Results and Discussion

West Nile Virus, a neurotropic virus is a single-stranded, positive sense RNA virus of Flaviviridae family, having approximately 11,000 nucleotides (Petersen and Roehrig, 2001) and is 45-50 nm in diameter (Mukhopadhyay et al., 2003). After invading USA through the east coast (New York City) in the summer of 1999, WNV continued its spread towards the northern and the western states, eventually reaching west coast (California) in 2003. Since then it has successfully over-wintered, amplified itself to the epidemic levels and subsequently spread to every County of state of California. In 2007, due to lack of effective WNV surveillance programme, it caused epidemic in California, resulting in declaration of state of medical emergency. This ability of WNV to spread to colder regions, e.g. to many Provinces of Canada up north; to find new reservoir/amplifying
hosts; and its ability to, not only establish itself, but also to amplify to alarming epidemic levels is a matter of grave concern for public health agencies. As there is no therapeutic treatment available to treat WNV related illness in its incidental hosts i.e. humans and horses, its effective surveillance becomes even more important matter of public health importance.

Present study was conducted in County of Riverside, California. This County comprises of 7,208 square miles (18,669 square kilometers) in the southeastern part (Latitude: 33° 46' 59" N/ Longitude: 116° 48' 12" W) of California State; stretches from Orange County on the western side to Colorado River on the east, which forms border with state of Arizona. Geographically, this county is mostly part of Mojave desert with most of the inhabitant cities located in the western part of this County. Topographically, these cities represent either semi-urban or rural kind of geographical set-up.

Since the epizootic cycle of WNV involves birds and mosquitoes, its surveillance was done in local mosquito and avian populations. Different methods/traps were used to collect mosquitoes of different physiological stages e.g. CO₂ baited EVS traps for host seeking female mosquitoes; fermented alfa-alfa
infused Gravid traps for ovipositioning gravid female mosquitoes; and Resting boxes to collect both blood-fed and gravid females. Gravid females were given special attention, in terms of testing for WNV, because as compared to host seeking females (which were mostly nulliparous), gravid females got a chance to amplify the virus in their bodies after getting an infected blood-meal.

Similarly, to do WNV surveillance in local avian population, free ranging wild birds, captive sentinel flocks and dead birds reported by public can be screened for WNV. Since free ranging wild birds are not stationary in their location, they didn’t tell us about the local foci of WNV amplification. To detect the activity of WNV in local neighborhood, six sentinel flocks of 10 white leghorn chickens each, were placed at fixed strategic locations in present study area. Seroconversion of these sentinel chickens, against WNV was definitive indicator towards the activity of WNV in their immediate neighborhood. After detecting a seroconverted chicken in these sentinel flocks, adulticiding efforts were mounted in their vicinity to halt the spread of infected adult mosquitoes. For this reason, helicopters were used for adulticiding (Fig. No. 3) and hovercrafts were used to do large scale larviciding (Fig. No. 4) of the wetlands and other larger water bodies.
For the same reason of detecting presence of WNV in the local avian population, screening of dead birds was also carried out. Whenever a dead bird report was received from the local residents of the study area, a service request (SR) was generated (Annexure 1) to collect it. All those dead birds, who were not infected with maggots or were dead for less than 24 hrs, were necropsized and tissue samples of heart, brain, lung, liver, kidneys, spleen, intestine, testes and ovaries were collected in 10% buffered formalin solution. After overnight fixing in 10% buffered formalin solution, they were sectioned as paraffin embedded tissue blocks and were screened with Immunohistochemistry (IHC) for presence of WNV antigen. Any bird which came out positive for WNV in even a single organ was considered WNV positive.

Currently, there are different testing methods to screen dead birds for WNV. In spite of all these molecular methods available to detect WNV, public health agencies still lack a screening method which should be cheap, quick, safe, with high sensitivity and specificity values. RT-PCR has the shortest turnaround time to screen WNV, but because of its expensive equipment, those public health agencies with limited resources
can not afford it. Like RT-PCR, Electron microscopy is even expensive. Virus isolation is very sensitive and specific, but because one has to work with live virus, thus it is potentially biohazardous for the laboratory workers. Vec Test and RAMP Test loose their sheen because of low sensitivity and specificity values (Stone et al., 2005). IHC method, which is a combination of three sciences: immunology, histology and chemistry, is the only diagnostic test which is cheap; safe as one works with fixed virus; has relatively high sensitivity and specificity, but as compared to RT-PCR screening, it has a drawback of long turnaround time. This turnaround time can be shortened by having in-house histology facility.

