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Powassan virus (POWV) Infection in Animals and Humans: A Review

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ABSTRACT

Powassan encephalitis is a rare but severe disease caused by infection with Powassan virus (POWV). It is a tick-borne *Flavivirus* (family Flaviviridae) having single stranded Ribonucleic Acid (RNA) which is positive sense in nature. The virus has highest case-fatality rates and is associated with a very high incidence of severe neurologic sequelae. Humans contract POWV infection accidentally when they are exposed to areas where the virus, arthropod vector (an Ixodid tick) and the vertebrate natural hosts coexist. Reported incubation periods for Powassan virus range from 8 to 34 days. The disease is associated with a reactive inflammatory cellular infiltrate (chronic) of lymphocytes and macrophages that include the abundance of perivascular inflammatory cells and multiple foci of parenchymal cells in grey matter. Basically two diagnostic approaches are considered. First one is the direct detection of the virus or viral RNA in the initial (viremic) phase of infection by virus isolation in mammalian cell culture or by reverse transcriptase polymerase chain reaction (RT-PCR). Second is the indirect detection of specific immunoglobulins (IgM and IgG antibodies) with serological methods such as Enzyme Linked Immunosorbent Assay (ELISA); Immunofluorescence Assay (IFA) or Neutralization Tests (NTs). Phylogenetic analysis is important for genogrouping of the virus. Oligomers targeting specific locations in the RNA genome of the flavivirus have been used at present for successful suppression of viral gene expression. Strict hygienic and biosafety measures including tick control is pre-requisite for prevention of disease. The present review will give an insight to the details of disease caused by this arbovirus that may often prove fatal, its epidemiology, diagnosis, prevention and control measures to be adopted.

Key words: Powassan virus, tickborne encephalitis, epidemiology, diagnosis, prevention, control

INTRODUCTION

Powassan encephalitis is a rare but severe disease caused by infection with Powassan virus (POWV). It is a member of Tick-borne Encephalitides (TBE) serogroup *Flavivirus* which is

maintained in a transmission cycle between *Ixodes cookei* and other Ixodid ticks and small and medium-sized mammals. In humans, POWV encephalitis is associated with a high case fatality rate and severe neurologic consequences in many survivors (Artsob, 1989; Hinten *et al*., 2008). POWV was first isolated in 1958 from the brain of a five-year-old boy from the town of Powassan, Ontario, who died of encephalitis (McLean and Donohue, 1959). POWV produces a non-suppurative, focal necrotizing meningoencephalitis in horses (Keane and Little, 1987; Whitley and Gnann, 2002).

ETIOLOGY

Powassan Virus (POWV) is a tick-borne *Flavivirus* (family Flaviviridae) having single stranded RNA which is positive sense in nature (Westaway *et al*., 1985). Its genome is 10,839 nucleotides long. The complete nucleotide sequence of the Powassan virus placed it as the most divergent member of the tick-borne serocomplex within the genus *Flaviviruses*, family Flaviviridae. On comparative analyses it significantly differed from the mosquito-borne flaviviruses (Mandl *et al*., 1993). Diverse nucleotide sequence analysis from 16 strains of POWV and deer tick virus (DTV) in a phylogenetic study, conducted to determine whether these viruses constitute distinct flaviviral populations transmitted by Ixodid ticks in North America, revealed two distinct genetic lineages. They could be defined by geographical and host associations (Ebel *et al*., 2001). The nucleotide and amino acid sequences of lineage one (comprising New York and Canadian POWV isolates) were highly conserved across time and space. But those of lineage two (comprising isolates from deer ticks and a fox) were more variable. The divergence between lineages was much greater than the variation within either lineage, or lineage two appeared to be more genetically diverse than lineage one. Application of McDonald-Kreitman tests to the sequences of these strains indicated that adaptive evolution of the envelope protein separated lineage one from lineage two. In humans, both lineages can cause Central Nervous System (CNS) infection. Using combination of molecular definition of species of virus within the genus *Flavivirus* and serological distinction in a 2-way cross-neutralization test, the lineage of DTV has been classified as a distinct genotype of POW virus (Beasley *et al*., 2001; Ebel *et al*., 2001; Kuno *et al*., 2001; Jenkins *et al*., 2002).

