The present study was undertaken to do surveillance of WNV in local avian population of Riverside County of California. In addition to this, for the first time, distribution of WNV in different organs of infected birds, species-wise and sex-wise, was studied. In Riverside County, ranch style of living is very popular, which has resulted in dense population of horses. Since invasion of California by WNV in 2003, its presence has been detected continuously every year in this County. As there is no definitive treatment or vaccine currently available for human use, the surveillance of this virus to protect the public health becomes extremely important.

Screening of WNV had been continuously going on in this County by collection and testing of local mosquito species with
RT-PCR; and by testing of sentinel chicken flocks for seroconversion against WNV with EIA. With exception of some studies from US east coast, the present study used immunohistochemistry technique for the first time, to screen and study the distribution of WNV in the organs of dead birds. It was found that, as compared to RT-PCR, IHC technique was equally efficacious and safe for lab workers for detecting WNV antigen in the organs of dead birds. It doesn’t require costly and highly specialized equipment. This cost factor is a very important consideration for those public health agencies having limited resources, thus opening a new window for developing and underdeveloped countries to undertake/initiate research on WNV. Additionally IHC gives an opportunity to examine the histological and pathological characteristics in the host tissues related with WNV infection.

Moreover formalin-fixed or paraffin embedded tissue samples can be archived, re-sectioned and re-stained for confirmation. In addition to that, this technique can also be used to screen archived tissues for new pathogens in future, thus depicting the previously unknown presence of an emerging disease.
The salient features/ findings of present study are further listed as follows

1. WNV was detected in birds belonging to 7 different Orders: Passeriformes, Falconiformes, Strigiformes, Columbiformes, Trochiliformes, Psittaciformes and Accipitriformes. While not a single bird belonging to Order Anseriformes was detected positive during present study, majority of the birds collected as well as the birds that came out WNV positive belonged to Order: Passeriformes; family: Corvidae. Like during the WNV outbreak in New York City, in 1999, American crow was the most frequently found WNV positive species in present study.

2. For 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.

3. No gross lesions on the examined internal organs were found to be specific in nature for WNV infection. With IHC staining, WNV antigen was detected in Brain, Lung, Kidney, Liver, Heart, Spleen, Ovaries, Testes and Intestine. The present study, using IHC technique, is the first one on the
Summary

US west coast to do surveillance of WNV in the dead birds and to study its epidemiology in the birds of Riverside County of California.

4. The pattern of distribution of WNV in different organs varied between different Orders of birds.

5. Microscopically, staining pattern in the spleen was diffuse type and the cells showing presence of WNV antigen were reticuloendothelial cells while Liver showed diffuse type of staining pattern with Kupffer cells constituting the majority of the stained cells for WNV antigen. On the other hand in kidney tissue it was multifocal and was centered around collecting ducts. WNV antigen was stained mostly in macrophages and in the walls of excretory tubules in tubular epithelial cells.

6. The duodenum showed intense focal staining pattern and WNV antigen was stained throughout the payer patches while lungs showed staining around airways, blood vessels and the stained cells were macrophages and endothelium of the vessels and airways. The staining was faint in heart, diffuse type and was scattered all over the myocardium. Stained cells were macrophages, myofibers and occasionally endothelial cells were also stained. Pericardium was also showing marked sections of intense
staining of macrophages.

7. In brain, WNV antigen was found in Purkinje cells, neurons and Glial cells.

8. In the ovaries, the parenchymal, interstitial and stromal cells stained for presence of WNV. Surprisingly, no WNV antigen was stained in the epithelium of the wall of oviduct and zona pellucida. Likewise in testes, the sertoli cells stained positive for WNV antigen and the staining pattern was focal type.

9. Overall, spleen, kidneys and liver were the organs which most frequently tested positive for WNV. So no single organ was found fully reliable for the sake of doing WNV surveillance among dead birds. Since with same input of time, labour and amount of reagents, multiple organs collected from same bird were conveniently screened for the presence of WNV. Hence for effective surveillance of WNV, screening of multiple organs is recommended.

