Introduction

West Nile Virus (WNV) is a positive sense single stranded RNA virus of family *Flaviviridae*, Genus *Flavivirus* (Heinz et al., 1999) having approximately 11,000 nucleotides (Petersen and Roehrig, 2001) and is 45-50 nm in diameter (Mukhopadhyay et al., 2003). In nature, WNV is reported to be maintained in enzootic cycle involving certain birds and mosquitoes (Hayes, 1989). In nature, 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 don’t amplify this infection, thus acting as dead end hosts. Migratory birds have been suggested to play a major role in WNV dissemination (Rappole et al., 2000). Baqar et al. (1993) studied that outbreaks of WNV infection coincided with the increased population of mosquitoes belonging to *Culex* species during
summer in temperate and during rainy seasons in tropical regions. WNV is of immense importance for public and livestock health, because; it is not only infecting the livestock mainly horses, but also causes neurological diseases in humans. So far, there is no treatment or vaccine available for WNV illness in humans, which itself needs further exploration in this direction.

West Nile virus was first isolated from a febrile patient in 1937 in the West Nile district of Uganda (Smithburn et al., 1940) in African continent. Since then, there have been documented outbreaks in Egypt, Israel, South Africa, parts of Europe, Asia and North America. The first recorded outbreak of WNV in North America occurred in New York City in the summer of 1999, followed by a state of medical emergency in 2007 in California.

In humans, infection with WNV is clinically presented as a range from asymptomatic infection to neuroinvasive form (CDC, 2009). Usually, WNV infection in humans is benign, generally causes a febrile illness- with or without a rash; but occasionally, WNV infection can lead to hepatitis (George et al., 1987), acute pancreatitis (Perelman and Stern, 1974) encephalitis (Flatau et al., 1981), acute flaccid paralysis/ poliomyelitis (Sejvar et al.,
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2003), rhabdomyolysis (Gupta et al., 2008), corneal scarring accompanied with neurological malformations in congenitally infected infants (Alpert et al., 2003) and rarely as hemorrhagic fever (Paddock et al., 2006). Surviving patients, which developed non-fatal CNS symptoms, continue to have sequel (headache, muscle weakness, difficulty walking and memory loss) for months after recovery from their acute illness (Ravindra et al., 2004). Murray et al. (2010) reported that resolution of symptoms plateau approximately after 2 years of infection and 60% of patients continued to report clinical symptoms even after 5 years of recovery. In another follow-up study of surviving WNV meningoencephalitis cases in New York, it was found that 60% of the surviving patients were still having detectable IgG antibodies in their sera, even after 1.5 year later (Roehrig et al., 2003). Luckily, since humans do not produce sufficient viraemias for the non-infected fresh biting mosquitoes to pick up the infection, they act as dead end hosts.

In horses, WNV related illness causes neurological disease; mostly as encephalomyelitis accompanied with ataxia, weakness of limbs, muscle fasciculations, fever, paralyzed or drooping lip, twitching face/muzzle, teeth grinding and blindness (Ostlund et
al., 2001; Snook et al., 2001); viraemias of low grade and short duration are produced, thus limiting the possibility to serve as amplifying host for the enzootic cycle of WNV (Bunning et al., 2001, Bunning et al., 2002).

Ironically, a recent report by Leger et al. (2011) regarding an WNV infection in a Killer whale points towards a hard fact that this virus has now entered into the animals of marine ecosystem, which is a matter of grave concern for the humans as well as other valuable marine animals. In the light of above report, it becomes critical to conduct further research on the underlying biological and geographic factor(s) that allows these pathogens to adapt to new hosts and environments.

To date, little is known about the site of WNV replication in the vertebrate hosts. Experimental infection of hamster model of WNV encephalitis has indicated that WNV persists in the brain and kidneys of experimentally infected golden hamsters (*Mesocricetus aurattus*) for several weeks after infection. Infectious WNV was cultured from the urine and CNS of experimentally infected golden hamsters (*Mesocricetus aurattus*) for upto 52 days post infection (Tonry et al., 2005).
Worldwide distribution of WNV has been reported from Algeria, Italy, Greece, Hungary, North America, Russia, Romania, Kazakhstan, Armenia, Georgia, Borneo, Azerbaijan, Botswana, Central Africa, Cyprus, Republic of Congo, Egypt, Ethiopia, Uganda, Kenya, Sudan Mozambique, Nigeria, Israel, Turkey, Morocco, Tunisia, Czech, Pakistan, Senegal, South Africa, Madagascar, Tajikistan, Uzbekistan, Middle East, Iraq, Syria, Lebanon, Southwest Asia, China, Malaysia, Philippines, Sri Lanka, Thailand, and India (Danis et al., 2011; Rizzo et al., 2009; Petersen and Roehrig, 2001; Platonov et al., 2001; Hubalek et al., 1999; Hubalek and Halouzka et al., 1999; Hayes et al., 1982).

Historically, it is further reported that first evidence for prevalence of WNV in India was documented in 1952 with detection of WNV related antibodies in human population (Banker, 1952; Smithburn et al., 1954). Since then WNV had been reported in Rajasthan, Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Gujarat, Vellore, Chandigarh, Madhya Pradesh and Orissa states (Paramasivan et al., 2003; Banerjee, 1996; Rodrigues et al., 1980; Banerjee et al., 1979; Dandawate et al. 1969; Carey et al., 1968). With respect to
remaining Indian states, Researcher was not able to find any published epidemiological study related with this virus.

