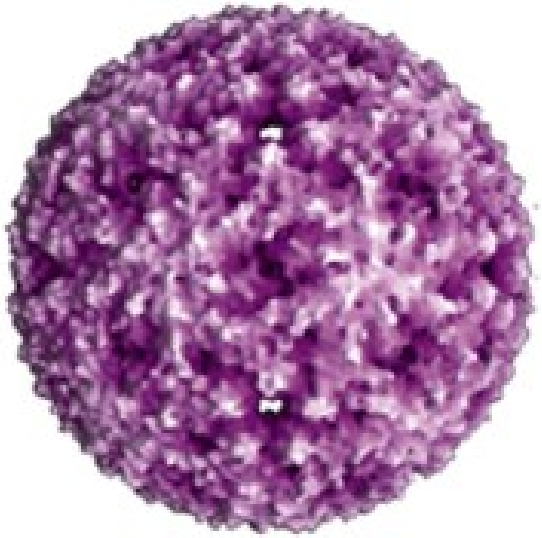
Picornaviridae



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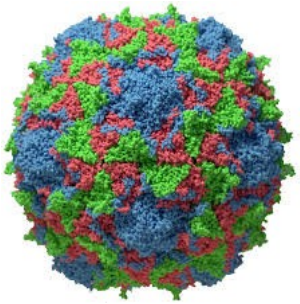


Poliovirus

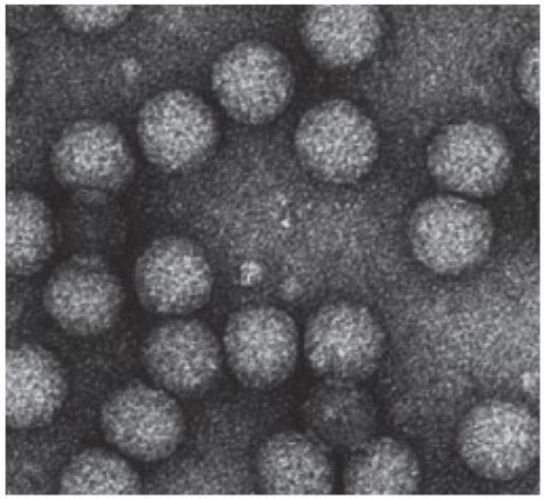
Classification of Picornaviridae

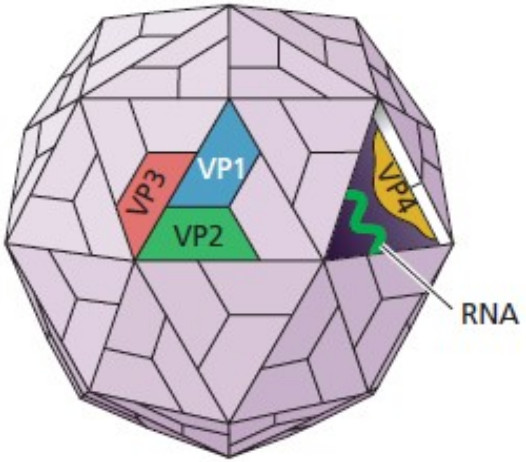
Genus	Species
Aphthovirus	*Foot-and-mouth disease virus*
	Equine rhinitis A virus
	Equine rhinitis B virus
Avihepatovirus	*Duck hepatitis A virus*
Cardiovirus	*Encephalomycarditis virus*
	Theilovirus
Enterovirus	*Bovine enterovirus*
	Human enterovirus A (Coxsackievirus, enterovirus)
	Human enterovirus B (Coxsackievirus, echovirus, enterovirus)
	Human enterovirus C (poliovirus, Coxsackievirus, enterovirus)
	Human enterovirus D (enterovirus)
	Porcine enterovirus B
	Simian enterovirus A
	Human rhinovirus A
	Human rhinovirus B
	Human rhinovirus C
Erbovirus	*Equine rhinitis B virus*
Hepatovirus	*Hepatitis A virus*
Kobuvirus	*Aichi virus*
	Bovine kobuvirus
Parechovirus	*Human parechovirus*
	Ljungan virus
Sapelovirus	*Porcine sapelovirus*
	Simian sapelovirus
	Avian sapelovirus
Senecavirus	*Seneca Valley virus*
Tremovirus	*Avian encephalomyelitis virus*
Teschovirus	*Porcine teschovirus*

