PICORNAVIRIDAE

Picornaviridae: an acronym from poliovirus, insensitivity to ether, coxsackievirus, orphan virus, rhinovirus, ribonucleic acid;

Also “pico” - small

“rna” - RNA

Baltimore group IV

Genera in Picornaviridae

- ***Aphthovirus*** - Foot and Mouth disease virus
- ***Enterovirus*** - enteroviruses of various species, polio virus (humans), hand, foot and mouth disease (humans), **Swine vesicular disease**
- **Avihepatovirus - Duck Hepatitis virus**
- ***Cardiovirus***
- ***Rhinovirus*** - various species, human "common cold" viruses
- ***Hepatovirus*** - hepatitis A (humans)
- ***Parechovirus- human***
- ***Erbovirus-*** *Equine rhinitis virus*
- ***Kobu virus- human and cattle***
- ***Teschovirus- swine***

Vesicular diseases in swine

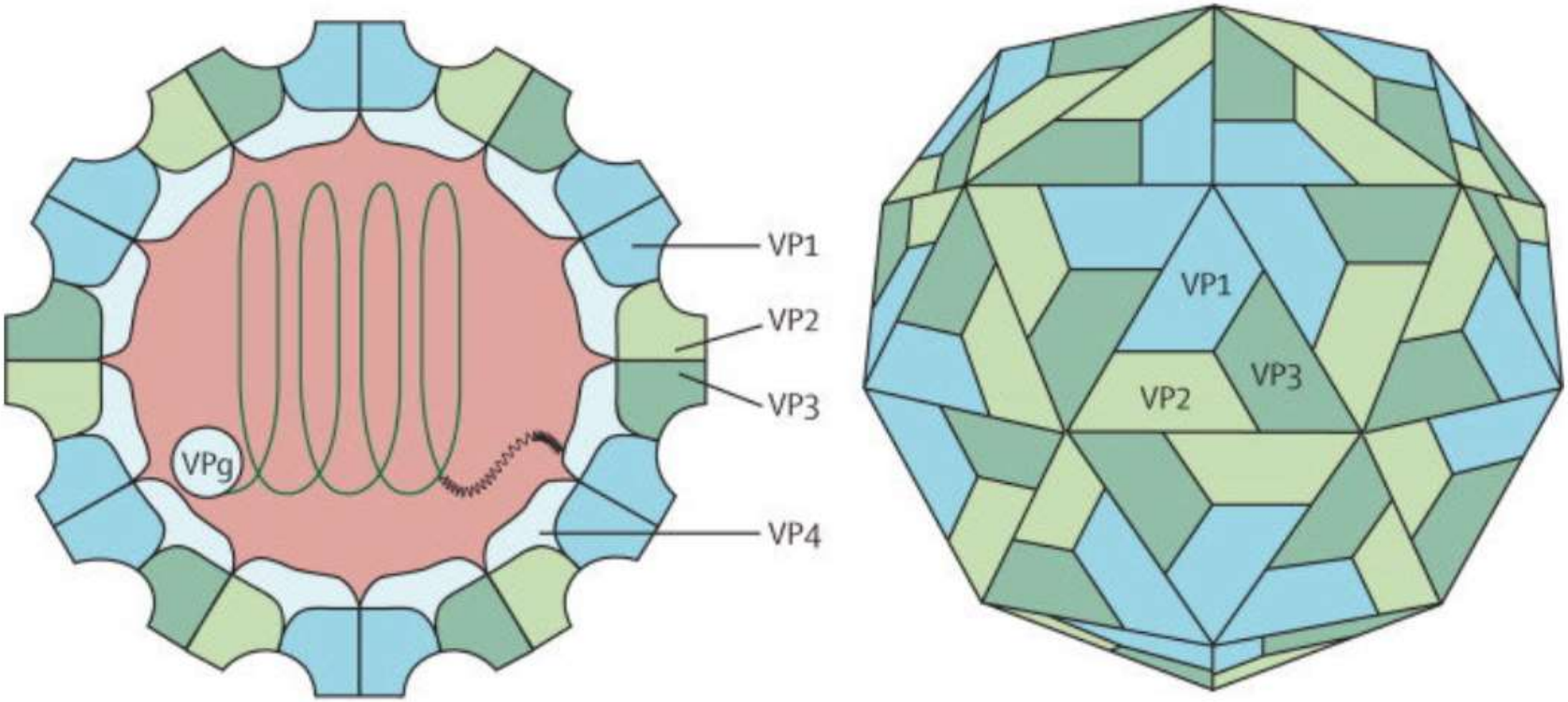
- FMD
- Swine vesicular disease – Enterovirus
- Vesicular stomatitis- Vesiculovirus (Rhabdoviridae)
- Vesicular exanthema of swine- Calicivirus

