

JAPANESE ENCEPHALITIS VIRUS



The Swine Health Information Center, launched in 2015 with Pork Checkoff funding, protects and enhances the health of the United States swine herd by minimizing the impact of emerging disease threats through preparedness, coordinated communications, global disease monitoring, analysis of swine health data, and targeted research investments.

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SUMMARY

IMPORTANCE

- Japanese encephalitis virus (JEV) is an arbovirus that causes encephalitis in humans mainly in Asia and the western Pacific. The virus also produces neurological disease in pigs and horses and reproductive failure in pigs.
- JEV emergence in the United States is concerning due to the presence of competent mosquito vectors and susceptible hosts.

PUBLIC HEALTH

- JEV is a zoonotic pathogen. It causes an estimated 70,000 cases of viral encephalitis annually, with a 20–30% mortality rate. However, humans are incidental (dead-end) hosts.
- Amplification of JEV in swine often precedes human epidemics.

INFECTION IN SWINE

- Most JEV infections are subclinical in pigs.
- In sows, infection with JEV before 60–70 days of gestation causes abortion, fetal mummification, stillbirth, or the birth of weak piglets. Infected boars develop reduced sperm motility, testicular edema, and temporary infertility. Piglets born alive may develop encephalitis with tremors and convulsions.

TREATMENT

- There is no available treatment for JEV infection in swine.

CLEANING AND DISINFECTION

- JEV does not survive well in the environment. The virus is inactivated at 40°C (104°F).
- JEV is inactivated by 1% hypochlorite, 3–8% paraformaldehyde, 2% glutaraldehyde, mM binary ethylenimine, ethanol, and isopropanol. JEV is also sensitive to ultraviolet light.

PREVENTION AND CONTROL

- JEV is reportable to the World Organization for Animal Health (WOAH).
- Mosquito control is essential for the prevention of JEV infection. Recommended practices include removing stagnant water around pig enclosures, using insecticides, installing insect screens, and using fans inside buildings where pigs are housed to disrupt mosquito activity.
- Swine vaccination is practiced in some parts of Asia.

TRANSMISSION

- JEV is transmitted by mosquitoes. The main vector in Asia is *Culex tritaeniorhynchus*. Wading ardeid birds (herons and egrets) are the primary natural reservoirs of the virus. Pigs act as amplifying hosts.
- Direct pig-to-pig transmission has been documented experimentally.
- Artificial insemination practices could facilitate JEV spread since infected boars shed virus in the semen.

PATHOGENESIS

- JEV replicates in the skin (keratinocytes) and is transferred to the lymph nodes within dendritic cells. Then, virus disseminates to tissues including the brain via the blood and nerves. Replication occurs in the central nervous system and lymphoid tissues, especially the tonsils.

DIAGNOSIS

- Virus isolation is the reference standard. Some of the molecular methods described for JEV include conventional and nested reverse transcriptase polymerase chain reaction (RT-PCR), quantitative RT-PCR (qRT-PCR), multiplex ligase detection-polymerase chain reaction and microarray (MLPM), multiplex ligation-dependent probe amplification (MLPA), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and antigen-capture enzyme-linked immunosorbent assay (ELISA). RNA has been recovered from oronasal swabs and oral fluids experimentally.
- Serological tests include virus neutralization, hemagglutination inhibition, complement fixation, IgM-capture ELISA, and indirect IgG and IgM ELISA. Tests with potential for on-site use include latex agglutination, lateral flow assay, and protein-based dipstick IgG ELISA.

EPIDEMIOLGY

- Pigs are the main amplifying host of JEV and seroconversion can reach 100%. In addition to humans, horses can become ill. Most other susceptible species are dead-end hosts and do not transmit JEV.
- Seasonal precipitation, humidity, and temperature changes are thought to influence JEV transmission. JEV is endemic in much of Asia and the western Pacific. However, in 2022 an outbreak occurred in people and pigs in Australia.

ETIOLOGY

- JEV belongs to the family *Flaviviridae*. Five genotypes have been described (GI–GV); GI-b is now the dominant JEV genotype throughout Asia, replacing GII and GIII strains.
- The E, prM, and NS1 proteins are immunogenic.

HISTORY IN SWINE

- Human JE epidemics have been described in Japan since the 19th century. The pig-mosquito transmission cycle was confirmed in the late 1960s.
- GIII strains were prevalent until the 1990s. GI has since become dominant among pigs throughout Asia.