EPIDEMIOLOGY

Powassan virus infection is one of the least common causes of arbovirus encephalitis reported in cases from the United States and Canada ranking behind LaCrosse; St. Louis and eastern and western equine encephalitis (Calisher, 1994; Hinten *et al*., 2008; Romero and Simonsen, 2008). However, Powassan virus and eastern equine encephalitis have the highest case-fatality rates and are associated with a very high incidence of severe neurologic sequelae (Calisher, 1994; Gholam *et al*., 1999; Hinten *et al*., 2008). Cases of Powassan encephalitis have been reported from Ontario, Quebec and New Brunswick in Canada; from Maine, New York, Michigan, Vermont, Wisconsin, Pennsylvania and Massachusetts in the United States; Russia; and Marburg-Biedenkopf in the Germany (Leonova *et al*., 1991; Calisher, 1994; Gholam *et al*., 1999; Muller *et al*., 2006; Hinten *et al*., 2008; Romero and Simonsen, 2008). The Michigan and Wisconsin cases are the first ever reported from the north-central United States recently (Hinten *et al*., 2008). Tick-borne encephalitides (TBE) including Powassan encephalitis is among the most important flaviviral infections of the CNS in Europe and Russia, with 10,000 people in Russia and approximately 3000 in other European countries being diagnosed annually. The lethality of infections in Europe is 0.5% and a post-encephalitic syndrome is seen in over 40% of patients affected often resulting in an intense impairment in quality of life (Gritsun *et al*., 2003;

Gunther and Haglund, 2005). By reverse transcriptase-polymerase chain reaction (RT-PCR) assay and virus isolation study (Brackney *et al.*, 2008), adult *Ixodes scapularis* and *Dermacentor variabilis* collected from Hayward and Spooner area of Wisconsin were found to be infected with the POWV. All the isolated viruses belonged to the Deer Tick Virus (DTV) genotype of POWV (Beasley *et al.*, 2001; Kuno *et al.*, 2001; Tokarz *et al.*, 2010). It suggests stable transmission of POWV in this focus. Surveillance serologic studies have been positive in up to 3% of the population in certain northern Ontario communities that is suggestive of infection (without encephalitis) can occur in humans (McLean *et al.*, 1962).

Humans contract the POWV infection accidentally when they are exposed to areas where the virus, the arthropod vector (an ixodid tick) and the vertebrate natural hosts co-exist (Nuttall *et al.*, 1994; Falco *et al.*, 1995; Brackney *et al.*, 2010). Woodchucks and snowshoe hares are the most commonly implicated natural hosts (Calisher, 1994). However, other animals including chipmunks, squirrel, coyotes, foxes, raccoons, skunks and dairy cattle have also shown serological evidence of infection (McLean *et al.*, 1960; Artsob *et al.*, 1986; Johnson, 1987; Hinten *et al.*, 2008). In addition, exposure to domestic cats and dogs whcih can act as portent of infected ticks, may widen the scope of POWV transmission. In a study on POWV infected deer ticks (*Ixodes scapularis*) (Alekseev *et al.*, 1996), nymphal deer ticks efficiently transmitted POW virus to naive mice after as few as 15 minutes of attachment. It suggests that unlike *Borrelia burgdorferi* and *Babesia microti*; *Anaplasma phagocytophilum*, there is no grace period between tick attachment and POW virus transmission (Telford *et al.*, 1997; Katavolos *et al.*, 1998; Ebel *et al.*, 1999; Ebel and Kramer, 2004). Experimental milk-borne transmission of POWV has also been demonstrated in the goats (Woodall and Roz, 1977; CDC, 2001; Lloyd-Smith *et al.*, 2009). In adult residents of Wisconsin, during the year 2006 and 2007, POWV infections had been confirmed by serology wherein initial identification by detection of IgM type of antibody has been done followed by confirmatory molecular diagnosis. Such reporting has increased the necessity of routine testing (confirmatory) for diagnosis of such arboviral infection properly. The role of public education regarding the ways of acquiring such infection has also been analysed recently in a study by Johnson *et al.* (2010).