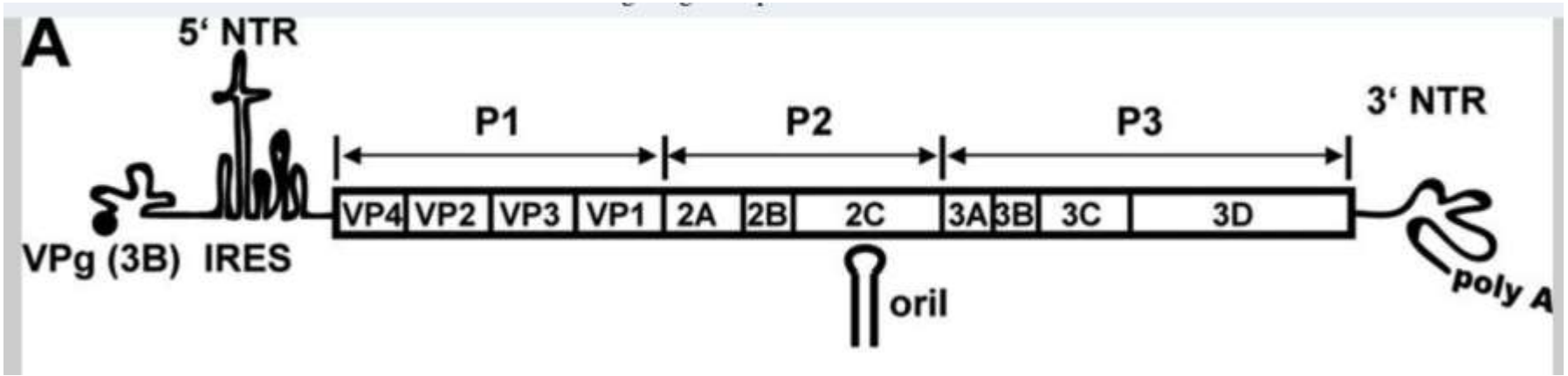
Structure

- **Non-enveloped**, relatively stable
- Capsid has icosahedral symmetry
- 60 capsomeres are there made of 4 viral proteins VP1, VP2, VP3 and VP4.
- All are derived from a single poly protein.
- Particle is 27-30nm in diameter and full length genome is 2500nm thus tightly packed in the virion.
- **Single stranded, positive sense RNA**
- RNA is infectious

Genome

- RNA lacks a cap at the 5′ end.
- On 5’ end a covalently attached small basic protein is there called **VPg** and on 3’ end there is a poly A tail.
- Genome has a long 600-1200 base untranslated region (UTR) at 5’end and shorter 3’ untranslated region of 50-100 bases.
- 5’UTR contains a clover leaf secondary structure also called as the **IRES (internal ribosome entry site)**. Internal ribosomal entry sites (IRESs) are regions in the mRNAs that allow the internal initiation of translation.
- Rest of the genome codes a single poly protein of around 2100-2400 aa.


VP1
VP2
VP3
VPg
VP4
VP1
VP2
VP3


A
5‘ NTR
P1
P2
P3
3‘ NTR
VP4
VP2
VP3
VP1
2A
2B
2C
3A
3B
3C
3D
VPg (3B)
IRES
oril
poly A

- The single open reading frame encodes a polyprotein.
- P1 encodes virion structural proteins
- P2 encodes proteins thought to participate in virus-host interactions required for genome replication.
- P3 encodes proteins thought to participate directly in genome replication.
- Polyprotein processing is mediated by protease activity residing in 2A, 3C, and/or 3CD proteins.

Susceptibility to inactivation

- pH
  - stable between pH 7 and 9
  - inactivated by:
    - 5% acetic acid or other acids
    - 1-2% sodium hydroxide or other alkalis
- Phenolic and quarternary ammonium compounds - not effective
- Oxidizing agents (bleach) effective when environment not contaminated with organic substances.
- Detergents increase effectiveness.
- The virus is sensitive to calcium chlorate lime, chloramine, formalin, potassium permanganate, and hydrogen peroxide solutions.

- It is rapidly killed on boiling.
- By Heat:
- in suspension 80^{o} C for 1 hr, 50℃ for 2 days, 37℃ for seven days
- stable when associated with dried organic matter.

Stability

- The virus is extremely resistant to photodynamic inactivation.
- It survives in sterile water at room temperature for a period of more than 100 days, in milk for 90 days, in faeces in the cold for more than 6 months, and in sewage for several months.
- It withstands exposure to 0.5-1 per cent phenol solutions.
- Aphtho virus is unstable below pH 7.0
- Rhino virus is unstable below pH 5.0
- Enterovirus is unstable below pH 3.0

Replication

- **Poliovirus-** a major model for studying RNA viruses
- Site: cytoplasm
- The cellular receptors used by different picornaviruses are diverse.
- Polioviruses, Coxsackie B viruses, and some rhinoviruses use members of the immunoglobulin (Ig) superfamily, while other picornaviruses attach via other cell surface molecules including integrins, heparin sulfate, low-density lipoproteins, or extracellular matrix-binding proteins.
- Some foot-and-mouth disease viruses, employ two receptors: initial binding involving heparin sulfate followed by high-affinity binding via integrins.

- Following attachment, entry and viral genome uncoating, the VPg protein covalently bound to the 5′ end of the RNA is removed by cellular enzymes, and translation of the RNA begins.

