Chapter 12
Japanese Encephalitis Virus

Samir Das, Rahul Kolhe, Arockisamy Arun Prince Milton, and Sandeep Ghatak

Abstract Viruses belonging to the genus *Flavivirus* of the family *Flaviviridae* are the established human pathogens and their zoonotic potential has escalated in the last few decades. They are transmitted by vectors and accordingly grouped as tick-borne flaviviruses and mosquito-borne flaviviruses. Viruses transmitted by ticks are closely related species of single sero-complex, whereas mosquito-borne flaviviruses are diverse. Yellow fever virus is the prototype of this genus. There are four important Flaviviruses associated with Japanese encephalitis sero-complex, which causes encephalitis epidemics world over. Examples include Japanese encephalitis, West Nile, Murray Valley encephalitis, and Saint Louis encephalitis viruses. Japanese encephalitis is a leading cause of neurological illness in children's aged below 15 years. It is transmitted by *Culex tritaeniorhynchus* mosquito. JEV is maintained in nature by ardeid birds and pigs (both domestic and wild pigs). There are two enzootic cycles of JEV transmission, i.e. pig-mosquito-pig and bird-mosquito-bird cycle. The ardeid birds are the natural reservoir maintaining the JE virus, whereas pigs are the amplifier host. The disease is endemic in South-East Asian countries, and the highest numbers of deaths are recorded in India. JEV is considered as emerging pathogen due to changing epidemiology. JEV is endemic in 24 countries, and most of them are Asian countries. JE is spreading in new area owing to climate change, expansion of vector range, increase in pig husbandry, and population explosion. Introduction of JE vaccines has curtailed down the incidence of JE to a great extent in several endemic countries. India has also recommended JE vaccination for children in the endemic regions. Although immunization of humans is an effective strategy for JEV prevention, its control is challenging due to the existence of different transmission cycles. Especially, risk from the ardeid birds is unpredictable and cannot be controlled. Few countries have attempted JE

S. Das (✉) · A. A. P. Milton · S. Ghatak
Division of Animal Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India

R. Kolhe
Department of Veterinary Public Health, KNP College of Veterinary Science, MAFSU, Shirwal, Maharashtra, India

Y. S. Malik et al. (eds.), *Animal-Origin Viral Zoonoses*, Livestock Diseases and Management, https://doi.org/10.1007/978-981-15-2651-0_12

vaccination in porcine population as a strategy for JE prevention but it is not universally adopted. Co-circulation of different flaviviruses in nature makes the diagnosis and prevention of JE challenging in the endemic countries. In the context of global warming and climate change, it is mandatory to consider JE in One Health paradigm.

Keywords Japanese encephalitis · Japanese encephalitis virus · Ardeid · Encephalitis · Acute encephalitis syndrome · Flavivirus · Zoonoses · *Culex tritaeniorhynchus*

12.1 Prologue

Japanese encephalitis (JE) is an emerging vector (mosquito) borne zoonotic disease caused by the Japanese encephalitis virus (JEV) responsible for encephalitis (acute inflammation of the brain) in horses and humans. Swine act as an amplifier host for JEV, and occationally JEV can cause abortions and stillbirths in pigs. Around three billion people are living in endemic zones and are at risk of JE. JEV is the reason for 35,000–50,000 human cases annually and is responsible for ~10,000 human deaths throughout the globe, though the majority of the cases come from Asian countries (Campbell et al. 2011; CDC 2019). The global incidence of JE is 1.8/100,000 which increases with the inflow of tourists travelling from epidemic areas to the non-endemic area bringing new infections and thus making it an international public health issue (Gao et al. 2019; Campbell et al. 2011). Of the infected cases, approximately 25–30% succumbs to death and about 30–50% people recovered and survived may suffer from permanent neurological sequelae. Living close to paddy cultivation, pig farming, and water lodging are some of the predisposing factors for increased risk of JE in humans. Such factors facilitate the close contact of vector and host. JE is a classic example of Flavivirus infection widespread across the countries of Asia, Western Pacific, and parts of Australia. The disease is hyperendemic in the worlds most populated countries, namely China and India. JE was once considered as a disease of children, but JEV can cause illness and deaths in adults. A survey conducted in Korea revealed that foreign expatriates living in Korea are at more risk of JE with more incidence rate as compared to the native people (Shin et al. 2018). The age factor is crucial in the JE epidemiology and consideration should be given to the adult age group while designing vaccination strategies. It was observed that median age Korean JE infected cases had been increased from 49.8 to 53 years from 2007 to 2015 (Sunwoo et al. 2016). Three JE cases of the US citizens who visited JE endemic countries, viz. Taiwan, China, and South Korea have suffered from clinical JE. All the three US citizens were healthy before their visits to the endemic nations and after return to home country suffered from the disease. Out of them, one died of the disease, and two were completely recovered without any neurological sequelae (Hills et al. 2014). Thus, differential diagnosis of JE is important in the travellers returning from the JE endemic countries.

Similarly, counselling on the prevention and control measures of JE must be done in the travellers. It is well known that JEV epidemiology is changing at a great pace. The complex of multiple factors like introduction in new geographic areas, non-vector risk of spread, age factor, currently available vaccines and vaccination protocol, agricultural practices, migration of birds, etc., makes JE as an important zoonotic disease with potential risk to humans globally (Connor and Bunn 2017).

12.2 Etiology

JE is a zoonotic viral disease of humans and horses. It is caused by the JEV which is a prototype species of JE sero-complex of mosquito-borne flaviviruses. The JEV has an RNA genome which is single-stranded and positive-sense. It is comprised of a short 5′ untranslated region, a single open reading frame (ORF), and a longer 3′ untranslated region. The polyproteins are encoded by the ORF which further cleaved into structural and non-structural proteins by host and viral proteases. Capsid protein (C), pre-membrane protein (PrM), and envelope protein (E) are the three structural proteins, whereas NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are seven non-structural proteins (Unni et al. 2011; Qiu et al. 2018; Solomon et al. 2003). The JEV belongs to the genus *Flavivirus* of family *Flaviviridae*. The virus is small-sized around 40–50 nm and is enveloped. Structurally it is spheroid with cubical symmetry, and recently, JEV capsid protein crystal structure has also been described (Poonsiri et al. 2019). The positive-sense single-stranded RNA has a genome size of 11 kb with one open reading frame (ORF) that encodes a polyprotein. The genome is covered by a capsid and a host-derived lipid bilayer. The ORF flanked with non-coding region 5′ and 3′ at either side is required for viral replication, transcription, and translation. Envelope protein is a major viral protein playing important role in the virulence, host cell entry, and humoral immune response. NS1 is linked with replication. NS2A is cleaved from NS1 by host proteases, and NS2B is a cofactor of viral serine protease. During virus assembly NS3 acts as a reservoir for viral proteins, NS4 is an important membrane component, and NS5 is an essential component of virus replication complex (Sahoo et al. 2008). Clinically and ecologically, JEV is much closely related to West Nile virus and Saint Louis encephalitis virus. It is assumed that all the closely related flaviviruses must have evolved from the common ancestor's way back some 10–20 thousand years and later separated and adapted different ecological positions (Solomon et al. 2000).

Nucleotide sequencing of the capsid (C), precursor membrane (PrM) and envelope (E) genes, and its further phylogenetic analysis revealed the existence of five genotypes (I, II, III, IV, and V) of JE virus and all these genotypes form a single serotype (Banerjee 1996; Uchil and Satchidanandam 2001; Desingu et al. 2017). These five genotypes have been isolated from different parts of the globe with genotype I being isolated from northern Thailand, Cambodia, and Korea; genotype II from southern Thailand, Malaysia, Indonesia, and Australia. Genotype III from China, India, Japan, Korea, Taiwan, Sri Lanka, Philippines; genotype IV from

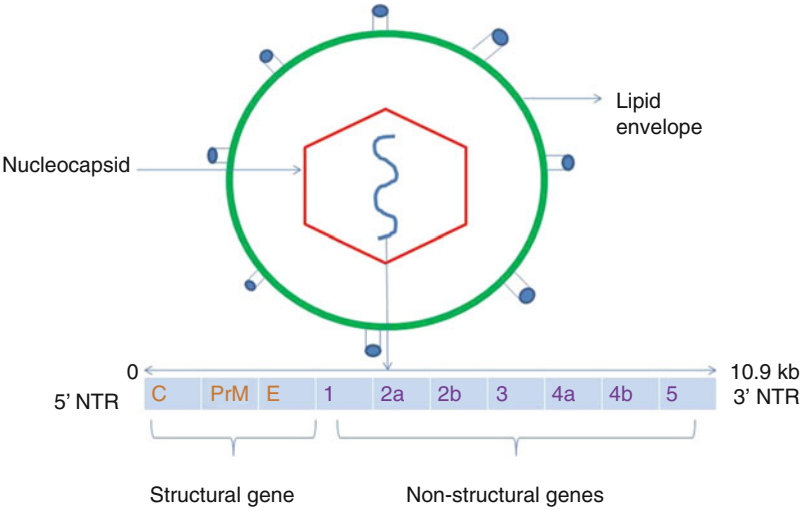

Fig. 12.1 Structure and organization of JE viral genome

Indonesia (Chen et al. 2019) and genotype V from Malaysia (Uchil and Satchidanandam 2001; Solomon et al. 2003). Earlier, genotype III was the most prevalent genotype in humans which is now replaced by genotype I and this shift has been observed in the major JE reporting countries, viz. China, Japan, and Korea (Simon-Loriere et al. 2017; Nga et al. 2004; Wang et al. 2007; Nitatpattana et al. 2008; Pan et al. 2011; Gao et al. 2019). The prevalent genotype I can be further categorized into genotype I-a and genotype I-b clades (Schuh et al. 2014). The earlier dominant genotype III may have less occupancy in Asia as compared to genotype I in present time, but genotype III has spread to other continents like Europe and Africa. Genotypes II and V which were endemic in Malaysia were also spreading to other parts, viz. genotype II to Australia, and genotype V to China and South Korea (Gao et al. 2019). Study on the origin and evolution of JEV suggested that genotype IV has ancient lineage than other genotypes. Similarly, phylogeny of E protein revealed Muar strain as genotype V. It was also stated that JEV genotypes I, II, and III must have diverged recently, while genotype IV diverged 350 (±150) years ago from common ancestors (Solomon et al. 2003) (Fig. 12.1 and Table 12.1).

12.3 History of JEV

The serological surveillance carried out by Mitamura and colleagues in Japan in the 1930s showed that various mammals, such as horses, pigs, goats, rabbits, and sheep, had antibody reactions against JEV (Morita et al. 2015). After that, in the year 1937, the virus was isolated from the brain of a horse suffering from encephalitis in Japan. The role of the mosquito vector (*Culex tritaeniorhynchus*) for JEV transmission was

Table 12.1 Genes and amino acids of the genome of JEV vaccine strain SA-14-14-2

Gene	Nucleotide sequence length (bp)	Amino acid
5′ Non-coding region	95	Nil
Capsid	381	127
Pre-membrane/membrane	501	167
Envelope glycoprotein	1500	500
Non-structural 1	1245	415
NS2a	492	164
NS2b	393	131
NS3	1857	619
NS4a	801	267
NS4b	411	137
NS5	2715	905
3′ Non-coding region	582	Nil
	10,973	3432

documented in the year 1938 (Mitamura et al. 1938; Erlanger et al. 2009). Genetic studies of JEV lead to the finding of its origin to the Malay Archipelago—an area between the mainlands of India, China to Australia. This ancient JE virus then evolved into the many present genotypes and travelled across Asian countries and is putting its foot to European counties also (Solomon et al. 2003; Schuh et al. 2013). Globally, the disease is endemic in parts of China, India, South Korea, Japan, Nepal, Vietnam, Indonesia, Philippians, Taiwan, Sri Lanka and is being reported from many other countries of Asia and spreading to Russia and European countries.

The first extensive research findings on the origin and evolution of JEV was published by Solomon et al. (2003). Their study suggested that JEV genotypes originated from Indonesia and Malaysia regions from the ancestor viruses. It was further stated that South-East Asian regions could be the hotspots for the emergence of viral pathogens. Historically, endemic genotype III has been replaced by genotype I in many parts of the JE endemic regions. Genotype V of JEV is not extensively reported and others that Muar strain isolated in 1952 in Malaysia, it was not reported at other places. The genotype V of JEV was detected in *Culex tritaeniorhynchus* mosquitoes in 2009 from China. This strain was designated as XZ0934. The study suggested the re-emergence of JEV genotype V in Asia (Li et al. 2011). A study from the Republic of Korea has also detected JEV genotype V for the first time in *Cx. Bitaeniorhynchus mosquitoes (*Takhampunya et al. 2011*).* JEV genotype I was found to be the predominant genotype in Asia as studied by Pan et al. (2011). As per this study, JEV genotypes diverged over some time in the following order as: JEV genotype IV, genotype III, genotype II, and genotype I. Gradual increase in the genetic diversity of genotype I is consistent and thus it is a predominant genotype at present in Asia. The estimated years of occurrence of genotypic diversions in the JEV recorded were 1695 years ago, 973 years ago, 620 years ago and 193 years ago in for the JEV genotypes IV, III, II, and I, respectively (Pan et al. 2011). Phylogenetic analysis of JEV genotypes based on the whole genomic sequencing

of all five genotypes revealed that 1930–1960 and 1980–1990 are the periods of peak genetic diversity and after 2000 it remains high (Gao et al. 2015). Clinical illness resembling the JE viral infection dates back in the nineteenth century. It was responsible for summer encephalitis in Japan, and first clinical case of JE was documented in 1871. Recurrent JE outbreaks were recorded during 1930s in Japan almost after every 10 years. In 1935, JEV was first time isolated from the brain which is known as Nakayama strain, a prototype strain of JEV. The role of vectors, reservoirs, and amplifiers was documented in the year 1938 (Tsai 1997).