Based upon testing of only kidney tissue of birds collected in present study, IHC had a sensitivity of 95.45% and specificity of 73.68% with positive predictive value (PPV) of 80.77% and negative predictive value (NPV) of 93.33%. The comparability of agreement between RT-PCR and IHC for positive birds was 95.45% which increased to 100% when multiorgan from same bird were screened with IHC. The Sensitivity, Specificity, PPV and PNV for birds of the Order Passeriformes were 95.24%, 64.29%, 80% and 90% respectively while for American crows, *Corvus brachyrhynchos* (the most sensitive bird to WNV showing the
Results and Discussion

highest mortality rate) were 95%, 54.54%, 79.17% and 85.70% respectively. Sample size for other Orders of birds was too small to draw any statistically significant conclusion with respect to Sensitivity, Specificity, PPV and PNV values of IHC method. Present comparison of IHC with RT-PCR in terms of Sensitivity, specificity, PPV and PNV was done for the first time by Sandhu et al. (2010).

4.1 Surveillance

During the study period of August 2007-2010, total of 198 birds belonging to 8 Orders were collected. Out of these 198 birds collected, 172 birds were found suitable for necropsy to collect tissues and for subsequent testing for WNV. Any bird that was found infected with WNV in even a single organ was considered positive. Out of these 172 dead birds tested, 83 birds belonging to 7 Orders (Passeriformes: 71; Falconiformes: 2; Strigiformes: 1; Columbiformes: 5; Trochiliformes: 2; Psittaciformes: 1; Accipitriformes: 1) were found positive for WNV infection. Species and sex of all the birds which were collected, tested and found positive for WNV is listed in Table No. 2. These birds were not part of any mass die-off. Majority of the birds collected (n= 164) as well as the birds who were found positive (n= 71) for WNV belonged to Order: Passeriformes.
Experimental studies had shown that American crows are most susceptible to WNV infection and dies within hours of getting infected (Weingartl et al., 2004). From present study, it is clearly evident that the maximum number of dead birds (n=64) collected in Riverside County of California were American crows, followed by House finches (n=20), Western scrub jay (n=15) and House sparrows (n=13). All these four species of birds belong to family Corvidae suggesting that in order to do surveillance of WNV in a geographical area, dead birds of this family can be reliably screened with IHC technique. Out of these 64 American crows collected, 56 were screened for WNV and it was found that ~70% (n=39) of them were positive for WNV. Similar high incidence of WNV, in American crows, was also reported by Kramer & Bernard (2001) and Mclean et al. (2002) during WNV outbreak in New York City in 1999 and 2000 respectively. As evident from Table No. 2, the proportion of WNV positive male birds (68.68%) in present study was double than the proportion of positive female birds (31.32%). Similar high proportion of WNV positive male birds was also reported by Steele et al. (2000) during WNV outbreak in New York in 1999.

During the present study period, 69 cases of human WNV related illness were also detected by the California Department of
Results and Discussion

Public Health. The age group and the pattern of clinical presentation of these 69 cases of WNV illness is depicted in Fig No. 5. In the absence of any travel history outside of California, medical history of organ transplant/blood transfusion, it was assumed that all these human cases of WNV were acquired locally in California via infected mosquito bites.

From Table No. 3 and Fig. No. 6, it is evident that ~6% (4/69) of the WNV illness cases were asymptomatic i.e. neither they were exhibiting clinical symptoms at the time of testing nor they developed them later on. These asymptomatic cases were blood donors and fall in the age group of 11-40 years (11-20 yrs: 1, 21-30 yrs: 1 and 31-40 yrs: 2). Out of these 4 asymptomatic WNV cases, 3 were females while 1 was a male. Furthermore, 71% (49/69) cases were clinically presented as West Nile neuroinvasive disease (WNND) and 23% (16/69) were presented as West Nile Fever (WNF). Although, as is evident from Fig No. 7, majority of WNV human cases (32/69) occurred in the age group of 41-60 years, there was no fatality recorded in this age group. At the same time 50% fatality rate (4/8) was observed in the age group of 81-90 years. The median age of human WNV related illness cases was 53 years (Range: 4-90 years).
Results and Discussion