In India, this virus is known to be active in mosquitoes (Rodrigues et al., 1980; Pavri and Singh, 1965), birds (Jamgaonkar et al., 2003; Rodrigues et al., 1981) and pigs (Ilkal et al., 1997; Ilkal et al., 1994). Rodrigues et al. (1985), George et al. (1984) and Kedarnath et al. (1984) found it associated with human encephalitis cases; Paul et al. (1970a) found it associated with human case of febrile illness; and it had been isolated from Frugivorous bat, *Rousettus leschenaultia* (Paul et al., 1970b). During a post sero-epidemiological study, Risbud et al. (1991) detected WNV neutralizing antibodies among humans at South Arcot district of Tamil Nadu state; and Jamgaonkar et al. (2003) found serological evidence for JEV and WNV in terrestrial wild birds in Kolar District of Karnataka. Carey et al. (1968) had also reported WNV neutralizing antibodies among children of Vellore in South India. WNV had also been isolated from sporadic cases of encephalitis and mosquitoes in South India (Dandawate et al., 1969). Work (1971) postulated a hypothesis of a zoogeographical interface of Japanese Encephalitis (JEV) and West Nile virus (WNV). This hypothesis
proposed the intermingling distribution of JEV and WNV at the south Indian peninsular region which was later corroborated by serological evidence of simultaneous detection of JEV and WNV in terrestrial wild birds in Kolar District of Karnataka (Jamgaonkar et al., 2003).

In southern India, WNV neutralizing antibodies were detected by Jamgaonkar et al. (2003) in Ardeidae birds mainly pond herons (*Ardeola grayii*) and cattle egrets (*Bubulcus ibis*). This indicated the possible involvement of Ardeidae bird in the natural cycle of WNV in India (Rodrigues et al., 1981). Horses were found to be incompetent as amplifying host of WNV in nature (Bunning et al., 2002). In southern India, domestic pigs were also shown to develop antibodies to WNV, but experimental studies showed that they are poor hosts (Teehee et al., 2005; Ilkal et al., 1994).

The identification of WNV-positive birds has been shown to be the earliest indicator of WNV in an area (Ceccaldi et al., 2004). American crows (*Corvus brachyrhynchos*) are the most sensitive sentinel species used to detect the presence of WNV in northern regions (Nir et al., 1965). Corvids infected with WNV
are usually found dead without any previously reported clinical signs, or die within 48 hours of the onset of clinical signs. Because of this acute onset and rapidly progressive nature of the disease, significant gross and histological lesions are rarely observed at necropsy (Hayes, 1989). Therefore, appropriate tissue collection and diagnostic testing are imperative for accurate diagnosis and usefulness in an effective WNV surveillance program.

Riverside County of California closely resembles the climatic & geographical characteristics, nature of bird & mosquito population of tropical countries like India, including the state of Punjab. Because of Ranch style of living, it has huge horse population with variety of outdoor activities as part of lifestyle. The horses, like humans, are incidental hosts and likewise there is no therapeutic treatment available to treat WNV related illness and eventually either horses die or have to be humanly euthanized. In the light of these facts, it is of immense necessity to do effective and timely surveillance of this virus to save the precious human lives and prevent the loss of valuable equine population. Its surveillance can be done in various sorts of fauna such as horses, birds, mosquitoes, etc. and a check list of the
infected birds needs to be made depending upon their susceptibility to WNV infection.

To date, few studies have been performed to determine appropriate tissue selection, test sensitivity, test specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for WNV surveillance. Immunohistochemistry (IHC) technique has been documented as a reliable and efficient method of identifying WNV in formalin-fixed avian tissues (Ellis et al., 2005). Turell et al. (2005) reported that IHC, using a polyclonal antibody, was comparable to Virus Isolation for the detection of WNV in birds. The objective of this study was to do screening of local dead birds for WNV infection with IHC and testing it’s usefulness for the sake of doing surveillance in the local geographical location of Riverside County, California. This is very important from public health point of view as we have to stay ahead of spread of this disease by mounting effective mosquito control programmes in the areas of detection before it spreads to epidemic level; and this surveillance method is also equally significant for other states of America as well as in other countries.
Another important issue taken up in this study was to compare the efficacy of IHC with RT-PCR for screening of dead birds for WNV surveillance. IHC method was chosen to do the screening of dead birds as this method is cheap, safe in terms of biohazard and equally efficacious as compared to doing screening with RT-PCR or Virus Isolation methods. As compared to setting up a RT-PCR lab, it is much more convenient to setup a IHC lab for doing WNV surveillance. It doesn’t require costly and highly specialized equipment (a very important consideration for those countries/states with limited resources); is not hazardous for laboratory workers as they work with fixed tissue samples and give them the opportunity to examine the architectural characteristics of tissues in relation to WNV infection. Additionally, formalin-fixed or paraffin embedded tissue samples can, not only be archived, re-sectioned and re-stained for confirmation, but can also be used to screen for new pathogens emerging in the future, thus depicting the previously unknown presence of an emerging disease. Therefore, appropriate tissue collection and diagnostic testing becomes important for accurate diagnosis and effective WNV surveillance program.
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. Keeping in view the above noted aspects of human and livestock health, it has been thought appropriate to conduct the present study with the following as objectives of the study:

**OBJECTIVES OF THIS STUDY**

1. To study epidemiology of WNV in birds of Riverside County of California.
2. To identify species and sex of birds found in Riverside County which test positive for WNV.
3. To study distribution of WNV in intracellular and extracellular areas of various organs of birds.
4. To establish the course of incidence of infection in reported human cases during the duration of this study, if any.
5. To forecast the presence of WNV in the county for the knowledge of public health agencies.

It is hoped that the present study shall initiate a further interest in scientific community to undertake more such studies, not only in American states but also in the tropical region of the
world which includes many sensitive countries suitable for the amplification and spread of this incurable virus.