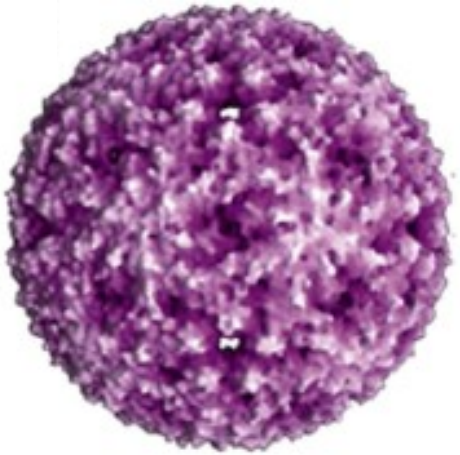
Properties of Picornaviruses

- Virions appear smooth and round in outline, nonenveloped, ,30 nm in diameter, and have icosahedral symmetry.
- All picornaviruses are single-stranded, positive-sense RNA viruses, 7-8 kb in length with a 5 ´-untranslated region (5 ´-UTR).
- The RNA is **uncapped**, but with a viral protein (VPg) covalently linked to the 5´ end and is polyadenylated at the 3' end.
- The length of the 5 ´-UTR in picornaviruses varies from approximately 500 to 1200 nt and contains one of at least five different **internal ribosome entry sites (IRESs) of cloverleaf-like structure.**
- Genome is **infectious**. Virion RNA act as mRNA that comprise a single ORF and is translated into a **polyprotein**, which is post-translationally processed by viral coded proteinases to yield (11-12) nonstructural and structural proteins.
- **Cytoplasmic replication** and extremely cytopathic.
- The name of the virus family was intended to convey the small size of the viruses (pico,[10-12]) and the type of nucleic acid that constitutes the viral genome (RNA).

