

GENERAL AND SYSTEMIC VIROLOGY (MICRO – 303)

Group I **ds** DNA viruses

POXVIRIDAE

Group II **ss** DNA viruses

PARVOVIRIDAE

CIRCOVIRIDAE

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POXVIRIDAE

Classification

The family “*Poxviridae*” comprised of two subfamilies, viz.

1. *Chordopoxvirinae*
2. *Entomopoxvirinae*

Chordopoxvirinae

Orthopoxvirus	Vaccinia virus Variola major (small pox) virus, variola minor (alastrim) virus Cowpox virus, buffalo poxvirus, camel poxvirus, horse poxvirus
Parapoxvirus	Bovine popular stomatitis virus (Bovine pustular dermatitis virus) Orf virus (contagious ecthyma virus) Milker’s nodule (paravaccinia or pseudo-cowpox) virus
Capripoxvirus	Sheep poxvirus, Goat poxvirus Lumpy skin disease virus (Neethling)
Lepropoxvirus	California myxoma virus Rabbit fibroma virus, Squirrel fibroma virus, Hare fibroma virus
Avipoxvirus	Fowl poxvirus, Turkey poxvirus, Quail poxvirus, Pigeon poxvirus
Suipoxvirus	Swine poxvirus
Molluscipoxvirus	Molluscum contagiosum virus of man
Yatapoxvirus	Yaba monkey tumour virus (zoonosis)

Entomopoxvirinae

Insects viruses	Infect insects
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General Properties

Poxviruses are the most complex and the largest “DNA” viruses and are just visible under the light microscope. The term “*pock*” was originally applied in the 15th century to the pustular eruption of skin associated with smallpox in man. The name “*smallpox*” was introduced to distinguish the disease from “*largepox*” or “*syphilis*”.

Size and Shape:

- a) Virions are Large, somewhat rounded, oval or *brick shaped particles*.
- b) They have a complex structure consisting of a central electron dense *nucleoid* which resemble a biconcave disc or *dumb-bell* with a rounded ‘*lateral body*’, occupying each concavity.
- c) The surface of double-layered outer membrane surrounding the virion contains numerous *protein threads* (9nm wide).
- d) The arrangement of these filaments sometimes gives the virion a characteristic appearance e.g. the “*ball of wool*” – particles of *orf virus*.

Nucleic acid:

- i) The bulk of nucleic acid is “*double stranded DNA*” with a very high mol. wt.
- ii) Genome (130kbp) encodes 150-300 proteins, 100 of these are contained in virion (enzymes) → involved in virus replication.

Resistance: Desiccated crusts retain infectivity for a year at room temperature, and purified virus resists 100°C dry heat for 5-10 min., but is destroyed by moist heat (60C) within 10 min.

Many members resist 50% glycerol and many commonly used antiseptics, but KMnO_4 and formalin will inactivate the virus.

Replication: Unique – *It takes place in the cytoplasm of the host.*

Poxviruses encode the enzymes required for mRNA and DNA synthesis as well as activities that other DNA viruses normally obtain from host cell.

After binding to receptors on cell surface, outer envelope of virus fuses with cell membranes → internalized → Outer membrane is removed → early gene transcription is started;

i) Early protein (*uncoatase*) dissolves the core membrane → Liberating viral DNA in cytoplasm → viral DNA replicates in electron-dense cytoplasmic inclusions (*Guannieri's inclusion bodies*) referred to as “*factories*”.

ii) Late viral mRNA is translated into structural and virion proteins.

iii) In poxviruses, unlike other viruses, the membranes assemble around the core factories. About 10,000 viral particles are produced by infected cell and are released upon lysis of cell.

1. ORTHOPOXVIRUS

VACCINIA VIRUS

Variolation: The ancient and dangerous practice of inoculating material from *smallpox* lesions into the arm to confer protection against the disease was prohibited in Britain in 19th century.

