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WEST NILE VIRUS EPIDEMICS : LESSONS FOR INDIA

During 1999 and 2000, epidemics of severe neurologic illness were reported in New York (USA) among humans, horses/and birds with unprecedented morbidity and mortality. The causative organism was identified as West Nile (WN) virus. By the end of the year 2000 the virus activity had spread to 12 states[1-3]. The WN virus responsible for the US outbreak (NY99) was found to be genetically related to a virus circulating in Israel from 1997 to 2000 (lsr98). First isolated from blood of an elderly woman with febrile illness in West Nile district in Uganda (currently Nile Province) in 1937[4], the virus caused severe outbreaks in the Middle East, European and African countries during 1950s and 70s. However, the increase in the frequency and severity of outbreaks in humans and animals particularly horses since mid 1990s in these countries and its incursion for the first time in USA, has caused much alarm as a re-emerging disease.

Japanese encephalitis (JE), WN and Dengue (DN) viruses belong to family flaviviridae. They are transmitted by mosquitoes in the genus Culex (JE,WN) and Aedes (DN). In India unlike JE and DN there has been no serious epidemic due to WN virus. Generally WN infection is said to run a mild, often-subclinical course. But, differential diagnosis using conventional serological tests is often difficult (due to antigenic sharing among the members of flaviviridae) and hence true magnitude of WN infection is not clearly known. In the recent WN fever outbreaks in the US and Europe, precise diagnostic techniques and bioinformatics tools not only established the identity of the virus but also traced its origin. This write-up briefly summarises information on the epidemiology of WN virus infection, examines the possibility of its emergence as a major public health problem in India and discusses the need for better diagnostic tools.

The West Nile Virus

West Nile virus contains single-stranded, positive-sense, RNA containing approximately 11,000 nucleotides enclosed in 12kDa capsid protein and host-derived envelope of membrane glycoproteins E and M, and 7 non structural proteins, NS. The E protein is the most important structural protein eliciting immunological responses. It also mediates virus-host cell attachment. Phylogenetic analysis shows that WN virus isolates from different geographical regions fall into lineage 1 or 2. Isolates belonging to lineage 1 only have been found associated with human encephalitis[5].

Transmission Cycle

Birds act both as carriers and amplifying hosts of WN virus in nature. Ornithophilic mosquitoes belonging mainly to Culex species act as vectors for transmission of infection from viraemic birds to a large spectrum of vertebrate hosts. *Cx. univittatus* complex (South Africa, Israel), *Cx. modestus* (France), *Cx. vishnui* complex (lndia and Pakistan), *Cx. pipiens pipiens* (Romania, USA) act as major vectors of WN virus. There is no evidence to suggest person to person/animal, or animal to animal/ person transmission. The virus multiplies in the mosquito vector and after an extrinsic incubation period of about 2 weeks, the vector becomes infective for active transmission to a susceptible host. Hibernating mosquitoes can carry the virus[6,7] and vertical transmission of the virus from infected female to her progeny has been reported[8]. Migratory birds playa major role in the WN virus dissemination. However, virus dissemination through infected mosquitoes or by illegally imported infected pet birds should also be considered a possibility.

WN Virus Epidemics

Since the original isolation of WN virus in 1937, notable outbreaks were recorded in Israel (1951-1954,1957), South Africa (1974), Romania and Morocco (1996), Tunisia (1997), Italy (1998) Russia, the United States, and Israel (1999), and Israel, France and the United States 2000[5]. The increase in the frequency of outbreaks, severe disease in humans and horses and high mortality rates in birds have emerged as the disturbing trends in the epidemiology of WN fever.

The Indian Scenario

Epidemiology of WN infection is not well known in India. Serological surveys during JE epidemics and in areas endemic to JE show that the virus is prevalent in India[10]. Generally WN virus infection runs a mild febrile course without any serious involvement of central nervous system. However, fatal cases of encephalitis in children due to WN virus have been recorded[11]. In very few cases WN virus isolates have been obtained from different hosts. Two isolations from *Cx. vishnui* and one from a human patient (GenBank Accession Nos.AF196535 AF196537 and AF196540 respectively) made by the scientists of the National Institute of Virology, Pune, have been sequenced and their genetic relationships show that these Indian strains belong to lineage 1. They cluster together, with a sequence identity of 97 and 98% for sequences of the E gene and NS5/3¢UTR, respectively[12] and appear as monophyletic sister clades to the European and African WN viruses.

