GENERAL AND SYSTEMIC VIROLOGY
(MICRO – 303)

Group I ..... **ds** DNA viruses

HERPESVIRIDAE

Subfamilies

Alphaherpesvirinae

Betaherpesvirinae

Gammaherpesvirinae

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HERPESVIRIDAE

(**Greek, *herpien* = to creep**)

Classification

The family '*Herpesviridae'* has been subdivided into three subfamilies.

1. *Alphaherpesvirinae*

This subfamily comprises 4 important genera;

Genus	Viruses
Simplexvirus	Human herpes virus 1, 2 (Herpes simplex 1,2 virus)
	Human herpes virus 3 (Vericella zoster virus)
	Bovine herpes virus 2 (Bovine ulcerative mammalitis V. / pseudo-lumpy skin disease V.)
	Cercopithecine herpes virus (Herpes virus of monkey)
Varicellovirus	BHV 1 (Infectious bovine rhinotracheitis, pustular vulvoginitis)
	BHV 5 (Meningoencephalitis in cattle – in south America)
	Suid HV 1 (Pseudorabies or Aujeszky's disease)
	Canine HV 1 (Canine herpes virus infection)
	Equine HV 1 (Equine rhinopneumonitis and abortion)
	Equine HV 3 (Equine coital exanthema)
	Equine HV 4 (Equine rhinopneumonitis)
	Feline HV 1 (Feline viral rhinotracheitis)
Iltovirus	Gallid HV 1 (*Infectious laryngotracheitis* in chicken and pheasants
	Anatid HV 1 (Duck herpes virus causes 'duck plaque')
Mardivirus	Gallid HV 2 (*Marek's disease*)

2. *Betaherpesvirinae*

This subfamily is of little veterinary importance; and contains three genera;

Genus	Viruses
Cytomegalovirus	Human herpesvirus 5
Muromegalovirus	Murid cytomegalovirus 1
Roseolovirus	Human herpesvirus 6

- ► Slow replication cycle (>24 hours) → means slow destruction of cells and their spread takes many weeks → Cultured cells become enlarged.
- ► Narrow host range
- ► Establish latency in monocytes/lymphocyte (T cells) of many tissues (lymphoreticular and secretory gland cells).
- ► Associated with respiratory tract infections of animals
- ► No virus is of significance in domestic and farm animals.
- ► Swine herpesvirus 2 – causes inclusion body rhinitis.

3. *Gammaherpesvirinae*

- ► Slow in cytopathogenecity.
- ► Replicate in lymphoid cells (B cells, epithelial cells and other cells) – generalized disease of lymphoid tissues and produce tumors.
- ► Latency is established in lymphoid tissues (immortalize lymphocytes *in vitro*)

Genus	Viruses
Lymphocrytovirus	Human HV 4 (Epstein Barr (Kissing disease) virus
	Kaposi Sarcoma virus (infects human)

Rhadinovirus	Alcelaphine herpesvirus 1 (Natural host: wild beast)
	Ovine herpesvirus 2 (Natural Host: sheep)

General Properties

Shape and size:
Large, isometric viruses containing linear *double-stranded DNA*.
Usually *enveloped* and ether sensitive;
Diameter of virion: 110 nm (naked) and 180-250 nm (enveloped).
Icosahedral symmetry; nucleocapsid consists of 162 hollow prismatic capsomers resembling short hexagonal (150) and pentagonal (12) tubular projections.

Sensitivity: Acid stable but heat sensitive (inactivated at 50C for 30 min).

Replication: Viral protein is synthesized in the cytoplasm but synthesis of viral DNA and assembly of the mature virion begins in the nucleus. The virus derives its envelop largely from the nuclear plasma membrane and an additional (outer) membrane forms at the cytoplasmic membrane.

Virulence: Herpes virus show marked changes in virulence when infecting other than natural hosts (e.g. latent B-virus in monkey is highly fetal in man).
Some viruses are tend to become oncogenic (e.g. Marek's disease virus).

Haemagglutination:
Not observed but hamster tissues infected with certain strains of *equine rhinopneumonitis virus* agglutinate horse and guinea pig RBCs.

Antigenicity:No antigen is common to the group, but some strains are closely related (H. simplex, B-virus, and *Aujeszky's disease virus*).

