

Japanese Encephalitis

*Japanese B Encephalitis,
Arbovirus B,
Mosquito-borne Encephalitis Virus*

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Importance

Japanese encephalitis is a mosquito-borne viral disease that affects horses, donkeys, pigs and humans. In countries where it is endemic, this virus causes reproductive losses in swine and encephalitis in horses. Birds, which are infected asymptotically, serve as important reservoir hosts. In humans, Japanese encephalitis can be a very serious disease: although most infections are asymptomatic, clinical cases tend to manifest as severe, often fatal encephalitis. Epidemics, which occur periodically in endemic regions, can cause significant morbidity and mortality in unvaccinated humans and animals. Approximately 4,000 people died during the 1924 epidemic in Japan, and nearly 2,500 died in South Korea in 1949. In Japan, close to 3,700 horses died in 1949. Sporadic cases also occur in susceptible humans and animals throughout the mosquito season.

During the last fifty years, Japanese encephalitis virus has gradually expanded its geographic range within Asia. It has also become endemic in parts of Australia and Indonesia. When this virus becomes established in a new region, major epidemics can occur. There is a possibility that Japanese encephalitis could become endemic in the United States. The West Nile virus, a closely related flavivirus, was introduced to the U.S. in the late 1990s and has become endemic in wild birds and native mosquitoes.

Etiology

Japanese encephalitis virus is an arbovirus (arthropod-transmitted virus) in the genus *Flavivirus* and family Flaviviridae. There is only one serotype but there are two subtypes of the virus (Nakayama and JaGar-01). Viral strains can also be grouped into four or perhaps five genotypes.

Japanese encephalitis virus is closely related to St. Louis encephalitis virus, Murray Valley encephalitis virus and West Nile virus; these viruses and a few others comprise the Japanese encephalitis serogroup of the flaviviruses.

Species Affected

The Japanese encephalitis virus causes disease in horses, donkeys, pigs and humans. This virus can also infect other domesticated animals including cattle, sheep, goats, dogs and cats, as well as wild mammals, reptiles, amphibians and birds; these infections are typically asymptomatic. Birds including herons and egrets, and swine are the most important maintenance hosts.

Geographic Distribution

Japanese encephalitis virus occurs throughout the temperate and tropical regions of Asia. Recently, this virus has also spread to Indonesia, northern Australia, Papua New Guinea and possibly Pakistan.

Transmission

Japanese encephalitis virus is usually transmitted by mosquitoes in the genus *Culex*. The specific mosquito vectors vary with the region; however, *Culex tritaeniorhynchus* is important in spreading this virus to humans and domesticated animals across a wide geographic range. *C. tritaeniorhynchus* breeds in rice paddies and connecting canals, and is active at twilight. Many other species of *Culex* including *C. vishnui* and *C. fuscocephala* can also transmit Japanese encephalitis virus. In some regions, *Aedes* mosquitoes have been implicated in transmission. The virus has also been isolated from mosquitoes in the genera *Anopheles* and *Mansonia*; however, their role in transmission has not been confirmed.

Most animals are infected when they are bitten by a mosquito. Lizards and bats can also be infected by eating infected mosquitoes. Boars transmit the virus in semen. Birds are the most important reservoir hosts, and usually maintain the virus cycle in nature. Although birds of the family Ardeidae (herons, egrets and bitterns) have been studied the most, other avian species may also be important in transmission. Swine are important amplifying hosts, as they are bitten by the same mosquitoes that bite horses and humans. With the possible exception of bats, most other species of mammals either do not develop viremia capable of infecting mosquitoes, or are unimportant in the epidemiology of the disease for other reasons. For example,

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although horse-to-horse transmission via mosquitoes has been demonstrated in the laboratory, too few susceptible horses are usually found in an area to maintain the virus, and the viremia in these animals may be low.

Humans are usually infected when they are bitten by a mosquito. Some cases are acquired in the laboratory or during tissue sample collection; Japanese encephalitis virus can be transmitted through mucous membranes or broken skin, inhaled in aerosols, or acquired by needlestick injuries. Although this virus is occasionally recovered from human blood, people are generally thought to be dead-end hosts.

Japanese encephalitis virus does not persist outside a living host. How the virus survives the winter in temperate climates is unknown. It may be maintained in mosquitoes, either by transovarial passage or during hibernation. Reptiles, amphibians or bats might also be able to carry the virus for long periods of time when they hibernate. Although there is some evidence for each of these hypotheses, their relative importance is unknown.

