JAPANESE ENCEPHALITIS VIRUS

Prepared for the Swine Health Information Center
By the Center for Food Security and Public Health,
College of Veterinary Medicine,
Iowa State University
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SUMMARY

Etiology

- Japanese encephalitis virus (JEV) is a zoonotic, vectorborne flavivirus closely related to St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, and dengue fever virus. It is the leading cause of epidemic viral encephalitis worldwide.
- JEV occurs as a single serotype with five known genotypes (GI–GV); genotypes GIb and GII are associated with temperate climates. GIb is believed to be the dominant JEV genotype in Asia.

Cleaning and Disinfection

- JEV is inactivated at temperatures over 40°C. It is sensitive to ultraviolet light and gamma radiation and does not survive well in the environment. High humidity is important for transmission as it influences vector (mosquito) behavior.
- Disinfection can be achieved with organic and lipid solvents, common detergents, iodine, phenol iodophors, 70% ethanol, 2% glutaraldehyde, 3–8% formaldehyde, or 1% sodium hypochlorite.

Epidemiology

- Pigs are the main amplifying host of JEV. Horses are occasionally affected. Many other animal species can be infected subclinically but do not contribute to disease transmission.
- Humans are usually considered dead-end hosts because they do not transmit the virus.
- JEV is endemic in much of Asia and the Pacific. The virus may be able to spread quickly upon introduction to new areas. In temperate climates, epidemics are most likely to occur in the summer and fall.
- Swine exhibit high and prolonged viremia and a natural infection rate of 98–100%. The mortality rate in infected piglets with no immunity to JEV is near 100%, while mortality in infected adult swine is close to zero.

Transmission

- JEV is a zoonotic disease persisting in nature through a cycle of transmission involving mosquitoes, some domestic and wild birds, domestic and feral pigs, and humans.
- Worldwide, known vectors include *Culex* mosquito species, primarily *Cx. tritaeniorhynchus,* as well as *Cx. fuscocephala, Cx. annulirostris, Cx. annulus, Cx. sitiens,* and potentially *Cx. quinquefasciatus.* Several of these species are found in the United States.
- In addition to mosquito-borne transmission, artificial insemination practices could potentially lead to JEV spread since infected boars can shed virus in the semen.

- Cyclical epizootics in swine often seem to precede JE outbreaks in humans.

Infection in Swine/Pathogenesis

- JEV causes reproductive failure in infected swine that have not developed immunity, with expected losses between 50 and 70%.
- Sows may give birth to stillborn, mummified, or darkened fetuses. There are no characteristic gross lesions in infected sows.
- Boars may experience reduced number and motility of sperm. Testicular edema and temporary infertility in boars has been observed.
- Piglets born alive commonly display tremors and convulsions, followed by death. Gross lesions of affected piglets include hydrocephalus, cerebellar hypoplasia, subcutaneous edema, and spinal hypomyelinogenesis.

Diagnosis

- Virus isolation is the reference standard. Infected tissues are the preferred sample.
- Newer tests that are faster, more efficient, and less expensive include:
    - Multiplex real-time polymerase chain reaction (PCR) assays
    - Reverse transcriptase loop-mediated isothermal amplification assays (RT-LAMP)
    - Antigen capture enzyme-linked immunosorbent assay (ELISA)
    - ELISA for the detection of IgM antibodies (IgM capture-ELISA)
- Serological diagnoses should be interpreted with caution, as maternal antibodies may be present for up to eight weeks in piglets, and cross-reactivity with other flaviviruses can also occur.

Immunity

- Natural infection contributes to long lasting immunity in adult pigs and surviving piglets. There may be some cross-protective antibody response in pigs exposed to closely related flaviviruses.
- Vaccination of swine can decrease amplification of the virus; however, complete control and eradication of JEV by vaccination is unlikely.
- A swine vaccine is being developed in the United States. Due to the presence of five known viral genotypes of JEV (GI–GV) and their various dispersal patterns in different climates, special attention must be paid to developing vaccines effective at neutralizing the appropriate strains in a given area.

