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*

Family	Genus	Species
Flaviviridae	**Flavivirus**	**Dengue virus**
	Hepacivirus	**Hepatitis C virus**
	Pestivirus	**Classical swine fever virus**
		Bovine viral diarrhea virus 1
		Bovine viral diarrhea virus 2
		Border disease virus

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

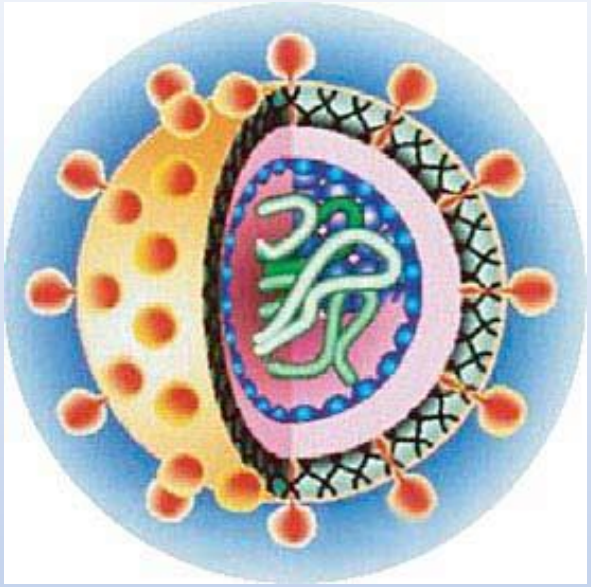
- **Flavivirus virions have enveloped particles.**

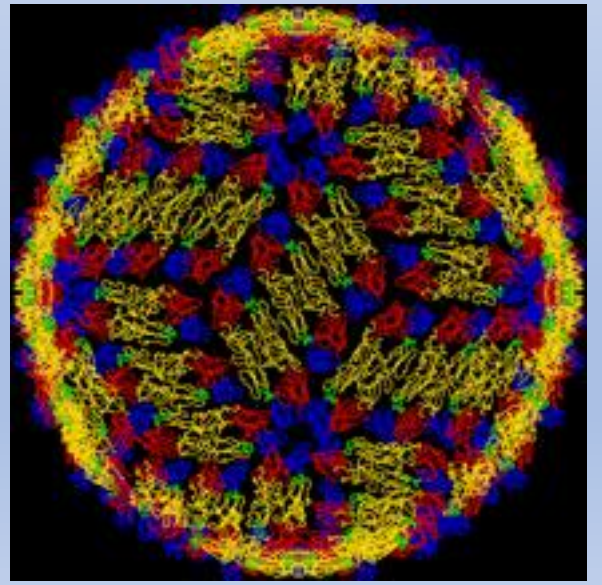
- **The enveloped icosahedral virions are composed of a lipid bilayer surrounding an icosahedral nucleocapsid. The envelope protein forms a very tight protective shell composed of 90 E dimers (180 subunits) that bind close to the inner capsid protein.**

- **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**





Flavivirus (1)

Characteristic	Description
Typical member	yellow fever virus-17D (X03700), species Yellow fever virus, genus Flavivirus
Virion	Enveloped, 40–60 nm virions with a single core protein (except for genus Pegivirus) and 2 or 3 envelope glycoproteins
Genome	9.0–13 kb of positive-sense, non-segmented RNA
Replication	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
Translation	Directly from genomic RNA containing a type I cap (genus Flavivirus) or an internal ribosome entry site (other genera)
Host range	Mammals (all genera); most members of genus Flavivirus are arthropod-borne
Taxonomy	Four genera containing 89 species

