JAPANESE ENCEPHALITIS

Aetiology Epidemiology Diagnosis Prevention and Control References

AETIOLOGY

Classification of the causative agent

Japanese encephalitis (JE) virus is a member of the family Flaviviridae, genus *Flavivirus*. Only a single serotype of JE virus has been identified and subtipification has been described. To date, five genotypes have been described based on the phylogenetic analysis of the viral envelope 'E' gene.

Resistance to physical and chemical action

Temperature:	Destroyed by heating for 30 minutes above 56°C; thermal inactivation point (TIP) is 40°C
pH:	Inactivated in acid environment of pH 1–3 (stable in alkaline environment of pH 7–9)
Chemicals/Disinfectants:	Inactivated by organic and lipid solvents, common detergents, iodine, phenol iodophors, 70% ethanol, 2% glutaraldehyde, 3–8% formaldehyde, 1% sodium hypochlorite
Survival:	Virus very labile and does not survive well in the environment; sensitive to ultraviolet light and gamma irradiation

EPIDEMIOLOGY

- JE presents two recognised epidemiologic patterns in Asia:
  - Late summer/early autumn-associated epidemic disease of northern temperate areas
    - large numbers of mosquitoes feed on Ardeid birds (spring season)
    - Ardeid birds migrate between rural and urban ecosystems introducing JE virus (spring season); these birds also amplify virus
    - increased vector activity leads to spill-over and infection of swine by mosquito vectors shared by birds and pigs
    - infection of pigs produces additional amplification of virus; also, large populations of vector-accessible swine with rapid generational overturn facilitate JE virus infection (summer)
    - with profusion of JE virus circulating, mosquitoes of horses and humans also transmit agent to these hosts; usually sporadic and localised epizootics/epidemics (late summer or early autumn)
  - Year-round endemic disease of southern tropical areas
    - continual cycle between birds, swine and mosquitoes
    - principal vectors: *Culex tritaeniorhynchus* and *Culex gelid*
    - minor sporadic outbreaks in horses and humans during monsoon season
- Overwintering of epizootic/epidemic JE virus has not been elucidated
  - introduction of JE virus strains from endemic areas
  - hibernating mosquitoes may maintain virus; also, through transovarial passage
  - maintenance in reptiles, amphibians or bats

Hosts

- Horses are the primary affected domestic animals of JE though essentially a dead-end host; other equids (donkeys) are also susceptible
- Pigs act as important amplifiers of the virus producing high viraemias that infect mosquito vectors
- The natural maintenance reservoir for JE virus are birds of the family Ardeidae (herons and egrets)
  - Although they do not demonstrate clinical disease, they do generate high viraemias upon infection, acting as an amplifying host
- Humans are vulnerable to this disease and this disease is a primary public health concern in Asia; humans are considered a dead-end host

- Other subclinically infected animals which likely do not contribute to spread include: cattle, sheep, goats, dogs, cats, chickens, ducks, wild mammals, reptiles and amphibians

Transmission

- Actual transmission of JE virus occurs by means of mosquitoes
    - principally *Culex* spp. mosquitoes; *Culex tritaeniorhynchus* is important as it has a wide host range that includes birds, horses, swine and humans
        - *C. tritaeniorhynchus* oviposits in flooded fields (fish ponds, rice paddies and ditches) and is most active at twilight hours
    - *Aedes* spp. mosquitoes have also been implicated
    - JE virus has been isolated from other species of mosquitoes (i.e. *Anopheles* and *Mansonia*) but their role is unclear
    - vertical transmission in mosquitoes has been documented
- JE virus cycles in Ardeid birds by means of mosquitoes
- Because of low titres and short duration of viraemia, humans and horses are usually inadequate to maintain the virus or have any important epidemiological impact

Sources of virus

- Water birds of the family Ardeidae; herons and egrets (also known as bitterns)
    - effective at geographical dispersal
- Mosquito vectors as described above
- Once infected, swine amplify JE virus and high titres in blood, provide more infectious agent to vectors
    - JE virus could be transmitted in boar semen

Occurrence

JE virus (JEV) is widespread in eastern, south-eastern and southern Asian countries and has spread to western India and to the western Pacific region including the eastern Indonesian archipelago, Papua New Guinea and Northern Australia. It is most often associated with areas of intensive rice farming and swine production. The epizootic/epidemic JE season in northern Asian temperate climates usually begins in May or June and ends around September or October. Endemic JE virus circulates year-round in tropical areas of Asia among birds, swine and mosquitoes. Some increase in incidence JE may occur in these endemic regions during monsoon season or areas of localised water use or collection.