IMMUNITY

- Natural infection results in long lasting immunity in adult pigs and surviving piglets.
- Vaccines are mostly based on GIII, leading to concern about efficacy.
- Some cross-protection is likely between strains and other flaviviruses.

GAPS IN PREPAREDNESS

- Introduction of JEV by an infected adult mosquito on aircraft is thought to be the highest risk for the United States. JEV could spread quickly and become endemic similar to West Nile virus.
- Although pig-to-pig transmission of JEV has been described, its role in natural transmission is unclear.

- JEV surveillance and continued research on the cross-protective characteristics of flaviviruses and effective vaccines is needed.

LITERATURE REVIEW: JAPANESE ENCEPHALITIS VIRUS

IMPORTANCE

Japanese encephalitis virus (JEV) is an arbovirus that causes encephalitis in humans mainly in Asia and the western Pacific. The virus also produces neurological disease in pigs and horses and reproductive failure in pigs. JEV emergence in the United States (and other areas outside of Asia) is a concern due to the presence of competent mosquito vectors and susceptible hosts. Most recently, JEV caused an outbreak in 2022 affecting people and pigs in Australia.

PUBLIC HEALTH

JEV is a zoonotic pathogen that cycles between mosquitoes, domestic and wild birds, domestic and feral pigs, and humans.² JEV is the greatest known cause of epidemic viral encephalitis worldwide,³ and more than one-third of the world population is at risk.⁴ JEV predominantly infects children and travelers from non-endemic areas without immunity to the virus.² An estimated 70,000 human cases of JEV occur annually, with a 20–30% mortality rate.¹ Up to 50% of survivors develop long-term neurological sequelae.¹ Humans are considered dead-end hosts.² General awareness of JEV may be low in developing areas; in Cambodia, for instance, very few households knew that JEV is transmitted to pigs and people through mosquito bites.⁵

Amplification of JEV in swine often precedes human epidemics.^{6,7} The density, size, and spatial organization of rice paddies, swine farms, and human communities influence human risk.^{3,8,9} However, JEV is not only a rural disease. Confirmed circulation in peri-urban environments exists in Cambodia,^{10,11} where identical JEV strains have been found in humans, pigs, and mosquitoes (*Culex tritaeniorhynchus*).¹² Dogs have been proposed as JEV sentinels in peri-urban and rural environments since they live with or near humans; they develop antibodies but do not transmit the virus to humans.¹³

Some studies suggest that proximity to pigs is related to increased JEV infection. Mosquitoes living near farm households with pigsties are more likely to be infected with JEV than those living near farm households without pigsties or intensive pig farms located 2 km away.¹⁴ Similarly, JEV prevalence in mosquitoes is highest in traps near pigs in urban Vietnam.¹⁵ In Hong Kong, the absence of human cases from 2006–2010 was linked to a sudden reduction in the number of pigs, while a 2011 resurgence was related to a new JEV strain.¹⁶ In regions with little to no pig farming, a secondary reservoir could explain JEV transmission.¹⁷ Examples include:

- Singapore: pig farming was abolished in 1992, and afterward, transmission of JEV nearly ceased. However, serosurveillance has since detected anti-JEV antibodies in migratory birds (2010–2017), wild boar (2014), and sentinel chickens (2013–2018).¹⁸
- Goa, India: despite few human JE cases in this region, about 7% of pigs were seropositive.¹⁹ The highest prevalence occurred in the post-monsoon season,²⁰ when mosquito populations were high.²¹

JEV expansion out of Asia is a concern (see *Geographic Distribution*). The virus was detected in pigs and people in four Australian states in 2022. Ultimately, 42 human cases and seven deaths were reported.²² Since then, surveillance shows that nearly 9% of people from New South Wales²³ and 3% from northern Victoria²⁴ were seropositive. JEV has also been isolated from Australian mosquitoes.²⁵ A nationwide cross-sectional study has been proposed to determine wider seroprevalence and identify risk factors.²⁶ Efforts to control mosquito breeding in and around pig farms and to vaccinate at-risk people have been implemented.