Cultivation: All typical *herpesviruses* grow readily in cell cultures and produce cytopathic effects including ballooning degeneration, Syncytial formation and Cowdry type A I/N inclusions. All spp. grow in embryonated eggs and form on pocks on CAM.
Direct cell to cell spread may occur giving rise to foci which enlarge to form visible plaques. Some possesses a broad host-cell susceptibility (e.g. ADV) while others show a limited cell susceptibility (e.g. ILT)

1. SIMPLEXVIRUS

BOVINE HERPES VIRUS 2
(Bovine Mammillitis Virus)

About Virus:
- *Bovine herpes virus 2* of the genus *Simplexvirus* and subfamily *Alphaherpesvirinae*.
- The virus is associated with bovine mammillitis disease.

General Properties:
- Morphologically virus is resembled to other members of the family.
- The virus has been reported in many countries of the world.
- Virus shows 15% homology with human herpes simplex and 6% homology with BHV-1 (RE analysis) but differs biologically
- Latency has been reported and virus can be preserved at -50C for a month, sensitive to chloroform/ether.

Susceptible Hosts:
- Natural – Cattle but, buffalo and giraffe may be infected.
- Guinea pig and rabbits can be infected experimentally.
- Recovered/latent infected animals may act as reservoir – carrier.

Transmission:
- In milking cows, the spread of the virus occurs mainly through contaminated milking machines and milker hands. Mechanical transmission by arthropods is also suggested.

Pathogenesis:
- The virus enters through skin injury/laceration.

- The virus localizes in teat, mammary gland epithelium. Sometimes viremia occurs and lead to generalized infection.
- Higher body temperature favors the local production of large amounts off highly active interferon, which can inhibit the virus replication.
- Conversely at subnormal body temperature (i.e. in the skin of the teats and udder) not only the interferon production is limited, but there INF produced is also of low specific activity → lesions mainly develop i.e. mammillitis.
- The disease is normally self limiting and lasts for 10-12 days.
- Secondary bacterial infection: Mastitis and Necrosis of the udder.
- Sometimes nodular lesions seen in different parts of skin → generalized skin lesion called "pseudo-lumpy skin disease" occurs in tropical and subtropical areas where susceptible wild ruminants act as subclinical reservoirs.

Lab Diagnosis:

- Disease can be confused with FMD, Cowpox thus, confirmatory diagnosis is necessary.
- The clinical materials of choice for lab diagnosis are vesicular fluid, biopsy tissue, paired serum samples.
- The direct visualization of virus can be done via EM, demonstration of I/N inclusions under light microscope in biopsy tissues by Geimsa staining.
- Serological tests like AGPT, ELISA, and VNT are useful for detection of virus specific antigen and antibodies.

Prevention and Control:

- No commercial vaccines are available.
- Self limiting infection – Topical antibiotics.
- Hygienic measures for milking machine and milking parlor.
- Antiviral drugs are also useful. Insect control.

2. VERICELLOVIRUS

BOVINE HERPES VIRUS 1

(Infectious Bovine Rhinotracheitis Virus)

About Virus:

Bovine herpesvirus – 1 of the genus *Vericellovirus* & subfamily *Alphaherpesvirinae*.

Associated disease:

- Virus causes a disease, *Infectious bovine rhinotracheitis* in dairy cattle.
- Antigenically related with *Equine herpesvirus-1* but IBRV and *infectious pustular vulovagintis virus*'s DNA sequence show differences (Respiratory subtypes + genital subtypes).
- Virus is sensitive to many chemicals like NaOH, phenol, chloroform.
- Virus can be inactivated by 5% formalin within 1 minute, but is stable at wide range of pH (6-9).Virus can be preserved at -70C for long period and at 4C for a few days.

Susceptible Hosts:

- Cattle of all ages
- Goats, swine, water buffalo may be infected.
- Wild ruminants may act as reservoir.
- Rabbit, hamsters may be infected experimentally.

Transmission:

- Direct contact, aerosol
- Semen of infected bulls.
- Latently infected animals spread the virus.

Pathogenesis:

- Portal of entry: are respiratory tract, conjunctiva, and genital tract.