Prevention and Control

- Control of mosquito vectors is a cornerstone of JEV prevention. Insecticides can be used to reduce the mosquito population. Stagnant water sources should also be removed.
- Efforts should also be made to decrease mosquito access to pigs via screening of barns and use of fans inside buildings.
- Vaccination of humans and pigs in endemic areas is a known method of JEV control.

Gaps in Preparedness

- There is currently no licensed swine JEV vaccine in the United States (although there is one in development). Uncertainty exists about the efficacy of existing GIII-based vaccines against emerging strains.
- There is currently no active JEV surveillance in the United States. If JEV is introduced in the United States, surveillance of swine herds will be essential to monitor the spread of disease and to limit reproductive losses and human epidemics.

- The capacity for JEV to establish natural transmission cycles with new vectors upon introduction into the United States is unknown. Competence studies with potential native vector and host species and a better understanding of their ecology could lead to a more targeted response, should the virus reach North America.

OVERVIEW

Japanese encephalitis is a zoonotic disease caused by a mosquito-borne virus similar to West Nile, dengue fever, St. Louis encephalitis, and Murray Valley encephalitis viruses. It is the leading cause of epidemic viral encephalitis worldwide. Japanese encephalitis virus (JEV) is endemic in much of Asia and the Western Pacific, causing severe encephalitis and death in a small percentage of infected humans, horses, and donkeys. Swine play an important role as amplifying hosts in a transmission cycle involving mosquito vectors and wading birds like herons and egrets. Due to their high levels of viremia, swine are considered a sentinel species, and a high seroprevalence in swine populations typically precedes human outbreaks. Infection occurs sporadically throughout the year in tropical climates, aided by increases in temperature, rainfall, and humidity. In temperate climates, viral epidemics typically occur during summer and fall months. Humans in rural areas are at greater risk, and the presence of rice paddies and swine farms near human communities are known to contribute to the spread of the disease.

JEV causes reproductive failure in infected swine that have not developed immunity, with expected losses between 50 and 70%. The most common clinical signs include stillbirths, abortions, or mummified fetuses that can be dark in color. Sows typically display no outward signs of infection, while boars may experience testicular edema and infertility. Mortality is rare in adult swine, while mortality in piglets with no immunity to the virus is almost 100%. Hydrocephalus and subcutaneous edema may be seen grossly in infected piglets that are born alive, and convulsions and death typically follow. Transmission occurs through the bite of infected mosquitoes or in the semen of infected boars.

In rare situations, human infections have been reported in the United States in travelers returning from endemic regions of the world. These events alone are unlikely to contribute to the spread of the virus, since humans, horses, and donkeys exhibit low viremia and are generally believed to be dead-end hosts incapable of transmitting the virus to mosquitoes. However, establishment of new vector-host transmission cycles in the United States, similar to West Nile virus and other related flaviviruses, is certainly a possibility. Several JEV vectors, including mosquito species *Culex pipiens, Cx. quinquefasciatus,* and *Cx. tarsalis,* are present in the United States. Introduction and infection in swine, birds, or potential unknown hosts exhibiting high viremia, in the presence of suitable mosquito vectors, could lead to the spread of JEV in North America.

Aside from supportive care, there is no specific treatment for Japanese encephalitis. Licensed human vaccines are available in the United States and abroad, and a swine vaccine is currently being developed in the United States. Pigs are occasionally vaccinated in some endemic countries, which may help to dampen the spread of the virus to other susceptible vertebrate hosts. Further, survivors of natural infection develop long-lasting immunity to the virus. Due to the presence of five known viral genotypes of JEV (GI–GV) and their various dispersal patterns in different climates, special attention must be paid to developing vaccines effective at neutralizing the appropriate strains in a given area.