The proportion of clinical presentation of WNND and WNF in present study was identical to proportion of WNND and WNF cases which were reported from WNV outbreak in Greece in 2010 (Danis et al., 2011) except that the median age of patients with WNND in present study was much lower (54 years) as compared to 72 years during the WNV outbreak in Greece. In present study, the overall fatality/case ratio observed in patients aged >70 years age group was of 50% which was nearly double than the case fertility ratio (29%) reported during a similar outbreak of WNV in Israel in 2000 (Chowers et al., 2001) and higher than the case fatality ratio of 35% within the same age group of >70 years age group reported from WNV outbreak in Greece in 2010 (Danis et al., 2011).

Clinically, as shown in Table No. 4, 71% (49/69) of these WNV illness cases in present study were presented as (WNND) which is close to double i.e. 41% of the total proportion of WNND cases reported during 1999-2008 by CDC (CDC 2010). This nearly two fold increase in the proportion of WNND type cases in present study area suggests that this virus might have evolved/deviated from the original strain which was introduced on the east coast of United States. Similarly in present study,
23% (16/69) of the WNV cases were presented clinically as West Nile fever (WNF) which is less than half (59%) of the proportion of the WNF cases which were reported by CDC during 1999-2009 (CDC 2010). Additionally, half of the WNV cases i.e. 46% (32/69) in present study occurred in the age group of 40-60 years while national occurrence during 1999-2008 reported half of the cases of WNV in the age group of >60 years (CDC, 2010).

The gender distribution of these WNV illness cases is presented as Fig No. 8 and Table No. 5. It was found that 61% of the WNV illness cases were males while 39% were females. The reason for higher proportion of WNV illness cases in males is that, as compared to females, they spend more time in outdoor activities thus potentially at more risk of getting bitten by an infected mosquito. Out of these total 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 (Table No. 3). As mentioned earlier, although the maximum number (n=32) of WNV illness cases fall in the 41-60 years age-group, but there was no fatality in this group while all the dead persons (50% fatality rate) belonged to the age-group of 81-90 years (Median age: 83.5 years; Range 82-90 years). The lowest survival rate
observed in this 81-90 year age-group was possibly because of weakened immune system due to old-age. Overall, in present study, the fatality rate (8.2%) among the WNND cases was consistence with reported national US fatality rate of 9%, during 1999-2008 (CDC 2010).

Since the origin of WNV strain responsible for epidemic in New York (NY-99) has been linked with the strain of WNV previously isolated from geese in Israel (Lanciotti et al., 1999), this variation in case fatality is observed because of different genetic structure of populations involved, variable background immunity against WNV and co-circulation of related flaviviruses in that geographical region. Similarly, role of genetic make-up and background immunity of human population involved was evident during the largest documented epidemic of WN viral disease in South Africa during 1973-74 involving thousands of human cases of WN fever but only a single case of WNND (McIntosh et al., 1976). Since WNV has been endemic in South Africa, so the human population co-evolved with this virus and was having adequate background immunity resulting in only one WNND case.

Clinical presentation of this disease in present study was
different from the previously reported presentations of WNV disease in humans in Egypt and Africa (Hayes, 1989). Since its introduction to the ‘virgin soil’ of United States in 1999, this virus is adapting to new environments, hosts and over-wintering mechanisms. During this course of adaptation to new circumstances, it is evolving/deviating from the original strain which got introduced in North America.

This change in the pattern of clinical presentation of WNV illness in present study i.e. more proportion of WNND presentation as compared to WNF; increased pathogenicity i.e. higher incidence of WNV illness in younger (40-60 years) age groups as compared to older (>60 years) age group as reported in previous reports; and comparatively younger age (41-60) of majority (42.9%) of the WNND cases in present study and higher case fatality rate among people aged >80 years strongly suggests that since the pathogenicity of WNV illness depend upon strain and Lineage of WNV; previous history of WNV activity in that area; the consequent level of background immunity in that population (including cross-immunity to other closely related flaviviruses) (Nemeth et al. 2009); immunologically naïve population; and genetic make-up of population, this varied pattern of pathogenicity of WNV related
illness observed in present study was because of either or all of above listed factors.