12.4 Host Range

The reservoir maintenance host of JE virus is ardeid birds, the reservoir amplifier host pigs, and the accidental dead-end host is human and horses. In equid, family donkeys are also susceptible to JEV. Bovine, ovine, caprine, dogs, cats, chickens, ducks, wild mammals, reptiles, bats, and amphibian can get sub-clinical infection, but they probably do not contribute to the spread of JEV (Yang et al. 2011; Xiao et al. 2018). The epidemiology and ecology of JEV are complex, and several epidemics were documented in the absence of amplifying host pigs. JEV has proven hosts as birds as reservoirs, pigs and Ardeidae birds as amplifiers, and human and equines as dead-end hosts. However, serological evidence of JEV antibodies in other animals like cattle, chickens, ducks, bats, small ruminants, dogs and cats, amphibians like frogs, monkeys, raccoons, etc., highlighted the need for exploring the role of animal species other than pigs and birds in the JE transmission (Shimoda et al. 2011; Bhattacharya and Basu 2014). The potential of bats in JEV transmission has already been documented (Mackenzie et al. 2008). Recently, a study from Malaysia has reported high prevalence of JE in dogs and followed by pigs, cattle, cats, and monkeys (Kumar et al. 2018).

12.5 Geographical Distribution

Infection due to JEV has been reported from several countries, viz. China, India, Japan, Bangladesh, Australia, Burma, Indonesia, Vietnam, South Korea, North Korea, Nepal, Sri Lanka, Pakistan, Philippines, Malaysia, Thailand, Taiwan, Timor-Leste, Papua New Guinea, Russia, Saipan, Singapore, Cambodia, Guam, Laos, Brunei. JE is considered as an emerging zoonosis and is rapidly spreading to new regions (Park et al. 2018; Zhao et al. 2018; CDC 2019; Yap et al. 2019). Here we will elaborate the JE scenario in those countries where JE incidences are more or emerging. More than 95% of JEV cases are seen from China and India, the two highest populous countries of the globe. The JE epidemiological pattern is epidemic and endemic in northern and southern parts of the world, respectively. Bangladesh, China, Taiwan, Japan, South Korea, India, Thailand are the countries where the

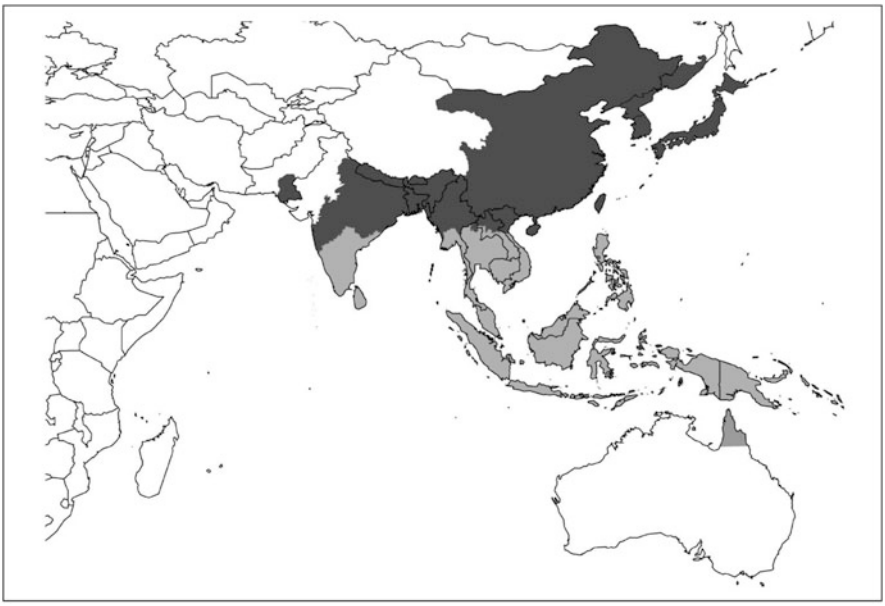

Fig. 12.2 Global distribution of Japanese encephalitis (Dark areas represent epidemic pattern of JE; light grey colour represents endemic pattern of JE)

epidemic pattern of JE is recorded. While endemic pattern is recorded in countries like Australia, Cambodia, Indonesia, Laos, Malaysia, Vietnam, Sri Lanka, and Timor-Leste (Wang and Liang 2015). As of today, JEV infection has been detected in 27 countries globally (Figs. 12.2 and 12.3).

India JE is endemic to the country and epidemics are reported from most of the Indian states, mainly from plain belts and emerging in hilly states. It is a major paediatric problem with cases of adults also coming forward (Kulkarni et al. 2018; Baruah et al. 2018). First clinical case of JE was reported from Vellore, Tamil Nadu in the year 1955 (Namachivayam and Umayal 1982) and thereafter India recorded the first major outbreak from Burdwan and Bankura districts of West Bengal in the year 1973 with 700 cases taking a toll of 300 human lives (Banerjee 1996). Since then, the virus is active in almost all parts of India, especially from rural parts of the country with regular reports from Uttar Pradesh, Bihar, Tamil Nadu, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Haryana, Kerala, West Bengal, Orissa, Goa, and Pondicherry (Kabilan et al. 2004). Gorakhpur district of Uttar Pradesh is the worst affected district in India. Major outbreaks of JE were reported in the years 1978, 1988, and 2005 with more than 1000 deaths in each of the outbreak (Tiwari et al. 2012). Presently, JE is not only endemic in many areas; it is also spreading to naive non-endemic areas, viz. hill states of North Eastern part of the country. Outbreaks recorded in Malkangiri, Orissa state and Manipur state in 2012 and 2016, respectively, are the examples. In India, 24 states/Union territories have

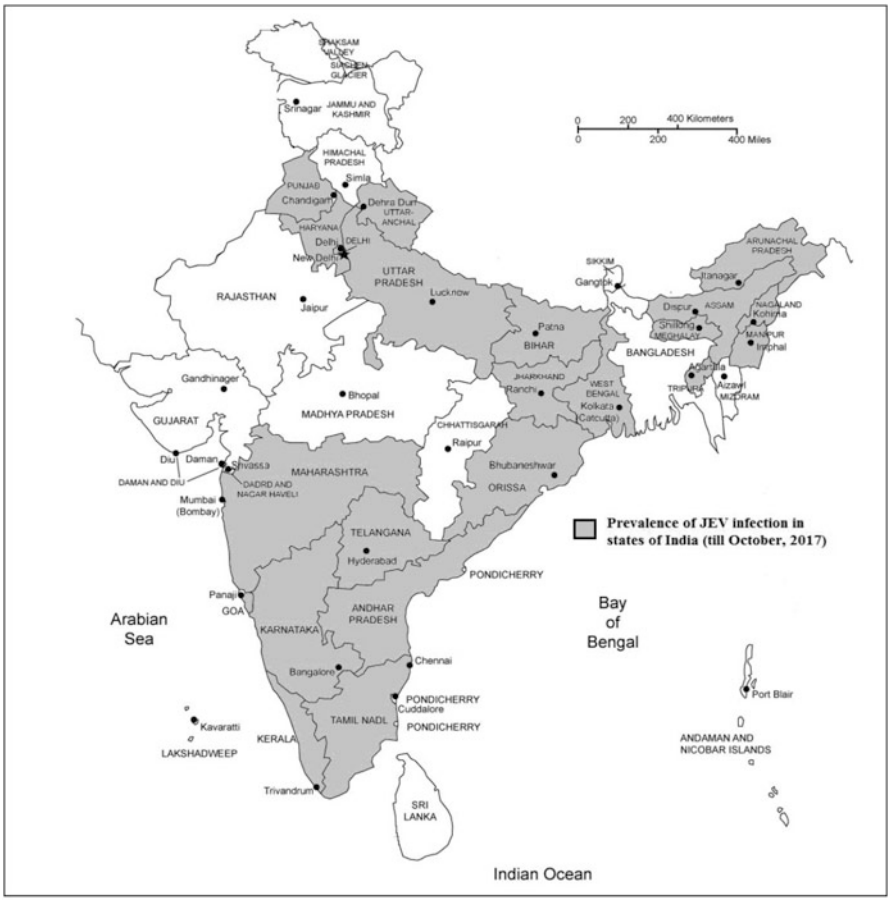

Fig. 12.3 Prevalence of Japanese encephalitis in India

reported JE (Rao et al. 2000). India is following strategies of human vaccination and vector control programme. There is no pig or equine vaccination available in India. In India, JE vaccination has been introduced in 2006 for children aged 1–15 years. This vaccine was included in the National Immunization Programme by Government of India in 2014. The districts where JE is endemic, the SA-14-14-2 JE vaccine are being used as a part of Universal Immunization Programme. More than 11 crore children's from identified JE endemic districts are immunized in India. Simultaneous detection of JEV genotype I and genotype III from cases of acute encephalitis syndrome (AES) in 2009 Gorakhpur outbreak was recorded by Fulmali et al. (2011). JE cases are mostly reported during monsoon and post-monsoon periods due to vector abundance.

Japan The name of JE is originated from Japan, and the first JEV Nakayama strain was isolated in 1935.The cases of JE have been drastically reduced in Japan in the last three decades after the implementation of National Surveillance of vaccine-preventable diseases since 1965. The figures of JE cases were above 1000 per year during the 1960s and surprisingly, during 1982–2004 only 361 cases were reported (Arai et al. 2008). The age-wise distribution revealed 78% of the JE cases were above 40 years of age (Matsunaga et al. 1999). Since 2005, inactivated Vero cell-derived JE vaccine (Beijing-1 strain) is in use. *C. tritaeniorhynchus* is the main vector in Japan and epidemics were reported from July to November. JE surveillance shows that JE is still prevalent in Japan but with combined efforts of vaccination, mechanization of rice cultivation, and mosquito control programme, the country has controlled JE to a great extent (Konishi et al. 2006; Ayukawa et al. 2004; Wang and Liang 2015; Nanishi et al. 2019). July to November is the peak season for JE occurrence in Japan, and it is predominantly observed in unvaccinated individuals only. Japan has set a very effective model of mass vaccination to control the JE. As per the South-East Asian Regional Office of the WHO, health education and training is the recommended strategy for JE prevention and control.

China China is one of the hyperendemic countries of JE. The disease is responsible for thousands of cases annually since 1943. The JEV was isolated in 1949, and until now numerous JEV strains were isolated from human, animals, mosquitoes, etc. Due to the endemic status and clinical spectrum, JE has been declared a notifiable disease in 1950. During 1950–2011 approximately two million JE cases were documented from 26 provinces of China. After the introduction of immunization the incidence has been declined significantly (Gao et al. 2014). China has developed P3 inactivated JE vaccine which was in use for the immunization. Later in 1988, the live-attenuated SA-14-14-2 vaccine was licensed and still used for JEV immunization. This vaccine is prepared by four companies in China for domestic as well as export purpose. The first report of JE from China came in the 1940s, with reporting of 10–15 cases per 100,000 in 1960–1979. Children upto15 years of age constitute the majority of JE cases in China. The peak JE season is from July to August and cases were seen even 1 month before and after the peak season. JE vaccination was introduced in China from the 1970s with mouse brain-derived JE vaccine (MBD) (P3 strain) and later with the live-attenuated SA 14-14-2 in 1989, and the same was used in National Immunization Programme since 2008 (Wang and Liang 2015). There were around 10,308 reported JE cases in 1996 which got decreased to 2541 cases in 2010 (Shi et al. 2019). JE is reported from the Tibet region also (Zhang et al. 2017).

South Korea Since 1993, JEV III has been completely replaced by genotype I in Korea. Genotype V was also isolated from *Culex* mosquito in this country. Due to vaccination policy adopted for infants, JE incidence is very low in Korea. Similarly, JE in swine is a notifiable disease of animals in Korea and sow vaccination is compulsorily done. The vaccine strain Anyang 300, G3 is being used for swine vaccination for the last 30 years (Nah et al. 2015). With the detection of the first human case of JE in 1946, the government started to include JE in national

surveillance system from 1949 and later in the year 1971 JE vaccination with the MBD was started with children with mass vaccination programme taking place since 1983 after a large outbreak in that year. These mass vaccination efforts could be seen in the decrease of JE annual cases from 100 to 1000 cases (before 1983) to 10 annual cases thereafter (Lee et al. 2012). August to October is the season of JE with *C. tritaeniorhynchus* as a principal vector (Wang and Liang 2015; Bae et al. 2018). JE sero-monitoring is mandatorily done in Korea, and since 2007, JE outbreak is not notified in pigs in Korea.

Vietnam The first isolation of JE virus was recorded in the year 1951. During the 1960s as high as 22 human cases per 100,000 were recorded, this later decreased to 1–8 per 100,000. JE surveillance is a part of their national surveillance system. In the year 1997, JE vaccination was initiated for children in the 12 high-risk districts with MBD vaccine which was later expanded to 65% of districts of Vietnam (Yen et al. 2010; Wang and Liang 2015).

Thailand In Thailand, JEV immunization began as a part of the childhood vaccination programme in the Northern provinces in 1990; this programme rapidly expanded to 36 provinces that had reported a persistent incidence of encephalitis. Study of Nitatpattana et al. (2008) conducted on the pigs and mosquitoes samples collected from the JEV confirmed human cases revealed co-circulation of genotype I and genotype III of JEV. It was further stated that genotype III is getting replaced by GI. Serological evidence was there since 1961. Encephalitis cases (JE included) were recorded in the database for routine disease surveillance in Thailand. Epidemics in Thialand were seen mainly from May to September, with record of sporadic cases (occurring throughout the year). *C. tritaeniorhynchus*, *C. gelidus*, and *C. fuscocephala* are the suspected vectors for transmission of JE in Thailand. Annually around 1500–2500 encephalitis cases were reported between 1970s and 1980s, which got decreased to 297–418 cases recorded during 2002–2008. JE was recorded more in children. The MBD JE vaccine was introduced in 1990 and at present after successful trial of chimeric live-attenuated vaccine strain (SA-14-14-2) in Thailand got recommended by WHO and had been in practice (Appaiahgari and Vrati 2010; Wang and Liang 2015).

