Review of literature

Sources for the review of literature were identified by searching indexed research databases mainly MEDLINE, PubMed Central; online search engines eg. Google scholar®; and back references from relevant articles and book chapters. To do online search for related literature, keywords like ‘West Nile virus’, ‘arthropod-borne flavivirus’, ‘arbovirus’, and ‘flavivirus’ were used, with reference to India and USA. Unpublished data, regarding the reporting of WNV infection in humans, from the National Arbovirus Surveillance system (ArboNET) of the Centers for Disease Control (CDC) and California Department of Public Health (CDPH) was also consulted.

West Nile virus is the most widespread neurotropic arbovirus (group B arbovirus; family Flaviviridae; genus
Flavivirus) in the world which is emerging, re-emerging, expanding its range globally, and is currently present in all continents, except Antarctica. Typical, WNV virion is between 45-50nm in diameter and contains a nucleocapside core surrounded by an envelope. Embedded in the viral envelope are the viral envelope (E) proteins and memberane (M) proteins which are responsible for many important properties of the virus such as host range, virulence, pathogencity of disease, tissue tropism and induction of immune responses (Chu et al.., 2007; Petersen and Roehrig, 2001). On the basis of cross-neutralization (Calisher et al.., 1989) and molecular genetic studies (Kuno et al.., 1998), it has been classified within the antigenic group of Japanese Encephalitis (JE). It is a single-stranded, positive sense RNA virus of approximately 11,000 nucleotides and is 45-50 nm in diameter (Mukhopadhyay et al.., 2003).

Historically speaking, the oldest evidence of WNV has been speculated in year 323 BC, when Alexander, the Great died in ancient Mesopotamian city of Babylon, on 10 June 323 BC (Marr and Calisher 2003). Direct evidence for their opinion comes from the recorded observation of historians, of his time, who recorded the entrance of King Alexander through the gates of city of
Babylon as follows “...when he arrived before the walls of the city he saw a large number of ravens flying about and pecking one another, and some of them fell dead in front of him.” (Plutarchus, 1995).

If this observation is included as part of the description of Alexander’s illness, West Nile virus encephalitis complicated by flaccid paralysis becomes most probable diagnosis. It is possible that, in the 3rd century BC, disease caused by West Nile virus arrived in Mesopotamia for the first time in recorded history, killing indigenous birds and an occasional human and causing only incidental febrile illnesses in many others. Similar pattern of mass-die offs of birds; preceding neuroinvasive illness in humans was observed when the old world strain of WNV got introduced in United States for the first time in 1999.

In the modern history, in 1937, WNV was isolated in the blood of a febrile patient in West Nile province of northern Uganda (Smithburn et al., 1940). The virus became recognized as a cause of sever human meningitis or encephalitis in elderly patients during an outbreak in Israel in 1957. WNV related illness in equines was first noted in Egypt and France in early 1960s. Since then, as is evident from the Table No.1, it has been
reported from different countries of the globe. This disease has become more evident and had caused major public health epidemics in Algeria (1994), Romania (1996), New York (1999), Hungary (2008), Italy (2009) and recently in Greece (2010): places that suffered epidemics of WN virus encephalitis. Epizootics of disease in horses occurred in Morocco in 1996, Italy in 1998, United States during 1999-2001 and France in 2000; and in birds in Israel during 1997-2001 and in the United States in 1999-2002. The emergence of WNV in America (Ebel et al., 2001; Asnis et al., 2000) and its impact on the health of humans, horses and birds, have caused global concern about the public health importance of this neurotropic virus.