Incubation Period

In horses, the incubation period is usually 8 to 10 days. Experimentally infected horses develop clinical signs after 4 to 14 days. In experimentally infected pigs, rising temperature and viremia can occur as soon as 24 hours after inoculation. The incubation period in pregnant swine is uncertain, but exposure early in gestation seems to increase the chance that the litter will be affected.

Clinical Signs

In horses, most infections are subclinical. Symptomatic cases vary in severity. Some horses have a mild illness with a transient fever, anorexia, lethargy, and congested or jaundiced mucous membranes. This syndrome usually lasts for 2 to 3 days, and the horse recovers without complications. Other horses develop encephalitis. In the milder form, the horse is lethargic and anorexic, with a fluctuating fever and neurological signs that commonly include difficulty swallowing, incoordination, transient neck rigidity, radial paralysis or impaired vision. Jaundice or petechial hemorrhages may be found on the mucous membranes. These horses often recover within a week. A more severe form, called the "hyperexcitable form," is characterized by high fever, aimless wandering, violent and demented behavior, occasional blindness, profuse sweating and muscle tremors. Although some horses recover, these symptoms are often followed by collapse and death in 1 to 2 days. The hyperexcitable form is uncommon, and occurs in less than 5% of symptomatic horses. In some horses, neurologic defects such as ataxia may persist after recovery.

In pigs, Japanese encephalitis is usually characterized by reproductive disease. The most common symptom is the birth of stillborn or mummified fetuses, usually at

term. Piglets born alive often have tremors and convulsions, and die soon after birth. Pregnant sows may also abort. Nonpregnant animals are usually asymptomatic or experience a transient febrile illness, but symptoms of encephalitis are occasionally seen in pigs up to six months of age. A wasting syndrome was the only symptom in one group of piglets with post-mortem evidence of nonsuppurative meningoencephalitis. In addition, disturbances of spermatogenesis can cause infertility in boars; although this is usually temporary, it can be permanent in severely affected animals.

Other domesticated animals can be infected but typically remain asymptomatic.

Post Mortem Lesions [Click to view images](#)

Only nonspecific post-mortem lesions are seen in horses, and there are no characteristic gross lesions in the brain. Diffuse nonsuppurative encephalomyelitis is seen microscopically. There is phagocytic destruction of nerve cells, perivascular cuffing and focal gliosis. The blood vessels are dilated and contain numerous mononuclear cells.

Mummified or stillborn fetuses can be found in litters from infected sows. Congenital neurologic defects including hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis may be seen in some litters. Experimentally infected piglets with encephalitis had swelling and edema of the brain.

Morbidity and Mortality

In temperate regions of Asia, the Japanese encephalitis season usually begins in May or June, and ends in September or October. Waterfowl are infected when mosquitoes appear in late spring, swine are infected somewhat later, and equine and human cases peak in late summer and autumn when the virus spills over into these hosts. In tropical regions, Japanese encephalitis virus circulates year-round in mosquitoes, birds and swine, but there may be seasonal peaks of disease associated with irrigation, rainfall or other factors that affect the local abundance of mosquitoes and vertebrate amplifying hosts. In some tropical areas, epidemics may be seen at the end of the rainy season.

Naïve pigs are highly susceptible to infection, and pregnant sows often abort or give birth to stillborn and mummified fetuses. During one epidemic in Japan, 50-70% of all pigs suffered reproductive losses. Affected piglets born alive often die; however, the mortality rate is close to zero in adult pigs. Swine are important amplifying hosts for humans and horses, and the number of swine in a region can affect the incidence of disease in other species. However, this varies with the type of husbandry practices, and modern pig farming does not necessarily increase the risk of infection. Wild and feral pigs may also serve as reservoir and amplifying hosts; in a recent study, 68% of wild boars in part of western Japan

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were seropositive. Where Japanese encephalitis is seasonal, serologic surveillance in pigs can be used to predict epidemics in humans.

In horses, cases usually occur sporadically or in small clusters, but epidemics may be seen when large numbers of susceptible animals exist. Inapparent infections are common in this species. Between 1948 and 1967, the morbidity rate in Asia was estimated to be approximately 0.045% (45 cases per 100,000 horses). Higher morbidity rates can be seen during outbreaks. During the 1948 epizootic in Japan, the morbidity rate in horses was 0.3% overall; however, in some areas, it was as high as 1.4%. The case fatality rate in horses is reported to be approximately 5% or less in some areas, and 5-15% in others. During severe outbreaks, the case fatality rate can be as high as 30-40%. Naïve animals are particularly susceptible. When one group of susceptible broodmares was introduced to an endemic area, a third of the mares died.

