Japanese Encephalitis: A Veterinary Perspective

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Abstract

Japanese encephalitis is one of the most important arboviral zoonoses caused by Japanese encephalitis virus of Flaviviridae family. The virus cause huge mortality in children in several Asian countries. The virus circulates among birds and pigs and gets transmitted to humans through mosquito bite. The children below 15 year of age are highly susceptible and affected children suffer from neurological sequel. The virus causes abortion in sows, encephalitis in horses, however other species of animals remain asymptomatic. The increased paddy cultivation, presence of pigs near human dwelling, change in climate and presence of unvaccinated human host contributes to the incidence of the disease. Therefore, vaccination of susceptible population, vector control, early diagnosis and treatment and continuous serosurveillance in animals are the keys to combat this crippling disease.

Keywords: Animals, Epidemiology, Japanese encephalitis, Zoonoses.

1. Introduction

Japanese encephalitis (JE) is a re-emerging mosquito-borne zoonotic flaviviral disease. It is the most important cause of epidemic encephalitis and childhood mortality worldwide with an estimated 50,000-67,900 cases and 10,000-20,000 deaths annually although official reports undoubtedly under estimate the true number of cases (Acha and Szyfres, 2003; Solomon et al., 2003; Yadav, 2006; Campbell et al., 2011). Almost half of the world population is estimated to live in countries where JE virus is endemic (Ghosh and Basu, 2009), thus making it a cause of public health concern worldwide. The disease in animals is limited to encephalitis in horses and reproductive disorders in pregnant sows. Besides causing serious public health implications, the JE virus causes abortion and still birth in swine which has hot profound impact on swine industry affecting the national economy.

Japanese encephalitis virus (JEV) exists in a zoonotic cycle that involves ardeid birds as reservoirs, mosquitoes as vector, pigs as amplifying host and human being as incidental and dead-end hosts (Acha and Szyfres, 2003). In pigs, comparatively high viraemia occurs and they contribute significantly to the perpetuation of the disease in rural settings without displaying any overt clinical signs except abortion and still birth in pregnant sows (Guerin and Pozzi, 2005). Moreover, the presence of pigs in the peri-domestic areas increases the chances of spillover over of infection to human, especially when the vector density is high. Pigs seroconvert 2-3 weeks before infection occurs in human and can be used as sentinels for monitoring the prevalence and enzootic transmission events of JEV in an endemic area.

In the present scenario, where ‘one health approach’ has become sine qua non of public health system, the zoonotic diseases like JE need careful attention of Veterinarian. This paper presents a review which elaborates the important aspects of JE with special attention on veterinary and public health aspect.

2. Etiology

JEV belongs to genus Flavivirus of family Flaviviridae and is transmitted between birds, pigs and some other domestic animals by Culex mosquitoes (Monath and Heinz, 1996). Human beings become infected only accidentally when bitten by infected mosquito (Acha and Szyfres, 2003). The phylogenetic data and the biological properties of different flaviviruses helped research workers to hypothesise that the genus Flavivirus evolved from ancestral virus in Africa within past ten thousand years (Billoir et al., 2000; Gritsun et al., 2003). In the evolution of this genus, 3 lineages had diverged and they are mosquito borne viruses, tick borne viruses and viruses with no known vector. The tick borne lineage diverged about 3,000 years ago, followed by the mosquito borne viruses (Gould et al., 1997). The mosquito borne lineage can be further classified into the yellow fever...
virus (YFV) group, which is the most divergent clad, transmitted by means of Aedes mosquitoes in the Old World and causing hemorrhagic disease in primates. A subsequent divergence in the genus gave rise to the dengue serological group and the Japanese encephalitis serological group, associated with Aedes and Culex mosquitoes, respectively (Porterfield, 1975; Gritsun et al., 2003). The Japanese encephalitis serological group comprises 8 virus species among which the major are Japanese encephalitis virus (JEV), West Nile virus (WNV), Murray Valley encephalitis virus (MVEV) and St. Louis encephalitis virus (SLEV) (Mackenzie et al., 2004).