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

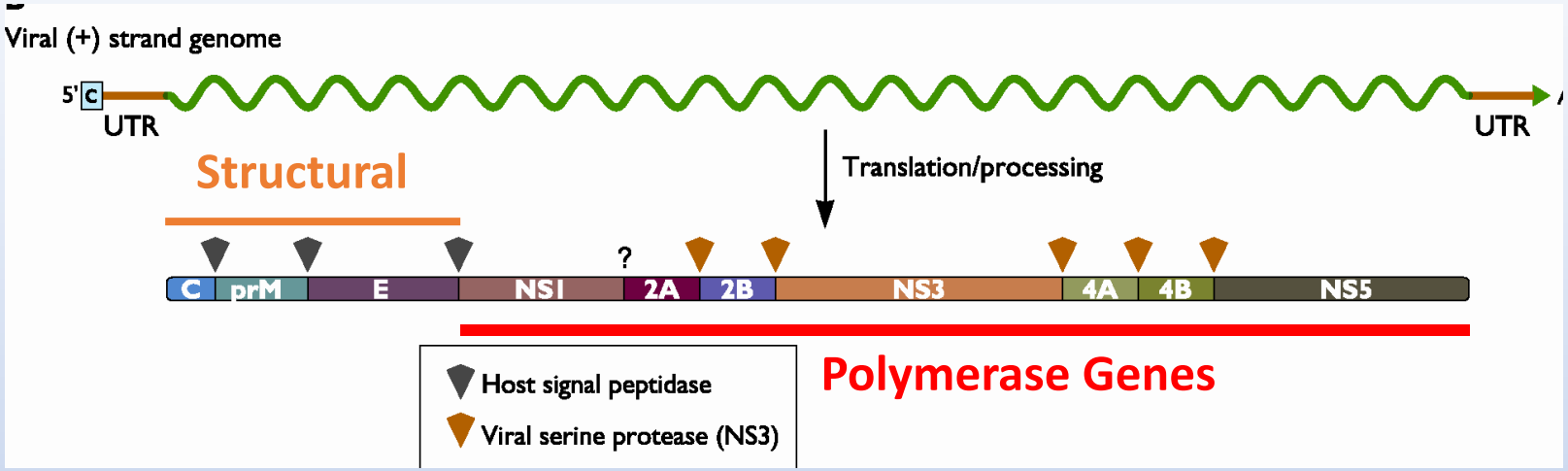
…can survive for months

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.