Diagnosis

Clinical

Japanese encephalitis should be suspected in horses with fever and neurological signs. In temperate regions, this disease is most common in the late summer and early autumn. The principal sign in pigs is the birth of a litter with a large number of stillborn, mummified or weak piglets.

Differential diagnosis

In horses, the differential diagnosis includes equine herpes myelencephalopathy, hepatic encephalopathy, bacterial or toxic encephalitis, equine protozoal myelencephalitis, rabies and viral encephalitides such as Murray Valley encephalitis and Western, Eastern and Venezuelan equine encephalomyelitis.

In pigs, other causes of SMEDI (stillbirth, mummification, embryonic death, and infertility) or encephalitis in newborns should be ruled out. These diseases include Menangle virus infection, porcine parvovirus infection, classical swine fever, porcine reproductive and respiratory syndrome, Aujeszky's disease (pseudorabies), blue eye (La Piedad Michoacan) paramyxovirus disease and porcine brucellosis, as well as a common hemagglutinating DNA virus infection that occurs in Japan. Coronavirus infections can also cause encephalitis in neonatal pigs.

Laboratory tests

A definitive diagnosis can be made by virus isolation. Japanese encephalitis virus can be isolated in chicken embryo, porcine or hamster kidney cells, African green monkey kidney (Vero) cells, the MDBK cell line or mosquito cell lines (e.g. C3/36). Tissue samples are also inoculated into 2 to 4 day old mice. The isolated virus can be recognized as a flavivirus by hemagglutination

inhibition or enzyme-linked immunosorbent assays (ELISAs). It can be confirmed as Japanese encephalitis virus by virus neutralization, reverse transcription polymerase chain reaction (RT-PCR) assays, or immunofluorescence for viral antigens. Virus isolation from sick or dead horses is often unsuccessful.

RT-PCR can also detect viral nucleic acids directly in tissues or blood. Immunohistochemistry has been used to identify viral antigens in the central nervous system (CNS). Histopathology is also helpful.

Serologic tests include virus neutralization, hemagglutination inhibition, ELISAs and immunofluorescence. Complement fixation is also used occasionally. A latex agglutination test has been described in swine. In endemic regions, serologic diagnosis usually depends on a significant rise in titer with paired acute and convalescent samples. A presumptive diagnosis may be made if a high titer is found in a single serum sample, but supportive evidence should be collected if possible. In horses, the detection of specific IgM and IgG in cerebrospinal fluid (CSF) is also good evidence of infection. In regions where other viruses in the Japanese encephalitis serogroup are present, cross-reactions can occur in serologic tests. These reactions can be differentiated by virus neutralization or with epitope-blocking ELISAs.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. Biocontainment conditions are required for all potentially infectious material from a Japanese encephalitis case. Human encephalitis has been seen after infection through a scratch. Biosafety level 3 practices are used during virus isolation.

Japanese encephalitis virus can be isolated from the corpus striatum, cortex, thalamus or spinal cord, and occasionally from the blood or CSF. At necropsy, samples for virus isolation should be taken from animals that have been dead for less than 12 hours, or from animals killed during the acute stage of the disease. Samples for virus isolation should be kept chilled and shipped on wet ice. If shipping will be delayed for 48 hours or more, the samples can be frozen and sent on dry ice.

The brain should be submitted from horses that die of encephalitis; half should be fixed in 10% formalin for histopathology, and the other half left unfixed for virus isolation. Alternatively, samples for virus isolation and histopathology can be collected from the cortex, midbrain and brainstem. In live horses, whole blood should be collected into heparin. Virus isolation can also be attempted from serum and/ or CSF. Virus isolation is rarely successful in live horses, as the viremia is usually

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short-lived. Paired acute and convalescent serum samples, taken 2 to 4 weeks apart, should be submitted for serology. Antibodies may also be found in CSF from acutely affected animals. In addition, a full range of tissues including the spleen, liver, kidney, lung and heart should be collected for histopathology to rule out other causes of encephalitis.