- Virus multiplies in the upper respiratory tract → rhinitis, laryngitis, tracheitis.
- Virus spread to maxiallary and mandibular branches of trigeminal nerve → go to brain → causes encephalitis, ataxia and incoordination
- There is 100% morbidity but Mortality is only 10%.
- Genital form of disease (IPVV) results after entry of the virus through genital tract and localization in the genitalia (vulva, vagina of cow, and prepuce sac of male) → there may be inflammatory changes (vulvitis, vaginitis and orchitis), focal necrosis, and yellow pustules which may slough.
- Pregnant animals may abort at 4-7 months of gestation.
- The virus may reach the mammary gland and causes mastitis.
- Virus may remain latent in trigeminal nerve. *Dexamethasone* can activate the virus in diseased animal and asymptomatic virus shedder.

Cultivation:

- Virus can be isolated in Primary bovine kidney cell culture

Laboratory Diagnosis:

- Virus can be procured from nasal discharge, conjunctiva and swabs from vulva, vagina, prepuce and paired serum samples.
- After postmortem, sample may be collected from liver, spleen, lymph node and brain.
- In aborted cases → fresh tissues (Cotyledons, fetal liver, lungs etc.) can be taken.
- Virus can be detected via EM, viral specific antigen detection and antibodies through AGPT, ELISA, FAT, VN, PCR, dot blot.
- Endonuclease fingerprinting --- differentiate IBRV, IPVV
- Detection of intranuclear inclusion bodies in liver tissues of aborted fetuses is a diagnostic feature.

Prevention:

- Live, attenuated, inactivated virus vaccines.
- Live vaccine can be given intranasally or intramuscularly.
- Calves be vaccinated by I/N (2-4 weeks) or I/M (6 month of age), booster after 6 month
- I/N vaccine is safer for pregnant animals as I/M vaccination may cause abortion.
- Live vaccine may produce latent infection in nervous tissue. Inactivated vaccine is safe but gives poor immunity.
- A combined vaccine containing inactivated BHV-I, killed pasturella multocida and killed parainfluenza-3 virus is available. It can be used in calves of 5 month age and produce immunity in 10-15 days post vaccination.

Prevent spread:

- Segregation of infected animals from the healthy one.
- Kulling of +ve breeding bulls.

EQUINE HERPES VIRUS 1

(Equine Rhinopneumonitis and Abortion)

About Virus:

Equine herpesvirus 1 of the genus *Vericellovirus* and subfamily *Alphaherpesvirinae*. The virus causes equine rhinopneumonitis and abortion in mares.

General Properties:

- Morphology of the virus is similar to the members of the family.
- The virus possess complement fixing, precipitating and HA antigens.
- The virus agglutinates equine RBCs.
- It does not survive long outside the host, may survive for several weeks at low temp and can be preserved for 1 year at -180C.
- The virus can be readily inactivated by lipid solvents, 0.35% formalin and by heating at 56C for 10 min.

Susceptible Hosts:

- Equines are the natural hosts. Unweaned hamsters can be infected experimentally.

Transmission:

- The virus spread by direct contact and indirect contact with infective nasal secretions, aborted fetuses, placentae. Recovered carrier animals may also play role in spreading of virus.

Pathogenesis:

- The virus enters by inhalation of aerosols.
- The site of predilection of the virus for vascular epithelium of nasal mucosa, lung, adrenal and thyroid.
- *Leukocyte associated viremia* may occur and in some cases, neurological disease due to involvement of CNS.
- Virus can pass through the placental barrier of pregnant animals and give rise to abortion
- Mares, infected at the end of the gestation may not abort but give birth to the live foal with extreme pulmonary hepatic lymphoreticular and adrenal damage.

Isolation:

- Virus can be isolated on equine kidney and equine dermal cell lines.

Diagnosis:

- Clinical symptoms, history of abortion – provisional diagnose may be conformed by lab diagnosis. The symptoms may be confused with other viral disease like *equine influenza* and *equine viral arteritis*.
- Materials of choice for lab diagnosis are nasopharyngeal swabs, aborted fetus liver, lung.
- Serological tests: ELISA, FAT, VNT

Prevention and Control:

- Live attenuated and inactivated vaccines are available commercially.
- Inactivated one is recommended to prevent abortion in pregnant mares.
- Foal should be vaccinated at 3-4 month of age with a booster at 2-3 month later.
- Adaptation of sound management and isolation of affected animals are important.