Picornavirus Structure

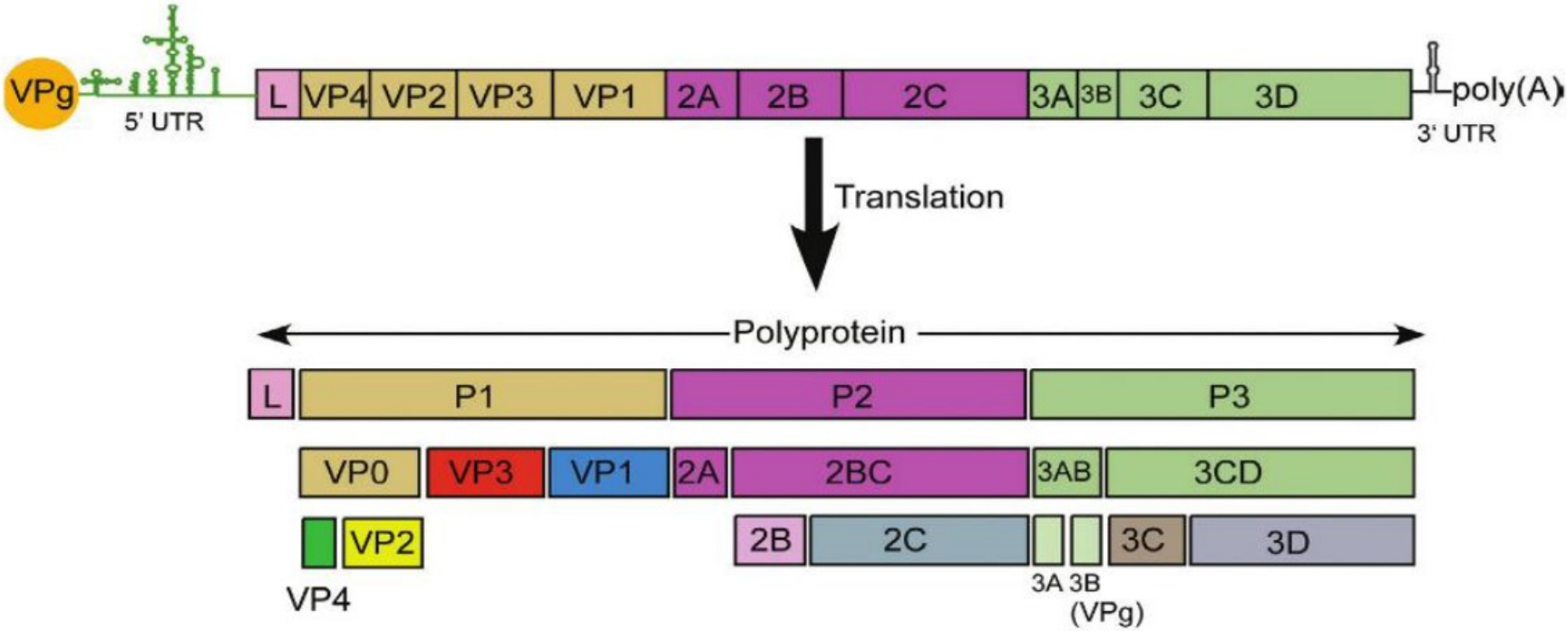






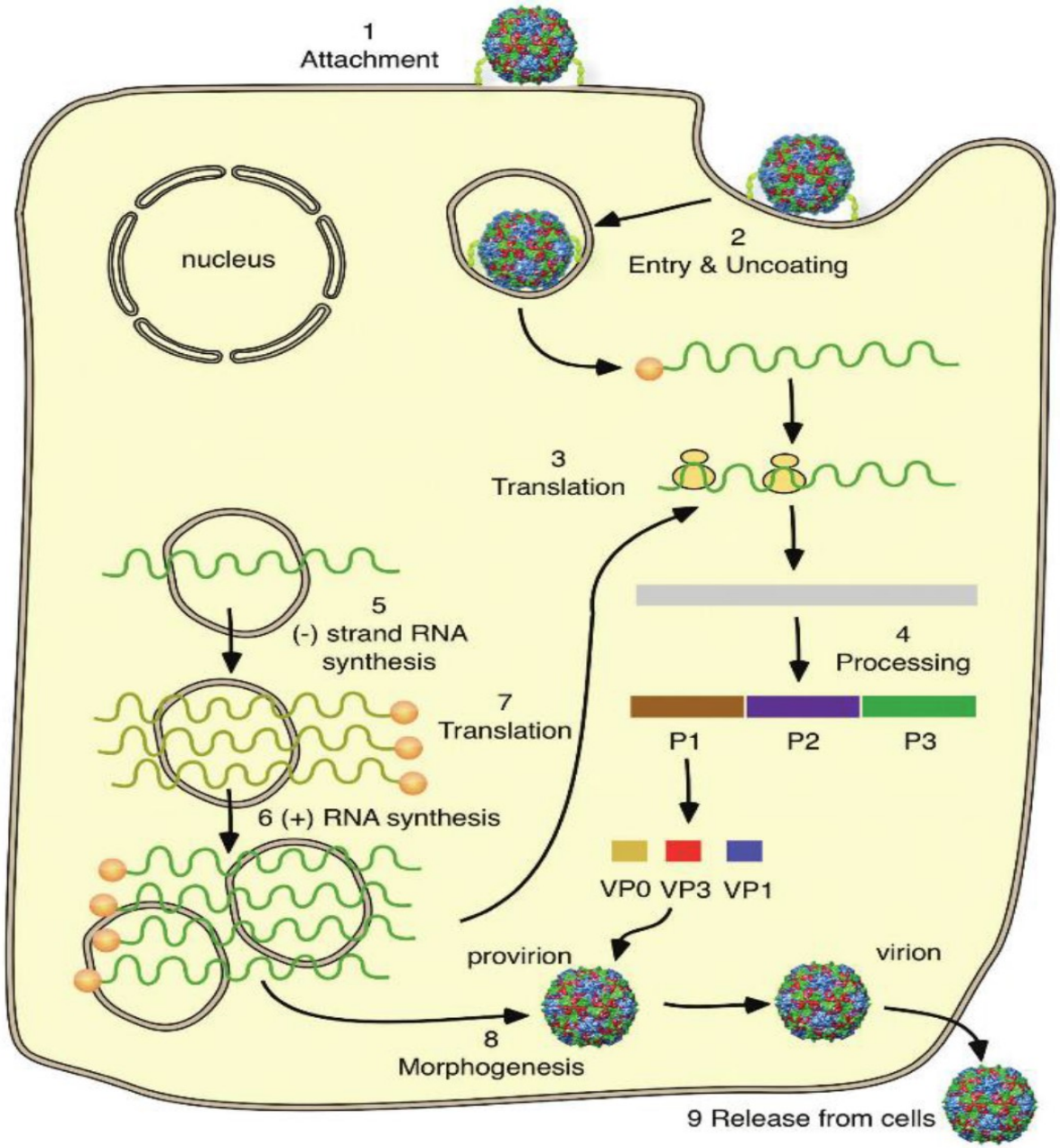
Virion structure. The electron micrograph shows negatively stained Foot and Mouth Disease (FMD) Virus. The capsid consists of 60 structural units (each made up of a single copy of **VP1**, **VP2**, **VP3**, and **VP4**, colored blue, green, red, and yellow, respectively) arranged in 12 pentamers. One of the icosahedral faces has been removed in the diagram to illustrate the locations of VP4 and the viral RNA.