Several diagnostic methods exist for detection of JEV. Virus isolation is possible from infected tissues, and the virus can be grown successfully in cell culture. Current trends in diagnostics are directed toward the development of faster, more efficient and inexpensive tests that require fewer materials and less technical skill. Recent attention has been given to multiplex real-time polymerase chain reaction (PCR) assays, reverse transcriptase loop-mediated isothermal amplification assays (RT-LAMP), antigen capture enzyme-linked immunosorbent assay (ELISA), ELISA for the detection of IgM antibodies, and immunochromatography. Serological diagnoses should be interpreted with caution, as maternal antibodies may be present for up to eight weeks in piglets, and cross-reactivity with other flaviviruses can also occur. If JEV were to become established in the United States, protective measures should center on vaccination of susceptible populations and control of mosquito vectors. Identifying potential native host and vector species prior to introduction of the virus could increase our ability to respond in a rapid and targeted

manner. Though the virus is primarily vector-borne, care must also be taken in the handling of samples of any infected individuals, as laboratory-acquired infection has been reported. Other precautions include placing screens on barns, using fans or insecticides to deter mosquitoes, and separation of amplifying swine hosts from human and equine populations. Availability of rapid diagnostic tests and a licensed swine vaccine, commercially available overseas, would also be beneficial. However, uncertainty about the efficacy of existing GIII-based vaccines against emerging strains calls for more comprehensive evaluation of cross-protective antibody responses among different genotypes. If JEV is introduced in the United States, continued surveillance of swine herds will be essential to monitor the spread of disease and to limit reproductive losses and human epidemics.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Japanese encephalitis virus (JEV) is an enveloped, single-stranded RNA virus, closely related to St. Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV), West Nile virus (WNV), and dengue fever virus, belonging to the genus *Flavivirus* in the family *Flaviviridae.* It is estimated to have emerged around 350 years ago in Southeast Asia.

1.2 Strain Variability

JEV occurs as a single serotype, though considerable antigenic variation is observed.[1] More than 50 strains have been isolated in Japan alone.[2] Five JEV genotypes have been described (GI–GV), however genotype relationship to phenotype or virus fitness is still unclear.[3] The basis for genotyping is phylogenetic analysis of the viral envelope *E* gene,[4] vitally important in viral attachment and entry into host cells.[5] A potential link does exist between JEV genotype and climate. GIa and GII are significantly associated with tropical climates, GIb and GIII with temperate climates, and GIV is confined geographically within Indonesia.[6] Despite its current isolation, GIV is believed to be ancestral to all other circulating genotypes and shows the largest antigenic and phylogenetic difference of all the genotypes.[7] GV has been found in both tropical and temperate climates and recently reemerged in Tibet and South Korea after remaining undetected for nearly 60 years.[6] Since its reemergence, GV has shown a high capacity for dispersal in a short time period and also warrants continued monitoring given its potential for high virulence.[8] GIb is currently believed to be the dominant JEV genotype throughout Asia.[6,9]

2. Cleaning and Disinfection

2.1 Survival

The thermal inactivation point of JEV is 40˚C, and the virus can be destroyed completely by heating for 30 minutes at 56 ˚C. JEV is labile, sensitive to ultraviolet light and gamma radiation, and does not survive well in the environment. The virus is stable in alkaline environments (pH 7–9) and inactive in acidic environments (pH 1–3).[4]

Seasonal precipitation, humidity, and temperature changes are thought to influence JEV transmission by affecting human agricultural practices and the life cycle of mosquito vectors. Higher absolute humidity is a key factor, known to influence longevity, mating, dispersal, and feeding behavior of mosquitoes. Environmental temperatures in the optimal range of 22–34˚C have been shown to increase mosquito density,[10] decrease larval development time, and reduce the extrinsic incubation period of the virus in mosquito vectors, affecting potential JEV transmission.[11]

In endemic areas, cases generally occur sporadically throughout the year with occasional increases during the rainy season. JEV exhibits more epidemic viral activity in northern temperate Asian climates, with disease outbreaks in summer months. Subtropical regions, such as Thailand and Vietnam, may see a combination of epidemic and endemic disease characteristics.[1] Year-round maintenance of JEV in temperate climates is thought to occur by overwintering mosquitoes, poikilothermic vertebrates, hibernating bats, and/or by vertical transmission from female mosquitoes to offspring.[6,9] Annual reintroduction into temperate climates by migrating birds, bats, or wind-borne mosquitoes is also a possibility,[12] though phylogeny studies of JEV genotypes suggest more local rather than widely dispersed cycles of transmission.[9] Genotypes in tropical climates are maintained year-round by traditional vector-host transmission cycles.[6]