Characterization of several POWV isolated from *I. scapularis* that have been collected from Bridgeport and North Branford has been done by phylogenetic analysis of the sequences of the envelop gene. It has been found that sequences of Powassan virus have segregated into major two groups which are termed as the Deer Tick Virus (DTV) and Powassan virus lineages. It has been found that the lineage from *I. cookie* is POW whereas that from *I. scapularis* is DTV lineage. The remaining of the viruses from *I. scapularis* are grouped with the lineage of DTV. The Bridgeport Powassan viruses are nearly identical to the strain of virus detected in human from New York and are clustered in the same group. Homogeneity has been observed in the viruses from North Branford and these have been grouped with viruses from Massachusetts as well as Connecticut (north-western) and Ontario. Such findings are suggestive of the fact that introduction of POWV has occurred independently into these geographical locations in Connecticut and focal maintenance is done in their environments, respectively (Stafford *et al.*, 2003; Ebel, 2010; Pesko *et al.*, 2010; USGS, 2011; Anderson and Armstrong, 2012).

The assumption that strains of POWV have been imported in Russia 100 years back is supported by the clustering of samples from Russia with the prototypic strain from Ontario isolated during the year 1958. The envelope as well as the non-structural-5 (NS-5) protein topology has shown DTV to be falling in the same lineage as POWV. This suggests that exploitation of various ecological niches is done by these two sympatric lineages (partial). This supports each lineage's association with a distinct cycle of transmission (Ebel *et al.*, 1999; Leonova *et al.*, 2009).

THE DISEASE

The reported incubation periods for Powassan virus range from 8 to 34 days (Gholam *et al.*, 1999). Smith *et al.* (1974) reviewed the first 5 known cases of Powassan virus encephalitis in humans. They observed clinical picture of prodromata including sore throat, sleepiness, headache and disorientation; encephalitis characterized by vomiting, respiratory distress, possible convulsions and prolonged, sustained fever. Lethargy was common throughout the acute phase; patients were occasionally semicomatose and showed some degree of paralysis (Tavakoli *et al.,* 2009). Hemiplegia was the most common manifestation of neurologic damage. However, recurrent severe headaches (Goldfield *et al.*, 1973), minor memory impairment (Fitch and Artsob, 1990) and damage to the cervical cord (upper part) resulting in paralysis as well as wasting of right shoulder muscles (Deibel *et al.*, 1975) were also reported. Neuropathological alterations were akin to an infectious viral meningoencephalitic pattern of changes, mainly affecting grey matter of entire brain. It is associated with a reactive inflammatory cellular infiltrate (chronic) of lymphocytes and macrophages, including the abundance of perivascular inflammatory cells and multiple foci of parenchymal cells in grey matter. The lymphocytic reactive population comprised of almost equal proportion of T and B lymphocytes (Gholam *et al.*, 1999).

In horses, Powassan virus causes non-suppurative, focal necrotizing meningoencephalitis (Keane and Little, 1987). The virus has been reported from Ontario and the eastern United States. In 1983, approximately 13% of horses sampled in Ontario were found seropositive for the virus. But in the estuary of the Kuban River, 0-2% horses/cattle were found positive for TBE when their sera tested by enzyme immunoassay (solid-phase); neutralization test; and hemagglutination-inhibition test (L'vov *et al.*, 2008). Powassan virus has not been isolated from any naturally infected domestic animal. Little *et al.* (1985) however have reported the experimental induction of neurologic syndrome within 8 days of intra-cerebral inoculation of POWV into the horses. Keane *et al.* (1988) produced the disease experimentally in ponies via intra-cerebral and intra-venous inoculation of POWV. Antibodies to POWV were detected in the sera of all animals but in Cerebrospinal Fluid (CSF) of a few animals. Isolation of POWV was done from brain and spinal cord of only the intra-cerebrally inoculated animal. Infected animals developed neurological signs of "a tucked-up" abdomen; head and neck tremors; sloppy and chewing movements. This results in foamy saliva, stiff gait, staggering and recumbency. The pathological lesions observed were non-suppurative encephalomyelitis, neuronal and focal parenchymal necrosis. Inspite of the POWV causing neurologic syndrome, the virus has not been isolated from the brain of field cases (Hinchcliff, 2007). A survey conducted on 115 paired equine serum and cerebrospinal fluid samples collected in Ontario over the 18 month period between August 1984 and January 1986 did not reveal the presence of hemagglutination-inhibition for antibodies to POWV antigen in any animal (Keane *et al.*, 1988). This suggests a very low incidence of infection by POW virus in horses.

