Flaviviridae

VMC 321

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Taxonomy

- Family: *Flaviviridae*
- Genus - *Flavivirus*
- Genus - *Pestivirus*

- *Species* - *Classical swine fever virus*
- *Species* - *Bovine viral diarrhea virus 1*

*Bovine viral diarrhea virus 1*
## Taxonomy: *Flaviviridae*

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
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<tr>
<td><em>Flaviviridae</em></td>
<td><em>Flavivirus</em></td>
<td>Dengue virus</td>
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<td></td>
<td><em>Hepacivirus</em></td>
<td>Hepatitis C virus</td>
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<td><em>Pestivirus</em></td>
<td>Classical swine fever virus</td>
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<td>Bovine viral diarrhea virus 2</td>
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<td>Border disease virus</td>
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</table>
Derivation of names

• Flavi: from Latin *flavus*, “yellow”.
• Pesti: from Latin *pestis*, “plague”.
• Hepaci: from Greek *hepar, hepatos*, “liver” and identifying letter from hepatitis C virus.
• Pegi: from persistent, and the original names of the GB viruses and hepatitis G, deriving from the initials of the original source, the surgeon “GB”
Flaviviruses

- Flavivirus virions have enveloped particles.
- The enveloped icosahedral virions are composed of a lipid bilayer surrounding an icosahedral nucleocapsid. The envelope protein forms a very tight protective shell composed of 90 E dimers (180 subunits) that bind close to the inner capsid protein.
- Genome is a single stranded positive sense RNA with a cap structure at the 5’ end. The 3’ end of the flaviviruses lack a poly(A) tail. Thus, the flaviviruses differ from picornaviruses at their 5’ and 3’ terminus.
- The viral proteins are produced from a single polyprotein that is cleaved by host and viral proteases. Thus the flavivirus protein expression strategy has some similarities to the expression strategies of the Picornaviridae.
- Transmitted mostly by mosquitos or ticks
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
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<tr>
<td>Typical member</td>
<td>yellow fever virus-17D (X03700), species Yellow fever virus, genus Flavivirus</td>
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<tr>
<td>Virion</td>
<td>Enveloped, 40–60 nm virions with a single core protein (except for genus Pegivirus) and 2 or 3 envelope glycoproteins</td>
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<tr>
<td>Genome</td>
<td>9.0–13 kb of positive-sense, non-segmented RNA</td>
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<tr>
<td>Replication</td>
<td>Cytoplasmic, in membrane vesicles derived from the endoplasmic reticulum (ER); assembled virions bud into the lumen of the ER and are secreted through the vesicle transport pathway</td>
</tr>
<tr>
<td>Translation</td>
<td>Directly from genomic RNA containing a type I cap (genus Flavivirus) or an internal ribosome entry site (other genera)</td>
</tr>
<tr>
<td>Host range</td>
<td>Mammals (all genera); most members of genus Flavivirus are arthropod-borne</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>Four genera containing 89 species</td>
</tr>
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</table>
Flavivirus (2)

• Most members of this genus, which includes 53 species
• arthropod-borne viruses, with distinct groups infecting mosquitoes or ticks.
• Mammals and birds are the usual primary hosts, in which infections range from asymptomatic to severe or fatal haemorrhagic fever or neurological disease.
Flavivirus (3)

• Important human pathogens include:
  • Yellow fever virus
  • Dengue virus
  • Zika virus
  • Japanese encephalitis virus
  • West Nile virus
  • Tick-borne encephalitis virus.
**Flavivirus (4)**

- Other members cause economically important diseases in domestic or wild animals.
- Diseases of veterinary importance:
  - Classical swine fever
  - Bovine viral diarrhoea
  - Border disease
Genus: *Pestivirus*
**Pestivirus:**

• Pestiviruses infect pigs and ruminants, including cattle, sheep, goats and wild ruminants

• Transmitted through contact with infected secretions (respiratory droplets, urine or faeces)

• Infections may be subclinical or cause enteric, haemorrhagic or wasting diseases, including the economically important
  • Bovine viral diarrhoea virus
  • classical swine fever virus
Genus: *Hepacivirus*
Genus: *Hepacivirus*