2.2 Disinfection
Disinfection can be achieved with organic and lipid solvents, common detergents, iodine, phenol iodophors, 70% ethanol, 2% glutaraldehyde, 3–8% formaldehyde, or 1% sodium hypochlorite.[4]

3. Epidemiology

3.1 Species Affected
Pigs are the main amplifying host of JEV, exhibiting high and prolonged viremia and a natural infection rate of 98–100%.[5] Reproductive failure is a trademark of the clinical disease in pigs, and total losses can reach 50% or more.[4]

JEV infection occurs in humans, predominantly children and travelers from non-endemic areas who have not developed immunity to the virus.[1] Sporadic encephalitis is observed in horses, and the virus is manifested either as severe encephalitis or more commonly as subclinical infection. In horses, the mortality rate is about 5%.[4] Neither humans nor horses are believed to contribute to virus transmission due to their relatively low titers and short-term viremia.[4]

Subclinical infection has been reported in cattle, sheep, goats, dogs, cats, chickens, ducks, wild mammals, reptiles and amphibians, but these species are not known to contribute to spread of the disease. [4]

3.2 Zoonotic Potential
JEV is a zoonotic disease persisting in nature through a cycle of transmission primarily between mosquitoes, some domestic and wild birds, domestic and feral pigs, and humans.[1] Humans can become infected with the virus, sometimes fatally, but are considered dead-end hosts. Amplification of JEV in swine often precedes human epidemics.[12] A recent multi-criteria decision analysis (MCDA) conducted in Australia found JEV, along with rabies, Nipah virus, and Eastern equine encephalitis, to be the highest priority diseases in the swine industry when considering zoonotic criteria alone.[13]

In regions where the virus exists, several key characteristics influence human infection risk: the density, size, and spatial organization of rice paddies, swine farms, and human communities.[3]

3.3 Geographic Distribution
JEV is endemic in much of Asia and the Pacific, encompassing regions occupied by nearly half of the human population. The greatest population density in endemic areas can be found in India and China, though the disease has been reported as far west as Pakistan, as far south as northern Australia and Papua New Guinea,[14] as far north as maritime Siberia, and as far east as the island of Saipan.[9]

The potential for JEV to spread quickly upon introduction into new areas, similar to closely related WNV in 1999, is a public health concern. Recent isolation of genetic material of JEV in birds and mosquitoes in Italy further substantiates the need for continued vigilance.[15]

3.4 Morbidity and Mortality
Rates of JEV infection are higher in animals than humans, and detrimental biological and economic consequences in swine production arise primarily from reproductive failure and high piglet mortality.[16] 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%.[4]

In humans, JEV is the greatest known cause of epidemic viral encephalitis worldwide,[3] and more than one-third of the world's population is at risk of infection.[5] The virus primarily affects children; however

less than 1% of JEV infections in humans actually progress to encephalitis.[17] An estimated 50,000 documented human cases of JEV occur annually, resulting in at least 15,000 deaths. Due to the lack of surveillance and data collection in many areas, the actual incidence may be much higher.[5] Up to half of those infected can suffer permanent neurologic damage.[4]

4. Transmission

JEV relies on vector-borne transmission. Wading ardeid water birds (such as herons and egrets) are the primary natural reservoirs of the virus, while pigs act as amplifying hosts.[14] Over 90 bird species are known to be amplifying and reservoir hosts of JEV, capable of wide virus distribution and introduction into new areas.[3] Additional proposed amplifying hosts include orangutans (in Borneo) and bats,[18] while raccoons, raccoon dogs, and flying foxes have also exhibited seropositivity for anti-JEV antibodies.[3] Known vectors include *Culex* mosquito species, primarily *Cx. tritaeniorhynchus,* as well as *Cx. fuscocephala, Cx. annulirostris,*[12] *Cx. annulus, Cx. sitiens,* and potentially *Cx. quinquefasciatus.*[3] Other important regional vectors include *Cx. vishnui* in India and *Cx. gelidus* in Indonesia.[18] In addition to the preferred *Culex* species, *Anopheles sinensis* and *Aedes albopictus* are known vectors,[10] along with several *Armigeres* and *Mansonia* species.[19] Potential natural JEV vectors, *Cx. pipiens, Cx. quinquefasciatus,* and *Ae. japonicus,*[20] are also known vectors of WNV in the United States.[21] Further, experiments with American mosquitoes exposed to JEV have confirmed the susceptibility of *Cx. pipiens, Cx. quinquefasciatus,* and *Cx. tarsalis* to JEV infection and their role as potential vectors in the United States.[7]