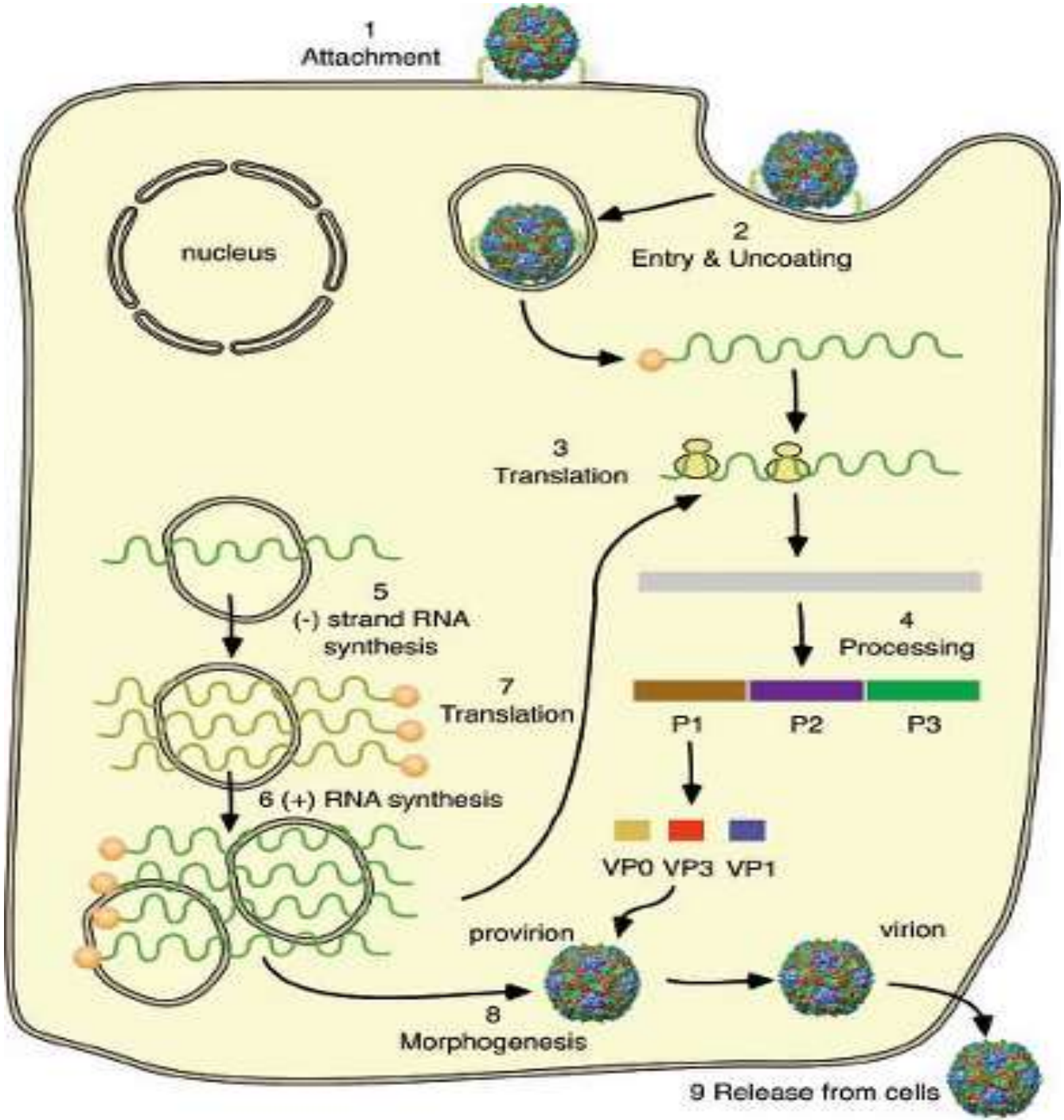
- Due to the absence of a 7-methyl guanosine cap, picornaviruses initiate translation in a **cap-independent manner**.

- Initiation of translation relies on binding of ribosomes to an internal ribosomal entry site (IRES) within the 5′ UTR.
- This region of RNA can fold to form cloverleaf structures that bind host proteins, allowing the initiation of viral protein and RNA synthesis.

- Because the poliovirus genome does not contain internal translational stop codons, a single polyprotein is generated and this is subsequently cleaved at specific sites by virus-coded proteinases to generate 11 or 12 discrete proteins.

- The structural proteins that make up the virion capsid (VP4, VP2, VP3, and VP1 respectively) are coded by the 5′ end of the genome,
- while the various cleavage products of proteins from the 3′ end provide replicative functions, including protease and RNA-dependent RNA polymerase activities and the genome-associated protein VPg.

- Virus replication takes place in a replication complex, consisting of RNA templates, the virus-coded RNA polymerase and several other viral and cellular proteins, all tightly associated with newly assembled smooth cytoplasmic membrane structures.
- Synthesis of the minus RNA strand is initiated at the 3′ end of the virion RNA, using the protein VPg or 3B as a primer.
- The completed minus strand is then used as the template for synthesis of the virion plus strand RNA.
- Newly synthesized plus strands and the structural capsid proteins then self-assemble into virions,
- Picornaviruses have evolved various ways to rapidly and preferentially inhibit the translation, and the synthesis, of cellular mRNA.