For the WNV related illness to manifest as WNND, this neurotropic virus has to cross the blood-brain barrier and infect the brain. As compared to young people, high incidence of WNND in the elderly people (>70 years age) suggests that certain factor in old age facilitate the entry/transportation of WNV across the blood-brain barrier. Deubel et al. (2001) and Chambers et al. (1998) reported that the WNV envelope protein ‘E’ mediates cell attachment and neuroinvasiveness and seems to be a primary virulence factor. Host factors that allow entry of WNV may include factors that promote virus entry into and replication in the endothelium at the blood-brain barrier. Such factors in elderly people, that enhance entry of WNV into the CNS, may act by disruption of cerebral endothelium (eg. Hypertension, cerebrovascular disease, diabetics) or an increase in magnitude and duration of viraemia (eg. immunosuppression, immunesenescence). Other possible mechanisms of viral entry into the CNS include axonal transport through olfactory neurons, cytokine-directed leukocyte diapedesis through endothelial tight junctions or viral shedding through the choroids plexus (Deubel et al., 2001). Based upon lab studies with SLE virus, probability
of neuroinvasion by WNV is correlated with level and duration of viraemia (Nathonson, 1980).

A WNV related death of one 17 year old girl, living in neighboring Menifee city is worth mentioning (Annexure- 2). She died with West Nile encephalitis after 4 years of getting infection. When she was 13 years old, she got WNV infection from bite of an infected mosquito. During next 4 years of sickness, when she was living in a vegetative state, she underwent umbilical cord blood stem-cell injections and hyperbaric treatments but without any improvement. Eventually, after 4 years of vegetative condition, she died of kidney failure (The Press Enterprise, 2008). This incident should be highlighted by public health agencies for doing public relation work regarding the severity of WNV related illness and highlighting the role of mosquitoes in the spread of this disease.

4.2 Distribution of WNV in various organs of dead birds

Gross examination of some of the WNV positive American crows showed subcutaneous hemorrhage (Fig No. 9) on the skull thus indicating signs of trauma. Because of WN related encephalitis, they lost depth of vision/perceptive, thus while flying they were not able to maneuver through obstacles. No
Results and Discussion

gross lesions on the examined internal organs were found to be specific in nature for WNV infection. Myocardial lesions included pallor and hemorrhage while splenomegaly was evident in some of them. With the IHC staining, the distribution of WNV was studied in the various collected organs of birds. WNV demonstrated non-restrictive tropism in affected birds, infecting almost all major organ systems. All the collected organs i.e. Brain, Lung, Kidney, Liver, Heart, Spleen, Ovaries, Testes and Intestine, who were screened for WNV antigen tested positive. Similar distribution of WNV antigen was also observed in the organs of birds of US east coast (Steele et al., 2000).

Distribution of WNV in different organs of birds of different orders, with special reference to family Corvidae (most sensitive to WNV infection with highest mortality rate) is listed in Table No. 6. With IHC, it is evident that between different orders of birds tested, the distribution of WNV among the different organs and the staining pattern within tissues varied greatly. In birds belonging to Order Passeriformes, WNV antigen was detected in all the tissues which were tested i.e. kidney, liver, spleen, lung, intestine, heart, brain, testes and ovaries. In this Order of birds, spleen was the organ which came out positive most frequently (97.5%) in the infected birds, followed closely by liver (92%)
and kidney (90%) tissues. Experimental studies have shown that in Order Passeriformes, birds belonging to family Corvidae are most sensitive to WNV infection. American crows are reported to have exhibited 100% mortality rate with experimental infections (Weingartl et al., 2004). When distribution of WNV was studied in this family, again spleen came out to be the most frequently (98%) detected positive organ, followed by liver, kidney and intestine (95% each) tissues. Additionally, as shown in Table No. 6 and Fig No. 10, all other tested organs also showed presence of WNV antigen. In this family Corvidae, brain tissue from WNV infected birds tested least frequently (36.5%) for WNV antigen. Although Panella et al. (2001) has reported that brain tissue was the most reliable organ in American crows for isolation of WNV with Virus Isolation and RT-PCR techniques, present study suggests that spleen, closely followed by kidney, liver and intestine are the organs of choice with IHC technique. Gibbs et al. (2005) also reported that detectability of WNV varies by tissue and is dependent on the diagnostic test employed.