From review of literature, it is evident that there is difference in the pathogenicity of illness caused by this virus. In humans, high fatality rate of 17% from Greece (Danis et al., 2011) and ~7% from Romania (Tsai et al., 1998) was reported; older age group of humans was involved in Romania (Tsai et al., 1998) and Greece (Danis et al., 2011; Papa et al., 2010), while mainly children were affected in Algeria (LeGuenno et al., 1996). West Nile and Japanese encephalitis viral genomic sequences had been detected in cerebrospinal fluid from acute
WN related encephalitis human cases in Karachi, Pakistan (Igarashi et al., 1994), USA (Lanciotti et al., 1999), Israel (Weinberger et al., 2001) and India (Kedarnath et al., 1984; George et al., 1984; Tsai et al., 1998). Serological evidence (WN specific IgM antibodies) indicating recent WNV infection was found by Thakare et al. (2002) in samples of human sera and CSF collected from different states of India such as Maharashtra, Rajasthan, Goa and Orissa. Of these clinical cases, 92% were presented as West Nile fever, while the rest 8% were presented as case of encephalitis. Above evidence pointed towards the variation in the pathogenicity of neuroinvasive and neurovirulent illness caused by this virus in different parts of world. In California and New York, high avian mortality had also been associated with human epidemics of WNV, which surprisingly was not observed in the Old world (Komar and Clark, 2006). This is because the birds of North America were naïve to exposure of WNV, were never exposed to this virus, thus having no prior immunity to this infection. For the sake of doing surveillance for WNV public health agencies effectively exploited this phenomenon of mortality of birds by screening dead birds for WNV (Eidson et al., 2001).
The variation in the pathogenicity of WNV related illness is attributed to different lineages of this virus. From the published studies, so far, WNV has been genetically divided into two lineages (Scherret et al., 2001; Berthet et al., 1997): Lineage 1 is found circulating in Middle east, Asia (including India), Africa (north, west, central parts), Europe (southern and eastern region), North America (Jia et al., 1999); while Lineage 2 is reported circulating in enzootic foci in west, central and eastern Africa and Madagascar. Recently, two WNV isolates; one isolated in 1997 from Culex pipiens mosquitoes in South Moravia region of Czech Republic in Europe (Bakonyi et al., 2005) and other isolated in 1998 from Dermacentor marginatus ticks in the north-west Caucasus Mountain valley (Prilipov et al., 2002) have been found genetically different from both WNV Lineages 1 and 2; hence these two isolates have been proposed to be classified as Lineage 3 and 4 respectively.

Bondre et al. (2007) studied the genetic status of 15 Indian isolates of WNV (Humans: 3, Fruit bat:1 and Mosquitoes:11; isolated over a period of 27 years between 1955-1982) and concluded that 13 out of these 15 Indian isolates can be grouped together to form a distinct genetic cluster. Based
upon this phylogenetic analysis, it was concluded that Indian WN virus isolates form a distinct genetic lineage, which this study proposed to classify as WNV Lineage 5. Two isolates (one from human and the other isolated from fruit bat) studied in this study were found to be phylogenetically belonging to WNV Lineage 1, thus giving evidence that WNV Lineage 1 is circulating in India. Kunjin virus, which is circulating in Australia, is a subtype of WNV Lineage 1.

WNV Lineage 1 had been held responsible to cause WNV related neuroinvasive disease (WNND) in human and horses. WNV Lineage 2 was postulated not to cause any case of human neuroinvasive illness but recent reports has documented fatal neurological disease, attributed to this WNV Lineage 2, in humans in South Africa (Venter and Swanepoel, 2010) and Greece (Papa et al., 2011a; Papa et al., 2011b); and horses in South Africa (Venter et al., 2009). Above reports are direct evidence that this Lineage 2 of WNV has evolved further to attain the ability to produce neuroinvasive disease in humans as well as horses. Secondly, this lineage has also extended its geographical distributions outside its historical hotspots in South Africa to all the way to Europe ie. in Greece (Papa et al. 2011a)
Like all members of JE virus complex, WNV is maintained in nature by an arthropod-borne transmission cycle. It is transmitted in an enzootic cycle (Fig 1) between ornithophilic mosquitoes, predominantly *Culex* species (vector), and amplifying host-birds (Komar, 2000; Ahmed et al., 1979; Baqar et al., 1993). Transmission of WNV between arthropods and birds is called rural or sylvatic transmission while between arthropods and other vertebrates is called urban transmission (Van der Muelen et al., 2005). The competence of a mosquito vector to transmit WNV varies widely between different species of mosquitoes. Worldwide, *Culex* spp. are found to be the primary vector for WNV; in Africa and Middle East the primary vector for WNV is *Culex* (*Cx.*) *univitattus* (Nir et al., 1968; McIntosh et al., 1976); while in North America it is the *Cx. pipiens* complex (Turell et al., 2005); and in Asia it is *Cx. vishnui* complex (Ahmed et al., 1979). Under field condition, WN virus has been isolated from other genera of mosquitoes too e.g. *Ochlerotatus* (CDC, 2000), *Aedes* (Holick et al., 2002) and *Culiseta* (Nasci et al., 2001).

