Japanese Encephalitis Virus (JEV) infection in different vertebrates and its epidemiological significance: a Review

Sajal Bhattacharya and Probal Basu

Abstract
The maintenance of Japanese Encephalitis Virus (JEV) in nature occurs in a cycle which includes mosquito vectors and the vertebrate hosts. Japanese encephalitis (JE) being primarily a zoonotic disease, animal hosts such as pigs and birds play an important role in the maintenance and amplification of the virus. JE antibody has been detected in several poikilothermic and homeothermic vertebrates, apart from the recognized reservoir/amplifying hosts. Emerging arboviral diseases such as Japanese Encephalitis, Chikungunya, Dengue generally stems from an animal reservoir, but there is inadequate information on the natural history of arboviruses especially its method of survival in the inter-epidemic period. Keeping in view the above perspective, an attempt has been made to review the various non-human vertebrates infected with JEV and to analyze their epidemiological significance. Some zoonotic potential might exist in the ectothermic animals as overwintering reservoir hosts for Japanese Encephalitis Virus (JEV). Mosquito species having predilection to animal blood could get the virus from a broad array of low viraemic ectothermic and endothermic vertebrates, but possibly fail to attain the infective stage and to acquire transmission competence due to inadequate titre level and short duration of viraemia in those vertebrates. These animals might have some complementary role in the maintenance of the enzootic cycle of the virus along with the recognized principal reservoir hosts i.e., pigs and birds. The principal and complementary vertebrate reservoir hosts keep the virus circulating perennially in nature and epizootic occurs at the time of higher than average level of virus circulation.

Keywords: JEV; Viraemia; Vertebrate Hosts; JE transmission, epidemiology

1. Introduction
Japanese encephalitis is a mosquito-borne viral disease. JEV belongs to the family Flaviviridae and is one of the major causes of viral encephalitis in human beings in Southeast Asia. JEV continues to invade other geographical areas and becoming a serious public health problem [1, 2]. Japanese encephalitis (JE) was first reported in Japan in 1871 [3]. The other important members of the same serological group are West Nile virus (WNV), St. Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV) [4]. These arboviruses cannot be transmitted mechanically, but require replication before transmission [5]. JE being primarily a zoonotic disease, animal hosts such as pigs and birds play an important role in the maintenance and amplification of the virus [6] and it appears as local outbreaks in humans under specific ecological conditions. In the natural cycle of JE, man is the dead-end host [7]. JE antibody has been detected in several poikilothermic and homeothermic vertebrates, apart from the recognized reservoir/amplifying hosts (Table. 1). During the invasion into new areas, JEV caused major epidemics which are often enabled by rice culture along with amplification in domesticated swine [8]. The invertebrate host, mosquitoes may carry the virus for life after getting the infection. JE virus has been isolated from different mosquito species belonging to the genus Culex, Anopheles, Mansonia but members of the Culex vishnui complex are found to be the major vectors of JEV [7, 9]. The vertebrate hosts after infections, usually recover, and rapidly eliminate the virus and develop a lasting immunity. Accordingly, the virus survives by virtue of alternation between vertebrate and invertebrate hosts in a “cycle”- man gets the infection tangentially on accidental intrusion in this pathway. Fortunately, however, the infection represents a blind alley terminating the chain of transmission. The consequence of infection in man, however may prove to be more serious than those of the natural host that has presumably benefitted from thousands of years of natural selection for genetic resistance.
Researchers demonstrated that a bird-mosquito-bird cycle occurs in JE [26]. It has been stated that some birds might be involved in the natural maintenance of JE virus. Viraemia with high titres has been reported in ducks, young chickens, pigeons and sparrows in India [15, 27]. Several workers have studied the serological evidence of JE virus in birds [28, 29, 30, 31, 32, 33, 34]. Researchers collected 127 serum samples from ten migratory and seven resident species of birds at Bharatpur, Rajasthan and conducted HI tests for antibodies against Group B arboviruses. 15 sera of migratory birds viz., *Anas clypeata*, *Anas crecca*, *Aythya fuligula*, *Anas strepera* were positive for antibodies against JE virus [31]. In Bankura District, West Bengal State, India, 104 birds sera were tested, only 8 showed the presence of JE antibodies [7, 30]. In Andhra Pradesh State, India, only 5.7 percent of ducks (3 out of 53) possessed N antibodies to JE virus. 146 bird sera collected from Asansol and Dhanbad, India. Neutralizing antibodies against JE virus were found in 9 out of 34 little egrets, 1 out of 15 cattle and 1 out of 13 paddy birds. 1 out of 4 crow sera and 3 out of 16 duck sera also showed antibody against JE virus. The scientists carried out a serological survey of birds during an outbreak of JE in Asansol and Dhanbad region of India. Neutralizing antibodies against JE virus were found in 11 out of 62 ardeid birds, 3 out of 35 ducks and 1 out of 4 crows [32]. Research revealed that there were a few reports in India, where fowl sera were found positive for JE antibody [34, 35, 36]. Similarly, antibodies have been shown in the sera of chicks, sparrows, parrots, and some migratory birds. The available information and evidences indicate that birds like pond herons, cattle egrets along with other animals are probably incriminated for maintenance and transmission of JE virus [36]. Interestingly, researchers suggested that ardeid birds *Ardea
grayii* (pond herons) and *Bubulcus ibis
coromandus* (cattle egret) [56] may be involved in the natural cycle of JE virus in India. It has been reported that neutralizing antibody to JE virus was detected in 179 sera out of 514 sera i.e., 34.8% of these two species in Andhra Pradesh State, India [33]. Scientists examined the sera of a number of birds belonging to different species collected from the JE affected Burdwan District of West Bengal State, India and showed JE antibodies in some of them [15, 32]. It has been reported that ducks, fowls and para-domestic sparrows possessed antibodies to JE virus in Bihar, India. It has also been demonstrated that JE virus activity in ducks and fowls was significantly higher compared to its activity in pigs in the same area as reported earlier, indicating the persistence of activity [34]. Some species of migratory birds such as *Egretta
garzetta* and *Nycticorax nycticorax* which follow a complex mode of migration over a vast geographical area might cause significant spread of JEV [38, 39].