Similar to Order Passeriformes, Order Columbiformes was the other Order whose members showed distribution of WNV in all the organs which were tested in present study. In this Order, 80% of the WNV infected birds were having their kidney tissue
Results and Discussion

Positive for presence of WNV antigen followed by liver (60%) and spleen (60%). In Order Trochiliformes, spleen and kidney were the two tissues who tested positive in all the WNV positive birds followed by brain, heart, testes and lungs. In this order WNV antigen was not stained in intestine, heart and ovaries. So in order to do screening of birds belonging to this order, testing of spleen and kidney tissue only will be sufficient to rule out WNV infection.

Likewise all the WNV positive birds belonging to Order Strigiformes were having 100% of their liver, kidney, ovary and intestine tissues positive for WNV which was different from the previous published pattern of distribution of WNV virus in these birds (Lopes et al., 2007). This above referred study reported primarily splenic and hepatic distribution of WNV, while in present study WNV distribution was dominant in liver, kidney, intestine and ovarian tissues of birds belonging to Order Strigiformes. So in order to screen birds belonging to this order, testing of these tissues will effectively rule out the WNV infection.

In both Orders of Falconiformes and Psittaciformes, spleen, kidney and testes were the organs which tested positive for WNV
antigen. But in Order Psittaciformes these three organs were positive in all the infected birds while in Order Falconiformes spleen tested positive in every infected bird while kidney and testes showed presence of WNV in 50% of the WNV positive birds.

Furthermore, in Order Accipitriformes, WNV antigen was stained in brain, heart and spleen of all the WNV positive birds. Hence from above distribution of WNV in different order of birds, it is evident that affinity of WNV for different organs of birds varies from order to order. Similar varied distribution of WNV with experimental infection of various birds of different orders was also reported by Komar et al. (2003).

In brain tissue, detection of WNV antigen varied from none in WNV positive birds belonging to Orders: Falconiformes, Strigiformes and Psittaciformes to 100% of of the brain tissues collected from positive birds belonging to Order Accipitriformes. It is worth mentioning that 36.5% of the brain tissue of birds belonging to family Corvidae (most sensitive to WNV infection) tested positive for WNV antigen with IHC technique which was consistent with the previous findings of Weingartl et al. (2004) in American crows and of Gibbs et al. (2005) in another member of family Corvidae i.e. Blue jays.
Similarly in present study, spleen, kidney and liver were the organs which tested positive for WNV antigen in almost all the order of birds, except that spleen was negative for WNV in birds belonging to Order: Strigiformes, kidney was negative for WNV in birds belonging to Order: Accipitriformes and liver was negative for WNV in birds belonging to Orders: Falconiformes, Psittaciformes and Accipitriformes. Wunschmann et al. (2005) also reported negligible to very low detection of WNV antigen in spleen, lungs, and brain tissue of birds belonging to Order: Strigiformes.

Interestingly, staining of WNV was observed in testes and ovaries of positive birds belonging to most of orders. Gibbs et al. (2005) also observed WNV antigen in the ovaries and testes of Blue jays found on US east coast. These findings suggest need for further investigation in the transmission of this virus from infected avian parents to their unborn off-springs.

In birds belonging to Order: Columbiformes, WNV antigen was detected in all the samples tissues i.e. kidney (80%), brain (20%), spleen (60%), lung (20%), intestine (20%), liver (60%), heart (40%), testes (33%) and ovaries (50%). Values in
Results and Discussion

Parenthesis denotes percentage positive for that organ out of total WNV positive birds belonging to that Order. In the present study, in organs of birds belonging to order Falconiformes, WNV antigen was detected only in spleen, kidney and testes. Similar distribution of WNV was also observed by Steele et al. (2000) in birds belonging to Order: Falconiformes during the outbreak of WNV in New York in 1999.