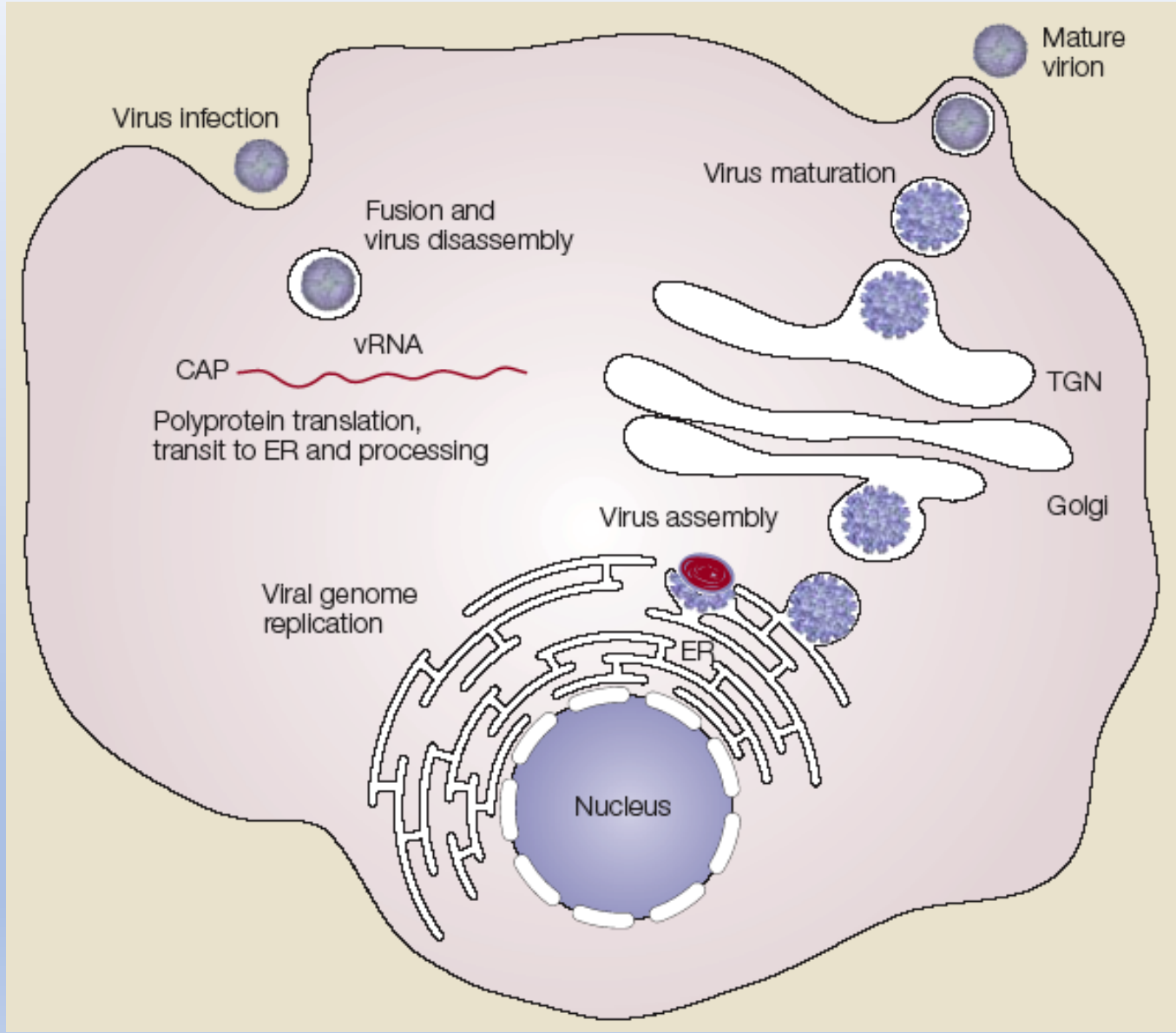
NS2B - Hydrophobic protein. Serves as cofactor for the NS3 protease.

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

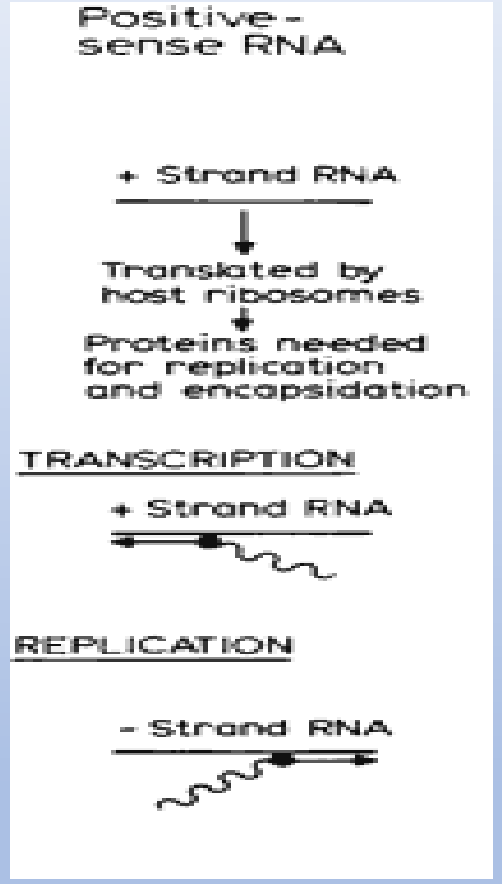
NS4A & B - Hydrophobic proteins that may anchor replication machinery in the Endoplasmic Reticulum.