Picornavirus genomic organization



Top: Schematic of the viral RNA genome, with the genome-linked protein **VPg** at the 5′ end, the 5′ untranslated region containing the **IRES**, the protein coding region, the 3′ untranslated region containing a pseudoknot, and the poly(A) tail. **L** is a **leader protein** encoded in the genomes of erboviruses, cardioviruses, and aphthoviruses but not other picornaviruses. Coding regions for the viral proteins are indicated. **Bottom:** Processing pattern of picornavirus polyprotein. Some genomes encode multiple copies of protein coding regions, e.g., there are three VPgs in the FMDV genome, two 2A motifs in Ljungan virus, and three 2A motifs in the duck hepatitis A virus genome.

Picornavirus Replication Cycle

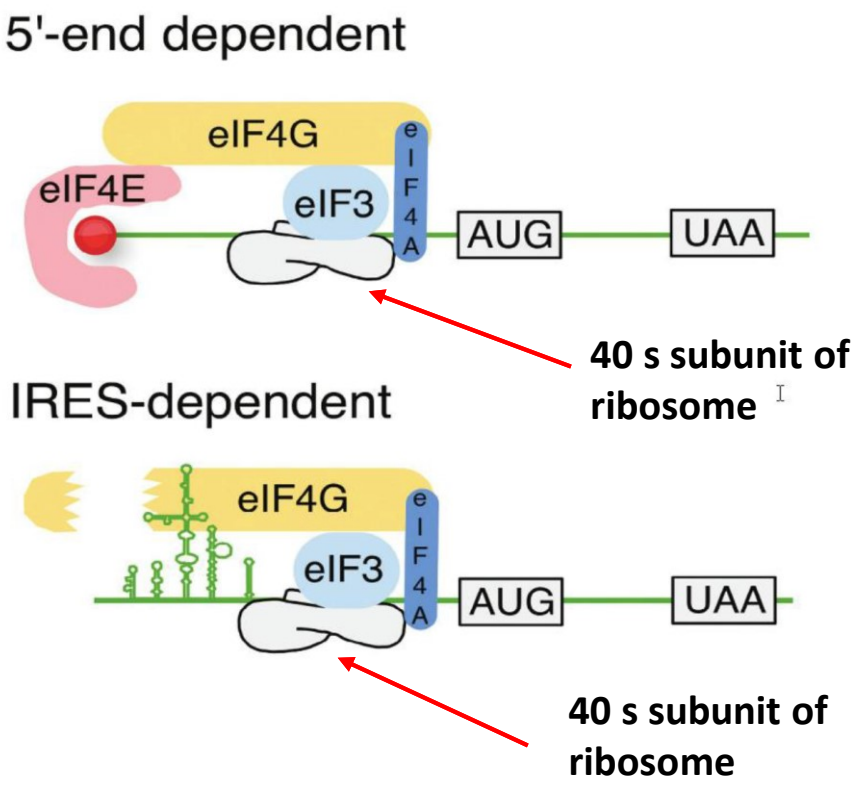


Picornavirus Replication Cycle

- Virus binds to a cellular receptor (1) and the genome is uncoated (2). VPg (virion protein, genome linked) is removed from the viral RNA, which is then translated (3).
- The polyprotein is cleaved nascently to pro-duce individual viral proteins (4). RNA synthesis occurs on membrane vesicles induced by viral proteins. Viral (+) strand RNA is copied by the viral RNA polymerase to form full-length (–) strand RNAs (5), which are then copied to produce additional (+) strand RNA (6).
- Early in infection, newly synthesized (+) strand RNA is translated to produce additional viral proteins (7).
- Later in infection, the (+) strands enter the morphogenetic pathway (8).
- Newly synthesized virus particles are released from the cell by lysis (9)

N.B* ** picornavirus replication can be very efficient, producing new virions after an eclipse period of less than 3 hours at yields of up to 106 virions per cell.

Models for Translation Initiation Complex Formation



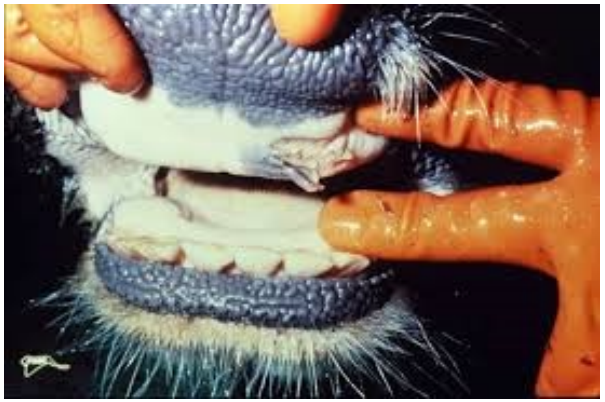
In 5′ end–dependent initiation, the 40S subunit is recruited to the messenger RNA (mRNA) through its interaction with eIF3, which binds eIF4G. The latter initiation factor is part of eIF4F, which also contains eIF4A, a helicase to unwind RNA secondary structure, and eIF4E, the cap-binding protein. Binding of eIF4E to the cap thus positions eIF4E at the 5′ end and positions the 40S subunit on the mRNA. **In IRES-dependent translation**, a 5′ end is not required. The eIF3–40S complex is believed to be recruited to the RNA by the interaction of eIF4G with the IRES.

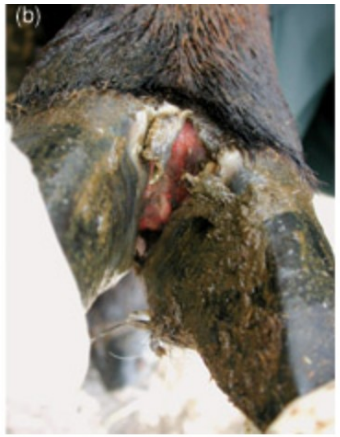
*Initiation of translation does not proceed by the well-established **Kozak scanning model.** Instead, ribosomal binding to viral RNA occurs in a region of the 5 UTR of the genome known as the **internal ribosome entry segment (IRES).** This segment of the viral genomic RNA is folded into cloverleaf-like structures, which bind specifically to host-cell proteins that play key roles in initiating the synthesis of viral protein and RNA.

Physico-chemical Properties of Picornaviruses

- Aphthoviruses and Rhinoviruses are highly labile and rapidly lose infectivity at pH values of less than 7.0, whereas the enteroviruses, hepatoviruses, cardioviruses, and parechoviruses are stable at pH 3.
- All Picornaviruses are resistant to the bile salts because they are free from lipids (non enveloped viruses).
- **Sodium carbonate** (washing soda) is effective against FMD virus, but is not effective against swine vesicular disease virus.
- Most picornaviruses are relatively **heat stable** at usual ambient temperatures. Some enteroviruses, for instance, may survive for several days, and often weeks, in feces.
- Aphthoviruses (FMDV) is inactivated in 30 minutes at 56ºC.
- The viruses are inactivated when exposed to direct sunlight.
- In slaughtered animals infected with FMDV, the virus rapidly inactivated due to lactic acid formation.