Evidence of vertical transmission, in mosquitoes, for
perpetuation of WNV in nature had come from isolation of this virus from field collected larvae and male mosquitoes of *Cx. univittatus* mosquitoes in Kenya (Miller et al., 2000); from larvae of *Cx. erythrothorax* in Utah, USA (Phillips and Christensen, 2006); and from diapausing adults of *Cx. pipiens* in United states (Anderson and Main, 2006; Nasci et al., 2001). Experimentally, vertical transmission of WNV has been demonstrated by *Cx. tritaeniorhynchus*, *Aedes (Ae.) Albopictus*, *Ae. aegypti* (Baqar et al., 1993); *Cx. pipiens* (Turell et al., 2005); and *Cx. tarsalis* (Goddard et al., 2003). Evidence of WNV in the over-wintering mosquitoes had been reported from New York (Nasci et al., 2001) and southern California (Reisen et al., 2006). In the light of above reports, it is inevitable to do screening of local mosquito species for presence of WNV. After detection of WNV in the local adult mosquito population, its amplification cycle can be quickly and effectively disrupted by doing adulticiding and larviciding with appropriate pesticides.

In addition to mosquitoes, experimental transmission of WNV has been demonstrated with ticks: *Argas arboreus* (Mumcuoglu et al., 2005; Abbassy et al., 1993); *Carios capensis* (Hutcheson et al., 2005); *Hyalomma marginatum*
(Formosinho et al., 2006); but neither ixodid ticks (Lawrie et al., 2004; Anderson et al., 2003; Reisen et al., 2007) nor fleas: *Orchopeas howardi* (Root et al., 2007) were able to transmit WNV in experimental studies. Furthermore, WNV had also been isolated from louse flies (*Icosta americana*; Diptera: Hippoboscidae) collected from raptors in United States (Farajollahi et al., 2005) and Canada (Gancz et al., 2004).

Further review of literature has also shown sufficient evidence to support the non-vector transmission of WNV; Bird to bird transmission through feco-oral route and by eating a infected mosquito (Komar et al., 2003), contact transmission between birds (Komar et al., 2003), mice (Odelola et al., 1977) and hamster (Sbrana et al., 2005); oral transmission in alligators after eating infected horse meat (Klenk et al., 2004). Documented evidence showed that humans had got WNV infection via blood transfusion (Montgomery et al., 2006); liver transplant (Rhee et al., 2011; Iwamato et al., 2003); intrauterine/ transplacental routes (CDC, 2002; Alpert et al., 2003); through breast feeding (Hinckley et al., 2007; CDC, 2002); occupational exposure of a veterinary student in South Africa while conducting necropsy on a WNV infected horse.
Murray et al. (2010) reported that WNV was secreted in the urine of human patients for up to 6.7 years after recovery from WNV illness; infectious WNV could be cultured for up to 52 days, each from the urine (Tonry et al., 2005) and the CNS (Xiao et al., 2001) of experimentally infected golden hamsters (Mesocricetus auratus); and up to 5.5 months from the CNS of experimentally infected macaques (Pogodina et al., 1983). Currently, screening of human blood donors is done with nucleic acid amplification tests to detect the WNV positive asymptomatic donors (Busch et al., 2005).