From this kind of distribution of WNV in different organs of birds, it is evident that for the purpose of screening dead birds for WNV, no single organ is reliable. To increase the chances of detection of WNV during routine surveillance, it was found that screening of multiple organs of same bird is much better as compared to relying upon a single organ. Multiple organs of same bird were conveniently screened for WNV on a single slide, with same amount of labor, time and reagents. Additionally it was found that even among the different positive organs, the distribution of this virus was not following a uniform staining pattern.

Table No. 7 depicts the nature, pattern and frequency of cellular distribution of WNV staining in different organs of positive birds. Microscopically, staining pattern in the spleen was
Results and Discussion

diffuse type and the cells showing presence of WNV antigen were reticuloendothelial cells (Fig No. 11). Liver showed diffuse type staining pattern with Kupffer cells constituting the majority of the stained cells (Fig No. 12). Staining of the kidney 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 (Fig. No. 13).

The duodenum showed intense focal staining pattern and WNV antigen was stained throughout the payer patches (Fig No. 14). Lungs showed staining around airways and blood vessels (Fig. No. 15) and the stained cells were macrophages and endothelium of the vessels and airways. The heart showed faint stating pattern which was diffuse type and was scattered all over the myocardium. Stained cells were macrophages, myofibers and occasionally endothelial cells were also stained (Fig No. 16B). Pericardium (Fig No. 16A) was also showing marked sections of intense staining of macrophages.

In brain, WNV antigen was found in Purkinje cells, neurons and Glial cells (Fig. No. 17). In the ovaries, the parenchymal, interstitial and stromal cells stained for presence of WNV (Fig. No. 18). Surprisingly, no WNV antigen was stained in the
epithelium of the wall of oviduct and Zona pellucida. It will be interesting to study the transmission of this virus to offspring through infected male as well as female parents. In testes (Fig. No. 19), the sertoli cells stained positive for WNV and the staining pattern was focal type. As observed in the present study, Weingartl et al. (2004) also reported similar type of WNV infected cells and staining pattern in organs like Kidney, spleen, lungs, liver, heart, brain, ovaries and testes. Since some organs showed focal or multifocal staining pattern rather than diffuse type, it is recommended to have proper sample size of the organs too so that focal staining patterns are not missed.

In year 2007, because of unusually high number of human WNV illness cases, a state of medical emergency was declared in California. Next year in 2008, the first indicator of WNV activity in present study area was a WNV positive dead American crow which was tested using IHC technique. This WNV positive crow was detected two months before the reporting of first WNV positive human case. Hence due to the surveillance programme of WNV among dead birds, activity of this virus was detected before it took epidemic form. Since WNV is vectored by mosquitoes, and as compared to humans birds live outdoor, they are significantly at more risk of getting bitten by an infected
mosquito. This aspect of WNV surveillance, by screening of dead birds, gave jump start to public health agencies for mounting up the necessary mosquito control measures which saved many precious human lives and equine wealth. Such advantage of getting early warning regarding WNV activity from dead bird screening, has also been documented in New York (Eidson et al., 2001) and Connecticut (Mostashari et al., 2003).

Worldwide, the distribution of this virus has closely followed the pattern of distribution of mosquito species. Since local abundance of mosquitoes is widely influenced by the changes in climate and environment (Reiter 2001; Kovats et al., 2001), these global changes in the climate will result in invasion of newer areas/geographical latitudes by mosquitoes. In Europe, summer temperature was found as one of the most important environmental variables modulating WNV activity in Europe (Savage et al., 1999). Epstein (2001) reported that high temperatures speed up the replication of WNV in mosquitoes along with speeding up the maturity of mosquitoes, which means mosquitoes will attain the infectivity in shorter life span as compared to low temperatures. Experimentally, it has been proven that high temperatures increase mosquito abundance, as well as increase the vector competency (Dohm et al., 2002;
Turell et al. (2001). Pats et al. (2003) showed that amplification of WNV occur under climatic conditions of warm winters followed by hot dry summers.