Nepal JE has been transmitted from northern India to Nepal, and the first case was detected in the Terai region in 1978. JE is presently endemic in Nepal and outbreaks are recorded every 2–3 years span. Nepal is a hilly country, and the cases were seen mainly in low hill relatively plain areas (Bhattachan et al. 2009). July to October is the main season for JE epidemics. Between 2005 and 2010, a total of 2040 JE cases with 205 deaths were recorded in Nepal. JE mass immunization with the live-attenuated SA-14-14-2 was carried in 2006 in the epidemic area which decreases the incidence rate to 1.3 per 100,000 (Wierzba et al. 2008). Pig vaccination was initiated in the Terai districts in 2001 with the live-attenuated virus (Wang and Liang 2015). In the last 25 years, over 26,000 cases and 5000 deaths are attributed to JE in Nepal. Out of 75 districts, JE cases were recorded in 54 districts (Ghimire and Dhakal 2015). Rice cultivation, pig farming, and other climatic factors favour the existence of JEV in Nepal.

Myanmar After serological evidence in 1968, the first JE outbreak was recorded in 1974 and July to October is the season of JE in Myanmar. Majority cases were in children and teenagers. *C. tritaeniorhynchus* is the vector being suspected and the seropositivity is seen in domestic animals and human in the country (Wang and Liang 2015).

Singapore The first JE cases were reported in 1952, and after that, 100 cases were recorded during the 1970s to the early 1980s, and another 12 cases during 1985–1992. In 1992 Singapore completely closed pig farming from the state and after which the incidence of JE decreased considerably with only 06 cases are reported from 1991 to 2005. However, JEV is still being circulated in the wild boars with sero-evidence and it has been isolated from mosquitoes and human blood indicating possible future threats (Wang and Liang 2015).

Indonesia JEV circulation was first documented through serosurvey in the island of Lombok in 1960 and the later virus was isolated from vector *C. tritaeniorhynchus* in 1972. In a hospital-based survey between 2001 and 2003, the JE incidence rate was recorded to be 8.2 per 100,000 in children below 10 years. Another hospital-based survey involving 15 hospitals covering 06 provinces during 2005–2006 confirmed the presence of JE cases in all provinces and throughout the year, with majority of cases in children under 10 years. JE is endemic in Indonesia with 32 of 34 provinces reporting JE cases which are occurring throughout the year with the peak in rainy season (Garjito et al. 2019). Sero-surveillance in pigs for JE antibodies revealed higher antibody rate in Bali Island than East Java (Wang and Liang 2015). Indonesia is looked close for JE as its location is geographically close to the place where ancient JE virus originated, i.e. Malay Archipelago.

Malaysia The first human record of JE goes back to 1942 in Malaysia with first human isolation in 1951. JE major outbreaks were recorded in the year 1974 and subsequently in 1988, 1992, and 1999. During the last major JE outbreak in 1999, there were 154 JE cases with 42 being confirmed and 56 deaths. The majority of the cases were confirmed from the pig handler working at the farms. The MBD JE vaccination programme introduced in the year 2001 for children under 15 years reduced the JE incidence from 9.8 to 4.3 cases per 100,000 children under 12 years of age. *C. tritaeniorhynchus* and *C. gelidus* are the main vectors in Malaysia (Wang and Liang 2015). Sarawak state is the most affected part of the country; otherwise JE is not regarded as a major public health issue in Malaysia.

Bangladesh The first report of the JE outbreak in Bangladesh came in 1977 with 22 cases and 07 deaths mainly affecting the children. After that, low seropositivity was recorded, and majority of cases are from a rural area. May to October is the season of JE in Bangladesh (Wang and Liang 2015).

Australia JE was first reported in 1995 from human in Torres Strait inhabitant in the mainly aboriginal population (Hanna et al. 1996). After that, sero-evidence was detected in pig population with isolation from mosquito also. *C. annulirostris* is the major vector in Australia. JEV had become endemic in the Torres Strait as per survey

reports conducted between 1995 and 2005 (van den Hurk et al. 2019). The MBD vaccine is used from 1995 and now carried exclusively for Torres Strait Islands residents, and visitors (Wang and Liang 2015).

Sri Lanka JE presence was recorded since 1968 in Sri Lanka, and the first JE outbreak was recorded in 1971 with first isolation in 1974. Thereafter, three major outbreaks were recorded between 1985 and 1987 and that too in the winter months. *C. tritaeniorhynchus* and *C. gelidus* were the suspected vector. JE immunization programme was launched in 1988 using the MBD and the live-attenuated SA14-14-2 vaccine, and later they carry forward only the MBD vaccine (Wang and Liang 2015).

12.6 Transmission

JE virus is maintained in nature between culicine mosquitoes (vector), ardeid birds (reservoir), and pigs (amplifier host). Human acts as an incidental dead-end host and infection is acquired through mosquito bite harbouring JEV contracted from pigs or birds. Birds like cattle egret and heron saw in the rice field are the maintenance reservoir of the virus (Acha and Syfrez 2003). Viremia range in egret goes up to $10^{2–4.2}$ plaque-forming units (PFU)/ml which show the potential viremia in egret, whereas in chicken (*Gallus domesticus*) the viremia is $10^{1.7}$ which is considered low to infect the vector (Nemeth et al. 2012; Preziuso et al. 2018).

Swine acts as amplifier host and plays a crucial role in the transmission of JEV as they develop high viral load with long viremia after natural infection with JEV and the vector gets enough opportunity to get the JEV lading to further transmission of virus to human living in their close proximity (Diallo et al. 2018). Pigs once infected carry the viremia for 05 days and thereafter become immune lifelong. But as the herd replacement of pigs is fast and every year newborn non-immune population is being built up, so the virus always gets a naïve population who is ready to be infected. Maternal antibodies in piglet can protect for up to 4 months against JE. The 4–6-month-old pigs which have now lost the maternal antibodies and are non-vaccinated are crucial for taking up the natural infection from the mosquitoes. Vector free transmission has also been recorded in experimentally infected pigs and mice (Chai et al. 2019). Equine and human are dead-end hosts as the viremia is transient with low viral load in the peripheral blood which is not sufficient to be carried over by vector (Niazmand et al. 2019). The virus had been isolated from bats (Liu et al. 2013) and ducks (Xiao et al. 2018).

Among mosquito, *Culex* is predominantly responsible in Asia, and *C. tritaeniorhynchus* is the commonest species responsible for the transmission of the disease (Fang et al. 2019). Other species of mosquito similar to *C. tritaeniorhynchus* which lays eggs in the paddy field can also harbour JE virus. JEV is persistent and non-pathogenic to susceptible cells of mosquitoes. In that respect, one has to consider the long co-evolution of flaviviruses in mosquitoes with persistent infection. During JE epidemic season the vector (mosquito) breeding

ground is being noticed in waterlogged rice fields, irrigation channels, ponds, drains, etc. (Gao et al. 2019; Pearce et al. 2018). The active mosquito time is dusk and dawn, and hence control measures like fogging will be effective at this period effectively. The mosquitoes have preferential biting to pigs and limited in cattle (Oliveria et al. 2018). Pig sero-surveillance is used for epidemiological studies but if seropositivity is seen in cattle than it indicates active infection in that area. Mosquito life span is from 10 days to 8 weeks and can hibernate up to 6 months, so once the mosquito gets the JE virus then it can carry it for long time. Vertical transmission is being recorded for JE Virus in mosquitoes (Rosen et al. 1989). In JE transmission *C. tritaeniorhynchus* is considered the most important vector mosquito. The mosquito and even this *C.tritaeniorhynchus* can survive the winters by hibernation and it can carry over the JE virus present in it which they acquired after feeding on a viraemic host before entering hibernation. This phenomenon of carrying over the virus to next winter through infected mosquito is known as overwintering (Karna and Bowen 2019). Experimentally infected *C. tritaeniorhynchus* and *C. quinquefasciatus* have shown to transmit the JE virus to susceptible hosts by overwintering phenomena (Hurlbut 1950; Mifune 1965). A mosquito can travel 1–2 km in still air condition but can be blown through high winds or through vehicular or aircraft transport can travel long distance (Ritchie and Rochester 2001). A study in China calculated potential dispersal of *C. tritaeniorhynchus* up to 200 km per (Ming et al. 1993). The saliva of infected mosquito contains very high concentration of virus up to $10^{4.2}$ SMIC-LD50/1 mL of saliva and virus diluent is being recorded (Takahashi 1976). Birds (ardeid and heron) usually have a viremia of $10^{3.5}$ suckling SMIC LD50/0.03 mL of blood (Buescher et al. 1959; Scherer et al. 1959b) which is sufficient to infect mosquito. Even the highly competent *C. tritaeniorhychus* can be infected with low doses of virus $10^{1.0–3.5}$ suckling mouse intracerebral (SMIC) LD50 (lethal dose 50%)/0.03 mL of blood after feeding on infected birds (Hale et al. 1957; Gresser et al. 1958; Hill 1970; Takahashi 1976; Burke and Leake 1988). Pigs have even high viremias of 10^6 SMIC LD50/mL of blood which last from 24 h post-infection to 05 or more days. Furthermore, almost all domestic pigs irrespective of breed and even wild boar are capable of infecting mosquitoes (Gresser et al. 1958; Scherer et al. 1959a, b).

JEV have been isolated from around 30 species of mosquitoes, with *C. tritaeniorhynchus* being the major vector along with involvement of some other species like *C. gelidus, C. vishnui, C. pseudovishnui, C. whitmorei, C. epidesmus, C. quinquefasciatus*, *Mansonia indiana, M. uniform, Anopheles subpictus, A. peditaeniatus,* etc. (Kanojia et al. 2003; Lindahl et al. 2012).

Two basic cyclic transmission pattern of JEV exists between mosquito, pig, and human, i.e. synchronous infection in pigs and asynchronous infection in pigs. In synchronous infection of pigs few mosquitoes infects few numbers of pigs say 20% in the initial first outbreak, and then gradually number of mosquitoes get the JEV infection and it infects almost all pigs say 100% and a large population of mosquito again suck the blood from this huge pig population infected with JEV and a huge build-up of mosquito with JEV is now ready to infect human being and this is the

stage for epidemics. Another asynchronous infection in pigs is when during initial first outbreak when few mosquitoes infect few pigs say 20% and then more mosquito starts building the JEV infections and ready to infect pigs but these mosquito are not getting enough chance to bite or infect new pig population because these pigs are being protected through various means of mosquito control or vaccination which does not allow more build-up of JEV infected mosquito and here human outbreak does not occur (Impoinvil et al. 2012).

Two basic epidemiological patterns of JE, namely epidemic and endemic, are being documented. Epidemic patterns demonstrate typical seasonal characteristics mainly in the summer/monsoon season with occasional outbreaks and are seen mostly in northern areas (Northern India, China, Japan, Korea, Nepal, Bangladesh, Bhutan, Taiwan, Pakistan, Northern Vietnam, Northern Thailand, and Russia). Endemic patterns seen in tropical region and shows sporadic JE cases throughout the year and found in southern areas (Southern India, Sri Lanka, Burma, Brunei, Australia, Cambodia, Indonesia, Malaysia, Laos, Papua New Guinea, Philippines, Singapore, Southern Vietnam, Southern Thailand, and Timor-Leste) (Wang and Liang 2015). Important factors in endemic area are rice farming in larger area following traditional farming practices, vector population density, and stagnation of surface water due to improper drainage due to flood, post-rainy season, break-down of municipality services, and lack of personal care against mosquito (Witt et al. 2011). The increase in JEV activity in newer areas has been attributed to the demographic pressure of human population, intensification and expansion of rice farming, increase in pig husbandry, and introduction of vector owing to climate change, deforestation, urbanization, and increasing regional and global trade (Mackenzie et al. 2008). Other reasons for JE outbreak and spread are the lack of potential vaccination programmes and proper surveillance in these areas (Fig. 12.4).