In swine, Japanese encephalitis virus can be isolated from the brains of affected fetuses or piglets that die with encephalitis. The sow has usually cleared the virus by the time an affected litter is born. In endemic areas, serologic diagnosis is complicated by antibodies from vaccination or previous exposure. In addition, seroconversion generally occurs in the sow before reproductive symptoms are seen, and rising titers may not be observed.

Recommended actions if Japanese encephalitis is suspected

Notification of authorities

Japanese encephalitis must be reported to state or federal authorities immediately upon diagnosis or suspicion of the disease.

Federal: Area Veterinarians in Charge (AVIC):

http://www.aphis.usda.gov/animal_health/area_offices/

State Veterinarians:

<http://www.aphis.usda.gov/vs/sregs/official.html>

Control

Japanese encephalitis vaccines can prevent disease in horses and pigs. Vaccines are protective for all genotypes. Vaccinating pigs can also decrease the amplification of the virus, and help protect horses and humans. However, Japanese encephalitis virus is also amplified in birds, and some infections will still occur. Stabling animals in screened barns can be partially protective, particularly during outbreaks. Peak mosquito biting activity is usually from dusk to dawn. Barn fans are helpful, as mosquitoes do not fly well in strong winds. The walls may also be sprayed with insecticides. Insect repellents can help protect individual animals. In some climates, horses may be rugged and hooded in lightweight permethrin-treated material. Environmental control of mosquitoes can reduce the number of vectors, but in many areas it is impractical. Whenever possible, pigs should be raised away from horses.

Japanese encephalitis virus does not survive well outside a living host or tissues. In the laboratory or other situations where disinfection is necessary, this virus can be inactivated with 70% ethanol, 2% glutaraldehyde, 3–8% formaldehyde, 1% sodium hypochlorite, iodine, phenol iodophors and organic solvents/detergents. It is also sensitive to heat, ultraviolet light and gamma irradiation.

Public Health

In parts of Asia, Japanese encephalitis is the most important viral encephalitis in humans. Approximately 20,000-50,000 cases are reported worldwide each year; this is thought to be an underestimate. The incidence of disease varies with the country and the season. In temperate regions, human infections are particularly common in late summer and fall. In tropical areas, infections can occur year-round. People in rural areas have the greatest risk of infection, but occasional cases are reported in cities. Vaccination, changes in agricultural practices and improved standards of living have reduced the incidence of disease in some countries.

The risk of clinical disease is affected by many factors including the strain of the virus, and the person's age and immunity to flaviviruses. Infections are generally more severe in infants and the elderly. Most human infections are asymptomatic: approximately one in every 500 to 1,000 infected people develops clinical signs. However, during some outbreaks, this ratio can be as high as 1 in 25. The initial signs, which begin after an incubation period of 6 to 14 days, are nonspecific; they may include a fever, chills, muscle aches and severe headache with vomiting. In children, the initial symptoms tend to resemble gastrointestinal disease with nausea, vomiting and abdominal pain. Although some people recover, in others a reduced level of consciousness is seen after a few days, with decreased alertness that can progress to coma. Painful stiffness of the neck is common. Convulsions also occur frequently, particularly in children. Some patients develop flaccid paralysis resembling polio, particularly in the upper limbs. In others, there may be tremors, rigidity and/ or abnormal movements. In symptomatic cases, the case fatality rate is approximately 15-30%. Approximately 50% of the survivors have permanent neurologic damage such as epileptic seizures, a Parkinsonian syndrome with tremors and rigidity, or cognitive or language impairment. Convalescence can be prolonged. Miscarriages have been reported in pregnant women who were infected for the first time during pregnancy.

Preventative measures include the use of insect repellents, insecticide-impregnated bed nets, and long-sleeved shirts and pants to discourage mosquito bites. Environmental modifications to decrease mosquito populations, including insecticide spraying, may be used in some areas. Several vaccines are available for humans. These vaccines are protective for all genotypes. In some countries, vaccination is routine in children. In non-endemic areas, laboratory workers at risk of infection should be vaccinated. Travelers should also be vaccinated before traveling to endemic areas, if they will be in danger of infection. Travelers to rural areas are at higher risk than those who visit only urban regions. The risk also varies with the season, duration of travel, activities and lodgings. There is no treatment other than supportive therapy.