Epizootics involving swine appear to be cyclical, consisting of two separate amplification cycles. During the first cycle, roughly 20% of pigs will become infected and develop antibodies within ten days. This is followed by a second cycle, one to two 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.[12] As each extrinsic 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.[10]

In addition to natural transmission cycles, attention must also be given to artificial insemination (AI) practices in modern swine production. 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.[22]

Laboratory-acquired JEV infection has been reported in humans, and work with the virus is restricted to Biosafety Level 3 (BSL-3) facilities and practices. Transmission can occur through needlesticks and potentially at mucosal surfaces if exposed to high concentrations of aerosolized virus.[23]

5. Infection in Swine/Pathogenesis

5.1 Clinical Signs

Reproductive failure is the most common clinical manifestation in swine. Sows may give birth to stillborn, mummified, or darkened fetuses, and boars may experience reduced number and motility of sperm.[4] Testicular edema and temporary infertility in boars has also been observed.[24] Piglets born alive commonly display tremors and convulsions, followed by death.[4]

5.2 Postmortem Lesions

Gross lesions of affected piglets include hydrocephalus, cerebellar hypoplasia, subcutaneous edema, and spinal hypomyelinogenesis.[4] Multifocal hepatic and splenic necrosis may also be seen grossly, while histological lesions are generally restricted to the central nervous system.[25] Serosal petecchiae, bicavitary

effusions, neuronophagia, glial nodules, and perivascular cuffing in the brain and spinal cord have also been observed.[24]

6. Diagnosis

6.1 Clinical History

Reproductive failure is the most common manifestation of the disease, and there are no characteristic gross lesions of infected sows. JEV infection prior to 60–70 days of gestation can cause abortion, fetal mummification or stillbirth, and encephalitis in young animals (up to six months of age).[24] In endemic areas, the breeding impact may actually be seen more in boars than sows. Some sows may be able to develop protective immunity prior to reaching sexual maturity, whereas earlier maturing boars are at risk of infertility if they have not yet developed protective immunity.[26]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Virus isolation, though time consuming, is the reference standard for a definitive diagnosis. To isolate the virus in laboratory animals, tissue homogenates from infected animals are first inoculated intracerebrally into two to four day old mice. If the mice show neurological signs, followed by death within 14 days, their brain tissue is collected for a second passage in mice. At this stage, if viral antigen collected from the brain tissue supernatant of infected mice is able to agglutinate red blood cells of one-day-old geese or chickens, it is then available for use in hemagglutination inhibition (HI) testing with JEV antiserum.[27]

Primary cultures can also be used, inoculated either with tissue directly from infected swine or brain suspension from inoculated mice.[27] A variety of cell lines are suitable; the *Aedes albopictus* mosquito cell line C6/36 is useful, as are chicken embryo, African green monkey kidney (Vero) cells, baby hamster kidney (BHK) cells, and porcine kidney (PSEK) cells.[24] Cytopathic effect may not be observed in C6/36 cells, requiring further culture or other diagnostics (such as detection of antigen or RNA). Following culture, indirect immunofluorescence can be used to identify the viral antigen using monoclonal antibodies to JEV specifically or flaviviruses in general. Isolated virus can also be identified by reverse transcription polymerase chain reaction (RT-PCR) or serological methods.[27]