12.7 Pathogenesis in Human

Humans are usually infected through bites of JEV infected mosquitoes. Incubation time and appearance of first symptoms take 5–15 days (Ghosh and Basu 2009). With limited details available for the early events in JE, it is anticipated that the virus infects local cell, viz. fibroblasts, endothelial cells, pericytes macrophages, and dermal dendritic cells in the skin where mosquito bite has occurred, and there the first round of virus amplification takes place. After that, the virus spreads to the brain, via newly produced virion particles, or by migratory infected immune cells, viz. dendritic cells and T-lymphocytes, which release infectious virions at their target location (Wang et al. 2017). How JEV crosses the blood–brain barrier and infects other brain cells is also not so well defined. Mouse model studies show that the virus enters the brain infect the neurons and tissue-damaging inflammation leads to breakage of the blood–brain barrier (Liu et al. 2018). Two possible mechanisms of how JEV enters the brain tissue in which first says endothelial cells of the brain capillaries may be infected with JEV, without being functionally affected, able to

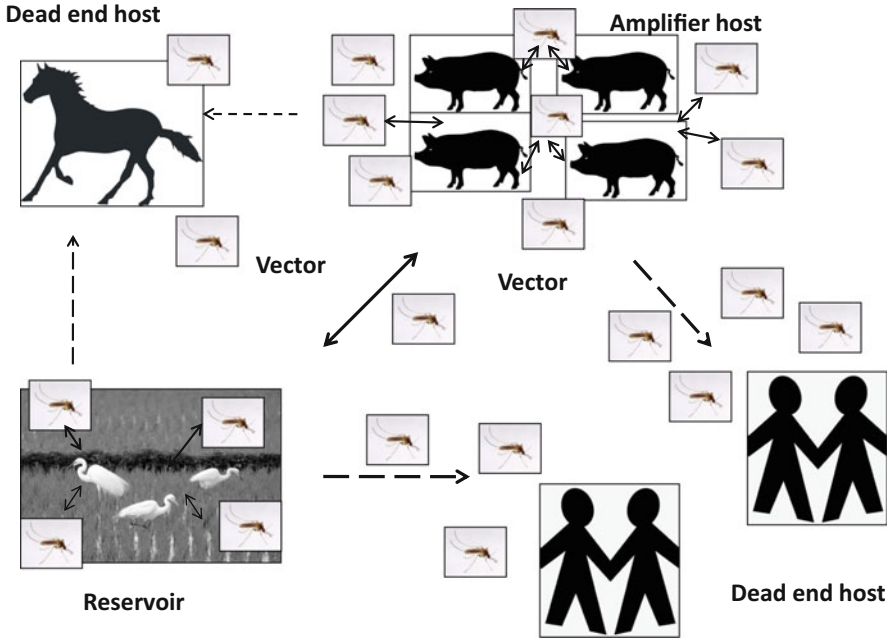

Fig. 12.4 Enzootic cycles of JEV transmission

sustain the blood–brain barrier and subsequently pass the infection to underlying microglial cells and astrocytes, which pass it further on to neurons. Secondly, JEV infected immune cells may enter through known physiological ways into the brain as in the healthy individual, that is, via the choroid plexus into the ventricular space from where they may spread the infection in the brain tissue. Thereafter, the breakdown of the blood–brain barrier may be only secondary to the infection of nerve tissue cells and after the anti-viral and inflammatory response (Filgueira and Lannes 2019). Usually, encephalitis is the most severe clinical appearance of JEV infection with a variety of first symptoms, including seizures, as well as acute sensory and neuromuscular functional deficiencies. In response to JEV infection in brain the immune cell responses may clear the infection with minimum damage in some patients but 20–40% of patients suffer from severe encephalitis and neuronal infection, and this immune response may damage key centres of the brain with long-term deficiencies or a fatal outcome. Pathological symptoms are mainly seen in brain with inflammation and congestion of grey matter showing confluent areas of haemorrhage and focal, punched out necrosis, infiltration of meninges and perivascular areas with mononuclear cells (Kumar et al. 2019). The cerebral cortex shows microglial infiltration with circumvascular necrolytic zones with total loss of neurons, whereas the white matter is fairly well preserved (Chauhan et al. 2017).

12.8 Clinical Symptom

The incubation period of 5–15 days is seen for JEV infection with the asymptomatic outcome or leads to febrile aseptic meningitis or encephalitis. The course of encephalitis and the illness can be divided into three stages—prodromal, acute encephalitic phase, and a convalescent phase. The symptoms start suddenly with a high grade of fever with a headache, which is occasionally associated with vomiting and diarrhoea. The patient and here most often children start showing seizures and that too tonic spasms, and in a matter of hours to few days the patient may go to coma and even death. Hyperventilation raised intracranial tension, shock, with death of patient is seen. Gastric haemorrhage is one of the common signs seen in seriously ill children during terminal stage. Around 33% of children affected with JE use to die during the acute stage, and many even could not reach the hospital because of very quick development of symptom and going to terminal stage in short period. Rest cases have equal fate of recovery or prolonged convalescence. These cases of prolonged convalesce show pronounced extrapyramidal signs, abnormal movements, gradually improving over weeks to months or may get lifelong implications. Clinically, JE is difficult to be distinguished from other encephalitis cases or with acute encephalitis syndrome. Therefore, laboratory testing and confirmation are advocated.

World Health Organization in 2006 defined acute encephalitis syndrome (AES) for surveillance purposes for JE endemic area which says clinically an AES patient should have at least one of the following condition (1) change in mental status (like confusion, disorientation, coma, or inability to talk); (2) seizures (not common to person or because of simple fever). Other findings include increased irritability, abnormal behaviour (WHO 2018).

WHO classified the AES cases into four categories:

1. Laboratory confirmed JE: An AES case which is confirmed to be JE based on the result of laboratory results.
2. Probable JE: An AES case from which there may be an adequate sample collection or even no sample collection but had occurred in the JE endemic geographical area during the outbreak.
3. AES—another agent: An AES case where other than JEV has been confirmed through laboratory testing.
4. AES—unknown: An AES case which came out to be negative to JE or another etiological agent through laboratory testing or that case has not been tested.

12.9 Disease in Animal

Horses manifest encephalitic disease accompanied by fever and there could be mortality also. Most often in horses, JE is seen as a sub-clinical and when clinical signs are present which is usually sporadic have three major manifestations—transitory, lethargic, or hyperexcitable. In transitory type syndrome, there is

moderate fever which lasts for a few days and can be accompanied by loss of appetite, incoordination, jaundice and the horse recovers in the next few days. Lethargic type syndrome will have a high fever for variable periods with pronounced neurological symptom, difficulty in swallowing, haemorrhagic petechiae over mucosa, and can go to even paralysis. These cases take longer time to recover which goes around 1 week or more. The third pattern is the hyperexcitable type having very high fevers, accompanied by heavy sweating, muscle tremors, pronounced neurological symptom, and loss of vision, coma, and death. Mortality in horses goes around 5% to as high as 30% with morbidity rates around 1%. Horses dying with JEV infection in post-mortem (PM) shows gross lesions in the central nervous system, viz. a diffuse non-suppurative encephalomyelitis with apparent perivascular cuffing; phagocytic destruction of nerve cells, perivascular cuffing and focal gliosis, blood vessels appear dilated with numerous mononuclear cells.

In pig herds, the disease is seen with large numbers of stillborn or weak piglets which are negative to the known abortion causes like brucellosis, swine fever, African swine fever, porcine reproductive and respiratory syndrome, etc. Reproductive disease manifestation is the most common in pigs with reproductive losses ranging between 50 and 70%. The reproductive manifestation can be abortions in sows, stillbirths or mummified foetuses, and in boars, there are sperm abnormalities. The piglets which are born with JE often display neurological symptoms and often die after birth with mortality rates as high as 100% in these piglets. It is worth to note that the adult non-immune pigs which usually do not die and after getting JE infection results in lifelong immunity. In swine, the PM sign in the mummified or stillborn foetuses shows dark appearance with neurologic damage; hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis, and subcutaneous oedema (Scherer et al. 1959c).

12.10 Diagnosis of JE

Virus Isolation The JEV can be isolated using a cell culture system, intracerebral inoculation of suckling mice, and mosquito inoculation. The virus isolation rate is usually less because of low circulating viral copies and fast development of neutralizing antibodies (Solomon et al. 1998a, b). Successful isolation goes with proper collection of biological sample at an appropriate time, i.e. brain tissue or biopsy sample during post-mortem/autopsy or from cerebrospinal fluid (CSF) of human within 4 days of the onset of symptom. JE had been isolated from pigs from blood, and CSF and mosquitoes also have been isolated. Isolation is usually carried out in one-day-old suckling mice or cell line like in porcine stable kidney cells, Vero cell line, mosquito cell line of *Aedes albopictus* clone C6/36 etc.

Molecular Techniques Molecular detection of JE viral genome by reverse transcriptase-polymerase chain reaction (RT-PCR) techniques are used in blood, cerebrospinal fluids, the brain tissue of human, pigs, and experimental animals like mice. It is even used to detect viral genome from vector mosquito. The success rate of detection of viral genome from human blood is less due to short duration of virus in blood and very low-level viremia. Though it can be detected from blood and/or CSF in around 0–25% of clinically affected cases which can be improvised to some extent up to 25–30%, if sample is collected within 3 days of the onset of infection (Dubot-Peres et al. 2015; Khalakdina et al. 2010; Touch et al. 2009; Yeh et al. 2010; Swami et al. 2008). The molecular assays hold good during the early stages of the infection when seroconversion has not occurred significantly to be detected by serological assays. However, as stated above RT-PCR is not very sensitive and it most often misses to detect the viral genome in actual JE cases. Therefore, if the PCR result is positive then the case can be regarded as JE positive but negative PCR result should not be treated as JE negative and it must be complemented with serology. Other techniques like real-time PCR, loop-mediated isothermal amplification (LAMP PCR), lateral flow test (LFT) are also available (Dhanze et al. 2019a). TaqMan real-time based RT-PCR assay has been developed for the detection of JEV in swine and mosquito (Pantawane et al. 2018; Shao et al. 2018) and other real-time based also been documented (Bharucha et al. 2018). Reverse transcription LAMP coupled with a lateral flow dipstick assay for the detection of JE virus has also been developed and is claimed to be specific (Deng et al. 2015). Whole-genome sequencing confirmation has also been approved in addition to the above stated molecular tests (WHO 2018).

Serological Techniques Serological techniques are widely used for the diagnosis of JE antibodies and are regarded to be the gold standard. The detection of virus-specific antibody in the CSF is more than other clinical samples. Hence CSF based diagnosis is advocated (Ravi et al. 2006). Various serological techniques like haemagglutination inhibition test (HAI), virus neutralization test (NT) were employed for assay of antibody of JE and were recommended by WHO, OIE, and reference laboratories. However both the test requires high level of expertise and its antigen production is limited to reference laboratories only, with requirement of handling the virus, requirement of red blood cells obtained from geese in HAI and is laborious. There is a want for the development of easy to use, specific and sensitive JEV serological kit. In this search, indirect ELISA had shown some promising result. The HAI based JEV diagnosis in the paired sera is the most preferred one till the 1990s, but nowadays immunoglobulin M (IgM) capture-based enzyme-linked immunosorbent assay test as indirect ELISA or popularly known as MAC-ELISA in CSF or serum is routinely practiced and gives confirmation for recent infection in human (Cha et al. 2014). The use of MAC-ELISA as the first-line diagnostic assay in human has also been recommended by the World Health Organization for the detection of acute infections, and for best result the sample collection should be

collected within 5 days after the onset of illness. If the first initial sera turn out to be negative by MAC-ELISA, then it can be repeated after 7–10 days. There are three commercial MAC-ELISA kits available by (1) XCyton Diagnostics Limited, India, (2) the Inbios kit (InBios International Inc., United States of America), and (3) a combo kit for dengue and JE marketed by PanBio, Australia (Lewthwaite et al. 2010; Johnson et al. 2016; Sirikajornpan et al. 2018).

Indirect ELISA for pigs have been developed and used but is limited to reference laboratories only. Few indirect ELISA using whole JEV antigen for the detection of IgG JEV antibodies and had comparable sensitivity with HAI and SNT were developed and used, but none of them is available commercially for larger use (Yang et al. 2006; Hamano et al. 2007; Kolhe et al. 2015). Most of the countries still preferred whole JEV antigen harvested from cell culture for indirect ELISA and using it for pig JEV sero-surveillance. Many authors have developed JEV peptide-based and expressions based ELISAs, and few of them are in the pipeline for commercialization but at present not available commercially (Dhanze et al. 2019b; Hua et al. 2010). Many companies are claiming JEV ELISA for pigs, but their sensitivity and specificity are not fully validated and had not been recommended by OIE or reference laboratories. With less option of a better commercial JEV kit, the researcher are using and reporting the JEV seropositivity with the available kits, but these need to be further validated (Pegu et al. 2019). There is a chance of cross-reactivity with other Flaviviruses, viz. West Nile and dengue; hence, these other *flaviviruses* should also be monitored along with JE (Maeki et al. 2018; Nealon et al. 2019).

12.11 Treatment

There is no specific treatment available for JE patients, and only symptomatic treatment is the option. Most of the cases require hospitalization with supportive care under close observation. Rest, ample fluids, antipyretic, and analgesic can be used to relieve symptoms. Severe cases may require management in intensive care unit with supports to maintain clear airways, breathing, circulation, raised intracranial pressure, electrolyte balance, fever, convulsions, and parenteral antibiotics to cover for bacterial infection (Turtle and Solomon 2018). Proper nursing care is of paramount importance to prevent aspiration pneumonia, bedsores along with nutritional care of the patient (Kumar et al. 2019). The use of steroids like dexamethasone in JE patients has been tried, but its effectiveness is debatable. The tetracycline group of drug—Minocycline having antibacterial plus neuroprotective advantage has shown beneficial in animal model.

12.12 Vaccines

A vaccine against Japanese encephalitis was first introduced during the 1930s which were inactivated mouse brain-derived JEV strains of Nakayama and/or Beijing-1 made by BIKEN Company of Japan. Initially these first vaccines were used successfully in control of JE in countries like Japan, Korea, and China. Later many advance vaccines came up which were based on inactivated cell culture vaccines, chimeric virus vaccines, recombinant adenovirus-based vaccines and few of them got successfully tested in animal and human clinical trials. At present there are good vaccine options in the market and have been in practice in endemic countries (Appaiahgari and Vrati 2010; Butler et al. 2017; Hegde and Gore 2017; Li et al. 2019).

12.13 Human Vaccines

(a) *Mouse brain killed vaccine*—the inactivated vaccine derived from mouse brain was the first to be introduced by BIKEN (Japan). The Nakayama and/or Beijing strains were used initially by different companies for this mouse brain inactivated JE vaccine and had been successful in the control programme of JE in countries like China, Korea. In the initial year, this vaccine was widely used, but with reports of its neural side effect, it was phased out slowly, and BIKEN had stopped its production since 2007.

(b) *P3 strain inactivated vaccine*—The JE Beijing P-3 strain is the most virulent strain of JE known. This was converted to a cell culture-derived, formalin-inactivated JE vaccine and is widely used in China since the 1960s through Chengdu Biologicals Corporation limited. Initially, in China with the use of this vaccine in infants, they could achieve a protection level of up to 76–90%, later this vaccine was replaced by the live-attenuated vaccines because of report of low efficacy, short-lived immunity, and requirement of the booster dose.