Laboratory-acquired JEV infection has been reported in humans, and work with the virus is restricted to BSL-3 facilities and practices. Transmission occurs through needle sticks and potentially at mucosal surfaces if exposed

to high concentrations of aerosolized virus.²⁷ Transmission of JEV via blood transfusion was reported in two patients for the first time in 2018.²⁸

INFECTION IN SWINE

CLINICAL SIGNS

JEV mainly causes subclinical infection in pigs. Reproductive failure is the most common clinical manifestation. Infection with JEV before 60–70 days of gestation can cause abortion, fetal mummification or stillbirth, or the birth of weak piglets.¹ In endemic areas, the breeding impact may be most significant in boars. Infected boars can experience reduced numbers and motility of sperm.¹ Testicular edema and temporary infertility in boars have also been observed.¹ Some boars regain fertility while others do not.²⁹ Piglets born alive may develop encephalitis, tremors, and convulsions.¹ In some cases, weak or stillborn piglets have abnormal folding in the brain or even the absence of brain tissue altogether.²⁹

An experimental study inoculated 3-week-old domestic pigs intravenously with GI-b, an emerging genotype (see *Etiology*). Non-specific clinical illness occurred (fever, weight loss, lethargy, depression) but resolved in one week. Mild ataxia was also seen in a few pigs.³⁰ Pigs with a feral phenotype developed signs similar to domestic pigs when inoculated with GI-b.³¹

No characteristic lesions are seen in sows. Lesions in boars may include thickening of the tunica vaginalis and epididymis and edema and inflammation of the epididymis, tunica vaginalis, and testis.¹ Affected piglets develop hydrocephalus, cerebellar hypoplasia, subcutaneous edema, and spinal hypomyelinogenesis.¹ Multifocal hepatic and splenic necrosis may also be seen grossly, while histological lesions are generally restricted to the central nervous system.^{1,32} Brain and spinal cord changes can include serosal petechiae, bicavitary effusions, neuronophagia, glial nodules, and perivascular cuffing.¹

TREATMENT

There is no available treatment for JEV infection in swine. Anti-flavivirus drugs have been reviewed by Pierson and Diamond.³³

CLEANING AND DISINFECTION

SURVIVAL

JEV does not survive well in the environment. The virus is inactivated at 40°C (104°F) and can be destroyed by heating for 30 min at 56°C (132.8°F). JEV is stable in alkaline environments (pH 7–9) and inactive in acidic environments (pH 1–3).¹

DISINFECTION

JEV is inactivated by 1% hypochlorite, 3–8% paraformaldehyde, 2% glutaraldehyde, mM binary ethylenimine, ethanol, and isopropanol. JEV is sensitive to ultraviolet light.¹

PREVENTION AND CONTROL

DISEASE REPORTING

JE is a World Organization for Animal Health (WOAH)-listed disease and must be reported internationally according to the *Terrestrial Animal Health Code*. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION AND CONTROL

Mosquito control is essential for the prevention of JE. Recommended practices include removing stagnant water around pig enclosures, using insecticides, installing insect screens, and using fans inside buildings where pigs are housed to disrupt mosquito activity.³⁴ However, the discovery of direct pig-to-pig transmission shows that JEV may spread and circulate even in areas without competent mosquito vectors.^{35,36}

The decline of JEV in endemic areas has been attributed to large-scale human immunization, immunization of pigs (see *Immunology*), separation of pigs from human communities, alterations in agricultural practices to reduce the presence of mosquitoes, and better overall standards of living.³⁷

TRANSMISSION

JEV is usually transmitted by mosquitoes. Wading ardeid birds (such as herons and egrets) are the primary natural reservoirs of the virus. Pigs act as amplifying hosts, developing high viremia that lasts for 2–4 days.^{1,37} Additional proposed amplifying hosts include orangutans (in Borneo) and bats.³⁸ Seropositive raccoons, raccoon dogs, and flying foxes have also been identified.³ Many species are most likely incidental (dead-end) hosts, including horses, cattle, buffalo, goats, sheep, dogs, and humans.¹

Mosquito vectors must be capable of acquiring JEV, transmitting the virus to susceptible hosts, and abundant in the environment.³⁹ *Culex tritaeniorhynchus*, found in rice fields, wells, ponds, ditches, and urban environments, is the primary JEV vector in Asia.⁴⁰ Others include *Cx. fuscocephala*, *Cx. annulirostris*,⁶ *Cx. annulus*, *Cx. sitiens*, and potentially *Cx. quinquefasciatus*.³ Important regional vectors include *Cx. vishnui* in India, *Cx. gelidus* in Indonesia,³⁸ and *Cx. annulirostris* in Australia.⁴¹ *Anopheles sinensis*, *Anopheles plumbeus*, and *Aedes albopictus* are also vectors,^{42,43} along with several *Armigeres* and *Mansonia* species.⁴⁴ Other mosquito species with a feeding preference for pigs could potentially spread pathogens like JEV.^{9,45}