RT-PCR is routinely used in diagnostic laboratories, and both conventional and real-time assays are available.[24] Several multiplex PCR (mPCR) assays have even been developed for simultaneous detection of six common diseases of swine—pseudorabies virus (PRV), porcine parvovirus (PPV), porcine circovirus (PCV) type 2, porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), and JEV—in a single reaction system.[28-30] Further reduction of cost and overall assay time is achieved with multiplex real-time PCR.[28,30,31] Simultaneous detection of three targets is possible with TaqMan® probes[28] and of six targets with EvaGreen® dye[31] using this assay. Sensitivity remains higher with single PCR; however multiplex tests have high specificity for each individual virus.[28-31]
An additional testing method of interest is multiplex ligase detection–polymerase chain reaction and microarray (MLPM). MLPM has been described for the simultaneous detection of PCV, PRRSV, CSFV, PPV, PRV, and JEV. This test shows a high degree of specificity, more rapid diagnostic time, and enhanced sensitivity compared to real time RT-PCR, as well as modification capacity to include additional pathogens.[32]

A nucleic acid detection method, the reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) assay, has been described for detection of viral RNA, though veterinary application is still limited.[27] One-step real time RT-LAMP assays are experimentally proven to have sensitivity equal to real time RT-PCR and greater than conventional RT-PCR in targeting the *NS1* gene of JEV.[33] Real time RT-LAMP also demonstrates greater sensitivity than conventional RT-PCR in targeting the highly conserved *NS3* gene.[34] Additional targets in RT-LAMP assays are the *E* genes of JEV genotypes I and III. This

method demonstrates a ten-fold increase in sensitivity compared to conventional RT-PCR.[16] Genotypes I and III can also be differentiated with RT-LAMP by targeting a single nucleotide polymorphism located in the capsid *C* gene,[35] and RT-LAMP coupled with a lateral flow dipstick (LFD) is able to detect multiple strains of JEV and eliminate the need for potentially unstable fluorescent dyes.[36] A high degree of specificity can be achieved with RT-LAMP assays, showing no cross-reactivity with other clinically and/or serologically related swine diseases.[16,33,34,36] Given the efficacy, simplicity, and speed of these assays, they are a potentially desirable option for field-level testing and accommodation of a large number of samples.

Flavivirus-specific monoclonal antibodies (MAb) can detect JEV antigen in serum, and immunohistochemistry can be used to detect JEV antigen in fetal tissues.[24] Antigen capture enzyme-linked immunosorbent assay (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. It is important to note that sensitivity is slightly higher with RT-PCR, and antigen capture ELISA may be unable to detect very low viral loads.[37]

6.3 Tests to Detect Antibody

A variety of serological tests are available to establish the prevalence of infection in a population or to make a diagnosis in a diseased individual.[27] Methods include immunofluorescent antibody (IFA), virus neutralization (VN), hemagglutination inhibition (HI), complement fixation (CF), and ELISA.[24]

The VN test is most specific, enabling the greatest differentiation between JEV and other flaviviruses. JEV, typically the Nakayama or JaGAr-01 strain, is propagated in cell culture using chicken embryo, Vero or BHK cells for the preparation of aliquots of virus-containing supernatant.[27] Epitope blocking ELISAs can also help to determine if cross-reactions have occurred in the presence of other viruses from the JEV serogroup.[5]

Hemagglutination inhibition is widely used, despite its cross-reactivity with other flaviviruses. A four-fold difference in titer between serum samples from acute and convalescent phases is sufficient for diagnosis of flavivirus infection, though not necessarily JEV.[27] A faster, more efficient potential alternative to HI is a newly developed immunochromatographic test for the detection of anti-JEV IgG in pig serum. For this test, domain III of the JEV E protein, known to be bound by anti-JEV antibodies, is expressed in soluble form in a bacterial expression system. This eliminates the need for erythrocytes and mouse brain-derived JEV antigen. Immunochromatographic assays have already been used for the detection of avian influenza, PRRSV, and PCV, as well as contagious diseases in humans.[38]

Complement fixation 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). The highest test serum dilution where no hemolysis occurs is the titer for this particular test, and a four-fold change in titer is considered to be significant for diagnosis.[27]

The IgM capture-ELISA (MAC-ELISA) is frequently used. IgM can be detected within two to three days and for up to three weeks post-infection in pigs.[24] To distinguish between antibodies from natural infection and antibodies to inactivated vaccines, ELISA detection of antibody to the nonstructural NS1 protein of the virus is utilized.[4]

