KM, Singh KRP. Isolation of West Nile virus from *Culex fatigans* mosquitoes from western India. *Indian J Med Res* 1965; 53 : 501-5.
21. Dandawate CN, Rajagopalan P K, Pavri K M, Work TH. Virus isolations from mosquitoes collected in North Arcot District, Madras State, and Chittoor District, Andhra Pradesh between November 1955 and October 1957. *Indian J Med Res* 1969; 57 : 1420-6.
22. Ilkal MA, Mavale MS, Prasanna Y, Jacob PG, Geevarghese G, Banerjee K. Experimental studies on the vector potential of certain *Culex* species to West Nile virus. *Indian J Med Res* 1997; 106 : 225-8.
23. Varma MGR. Preliminary studies on the infection of Culicine mosquitoes with the Tamil Nadu strain of West Nile virus. *Indian J Med Res* 1960; 48 : 537-48.
24. Mishra AC, Mourya DT. Transovarial transmission of West Nile virus in *Culex vishnui* mosquito. *Indian J Med Res* 2001; 114 : 212-4.
25. Rodrigues FM, Guttikar SN, Pinto BD. Prevalence of antibodies to Japanese encephalitis and West Nile viruses among wild birds in the Krishna- Godavari Delta, Andhra Pradesh, India. *Trans R Soc Trop Med Hyg* 1981; 75 : 258-62.
26. Schmidt JR, El Mansoury HK. Natural and experimental infection of Egyptian equines with West Nile virus. *Ann Trop Med Parasitol* 1963; 57 : 415-27.
27. Guillon JC, Qudar J, Joubert L, Hannoun CL. Lésions histologiques du système nerveux dans l'infection à virus West Nile chez le cheval. *Ann Inst Pasteur* 1968; 114 : 539-50.
28. Joubert L, Qudar J, Hannoun C, Chippaus M. Reproduction expérimentale de la méningo-encéphalomyélite du cheval par l'arbovirus West Nile. 3. Relations entre la virologie, la sérologie et l'évolution anatomo- clinique. Conséquences épidémiologiques et prophylactiques. *Bull Acad Vet Fr* 1971; 44 : 159-67.
29. Qudar J, Joubert L, Lapras M, Guillon JC. Reproduction expérimentale de la méningo-encéphalomyélite du cheval par l'arbovirus West Nile. II Etude anatomo- clinique. *Bull Acad Vet Fr* 1971; 44 : 147-58.
30. Bunning ML, Bowen RA, Cropp CB, Sullivan KG, Davis BS, Komar N, *et al.* Experimental infection of horses with West Nile virus. *Emerg Infect Dis* 2002; 8 : 380-6.
31. Geevarghese G, Shaikh BH, Jacob PG, Bhat HR, Pavri KM. Domestic pigs as sentinels to monitor the activity of Japanese encephalitis & West Nile viruses in Kolar District, Karnataka. *Indian J Med Res* 1987; 86 : 413-8.
32. Ilkal MA, Prasanna Y, Jacob PG, Geevarghese G, Banerjee K. Experimental studies on the susceptibility of domestic pigs to West Nile virus followed by Japanese encephalitis virus infection and vice-versa. *Acta Virol* 1994; 38 : 157-61.
33. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med* 1940; 20 : 471-92.
34. Petersen LR, Roehrig JT. West Nile virus: A reemerging global pathogen. *Emerg Infect Dis* 2001; 7 : 611-4.
35. Platonov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, Lanciotti RS *et al.* Outbreak of West Nile Infection, Volgograd region, Russia, 1999. *Emerg Infect Dis* 2001; 7 : 128-32.
36. Banker DD. Preliminary observations on antibody patterns against certain viruses among inhabitants of Bombay city. *Indian J Med Sci* 1952; 6 : 733-46.
37. Smithburn KC, Kerr JA, Gatne PB. Neutralizing antibodies against certain viruses in the sera of residents of India. *J Immunol* 1954; 72 : 248-57.
38. Risbud AR, Sharma V, Mohan Rao CVR, Rodrigues FM, Shaikh BH, Pinto BD, *et al.* Post-epidemic serological survey for JE virus antibodies in South Arcot district (Tamil Nadu). *Indian J Med Res* 1991; 93 : 1-5.
39. Work TH. On the Japanese B- West Nile virus complex or an arbovirus problem of six continents. *Am J Trop Med Hyg* 1971; 20 : 169-86.
40. Gajanana A, Thenmozhi V, Samuel PP, Reuben R. A community based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. *Bull World Health Organ* 1995; 73 : 237-44.
41. Hammam HM, Clarke DH, Price WH. Antigenic variation of West Nile virus in relation to geography. *Am J Epidemiol* 1965; 82 : 40-55.
42. Gaidamovich SY, Sokhey J. Studies on antigenic peculiarities of West Nile virus strains isolated in the USSR by three serological tests. *Acta Virol* 1973; 17 : 343-50.
43. Besselaar TG, Blackburn NK. Antigenic analysis of West Nile virus strains using monoclonal antibodies. *Arch Virol* 1988; 99 : 75-88.