2.1 Structure and Genome of the JEV

JEV is a 40-50 nm enveloped, positive sense single-stranded RNA virus, with an isometric 30 nm nucleocapsid core. The envelope is spiked with a mature membrane (M) protein and a glycosylated envelope (E) protein, which comprises of 3 domains (I, II and III) that are involved in antigenic properties, cell receptor binding and penetration of the virion into the host-cell. The 10,976 bases long single-stranded viral RNA encodes an uninterrupted open reading frame that is translated into a polyprotein precursor and eventually processed into capsid (C), membrane (M/prM) and envelope (E) structural proteins and into seven non-processed into capsid (C), membrane (M/prM) and envelope (E) structural proteins and into seven non-processed into capsid (C), membrane (M/prM) and envelope (E) structural proteins and into seven non-processed into capsid (C), membrane (M/prM) and envelope (E) structural proteins and into seven non-processed into capsid (C), membrane (M/prM) and envelope (E) structural proteins (Sumiyoshi et al., 1987; Solomon et al., 2003; Weaver and Barrett, 2004).

The flavivirus C protein (12-13 kDa) is surrounded by a lipid bilayer composed of the two membrane glycoproteins, E (50 kDa) and prM (22 kDa), the latter being replaced by a shorter M protein (8 kDa) in case of extracellular virions. The flavivirus E protein is a class II fusion protein and is host cell derived. The M protein is first synthesised as a precursor protein, prM and cleaved before the release of the virus to the mature M protein. In some instances the prM may not be cleaved completely and act as an additional target on virions for neutralizing antibodies (Bray and Lai, 1991).

The viral E protein is a major antigen responsible for eliciting neutralizing antibody response and protective immunity in host (Konishi et al., 1991; McMinn, 1997). The E protein contains three antigenic domains A (II), B (III) and C (I) (Heinz and Roehrig, 1990; Roehrig et al., 1998). The A domain is linearly discontinuous being divided by the C domain. The A domain contains five disulphide bonds and the epitopes are conformationally dependent virus neutralizing epitopes. The B domain also contains a disulphide bond which is required for the antigenicity of this domain. The three dimensional molecular structure of the Tick borne encephalitis virus (TBEV) has been deciphered (Rey et al., 1995), and rest of the flaviviruses are believed to have a similar structure, as the E protein cysteine residues are conserved among all of them. The E protein is folded into three domains (I, II and III) that correlate with the C, A and B domains. The domain III is supposed to be the receptor binding domain. The host cell receptors have not yet been identified and the heat shock protein 70 is thought to be the possible receptor. The domain II is the dimerization unit, and domain I has a central β barrel and links the two domains (II and III).

The non structural proteins play important role in viral genome replication and expression. The NS1 protein (48 kDa) is a secreted glycoprotein and contains 2 or 3 conserved N-linked carbohydrates (Chambers et al., 1990) and six conserved disulphide bridges (Wallis et al., 2004). The NS2B and NS3 proteins together work as a virus specific protease and cleave polyprotein to generate 3 structural and 7 non-structural proteins (Chambers et al., 1990; Yusof et al., 2000). The NS3 also has helicase activity (Matusan et al., 2001). Both NS4A and NS4B have a role in viral replication. The NS5 protein is the viral RNA dependent RNA polymerase.

2.2 Serotype and Genotypes of the JEV

All known JEV isolates comprise a single serotype and this fact is supported by phylogenetic analysis performed for different genome regions of Japanese encephalitis virus (Tsarev et al., 2000). Major alterations in the genome of resulting viral variants frequently occur in the envelope (E) protein (Deubel et al., 1993). To date, five genotypes of JEV have been described based on phylogenetic analysis of the viral E gene (Williams et al., 2000; Uchil and Sachidanandam, 2001; Solomon et al., 2003).

Most virus strains of genotype I were isolated from northern Thailand, Cambodia and Korea; those from genotype II were isolated from southern Thailand, Malaysia, Indonesia and Australia; those from genotype III were isolated from areas of Asia that are largely temperate, such as Japan, Korea, China, Taiwan, Philippines, India and Sri Lanka; and those from genotype IV have only been isolated from Indonesia (Chen et al., 1992). The Muar strain, isolated from Malaysia in 1952 is the only known representative of the proposed fifth genotype (Uchil and Sachidanandam, 2001).

The genotype III is the most widely distributed genotype with the prototype Nakayama strain also belonging to it (Mackenzie et al., 2004). It is widely distributed in Asian countries, including India. However, during the past decade, genotype I has been
3. Epidemiology

JE virus is transmitted to host by the bite of an infected mosquito. The virus is maintained in nature by a complex natural cycle involving vertebrate species, which act as reservoir and amplifier hosts.