Vaccination: In 1798, Jenner reported that artificial inoculation of *cowpox* material protects against *smallpox*. *Vaccinia* virus which is used today as *smallpox* vaccine differs in some respects from typical *cowpox* virus.

There is a dose immunological relationship between *vaccinia* and *variola* viruses, and *smallpox* vaccine is prepared by inoculation with *vaccinia* of the shaved and scarified skin of the abdominal wall of sheep or calves.

Virus properties: are common to all members of the ‘*orthopoxvirus*’ group of which *vaccinia* is the type species.

COW POX VIRUS

The virus is closely related to *vaccinia*, yet distinct antigenically.

Associated disease:

Virus causes a disease of cattle affecting principally the skin of the udder and teats. It has a wide host range both in domestic and laboratory animals; and is an occupational disease of man producing lesions on the hands, arms, face & eyes.

The disease is now rare, and little is known about its present geographical distribution, but sporadic cases still occur in the UK.

Cultivation: The properties of virus are similar to those of *orthopoxviruses*. Growth occurs in Cell cultures and hemorrhagic pocks are produced on CAM of fertile hen's eggs.

Haemagglutination:

The haemagglutinin is separable from the virion and agglutinate avian *RBCs*.

Immunity: Infection provokes a solid durable immunity.

MOUSEPOX (ECTROMELIA) VIRUS

Associated disease:

Infectious ectromelia is a highly contagious, often fetal, generalized infection that is frequently carried in a latent form by individual mice and is readily activated by experimental procedure and other conditions of stress.

Early lesions on the lips, feet, and tail take the form of edematous swelling which become vesicles and later scabs. Secondary cutaneous eruptions occur 10-

14 days later. Mortality rates may be as high as 80-90% and postmortem examination may show necrotic areas or mottling of the liver and spleen.

Virus is shed in the urine and feces for several months after recovery and the infection is spread by direct contact with skin lesions or by the resp. tract.

Cultivation: Growth occurs in cell cultures and on the CAM of the fertile hen's eggs with the formation of the numerous small white pocks.

2. PARAPOXVIRUS

ORF VIRUS

Associated disease:

Orf, contagious ecthyma or contagious pustular dermatitis (CPD) is a viral dermatitis of sheep and goat, affecting primarily the lips of the young animals. Man may also become infected. The disease has a worldwide distribution and is especially prevalent where intensive husbandry of sheep and goats is practiced.

Virus Properties:

Mature virions of *orf, bovine papular stomatitis* and *milker's nodule* (pseudo-cowpox) have a similar, characteristic morphology and differs from the normal appearance of poxviruses. They are slightly smaller and more oval in outline.

Resistance: Virus resists desiccation and can survive outside the body for many months in dried scabs or at room temperature in the laboratory for upwards of 15 years. It is moderately sensitive to ether but is inactivated by chloroform and by a temperature of 60C for 30 minutes.

Cultivation: *Orf* virus does not grow in fertile hen's eggs but can be propagated serially in rabbits with difficulty. Growth occurs in various tissue culture systems including sheep testis, sheep kidney and human amnion cells with CPE: Formulation of multiple compact granular acidophilic I/C inclusions.

Pathogenicity:

Young lambs and kids are mostly affected. The morbidity rate is generally very high (upto 100%) but the mortality rate is low except among very young lambs in overcrowded conditions.

In natural cases, the **portal of entry** of the virus is probably through small wounds or abrasions of the skin. Vesicles and pustules mainly occur on the lips and mouth, and occasionally the eyes, interdigital regions, coronet of the foot; as well as on the teats and udder of the adult sheep. Healing may take place after 2-4 weeks.

In human infections, the lesions are generally firm, painless, bluish-red nodules without vesication. *Lymphadenitis* may be present but the lesions tend to heal spontaneously within a few weeks without scar formation.

Haemagglutination:

Orf and other *parapoxviruses* do not produce haemagglutination.

Diagnosis: A confirmatory diagnosis can be readily obtained by demonstration of the characteristic virus particles in scab material examined under electron microscope
Serological tests: AGPT, CFT and other recent more sensitive techniques.