1
Attachment
nucleus
2
Entry & Uncoating
3
Translation
4
Processing
P1
P2
P3
5
(-) strand RNA
synthesis
6 (+) RNA synthesis
7
Translation
VP0 VP3 VP1
provirion
8
Morphogenesis
virion
9 Release from cells

Foot and Mouth disease (FMD)

- By FMDV - the type species of the ***Aphthovirus*** genus.
- Highly infectious disease of **cloven-footed animals** (e.g., cattle, goats, pigs, and sheep)
- Systemic disease with high fever, vesicles on epithelial surfaces
- Not usually fatal in adults but causes economic losses
- Can be fatal in young animals – myocarditis.
- In 1897, **Loeffler and Frosch** demonstrated that a filterable agent caused FMD. This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology.
- FMD is in the OIE **list A** of infectious diseases of animals

Serotypes

- Basically 7 serotypes –
- O, A, C, South African territories (SAT)-1, SAT-2, SAT-3, and Asia-1.
- **Currently, FMDV serotypes O, A, Asia1, Southern African Territories (SAT)-1, SAT-2, and SAT-3 are the six antigenically distinct serotypes globally prevalent.**
- **FMDV serotype C has not been reported from any part of the globe since 2004**
- **Serotypes O, A, and Asia1 are currently prevalent in India.**
- **Serotype O is responsible for the majority of FMD outbreaks in the country, followed by serotypes Asia1 and A.**

Susceptible species

- Domestic ruminants (cattle, buffalo, sheep, goats, camelids)
- Wild or exotic ruminants (African buffalo, various antelope and deer species)
- Others (pigs, rabbits, mice, guinea pigs, chickens, humans)

- **Pigs (amplifying hosts) -** secrete large amounts of virus in breath, air borne spread
- **Cattle (sentinel or indicator hosts) -** highly sensitive to infection by respiratory route
- **Sheep (maintenance hosts) -** mild-asymptomatic disease, can spread through flocks before detection

Important factors for spread of infection

- Short incubation period
- Release of virus prior to appearance of clinical signs
- Massive quantities of virus released
- Antigenic variation (7 serotypes with no cross protection and many antigenic variants with limited cross reactivity)
- Extended survival in the environment
- Multitude of routes of virus transmission
- Minimal size of the infective dose
- Aerosol transmission possible up to 250 km depending on strain and environmental conditions (10km, 170km, 250km reported over water)
- **Agroterrorism**

Transmission

- FMD is found in all excretions and secretions from infected animals.
- These animals breathe out a large amount of aerosolised virus, which can infect other animals via the respiratory or oral routes.
- The virus may be present in milk and semen for up to 4 days before the animal shows clinical signs of disease.

Routes of transmission

- Aerosols/Airborne, Inhaled
- Direct or indirect contact-droplets,
- Ingestion
- Artificial Insemination
- Vectors (vehicles, equipment, or humans)
- Carrier state