- This genus includes hepatitis C virus (HCV)
- major human pathogen causing chronic liver disease, including cirrhosis and cancer.
- Other viruses infect:
  - Horses
  - Rodents
  - Bats
  - Cows
  - Primates
- Infections are typically persistent and target the liver.
Morphology of virion

- Size: 40–60 nm in diameter
- Shape: spherical in shape with a lipid envelope
- Capsid: comprise of a single protein
- Envelope: contains two or three virus-encoded membrane proteins.
Genome of virion

- Genomes are positive-sense ssRNA approximately for members of the genera
  - *Flavivirus*: 9.2–11.0
  - *Pestivirus*: 11.3–13.0
  - *Hepacivirus*: 8.9–10.5
  - *Pegivirus*: 8.9–11.3 kb.

- All members of the family lack a 3′-terminal poly(A) tract.
- Only the genomes of members of the genus *flavivirus* contain a 5′-terminal type I cap structure.
- Others possess an internal ribosomal entry site (IRES).
CSFV

• Family: *Flaviviridae*

• Genus: *Pestivirus*

• Species: *Classical Swine Fever Virus*
• Enveloped viruses, 40–60 nm in diameter
• single-stranded, positive-sense RNA 9.0–13 kb.
• Genome sizes vary from 9.5 kb (hepatitis C) to 10.7 kb (flaviviruses) to 12.5 kb (pestiviruses).
• Mature virions accumulate within cisternae of the endoplasmic reticulum.
• *Flaviviridae* consist of group of arboviruses includes yellow fever virus and dengue viruses.
• Most members are transmitted by blood-sucking arthropods.
• Hepatitis C virus has no known vector.
Classical Swine Fever Virus (CSFV)
CSFV

• Small size (diameter 45 nm)

• Single-stranded, linear, positive-sense RNA(10-12 kb)

• Only one serotype of the virus

• Closely related antigenically to
  ✓ BOVINE VIRAL DIARRHOEA and
  ✓ BORDER DISEASE VIRUSES
Classical Swine Fever

...can survive for months in refrigerated meat and for years in frozen meat
Flavivirus Genome Organization and Protein Functions

C - Nucleocapsid protein that forms the enveloped icosahedron.

prM - Structural glycoprotein. Cleaved to pr and Membrane protein by furin.

E - Envelope Protein

NS1 - Nonstructural glycoprotein required for RNA replication.

NS2A - Hydrophobic protein that anchors replication machinery in the Endoplasmic Reticulum.

NS2B - Hydrophobic protein. Serves as cofactor for the NS3 protease.

NS3 - Serine protease; Helicase; component of capping enzyme.

NS4A & B - Hydrophobic proteins that may anchor replication machinery in the Endoplasmic Reticulum.

NS5 - RNA polymerase contains capping enzyme activity.
Replication
Flaviviruses - the genome acting as a single polycistronic mRNA, is translated into a single polyprotein which is subsequently cleaved to give the individual viral polypeptides.
• Suitable cell lines- Porcine kidney cell line PK15
• Sheep fetal thymoid (SFT-R) cell line
• Swine Testis Endothelial (STE) cell line
• MDBK, bovine turbinate cells
Hog Cholera
(Classical Swine Fever)
Definition of hog cholera:

• highly contagious viral disease of swine

• can cause acute, chronic, or congenital disease

• considered a foreign animal disease
Epidemiology of HCV:

• Hosts:
  • pigs and wild boar

• Incubation period:
  • usually 3-4 days, but can range from 2-14 days

• Distribution:
  • occurs in much of Asia, Central and South America, and parts of Europe and Africa
  • disease has been eradicated from about 16 countries, including Australia, Canada, and the United States (1978 - after a 16 year long effort)
Epidemiology of HCV:

• Transmission:
  • Blood, tissues, semen, secretions, and excretions of infected animals contain HCV
  
  • transmission usually occurs via oral route
  
  • can also occur via the conjunctiva, mucous membranes, skin abrasions, insemination, common needles, and contaminated instruments
Epidemiology of HCV:

• Transmission:
  • Feeding raw or insufficiently cooked waste food containing infected pork scraps can be a potent source of HCV
  • Mechanical vectors can spread HCV
    • farm visitors - on their person or their clothes
    • vehicles
    • insects and birds
Epidemiology of HCV:

• Transmission:
  • Transplacental infection with a lowly virulent strain of HCV can result in persistently infected piglets
  • These piglets will persistently shed the virus for months before succumbing to the disease
Forms of the disease:

- Acute form
- Chronic form
- Congenital form
Host Range

Domestic swine and European wild boar are the only natural reservoir of classical swine fever virus.

http://www.krykiet.com/polish_wildlife.htm
Host Range

Collared Peccary is only mildly susceptible.
Transmission

- **Direct Transmission**
  - Contact between sick and healthy animals: all tissues, excretions, secretions, semen and blood (oronasal).
  - Transplacental infection: Carrier Sow Syndrome.
  - Airborne spread to neighbors possible if high density pig farms.

