ABSTRACT

West Nile Virus (WNV) is a positive sense single stranded RNA virus (Family: Flaviviridae, Genus: Flavivirus) having approximately 11,000 nucleotides and is 45-50 nm in diameter. It was first isolated from a febrile patient in 1937 in the West Nile district of Uganda in African continent. Since then, there have been documented outbreaks in Egypt, Israel, South Africa, parts of Europe, Asia and North America. In nature, WNV is reported to be maintained in enzootic cycle involving certain birds and mosquitoes. Birds act both as carriers and amplifying hosts of WNV. Ornithophilic mosquitoes act as vectors for transmission of WNV infection from viraemic birds to non-infected birds and large spectrum of vertebrate hosts, including mammals. Although humans and horses fall sick with WNV infection, they act as dead end hosts.

Since so far, there is no treatment or vaccine available for WNV illness in humans, WNV is of immense importance for public health. So, in present study, it was decided to compare the efficacy of IHC with RT-PCR, in terms of screening dead birds, for doing surveillance of WNV. Present research work was carried out in the northwestern part of Riverside County in California, USA which closely resembles the climatic & geographical characteristics, nature of bird & mosquito population of tropical countries like India, including the state of Punjab. Dead birds reported by public were collected over ice, necropsied under biological cabinet in a negatively pressurized room and brain, heart, liver, spleen, kidney, testes, ovary, intestine and lung were collected for making histological sections. These tissue sections were screened for WNV using Immunohistochemistry (IHC) technique. With exception of some studies from US east coast, the present study used IHC technique for the first time, to screen and study the distribution of WNV in the organs of dead birds in this part of California. As compared to RT-PCR, IHC technique was found to be equally efficacious for detecting WNV antigen in the organs of dead birds. Out of birds belonging to 8 different Orders (Passeriformes, Falconiformes, Strigiformes, Columbiformes, Trochiliformes, Psittaciformes, Accipitriformes and Anseriformes) which were tested, birds belonging to 7 orders (except Order Anseriformes) were found infected with WNV. Majority of the birds that came out WNV positive belonged to Order: Passeriformes; family: Corvidae. American crow was the most frequently found WNV positive species in this study. Total of 198 dead birds belonging to 26 species were collected. Out of these collected birds, 172 birds were screened for WNV. During screening of these 172 birds, 83 (48.30%) birds belonging to 25 species were found positive, comprising of 57 (68.68%) males and 26 (31.32%) females. No gross lesions on the examined internal organs were found to be specific in nature for WNV infection. WNV antigen was detected in Brain, Lung, Kidney, Liver, Heart, Spleen, Ovaries, Testes and Intestine. The pattern of distribution of WNV in different...
organs varied between different Orders of birds. Overall, spleen, kidneys and liver were the organs which most frequently tested positive for WNV. During the study period, 69 cases of human WNV illness (West Nile Neuroinvasive disease (WNND): 71%, West Nile fever (WNF): 23%, Asymptomatic infection: 6%) were reported. Gender wise, 61% of the WNV illness cases were males while 39% were females. From these 69 cases, 4 persons (Male: 1, Female: 3), all exhibiting WNND symptoms, eventually died (Fatality rate: 2.5% among males and 11.11% among females). All the dead persons fall in the 81-90 year age-group. California closely resembles the climatic & geographical characteristics with Northern India including Punjab. Nature of bird and mosquito population of state of Punjab almost resembles with composition of nature of bird and mosquito population of California. Lessons learned in doing surveillance of this virus in California can be easily replicated in Punjab to set up a disease surveillance laboratory. Since WNV is trotting globally, as is evident from the plethora of published reports, there is a dire need to set up a globally centralized database which can serve as a platform for closer interactions between physicians, veterinarians and scientists.

Key words: West Nile virus, Immunohistochemistry, American Crows, RT-PCR, Arbovirus surveillance, Neuroinvasive disease