Properties of Foot and Mouth Disease Virus (FMDV)





- Foot-and-mouth disease virus was the first animal virus to be discovered, by Loeffler and Frosch in 1898. Poliovirus was isolated 10 years later.
- FMD is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. There are seven serotypes of FMD virus (FMDV), namely, **O**, **A**, **C**, **SAT 1**, **SAT 2**, **SAT 3** and **Asia 1.** Infection with any one serotype does not confer immunity against another. Within serotypes, many strains can be identified by biochemical and immunological tests.
- FMDV infect **cloven-hoofed animals** (cattle- buffalo- pig- sheep- goat- deer).
- Typical cases of FMD are characterized by a vesicular condition of the feet, buccal mucosa and, in females, the mammary glands. Clinical signs can vary from mild to severe, and fatalities may occur, especially in young animals.

FMDV

What are the reasons for the rapidity of spread in such fully susceptible populations ??

- **The highly infectious nature of the virus**
- **The production of high titers of virus in respiratory secretions**
- **The large volumes of droplets and aerosols of virus shed by infected animals**
- **The stability of virus in such droplets**
- **The rapid replication cycle with very high virus yields**
- **The short incubation period**
- **The involvement of sheep or other animals that show minimal signs of infection may also contribute to rapid spread of the virus.**

FMDV may persist in the pharynx of some animals for a prolonged period after recovery. Virus may be detectable for extended periods in cattle (perhaps up to **2 years**) after initial exposure to infection**; in sheep,** for about 6 months.

Lab diagnosis of FMDV

Sampling

The preferred sources of samples are:

- **Early in the infection**, samples should include vesicular fluid, epithelial tissue from the edge of recently ruptured vesicles, blood (in anticoagulant), milk, and serum.
- **In more advanced cases**, esophageal/pharyngeal fluids collected with a probang.
- **Typically**, these samples are diluted immediately with an equal volume of VTM (7.2-7.6 PH) containing a protein stabilizer such as 10% fetal bovine serum or 50% glycerol. From dead animals, additional tissue samples may be collected from lymph nodes, thyroid, and heart.

Viral isolation

The preferred susceptible hosts for viral isolation:

Susceptible lab animals:

-Unweaned **mice** are an alternative to cell cultures and should be 2–7 days old and of selected inbred strains. Some field viruses may require several passages before they become adapted to mice.

Cell culture

- Primary cultures of bovine, porcine, or ovine kidney are more sensitive than established cell lines such as **BHK-21** or **IB-RS-2** cells.

- **The CPE** including cellular rounding, degeneration and cytoplasmic granularity that followed by complete cell lysis 24-48 hr post inoculation.

Lab diagnosis of FMDV

Viral identification

- **The preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the ELISA (Antigen capture ELISA).**
- **Nucleic acid analysis (RT-PCR assays)**

-Rapid differentiation of the agents causing vesicular disease is available using multiplex RT-PCR assays, and RT-PCR tests can also be used to identify specific serotypes of foot-and-mouth disease virus.

- **Serodiagnosis**
- **VNT**

-Virus neutralization assays have been a mainstay in the serological diagnosis of foot-and-mouth disease virus

- **ELISA**

-ELISA tests that detect antibodies to the nonstructural proteins of foot-and-mouth disease virus have been developed in an attempt to distinguish animals vaccinated with killed vaccines from those naturally infected with the virus (DIVA).

FMDV

Immunity, Prevention, and Control

- Recovery from clinical FMD is correlated with the development of a virus-specific antibody response. The early **IgM** antibodies neutralize the homologous type of virus and may also be effective against heterologous types. In contrast, the **IgG** produced during convalescence is type-specific and, to varying degrees, subtype-specific.
- Cattle that have recovered from foot-and-mouth disease are usually immune to infection with the same virus serotype for **a year or more, but not lifelong**.
- As seen with natural infections, a vaccine strategy based on a single serotype will not work to control infections by the other serotypes. Even within a serotype, antigenic variation may make a vaccine less effective than is necessary to prevent infection.
- **Control of FMD in Egypt** relies mainly on quarantine and mass vaccination, Obligatory vaccination of all ruminants every six months and of dairy cows every four months is performed using a trivalent vaccine which is prepared from local isolates and contains strains of serotypes A, O and SAT2.