Control: The scarification of a live virus vaccine consisting finely powdered dried infected scabs suspended as a 1% concentration in 50% glycerol-saline --- will induce a satisfactory level of immunity.

Annual vaccination may be necessary in endemic areas.

BOVINE PAPULAR STOMATITIS VIRUS

(Bovine Pustular Dermatitis Virus)

Associated disease:

Generally a mild, usually non-febrile disease occurring mostly in young cattle, and which is characterized by the presence of lesions in the epithelial cells of the oral

mucosa, muzzle and external nares. In many cases pathognomonic ring-shaped sores appear due to peripheral extension of the lesions forming concentric rings.

Cultivation: Embryonated eggs: No growth

Tissue culture: Primary sheep testis, bovine testis and human amnion.

MILKER'S NODULE VIRUS

(Pseudo-Cowpox Virus)

Associated disease:

A benign disease causing proliferative lesions on the udder and teats of lactating cows. It is probably the commonest viral infection of the udder and teats of cattle and is much more prevalent than true cowpox. Milking cows are most commonly affected but man frequently acquired the infection (Milker's nodule).

3. AVIPOXVIRUS

FOWL POX VIRUS

Associated disease:

Fowl pox occurs mainly in two forms: Most commonly it is a cutaneous infection of epithelial tissues of the non-feathered portions of the skin characterized by the formation of *wart-like nodules* on the comb, wattles, oral commissures, eyelids, feet and legs; alternatively it appears as an infection of the mucus membranes of the mouth, nose and eyes giving rise to *diphtheritic pseudo-membranes* in the mouth, pharynx and larynx.

Strains: There are 4 main strains of the virus, namely; *fowl pox*, *turkey pox*, *pigeon pox* and *canary pox*.

Antigenicity: All avian poxviruses are closely related immunologically but most show a considerable degree of host modification. For example, fowl poxvirus can be transmitted to pigeons by parenteral inoculation only with difficulty; and pigeon pox virus which is only slightly pathogenic for domestic poultry, immunizes chickens against fowl pox and constitutes a good vaccine for this purpose.

Resistance: Avian poxviruses are very stable and can remain viable in dried swabs for many months or even years, are highly resistant to 1% phenol or 1/1000 formaline, but are rapidly destroyed by *caustic potash* or *caustic soda*.

Cultivation: Avian poxviruses grow readily in fertile hen's eggs producing large white pocks on CAM and large acidophilic I/C inclusions (*Bollinger bodies*).

Cell culture: Most strains replicate without cellular changes, but a marked c.p.e. is obtained in whole chick embryo (fibroblast) cell cultures.

Transmission:

Direct contact with infected birds through abrasions and wounds caused by pecking etc. by indirect contact with exudates and contaminated utensils etc. or through transfer by biting insects.

Diagnosis: Histologically examination of lesions for Bollinger bodies. Serum neutralization tests in eggs (depression in pock count) and agar gel diffusion.

Control: Recovered birds develop a solid lifelong immunity.

Vaccines commonly employed to control fowl pox consisting of either fowl pox virus or pigeon pox virus grown in embryonated hen's eggs (CAM), and administered by the feather follicle method.

4. LEPRIPOXVIRUS

RABBIT MYXOMA VIRUS

Associated disease:

Virus causes an infectious disease of rabbits (*myxomatosis*), named after the mucinous nature of the exudate from the cut surface of the tumor-like swellings which develop in the skin of infected animals.

The infection is mild in the natural host (local wild rabbits of Uruguay and Brazil) but gives rise to a highly fatal disease in wild and domestic rabbits in Australia and Europe. Hares are rarely affected.

Sensitivity: *Myxoma* virus is unusual in that it is ether sensitive and *sodium deoxycholate* resistant. The virus has the general properties of *orthopoxviruses* in most respects.