American mosquitoes exposed to JEV are susceptible to infection.^{46,47} Some natural JEV vectors (*Cx. pipiens*, *Cx. quinquefasciatus*, and *Ae. japonicus*)⁴⁸ are also vectors of West Nile virus in the United States.⁴⁹ Environmental temperatures in the range of 22–34°C (71.6–93.2°F) have been shown to increase mosquito density,⁴² decrease larval development time, and reduce the extrinsic incubation period of the virus in mosquito vectors, affecting potential JEV transmission.⁵⁰

Experimentally, pigs have been co-infected with JEV and mosquito salivary gland extract (*Cx. quinquefasciatus*) to mimic natural transmission. In these animals, febrile illness and duration of nasal shedding were reduced, but there was no effect on viremia or pathogenesis.⁵¹

Non-vectorborne JEV transmission in pigs has been documented by Ricklin et al.³⁵

- Two sentinel pigs were placed in contact with three pigs that had been infected intravenously with JEV. Viremia was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in both contact pigs for 2–4 days but at a lower level than in needle-infected animals.³⁵
- Six sentinel pigs were housed with two needle-infected pigs. Viremia was detected by qRT-PCR in only one contact pig and lasted three days; values were similar to those found in needle-infected pigs.³⁵
- Nine pigs were oronasally inoculated with three doses of JEV (10^3 , 10^5 , or 10^7 TCID₅₀). By qRT-PCR, viremia was detected in all groups for about four days. JEV RNA was also found in most oronasal swabs between 3–6 days post-infection.³⁵
- Nine pigs were oronasally inoculated with three doses of JEV (10^1 , 10^2 , or 10^3 TCID₅₀). All pigs became infected and were viremic for 5–6 days. Viral RNA was detected in oronasal swabs as early as three days post-infection; by seven days post-infection, all swabs were positive.³⁵

Through all experiments, the highest levels of viral RNA were consistently found in the tonsil, where persistence

was documented for at least 25 days.³⁵ A further study confirmed that high viral RNA loads are found in the tonsils and that JEV can persist for up to 46 days.³⁶ Isolation of JEV RNA from oronasal swabs was also replicated by further work. Three 5-week-old control pigs challenged with JEV GIII shed virus oronasally from 4–10 days post-infection. Low amounts of JEV RNA were also detected in oronasal swabs from pigs infected with a GI strain or vaccinated with TRIP/JEV.prME (see *Immunology*).³⁶

A more recent experimental study found that viremia developed at three dpi (following intravenous inoculation with GI-b), neutralizing antibodies developed by 28 dpi, and virus was shed in nasal secretions. Similar to other JEV strains, tissue tropism for nervous and lymphoid tissues was seen.³⁰ Another experimental study found that nasal epithelium could be a route of entry for JEV in pigs.⁵² Aerosol transmission has been demonstrated experimentally in mice.⁵³

Artificial insemination practices in modern swine production could potentially lead to JEV transmission. JEV is known to cause inflammation of the genital tract in boars, which can lead to subsequent shedding of the virus in semen and possible transmission to breeding sows.⁵⁴ Experimentally, JEV causes persistent infection in the lower and upper reproductive tract of female pigs.⁵⁵

PATHOGENESIS

Following a bite from an infected mosquito, JEV replicates in the skin (keratinocytes) and is then transferred to the lymph nodes within dendritic cells.¹ From there, virus disseminates to many different tissues, including the brain, via the blood and nerves.⁵⁶ Replication occurs in the CNS and lymphoid tissues, especially tonsil, where viral persistence has been documented for up to 46 days.^{35,36,57} In immunocompromised hosts, cell and tissue tropism and viral replication are enhanced.¹

DIAGNOSIS

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

Virus isolation is the reference standard for definitive diagnosis. JEV can be grown in 2–4-day-old mice, chicken embryos, or cell lines, including African green monkey kidney (Vero) cells, baby hamster kidney (BHK) cells, porcine kidney (PSEK) cells, and the *Aedes albopictus* mosquito cell line C6/36.¹ A pig tonsil epithelial (PT) cell line has recently been established for JEV research.⁵⁸ Indirect immunofluorescence can be used to identify viral antigen using JEV-specific monoclonal antibodies or monoclonal antibodies for flaviviruses in general. Isolated viruses can also be identified by RT-PCR or serological methods.