44. Morvan J, Besselaar T, Fontenille D, Coulanges P. Antigenic variations in West Nile virus strains isolated in Madagascar since 1978. *Res Virol* 1990; 141 : 667-76.
45. Mathiot CC, Georges AJ, Deubel V. Comparative analysis of West Nile virus strains isolated from human and animal hosts using monoclonal antibodies and cDNA restriction digest profiles. *Res Virol* 1990; 141 : 533-43.
46. Damle RG, Yeolekar LR, Rao BL. Strain analysis and epitope mapping of West Nile Virus using monoclonal antibodies. *Acta Virol* 1998; 42 : 389-95.
47. Kulkarni AB, Goverdhan MK, Pavri KM. Passive transfer of immunity to Japanese encephalitis and West Nile viruses from actively immunized mothers to infant mice. *Curr Sci* 1983; 52 : 663-6.
48. Goverdhan MK, Kulkarni AB, Gupta AK, Tupe CD, Rodrigues JJ. Two-way cross-protection between West Nile and Japanese encephalitis viruses in Bonnet macaques. *Acta Virol* 1992; 36 : 277-83.

49. Tesh RB, Amelia PA, da Rosa T, Guzman H, Araujo TP, Xiao SY. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. *Emerg Infect Dis* 2002; 8 : 245-51.
50. Umrigar MD, Pavri KM. Comparative biological studies on Indian strains of West Nile virus isolated from different sources. *Indian J Med Res* 1977; 65 : 596-602.
51. Umrigar MD, Pavri KM. Comparative serological studies on Indian strains of West Nile virus isolated from different sources. *Indian J Med Res* 1977; 65 : 603-12.
52. Li W, Brinton MA. The 3' stem loop of the West Nile virus genomic RNA can suppress translation of chimeric mRNA. *Virology* 2001; 287 : 49-61.
53. Westaway EG. Flavivirus replication strategy. *Adv Virus Res* 1987; 33 : 45-90.
54. Porter KR, Summers PL, Dubios D, Puri B, Nelson W, Henchal E, *et al*. Detection of West Nile virus by the polymerase chain reaction and analysis of nucleotide sequence variation. *Am J Trop Med Hyg* 1993; 48 : 440-6.
55. Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A, *et al*. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *Am J Trop Med Hyg* 1999; 61 : 600-11.
56. Berthet FX, Zeller HG, Drouet MT, Rauzier J, Digoutte JP, Deubel V. Extensive nucleotide changes and deletions within the envelope glycoprotein gene of Euro-African West Nile viruses. *J Gen Virol* 1997; 78 : 2293-7.
57. Ebel GD, Dupuis II AP, Ngo K, Nicholas D, Kauffman E, Jones SA, *et al*. Partial genetic characterization of West Nile virus strains, New York, 2000. *Emerg Infect Dis* 2001; 7 : 650-3.
58. Scherret JH, Poidinger M, John S, Broom AK, Deubel VW, Lipkin I, *et al*. The relationships between West Nile and Kunjin viruses. *Emerg Infect Dis* 2001; 7 : 697-705.
59. Anderson JF, Vossbrinck CR, Andreadis TG, Iton A, Beckwith WH III, Mayo DR. A phylogenetic approach to following West Nile virus in Connecticut. *Proc Natl Acad Sci USA* 2001; 98 : 12885-9.
60. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. *Lancet* 1998; 352 : 767-71.
61. Update: West Nile virus activity-Northeastern United States, January-August 7, 2000. *MMWR Morb Mortal Wkly Rep* 2000; 49 : 714-8.
62. Albagal C, Chaimoff R. A case of West Nile myocarditis. *Harcfach* 1959; 57 : 274.
63. Perelman A, Stern J. Acute Pancreatitis in West Nile fever. *Am J Trop Med Hyg* 1974; 23 : 1150-2.
64. Pogodina VV, Frolova MP, Malenko GV, Fokina GI, Koreshkova GV, Kiseleva LL, *et al*. Study on West Nile virus persistence in monkeys. *Arch Virol* 1983; 75 : 71-86.
65. Rodrigues JJ, Gadkari DA, Shaikh N, Pavri KM. A case of West Nile virus encephalitis. *J Assoc Physicians India* 1985; 33 : 500.
66. Banerjee K. Emerging viral infections with special reference to India. *Indian J Med Res* 1996; 103 : 177-200.
67. Damle RG. *Preparation and characterization of some monoclonal antibodies raised against West Nile virus*. M.Sc., thesis, Pune University, Pune. Maharashtra. India. 1999.
68. Carey DE, Rodrigues FM, Myers RM, Webb JKG. Arthropod-borne viral infections in children in Vellore, south India, with particular reference to dengue and West Nile viruses. *Indian Pediatr* 1968; 5 : 285-96.
69. Banerjee K, Gupta NP, Goverdhan MK. Viral infections in laboratory personnel. *Indian J Med Res* 1979; 69 : 363-73.
70. Tber AA. West Nile fever in horses in Morocco. *Bull Office Int des Epizooties* 1996; 108 : 867-9.
71. Cantile C, Di Guardo G, Eleni C, Arispici M. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet J* 2000; 32 : 31-5.