Intensity of WNV transmission in a geographical region also depends upon some extrinsic factors like Temperature and Rainfall (Epstein, 2001), density of host populations (Kramer and Ebel, 2003), host heterogeneity (Kilpatrick et al., 2006); pattern of land use (Marsh and Gross, 2001); and spatial distribution of economic conditions of community (Harrigan et al., 2010). So far, there is no published evidence of man to man/animal or animal to animal/man transmission of WNV.
Rappole et al. (2000) has suggested the role of migratory birds in long distance movement of WNV but this looks questionable, as it is doubtful that an infected bird can fly thousands of kilometers of distance without reproducing the disease. Long distance migration is a very stressful event, thus it is unlikely that the latent WNV infection, if any, carried by a migrating bird will not flare up under these conditions of extreme stress. Experimental infection of tagged birds, before the start of migration season, and subsequent capture of those tagged birds at the end of migration route can shed light on the role of migrating birds in transmission of WNV; but because of ethical and other environmental/legal issues, as infected birds can not be freely released in nature, this hypothesis is yet to be investigated. Reisen et al. (2010) has also postulated that migratory birds entering California rarely exhibited a history of WNV infection and that most of migratory birds probably became infected after entering California.

Since it’s introduction in North American continent in the year 1999, approximately 59 species of mosquitoes and 284 species of birds have been found infected with WNV (Hayes et al., 2005). In spite of detection of this virus from so many
species of birds, the identity of the specific avian reservoir for WNV remains elusive. Hayes *et al.* (2005) further reported that passerine birds, charadriiform birds, and at least two species of raptors (American Kestrel and Great Horned Owl) were found to be more competent hosts than other species evaluated from the following orders: Anseriformes, Columbiformes, Galliformes, Gruiformes, Piciformes and Psittaciformes.

(Del et al., 2006). This recent evidence of WNV infection in the marine world is a matter of grave concern as it reflects the ability of this virus to adapt to various versatile ecosystems for its survival and spread. Role of marine animals in the maintenance, amplification, shedding and transportation of this virus within the aquatic world need to be further explored for deeper understanding of the ecology of this virus. In spite of it’s isolation from such a wide spectrum of vertebrate hosts, the exact site of WNV replication in the vertebrates is still unknown.

Review of published literature has further revealed serological evidence of WNV infection in black bear, Ursus americanus (Farajollahi et al., 2003); brown bear, Ursus arctos (Madic et al., 1993); Camel, Goat (Olaleye et al., 1990); Assmule, Cattle, Sheep, Dog (Ozkul et al., 2006); Ring-tailed lemur, Lemur catta (Sondgeroth et al., 2007); Cat (Kile et al., 2005); Baboon, Papio hamadryas, Pig-tailed macaque, Macaca nemestrina, Rhesus macaques, Macaca mulatta (Ratterree et al., 2003); Cattle (Ulloa et al., 2003); Pig (Ratho et al., 1999); Rabbits, Oryctolagus cuniculus (Le Lay-Rogues et al., 1990); Turtles (Nir et al., 1968); coyotes, Canis latrans (Miller et al., 2009); Big brown bats, Eptesicus fuscus (Bunde et al., 2006);
North American bats (Davis et al., 2005); and Bottlenose dolphins, *Tursiops truncates* (Schaefer et al., 2009). Experimental studies has shown that sufficient level of viraemia, for the feeding mosquitoes to pick up the WNV infection, is not produced in horses (Bunning et al., 2002), chicken (Komar et al., 2003), dogs and cats (Austgen et al., 2004) and cattle (Ilkal et al., 1988), hence luckily they do not play any role in amplification of WNV.