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Internet Resources

- Centers for Disease Control and Prevention (CDC) Japanese Encephalitis Home Page
<http://www.cdc.gov/ncidod/dvbid/jencephalitis/index.htm>
- Public Health Agency of Canada. Material Safety Data Sheets
<http://www.phac-aspc.gc.ca/msds-ftss/index.html>
- The Merck Veterinary Manual
<http://www.merckvetmanual.com/mvm/index.jsp>
- United States Animal Health Association.
Foreign Animal Diseases
http://www.vet.uga.edu/vpp/gray_book02/fad/index.php
- World Organization for Animal Health (OIE)
<http://www.oie.int>
- OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/eng/normes/mmanual/a_summry.htm
- OIE Terrestrial Animal Health Code
http://www.oie.int/eng/normes/mcode/A_summry.htm

References

- Acha PN, Szyfres B [Pan American Health Organization (PAHO)]. Zoonoses and communicable diseases common to man and animals. Volume 2. Chlamydioses, rickettsioses, and viroses. 3rd ed. Washington DC: PAHO; 2003. Scientific and Technical Publication No. 580. Japanese encephalitis; p.172-9.
- Agriculture, Fisheries and Forestry – Australia (AFFA). Japanese encephalitis. *Aust Vet J.* 2003;81:658-9.
- Centers for Disease Control and Prevention. Inactivated Japanese encephalitis virus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 1993;42:1-15.
- Diagana M, Preux PM, Dumas M. Japanese encephalitis revisited. *J Neurol Sci.* 2007;262:165-70.
- Guérin B, Pozzi N. Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. *Theriogenology.* 2005;63:556-72.
- Hamano M, Lim CK, Takagi H, Sawabe K, Kuwayama M, Kishi N, Kurane I, Takasaki T. Detection of antibodies to Japanese encephalitis virus in the wild boars in Hiroshima prefecture, Japan. *Epidemiol Infect.* 2007;135:974-7.
- Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2003. Equine encephalomyelitis. Available at: <http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/100900.htm>. Accessed 19 Nov 2007.
- Kitai Y, Shoda M, Kondo T, Konishi E. Epitope-blocking enzyme-linked immunosorbent assay to differentiate west nile virus from Japanese encephalitis virus infections in equine sera. *Clin Vaccine Immunol.* 2007;14:1024-31.
- Lian WC, Liao MY, Mao CL. Diagnosis and genetic analysis of Japanese encephalitis virus infected in horses [sic]. *J Vet Med B Infect Dis Vet Public Health.* 2002;49:361-5.
- Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med.* 2004;10:S98-109.
- Oya A, Kurane I. Japanese encephalitis for a reference to international travelers. *J Travel Med.* 2007;14:259-68.
- Petersen LR, Marfin AA. Shifting epidemiology of Flaviviridae. *J Travel Med.* 2005;12:S3-11.
- Public Health Agency of Canada. Material Safety Data Sheet – Japanese encephalitis. Office of Laboratory Security; 2001 Mar. Available at: <http://www.phac-aspc.gc.ca/msds-ftss/msds173e.html>. Accessed 15 Nov 2007.
- Rosen L. The natural history of Japanese encephalitis virus. *Annu Rev Microbiol.* 1986;40:395-414.
- See E, Tan HC, Wang D, Ooi EE, Lee MA. Presence of hemagglutination inhibition and neutralization antibodies to Japanese encephalitis virus in wild pigs on an offshore island in Singapore. *Acta Trop.* 2002;81:233-6.
- Shope RE. Japanese encephalitis. In: Foreign animal diseases. Richmond, VA: United States Animal Health Association, 1998. Available at: http://www.vet.uga.edu/vpp/gray_book02/fad/jen.php. Accessed 19 Nov 2007.
- Ting SH, Tan HC, Wong WK, Ng ML, Chan SH, Ooi EE. Seroepidemiology of neutralizing antibodies to Japanese encephalitis virus in Singapore: continued transmission despite abolishment of pig farming? *Acta Trop.* 2004;92:187-91.
- Tsai TF. Congenital arboviral infections: something new, something old. *Pediatrics.* 2006;117:936-9.
- World Organization for Animal Health [OIE]. Manual of diagnostic tests and vaccines for terrestrial animals [online]. Paris: OIE; 2004. Japanese encephalitis. Available at: http://www.oie.int/eng/normes/mmanual/A_00092.htm. Accessed 15 Nov 2007.
- Yamada M, Nakamura K, Yoshii M, Kaku Y. Nonsuppurative encephalitis in piglets after experimental inoculation of Japanese encephalitis flavivirus isolated from pigs. *Vet Pathol.* 2004;41:62-7.