EQUINE HERPES VIRUS 4

The virus causes a mild form of disease "*rhinopneumonitis*" and virus remain latent in trigeminal ganglion. Abortion may occur.

EQUINE HERPES VIRUS 3

Associated disease:

- The virus is associated with "*equine coitus exanthema*".
- It also causes a benign vulvo-vaginitis – but secondary bacterial infections are common.

Transmission:

- The virus is transmitted by *Coitus* and *Flies* feeding on infected vaginal secretions.

Pathogenesis:

- The infection may be subclinical, no fever etc.
- Without complications, the course of virus is less than 2 weeks.
- Ulcers heal within 2-3 weeks leaving white spots of unpigmented skin that persist for whole life.

Diagnosis:

- Virus can be diagnosed through isolation and identification of virus.

Prevention:

- No vaccine is available.
- Affected horses should be isolated
- Ulcers heal without treatment
- Mating must be stopped until ulcers heal up completely.

CANINE HERPES VIRUS

Virus is associated with generalized infection is canines; which may prove fetal in young dogs but older animals may suffered from *Kennel cough* infection.

FELINE HERPES VIRUS

The virus is associated with a disease, "*viral rhinotracheitis*" in felines (cats).

SUID HERPES VIRUS 1

(Pseudo-rabies Virus)

Associated disease:

- Virus causes pseudorabies disease in pigs which is also named as "*Aujeszky's disease*".
- The name pseudorabies was given due to its similarity in clinical signs with rabies.

Host Susceptibility:

- Pigs are considered to be the primary hosts and they may act as reservoir.
- Other species such as cattle, sheep, goat, dogs, and cats are susceptible.
- Cattle are considered as the dead end host.

Transmission:

- The virus spreads mainly by direct contact with infected pigs and ingestion.

Pathogenesis:

- The natural entry of the virus is via skin injury and also via respiratory route due to inhalation of aerosols. Infection occurs by oral route mostly in carnivores.
- After entry, there is viremia and localization of virus occurs in various organs.
- Virus multiplies initially in the tonsil, pharynx, trachea and esophagus.
- Entry of virus by the skin injury results in the involvement of peripheral nerve where the virus multiplies and passes centripetally to the CNS.
- Multiplication of virus occurs in nerve ganglia, medulla and pons → staggering gait and meningoencephalitis.
- The virus can cross the placental barrier and cause transplacental infection → abortion.

Cultivation:

- Embryonated egg: via CAM → Pocks after 3-4 months.

Diagnosis:

- The virus specific antibodies are detectable at 7 to 10 days after infection.
- Demonstration of virus specific abs in serum samples by passive HA, ELISA, & VNT.

Vaccines:

- Avirulent vaccines and inactivated egg adapted vaccines are available.

3. ILTOVIRUS

GALLID HERPES VIRUS 1

(Infectious Laryngotracheitis Virus)

About Virus:

- *Infectious laryngotracheitis virus* (Gallid herpesvirus 1) of the genus *Iltovirus* and subfamily *Alphaherpesvirinae*.

General Properties:

- Morphologically the virus is similar to the other members of the family.
- Antigenically the virus is homogenous.

Host Susceptibility:

- Fowls are the natural hosts. Chickens of all ages are susceptible, but pheasant, ducks and turkeys may be infected experimentally.

Transmission:

- Spread of virus occurs by direct contact with infected birds and indirect contact with contaminated feed and water. The virus is not transmitted within eggs from infected chickens. Wild birds can spread virus mechanically.

Pathogenesis:

- The virus enters commonly by inhalation of aerosols and occasionally via ingestion.
- After entry, the virus localizes in the upper respiratory tract and multiplication in trachea and larynx causes inflammation followed by hemorrhages, ulceration and necrosis.
- Latent virus may seen in trigeminal ganglion. No viremia occurred.
- Locally there is thickening of mucosa, hemorrhages, congestion and heart failure may be attributed. Morbidity – 100% and Mortality 20-60%.