Level of physical activity by humans and horses in outdoor environments directly influences the potential of exposure to this virus. Review of literature has showed that people who are more involved in outdoor activities, that too during the dawn and dusk hours of the day, have higher chances of getting exposed to mosquito bites thus putting them at higher risk of getting WNV infection from a infected mosquito bite.

To conduct surveillance for WNV in a particular geographical area, testing of birds (dead as well as live ones), mosquitoes, vertebrates e.g. squirrels and horses give a reliable indication regarding the local presence and activity of this virus. Testing of birds can further be classified as captive (sentinel
flocks), free ranging (wild birds) and screening of dead birds. For arbovirus surveillance programmes, captive and free-ranging birds have been used for decades as living sentinels. The limitation of using free-range wild birds is that, as they are flying around freely, they do not point toward local foci of WNV amplification, while captive birds, being stationary, point towards the local foci of amplification of this virus. Typically, captive chickens or pigeons are used worldwide, for WNV surveillance as experimental infections has shown that both these species produce antibodies after getting exposed to this virus without becoming highly infectious for *Cx. pipiens* vectors (Longevin *et al.*, 2001; Komar, 2001; Gruwell *et al.*, 2000).

Similarly, although infected dead birds could have picked up WNV infection from anywhere but they certainly indicate toward the introduction of infection in the local geographical location, which can be further corroborated with testing mosquito pools collected from that neighborhood. Ward *et al.* (2006) reported that radio-marked crows infected with WNV had traveled up to 4 km per night during the experimental 5 days post infection period before they died.
In 1999, during the outbreak of fatal neurologic WNV disease in New York City, dead American crows were the first causalities to the introduction of this virus in the city. Additionally to humans, this fatal neurologic disease was also reported in horses and exotic as well as native birds of North America (Komar et al., 2001; Steele et al., 2000). After the above outbreak of WNV in New York, Eidson et al. (2001) evaluated the usefulness of dead bird surveillance programme for detecting the geographic spread of WNV and concluded that dead bird surveillance was helpful on the east coast of New York state and with proper participation of public and local public health agencies it can serve as satisfactory early warning system for detection of WNV. Such findings point toward the relevance of using avian as well as local equine population to do forecast for the presence of WNV in a local geographical area. Since, in USA, birds showed high mortality with this virus, screening of dead birds played important role for doing WNV surveillance. Thus, in addition to doing WNV surveillance in local mosquito population and in sentinel chicken flocks, screening of dead birds can be conveniently incorporated in the present WNV surveillance setup.

To do surveillance of WNV in a geographical area,
screening of mosquitoes is done, primarily with RT-PCR method. Different physiological stages of female mosquitoes ie host-seeking, gravid and blood-fed stages are collected using specific mosquito traps. Among these three physiological stages, gravid mosquitoes are most useful for conducting surveillance of WNV as after getting an infected blood-meal, they get time to amplify it in their body.

Unfortunately, as there is no specific treatment currently available to treat WNV infection in humans, it’s surveillance in a timely and effective manner becomes even more important. Review of literature has showed that ongoing clinical trials with some antiviral agents and therapies to treat WNV related illness had shown promising results. Intravenous administration of immunoglobulin in humans, which were harvested from a plasma donor having high level of WNV related antibodies had been successfully used to treat WNV related encephalitis (Shimoni et al., 2001) and acute flaccid paralysis (Sejvar et al., 2003; Walid and Mahmoud, 2009). Another clinical trial tested the efficacy and safety of interferon therapy to treat WNV related meningoencephalitis (Samuel and Diamond, 2005). So far the only line of treatment available to treat WNV related illness is
supportive treatment i.e. respiratory support, management of cerebral edema, prevention of secondary bacterial infection and appropriate physiotherapy during post-recovery period.