72. Ostlund EN, Crom RL, Pedersen DD, Johnson DJ, Williams WO, Schmitt BJ. Equine West Nile encephalitis, United States. *Emerg Infect Dis* 2001; 7 : 665-9.
73. Beaty BJ, Calsher CH, Shope RE. Arboviruses In : Lennette EH, Lennette DA, Lennette ET, editors. *Diagnostic procedure for viral, rickettsial and chlamydial infections*, 7<sup>th</sup> ed. Washington, DC : American public Health Association; 1995 p. 204-5.
74. Gadkari DA, Shaikh BH. IgM antibody capture ELISA in the diagnosis of Japanese encephalitis, West Nile and dengue virus infections. *Indian J Med Res* 1984; 80 : 613-9.
75. Kulas KE, Demarest VL, Franchell CS, Wong SJ. Use of an arboviral immunofluorescent assay in screening for West Nile virus. *Ann Acad Sci* 2001; 951 : 357-60.
76. Pavri KM, Shaikh BH. A rapid method of specific identification of Japanese encephalitis- West Nile subgroup of arboviruses. *Curr Sci* 1966; 35 : 455-6.
77. Dandawate CN, Ghosh SN. Kinetic complement fixation test for rapid identification of Japanese encephalitis and West Nile viruses. *Curr Sci* 1974; 43 : 674-6.
78. Odelola HA. Application of single - radial - haemolysis for the detection of antibodies to togaviruses. *Arch Virol* 1979; 60 : 325-8.
79. Rodrigues FM, Vidyasagar J, Singh BP, Ghosh SN, Guttikar SN, Joshi. MV, *et al*. The 1973 epidemic of Japanese encephalitis in West Bengal: a serological survey of domestic animals. *Indian J Med Res* 1976; 64 : 973-80.
80. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, *et al*. Rapid detection of West Nile virus from human clinical specimens, field collected mosquitoes, and avian samples by a TaqMan reverse Transcriptase- PCR assay. *J Clin Microbiol* 2000; 38 : 4066-71.

81. Johnson DJ, Ostlund EN, Pedersen DD, Schmitt BJ. Detection of North American West Nile virus in animal tissue by a reverse transcription-nested polymerase chain reaction assay. *Emerg Infect Dis* 2001; 7 : 739-41.
82. Shi PY, Kauffman EB, Ren P, Felton A, Tai JH, Dupuis II AP, *et al.* High- throughput detection of West Nile virus RNA. *J Clin Microbiol* 2001; 39 : 1264-71.
83. Lanciotti RS, Kerst AJ. Nucleic acid sequence- based amplification assays for rapid detection of West Nile and St. Louis encephalitis viruses. *J Clin Microbiol* 2001; 39 : 4506-13.
84. Gubler DJ, Campbell GL, Nasci R, Komar N, Petersen L, Roehrig JT. West Nile virus in the United States: Guidelines for detection, prevention, and control. *Viral Immunol* 2000; 13 : 469-75.
85. Laird M, Miles JW, editors. *Integrated mosquito control methodologies*, 2 vols, London : Academic Press, Inc.; 1983-1985.
86. Haahr S. The occurrence of virus and interferon in spleen, serum and brain in steroid-treated mice under experimental infection with West Nile virus. *Acta Pathol Microbiol Scand* 1969; 75 : 303-12.
87. Vargin VV, Zschiesche W, Semenov BF. Effects of tilorone hydrochloride on experimental flavivirus infections in mice. *Acta Virol* 1977; 21 : 114-8.
88. Azarova IA, Mishaeva NP, Votiakov VI, Golovneva GP. Search for inhibitors of West Nile virus among antibiotics. *Antibiot Khimioter* 1992; 37 : 29-31.
89. Amvros'eva TV, Votiakov VI, Andreeva OT, Vladyko GV, Nikolaeva SN, Orlova SV, *et al.* New properties of trental as an inhibitor of viral activity with a wide range of activity. *Vopr Virusol* 1993; 38 : 230-3.
90. Ben-Nathan D, Maestroni GJ, Lustig S, Conti A. Protective effects of melatonin in mice infected with encephalitis viruses. *Arch Virol* 1995; 140 : 223-30.
91. Phillips DA, Aaskov JG, Atkin C, Wiemers MA. Isolation of Kunjin virus from a patient with a naturally acquired infection. *Med J Aust* 1992; 157 : 190-1.
92. Shimoni Z, Niven MJ, Pitlick S, Bulvik S. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. *Emerg Infect Dis* 2001; 7 : 759.
93. Arroyo J, Miller CA, Catalan J, Monath TP. Yellow fever vector live virus vaccines: West Nile virus vaccine development. *Trends Mol Med* 2001; 7 : 350-4.
94. Mackenzie JS, Smith DW, Hall RA. West Nile virus: is there a message for Australia? *Med J Austr* 2003; 178 : 5-6.

*Reprint requests:* Shri R. Paramasivan, Senior Research Officer, Centre for Research in Medical Entomology (ICMR)  
No-4 Sarojini Street, Chinna Chokkikulam, Madurai 625002, India  
e-mail : rpsivan 2000@yahoo.co.in