For more recent, detailed information on the occurrence of this disease worldwide, see the *OIE World Animal Health Information Database* (WAHID) interface [http://www.oie.int/wahis/public.php?page=home]

DIAGNOSIS

Incubation period has been experimentally determined to be 4–14 days with an average range of 8–10 days in horses. Inapparent infection is very common in this species. Swine manifested signs of disease as early as 3 days but viremia and accompanying fever may appear within 24 hours post-inoculation.

Clinical diagnosis

During epizootic periods or in enzootic areas, a presumptive diagnosis of JE can be based on horses that manifest encephalitic disease accompanied by fever. Under similar circumstances, a tentative diagnosis of JE in swine may be made with the presentation of large numbers of still born or weak piglets. A definitive diagnosis of JE in horses depends on the isolation of the causal virus. The isolation rate of virus from diseases or dead horses is usually very low. Clinical, serological and pathological findings are of assistance in diagnosis.

In horses:

- Subclinical disease is most common
- Clinical signs, if present, vary; disease usually presents itself in sporadic or localised clusters
- Three syndromic manifestations have been described:

    - Transitory type syndrome: moderate fever lasting 2–4 days accompanied by inappetence, impaired locomotion, congested or jaundiced mucosa; most commonly with swift recovery of 2–3 days
    - Lethargic type syndrome: variable febrile periods (as high as 41°C), with a pronounced stupor, bruxism and chewing motions, difficulty in swallowing, petechiation of mucosa, advanced incoordination, evidence of neck rigidity, impaired vision, paresis and paralysis; recovery usually occurs within about a week
    - Hyperexcitable type syndrome: high fevers (41°C or higher) accompanied by profuse sweating and muscle tremors, aimless wandering, behavioural changes manifested by aggression, loss of vision, collapse, coma and death; neurologic sequelae may result; 5% mortality in horses (up to 30%)
- Morbidity rates reported from field cases vary from less than 1% to 1.4 %
- Case fatality rate in outbreaks can vary from 5 to 15% but can reach 30–40% in more severe epizootics

In swine:

- Most commonly JE manifests as a reproductive disease; reproductive losses can reach 50–70%
    - abortions in sows
        - stillbirths or mummified foetuses; usually at term
    - reduced number and motility of sperm in boars
- Live born piglets most often demonstrate neurologic signs of tremors and convulsions and may die soon after birth
- Mortality in non-immune, infected piglets can approach 100%
- Mild febrile disease or subclinical disease in non-pregnant females
- Natural infection results in long lasting immunity
- Mortality rate is near zero in adult swine

Case fatality rates in humans can reach 25% and 50% of cases result in permanent neurologic damage; psychiatric disturbances, ataxia, and catatonia

Lesions

- In horses, post-mortem gross lesions of the central nervous system associated with JE are, in general, nonspecific
    - histopathologic examination reveals 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 swine, litters of infected sows contain mummified or stillborn fetuses; some foetuses dark in appearance
    - evidence of congenital neurologic damage; hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis observed in some litters
    - subcutaneous oedema

Differential diagnosis

In horses:

- Other equine viral encephalitides
    - Western equine encephalitis (WEE)
    - Eastern equine encephalitis (EEE)
    - Venezuelan equine encephalitis (VEE)
    - Murray Valley encephalitis
    - West Nile encephalitis
- African horse sickness
- Borna disease
- Viral equine rhinopneumonitis
- Equine infectious anaemia
- Acute babesiosis
- Equine herpes myelencephalopathy
- Hepatic encephalopathy
- Rabies

- Tetanus
- Botulism
- Bacterial or toxic encephalitis
- Equine protozoal myelencephalitis
- Cerebral nematodiasis or protozoodiasis
- Leucoencephalomalacia (*Fusarium moniforme*)

In swine:

- Menangle virus infection
- Porcine parvovirus infection
- Classical swine fever
- Porcine reproductive and respiratory syndrome
- Aujeszky's disease (aka pseudorabies)
- La Piedad Michoacan paramyxovirus (blue eye paramyxovirus)
- Haemoagglutinating encephalomyelitis
- Encephalomyocarditis virus
- Porcine brucellosis
- Teschen/Talfan
- Water deprivation/excess salt
- any other causative agent of SMEDI (stillbirth, mummification, embryonic death, and infertility) or encephalitis in newborns
- Coronavirus infections