RT-PCR is routinely used in diagnostic laboratories,⁴⁰ but methods have been hampered by the short period of viremia and low sensitivity.⁵⁹ Numerous variations have been described for conventional and nested RT-PCR and qRT-PCR as reviewed by Bharucha and colleagues.⁵⁹ For swine, multiplex RT-PCR assays have been developed for JEV and other swine pathogens, including pseudorabies virus, porcine parvovirus, porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, classical swine fever virus, *Actinobacillus pleuropneumoniae*, and *Mycoplasma hyopneumoniae*.⁶⁰⁻⁶⁸

Additional molecular techniques have been described for JEV, including:

- Multiplex ligase detection–polymerase chain reaction and microarray (MLPM) for detection of PCV, PRRSV, CSFV, PPV, PRV, and JEV⁶⁹
- Multiplex ligation-dependent probe amplification (MLPA) for detection of PCV, PRRSV, CSFV, PPV, PRV, and JEV⁷⁰
- Droplet digital PCR (ddPCR, JEV only)⁷¹
- Reverse transcription loop-mediated isothermal amplification (RT-LAMP) detecting the *NS1* gene,⁷² *NS3* gene,⁷³ *E* gene,⁷⁴ *C* gene^{75,76}
- RT-LAMP plus lateral flow dipstick (LFD) to detect multiple JEV strains⁷⁷

- Rapid Visual CRISPR (RAVI-CRISPR) assay to detect the *C* gene, based on RT-LAMP and CRISPR/Cas12a⁷⁸
- Reverse transcription recombinase-aided amplification (RT-RAA)⁷⁹

RT-LAMP assays are highly specific and do not cross-react with other clinically and/or serologically related swine diseases.^{72-74,77} Given the efficacy, simplicity, and speed of these assays, they are an option for on-site testing in an outbreak situation.

Flavivirus-specific monoclonal antibodies (MAb) can detect JEV antigen in serum, and immunohistochemistry can be used to detect JEV antigen in fetal tissues.¹ Antigen capture enzyme-linked immunosorbent assay (Ag-ELISA) has been developed for simple and rapid clinical detection of JEV infection as an alternative to virus isolation and RT-PCR. MAb against the E protein is used for its high specificity to JEV, followed with a polyclonal antibody (PcAb) conjugate to enhance sensitivity and detection of multiple strains. Ag-ELISA may be unable to detect very low viral loads.⁸⁰

TESTS TO DETECT ANTIBODY

Serological tests can be used to determine JEV prevalence in a population or to make a diagnosis in a diseased individual. Paired samples must be used to demonstrate a rise in titer from the acute to convalescent phases of disease.⁸¹ WOAHP recommends that diagnosis by serology be confirmed by virus isolation.

Serological methods for JEV include:

- Virus neutralization (VN), also known as the plaque reduction neutralization test (PRNT), is difficult and time-consuming, but it is the most specific serological test for JEV. VN is the gold standard for JEV diagnosis.⁸¹
- Hemagglutination inhibition (HI) is widely used despite cross-reactivity with other flaviviruses. A four-fold rise in titer is sufficient for diagnosis of flavivirus infection, though not necessarily JEV.⁸¹
- Immunochromatographic tests have been described for the E protein⁸² and NS1.⁸³
- IgM capture-ELISA (MAC-ELISA) detecting anti-NS1 antibody is useful for recent JEV infections (within 2–3 days and up to three weeks post-infection).¹ Indirect IgG ELISA⁸⁴ and IgM ELISA⁸⁵ have been described using recombinant NS1 protein as antigen, as well as a mAb-based blocking ELISA.⁸⁶
- Complement fixation (CF) is occasionally used for diagnosis by combining test serum, antigen extracted from inoculated mouse brains with acetone/ether, and pooled fresh guinea pig serum (complement). A fourfold change in titer is required for diagnosis.⁸¹
- Indirect immunofluorescence has been used to detect anti-JEV IgG or IgM in humans, with similar sensitivity and specificity to existing serological methods.⁸⁷

The interpretation of serological results, especially in older pigs, must consider vaccination history and age since maternal antibody can persist for up to eight weeks.¹ There is some cross-reactivity with other flaviviruses for all JEV serological tests. Epitope-blocking ELISAs can help to determine if cross-reactions have occurred (i.e., presence of other viruses from the JEV serogroup).⁴

Experimentally, protein biochip technology has been described that simultaneously detects antibodies to CSFV (E2 protein), PPV (VP2 protein), JEV (domain III of the E protein), and PRRSV (N protein).⁸⁸

Tests with potential on-site application must be quick, low cost, and simple (requiring no special equipment or skills). Tests designed with these factors in mind include:

- Latex agglutination using recombinant NS1 protein as antigen^{89,90}
- Lateral flow assay (LFA) using recombinant NS1 protein as antigen⁹¹
- Protein-based dipstick IgG ELISA using recombinant NS1 protein as antigen⁹²

SAMPLES

Infected tissues (brain, spleen, liver, placenta) are preferred for virus isolation.¹ Four to six fetuses should be sampled to avoid missing a diagnosis.^{1,32} Samples appropriate for RT-PCR include cerebrospinal fluid, sera, tissue culture supernatants, and tissue homogenates.¹ Ag-ELISA detects JEV in cerebrospinal fluid, brain tissue, and mosquito homogenate.⁸⁰ Detection of anti-JEV antibodies in serum, thoracic, or abdominal fluid of stillborn piglets is considered diagnostic.³²

RNA has been recovered from oronasal swabs in pigs experimentally infected with JEV and contact pigs.³⁵ Further, RNA has been found in oronasal swabs from control pigs challenged with JEV, previously infected with JEV, and inoculated with an experimental vaccine.³⁶ JEV is also found in oral fluids in experimentally infected pigs.⁹³

EPIDEMIOLOGY

SPECIES AFFECTED

Wading ardeid birds (such as herons and egrets) are the primary natural reservoirs. Other reservoirs may exist (see *Transmission*). Domestic pigs and wild boar are the main amplifying hosts of JEV, exhibiting high and prolonged viremia and a natural infection rate of 98–100%.⁴ Sporadic infection occurs in horses, resulting in severe encephalitis or subclinical infection. In horses, the mortality rate is about 5%. Rarely has JEV-associated encephalitis been described in cattle.⁹⁴⁻⁹⁶ Subclinical infection has been reported in nonhuman primates, sheep, goats, dogs, cats, chickens, ducks, wild mammals, reptiles, and amphibians. However, it is not known if these species contribute to the spread of the disease.

GEOGRAPHIC DISTRIBUTION

JEV is endemic in Asia and the western Pacific region. JEV occurs as far west as Pakistan, as far south as northern Australia and Papua New Guinea,³⁷ as far north as Siberia, and as far east as the island of Saipan.⁹⁷ The virus seems to be circulating in Africa, but to what extent is unclear.^{98,99} In early 2022, JEV was detected in domestic pigs, feral swine, horses, humans, and one alpaca in southern and eastern Australia.^{22,29}

Seasonal precipitation, humidity, and temperature changes are thought to influence JEV transmission by affecting human agricultural practices and the life cycle of mosquito vectors. High absolute humidity is a key factor known to influence mosquitoes' longevity, mating, dispersal, and feeding behavior.¹⁰⁰ JEV may spread to new areas through infected reservoir birds, windborne mosquitoes, or infected mosquito hitchhikers on ships or aircraft.¹⁰¹

In endemic areas, cases generally occur sporadically throughout the year, with occasional increases during the rainy season (e.g., Cambodia). JEV exhibits more epidemic viral activity in northern temperate Asian climates, with disease outbreaks in summer months (e.g., north India, Sri Lanka, Japan). Subtropical regions, such as Thailand, Vietnam, Laos, and Malaysia, may see a combination of epidemic and endemic disease characteristics, where JEV circulates year-round but peaks during the hot, rainy seasons.^{2,7}

Year-round transmission occurs in tropical regions.¹⁰² Maintenance of JEV in temperate climates might occur through overwintering mosquitoes, poikilothermic vertebrates, hibernating bats, or vertical transmission from female mosquitoes to offspring.^{97,102} Annual reintroduction into temperate climates by migrating birds, bats, or windborne mosquitoes is also a possibility.⁶ JEV infection has been documented in wild boar during winter in Japan.¹⁰³ Furthermore, JEV persists in swine tonsils for weeks to months, a possible new mechanism for virus overwintering in pigs.^{35,36,104}

Epizootics involving swine are cyclical, consisting of two separate amplification cycles. During the first cycle, roughly 20% of pigs become infected and develop antibodies within ten days. This is followed by a second cycle, 1–2 weeks later, in which mosquitoes transmit the virus to remaining naïve pigs, effectively raising the rate of seroconversion to almost 100%. Clinical cases in humans typically occur following this cycle of amplification in swine.⁶ Since the incubation period in mosquitoes ranges from 5–15 days, it can take up to 30 days for the virus to complete its infection process in humans.⁴²

Direct pig-to-pig transmission of JEV has been documented experimentally,³⁵ but whether it impacts viral maintenance in the absence of mosquitoes is unknown (see *Transmission*).³⁹ Open manure lagoons could possibly serve as a breeding ground for mosquitoes and attract waterfowl.^{9,29} It has been suggested that mechanical ventilation of swine buildings may reduce the risk of JEV infection in pigs.²⁹

MORBIDITY AND MORTALITY

In swine, production losses arise primarily from reproductive failure and high piglet mortality.⁷⁴ The mortality rate in infected piglets with no immunity to JEV is near 100%, while mortality in infected adult swine is close to zero. Reproductive losses can reach 50–70%.