Diagnosis:

- Field diagnosis can be made on the basis of clinical symptoms and lesions.
- Histologically: demonstration of I/N inclusions
- Immunologically: FAT, AGPT, ELISA
- Isolation: Embryonated eggs → Pock lesion on CAM

Prevention and Control:

- Vaccination is effective method to prevent the disease in endemic areas.
- Live virus vaccines are available and can be given eye drop instillation, in drinking water.
- Complete depopulation and disinfection of infected premises and introduction of healthy stock after about a month are the ideal methods to eliminate disease.

4. MARDIVIRUS

GALLID HERPES VIRUS 2

(Marek's Disease Virus)

About Virus:

- *Marek's disease virus* (Gallid herpesvirus 2) belongs to the genus *Mardivirus* of the subfamily *Alphaherpesvirinae*.
- The Marek's disease virus is of two types as follows;
  - i) *Type* 1 MD virus – Oncogenic and Pathogenic in chickens
  - ii) *Type* 2 MD virus – Non-oncogenic and Non-pathogenic in chickens.

General Properties:

- Morphological features of the virus are similar to the other family members.
- All strains of Marek's disease virus are antigenically identical but the virus show cross reaction with other *herpes* virus such as *pseudorabies* virus and *IBR* virus.

Host Susceptibility:

- Chicken – natural host. Avian species are susceptible only. Birds of 2-5 months age are usually affected.
- Pheasants, duck, swans, goose and pigeons are susceptible experimentally.

Transmission:

- High concentration of virus present in the feather follicle of infected birds and released in dander from the feather follicle.
- The dust and litters from poultry houses remain infectious for 6 weeks or longer.
- Virus may present in nasal, tracheal secretions and in feces of infected birds.
- There is no vertical transmission.

Clinical Symptoms:

- The clinical symptoms of the disease can be described in three forms;
  - i) *Neural* (neurolymphomatosis)
  - ii) *Visceral* (acute Marek's disease)
  - iii) *Ocular* (ocular lymphomatosis)

Pathogenesis:

- Entry of virus occurs by the respiratory route and occasionally by ingestion.

- Disease development is influenced by; virus strain, dose and route of infection, age, sex and immune status and genetic susceptibility of the host.
- In susceptible birds, virus first multiplies in the epithelial cells of resp. tract. → cell associated viremia occurs involving macrophages.
- Then, lymphoid cells of thymus, bursa of fabricious, bone marrow, and spleen cause damage of the lymphoid cells and immunosuppression.
- Lympho proliferative condition affects the peripheral nerves such as sciatic and brachial nerves.
- The virus mostly transformed T-lymphocytes (Visible tumor) and viral DNA can be demonstrated in the transformed cells.
- Subclinical Infection: is a common feature and subclinically infected birds remains as life long carriers and shedders of the virus.

Immune Response

- The virus exerts immuno-suppressive effect on the host.
- Both HI (Humoral immunity) and CMI (Cell mediated immunity) make a complex for initiating a cascade of immunological consequences in response to viral infection.

Diagnosis:

- The clinical symptoms and lesions are indicative of the disease.
- For laboratory conformation clinical samples from buffy coat, feather follicle and pieces of visceral organs like spleen, liver and gonads showing lesions are to be collected.
- **MASTA test**: Masta stands for, Marek's disease Associated Surface Tumor Antigen
  It is one of the sensitive test for diagnosis of disease includes detection of MAST by FAT.
- The virus antigen can also be detected in the feather follicles of affected birds by AGPT and ELISA.

Isolation/Cultivation:

- *Cell Culture*: Chicken kidney cell (CKC) culture, Duck embryo fibroblasts (DEF) and chicken embryo fibroblasts (CEF). → Virus produced CPE and I/N inclusion bodies.
- *Embryonated Egg*: via CAM → production of pock lesions at 7 days and by yolk sac route pock lesions at 14 days of post infection.

Prevention and Control:

- The effective way to prevent Marek's disease is by vaccination.
- The *herpesvirus* of turkey (HVT) which shows about 95% homology in DNA-DNA hybridization is commonly used as vaccine for Marek's disease – given to 1 day old chick by I/M or S/C.
- Bivalent (Gallid HV 3 and HVT) vaccine is recommended for control of very virulent strain of Marek's disease virus.
- Condemn birds with tumor or skin lesions should be slaughtered.
- Adopt hygienic measures and Breeding for disease resistant flock is an approach for control of the disease.