2.3 Mammals

2.3.1 Pigs

Several researchers very conclusively proved that pigs are important hosts playing an important role in the epidemiology of JE in many places such as Malaya, Singapore, Japan, Taiwan, Thailand [39, 40, 41, 42]. Wild boars have been surmised to be the origin of sporadic human cases of JE in Singapore [43]. In India, experimental transmission studies and serological studies also depicted important role of pigs in the dissemination of JE [44, 46, 47, 48].

2.3.2 Cattle

Interestingly serological examination of bovine sera has also
shown a high percentage of JE antibody prevalence to them both in South India and in Eastern India with previous history of JE epidemics [46, 47]. They showed their importance in JE epidemiology, even-though the bovines do not really exhibit long viraemia and thus presumably not helping JE transmission. These hosts may help in deflecting the invertebrate hosts and arrest the dissemination of JE [7, 48].

2.3.3 Bats
Studies in laboratory setup and natural condition reveals that bats might have the potential to transmit JEV [49, 50]. In Japan, isolation of JE virus from bats has been reported [51]. JEV has been also found and isolated from two species of bat in China [52]. Bats would also be ideal hosts for overwintering of arboviruses [53]. As a result of the nomadic behavior of flying fox, they are thought to be a potential reservoir or amplifying host of JEV [53, 54, 55].

2.3.4 Horses and Mules
Epizootics among horses by the JE virus have been observed in Japan. JEV dissemination among equines was found to be extensive with clinical illness and deaths following an epidemiological study in Japan [37]. Serological evidence of JE infection among mules in the Eastern Himalayan region has also been reported [37].