3.1 Reservoir and Host Range

The ardeid birds like cattle egrets and pond herons are the natural reservoir of the virus and play a definite role in maintenance of JEV in nature (WHO, 2006). Antibodies against JEV has been demonstrated in various species of domestic and wild birds (Acha and Szyfres, 2006). Pigs are the important vertebrate hosts which act as amplifier of the virus. In pigs, viraemia occurs with a high titre and they contribute significantly to the perpetuation of the virus in nature. Several researchers have demonstrated vertical transmission of JE virus in mosquito vectors (Rosen et al., 1983). However, it will be necessary to demonstrate that this mechanism also exists in nature.

The mechanism by which virus survives the winter in temperate climate is still not fully understood. It has been experimentally observed that JE virus can be maintained during winter in hibernating lizards and snakes (Doi et al., 1983). However, it will be necessary to demonstrate that this mechanism also exists in nature. Several researchers have demonstrated vertical transmission of JE virus in mosquito vectors (Rosen, 1986). JE virus has also been isolated from the mosquito larvae, supporting vertical transmission and overwintering in mosquito eggs (Rosen et al., 1989). Therefore, vertical transmission in mosquitoes might be an additional mechanism by which virus maintains itself in nature.

3.2 Modes of Transmission

The infection is transmitted among birds, pigs and human through the bite of infected culicine mosquitoes. Female mosquitoes become infected after feeding on viremic host and can transmit the virus to other hosts after an extrinsic incubation period of 9 to 12 days. The mosquitoes remain infected for life (WHO, 2006). Mosquitoes belonging to C. vishnui group, which includes C. tritaenioryynchus, C. vishnui and C. pseudovishnui, are the most common vectors of JEV in India. The virus is mainly transmitted by C. tritaenioryynchus mosquito that breeds in paddy fields and drainage ditches, thus making the disease a major concern in rice growing areas. The average life period of mosquito is about 21 days. Culex mosquito can fly for a distance of 1-3 kms or even more. These mosquitoes are more active at twilight and don’t go indoors. Hitherto, JEV has been isolated from 16 species of mosquitoes (Culex-10, Anopheles-3 and Mansonia-3) in India. However, it has been opined that mere presence of virus in Anopheles and Mansonia mosquitoes does not qualify them to be a potent vector of JEV (ICMR, 2000).

The seasonal incidence of JE virus varies greatly influenced by climate, geography and immune status of host population (Gubler, 2002). There are 2 distinct epidemiological patterns of JE. In temperate zones, such as the northern part of the Korean peninsula, Japan, China, Nepal, and northern India, large epidemics occur in the summer months while in tropical areas of southern Vietnam, southern Thailand, southern India, Indonesia, Malaysia, the Philippines, and Sri Lanka, cases occur more sporadically and peaks are usually observed during the rainy season (Vaughn and Hoke, 1992). The seasonal incidence of JE varies in different parts of India (Reuben and Gajanana, 1997). In northern India most of the human cases are reported between May and October, while the season may be extended or year-round in some areas, especially in southern India but often intensifies during rainy season (CDC, 2012). Two seasonal peaks of JE are recorded in some areas of southern India, where double paddy crops are being cultivated (Gajanana et al., 1997).
4. Disease in Animals

A large number of vertebrate animals like cattle, sheep, goat, dog, cat and chicken seem to be infected with JE virus subclinically and thus elicit no clinical signs (Pant et al., 2006). Birds and pigs are effective viraemic hosts and help in perpetuation of the disease in nature.

In pigs, viraemia occurs with a high titre and lasts for 2–4 days without displaying any overt clinical signs except for abortion and still birth in pregnant sows (Acha and Szyfres, 2003; Guerin and Pozzi, 2005).

In equines, the infection is inapparent though some cases may show encephalitis, pyrexia, depression, photophobia, muscle tremors and ataxia (Burke and Monath, 2001; Acha and Szyfres, 2003). The affected horse may collapse, fall in a coma and eventually die. Mortality rate due to JE in equines range between 5 per cent and 30 per cent (Gulati et al., 2011).

5. Prevalence/Incidence of JE in Animals and Birds

Although JEV infects several species of animals and birds, the major role in transmission is being played by birds and pigs only (Yang et al., 2007). JE is responsible for economic losses in Asian countries by causing abortion and neonatal mortality in pig population. Moreover, for effective surveillance of JE, it is necessary to ascertain its seroprevalence in animals and birds.