Laboratory diagnosis

Samples

Identification of the agent

- A complete set of tissues in 10% formalin from recently dead animals
- Brain, spinal cord and/or cerebrospinal fluid
- Thorasic fluid obtained from aborted fetuses up to 70 days of age

Serological tests

- Heparinised blood or serum of febrile animals in an early stage of infection and closely associated with clinical encephalitic cases
    - paired sera, if the animal survives
    - one sample at time of fever and convalescent phase serum sample should be collected 4–7 days after the collection of the first acute phase sample or at the time of death
- Thorasic fluid obtained from aborted fetuses over 70 days of age

Procedures

Identification of the agent

- Virus isolation in laboratory animals
    - inoculated mice are kept under clinical observation for 14 days; clinical signs observed and brains of dead or moribund mice are collected further passage, may develop, but anorexia becomes evident by the disappearance of the white milk spot on the abdomen
    - sucrose/acetone-extracted antigen is prepared from the infected mouse brains of a second passage in mice
    - antigen is checked for its ability to agglutinate goose red blood cells; if the antigen is able to haemagglutinate red blood cells, it is used in a haemagglutination inhibition (HI) test using a Japanese encephalitis antiserum
- Virus isolation in cell culture
    - primary cultures of chicken embryo, African green monkey kidney (Vero), baby hamster kidney (BHK) cells, or the C6/36 mosquito cell line may be used for virus isolation
    - specimens, such as brain and blood taken from animals suspected of being infected, and the brain suspension from mice after inoculation, are inoculated onto cell cultures

- Monoclonal antibodies specific to flavivirus and Japanese encephalitis virus are used to identify the virus in the indirect fluorescent antibody test
- RT-PCR assay can also be used for identification of JEV in clinical specimens or cell culture fluid using appropriate primers specific for JEV

Serological tests

- Serological tests are useful to determine the prevalence of infection in an animal population, the geographical distribution of the virus, and the degree of antibody production in vaccinated horses
- If serology is to be used for the diagnosis of the disease in individual horses, it should be remembered that horses in an endemic area may have been inapparently infected with the virus or may have been immunised with a vaccine
- Diagnosis requires a significant rise in antibody titre in paired sera collected during the acute and convalescent phases; specificity of each serological test should also be considered
- A latex agglutination test to detect swine antibodies to Japanese encephalitis has recently been described
- An ELISA for antibodies to a nonstructural protein (NS1) of JEV can be used to differentiate antibodies following natural infection from those induced by inactivated vaccines
- In some regions of the world, there is a need to perform additional tests for related viruses before an unequivocal diagnosis of Japanese encephalitis can be made
    - The presence of antibody to other flaviviruses can make serological diagnosis of Japanese encephalitis difficult
    - There is some cross reactivity with other flaviviruses on all the tests; the plaque reduction virus neutralisation test is the most specific
- Laboratory diagnostic methodologies have been described
    - Virus neutralisation
    - Haemagglutination inhibition
    - Complement fixation
    - ELISA

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapter 3.1.10 Japanese encephalitis in the latest edition of the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading "Diagnostic Techniques".

PREVENTION AND CONTROL

Sanitary prophylaxis

- Housing animals in-doors in screened stabling can provide protection from mosquitoes
    - Especially during active JE outbreaks and during peak vector activity (usually dawn to dusk)
    - Insecticides, repellents and fans also provide protection
- Vector control reduces transmission
- Immunisation of swine as they are JE virus amplifier
- If practical, swine should not be raised near horses

Medical prophylaxis

- Vaccine is available for both horses and swine, and also for humans
    - Two types of vaccine: modified live (produced in hamster or swine kidney tissue culture or hamster lung (HmLu) cell line) or inactivated (prepared in mouse brain, chick embryo or cell lines, e.g. Vero cells)
- Vaccination of swine prevents reproductive disorders and directly impacts JE viral amplification; especially in enzootic areas
    - vaccinated breeding sows and boars; protects animals, reduces amplification, ensures healthy litters and decreases likelihood of aspermia
    - drawbacks include high turnover of swine populations requires regular vaccination of newborn pigs which is costly and due to maternal antibodies effectiveness of live attenuated vaccines is hampered
- Vaccine also protects horses from clinical disease and possible sequelae

For more detailed information regarding vaccines, please refer to Chapter 3.1.10 Japanese encephalitis in the latest edition of the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animal*s under the heading "Requirements for Vaccines".

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the *OIE Terrestrial Animal Health Code*.

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department (scientific.dept@oie.int). Last updated December 2019.