Transmission

Aerosol droplets spread from infected animal

Sheds 400,000,000 virus particles per day

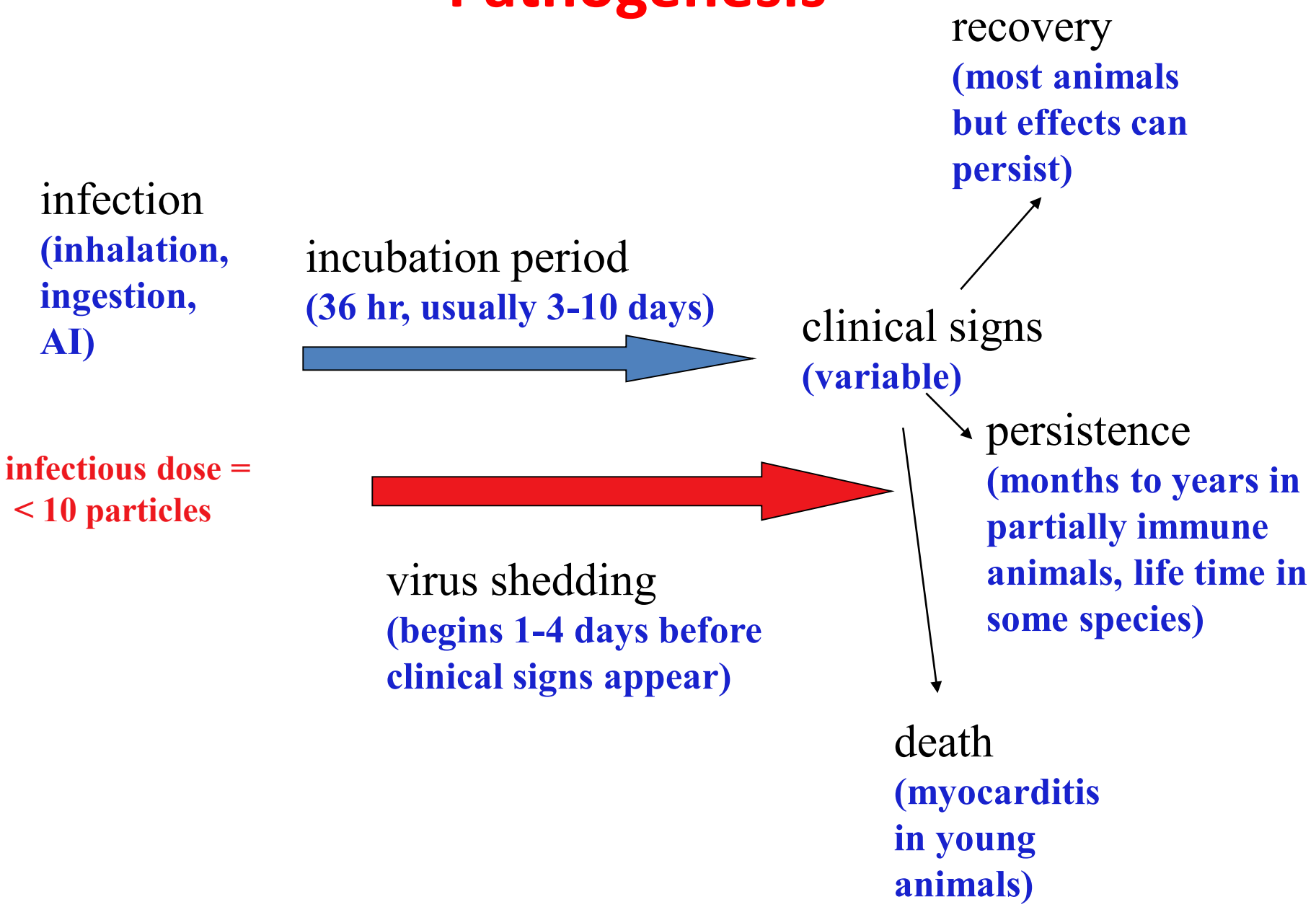
Only takes 10-12 virus particles to infect one cow

Transmission by humans

- A person in contact with infected animals may retain and exhale virus for up to 36 hours and serve as source of infection.

- Humans serve as a mechanical vector when moving from infected animals to susceptible animals.

Pathogenesis



Pathogenesis

- The pharynx is the primary site of infection, unless the virus enters the skin through a wound.
- The virus needs access to live cells on the surface and does not enter through cornified tissue
- After initial replication, the virus enters the circulation and disseminates to the areas of amplification such as the skin, tongue, and mouth (spread to **stratified squamous epithelium-target cells**).
- Virus can be detected in most cells during viremia but vesicles or blisters form in areas of rapid cell growth and where friction occurs naturally such as in the mouth or between toes

Progression of disease

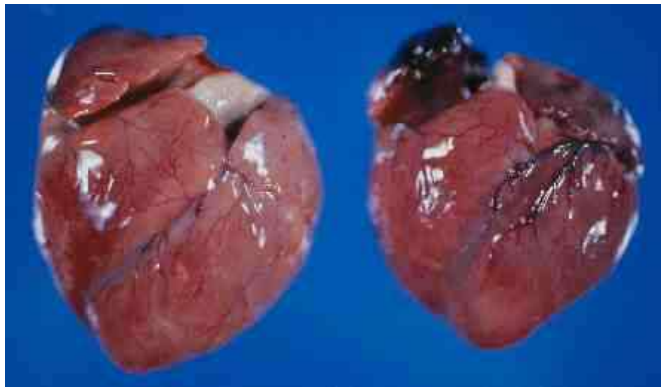
- Blisters at infection site initially appear as blanched area in the epithelium
- Area fills with serous fluid forming a vesicle
- Vesicles enlarge and coalesce
- Vesicles crack or rupture leaking fluid
- The epithelium necroses off leaving raw ulcer or erosion
- Grey fibrinous coating forms over lesions
- The coating becomes discolored, yellow, brown, green
- As epithelium is restored, lines of demarcation are evident
- Sometimes but not always, permanent scars form

Progression of disease

- When blisters are present, cattle salivate profusely with ropy viscous material hanging from mouth
- Also severe lacrimation and nasal discharge
- When vesicles rupture, fever ends followed by end of viremia
- Start to finish, signs last 15-30 days.
- Recovered animals are permanently unthrifty