The interpretation of serological results, especially in older pigs, must consider vaccination history and age; maternal antibody can persist for up to eight weeks.[24] It is also important to note that there is some level of cross reactivity with other flaviviruses for all of the serological tests. The OIE recommends that diagnosis by serology be confirmed by virus isolation.[27]

6.4 Samples

6.4.1 Preferred Samples

Infected tissues are preferred for virus isolation, as isolation from blood and cerebrospinal fluid is rarely achieved.[24] Due to the uneven spread of viral pathogens among fetuses *in utero*, it is important to sample tissue from an adequate number of fetuses. Generally, four to six should be sampled to avoid missing a diagnosis.[25] The OIE recommends brain or spinal cord be sampled,[27] though spleen, liver, or placental tissues from stillborns, neonates, or fetuses may also be acceptable.[24]

RT-PCR can identify the virus in cerebrospinal fluid, sera, and tissue culture supernatants.[24] Recent mPCR tests for detection of multiple diseases of swine have used tissue homogenates from lung, spleen, kidney, and lymph nodes of aborted fetuses or pigs displaying clinical signs of disease.[29,30] The ability to detect low levels of the virus in blood samples of newly infected swine can now be achieved with the real time RT-LAMP assay.[34]

Antigen capture ELISA is able to detect JEV in cerebrospinal fluid, brain tissue, and mosquito homogenate,[37] and detection of anti-JEV antibodies in serum, thoracic, or abdominal fluid of stillborn piglets is also considered diagnostic.[25]

Ideally, any serological tests should include paired samples to demonstrate a rise in titer from acute to convalescent phases of disease.[27]

6.4.2 Oral Fluids

The use of oral fluids as a diagnostic specimen has not been evaluated for JEV.

7. Immunity

7.1 Post-exposure

Natural infection contributes to long lasting immunity in adult pigs and surviving piglets.[4] The host immune system primarily targets the E protein, a viral envelope glycoprotein consisting of three structural domains.[39]

7.2 Vaccines

Currently, complete control and eradication of JEV by vaccination is unlikely.[27] Vaccination of swine can decrease amplification of the virus; however, regular vaccination of piglets can be cost prohibitive and maternal antibodies can decrease effectiveness of the existing live-attenuated vaccine.[4] Existing live-attenuated swine and human vaccines are GIII-derived and elicit greater GIII strain-specific neutralizing antibodies. The E protein of JEV is critical in neutralizing antibody responses and is also the basis for separation of JEV into different genotypes. Some level of protection using current vaccines is still observed against GI strains, possibly aided by a T cell response induced by vaccination.[40] There is still no specific treatment for Japanese encephalitis, and alternative vaccination methods are being developed. Despite their limitations, vaccines can help to prevent JEV infection in humans and animals.[41]

Efforts have been made to develop bivalent and multivalent vaccines as a time and cost-saving measure. An experimental recombinant pseudorabies virus (PRV) vaccine expressing the NS1 protein of JEV has been shown to induce long lasting antibody against JEV as well as cell-mediated immunity in piglets.[42] Pigs receiving simultaneous intradermal and oronasal vaccination with an experimental chimeric classical swine fever (CSF)-JEV virus replicon particle (VRP) exhibit more rapid anti-JEV IgG and neutralizing antibody responses than those vaccinated subcutaneously with a commercial inactivated vaccine. Viremia is also undetectable or transient in challenge studies with the VRP vaccinated pigs. The VRP in this case

is a non-infectious recombinant CSF virus with a deletion of the original gene coding for its envelope protein. In its place is a gene expressing domain III of the antigenic JEV E protein.[43]

Inactivated and live-attenuated vaccines are available in some countries for pregnant sows to increase chance of live births and for breeding boars to protect against reduction or deformity of sperm.[4] The live-attenuated vaccine has shown greater efficacy than the killed vaccine in both experimental and naturally occurring infection,[24] and an existing trivalent vaccine offers combined protection against JEV, PPV, and Getah virus (GETV).[2] Due to the genotypic shift from predominantly GIII to GI in large parts of Asia in the last decade, efficacy of some existing vaccines may need to be reevaluated. In a study of weaned piglets receiving live-attenuated JEV vaccine protective against GIII, only limited cross-reactive protection against GI was observed.[40] It may be possible to increase the immunogenicity of existing vaccines with the use of novel adjuvants. Specifically, an inactivated JEV GI vaccine containing the cytokine recombinant porcine granulocyte-monocyte colony stimulating factor (reporGM-CSF) has been shown to enhance humoral immunity in pigs.[44]