(c) *Live-attenuated vaccine*—The field isolates of JE SA14 was a relatively weak strain in term of virulence and later converting it to a live-attenuated strain avirulent strain through serial passaging in hamster kidney cell line leads to the development of a better vaccine which is known as SA-14-14-2 strain. This single-dose vaccine produced by Chengdu Biologicals is one of the popular vaccines and is used even nowadays. This has been approved by World Health Organization. China had been using this vaccine since 1998, and country like Nepal had tried in human population with satisfactorily good efficacy since 1999 and is used in India also since 2006. The efficacy of vaccine is good in various independent studies, in Nepal an efficacy of 99.3% (same year), 98.5% (after 1 year), and 96.2% (after 5 years of vaccination) was recorded and an efficacy of 94.5% after 6 months was recorded in India. The vaccine safety profile was also recorded to be good with the development of minor post-vaccination symptom

as low-grade fever, local reactions, or irritability in 5–10% of recipients (Turtle et al. 2017; Yun et al. 2016). The environmental safety of SA-14-14-2 inactivated vaccine was checked and was found to be still safe (Liu et al. 2019).

(d) *Vero cell-derived*—inactivated JE vaccine derived from Vero cell line have also come up and had been successfully used in many countries and is available in current time also. One such is available in India as JENVAC developed by National Institute of Virology, Pune an institute of Indian Council of Medical Research using the Kolar strain (821564 XZ) which was isolated from Kolar a place in Karnataka state during the early 1980s. JENVAC is used in current practice with approval from the Drug Controller General of India and marketed by Bharat Biotech Limited.

(e) *The IC51 Vaccine*—IXIARO®—is a new generation Vero cell line derived formalin-inactivated vaccine using the SA-14-14-2 strain and is manufactured by Intercell AG (Vienna, Austria) and distributed by Novartis (Amicizia et al. 2018). This vaccine had received US Food and Drug Administration approval for use in children and adults 17 years of age or older and the vaccine was later also approved in Europe and Australia. The vaccine is available in the name of JEEV (Biological E. Ltd., Hyderabad, India) and used in current practice. This is a two-dose vaccine given on 0 and 28th day and is applicable for both children and adults.

(f) *Chimeric vaccine*—This is also a new generation vaccine and is recently cleared the human clinical trials. The vaccine was developed by Acambis, Cambridge, UK using the live-attenuated Yellow fever Virus 17 D clone with an inset of pre-membrane and envelop genes of attenuated SA-14-14-2 JE virus in between the core and nonstructural genes yellow fever virus making it a live chimeric vaccine. Its phase II trials have shown a seroconversion of 94% with single-dose, and phase III trial are also completed in Thailand and is marketed as IMOJEV and THIAJEV (Appaiahgari and Vrati 2010; Chin and Torresi 2013). The phase IV vaccine trial is also successful for this vaccine (Chotpitayasunondh et al. 2017). This vaccine is successfully used in adult and even older persons and is recommended by WHO (Table 12.2).

12.14 Animal Vaccines

(a) *Horse vaccination*—As JE affects horses as they act as a dead-end host, so there was a need to protect the racing horses and horses with high values. It also serves as a model for vaccine trial even before the use of the vaccine in human and the first horse vaccination took place in the year 1948 with mouse brain-derived JE vaccines (Nakamura 1972). Horse vaccination has reduced JE cases in horses, 337.1/100,000/year in Japan from 1948 peak outbreak to 29.74 cases/100,000/year in 1960 again further reduction to 3.33 cases/100,000/year in 1967 (Goto 1976; Nakamura 1972) along with the advancement in vaccine. Countries such

Table 12.2 JE vaccines commercialized for human use

Type of vaccine	Virus strain/type	Substrate	Manufacturer/trade name/country of origin
Inactivated	Nakayama-NIH; wild-type	Mouse brain	BIKEN, Japan
	Beijing-1 (P-1); wild-type		Japan, Korea
	Beijing-3 (P-3)	Primary hamster kidney	China
	Beijing-1	Vero cell	Japan, BIKEN
	Beijing-3 (P-3)		JEBIKV, China
	SA14-14-2		1C51—Intercell, IXIARO—Valneva, JEEV-Biological E limited, India
	Kolar-821564XY		JENVAC, India, Bharat Biotech
Live attenuated	SA14-14-2	Primary hamster kidney	China, Chengdu Biological Products
Chimeric-live-attenuated	YFV 17D containing JEV proteins	Vero cells	ChimeriVax-JE; JE-CV Acambis/ Sanofi-Pasteur (IMOJEV, THAIJEV)

as Singapore and China are also using the horse vaccine and have reported the reduction of JE cases in horses (Ellis et al. 2000). In Hong Kong, thoroughbred racing horses are vaccinated when purchased from endemic countries (Ellis et al. 2000).

(b) *Pig vaccination*—Pig vaccinated with JE does not allow high viremia and hence breaks the transmission cycle of JEV (Sasaki et al. 1982). JE vaccination in pigs is helpful in term of reduction of stillbirth in the farms. In a Taiwan study, it was proved that the JE vaccinated sows give birth to healthy piglet around 92% healthy piglet, but in the unvaccinated group, 31.6–54.1% piglets are born as stillbirths (Hsu et al. 1972; Rosen 1986). These JE vaccinated pigs are now not acting as amplifier host and did not infect mosquitoes helping in the protection of human and horses (Sasaki et al. 1982). The vaccination of pigs is an effective tool to control JE but is not widely practiced across the countries because of high turnover in pig populations, pigs with 3 month gestation period can give at least three crops per year with an average of 8–10 piglets would give a new naïve population every year and to vaccinate this huge new population is costly and require huge manpower and efforts. Moreover, the effectiveness of the live-attenuated vaccines is decreased in young pigs because of maternal antibodies (Wada 1987). There is one more hurdle where it is said that natural infection of pigs with JEV develops lifelong immunity, but with the man-made vaccine the immunity is short. The JE vaccines are available only in few countries and they have been practicing it in field like Japan, Taiwan, etc. The pig vaccine for JE is not available commercially so many endemic countries like India who contributes a high number JE cases are unable to apply this strategy (García-Nicolás et al. 2017).

12.15 Prevention

Prevention of JE in the endemic area requires a multi-approach strategy with vaccination, vector control, change of rice field irrigation system, minimizing pig-human interaction, etc. The country like Japan had been able to control JE infection with human and pig vaccination, mechanization of rice cultivation, vector control strategy, etc. Even vaccination of racehorses is also practiced in some countries. Measures to prevent JE should be targeted for vector control, in the reservoir host pig, and the protective measures in human.

1. *The Vector Mosquito control*
   Though vector control for JE is one of the practical solutions in most of the country, especially in Asian countries with huge human and animal population. It not only solves JE but many other mosquito-borne illnesses. WHO had narrated that mosquito is one of the biggest enemies to the human race, but its control is not so easy and somewhat expensive in most of the Asian countries which are either developing or underdeveloped with the huge population of both human and animals spread over a large geographical area. If properly implemented it can break the JE cycle and can control the outbreaks. Application of larvicides to rice fields, natural insecticide of *Azadirachta indica* can be applied to rice fields, placing larvivorous fish like *Gambusia affinis* in rice paddies are some of the ways. Fogging in dawn-dusk when *Culex* activity is highest should be done. Insecticide-treated mosquito nets can be used in pig sheds. Cattle are also used as a damping host for JE virus as being a dead-end host it can divert the *Culex* population from pig and human, the approach better known as zooprophylaxis. Mechanization of rice field with frequent changing of the water in rice field destroys the breeding ground for mosquito and is an effective way but limited to developed countries only, as most of the agriculture in Asian country is monsoon fed. Elevation of general hygiene practices is needed in rural as well as urban cities. The role of municipality in cleaning garbage laden waterways especially in cities is needed. The mass awareness programme from radio, television, print media, social media can make a major change and attitude change by citizen is needed to win this war against mosquito, ultimately JE and other mosquito-borne diseases. Personal protection measures against mosquito bites like use of mosquito nets during bedtime, use of mosquito repellants, and protective clothing would be useful.
2. *Preventive measures towards reservoir host pig*
   Vaccination against JEV is one of the effective strategies which has been used by some countries but is not practiced in most of the countries because of the lack of pig vaccine and high cost involved in implementation. The pig farmers should be given awareness that pig farm should be away from human houses. In most of the village setting, in developing or underdeveloped countries the pig and human house are almost common. The pig lives here side by side of the residential premises or below the same house. Government has to give incentives to these farmers of low socio-economic group to make their new pig farm away from

human house. There is need to adopt mosquito control programme in pig farms also and where it is economically not feasible especially in rural area then also the villagers should be advocated to use their indigenous low-cost knowledge for keeping mosquito away like burning of Neem leaves, etc. The government mechanism has to undertake the responsibility of fogging in this area where the farmer cannot afford it.

3. *Control strategy directed to human*
   In human, vaccination is the most helpful control measure, and earlier the vaccination that was oriented to children below 12 or 15 years of age is now applicable to adult also (Kumar 2014). Even though human vaccination will decrease human JE cases, but the virus would be maintained in the reservoir host (pig, ardeid birds) and vector mosquito and the non-vaccinated group would always be prone to the JE infection. Hence, for effective JE control and prevention programme one health approach is must with the simultaneous effort of all the departments, viz. animal husbandry, medical, municipality, irrigation, agriculture, fishery acting together to curb the menace of JEV. Countries like Japan, China, and Korea been practicing JE human vaccination since long but many countries like India, Nepal have started in the last decades only and have to go a long way to effectively vaccinate the whole population. JE vaccination is also advised for travellers who are going to endemic countries (Connor et al. 2019).

12.16 Current Scenario and Conclusions

The disease burden of Japanese encephalitis is more in Asian countries. Due to variations in the diagnostic procedures being followed globally, the true incidence of JE is not well estimated. According to the earlier estimates, approximately 68,000 JE cases occur annually, and only 10% cases are actually reported to the World Health Organization. The vast majority of people (~3 billion) from South-East Asia and Western Pacific are at the risk of JE infection. Accordingly, people from 27 countries are at the risk of JE. Depending on the annual incidence and vaccination strategies, the JE endemic countries have been grouped into high, medium, and low. For example, Korea, Taiwan, Japan, China, and India are examples of countries with a high incidence of JE. The vaccination programmes for JE are also varyingly implemented in different endemic countries. JE vaccination is being implemented since long in countries like Japan, Korea, and Taiwan. China started JE vaccination programme in 1981, but JE as a routine vaccine is implemented since 2008. In India, JE vaccination has been introduced in 2006 for children aged 1–15 years. This vaccine was included in the National Immunization Programme by Government of India in 2014. The districts where JE is endemic, the SA-14-14-2 JE vaccine are being used as a part of Universal Immunization Programme (Tandale et al. 2018). More than 11 crore children's from identified JE endemic districts are immunized in India. Climate change may pose significant impact on the JEV transmission. Identification of JEV in Tibet and Australia proves that the prediction of JEV

transmission is very difficult in the context of global warming and climate change. Although JE is considered a paediatric disease, it has also been recorded in adults with significantly high proportion. Its increasing trend in the adult further suggests for revisions in the JE prevention strategies at national and international level in the JE endemic countries. The current endemic region of JE encompasses the entire South Asia, Southeast Asia, eastern Russian Federation, Australia, Saipan, and Papua New Guinea is the globally identified endemic regions of JE.

JEV infects the CNS which causes neuroinflammation and neuronal death. Personal factors are important in the development of clinical illness in the case of humans. Age factor is very important, and neuro invasiveness is multifold in people aged above 50 years. Similarly, risk of neurological sequelae is also more in the younger age. JE infection risk also increases during pregnancy. The epidemiology of JEV is complex and unpredictable, and its transmission by non-vector route cannot be ruled out. A study has been demonstrated that JEV can be transmitted between infected and susceptible pigs even in the absence of mosquitoes. In the same study it was revealed that infectious virus dose for pigs could be as low as 10 $TCID_{50}$ per animal and mucosal virus shedding (oronasal transmission) could be the important source of virus transmission in pigs without involvement of vector.

Vaccination is the only long-term strategy for prevention and control of JE infection. At present, there are more than 15 vaccines being used for JE immunization. They are grouped into four major classes, viz. inactivated mouse brain-derived JE vaccines; inactivated Vero cell culture-derived JE vaccines; live-attenuated SA-14-14-2 JE vaccines; and live recombinant JE vaccine. WHO has recommended to gradually reduce the use of mouse brain-derived JE vaccine due to its safety concern. Virus strains used for the preparation of JE vaccines are Beijing-1, Beijing P-3, Kolar strain, SA 14-14-2 strain and recombinant vaccine using structural and non-structural genes of SA 14-14-2 virus and yellow fever 17D virus, respectively. Immunization schedule and dose regimes are also different for different group of vaccines. For example, a single dose of the live-attenuated SA-14-14-2 JE vaccine in the children aged 9 months and above will give protection for 5 years. Recombinant JE vaccine (JE-CV) is a two-dose vaccine which also gives protection up to 5 years. A two-dose inactivated Vero cell-derived JE vaccines are being used in the USA, Australia, India, and New Zealand. Use of recombinant JE-CV was licensed since 2012 in Australia and Thailand. All the JE available vaccines are based on the genotype 3 of the JEV. In India JE vaccine derived from Vero cells is manufactured by Bharat Biotech. The first JE vaccine was prepared from the Nakayama strain of JEV. It was known as mouse brain-derived inactivated JE vaccine marketed as JE-VAX. This was the only vaccine available internationally for the prevention of JE for several decades. Later it was produced in several Asian countries like India, Japan, Korea, Taiwan, Thailand, and Vietnam. In 1988, China licensed the use of the live-attenuated SA14-14-2 JE vaccine for commercial use. This vaccine is highly immunogenic, widely used and now licensed in several Asian countries like South Korea, Nepal, India, Sri Lanka, Cambodia, Laos, Myanmar, and Thailand. In 1998 China licensed another vaccine for domestic use which is a Vero cell-derived Beijing-3 JE vaccine. In Japan, similar type of vaccine prepared from Beijing-1

strain of JEV is available under trade names JEBK V and ENCEVAC licensed in 2009 and 2011, respectively. IC51 is a new type of inactivated JE vaccine derived from Vero cells using SA-14-14-2 virus strain is in use since 2009 in the USA, Europe, Canada, Australia, India, Switzerland, and Hong Kong. It is marketed under trade names IXIARO, JESPECT, and JEEV (Yun and Lee 2014). The safety and immunogenic potential of chimeric vaccine produced using Yellow Fever Virus (YFV) 17D is now well-proven. With the advent of recombinant DNA technology, precursor membrane protein (prM) and envelop (E) proteins of SA-14-14-2 strain of JEV are expressed in the YFV. This vaccine is also a type of Vero cell-derived vaccine which is commercially available under trade names IMOJEV, JE-CV, and THAIJEV. Future JE vaccine development should be focused on the circulating genotypes of the JEV. Currently, genotype I is widely circulating JEV genotype which has replaced genotype III. Unfortunately, all the available JE vaccines at present have been derived from genotype III of the JEV strains, namely Nakayama, Beijing-1, Beijing-3, and SA-14-14-2. Several approaches are being explored to develop new JE vaccines using recombinant technology and expression of immunodominant proteins in poxviruses and also plasmid DNA vaccines.