Initially, speculations have been made on the basis of several reports that POWV lineage II may prove to be less pathogenic than strains belonging to lineage I. But subsequently, through several works it has been indicated that POWV lineage II may be responsible for causing illness in human. There is however poor description regarding the prevalence of exposure to POWV among human residents of areas infested with deer-tick along with the relationship between inoculums of virus and pathogenesis of POWV. In order to produce illness in humans, delivery of a large inoculum of virus is required over several hours or days. In the salivary glands of tick, POWV infection has been detected. It has also been suggested that during the earliest stage of feeding POWV is present in the salivary secretions of tick and may immediately be inoculated (Ebel and Kramer, 2004).

DIAGNOSIS

Since TBE shows clinical and laboratory findings similar to other CNS diseases (e.g., herpes simplex encephalitis) that may require special treatment, specific and differential diagnosis of the disease is necessary. Patient's clinical features often form the basis for preliminary diagnosis along with information regarding travelling date and place and the history of epidemiology regarding the location of occurrence of infection. In case of high fatality, amplification of nucleic acid, histopathology as well as immunohistochemistry and culture of the virus from autopsy tissues are proven to be useful but it must be kept in mind that only few specialized laboratories are capable of carrying out such tests (www.cdc.gov/powassan/diagnostic-testing.html). Basically, two diagnostic approaches are considered. First one is the direct detection of the virus or viral RNA in the initial (viremic) phase of infection by virus isolation in mammalian cell culture or by RT-PCR. Second one involves indirect detection of specific IgM and IgG antibodies with serological methods such as Enzyme Linked Immunosorbent Assay (ELISA), Immunofluorescence Assay (IFA) or neutralization tests (NTs). As patients usually approaches doctors when neurological symptoms are manifested, virus isolation and RT-PCR are then of minor importance for the TBE diagnosis as already the virus may be cleared from the blood and CSF (Mantke *et al*., 2007). Therefore, the diagnosis of TBE is based mainly on serological methods that have been developed towards higher specificity and sensitivity in the last decade, as previously described (Holzmann, 2003; Sonnenberg *et al*., 2004; Gunther and Haglund, 2005).

Serologic diagnostic testing can be performed using immunoglobulin M (IgM)-capture enzyme-linked immunosorbent assays (MACELISA) on serum and Cerebrospinal Fluid (CSF) and plaque-reduction neutralization tests (PRNT) on serum. This depends on the availability of specimens (Beaty *et al*., 1995; Martin *et al*. 2000). Serologic tests were performed at the Wadsworth Center, New York State Department of Health. Microsphere Immunoassay (MIA) has been described to detect total antibodies (IgG+IgA+IgM) to recombinant envelope and nonstructural protein 5 (NS5) protein of West Nile virus; and recombinant envelop protein of deer tick virus (DTV) (Wong *et al*., 2003; Wong *et al*., 2004). An approach to improve the specificity of the ELISA was achieved using sub viral particles (Sps) as antigens in a SP-IgG and SP-IgM ELISA (Obara *et al*., 2006). Recombinant prM/E proteins of the TBE virus were co-expressed in mammalian cells whereby SPs were released in culture medium. The same structure has been shown by the SP E protein as in the virion (complete). But genomic RNA is not present in SPs and could be handled in laboratories without the need for facilities of high containment. ELISAs (SP-IgG and SP-IgM) showed the same specificity as that of neutralization test (NT).