Table 1: Detection of JE antibody from different vertebrate

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3. Non-human Vertebrate Reservoirs and their possible role in JE epidemiology
Animal hosts such as pigs and birds are recognized animal reservoirs and play an important role in the maintenance and amplification of the JE virus [5, 6, 14]. Viraemia has been exhibited by several ardeid avian species upon experimental and natural infections. The ubiquitous nature of avian species that often involves the sharing of urban and suburban habitats with both the human and mosquitoes makes them more pertinent in the context of JEV transmission [57]. Viraemic birds might be responsible for JEV introduction and spread in India [58] and Taiwan [59]. Perennial transmission of JEV in its maintenance cycle has been demonstrated in West Bengal State, India. The rise of antibody prevalence in the wild bird population suggests a gradual infection of the birds that hatch in summer. The influx of young non immune birds and possibly new born mammals into the animal population could be accompanied by a higher than average level of virus circulation, which by spillover of the virus in the human population would explain the episodic occurrence of infections of humans with a peak in the rainy season [15]. It has been observed that certain avian species play a significant role in shaping the JEV epidemiology and ecology in North America. Researchers found that several passerine species might serve as amplifying hosts of JEV. This avian species possibly contributed to the maintenance and spread of JEV in North America because of their relatively high density and the exploitation of a variety of habitats [57]. However, pigs are considered as principal amplifying hosts for JEV [8]. High rate of natural infection (about 98-100%) along with high viraemia and a high birth rate are the significant attributes which support the notion that the pigs serve as amplifying hosts in the JEV transmission [59]. The industrialization of pig farming which has generally developed for several decades in many nations (China, Myanmar, Thailand and Vietnam) [17] has enhanced the amplification of the JEV within dense pig herds, and so contributed to the increasing risk of JEV transmission. Thus, the high proximity between humans and livestock became the primary risk factor for human infection [60]. In the case of epizootic arboviral infections like JEV, the repeated intersection between competent vectors and the population of the vertebrate host within a susceptible environment is necessary for the amplification of arboviruses to achieve an epidemic level [5]. The host which exhibit prolonged viraemic periods and high titre viraemia that cause them to show high infectivity to vector mosquitoes are considered as highly competent [61]. Researchers reiterated that minor genetic mutations in the virulent strains of JEV might result in the increase of the host and vector fitness without altering clinical presentations [58]. About 68, 000 JE cases are reported each year, which results in 10,000 to 15,000 deaths in more than 20 Australasian countries [39]. The contribution of the vertebrate host to its vector mostly relies upon their frequency of host-vector contact, their abundance and competence for vector infection [62]. Researchers reiterated that the hosts might influence the ecology of arboviral transmission, cross-protective immunity and antibody-dependent enhancement, pathogen spill-over and dispersal [63]. Certain mosquito species of the genus Culex are considered as the most significant enzootic vectors [64, 65, 66]. Becoming infected and gaining the ability to transmit the arbovirus by the vector mosquito requires a convoluted set of events which include detection of appropriate host during blood-meal. Then the virus replicates in the haemocoel and the adequate replication of virions develops a suitable dose of infection for a susceptible host in the next blood meal [67, 68]. In this context, the viraemia that infects 1-5% of the mosquito vectors should be considered as a “threshold” [69]. JE vectors are found to be the opportunistic feeders in West Bengal, a JE endemic state in India [70]. JE antibody has been detected in different non-human vertebrates (Table 1). Reptiles which exhibit low viraemic levels than pigs and birds cause fewer mosquitoes to be infected. The risk of transfer of viruses between reptiles and human is negligible owing to the thermoregulatory differences between above mentioned poikilothermic vertebrates and mammalian hosts which would limit the suite of pathogens able to grow in both temperature regimes. However, some zoonotic potential exist, when certain reptiles act as reservoirs for arboviruses that are pathogenic to humans such as WNV [71]. “Reptiles and amphibians might represent overwintering reservoir hosts for Eastern Equine Encephalitis Virus (EEEV)” [72]. Chelonia and other reptiles infected by bites of mosquito vectors develop cyclic viraemia without injury. The ectothermic animals maintain inapparent arbovirus (e.g., Flavivirus) infection during hibernation and they might play role as reservoirs for...
such viruses [73]. Although the JEV infection is found in diverse non-human vertebrates in natural and laboratory condition, additional evidences like titre level duration, vector predilection, host susceptibility and immune response of the newborn host population are needed to recognize those animals to be the potential reservoirs/amplifying hosts of the virus. In natural condition, low viraemia could cause the transmission and infection to mosquitoes; however, this depends on low viraemic host numbers and dose-infection-transmission relationship, duration of viraemia and the number of the vector mosquitoes biting these low viraemic hosts. There are several examples of viruses including Dengue virus that transfer between hosts to gain new host ranges and they cause outbreaks in those new hosts [74]. Interestingly, Lord et al., 2006 [62] stated that hosts with low viraemic levels might have a greater role in spreading arbovirus than high viraemic hosts. The significant effects of global climate change may act as a selective intruder in the known path of the disease transmission cycle and can change the disease dynamics.

4. Conclusion

JE antibody has been detected in several poikilothermic and homeothermic vertebrates apart from recognized reservoir/amplifying hosts. The finding of the JE antibody however, is not itself a proof that the animal is a reservoir host of JE virus. But animal and arbovirus interaction is a dynamic phenomenon and with the passage of time some of the above mentioned vertebrates from which JE antibodies had been detected could transform in epidemiologically important ways and subsequently may attain the status of a potential reservoir/amplifying hosts of JE virus, especially in the context of changing climate and environment. Global warming is likely to reshape the ecology of many vector mosquitoes, reservoir animals and the associated virus as well. Change of climate and the effect it causes may bring genetic changes in the virus. The climate change might be associated with enhanced transmission potential, elongation of transmission season, shortening of the incubation time of the pathogen and the changes in the distribution and breeding behaviour of vector and reservoir/amplifying hosts. Some zoonotic potential might exist in the ectothermic animals as overwintering reservoir hosts for Japanese Encephalitis Virus (JEV). However, thermoregulatory differences with the homeothermic vertebrates could be a limitation to the ectothermic animals to attain the status of the potential reservoir host/s. Interestingly, low viraemic hosts could infect the mosquito vectors. Major mosquito vectors of JE are facultative feeders and act as bridge vector/s. The virus spillover to human at the time of higher than average level of virus circulation in nature i.e. during the epizootic cycling of virus between mosquito vectors and animal hosts. Mosquito species having a predilection to animal blood could get the virus from a broad array of low viraemic ectothermic and endothermic vertebrates, but possibly fail to attain the infective stage and to acquire transmission competence due to inadequate titre level and short duration of viraemia in those vertebrates. These animals might have some complementary role in the maintenance of the enzootic cycle of the virus along with the recognized principal reservoir hosts i.e., pigs and birds. The principal and complementary vertebrate reservoir hosts keep the virus circulating perennially in nature and epizootic occurs at the time of higher than average level of virus circulation. Transovarial transmission of JEV by mosquitoes has also been reported. This is possibly another important mechanism for the maintenance of the JE virus in nature, especially in the inter-epidemic period. A comprehensive study encompassing virus isolation attempts from the above mentioned suspected wild animals, monitoring the duration and level of viraemia, vector predilection and infection rate of the vector/s in different seasons are required to prove the above hypothesis.

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