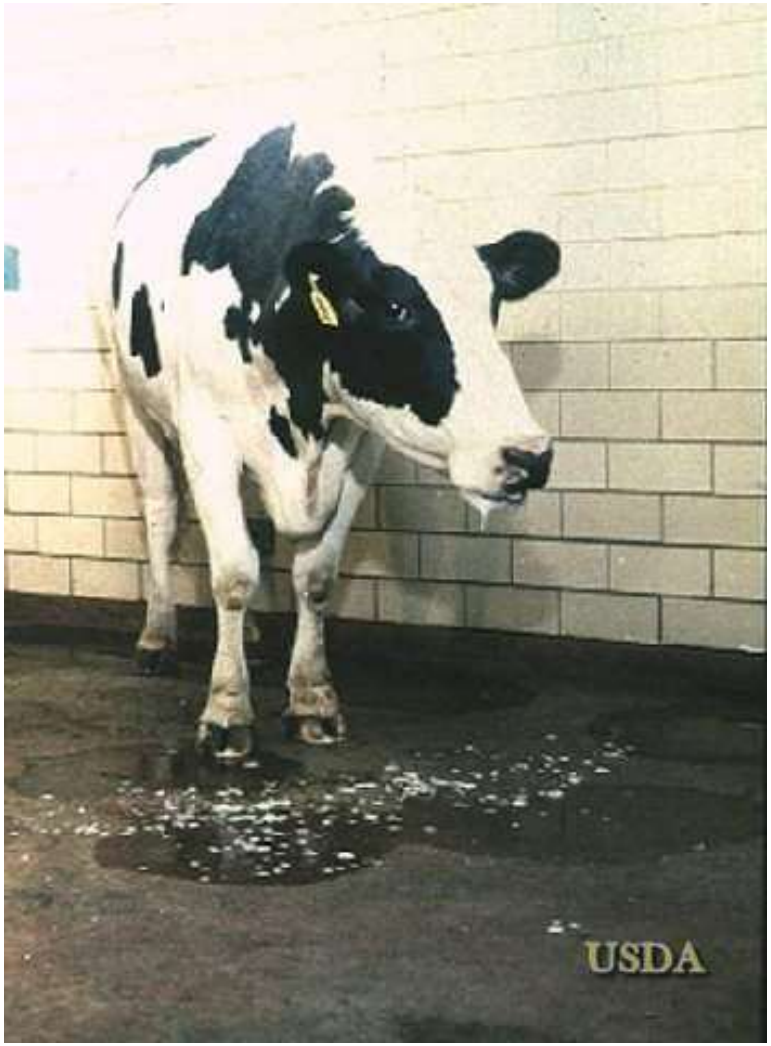
Infection of heart muscle

- In young animals (up to 6 months of age in cattle)
- 50 % mortality by myocardial damage in young animals
- In calves, myocarditis is considered a fatal form of FMD that occurs without developing the characteristic blister lesions noted in adult cattle
- The acute myocarditis of young animals is distinguished by hyaline degeneration, necrosis of muscle fibers and an intense infiltration mainly of lymphocytes.
- The mottled myocardial lesions referred to as **“tiger-heart”** lesions are useful in diagnosis



General Clinical Signs

- Listless
- Lifting feet alternately
- Lameness
- Clear nasal discharge progressing to mucopurulent



Clinical Signs in Cattle

- Fever (103°-106°F)
- Depression
- Anorexia
- Milk production ceases
- Blisters start to form in the mouth
- Excessive ropy, viscous salivation
- Smacking of the lips and sucking of the sore tongue is characteristic
- Blisters rupture within 24 hrs leaving raw, painful ulcers
- Mouth lesions usually heal in 10 - 14 days
- Mastitis
- Abortion in pregnant cows.

Clinical Signs in Cattle

Blisters form on:

Mouth

Tongue

Dental pad

Gums

Soft palate

Muzzle

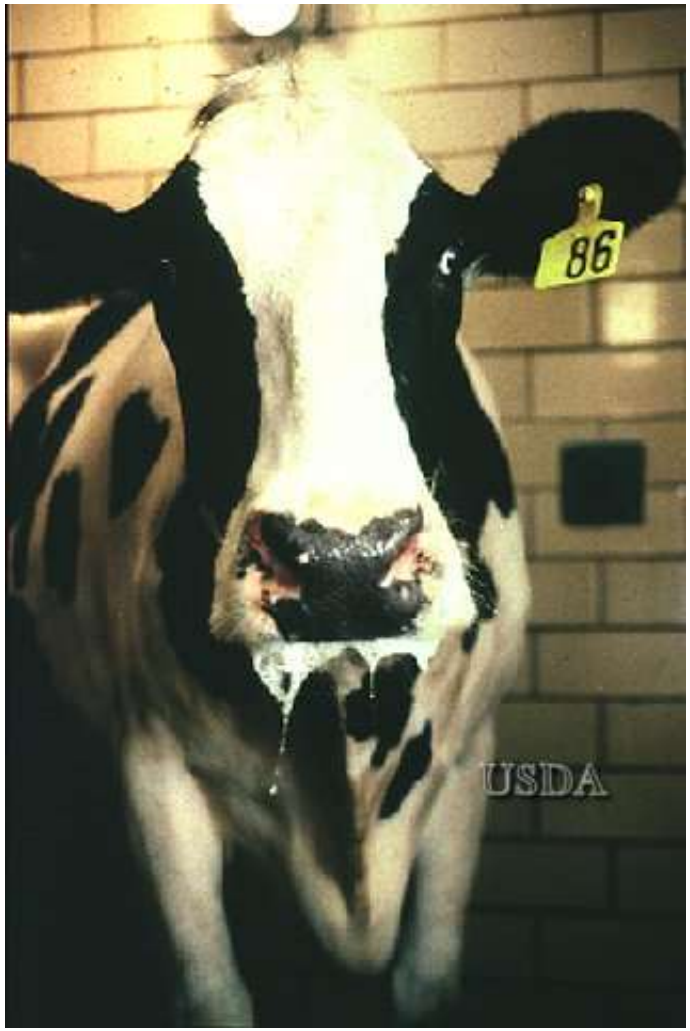
Nostrils

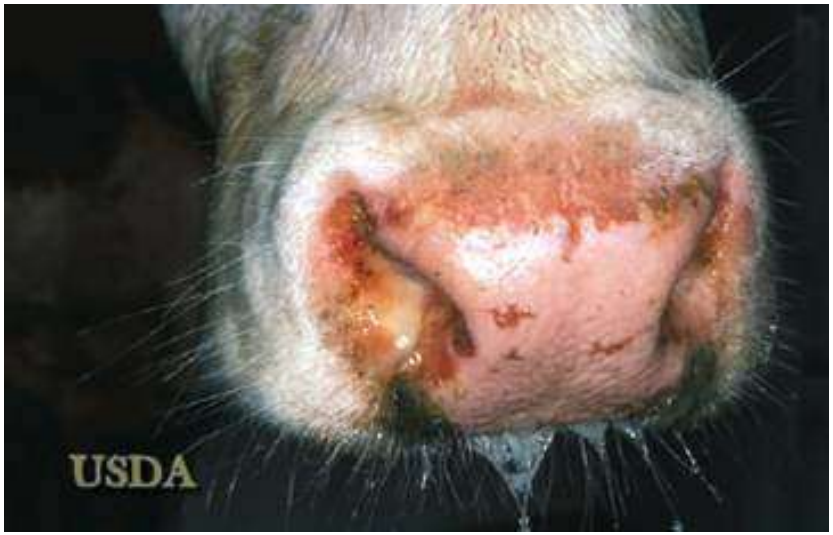
Feet

Inter-digital space

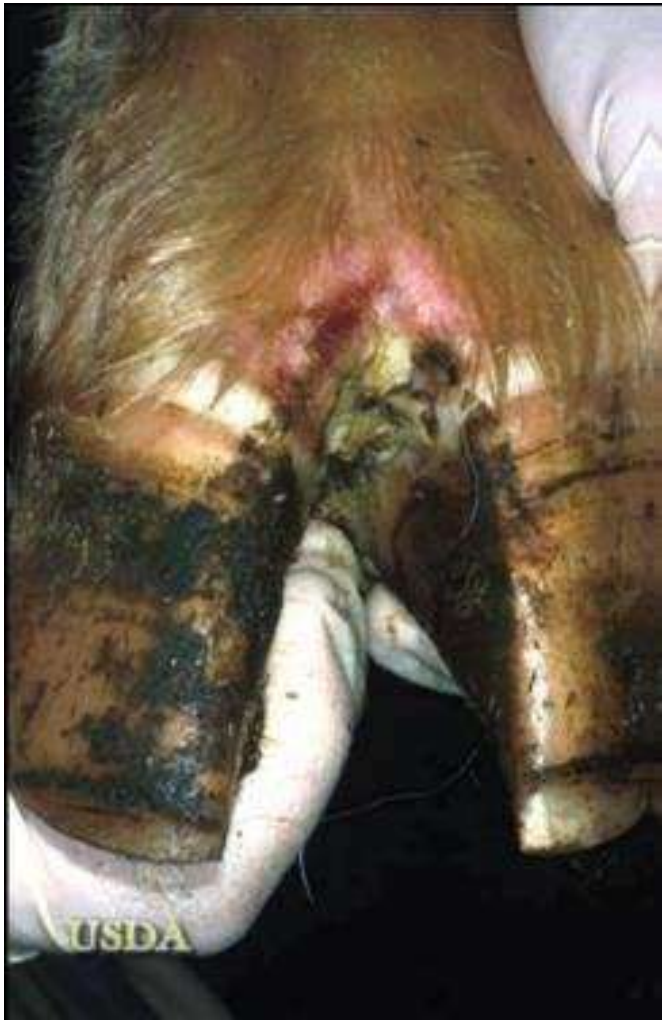
Coronary band

Teats





Foot-and-Mouth Disease



Foot-and-Mouth Disease

Lameness

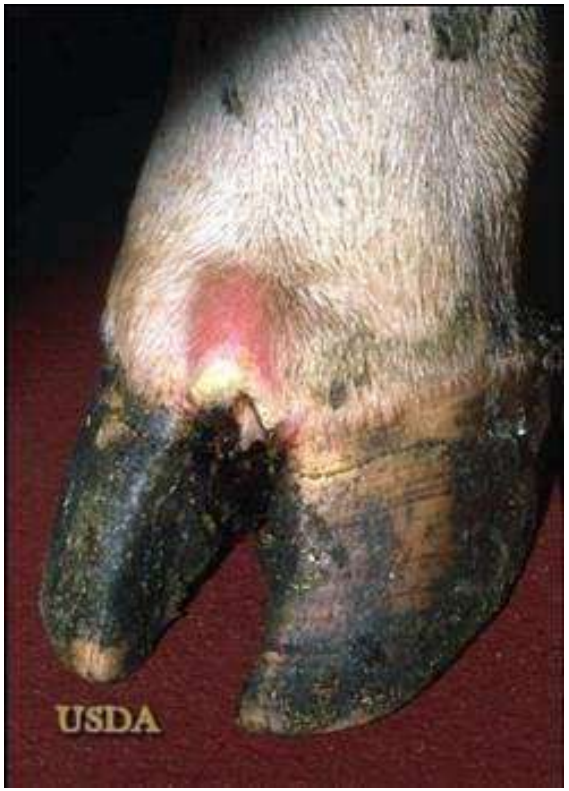
•Coronary band lesion first appears blanched

•Blisters form between the digits

•Stamping and shaking of feet

•Trembling

•Excretion of virus from foot lesions tends to last a day or two longer than from mouth lesions, so foot lesions may be a better source of virus for diagnostic purposes in older cases.



Prevention

- In countries with endemic FMD
  - vaccination
  - vaccination and slaughter
- FMD free countries
  - prevent introduction
  - in face of outbreak
    - test and slaughter
    - ring-vaccination and slaughter
    - ring-vaccination and slaughter only sick animals

- Both Polyvalent and Monovalent Vaccines are available.
- Monovalent vaccines provide immunity for longer periods.
- Vaccines are cell culture based.
- In endemic areas otherwise vaccination is done every six months.
- Under greater infection pressure four monthly vaccination schedules can be followed.

Vaccines

- Killed/Inactivated Oil Adjuvanted vaccine widely used in all species
- **Raksha**
- **Raksha-Ovac-trivalent –against FMD 0, A, Asia**
- **Raksha-Biovac- FMD and HS**
- **Raksha-Triovac-** FMD, HS and BQ

- **Futvac**

Problems with vaccination

- no cross protection if wrong serotype
- short-lived immunity
- partial protection if variant
  - does not prevent infection
  - persistent infection
- cannot distinguish between vaccinated and infected animals
- detection easier if no vaccination

Diagnosis

- Clinical signs - can be confused with other vesicular diseases
- Laboratory
  - sample collection
    - vesicle fluid, skin at edge of ruptured vesicle, excretions and secretions
    - inoculated onto susceptible cells
    - Serum neutralization test
    - Complement fixation test
    - ELISA

Laboratory Diagnosis

For virus	For antibodies
ELISA	Virus neutralization
Virus isolation	Agar gel immunodiffusion
PCR*	

Duck Viral Hepatitis (DVH)