Haemagglutination:

None

Antigenicity: *Myxoma* virus is closely related immunologically to *Shope's rabbit fibroma* virus which can be used for prophylactic vaccination of domestic rabbits against fetal *myxomatosis*.

Pathogenicity:

Mortality rate in susceptible rabbits is usually greater than 99%.

Lesions include blepharo-conjunctivitis with purulent discharge, swelling of the nose, muzzle, anal and genital openings and subcutaneous gelatinous swellings.

Death may occur within 3-5 days.

Transmission: Virus spreads with difficulty via respiratory tract and by contact; but rabbit to rabbit spread is mostly due to mechanical transmission of the virus by rabbit lice or fleas. (Rabbit flea; *Spilopsyllus cuniculii* is the principal vector).

Diagnosis: Histological examination of skin lesions reveals "myxoma cells and cytoplasmic inclusions. Virus can be isolated on the CAM and identified by *neutralization tests*.

5. CAPRIPOXVIRUS

SHEEP POX VIRUS

Associated disease:

Virus causes a highly contagious febrile and often fatal illness characterized by epithelial hyperplasia and micro-vesicle formation in the epidermis which is particularly noticeable in the more exposed parts of the body. It is the most severe pox disease of domestic animals and is prevalent in all over the world but it was eradicated from Britain in 1850.

Virus Properties:

Mature particles are smaller and more elongated than other poxviruses. They are extremely stable and survive in dried scabs in unused sheep pens or in grazed pastures for many months.

Cultivation: Embryonated eggs: No growths but some strains have been adapted to eggs by serial passage for vaccine production.

Tissue cultures: Various types of sheep and goat tissue cultures support growth.

Monolayer cell culture exhibit **CPE**: Intracytoplasmic inclusions and nuclear deg.

Antigenicity: A specific CFT can be used to demonstrate antibodies in the sera of recovered animals. Agar gel precipitation test (AGPT) suggests that some strains of *sheep pox virus* share a common antigen with *goat pox virus*. There does not appear to be any antigenic relationship with other poxvirus groups.

Pathogenicity:

Mortality rates vary from 5-50% or even higher, depending on the breed susceptibility and type of strain of virus involved. Apart from generalized lesions affecting the skin, there is tissue and occasionally, caseous nodules in the lungs.

Immunity: Recovered sheep develop a solid, long-lasting immunity.

Transmission:

Transmission is probably due to contact with infected animals or with objects contaminated with the virus. Air-borne infections may also be important.

Diagnosis: Serologically by various laboratory tests such as Agar gel diffusion test and neutralization test.

Control: Vaccination by scarification (*ovination*) or *intra*dermal injection of glycerol-preserved “*lymph*” obtained from artificially induced cases has been used to stimulate a solid long-lasting active immunity.
More recently, live attenuated calf kidney cell-culture vaccines have been developed and give good protection in sheep for upto 1 year.

PARVOVIRIDAE

The name of the family is derived from a Latin word **parvus**, which means small; thus virus has a very small DNA genome.

Classification

The family is subdivided into two subfamilies;

1. Parvovirinae
2. Densovirinae

PARVOVIRINAE

Genus	Viruses
<i>Parvovirus</i> (Replicate without helper virus in susceptible cells)	Bovine parvovirus
	Feline parvovirus (Feline Pan-leukopenia virus)
	Canine parvovirus
	Porcine parvovirus
	Chicken parvovirus
<i>Dependovirus</i>	Bovine, canine, equine & porcine adenoassociated virus
<i>Erythrovirus</i> (Replicate only with a helper virus present)	Human parvovirus B19 (Erythema infection in children)

DENSOVIRINAE

The members of this subfamily are insect viruses and are not of veterinary importance.