Genetic manipulation of JEV RNA is being explored to produce recombinant viruses from cloned DNA using reverse genetics. Due to the high cost of JE vaccine production and biosafety levels required for handling the JEV (BSL-3). Similarly, co-circulation of different related flaviviruses challenges vaccination and development due to cross-reactivity. In recent past research has been focused on the use of virus-like particles (VLPs). However, the VLP based vaccines are either in the pre-clinical or clinical stage of development. VLPs have great potential for future safe JE vaccine as they do not contain genetic material. Using mammalian and insect host systems; baculovirus, vaccinia virus and plasmid, retrovirus as vectors JEV VLPs are being produced to express prM and E proteins (Krol et al. 2019). Different JEV genotypes have been distributed to different geographic regions. Thus antigenic variation will exist in nature in JEV. It will pose some degree of impact on the prevention and control of this disease.

Acknowledgements All the authors of the manuscript thank and acknowledge their respective Institutes.

Conflict of Interest There is no conflict of interest.

References

Acha PN, Szyfrez B (2003) Zoonoses and communicable diseases common to man and animals. Vol. III, Chlamydioses, Rickettsioses and Viroses, 3rd edn. PAHO, New York, pp 172–176

Amicizia D, Zangrillo F, Lai PL, Iovine M, Panatto D (2018) Overview of Japanese encephalitis disease and its prevention. Focus on IC51 vaccine (IXIARO®). J Prev Med Hyg 59:E99–E107

Appaiahgari MB, Vrati S (2010) IMOJEV®: a yellow fever virus-based novel Japanese encephalitis vaccine. Expert Rev Vaccines 9(12):1371–1384. https://doi.org/10.1586/erv.10.139

Arai S, Matsunaga Y, Takasaki T, Tanaka-Taya K, Taniguchi K, Okabe N, Kurane I, Vaccine Preventable Diseases Surveillance Program of Japan (2008) Japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. Jpn J Infect Dis 61(5):333–338

Ayukawa R, Fujimoto H, Ayabe M et al (2004) An unexpected outbreak of Japanese encephalitis in the Chugoku district of Japan, 2002. Jpn J Infect Dis 57(2):63–66

Bae W, Kim JH, Kim J, Lee J, Hwang ES (2018) Changes of epidemiological characteristics of Japanese encephalitis viral infection and birds as a potential viral transmitter in Korea. J Korean Med Sci 33:e70. https://doi.org/10.3346/jkms.2018.33.e70

Banerjee K (1996) Emerging viral infections with special reference to India. Indian J Med Res 103:177

Baruah A, Hazarika RA, Barman NN, Islam S, Gulati BR (2018) Mosquito abundance and pig seropositivity as a correlate of Japanese encephalitis in human population in Assam, India. J Vector Borne Dis 55(4):291–296. https://doi.org/10.4103/0972-9062.256564

Bharucha T, Sengvilaipaseuth O, Vongsouvath M, Davong V, Panyanouvong P, Piorkowski G, Garson JA, Newton PN, de Lamballerie X, Dubot-Peres A (2018) Development of an improved RT-qPCR Assay for detection of Japanese encephalitis virus (JEV) RNA including a systematic review and comprehensive comparison with published methods. PLoS One 13:e0194412

Bhattachan A, Amatya S, Sedai TR, Upreti SR, Partridge J (2009) Japanese encephalitis in hill and mountain districts, Nepal. Emerg Infect Dis 15(10):1691–1692

Bhattacharya S, Basu P (2014) Japanese encephalitis virus (JEV) infection in different vertebrates and its epidemiological significance: a review. Int J Fauna Biol Stud 1:32–37

Buescher EL, Scherer WF et al (1959) Ecologic studies of Japanese encephalitis virus in Japan. IV. Avian infection. Am J Trop Med Hyg 8:678–688

Burke DS, Leake CJ (1988) Japanese encephalitis. CRC Press, Boca Raton

Butler S, Sutter D, Maranich A (2017) Tolerability of Japanese encephalitis vaccine in pediatric patients. J Pediatric Infect Dis Soc 6:149–152. https://doi.org/10.1093/jpids/piw029

Campbell GL et al (2011) Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 89:766–774, 774a–774e

Centers for Disease Control and Prevention (2019) National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). https://www.cdc.gov/japaneseencephalitis/maps/index.html

Cha GW, Ch JE, Ju YR, Hong YJ, Han MG, Lee WJ, Choi EY, Jeong YE (2014) Comparison of four serological tests for detecting antibodies to Japanese encephalitis virus after vaccination in children. Osong Public Health Res Perspect 5:286–291. https://doi.org/10.1016/j.phrp.2014.08.003

Chai C, Palinski R, Xu Y, Wang Q, Cao S, Geng Y, Zhao Q, Wen Y, Huang X, Yan Q et al (2019) Aerosol and contact transmission following intranasal infection of mice with Japanese encephalitis virus. Viruses 11:E87. https://doi.org/10.3390/v11010087

Chauhan PS, Khanna VK, Kalita J, Misra UK (2017) Japanese encephalitis virus infection results in transient dysfunction of memory learning and cholinesterase inhibition. Mol Neurobiol 54:4705–4715. https://doi.org/10.1007/s12035-016-9963-6

Chen C, Zhao T, Jiang Y, Li C, Wang G, Gao J, Dong Y, Xing D, Guo X, Zhao T (2019) Vector mosquito ecology and Japanese encephalitis virus genotype III strain detection from *Culex tritaeniorhynchus* and pig in Huaihua, China. Vector Borne Zoonotic Dis 19(12):933–944. https://doi.org/10.1089/vbz.2019.2453

Chin R, Torresi J (2013) Japanese B encephalitis: an overview of the disease and use of ChimeriVax-JE as a preventative vaccine. Infect Dis Ther 2:145–158. https://doi.org/10.1007/s40121-013-0018-2

Chotpitayasunondh T, Pruekprasert P, Puthanakit T, Pancharoen C, Tangsathapornpong A, Oberdorfer P, Kosalaraksa P, Prommalikit O, Tangkitthaworn S, Kerdpanich P, Techasaensiri C, Korejwo J, Chuenkitmongkol S, Houillon G (2017) Post-licensure, phase IV, safety study of a live attenuated Japanese encephalitis recombinant vaccine in children in Thailand. Vaccine 35(2):299–304. https://doi.org/10.1016/j.vaccine.2016.11.062

Connor B, Bunn WB (2017) The changing epidemiology of Japanese encephalitis and new data: the implications for new recommendations for Japanese encephalitis vaccine. Trop Dis Travel Med Vaccines 3:14

Connor BA, Hamer DH, Kozarsky P, Jong E, Halstead SB, Keystone J, Mileno MD, Dawood R, Rogers B, Bunn WB (2019) Japanese encephalitis vaccine for travelers: risk-benefit reconsidered. J Travel Med 26(5). https://doi.org/10.1093/jtm/taz037

Deng J, Pei J, Gou H, Ye Z, Liu C, Chen J (2015) Rapid and simple detection of Japanese encephalitis virus by reverse transcription loop-mediated isothermal amplification combined with a lateral flow dipstick. J Virol Methods 213:98–105

Desingu PA, Ray PK, John JK, Das T, Dubal ZB, Rajak KK, Singh RK, Saikumar G (2017) First complete genome sequence of genotype III Japanese encephalitis virus isolated from a stillborn piglet in India. Genome Announc 5:e01503–e01516

Dhanze H, Bhilegaonkar KN, Kumar C, Kumar MS, Singh P, Kumar A (2019a) Development and evaluation of lateral flow assay for sero-diagnosis of Japanese encephalitis in swine. Anim Biotechnol 15:1–7. https://doi.org/10.1080/10495398.2019.1602539

Dhanze H, Bhilegaonkar KN, Rawat S, Chethan Kumar HB, Kumar A, Gulati BR, Mishra BP, Singh RK (2019b) Development of recombinant nonstructural 1 protein based indirect enzyme linked immunosorbent assay for sero-surveillance of Japanese encephalitis in swine. J Virol Methods 24:113705. https://doi.org/10.1016/j.jviromet.2019.113705

Diallo AO, Chevalier V, Cappelle J, Duong V, Fontenille D, Duboz R (2018) How much does direct transmission between pigs contribute to Japanese encephalitis virus circulation? A modelling approach in Cambodia. PLoS One 13:e0201209. https://doi.org/10.1371/journal.pone.0201209

Dubot-Peres A, Sengvilaipaseuth O, Chanthongthip A, Newton PN, de Lamballerie X (2015) How many patients with anti-JEV IgM in cerebrospinal fluid really have Japanese encephalitis? Lancet Infect Dis 15(12):1376–1377. https://doi.org/10.1016/S1473-3099(15)00405-3

Ellis PM, Daniels PW et al (2000) Japanese encephalitis. Vet Clin North Am Equine Pract 16 (3):565–578. x–xi

Erlanger TE, Weiss S, Keiser J, Utzinger J, Wiedenmayer K (2009) Past, present, and future of Japanese encephalitis. Emerg Infect Dis 15(1):1–7. https://doi.org/10.3201/eid1501.080311

Fang Y, Zhang Y, Zhou ZB, Xia S, Shi WQ, Xue JB, Li Y, Wu JT (2019) New strains of Japanese encephalitis virus circulating in Shanghai, China after a ten-year hiatus in local mosquito surveillance. Parasit Vectors 12:22. https://doi.org/10.1186/s13071-018-3267-9

Filgueira L, Lannes N (2019) Review of emerging Japanese encephalitis virus: new aspects and concepts about entry into the brain and inter-cellular spreading. Pathogens 8(3). https://doi.org/10.3390/pathogens8030111 (review)

Fulmali PV, Sapkal GN, Athawale S, Gore MM, Mishra AC, Bondre VP (2011) Introduction of Japanese encephalitis virus genotype I, India. Emerg Infect Dis 17(2):319–321

Gao X, Li X, Li M, Fu S, Wang H, Lu Z et al (2014) Vaccine strategies for the control and prevention of Japanese encephalitis in Mainland China, 1951–2011. PLoS Negl Trop Dis 8(8): e3015

Gao X, Liu H, Li M, Fu S, Liang G (2015) Insights into the evolutionary history of Japanese encephalitis virus (JEV) based on whole-genome sequences comprising the five genotypes. Virol J 12:43

Gao X, Liu H, Li X, Fu S, Cao L, Shao N, Zhang W, Wang Q, Lu Z, Lei W et al (2019) Changing geographic distribution of Japanese encephalitis virus genotypes, 1935–2017. Vector Borne Zoonotic Dis 19:35–44. https://doi.org/10.1089/vbz.2018.2291

García-Nicolás O, Ricklin ME, Liniger M, Vielle NJ, Python S, Souque P, Charneau P, Summerfield A (2017) A Japanese encephalitis virus vaccine inducing antibodies strongly enhancing in vitro infection is protective in pigs. Viruses 9:E124. https://doi.org/10.3390/v9050124

Garjito TA, Prihatin MT, Susanti L, Prastowo D, Rofiatus Sa'adah S, Taviv Y, Satoto TBT, Waluyo J, Manguin S, Frutos R (2019) First evidence of the presence of genotype-1 of Japanese encephalitis virus in *Culex gelidus* in Indonesia. Parasit Vectors 12:19. https://doi.org/10.1186/s13071-018-3285-7

Ghimire S, Dhakal S (2015) Japanese encephalitis: challenges and intervention opportunities in Nepal. Vet World 8(1):61–65

Ghosh D, Basu A (2009) Japanese encephalitis – a pathological and clinical perspective. PLoS Negl Trop Dis 3:e437. https://doi.org/10.1371/journal.pntd.0000437

Goto H (1976) Efficacy of Japanese encephalitis vaccine in horses. Equine Vet J 8(3):126–127

Gresser I, Hardy JL et al (1958) The growth curve of Japanese encephalitis virus in the vector mosquito of Japan, *Culex tritaeniorhynchus*. Jpn J Exp Med 28:243–248

Hale JH, Colless DH et al (1957) Investigation of the Malaysian form of *Culex tritaeniorhynchus* as a potential vector of Japanese B encephalitis virus on Singapore Island. Ann Trop Med Parasitol 51(1):17–25

Hamano M, Lim CK, Takagi H, Sawane K, Kuwayama M, Kishi N, Kurane I, TTakasaki T (2007) Detection of antibodies to Japanese encephalitis virus in the wild boars in Hiroshima prefecture, Japan. Epidemiol Infect 135(6):974–977