10. During the study period, 69 cases of human WNV illness were reported. Out of these 69 cases, ~6% cases were asymptomatic infections. Clinically, 71% of WNV cases were presented clinically as West Nile Neuroinvasive disease (WNND) while 23% were presented as West Nile fever (WNF). Gender wise, 61% of the WNV illness cases
were males while 39% were females.

11. 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. The lowest survival rate observed in this 81-90 year age-group was possibly because of weakened immune system due to old-age.

12. The present study reveals another aspect of higher overall case fatality ratio of 50% (in 81-90 years age group) which is double than the US national case fatality ratio of 29% reported for the same age group over the time period of 1999-2008, double than the case fatality ratio in outbreak in Israel. The median age of human patients with WNND was much younger (54 years) as compared to median age of 72 years reported from outbreak in Greece. This suggest that this virus, since its introduction on this ‘virgin soil’ of North America has evolved/diverged into a different strain.

13. As indicated in present study, *Culex* spp. of mosquito plays the role of devil for amplification and spread of WNV, Public health agencies should focus on educating public on the importance of reducing/eliminating the mosquito breeding sources. In recent attempt to extend the vector control
services of Northwest Mosquito and Vector Control District to neighboring incorporated areas of Riverside County, a poll to implement a meager service fee of 8 US dollar/year was rejected by residents, that too of most developed country, because of lack of awareness regarding importance of mosquito control.

In this study, the predominant tissues which stained positive for WNV antigen were spleen, kidney and liver while in previous studies during outbreak in New York (Steele et al., 2000) the most frequently stained organs were brain and heart. This change in affinity of this virus for different organs, over a time period from 1999 to 2010, suggests that this virus is evolving/diverging from the originally introduced strain. Since the RNA viruses have very high rate of mutation, and as WNV is a RNA virus, it is suggested that its surveillance should be done more frequently with improved primers. In the absence of any definitive treatment for WNV related illness, such endeavors shall help the public health agencies to take proper measures for bringing necessary awareness regarding prevention of mosquito bites and controlling mosquito population.

California closely resembles the climatic & geographical
characteristics with Northern India including Punjab
(California:: Latitudes: 33° 46’ 59” N/ Longitude:116° 48’
12” W, Punjab:: Latitude: 30° 56’ N/ Longitude:75° 52 E).
Nature of bird and mosquito population of state of Punjab
almost resembles with composition of nature of bird and
mosquito population of California. In the light of above, it
is proposed to set up a Surveillance/Diagnostic/Referral lab
in Northern India and any other tropical country having
same physical and geographical features, to do routine
surveillance and confirmatory diagnosis of WNV infection.

This virus has defied the previous conventional
understanding of the scientific community that
underdeveloped or developing countries are hotbeds for
perpetuation of majority of diseases. Since from the
published reports, it is evident that its presence has been
reported not only from Africa or Asia but also from the
most advanced nations in Europe and North America. The
alarming speed with which it was able to establish itself in
the cooler latitudes and cause epidemics in humans and
horses, is a matter of grave concern. As it is a mosquito-
borne disease and with the globalization of world trade, the
speed with which an infected mosquito can travel long
distances is phenomenal, hence this aspect of disease
transmission ecology can not be neglected. Similarly, quarantine of certain imported products is not practically feasible.

The present study, using IHC technique, is the first one on the US west coast to do surveillance of WNV in the dead birds and to study its epidemiology in the birds of Riverside County of California and thus is a fundamental contribution to the understanding of ecology of this neurotropic virus.

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 to join hands in their efforts to fight this disease which is currently having no definitive treatment or vaccine for human use. In the light of present study, certain suggestions for further research in this area are as under

To predict the movement of zoonotic pathogens, further studies need to be focused on understanding the role of avian migration in the long-term perpetuation and spread of WNV.

Increased understanding of impact of climate change on
temporal and spatial distribution of vectors and the pathogens will improve our ability to do better risk prediction.

As the exact site of replication of this virus in vertebrate hosts is still elusive, further research need to be done in this direction.

Genetic studies to sequence the genome of primary vector of this virus, *Culex* species mosquitoes and different strains of WNV, isolated over space and time, will add to our understanding of vector-virus-vertebrate interactions, evolution and adaptation ability of this virus.