<i>Densovirus</i>	Infect insects
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General Properties

- ▶ Members of the family are the smaller DNA viruses.
- ▶ The virions are naked with cubical symmetry, 18-24 nm in diameter.
- ▶ The genome of the viruses is linear, *single stranded DNA* (4500-5500 nucleotides)
- ▶ There are *palindromic sequences* at both ends of genome.
- ▶ The capsid is composed with 32 capsomers and three major structural proteins viz. VP1, VP2 and VP3 have identified. The DNA is infectious

Physiochemical Properties

- ▶ Parvoviruses are stable at pH 3 – 9.
- ▶ They are relatively heat stable, resistant at 70C for 30 min, remain viable in fomites for longer periods and can be preserved well in 50% glycerol.

Replication

- ❖ The virus multiplies in the nucleus and is dependant on certain helper functions provided by the host cell or by another co-infecting virus.
- ❖ Disrupted fragments and empty shells are a characteristic feature. Two kinds of particles are produced in the nuclei, empty with vacant centers/densely stained.
- ❖ The bulk of complete virions are found on extranuclear/chromatin fibers forming linear or *trabecular beam* aggregates.

- ❖ Dependoviruses of the family are defective and completely dependent on an unrelated adenovirus for their replication.

Antigenicity:

- The antigenic properties of *parvoviruses* have not been well characterized.
- Haemagglutinin is present and involved in production of specific haemagglutination inhibition antibodies.

Pathogenicity:

- Parvoviruses primarily causes enteritis in most of the animal species, but they also cause generalized infection and reproductive disorders.
- The primary mode of entry of the viruses is the **oropharyngeal** route.
- *Transplacental* infection also occurs.
- Feline parvovirus causes pan-leukopenia, enteritis and cerebral hypoplasia.
- Parvovirus causes hepatitis and Myocarditis in goose and duck.

Immunity:

- Development of neutralizing antibodies could be detected within 3 to 5 days pi.
- The immunity that develops after natural infection appears to be life long.
- Natural passive antibody in pups and kittens can be detected and the antibody gives protection for few weeks.
- Generation of *cytotoxic T* cells after natural infection & vaccination has been report.

Prevention & Control:

- Attenuated and inactivated vaccines are available against parvoviral infections of porcine, canine and feline.

1. PARVOVIRUS

FELINE PAN LEUKOPENIA VIRUS

- The disease is most common in kittens about the period of weaning.
- All ages are susceptible
- Transmission is via oral or inhalation
- Incubation period = 5 days
- At 5-6 days → Leukopenia occur (100 WBCs/cm^3) → (less than 1000 WBCs/cm^3).
- Following initial proliferation in pharyngeal lymphoid tissue, virus is distributed to all organs via blood stream with infection and lysis of cells – having appositional receptors on which virus act as mutagens.
- Rapidly dividing intestinal epithelial cells in the crypts are also very susceptible → non-absorptive villi or they may lose – hence rapid accumulation of fluid & ingesta in the gut lumen leading to diarrhea and ultimately animal suffers from dehydration which result into death.
- Gut wall is thickened into rigid – hose like structure feces are also infected.
- Last 2 weeks of pregnancy – cerebellum (motor functions) damage of fetus.
- Newborn kitten has ataxia for at least a week after birth.
- If motor neurons are destroyed by feline parvovirus are not replace hence kitten remains ataxic permanently.

CANINE PARVOVIRUS (CPV)

- ▶ Pathogenesis is like that of 'Feline pan leucopenia virus'.
- ▶ Domestic dog, coyotes and wolves are susceptible to infection by CPV type 2.

- ▶ The virus spread by direct contact with infected animal and indirectly through excretions of the animals.
- ▶ Infected animals excrete the virus in high concentration in the feces.
- ▶ Entry of virus occurs by the oral or oronasal route.
- ▶ Myocarditis due to canine parvovirus infection is usually recognized as an acute disease of pups characterized by sudden death.
- ▶ The virus haemagglutinate pig and rhesus monkey erythrocytes (RBCs).