5.1 Global Scenario

Studies in Japan revealed that disease is endemic in the pig population, with virus isolation from stillborn pigs even during non-endemic winter seasons (Takashima et al., 1988; Arai et al., 2008). A high seroprevalence of 83 per cent among wild boars and 59 per cent among raccoons was reported in Japan (Ohno et al., 2009). In 1948 epidemic, morbidity rate was 337.1 per 10,000 equines in Japan due to genotype III of JEV (Acha and Szyfres, 2003). However, no cases in horses have been reported since 1986 due to effective vaccination except one case in 2003 due to genotype I that too in unvaccinated horse (Yamanaka et al., 2006). Seroprevalence of 17 per cent was found in dogs in Japan, revealing that they can be used as sentinels to ascertain JEV infection (Shimoda et al., 2010).

A serological study during the period of 2002 to 2003 revealed 55 per cent prevalence of JE in pigs in Nepal (Pant et al., 2006). In a similar study conducted by Pant (2006) during the period of 2003 to 2004, seroprevalence in pigs, ducks and horses was found to be 48.11 per cent, 26.79 per cent and 50.0 per cent, respectively. A recent cross sectional serosurvey in Nepal demonstrated 11 per cent of the pigs were positive for antibodies to JEV (Ghimire et al., 2014).

The JEV infection was reported in 18.5 per cent stillborn piglets in China during 2009 - 2010 (Liu et al., 2013). The prevalence in pigs was found to be in range of 49-70 per cent with round the year infection of pigs in Taiwan (Chang, 2002).

In a study conducted from 2005 to 2006 in Korea, the prevalence was found to be 12.1 per cent in goats with higher seropositivity in animals of above 2 years age (Yang et al., 2007). In other study antibodies to JEV was demonstrated in 51.3 per cent of cattle and 2.9 per cent of aborted pig foetuses in Korea (Lim et al., 2007).

In Australia, the first JE outbreak occurred in 1995 and serosurvey showed the simultaneous evidence of infection in pigs, horses and dogs (Hanna et al., 1999).

5.2 Indian Scenario

JE is endemic in India and several studies have been conducted to assess its seroprevalence in animals and birds (Angami et al., 1989; Mall et al., 1995; Kumanan et al., 2002; Raut et al., 2003; Kolhe et al., 2011). Studies conducted for detection of JE antibodies in sera of birds revealed that pond heron and cattle egret play a definite role in maintenance of JEV in India. In different parts of country, 12 to 44 per cent of pig population have been found to be seropositive for JE and besides birds and pigs, bovine and bats have also been found positive for JE antibodies (WHO, 2006).

In a study conducted in Bareilly region of U.P., the highest HI positivity against JE was found in dogs (55.77%) followed by pigs (40%), horses (37.65%), buffaloes (21.92%), goats (17.86%), sheep (2.38%) and cattle (1.98%) (Mall et al., 1995). In Chandigarh, the seroprevalence of JE in pig was 30.3 per cent by haemagglutination inhibition (HI) antibodies and 12.5 per cent by complement fixation (CF) antibodies (Ratho et al., 1999). In Tamil Nadu, seroepidemiological studies revealed highest incidence of JE in pigs (26.4%), followed by birds (9.37%), cattle (6.86%) and buffaloes (5.06%) (Kumanan et al., 2002). Seroprevalence of JE in pigs in Haryana was found to be 18 per cent (Nagaleelavathi et al., 2008). In another study, IgG and IgM antibodies to JEV were detected in 27.66 per cent and 28.89 per cent pigs, respectively (Kolhe, 2008).

A comprehensive JE sero- surveillance in horses was conducted between 2006 to 2009 in 13 states of India including Jammu and Kashmir (J & K), Himachal Pradesh, Punjab, Haryana, Rajasthan, Gujarat,
Uttarakhand, Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Karnataka, Sikkim and Manipur. Out of 3,286 equine serum samples tested, 327 (10%) were positive for JE antibodies according to both HI and VNT results (Gulati et al., 2011). None of the equines from the Northern hill States of J&K, Himachal Pradesh, or Sikkim were found positive for JE antibodies while maximum seroprevalence was found in equines from Manipur (91.7%), followed by Gujarat (18%) and Madhya Pradesh (14.4%).

With respect to birds, the prevalence of JE using I-ELISA in Guinea fowl and broilers was accessed as 12 per cent and 11 per cent, respectively (Kolhe et al., 2011).