Hanna JN, Ritchie SA, Phillips DA et al (1996) An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. Med J Aust 165(5):256–260

Hegde NR, Gore MM (2017) Japanese encephalitis vaccines: immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccin Immunother 13:1–18. https://doi.org/10.1080/21645515.2017.1285472

Hill MN (1970) Japanese encephalitis in Sarawak: studies on adult mosquito populations. Trans R Soc Trop Med Hyg 64(4):489–496

Hills SL, Stoltey J, Martínez D, Kim PY, Sheriff H, Zangeneh A, Eilerman SR, Fischer M (2014) A case series of three US adults with Japanese encephalitis, 2010–2012. J Travel Med 21 (5):310–313

Hsu ST, Chang LC et al (1972) The effect of vaccination with a live attenuated strain of Japanese encephalitis virus on stillbirths in swine in Taiwan. Bull World Health Organ 46(4):465–471

Hua R, Chen N, Qin C, Deng Y, Ge J, Wang X, Qiao Z, Chen W, Wen Z, Liu W, Hu S, Bu Z (2010) Identification and characterization of a virus-specific continuous B-cell epitope on the PrM/M protein of Japanese encephalitis virus: potential application in the detection of antibodies to distinguish Japanese encephalitis virus infection from West Nile virus and Dengue virus infections. Virol J 7:249. http://www.virologyj.com/content/7/1/249

Hurlbut HS (1950) The transmission of Japanese B encephalitis by mosquitoes after experimental hibernation. Am J Hyg 51:265–268

Impoinvil DE, Baylis M, Solomon T (2012) Japanese encephalitis: on the one health agenda. In: Mackenzie J, Jeggo M, Daszak P, Richt J (eds) One health: the human-animal-environment interfaces in emerging infectious diseases, vol 365: Current topics in microbiology and immunology. Springer, Berlin

Johnson BW, Goodman CH, Jee Y, Featherstone DA (2016) Differential diagnosis of Japanese encephalitis virus infections with the Inbios JE Detect™ and DEN Detect™ MAC-ELISA Kits. Am J Trop Med Hyg 94:820–828. https://doi.org/10.4269/ajtmh.15-0631

Kabilan L, Rajendran R, Arunachalam N, Ramesh S, Srinivasan S, Philip Samuel P, Dash AP (2004) Japanese encephalitis in India: an overview. Indian J Pediatr 71:609

Kanojia PC, Shetty PS, Geevarghese G (2003) A long-term study on vector abundance & seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. Indian J Med Res 117:104–110

Karna AK, Bowen RA (2019) Experimental evaluation of the role of ecologically-relevant hosts and vectors in Japanese encephalitis virus genotype displacement. Viruses 11:32. https://doi.org/10.3390/v11010032

Khalakdina A, Shrestha SK, Malla S, Hills S, Thaisomboonsuk B, Shrestha B et al (2010) Field evaluation of commercial immunoglobulin M antibody capture ELISA diagnostic tests for the detection of Japanese encephalitis virus infection among encephalitis patients in Nepal. Int J Infect Dis 14(Suppl 3):e79–e84

Kolhe RP, Bhilegaonkar KN, Dubbal ZB, Simranpreet K, Samir D, Agarwal RK (2015) Indirect ELISA for serosurveillance of Japanese encephalitis in pigs. Indian J Animal Res 49 (3):343–349

Konishi E, Shoda M, Yamamoto S, Arai S, Tanaka-Taya K, Okabe N (2006) Natural infection with Japanese encephalitis virus among inhabitants of Japan: a nationwide survey of antibodies against nonstructural 1 protein. Vaccine 24(16):3054–3056

Krol E, Brzuska G, Szewczyk B (2019) Production and biomedical application of Flavivirus-like particles. Trends Biotechnol 37(11):1202–1216. https://doi.org/10.1016/j.tibtech.2019.03.01

Kulkarni R, Sapkal GN, Kaushal H, Mourya DT (2018) Japanese encephalitis: a brief review on Indian perspectives. Open Virol J 12(Suppl 2, M8):121–130. https://doi.org/10.2174/1874357901812010121

Kumar R (2014) Prevention, diagnosis, and management of Japanese encephalitis in children. Pediatric Health Med Ther 5:99–110

Kumar K, Arshad SS, Selvarajah GT, Abu J, Toung OP, Abba Y, Bande F, Yasmin AR, Sharma R, Ong BL, Rasid AA, Hashim N, Peli A, Heshini EP, Shah AKMK (2018) Prevalence and risk factors of Japanese encephalitis virus (JEV) in livestock and companion animal in high-risk areas in Malaysia. Trop Anim Health Prod 50(4):741–752

Kumar M, Mishra K, Rajendiran R, Jain A, Sharma N (2019) The double doughnut sign on brain magnetic resonance imaging caused by Japanese encephalitis. J Emerg Med S0736-4679 (19):30252–30255. https://doi.org/10.1016/j.jemermed.2019.03.045

Lee DW, Choe YJ, Kim JH et al (2012) Epidemiology of Japanese encephalitis in South Korea, 2007–2010. Int J Infect Dis 16(6):e448–e452

Lewthwaite P, Shankar MV, Tio PH, Daly J, Last A, Ravikumar R, Desai A, Ravi V, Cardosa JM, Solomon T (2010) Evaluation of two commercially available ELISAs for the diagnosis of Japanese encephalitis applied to field samples. Tropical Med Int Health 15(7):811–818. https://doi.org/10.1111/j.1365-3156.2010.02537.x

Li MH, Fu SH, Chen WX, Wang HY, Guo YH, Liu QY, Li YX, Luo HM, Da W, Duo Ji DZ, Ye XM, Liang GD (2011) Genotype V Japanese encephalitis virus is emerging. PLoS Negl Trop Dis 5(7):e1231

Li G, Jin H, Nie X, Zhao Y, Feng N, Cao Z, Tan S, Zhang B, Gai W, Yan F, Li L, Zhang Y, Cao Z, Li N, Gao Y, Yang S, Xia X, Wang H (2019) Development of a reverse genetics system for Japanese encephalitis virus strain SA14-14-2. Virus Genes 55(4):550–556. https://doi.org/10.1007/s11262-019-01674-y

Lindahl J, Chirico J, Boqvist S, Thu HTV, Magnusson U (2012) Occurrence of Japanese encephalitis virus mosquito vectors in relation to urban pig holdings. Am J Trop Med Hyg 87 (6):1076–1082. https://doi.org/10.4269/ajtmh.2012.12-0315

Liu S, Li X, Chen Z, Chen Y, Zhang Q, Liao Y, Zhou J, Ke X, Ma L, Xiao J et al (2013) Comparison of genomic and amino acid sequences of eight Japanese encephalitis virus isolates from bats. Arch Virol 158:2543–2552. https://doi.org/10.1007/s00705-013-1777-5

Liu K, Xiao C, Wang F, Xiang X, Ou A, Wei J, Li B, Shao D, Miao D, Zhao F et al (2018) Chemokine receptor antagonist block inflammation and therapy Japanese encephalitis virus infection in mouse model. Cytokine 110:70–77. https://doi.org/10.1016/j.cyto.2018.04.022

Liu X, Jia L, Nie K, Zhao D, Na R, Xu H, Cheng G, Wang J, Yu Y, Li Y (2019) Evaluation of environment safety of a Japanese encephalitis live attenuated vaccine. Biologicals 60:36–41. https://doi.org/10.1016/j.biologicals.2019.06.001

Mackenzie J, Childs J, Field H, Wang L, Breed A (2008) The role of bats as reservoir hosts of emerging neurological viruses. In: Reiss CS (ed) Neurotropic viral infections. Cambridge University Press, New York, pp 382–407

Maeki T, Tajima S, Kyaw AK, Matsumoto F, Miura K, Yamashita A, Yoshikawa A, Negishi K, Noguchi Y, Tadokoro K et al (2018) Comparison of neutralizing antibody titers against Japanese encephalitis virus genotype V strain with those against genotype I and III strains in the sera of Japanese encephalitis patients in Japan in 2016. Jpn J Infect Dis 71:360–364

Matsunaga Y, Yabe S, Taniguchi K, Nakayama M, Kurane I (1999) Current status of Japanese encephalitis in Japan. Kansenshogaku Zasshi 73(2):97–103

Mifune K (1965) Transmission of Japanese encephalitis virus to susceptible pigs by mosquitoes of Culex tritaeniorhynchus after experimental hibernation. Endem Dis Bull Nagasaki Univ 7:178–191

Ming JG, Jin H, Riley JR, Reynolds DR, Smith AD, Wang RL et al (1993) Autumn southward 'return' migration of the mosquito C. tritaeniorhynchus in China. Med Vet Entomol 7:323–327

Mitamura T, Hazato H, Kitaoka M, Watanabe S, Okubo K, Ichikawa O, Tenjin S, Hayashi T, Tomizawa T (1938) Neutralizing antibody against various encephalitis viruses in sera collected from normal looking persons and domestic animals in both endemic and nonendemic (Hokkaido and Saghalien) areas. Takyo Ijishinshi 63:802, (In Japanese)

Morita K, Nabeshima T, Buerano CC (2015) Japanese encephalitis. Rev Sci Tech Off Int Epiz 34 (2):441–452

Nah J-J, Yang D-K, Kim H-H, Song J-Y (2015) The present and future of veterinary vaccines for Japanese encephalitis in Korea. Clin Exp Vaccine Res 4(2):130–136

Nakamura H (1972) Japanese encephalitis in horses in Japan. Equine Vet J 4(3):155–156

Namachivayam V, Umayal K (eds) (1982) Proceedings of the National Conference on Japanese Encephalitis. Indian Council of Medical Research, New Delhi, pp 30–33

Nanishi E, Hoshina T, Sanefuji M, Kadoya R, Kitazawa K, Arahata Y, Sato T, Hirayama Y, Hirai K, Yanai M, Nikaido K, Maeda A, Torisu H, Okada K, Sakai Y, Ohga S (2019) A nationwide survey of pediatric-onset Japanese encephalitis in Japan. Clin Infect Dis 68 (12):2099–2104. https://doi.org/10.1093/cid/ciy816

Nealon J, Taurel AF, Yoksan S, Moureau A, Bonaparte M, Quang LC, Capeding MR, Prayitno A, Hadinegoro SR, Chansinghakul D et al (2019) Serological evidence of Japanese encephalitis virus circulation in Asian children from dengue-endemic countries. J Infect Dis 219:375–381. https://doi.org/10.1093/infdis/jiy513

Nemeth N, Bosco-Lauth A, Oesterle P, Kohler D, Bowen R (2012) North American birds as potential amplifying hosts of Japanese encephalitis virus. Am J Trop Med Hyg 87(4):760–767. https://doi.org/10.4269/ajtmh.2012.12-0141

Nga PT, Parquet MC, Cuong VD, Ma SP, Hasebe F, Inoue S, Makino Y, Takagi M, Nam VS, Morita K (2004) Shift in Japanese encephalitis virus (JEV) genotype circulating in northern Vietnam: implications for frequent introductions of JEV from Southeast Asia to East Asia. J Gen Virol 85:1625

Niazmand MH, Hirai T, Ito S, Habibi WA, Noori J, Hasheme R, Yamaguchi R (2019) Causes of death and detection of antibodies against Japanese encephalitis virus in Misaki feral horses (Equus caballus) in southern Japan, 2015–17. J Wildl Dis 55(4):804–811

Nitatpattana N, Dubot-Pérès A, ArGouilh M, Souris M, Barbazan P, Yoksan S, de Lamballerie X, Gonzalez J-P (2008) Change in Japanese encephalitis virus distribution, Thailand. Emerg Infect Dis 14(11):1762–1765

Oliveira ARS, Piaggio J, Cohnstaedt LW, McVey DS, Cernicchiaro N (2018) A quantitative risk assessment (QRA) of the risk of introduction of the Japanese encephalitis virus (JEV) in the United States via infected mosquitoes transported in aircraft and cargo ships. Prev Vet Med 160:1–9. https://doi.org/10.1016/j.prevetmed.2018.09.020

Pan X-L, Liu H, Wang H-Y, Fu S-H, Liu H-Z, Zhang H-L, Li M-H, Gao X-Y, Wang J-L, Sun X-H, Lu X-J, Zhai Y-G, Meng W-S, He Y, Wang H-Q, Han N, Wei B, Wu Y-G, Feng Y, Yang D-J, Wang L-H, Tang Q, Xia G, Kurane I, Rayner S, Liang G-D (2011) Emergence of genotype I of Japanese encephalitis virus as the dominant genotype in Asia. J Virol 85(19):9847–9853

Pantawane PB, Dhanze H, Ravi Kumar GVPPS, Grace MR, Dudhe NC, Bhilegaonkar KN (2018) TaqMan real-time RT-PCR assay for detecting Japanese encephalitis virus in swine blood samples and mosquitoes. Anim Biotechnol 30:267–272

Park SL, Huang YS, Lyons AC, Ayers VB, Hettenbach SM, McVey DS, Burton KR, Higgs S, Vanlandingham DL (2018) North American domestic pigs are susceptible to experimental infection with Japanese encephalitis virus. Sci Rep 8:7951

Pearce JC, Learoyd TP, Langendorf BJ, Logan JG (2018) Japanese encephalitis: the vectors, ecology and potential for expansion. J Travel Med 25:S16–S26

Pegu SR, Choudhury M, Rajkhowa S Sarma DK, Gulati BR (2019) Molecular characterization and pathological studies of Japanese encephalitis virus in pigs of Assam 7(1):874–878

Poonsiri T, Wright GSA, Solomon T, Antonyuk SV (2019) Crystal structure of the Japanese encephalitis virus capsid protein. Viruses 11(7). https://doi.org/10.3390/v11070623