CIRCOVIRIDAE

Classification

The family '*Circoviridae*' comprised of following two genera;

Genus	Viruses
Gyrovirus	Chicken infectious anemia virus (CIAV)
Circovirus	Porcine circovirus type 1, 2 Beak and feather disease virus

General Properties:

- ▶ Non-enveloped (naked), icosahedral symmetry capsid.
- ▶ Non-segmented *single stranded DNA* have circular genome configuration.
- ▶ Virions measures 17-22 nm in diameter and capsid composed of 32 capsomers.
- ▶ Three viral proteins viz. VP1, VP2 and VP3 has been identified.

Physiochemical Properties:

- ▶ Members of the family have the ability to resist inactivation by exposure to heating at 56C for 1 hour, pH 3 and lipid solvents.
- ▶ But the viruses can be inactivated by heating at 100C for 15 min and exposure to 1% glutaraldehyde, 1% iodine and 1% sodium hypochlorite solution.

Replication:

- ▶ Viruses replicate in the nucleus of dividing cells of hemolymphatic system.

Cultivation:

- ▶ The viruses can be propagated in Chicken embryos by Yolk sac route, in continuous cell lines or in susceptible hosts.
- ▶ In chicken embryos, viruses cause death of the embryo.

Antigenic Properties:

- ▶ The structural proteins VP₁ and VP₂ are involved with antigenicity of the viruses.
- ▶ The VP3 protein of viruses is reported to be poorly immunogenic.

Immunity:

- ▶ Humoral immunity primarily involved in protection, but the role of CMI in protection against the disease caused by members of the family cannot be ruled out.

1. GYROVIRUS

CHICKEN INFECTIOUS ANEMIA VIRUS

Causative agent:

Chicken infectious anemia virus, belongs to the genus "*Gyrovirus*" of family "*Circovirida*".

Host susceptibility:

Chicken of all ages are susceptible to the virus infection

Mode of spread:

The virus can be spread both vertically and horizontally. The virus passed vertically to the offspring, which in turn develop clinical signs. Horizontally the virus spreads through direct contact with vertically infected chicks or through ingestion of feed contaminated with feces of infected birds.

Pathogenesis:

After entry, the chicken infectious anemia virus initially affects the haematopoietic precursor (haemocytoblasts) and thymic precursor (lymphoblasts) cells in the bone marrow and thymus cortex and subsequently spread to other tissues.

The virus replicates in the marrow haematopoietic precursor cells and thereby causes *immunosuppression* and transient *sever anemia* with atrophy of lymphoid tissue. Mortality rate is about 10-50%.

Clinical Symptoms:

Anemia is noticeable in the non-feathered areas particularly on comb, wattles, eyelids and legs. Bird may show inconsistent symptoms such as depression, paleness, weakness, anorexia and stunted growth.

The gross lesions in lymphoid tissue and bone marrow are most characteristic.

Isolation & Cultivation:

Embryonated Eggs: Isolation of virus can be made by me /M or I/P inoculation of whole blood into day old SPF chick or in embryonated chicken egg via yolk sac.

The virus causes mortality of chicken embryos within 16-20 days of incubation.

Cell Lines: Continuous cell lines can also be used for isolation purposes.

Diagnosis:

Detection of virus specific antigen can be made by FAT, IP and abs by ELISA. ELISA is the test of choice for sero epidemiology of the disease.

Prevention & Control:

It is very difficult to maintain laying hens from Chicken Infectious Anemia Virus.

Live vaccines are available that should be given to Abs-free breeder flock prior to start of egg laying.

Development of genetically modified virus vaccine may help to control the disease successfully. Antibiotics may be used to treat the secondary infection.

2. CIRCOVIRUS

BEAK AND FEATHER DISEASE VIRUS

Susceptible Hosts:

Psittacoses, Cockatoos are particularly susceptible.

Mode of Spread:

Direct and indirect contact, ingestion of contaminated feed, and by inhalation

Pathogenicity:

The virus specifically infects the cells of immune system and those cells that produce beak and feather. Virus is associated with beak deformities and abnormal feathering. Affected birds often die

Prevention & Control:

No vaccine available but other control measures are good management, careful purchase and quarantine.