The vector *Cx.vishnui*, in which natural infection of WN virus has been recorded, is mainly zoophilic and only a small proportion feeds on birds[13]. This species breeds profusely in paddy field waters and is a vector of JE virus also. Experimentally, *Cx.tritaeniorhynchus, Cx.pseudovishnui* and *Cx.univittatus* have also been shown to support WN virus multiplication[14]. In birds death due to WN virus has not been reported. Fatal cases were seen in children[11] unlike in the older age groups in the recent outbreaks in other countries. The role of birds and other mammalian vertebrates in the transmission of WN virus is not clear. Demonstration of acquired antibodies in pigs indicates that they could get infected in nature[15,16]. But, in experimental studies pigs developed antibodies against the virus but not viraemia[17]. In case of JE, birds act as carriers and pigs as amplifying hosts whereas in case of WN birds act both as amplifying hosts and carriers. Therefore, very little is known about the type of birds involved in India and their susceptibility to WN virus.

Despite the presence of mosquito vectors in abundance, and potentially neurovirulent strains of the virus (lineage 1) there has been no serious epidemics due to WN virus in India comparable to JE outbreaks. Reason(s) for this situation is not clear, but presence of other flaviviruses in India could be an important factor for limiting the spread of WN or reducing its severity. Studies involving JE and WN viruses in bonnet macaques[18], JE and WN viruses in domestic pigs[17] and JE, St.Louis encephalitis and Yellow fever viruses in hamsters[19], have shown cross protection between members of flavivirus group. Interference to oral superinfection by Thogoto virus in tick vector[20] and heterologous interference in *Aedes albopictus* cells infected with alphaviruses[21] have also been reported. In India it is not uncommon to detect co-circulation of more than one flaviviruses in endemic areas and persons living in endemic areas possess antibodies to one or more flaviviruses due to natural infection. Mosquitoes in the *Cx.vishnui* group being vectors for both JE and WN viruses, dual infection with JE and WN viruses may lead to interference. Thirdly, as vectors of WN virus are mainly zoophilic and only a small proportion feed on birds[13], probability of a vector feeding on a viraemic bird is very small. Further, probability of an infective mosquito feeding on a clean bird for virus amplification is also very small.

Also, being mainly zoophilic and zoophagic, there may be depletion of the virus in an infective female mosquito when she feeds on mammalian hosts. However, we need field epidemiological data to support laboratory observations.

Surveillance

For surveillance of WN virus in the US, the dead crows have served as "neon needles in a haystack"; indicators of viral activity and the dead crow density closely associated with the number of human cases[22]. Serosurveys and experimental data indicate that both chickens and pigeons can serve as useful captive sentinels; they develop antibodies after infection without becoming infectious to *Cx. pipiens* vectors[23,24]. In India, chickens have been found to be poor surveillance sentinel for JE as natural antibodies have not been found in chickens after JE outbreaks. Hence chicken can be examined if they can serve as sentinels for WN infection and as an adjunct to other differential diagnostic parameters for WN and JE infections in epidemic situations. Adult mosquito surveillance could provide the local health departments information on species' presence, density, seasonal fluctuations and virus infection. A surveillance system is being developed in the US with a view to provide basic information on the ecology of WN virus which in combination with information about landscape characteristics and weather conditions, over space and time could aid in developing forecasting models.

Conclusions

In India, mostly serological tests, for the detection of IgM and neutralizing antibodies, have been used to investigate epidemics of JE and dengue. Only some laboratories have attempted virus isolation and highly specific and sensitive tests based on polymerase chain reaction (PCR) which are confirmatory. Perhaps none have tried sequencing the virus isolates for phylogenetic analysis and other studies. IgM-capture ELISA is of limited value for diagnosis of cross-reacting flaviviruses and neutralization and virus isolation tests are time consuming. In fact preliminary diagnosis employing serological tests indicated the New York outbreak to be due to St.Louis encephalitis virus[25]. Only after virus isolation, and sequencing and phylogenetic analysis of the isolates, a definitive diagnosis could be made as WN epidemic and the origin of the epidemic strain could be traced. Monoclonal antibody-based ELISA[26] and reverse transcriptase (RT)-PCR[9] are suitable for large-scale screening of field collected mosquitoes for the virus. Fluorescent DNA probes in a 5' exonuclease assay (TaqMan)[9] offers the advantage over traditional RT-PCR of increased sensitivity and higher throughput. In the absence of application of precise diagnostic tools, magnitude of the disease burden and seriousness of the problem cannot be assessed. This has been the case with WN infections in India. If the laboratory findings on the interference between JE and WN viruses are confirmed in field situations, the presently available effective JE vaccine may be helpful for controlling WN infections also.

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