Seroprevalence of JEV in pigs varies regionally. In monitored swine, seroconversion can reach 100%.^{10,11} Ladreyt and colleagues⁷ summarized JEV seroprevalence studies in swine and found that global prevalence ranged from about 3% to 74%. Newer estimates of seropositivity include 93% in southern India,¹⁰⁵ 35% in Nigeria,⁹⁸ 96% in Bali,¹⁰⁶ and 19% in Hunan Province, China.¹⁰⁷ Additionally, a meta-analysis in pigs and other animals found that JEV prevalence was highest in Southeast Asia (39%), followed by East Asia (35%) and South Asia (15%), with an overall prevalence of 26%.¹⁰⁸ Data on antibody detection via ELISA or HI (without PRNT confirmation) should be interpreted with caution since it could be confounded by cross-reactivity with other flaviviruses.⁷

ETIOLOGY

CHARACTERISTICS OF FLAVIVIRUSES

JEV is a small (40–60 nm), enveloped, single-stranded RNA virus belonging to the family *Flaviviridae*. The family contains four genera: *Orthoflavivirus*, *Pestivirus*, *Hepacivirus*, and *Pegivirus*.¹⁰⁹ JEV belongs to the genus *Orthoflavivirus*, along with dengue virus, West Nile virus, yellow fever virus, Zika virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, and others. Speciation of flaviviruses is based on nucleotide sequencing and antigens but also considers geographic distribution, vector types, host types, disease types, and ecology.¹⁰⁹

Flaviviruses contain a single long open reading frame encoding for three structural proteins: capsid (C), envelope (E), and membrane (prM).^{1,109} prM and E are involved in viral entry and virion assembly.¹ During egress, prM is enzymatically cleaved to produce the M protein.¹ Flaviviruses also encode for seven nonstructural proteins that play a role in viral protein processing, RNA synthesis, and evasion of innate immunity (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).^{1,109} The E, prM, and NS1 proteins are immunogenic.¹

CHARACTERISTICS OF JAPANESE ENCEPHALITIS VIRUS

There is a single JEV serotype. Five genotypes have been described, GI–GV. Genotype relationship to phenotype or virus fitness is unclear.³ The basis for genotyping is phylogenetic analysis of the *E* gene, which is required for viral attachment and entry into host cells.⁴ Global distribution of JEV genotypes includes the following:

- GI-a: Cambodia, Thailand, Vietnam, India, Singapore, Australia
- GI-b: Vietnam, China, Japan, Korea, Taiwan
- GII: Indonesia, Malaysia, southern Thailand, Papua New Guinea, Australia
- GIII: throughout Asia
- GIV: Indonesia, Australia
- GV: Malaysia, China (Tibet), South Korea

Overall, JEV genetic diversity appears to be increasing over the past ten years.¹²² GIII was the most frequently isolated genogroup until the 1990s. GI-b is now believed to be the dominant JEV genotype throughout Asia, replacing GII and GIII strains.^{97,102,110-112} Local transmission leads to GI genetic diversity,¹¹³ and new variations are being described.^{114,115} However, GIII strains are still detectable,¹¹⁶⁻¹¹⁹ and regional co-circulation of GI and GIII strains has been documented in China.¹²⁰

GIV is believed to be ancestral to other circulating genotypes and shows the largest antigenic and phylogenetic difference of all the genotypes.⁴⁶ Previously confined to Indonesia, it has recently spread to Australia.^{22,29} GV reemerged in Tibet and South Korea after remaining undetected for nearly 60 years.¹⁰² GV has shown a high capacity for dispersal in a short time, and warrants continued monitoring given its potential for high virulence.¹²¹

HISTORY IN SWINE

Human JEV epidemics have been described in Japan since the 19th century.³³ The pig-mosquito transmission cycle was first investigated in 1964 and confirmed by 1969.⁷ As previously described, GIII strains were prevalent until the 1990s. Since then, GI has become dominant among pigs throughout Asia. However, GIII and GIV strains continue to circulate.¹¹⁶