As the mosquitoes will invade and establish in newer territories, so will happen with the distribution and emergence of infections vectored by them. A good example of effect of climate change on the distribution of vector-borne diseases is El Nino oscillation cycle. El Nino effect is a cyclic climatic event that is associated with heat waves and draught in southern Africa and Asia, while flooding on the coasts of South America and Central Africa. Sutherst (2004) concluded that outbreak of another mosquito-borne virus (Rift Valley fever) in Kenya in 1997-98 was as a result of flooding caused due to El Nino effect.

Invasion and subsequent adaptation of WNV on the ‘virgin soil’ of North American continent is a grim reminder of the ability of this RNA virus to adapt rapidly to new environments (Hayes 1999) and thus further stresses on the importance of local mosquito control measures and timely surveillance of emerging pathogens. Since WNV is vectored by number of mosquito species, so its ability to establish in new areas is marvelous, as has been proven after its introduction to United States and
Canada. Public health agencies should stress 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 was conducted among the residents to offer the vector control services at a meager service fee of 8 US dollars/household/year. Sadly, because of lack of awareness among residents regarding mosquito-borne diseases, these residents of most developed nation, voted against the introduction of this service fee citing that it was expensive. They fail to realize that as compared to the cost of human lives, the cost of 8 dollars per year is nothing, that too, when there is no treatment or vaccine for WNV related illness. Cost of hospitalization and medicare for WNV related illness in dead-end hosts i.e. human and horses itself is very high irrespective of the outcome of this disease.

Currently, the only way to fight this disease is by mosquito control which can only be achieved by active participation of the community. Proactive or reactive mosquito control is perhaps the only option for protecting the public health, with the possible
exception of personal protection through the use of mosquito repellents/nets. Proactive mosquito control is initiated when we get our first non-human case of WNV while reactive mosquito control is initiated when we get our first human case of WNV. Proactive mosquito control is considered more beneficial as public health agencies stays ahead of the incidence of human cases. Proactive mosquito control programme require a permanent well-trained staff and infrastructure to apply appropriate larvicidal products and then additional surveillance to evaluate the efficacy of this programme; rely on detailed and timely surveillance for WNV in the local geographical region which requires thorough knowledge of mosquito biology, landscape ecology, virus epidemiology and infrastructure to do various diagnostic/screening tests.

Reactive or emergency control depends upon passive case surveillance which, in turn, relies on the timely diagnosis and reporting of human cases to focus adulticide applications in time and space to interrupt transmission and prevent new infections. A major drawback to reactive mosquito control is that before this control is initiated, WNV has already amplified to epidemic levels; many human infections have already occurred but are not recognized because of delayed onset of symptoms (7-14 days
Results and Discussion

incubation period); slow laboratory confirmations; and delayed reporting to public health agencies (Reisen and Brault 2007). In the absence of an effective human vaccine against WNV, the cornerstones of WNV disease prevention will continue to be:

1) community-level mosquito control by adulticiding, larviciding and reduction of mosquito breeding sources;
2) Peridomestic measures eg repairing and installing door/window screens, reducing breeding sites in the backyard;
3) personal protection from mosquito bites by wearing appropriate clothing, using mosquito repellents/nets and avoiding outdoor exposures to mosquito bites

Another area which needs further research is to evaluate whether human population of North America (who has not co-evolved with WNV) will be able to develop adequate levels of background immunity with subsequent exposure to this virus over long period of time. Development and duration of acquired immunity depends upon virulence of pathogen, immuno-competence of host and the prevalence of antigenically related viruses in the given population. In serological studies conducted in Greece, in 1970s, WNV antibodies were detected in variety of mammals mainly humans, horses, cattle, goats and rabbits (Pavlatos and Smith, 1974). Further studies in 1980s identified
Results and Discussion

WNV antibodies in humans living in northern Greece (Antoniadis et al., 1990). In contrast to above studies, during 2005-07, a survey of 9590 blood donations and 115 CSF samples from aseptic meningitis in humans, in Greece, tested negative for WNV with Nucleic acid Amplification Test (Kantzanou et al., 2010).