- **Indirect Transmission**
  - Feeding uncooked garbage with infected meat.
  - Fomites: vehicles, equipment, boots, clothes.

How is CSFV often introduced into a new country?  **Garbage!**

How does CSFV travel once established?  **Movement/Fomites**
Route of Infection of CSF

- Ingestion: e.g. contaminated swill
- Contact with the conjunctiva
- Mucous membranes
- Skin abrasions
- Insemination
  - Contaminated semen caused 1967 outbreak in the Netherlands

All secretions and excretions are infectious
Environmental Persistence: Moderately Fragile

• Sensitive to desiccation & UV

• Stable at pH 3-11
Environmental Persistence: Moderately Fragile

• **Survival in Pork Products**
  - Up to 85 days in chilled pork.
  - >4 years in frozen pork.
  - 313 days in Parma hams and 140 - 252 days in Serrano and Iberian hams.
  - Readily killed by cooking e.g. 30 min 65°C.
Incubation

• 3-4 days average, 3-15 days range depending on strain, route and dose

• 2-14 days (O.I.E.)

• The **Severe Acute** form:
  
  2-6 days incubation; death at 10-20 days post infection
Syndromes

*Virulence*, immune status, age, breed, and pregnancy figure in the clinical picture.

- Highly virulent strains: prevalent decades ago - causes **Peracute and Classic Acute disease**
Syndromes

• Moderately virulent strains: prevalent today - causes **Subacute Disease**

• Low virulent strains:
  prevalent today - causes **Chronic Disease** and **Carrier Sow Syndrome/Persistent Infection**
Syndromes

• Seroconversion only after 2-3 weeks
  • CSF virus is immunosuppressive like BVD in cattle

• Cellular tropism of virus
  • Endothelial, lymphoreticular, macrophages, some epithelial
Acute Disease
(Classic Disease)

Mortality: approaches 100%.

Viral shedding: 10-20 days until antibodies
Acute Disease

**Clinical Signs**

High Fever: 106-108°F (>41°C)
Depression
Conjunctivitis
Constipation, then Diarrhea
Skin hemorrhages/Cyanosis
Acute Disease

Pile up for warmth
Anorexic and gaunt
Staggering gait
Convulsions
Abortion
Death 10-20 days post infection
Acute Disease

- Pathology
  - Severe tonsilitis
  - Severe leukopenia
  - Hemorrhagic swollen lymph nodes
  - Hemorrhages renal cortex
  - Petechiation of the bladder, larynx, epiglottis, heart, intestinal mucosa, skin
  - Splenic infarcts
  - Necrotic gastroenteritis
  - Encephalitis
Clinical signs of acute infection:

• Fever of 106 to 108 degrees Fahrenheit
  • may see huddling of pigs in warmest area of pen
• Lethargy and anorexia
• Intermittent vomiting of yellow fluid containing bile
• Transient constipation followed by diarrhea
• Conjunctivitis with encrustation around the eye
• Coughing and dyspnea
Clinical signs of **acute infection**:  

- Terminal stages of infections:  
  - Hemorrhagic lesions of the skin, especially on the abdomen and inner aspects of the thighs  
  - Cyanosis of the skin, especially the extremities  
    - ears, limbs, tail, snout  
  - Ataxia and paresis due to posterior weakness  
  - Convulsions may occur shortly before death  
  - Death usually occurs within 5 to 15 days of onset  
  - Mortality can approach 100% in young pigs
Subacute Disease

Mortality: reduced

Viral shedding: until death.
Subacute Disease

Clinical signs with subacute disease are similar to acute disease, but considerably less severe.