In 2022, a JEV outbreak occurred in Australia, expanding into the eastern and southern regions and impacting 60% of the swine industry. Movement of infected birds and mosquitoes was suspected to play a role in transmission, since many affected sites were located near a river or in a river basin.²⁹ Temperature and precipitation during that period were also favorable for JEV transmission.¹²³

IMMUNITY

POST-EXPOSURE

Natural infection contributes to long-lasting immunity in adult pigs and surviving piglets. Protective antibodies recognize proteins E and NS1.^{33,124} Both CD4+ and CD8+ T cells also have a role in flavivirus immunity.³³ Some sows and boars develop protective immunity prior to reaching sexual maturity, which prevents clinical disease (reproductive failure in sows, infertility in boars).¹²⁵ As described by Ladreyt and colleagues,⁷ anti-JEV antibodies appear 3–7 dpi after experimental infection and are detectable for 7–35 dpi (study endpoint).⁷

VACCINES

Most vaccine development is focused on humans.¹ Vannice and colleagues have described the status of JE vaccines and vaccination programs.¹²⁶ There is an inactivated whole virus JEV vaccine that is FDA-approved for emergency use in humans. There is no approved swine vaccine in the United States. However, in an outbreak, vaccination of pigs may reduce the risk of human disease in JEV-endemic areas.¹

Regionally approved swine JEV vaccines are available for local use in Asia.³⁹ Swine vaccines for JEV are mostly GIII-based. Since GI has largely replaced GIII, there is concern over vaccine efficacy. In naïve gilts inoculated with a GIII live-attenuated vaccine, effectiveness against GI ranged from about 65% to 75%.¹²⁷ In mice, there is partial cross-protection between GI and GIII strains. Administration of an inactivated GI vaccine led to 50–90% protection against a GIII challenge, while administration of an inactivated GIII strain led to 60–80% protection against a GI challenge.¹²⁸

Worldwide, swine vaccination is not widely practiced due to cost and the limited time window for administration. Based on temperature variations and month of birth, for example, it was predicted that only 80% of pigs born from July to September in Vietnam would reach immunity by sexual maturity.¹²⁹

CROSS-PROTECTION

Cross-protective antibody responses have been induced experimentally in pigs exposed to closely related flaviviruses.¹ Flavivirus cross-reactive epitopes are found predominantly in domain II of the E protein.¹²⁴ However, neutralizing epitopes have been mapped in domain III, showing a high level of conservation between multiple strains of JEV and West Nile virus, suggesting some level of cross-protection.¹³⁰ Experimentally, no cross-neutralizing activity between anti-GI and anti-G3 antibodies was observed in guinea pigs infected with two different Japanese strains of JEV.¹³¹

GAPS IN PREPAREDNESS

A risk assessment found that the introduction of JEV into the United States could occur via infected mosquitoes, viremic animals (including viremic birds), infected biological materials or animal products, infected humans, or contaminated vaccines. The highest risk involves infected adult mosquitoes entering via aircraft.^{132,133}

Regions most at risk have climates similar to JEV-endemic areas, such as California and most of the Eastern United States (except Southern Florida and the far Northeast). Like West Nile virus, JEV could spread quickly and become endemic after only a few years.¹³² Evidence suggests that JEV is already circulating in areas outside of Asia, including Europe,¹³⁴ Africa,^{98,99} and Australia.²⁹ Reasons why JEV has not yet emerged in the United States could include:

- High susceptibility of JEV to heat, UV light, and common detergents
- Low numbers of mosquitoes and hosts near airports and seaports
- Short period of viremia (3–4 days) in pigs and ardeid birds
- Lack of contact between hosts (ardeid birds, pigs) and vectors (mosquitoes)
- Cross-protection between JEV and endemic flaviviruses like West Nile virus
- Limited infection capability of mosquitoes during establishment

Although pig-to-pig transmission has been described, its role in natural JEV infection is unclear.³⁹ Modeling indicates that pig-to-pig transmission helps explain swine seroprevalence data in Cambodia.¹³⁵ Further studies on JEV transmission are needed. Improved surveillance of people, pigs, and mosquitoes and the development of rapid, specific diagnostic tests would increase outbreak readiness in the United States.^{9,136} Additionally, continued research on the cross-protective characteristics of flaviviruses and effective vaccines will help prepare for JEV.⁶

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