Similarly in the endemic areas in Africa continent (Egypt), Hayes (1989) reported overall prevalence of background immunity to WNV as nearly 50% in children and 90% in adult humans; in European human population Tsai et al., (1998) observed very low (2-4%) immunity while in United States, post epidemic period of 1999-2000 it was virtually absent (0-1%) in New York (Komar et al., 1999) and Connecticut states. This variation in the background immunity developed in different human populations against WNV indicates that in addition to geographical locations, its the genomic composition of population involved which influences the level and duration of background immunity against WNV. In the absence of any definitive treatment and vaccine for WNV related illness in humans, this absence of development of natural background immunity in North American human population is a matter of grave concern, which further demands timely and efficient...
Results and Discussion

diagnostic/surveillance techniques. Due to this hard fact, it becomes very important for the public health agencies to do surveillance for this virus and mount mosquito control measures as and when any presence of this neurotropic virus is detected to avoid future loss of precious human lives and equine wealth.

Some of the salient features of the present study are that the surveillance and distribution of this virus in various organs of birds has been thoroughly done for the first time in County of Riverside. It has also been noticed that the strain affecting the birds and human in California seems to be more virulent than the strains responsible for outbreaks in Israel, Greece and New York. In present study the median age of human WNV patients with WNND presentation was 54 years as compared to median age of 72 years in WNV outbreak in Greece. Secondly, the case fatality rate in WNV related outbreaks was 29% in Israel, 35% in Greece while in present study it was 50% in humans aged >70 years. Hence it indicates that this strain of WNV in present study was more virulent as it made more younger people sick, proportion of WNND presentation was higher and in old people the survival rate was lower as compared to above mentioned other two outbreaks. Thus as this strain is spreading from other areas, it’s virulence is getting increased because of either or all of below
Results and Discussion

listed factors ie different genetic make-up of the populations involved, lack of cross-immunity from other related flaviviruses, absence of background immunity from this geographical region, adaptation to new species of birds for amplification, type of Lineage of WNV involved, etc.

Another salient feature of this study is that for the first time distribution of this virus has been evaluated in different organs of wildly infected birds belonging to 26 species of birds, 7 Orders. The only other study (Komar et al., 2003) which studied the transmission ecology of this virus in 25 different species of birds on US east coast was conducted with respect to identifying the competent amplifying reservoir host from level and duration of viraemia observed after experimental infection.

Globally, the distribution of WNV is directly related with the distribution of its vector i.e. mosquitoes. The distribution of mosquitoes is further influenced by the climatic and geographical characteristics present on similar latitudes and longitude across the globe (McMichael, 2003). So to combat this disease, the best weapon which we currently have is vector control. We need to stress on the efforts of public health agencies to educate the community regarding the various means of mosquito control.
Present study has proven that IHC technique has comparative sensitivity and specificity with RT-PCR method in doing screening of dead birds for WNV thus helping immensely to resources starved public health agencies of developing countries in understanding fundamental ecology of this neurotropic virus.

The present study, as evident from Fig No. 10, revealed that out of the 8 orders of birds which were screened for WNV, 2 Orders of birds (including birds of family Corvidae) showed presence of this virus in all the tested organs; 4 Orders showed presence of this virus primarily in 3-4 tested organs; while not a single bird belonging to Order Anseriformes was detected positive. During this study period, only two dead birds belonging to Order Anseriformes were reported by public and they were found to be dead from botulism. It needs to be explored further, through experimental studies, that why WNV positive dead birds belonging to this Order were not found during this study period. During the WNV outbreak in New York City, in 1999, Steele et al. (2000) reported that WNV positive dead birds belonging to this Order were found. This indicates that there might be different WNV strain involved in present study area. It is suggested that in order to do effective surveillance of WNV using dead birds, birds belonging to family Corvidae should be preferred for screening,
followed by birds belonging to other Orders. At the same time, to do effective screening of dead birds for WNV with IHC technique, multiple organs from the same bird should be tested, as has been done in the present study.

Another pressing aspect which came to light during this study is that still public need to be made aware about the importance of mosquito control. Even the residents of most advanced nation opted against the mosquito control in order to save meager vector control service fee. They need to be enlightened on the benefits of vector control verses cost of hospitalization that too for a disease for which no treatment and vaccine is currently available.

As evident from review of literature (Table No. 1), various epidemics have been summarized and this virus is still invading new territories. Therefore, there is an urgent need to establish a globally centralized database for surveillance of this disease and for closer interaction between veterinarians, physicians and scientists by sharing their data so that concrete and timely efforts could be taken to fight this disease.