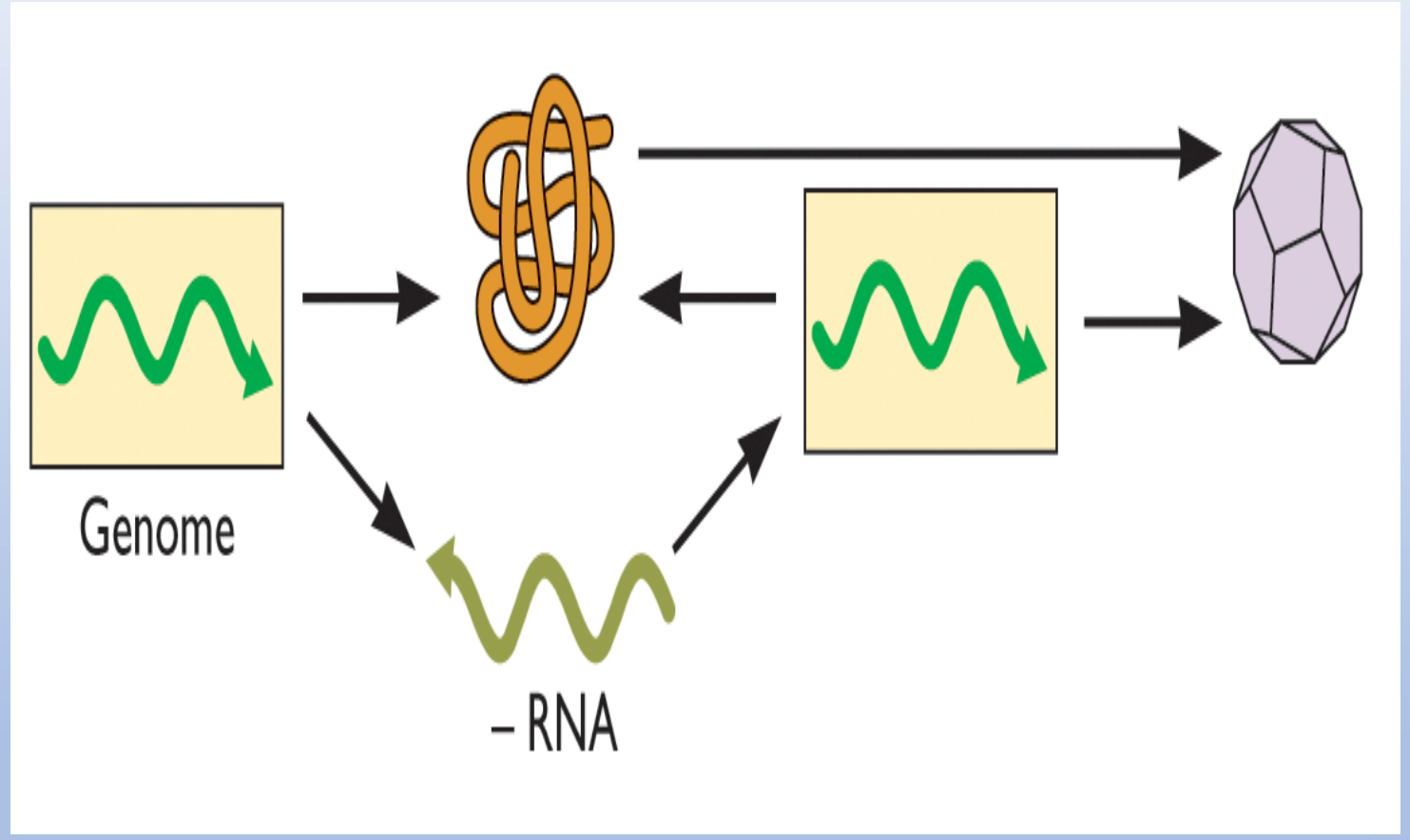
NS5 - RNA polymerase contains capping enzyme activity.

Replication



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

Epidemiolgy 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

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

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

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

Oronasal Route

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^{o}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 in Sows
  - Usually mild (fever)
  - or subclinical.