6. Diagnosis in Animals

Disease history, virus exposure and clinical features cannot help to diagnose JE specifically; hence diagnosis must rely on laboratory confirmation (WHO, 2007). Different approaches for diagnosis of JE are described below.

6.1 Virus Isolation

The suitable clinical specimens for virus isolation are blood, serum, CSF, brain and spinal cord in equines, while blood and aborted foetuses in case of pigs are useful. The JEV can be isolated using cell culture system, intra-cerebral inoculation of suckling mice and mosquito inoculation. Isolation of JEV is comparatively difficult, reasons being instability of virus in environment or the rapid development of neutralizing antibodies.

6.2 Molecular Approaches

The low success rate associated with virus isolation and the need for confirmation and typing of virus to provide definitive early diagnosis has led to the development of nucleic acid based assays viz., reverse transcription PCR (RT-PCR), real time RT-PCR, reverse transcription loop mediated isothermal amplification (RT-LAMP), RT-PCR has been used for detection of JEV from blood, brain and CSF (Lian et al., 2002). However, the RT-PCR is less sensitive than real time RT-PCR and RT-LAMP.

6.3 Serological Approaches

JE is most commonly diagnosed by detection of specific antibodies in serum or CSF. Different serological tests viz., plaque reduction neutralization test, virus neutralization test, haemagglutination inhibition test, enzyme linked immunosorbert assay and latex agglutination assay can be employed for diagnosis of JE in animals (OIE, 2010).

The HI was widely used for diagnosis of JE but cross reactivity among different flaviviruses and need for constant supply of goose RBCs has limited the application of HI. The Plaque Reduction Neutralisation Test (PRNT)/VNT are the gold standard for the serological diagnosis of flaviviral infections. But these assays are labour intensive, require skilled personnel, time consuming and poses risk of handling live virus. Therefore, PRNT/VNT is recommended for reference laboratories only, that too when it is difficult to differentiate samples using ELISA. (WHO, 2007)

Currently, ELISA has been widely accepted and used for diagnosis of JEV infection in both animals and humans. Although ELISA is suitable for high throughput screening of large number of samples, it is not readily applicable at the point of care. To overcome these drawbacks lateral flow assay has been developed which is simple to perform, rapid, cheap, and does not require special skills or expensive equipments (Peters, 2012).

7. Prevention and Control of JE

Since there is no specific treatment available for JE, effective prevention and control strategies must be designed to protect humans from getting infected. The prevention and control strategies must be multipronged and should address several important factors viz., mosquito control and intensive vaccination of humans, control of amplifying host, regular veterinary surveillance and public awareness.

Mosquito control aims at reducing mosquito density thereby protecting humans from mosquito bite. Several approaches including residual sprays, fogging and biological control by larvivorous fishes, environmental sanitation and proper irrigation management of paddy fields and use of personal protective measures are helpful in avoiding mosquito breeding and subsequent risk of mosquito bite.

Vaccination of children is one of the most effective measures in prevention of the disease. In India the childhood vaccination using SA14-14-2 has been implemented in endemic areas of JE since 2006 and has already covered 132 districts (NVBDCP, 2014).

Control of amplifying host involves slaughtering and vaccination. Vaccination of pigs could be an important strategy since it prevents pigs getting infected, decreases amplification of virus and reduces risk of abortion. But is suffers from drawbacks viz., vaccination is costly and requires immunization of large number fresh stock of piglets in each breeding season. Even though pigs are vaccinated, the circulation of virus may still continue among birds. On the other hand slaughtering of pigs is not feasible since it affects livelihood of pig farmers. For pigs, both
inactivated and live-attenuated vaccines derived from cell cultures are used in Japan and Taiwan. Infected mouse brains or cell culture derived formalin-inactivated/attenuated vaccines are used for horses, in Japan (OIE, 2010).

Continuous vector surveillance and sero-monitoring in pigs are very essential in determining the virus activity in endemic areas (NVBDCP, 2014). Increased vector activity coupled with detection/isolation of virus in mosquitoes and or sudden appearance of antibodies in pigs against JEV can be used to forecast JE outbreak in humans.

At last public health awareness programmes are very much necessary to sensitize the general population on how to reduce the risk of acquiring JE. People should be made aware of importance of vaccination, sanitation and personnel protection to combat JE successfully.

8. Future Directions

References


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