Preziuso S, Mari S, Mariotti F, Rossi G (2018) Detection of Japanese encephalitis virus in bone marrow of healthy young wild birds collected in 1997–2000 in Central Italy. Zoonoses Public Health 11:798–804

Qiu X, Lei Y, Yang P, Gao Q, Wang N, Cao L, Yuan S, Huang X, Deng Y, Ma W et al (2018) Structural basis for neutralization of Japanese encephalitis virus by two potent therapeutic antibodies. Nat Microbiol 3:287–294

Rao JS, Misra SP, Patanayak SK, Rao TV, Das Gupta RK, Thapar BR (2000) Japanese encephalitis epidemic in Anantapur district, Andhra Pradesh (October–November, 1999). J Commun Dis 32:306

Ravi V, Desai A, Balaji M, Apte MP, Lakshman L et al (2006) Development and evaluation of a rapid IgM capture ELISA (JEV-Chex) for the diagnosis of Japanese encephalitis. J Clin Virol 35:429–434

Ritchie SA, Rochester W (2001) Wind-blown mosquitoes and introduction of Japanese encephalitis into Australia. Emerg Infect Dis 7(5):900–903

Rosen L (1986) The natural history of Japanese encephalitis virus. Annu Rev Microbiol 40:395–414

Rosen L, Lien JC, Shroyer DA, Baker RH, Lu LC (1989) Experimental vertical transmission of Japanese encephalitis virus by Culex tritaeniorhynchus and other mosquitoes. Am J Trop Med Hyg 40(5):548–556

Sahoo GC, Dikhit MR, Das P (2008) Functional assignment to JEV proteins using SVM. Bioinformation 3(1):1–7

Sasaki O, Karoji Y, Kuroda A, Karaki T, Takenokuma K, Maeda O (1982) Protection of pigs against mosquito-borne Japanese encephalitis virus by immunization with a live attenuated vaccine. Antivir Res 2(6):355–360

Scherer WF, Buescher EL et al (1959a) Ecologic studies of Japanese encephalitis virus in Japan. III. Mosquito factors. Zootropism and vertical flight of Culex tritaeniorhynchus with observations on variations in collections from animal-baited traps in different habitats. Am J Trop Med Hyg 8:665–677

Scherer WF, Moyer JT et al (1959b) Ecologic studies of Japanese encephalitis virus in Japan. VI. Swine infection. Am J Trop Med Hyg 8:698–706

Scherer WF, Moyer JT et al (1959c) Immunologic studies of Japanese encephalitis virus in Japan. V. Maternal antibodies, antibody responses and viremia following infection of swine. J Immunol 83:620–626

Schuh AJ, Guzman H, Tesh RB, Barrett ADT (2013) Genetic diversity of Japanese encephalitis virus isolates obtained from the Indonesian archipelago between 1974 and 1987. Vector Borne Zoonotic Dis 13:479–488. https://doi.org/10.1089/vbz.2011.0870

Schuh AJ, Ward MJ, Leigh Brown AJ, Barrett AD (2014) Dynamics of the emergence and establishment of a newly dominant genotype of Japanese encephalitis virus throughout Asia. J Virol 88(8):4522–4532. https://doi.org/10.1128/JVI.02686-13

Shao N, Li F, Nie K, Fu SH, Zhang WJ, He Y, Lei WW, Wang QY, Liang GD, Cao YX et al (2018) TaqMan real-time RT-PCR assay for detecting and differentiating Japanese encephalitis virus. Biomed Environ Sci 31:208–214. https://doi.org/10.3967/bes2018.026

Shi Q, Song X, Lv Y, Huang X, Kou J, Wang HW, Zhang H, Cheng P, Gong M (2019) Potential risks associated with Japanese encephalitis prevalence in Shandong Province, China. Vector Borne Zoonotic Dis 19(8):640–645. https://doi.org/10.1089/vbz.2018.2416

Shimoda H, Tamaru S, Morimoto M, Hayashi T, Shimojim AM, Maeda K (2011) Experimental infection of Japanese encephalitis virus in dogs. J Vet Med Sci 73:241–1242. https://doi.org/10.1292/jvms.11-0142

Shin E-S, Park O, Kong I-S (2018) Review of the incidence of Japanese encephalitis in foreign-born and Korean nationals living in the Republic of Korea, 2007–2016. Osong Public Health Res Perspect 9(3):126–129

Simon-Loriere E, Faye O, Prot M et al (2017) Autochthonous Japanese encephalitis with yellow fever coinfection in Africa. N Engl J Med 376(15):1483–1485

Sirikajornpan K, Nisalak A, Thaisomboonsuk B, Buddhari D, Yoon IK, Ellison D, Macareo L, Fernandez S (2018) Comparison of anti-DENV/JEV IgG-mAb enzyme-linked immunosorbent assay and hemagglutination inhibition assay. Southeast Asian J Trop Med Public Health 49:629–638

Solomon T, Kneen R, Dung NM, Khanh VC, Thuy TT, Ha DQ, Day NP, Nisalak A, Vaughan DW, Whit NJ (1998a) Poliomyelitis-like illness due to Japanese encephalitis virus. Lancet 351:1094

Solomon T, Thao LT, Dung NM, Kneen R, Hung NT, Nisalak A, Vaughn DW, Farrar J, Hien TT, White NJ, Cardosa MJ (1998b) Rapid diagnosis of Japanese encephalitis by using an immunoglobulin M dot enzyme immunoassay. J Clin Microbiol 36(7):2030–2034

Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT (2000) Japanese encephalitis. J Neurol Neurosurg Psychiatry 68:405–415

Solomon T, Ni H, Beasley DWC, Ekkelenkamp M, Cardosa MJ, Barrett ADT (2003) Origin and evolution of Japanese encephalitis virus in Southeast Asia. J Virol 77:3091–3098

Sunwoo JS, Jung KH, Lee ST et al (2016) Re-emergence of Japanese encephalitis in South Korea, 2010–2015. Emerg Infect Dis 22(10):1841–1843. https://doi.org/10.3201/eid2210.160288

Swami R, Ratho RK, Mishra B, Singh M (2008) Usefulness of RT-PCR for the diagnosis of Japanese encephalitis in clinical samples. Scand J Infect Dis 40(10):815–820. https://doi.org/10.1080/00365540802227102

Takahashi M (1976) The effects of environmental and physiological conditions of Culex tritaeniorhynchus on the pattern of transmission of Japanese encephalitis virus. J Med Entomol 13(3):275–284

Takhampunya R, Kim HC, Tippayachai B, Kengluecha A, Klein TA, Lee WJ, Grieco J, Evans BP (2011) Emergence of Japanese encephalitis virus genotype V in the Republic of Korea. Virol J 8:449

Tandale BV, Khan SA, Kushwaha KP, Rahman H, Gore MM (2018) Effectiveness of Japanese encephalitis SA 14-14-2 live-attenuated vaccine among Indian children: retrospective 1:4 matched case-control study. J Infect Public Health 11:713–719. https://doi.org/10.1016/j.jiph.2018.04.011

Tiwari S, Singh RK, Tiwari R, Dhole TN (2012) Japanese encephalitis: a review of the Indian perspective. Braz J Infect Dis 16(6):564–573. https://doi.org/10.1016/j.bjid.2012.10.004

Touch S, Hills S, Sokhal B, Samnang C, Sovann L, Khieu V et al (2009) Epidemiology and burden of disease from Japanese encephalitis in Cambodia: results from two years of sentinel surveillance. Trop Med Int Health 14:1365–1373

Tsai TF (1997) Factors in the changing epidemiology of Japanese encephalitis and West Nile fever. In: Saluzzo JF, Dodet B (eds) Factors in the emergence of arbovirus diseases. Elselvier, Paris, pp 179–189

Turtle L, Solomon T (2018) Japanese encephalitis—the prospects for new treatments. Nat Rev Neurol 14:298–313. https://doi.org/10.1038/nrneurol.2018.30

Turtle L, Tatullo F, Bali T, Ravi V, Soni M, Chan S, Chib S, Venkataswamy MM, Fadnis P, Yaïch M et al (2017) Cellular immune responses to live attenuated Japanese encephalitis (JE) vaccine SA14-14-2 in adults in a JE/Dengue co-endemic area. PLoS Negl Trop Dis 11:e0005263. https://doi.org/10.1371/journal.pntd.0005263

Uchil PD, Satchidanandam V (2001) Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. Am J Trop Med Hyg 65:242

Unni SK, Růžek D, Chhatbar C, Mishra R, Johri MK et al (2011) Japanese encephalitis virus: from genome to infectome. Microbes Infect 13:312–321

van den Hurk AF, Pyke AT, Mackenzie JS, Hall-Mendelin S, Ritchie SA (2019) Japanese encephalitis virus in Australia: from known known to known unknown. Trop Med Infect Dis 4(1):38. https://doi.org/10.3390/tropicalmed4010038

Wada Y (1987) Strategies for control of Japanese encephalitis in rice production systems in developing countries. In: Vector-borne disease control in humans through rice agroecosystems management: proceedings of the Workshop on Research and Training Needs in the Field of Integrated Vector-Borne Disease Control in Riceland Agroecosystems of Developing Countries, 9–14 March 1987. International Rice Research Institute in collaboration with the WHO/FAO/UNEP Panel of Experts on Environmental Management for Vector Control

Wang LH, Fu SH, Wang HY, Liang XF, Cheng JX, Jing HM, Cai GL, Li XW, Ze WY, Lv XJ, Wang HQ, Zhang DL, Feng Y, Yin ZD, Sun XH, Shui TJ, Li MH, Li YX, Liang GD (2007) Japanese encephalitis outbreak, Yuncheng, China, 2006. Emerg Infect Dis 13(7):1123–1125. https://doi.org/10.3201/eid1307.070010

Wang H, Liang G (2015) Epidemiology of Japanese encephalitis: past, present, and future prospects. Ther Clin Risk Manag 11:435–448

Wang P, Li M, Lu W, Zhang D, Hu Q, Liu Y (2017) DC-SIGN promotes Japanese encephalitis virus transmission from dendritic cells to T cells via virological synapses. Virol Sin 32:495–502. https://doi.org/10.1007/s12250-017-4034-3

Wierzba TF, Ghimire P, Malla S et al (2008) Laboratory-based Japanese encephalitis surveillance in Nepal and the implications for a national immunization strategy. Am J Trop Med Hyg 78 (6):1002–1006

Witt CJ, Richards AL, Masuoka PM et al (2011) The AFHSC-division of GEIS operations predictive surveillance program: a multidisciplinary approach for the early detection and response to disease outbreaks. BMC Public Health 11(Suppl 2):S10. https://doi.org/10.1186/1471-2458-11-S2-S10

World Health Organization (2018) Japanese Encephalitis. Vaccine-Preventable Diseases Surveillance Standards. https://www.who.int/immunization/monitoring_surveillance/burden/vpd/WHO_SurveillanceVaccinePreventable_10_JE_R2.pdf?ua=1

Xiao C, Wang X, Cui G, Pang L, Xu J, Li C, Zhang J, Liu K, Li B, Shao D et al (2018) Possible pathogenicity of Japanese encephalitis virus in newly hatched domestic ducklings. Vet Microbiol 227:8–11. https://doi.org/10.1016/j.vetmic.2018.10.016

Yang DK, Kim BH, Lim SI, Kwon JH, Lee KW, Choi CU, Kweon CH (2006) Development and evaluation of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine. J Vet Sci 7(3):271–275

Yang D-K, Oh Y-I, Kim H-R, Lee Y-J, Moon O-K, Yoon H, Kim B, Lee K-W, Song J-Y (2011) Serosurveillance for Japanese encephalitis virus in wild birds captured in Korea. J Vet Sci 12:373–377. https://doi.org/10.4142/jvs.2011.12.4.373

Yap G, Lim XF, Chan S, How CB, Humaidi M, Yeo G, Mailepessov D, Kong M, Lai YL, Okumura C, Ng LC (2019) Serological evidence of continued Japanese encephalitis virus transmission in Singapore nearly three decades after end of pig farming. Parasit Vectors 12 (1):244. https://doi.org/10.1186/s13071-019-3501-0

Yeh JY, Lee JH, Seo HJ, Park JY, Moon JS, Cho IS, Lee JB, Park SY, Song CS, Choi IS (2010) Fast duplex one-step reverse transcriptase PCR for rapid differential detection of West Nile and Japanese encephalitis viruses. J Clin Microbiol 48(11):4010–4014. https://doi.org/10.1128/JCM.00582-10

Yen NT, Duffy MR, Hong NM, Hien NT, Fischer M, Hills SL (2010) Surveillance for Japanese encephalitis in Vietnam, 1998–2007. Am J Trop Med Hyg 83(4):816–819

Yun S-I, Lee Y-M (2014) Japanese encephalitis: the virus and vaccines. Hum VaccinImmunother 10(2):263–279

Yun SI, Song BH, Polejaeva IA, Davies CJ, White KL, Lee YM (2016) Comparison of the live attenuated Japanese encephalitis vaccine SA14-14-2 strain with its pre-attenuated virulent parent SA14 strain: similarities and differences in vitro and in vivo. J Gen Virol 97:2575–2591. https://doi.org/10.1099/jgv.0.000574

Zhang H, Rehman MU, Li K, Luo H, Lan Y, Nabi F, Zhang L, Iqbal MK, Zhu S, Javed MT et al (2017) Epidemiologic survey of Japanese encephalitis virus infection, Tibet, China, 2015. Emerg Infect Dis 23:1023–1024. https://doi.org/10.3201/eid2306.152115

Zhao S, Lou Y, Chiu APY, He D (2018) Modelling the skip-and-resurgence of Japanese encephalitis epidemics in Hong Kong. J Theor Biol 454:1–10. https://doi.org/10.1016/j.jtbi.2018.05.017