- Typically associated with an acute, contagious infection in susceptible ducklings **less than 6 weeks of age** and frequently under 3 weeks of age.
- **It does not occur in older birds.**
- The disease, DVH, has traditionally been subdivided into types I, II and III.
- **DVH type I** can be caused by at least three different genotypes of duck hepatitis A virus (DHAV) virus, a member of the genus ***Avihepatovirus***, of the family Picornaviridae.
- The most pathogenic and widespread is DHAV type 1 (DHAV-1), which was formerly designated as duck hepatitis virus type 1.

- DHAV-2 and DHAV-3 are two additional genotypes within the genus Avihepatovirus that have subsequently been identified as additional aetiological agents of DVH in ducklings.

- **DVH type II** is caused by **duck astrovirus type 1 (DAstV-1),** a member of the Astroviridae family. DAstV-1 has been reported primarily in the **United Kingdom**. It has been reported in ducklings from 10 days to 6 weeks of age, and causes pathological changes similar to those of DHAV-1.

- **DVH type III** is caused by **duck astrovirus type 2 (DAstV-2),** a member of the Astroviridae. It is considered distinct from DAstV-1 and has been reported only in the **United States of America.** It causes similar liver lesions in young ducklings, but is less virulent than DHAV.

Signs

- The clinical disease is characterised by lethargy and ataxia followed by opisthotonos and death.
- Ducklings lose their balance, fall on their sides and kick spasmodically prior to death.
- The whole disease sequence is rapid and can take as little as 1–2 hours.
- Practically all mortality in a flock will occur within 3–4 days, with most deaths on the second day

- **Post-mortem lesions**
- Liver swollen with Punctate/diffuse haemorrhages.
- Kidneys and spleen swollen.
- Microscopically - hepatocyte necrosis, bile duct hyperplasia

Diagnosis

- History, lesions, SN serology, isolation in CE (causes stunting of 9 day embryo).
- Differentiate from Duck plague (viral enteritis), Duck septicaemia (anatipestifer), coccidiosis, Newcastle disease, Influenza and a 'Type II Variant' hepatitis caused by Astrovirus

- DHAV-1 infections can be controlled by the use of live attenuated virus vaccines and an inactivated virus vaccine