Sadly, like the non-availability of any specific treatment for WNV, there is no vaccine currently available in the market for human use; but the good news is that there are 4 licensed WNV vaccines available for use in horses (Fort Dodge Animal Health, USA: Killed virus; Merial, USA: Recombinant canarypox virus; Intervet, USA: Chimeric virus-WNV and Yellow fever virus; Kimron Veterinary Institute: Killed virus) and one for use in domestic geese (Fort Dodge Animal Health, USA: Recombinant DNA plasmid) (Kramer at al, 2008; Kramer et al., 2007; Samina et al., 2005). Some experimental studies, with limited success, had been conducted to develop WNV vaccine for humans (Lieberman et al., 2007; Mason et al., 2006; Morrey et al., 2006; Seregin et al., 2006; Ledizet et al., 2005; Oliphant et al., 2005; Yamshchikov et al., 2004; Hall et al., 2003; Davis et al., 2001; Goverdhan et al., 1992); and dogs & cats (Karaca et al., 2005).

Review of literature suggests us that WNV has the ability to adapt to newer environments, ecosystems and newer species of
bird hosts. After its detection in New York, in the summer of 1999, WNV activity was reported from Canada for the first time in August 2001, where it was found in dead birds and mosquito pools collected from southern part of Ontario province. Then in 2002, Canada reported its first confirmed human cases in parts of Quebec and Ontario provinces. WNV was also found in birds, horses or mosquitoes in Nova Scotia, Quebec, Ontario, Manitoba and Saskatchewan provinces (Public Health Agency of Canada, 2005). This northward and westward spread of this virus not only points towards its ability to successfully over winter but also to adapt to new host species of birds. From public health point of view, this spread of WNV from east coast to west coast of North American continent is a matter of grave concern. Subsequently, in 2003 WNV invaded California, successfully amplified to epidemic levels in 2007, causing high mortality in avian hosts and eventually spreading to its every County.

The objective of this study was to study the epidemiology of WNV in the birds of Riverside County of California and to screen the dead birds for distribution of this virus in their different organs, and to detect the amplification of this virus in the study area for the knowledge of public health agencies.
Efficient WNV surveillance is equally significant for the other states of America as well as in other countries, as public health agencies 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.

Steele et al. (2000) reported that the level of pathogenicity of this virus on different avian species of east coast of USA varied from species to species. Similarly, this study also reported that the pattern of detection of WNV in the different organs of native birds found on the east coast of United States was not uniform for all species of birds. Hence the choice of tissue from dead birds, for screening of WNV, will varies from species to species. Different molecular diagnostic methods e.g. Virus Isolation (Ellis et al. 2005), electron microscopy (EM), RT-PCR (Lanciotti et al. 2000), Immunohistochemistry staining (Steele et al. 2000) are available for screening of dead birds for WNV. Most of these diagnostic techniques have certain drawbacks. Although Virus Isolation is the most reliable direct method for detection of WNV, but as laboratory workers are working with live virus, it is very hazardous and biohazard safety level 3 is required for this method.
Electron Microscopy is very cost prohibitive and most of Public health agencies of the developing nations can’t afford it. Similarly setting up a RT-PCR lab is also expensive. Ellis et al. (2005) reported that IHC staining method was approximately equal with Virus isolation in their ability to detect West Nile virus. Sandhu et al. (2010) reported that based upon testing of only kidney tissue of American crows, 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%. Similarly, Ellis et al. (2005) also reported that for screening of same dead bird, with virus isolation method, brain tissue was the tissue of choice for optimal WNV diagnostic ability, while with IHC, heart tissue was the tissue of choice. In this study, IHC method was chosen to do the screening of dead birds; as this method is cheap; has faster turn around time; safe in terms of biohazard (since live virus is not present in formalin-fixed tissues); equally efficacious as compared to doing screening with RT-PCR or Virus Isolation methods; and demonstrate the site of WNV replication within the tissue architecture; allows visualization of the histopathological changes associated with the virus replication. Therefore, appropriate tissue collection and
Review of literature

diagnostic technique are imperative for accurate diagnosis and effective WNV surveillance program.