Live-attenuated vaccines can be difficult and costly to produce and carry associated biosafety concerns. Efforts are underway to develop stable cell lines capable of continuously expressing JEV virus-like particle (VLP) antigens that can effectively induce neutralizing antibodies to JEV. One such cell line, BJ-ME, produced JEV VLPs able to induce high titers of neutralizing antibodies and complete protection in mice exposed to lethal JEV challenge.[41] Experimental DNA vaccines have also shown some ability to induce high antibody titers and have potential to be advantageous in swine production.[24] Proteins expressed from plasmids encoding the JEV *prM* and *E* genes associate to form particles, similar to VLPs, effective in eliciting neutralizing antibody and cytotoxic T lymphocyte (CTL) responses in mice. These same plasmids also elicit a higher neutralizing antibody response than the inactivated vaccine in swine, with antibodies persisting for up to eight months post-vaccination.[45]

7.3 Cross-protection

Cross-protective antibody responses have been induced experimentally in pigs exposed to closely related flaviviruses, suggesting the potential for prevention or inhibition of JEV infection in areas where multiple flaviviruses are endemic.[24] However, the coexistence of JEV with MVEV in Papua New Guinea and WNV in Pakistan suggests that adequate numbers of susceptible hosts may exist to accommodate multiple viruses.[12] Of the three domains of the immunogenic E protein component of the JEV viral envelope, flavivirus cross-reactive epitopes are found predominantly in domain II.[39] However, neutralizing epitopes have been mapped in domain III, showing a high level of conservation between multiple strains of JEV and WNV, suggesting some level of cross-protection.[46]

8. Prevention and Control

Successful decline of JEV in endemic areas has been attributed to large scale human immunization, immunization of pigs, separation of pigs from human communities, alterations in agricultural practices to reduce the presence of mosquitoes, and better overall standards of living.[14] Other examples of preventative measures include removal of stagnant water around pig enclosures, insecticides, insect screens, and use of fans inside buildings where pigs are housed to disrupt mosquito activity.[47]

In pigs that do become infected, alpha interferon (IFN-α) shows promise as an antiviral treatment method able to experimentally increase expression of interferon-stimulated genes with strong anti-JEV effects.[48]

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

JEV is covered in Chapter 8.9 of the 2015 OIE TAHC (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/chapitre_japanese_encephalitis.pdf).

There are recommendations for importation of horses from countries or zones infected with JEV, but no recommendations for importation of swine.

10. Gaps in Preparedness

There is currently no active JEV surveillance or a licensed swine vaccine for JEV in the United States,[49] though Harrisvaccines has a JEV vaccine in the research and development phase.[50]

The capacity for JEV to establish natural transmission cycles with new vectors upon introduction into the United States is unknown. Modern swine production, housing pigs indoors and away from preferred mosquito feeding grounds, may be enough to reduce or prevent amplification. If so, the main concern may be domestic swine with access to the outdoors and feral swine. As seen in Australia, the presence of existing flaviviruses, incompetent local vectors, or alternative hosts developing low to no viremia may also aid in dampening the spread of the virus. Competence studies with potential native vector and host species and a better understanding of their ecology could lead to a more targeted response, should the virus reach North America. Furthermore, continued research of the cross-protective characteristics of flaviviruses and the availability of diagnostic assays and effective vaccines will further ensure our preparedness for this potential threat.[12]

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Authors, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Tracy Lambert, BS; 2nd year student,
- Kristin Killoran, PhD; 2nd year student
- Kerry Leedom Larson, DVM, MPH, PhD; Veterinary Specialist

Reviewers, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Pamela Zaabel, DVM; Veterinary Specialist
- James A. Roth, DVM, PhD; Director

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