Clinical Signs

‘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

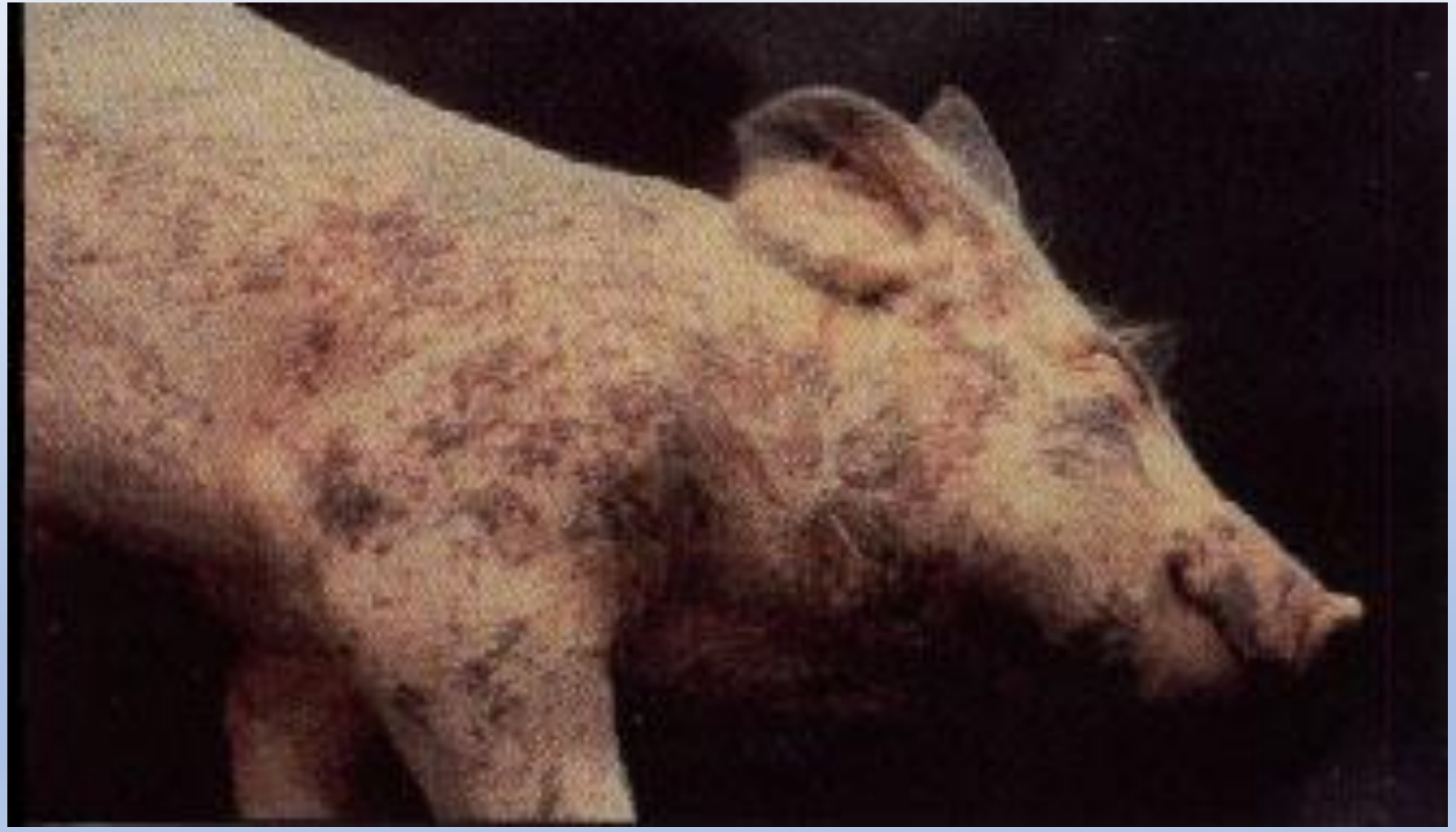
Gross lesions of acute infection:

- 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 dysmyelinogenesis
- 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

Laboratory 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. quaratines 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^{rns} ELISA

Acknowledgement

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Thank you!