Saksida *et al*. (2005) have described a modified nested RT-PCR that targets the highly conserved NS5 region of the viral genome. It was shown that prior to the appearance of antibody, RNA of TBE virus was detectable in every sample (blood and serum) collected. But CSF samples (only 3% tested positive) were found to be inappropriate for the molecular diagnosis of TBE when using this assay. RT-PCR assay may also be used for the early and differential detection of viral RNA in patients presented with febrile illness following a tick bite (Puchhammer-Stockl *et al*., 1995). This is particularly true in regions where different tick-transmitted diseases (e.g., Lyme disease, ehrlichiosis) are endemic and the therapeutic approaches for such diseases differ considerably. Detection of viremia by TBE virus-specific PCR has also been suggested for patients with febrile illness signs or when there is thrombocytopenia or leukocytopenia and a history of a tick bite recently wherein infection due to TBE virus requires to be suspected (Schultze *et al*., 2007; El Khoury *et al*., 2013). A quantitative real-time RT-PCR could be used in such clinical cases by

targeting the 3'-noncoding region of the viral genome (Schwaiger and Cassinotti, 2003). This method is highly sensitive and specific and enables the quantification of even low viral loads in different sample types such as serum or CSF. This helps to detect acute infection as well as for confirming by post-mortem and suspensions of tick for virus prevalence testing in the vectors. Ruzek *et al.* (2007) have developed a multiplex RT-PCR that is able to discriminate among TBE virus subtypes. This assay is based on the unique combination of oligonucleotide primers targeting the subtype-specific 'signature' positions of the E protein in the TBE virus genome. A molecular detection assay has been described to test for many flaviviruses using species-specific and group-specific primers in a single reaction (Dyer *et al.*, 2007). This real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay combines flavivirus species-specific and group-specific TaqMan primers and probes into a single tube subjected to a standardized thermocycling conditions. Specific detection of St. Louis encephalitis and West Nile virus; and consensus sequences for tick-borne encephalitis complex groups like Russian Spring-Summer encephalitis and Central European encephalitis viruses can be done. The assay also contains group-specific primers for Dengue I, II, III and IV viruses. All flavivirus species and sample types can be detected by the multiplex assay which is as sensitive as to detect low virus titer samples as the single-virus assay. Thus it is useful for screening of samples for the presence of many flaviviruses of interest while saving labor and reagents and without sacrificing sensitivity (Labuda *et al.*, 1993; Calisher, 1994; Costero and Grayson, 1996; Dyer *et al.*, 2007). For POWV it must be kept in mind that testing is not provided by commercial laboratories rather it is provided by the Centers for Disease Control and Prevention. Testing by serology like enzyme immunoassay along with confirmation by plaque reduction neutralization testing is preferred over POWV RT-PCR. Their usefulness is moreover limited by viremic periods of flaviviruses which is short (Lanciotti, 2003; Hinten *et al.*, 2008; Birge and Sonnesyn, 2012).

By lineage like prototype versus virus of deer tick, identification of infection due to few POWV is done but for several cases the lineage is unknown. Exposure to *I. scapularis* however, has been reported in many patients and sequencing technology can be used to identify the viruses from tick pools that are POWV-positive. The nieage distribution however, still remains a little unclear and in most of the instances cases go undetected without specific efforts of POWV surveillance (Brackney *et al.*, 2008; Neitzel *et al.*, 2013).

PREVENTION, TREATMENT AND CONTROL

Recent advances in virology, synthetic organic chemistry and the discovery of RNA interference (RNAi) have provided the basis for advances in the development of antisense-based approaches to address infections due to flaviviruses. Oligomers (several types of antisense structure) targeting specific locations in the RNA genome of the flavivirus have been used at present for successful suppression of viral gene expression and thereby inhibit replication of flavivirus. RNA (double-stranded) containing sequence of virus and designed to induce the endogenous cellular machinery of RNAi has also been shown capable of potentially interfering with flavivirus production and transmission (Stein and Shi, 2008).