*As with Acute CSF, the disease is clinically and pathologically consistent with a generalized septicemia*
Subacute Disease

Fever for 2-3 weeks
105-106°F
(>41°C)

Death within 30 days post infection
Chronic Disease

Low virulent strain or infection of vaccinated herd.

Three clinical phases:

- Initial: resembles Subacute
  - Fever
  - Anorexia
  - Depression
  - Leukopenia
- Second: improve, look ~normal
- Final: ‘runts’ with ‘Initial’ Phase signs.
Lesions

- Button ulcers in cecum and colon (caused by bacteria)
- Calcification rib cartilage
- Glomerulonephritis
‘Carrier Sow Syndrome’

Mortality: In pregnant sow disease goes unnoticed. Sow may shed virus for months especially at farrowing.

High Mortality: In piglets infected congenitally or post-natally. Piglets look healthy at birth, shed virus for 6-12 month before dying.
‘Carrier Sow Syndrome’

Clinical Signs

• Clinical Signs in Sows
  • Usually mild (fever)
  • or subclinical.
‘Carrier Sow Syndrome’

• Clinical Signs in Piglets
  • Stillbirths, deformities, mummies,
  • born dead, or congenital tremors.
  • Some are born healthy:
  • become persistent shedders to maintain CSF in breeding herd;
  • are immuno-tolerant but will eventually die of ‘late onset’ disease at 6-12 months of age.
Congenital Form of CSF

- Weak "Shaker" piglets
- Persistently infected
  - Viremic - seronegative piglets
- Life-long viremia
- Will in time lead to complications and death
Gross lesions of **acute infection:**

- Swollen, edematous, and hemorrhagic lymph nodes
  - esp. submandibular and pharyngeal lymph nodes
- Splenic infarcts
- All serous and mucosal surfaces may have petechial or ecchymotic hemorrhages
- Peritonitis, pleuritis, and pericarditis
  - straw-colored fluid
Gross lesions of **acute infection**:

- Petechial and ecchymotic hemorrhages on:
  - skin
  - surface of the kidneys
  - surface of the small and large intestine
  - larynx
  - heart
  - epiglottis
  - fascia lata of the back muscles
Petechial hemorrhages - skin:
Other lesions of **acute infection**:

- Leukopenia and thrombocytopenia

- Encephalomyelitis with microgliosis and perivascular cuffing is found in brains from about 75% of pigs acutely infected with HCV
Clinical signs of **chronic infection**:

- Prolonged and intermittent periods of:
  - anorexia
  - fever
  - dullness
  - alternating diarrhea and constipation for up to a month
  - alopecia
Clinical signs of chronic infection:

- May have a disproportionately large head relative to their small trunk
- Apparent recovery with eventual relapse
- All chronically infected pigs will die due to complications arising from HCV infection
Gross lesions of chronic infection:

• Lesions can be similar to those found in the acute form of infection, but are generally less severe.
• Button ulcers in the cecum and large intestine due to secondary bacterial infection are common
• Generalized depletion of lymphoid tissue
• Hemorrhagic lesions may not be present in chronically infected pigs
Clinical signs of **congenital infections**:

- **Highly virulent strain:**
  - abortion
  - birth of diseased pigs that die shortly after birth

- **Less virulent strain:**
  - mummification
  - stillbirth
  - birth of weak, “shaker” pigs (congenital tremor)
Clinical signs of congenital infections:

• If infected with a lowly virulent strain during fetus’s 1st trimester of life, piglets may:
  • not produce neutralizing antibody to the virus
  • experience life-long viremia and persistently shed the virus
  • have few clinical signs for the first few months of life, then develop anorexia, depression, diarrhea conjunctivitis, dermatitis, runting, and ataxia
  • ultimately end up recumbent and die
Gross lesions of congenital infections:

- Cerebellar hypoplasia
- Microencephaly
- Pulmonary hypoplasia
- Central dysmyelination
- Thymus atrophy
- Deformities of the head and limbs
- Petechial hemorrhages of the skin and internal organs towards the end of the disease process
Differential diagnosis:

• African Swine Fever
  • distinguished from hog cholera only via laboratory examination

• Erysipelas
• Salmonellosis
• Colisepticemia
• Thrombocytopenic purpura
• Acute pasteurellosis
• Infection with BVDV
Diagnosis of hog cholera:

• Specimens that should be collected and sent to the lab for virus isolation and antigen detection include:
  • tonsils (best)
  • submandibular and mesenteric lymph nodes
  • spleen, kidney, brain, and distal ileum

• For living cases, collect:
  • tonsil biopsies and blood in EDTA

• DO NOT freeze samples - interferes with some of the tests
Diagnosis

• History
• Clinical signs
• Post Mortem findings
  • Hemorrhage in lymph nodes, kidneys, tonsils, etc.
  • Splenic infarcts: nearly pathognomonic
• Histopathology
  • Degeneration and necrosis of endothelial cells
  • LN: lymphocytic depletion & reticular hyperplasia
• Laboratory testing: required for confirmation
Labortory Testing for CSF

• Virus isolation
  • In Swine Cell Cultures; Inoculation in Live Pigs to confirm.

• Antigen Detection
  • Direct Fluorescent Antibody Test (DFAT)
  • Monoclonal antibody-Avidin Biotin Complex (ABC)

• Nucleic Acid Detection
  • Polymerase Chain Reaction (PCR) – Conventional & Real-time

• Antibody Detection
  • ELISA
  • Immunoperoxidase Test (IPT)
  • Virus Neutralization Test
Differential Diagnosis

• African Swine Fever
• Pasteurella
• Haemophilus
• Salmonellosis (septicemic)
• Erysipelas
• Eperythrozoonosis
Differential Diagnosis

• Poisoning, e.g. Coumarin (hemorrhage), Salt (CNS)
• Pseudorabies virus (PRV)
• Porcine Reproductive and Respiratory Syndrome (PRRS)
• Porcine Dermatitis and Nephropathy Syndrome (PDNS)
• Post-weaning Multisystemic Wasting Syndrome (PMWS)
Laboratory diagnosis:

• Direct IFA on cryostat sections of organs or impression smears of biopsy material
• ELISA - blood antibody test
• RT-PCR
• Virus isolation in cell culture
  • detect virus by immunoperoxidase or immunofluorescence using labeled hog cholera antibody
For the identification of the CSF agent it lists the following test procedures:

- Fluorescent antibody test (FAT) for the detection of CSF antigen in cryostat sections
- Immunoperoxidase staining using monoclonal antibodies for differentiation of pestiviruses (in cryostat sections)
- Antigen-capture ELISAs
- Virus isolation in cell culture
- Reverse transcription-polymerase chain reaction (RT-PCR).
For serological tests, the methods are:

- Fluorescent antibody virus neutralisation test (VNT)
- Neutralising peroxidase-linked assay (NPLA)
- Antibody ELISAS.
Why prevent hog cholera?

• high death rates and severe illness cause significant production losses
• loss of productivity leads to an increase in the cost of food products obtained from swine
• lose economically important export markets until eradication is again achieved
  • 1997: total value of exported U.S. pork products exceeded $1 billion
• Re-eradication can be very costly
  • 1997 outbreak in the Netherlands cost $2 billion
Prevention and control:

• Affected pigs must be culled and the carcasses must be buried or burned

• Vaccination is used to reduce the number of outbreaks in countries where hog cholera is enzootic

• Vaccination is generally prohibited in countries which are free of disease or where eradication is in progress and nearing success
Prevention and control:

• Other prophylactic measures include:
  • quarantining incoming pigs before introducing them to the herd
    • U.S. quarantines swine imported from affected countries for 90 days at a facility in Key West, FL
  • keeping a good pig identification and recording system
  • strict adherence to waste food cooking laws
  • structured serological surveillance of breeding sows and boars to detect subclinical infections
  • maintaining a strict import policy for live pigs, as well as fresh and cured pork
Vaccination:

• Modified live vaccines:
  • Lapinized Chinese strain
  • Japanese guinea pigs cell culture-adapted strain
  • French Thiverval strain

• All three are innocuous for pregnant sows and piglets over 2 weeks of age
• All three are considered equally effective
Immunity & Vaccines for CSF

• Good immunity post-infection
• MLV vaccines available
  • Lapinized vaccines
  • Cell culture vaccines
  • Yearly dose (Safe in pregnant gilts)
• Marker sub-unit vaccines
  • DIVA strategy
    • E2 Vaccine and E\text{ms} ELISA
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Thank you!