There is no vaccine or specific therapy for POWV disease. Intravenous injection of acyclovir is important for the treatment of severe encephalitis assuming that the patients are suffering from encephalitis due to herpes simplex viral infection (Ralph, 1999). The prevention is best accomplished by protection from tick bites. Preventing tick infestation of family pets can also

prevent ticks from entering the home. *I. cookei* are often found on woodchucks and skunks (Fish and Dowler, 1989) and appear to be the primary POWV vector. Thus environmental controls reducing human contact with small and medium-sized mammals and their burrows can reduce exposure to POWV-infected ticks (Wilhams *et al.,* 2009). Persons should keep areas adjacent to their home clear of bush, weeds, trash and other elements that could support small and medium-sized mammals. When removing rodent nests, they should avoid direct contact with nesting materials and use sealed plastic bags for disposal and to prevent direct contact with ticks. For prevention of POWV, repellants are found to be important tools because of the severity as well as shorter attachment time of tick that is needed for the transmission of the virus. It is also advised to wear long pants along with clothings that are light-coloured; and checking for the presence of ticks thoroughly after time is spent in the woods. From late spring until the middle of the summer these precautions are mostly important and their importance increases again in the months of fall when the activity of black legged ticks increases. If there is development of fever and chills; rashes; headache as well as body aches; alteration in mental status along with other signs as well as symptoms of tick-borne illness, medical care must be taken into consideration. This is especially important after performing outdoor activities in tick-infested areas. 0.5 per cent permethrin along with 20-30 percent Diethyl Ethelene (DEET) containing insect repellent is found to be effective for repelling ticks but manufacturer' direction must be strictly followed. Residual insect repellant must be removed by taking a shower after coming in from out door. Awareness of POWV disease also should be promoted among clinicians, laboratory diagnostic staff and public health staff and prevention strategies for tick-bite need to be emphasized for the general public (Hinten *et al.*, 2008).

Keeping in view the importance of One Health, One Medicine, One World concept and scenario of global warming with increasing vector populations efforts need to be made for applying rapid and advanced diagnosis and surveillance systems (Lanciotti, 2003; Mantke *et al.*, 2007; Schmitt and Henderson, 2005; Belak, 2007; Dyer *et al.*, 2007; Stein and Shi, 2008; Pesko *et al.*, 2010; USGS, 2011; Deb and Chakraborty, 2012; Deb *et al.*, 2013, Dhama *et al.*, 2012, Dhama *et al.*, 2013a, b, c, Dhama *et al.*, 2014; Hayasaka *et al.*, 2013), effective and novel vaccine regimens (Meeusen *et al.*, 2007; Dhama *et al.*, 2008, Dhama *et al.*, 2013d) and emerging/novel and alternative therapeutics (Dhama *et al.*, 2013e, f, g; Mahima *et al.*, 2012; Tiwari *et al.*, 2014) against POWV. Apart from these, suitable prevention and control strategies with strict biosecurity practices must be given due emphasis to combat Powassan virus and its ill effects.

CONCLUSION AND FUTURE PERSPECTIVES

Powassan virus has the highest case-fatality rates and is associated with a very high incidence of severe neurologic sequelae which has increased the importance of this disease. It has been seen that no specific antiviral therapy is available for Powassan viral infection. Prevention therefore is of paramount importance for the control of morbidity as well as mortality associated with such illness. Vaccines are however available for certain arboviruses present in North America to be used in a restricted fashion in groups at high-risk but currently vaccines are not available for the prevention of POWV infection in particular. Awareness regarding the arthropod vector; the vertebrate hosts (natural) and the seasonality of transmission potential is found to be helpful to design measures of prevention against infection. Along with this the history of epidemiology is essential for the diagnosis of disease and has also got implication immediately for the differential

diagnosis as well as management. Addition of information to surveillance data is also of utmost importance. With the development of advanced diagnostic assays like ELISAs (SP-IgG and SP-IgM) and real-time quantitative RT-PCR the diagnosis of the disease has become more specific and quick. Promotion of awareness among clinicians; laboratory diagnostic and public health staff is essential for disease prevention. From a therapeutic standpoint on a more optimistic note, the technical resources as well as expertise that have added to several novel anti-HIV drug developments can be applied to the development of antiviral agents against this particular arboviral infection.

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