INTRODUCTION

This class is a revision of General Virology. Details like structure, classification, repliation and viral interference will be covered.

MODULE-2: PARAMIXOVIRUSES - NEWCASTLE DISEASE

Learning objectives

Introduction about

- Group V viruses – Negative sense single stranded RNA viruses
- Paramyxoviruses
    - Classification
    - Important diseases – Newcastle disease, rinderpest, PPR, canine distemper, phocine distemper
- Avulavirus
    - Newcastle disease virus – NDV, APMV-1
    - About the virus
    - About the disease
    - About the diagnosis
    - About the vaccines
    - About the control

NEWCASTLE DISEASE
INTRODUCTION

- Newcastle disease (ND) is caused by avian paramyxovirus type I (APMV-1). The infection is considered as one of the very severe viral diseases of birds. The disease affects almost all poultry.
- The infection causes severe economic losses to farmers. The infection is also endemic in most of the countries in the world.

NEWCASTLE DISEASE
GENERAL ASPECTS

Deals about

- Classification,
- Morphology,
- Physicochemical properties,
- Strains etc.

NEWCASTLE DISEASE
CLASSIFICATION

- Order - Mononegavirales

- Family – Paramyxoviridae
- Subfamily - Parmyxovirinae
- Genus - Avula virus
- Species - Avian Paramyxovirus 1 (APMV-1)

- There are nine different species under the genus Avula virus and they have been numbered 1-9 indicated as APMV-1 to APMV-9. Strains of APMV-1 causes Newcastle disease. APMV-1 cross reacts with APMV-2 and APMV-3.

NEWCASTLE DISEASE
MORPHOLOGY

- APMV-1 are single stranded, non-segmented and negative sense RNA viruses. They are enveloped viruses and are ether sensitive. They possess helical symmetry. The surface has number of projections (HN Protein). They measure 150-300 nm.
- The virus contains six genes encoding six major structural proteins:
  - Nucleoprotein (NP),
  - Phosphoprotein (P),
  - Matrix protein (M),
  - Fusion protein (F),
  - Hemagglutinin-neuraminidase (HN) and
  - RNA-dependent RNA polymerase (L).

- Apart from the six proteins APMV-1 also contains two proteins V and W.
  The RNA together with NP, P, and L proteins forms the ribonucleoprotein complex (RNP), which serves as a template for RNA synthesis. The hemagglutinin/neuraminidase (HN) is important for the attachment and release of the virus from the host cells in addition to its serologic identification.
- The other very important surface protein is the fusion (F) protein, which has a critical role in the pathogenesis of the disease.

NEW CASTLE DISEASE
SYNONYMS

- Pseudo fowlpest, Ranikhet disease, Vellaikalichal (vernacular)

NEWCASTLE DISEASE
STRAINS AND SEROTYPE

Strains and serotype

- Though there are many strains all strains are immunologically same. Some of the important virus strains are as follows:

Pathotype	Strains
Velogenic	Hertz 33, Texas GB
Mesogenic	K, R2B
Lentogenic	F, LaSota, Ulster 2C
Avirulent	V4

NEWCASTLE DISEASE
OIE LISTING AND RISK GROUP

- OIE Listing: List A infection (ND and highly pathogenic avian influenza are the only two poultry diseases specified as List A.
- Risk group(Animal Pathogen): Group II

NEWCASTLE DISEASE
HA PROPERTY

- APMV-1 causes agglutination of chicken and human O erythrocytes. The agglutination reaction is not permanent and after 30 minutes as a result of loss of receptor the agglutination is lost and this phenomenon is referred as elution.

NEWCASTLE DISEASE
CULTIVATION

- APMV-1 can be readily cultivated in embryonated eggs. The preferred route is allantoic cavity route. 9-11 days old embryos are preferred for cultivation.
- Virulent strains cause death of embryos in 24 hours with haemorrhages through out the body particularly in occipital region. Lentogenic strains do not cause death.
- Presence of virus is usually confirmed by HA and HI test using specific serum. The virus also grows in primary cells like Chicken embryo fibroblast and pig kidney cells. The virus also grows in BHK21 cell line.

NEWCASTLE DISEASE
PATHOGENESIS

- Dealt as hosts affected, distribution, transmission, symptoms and lesions.

NEWCASTLE DISEASE
HOST AFFECTED

- ND has been reported at least 241 species of birds representing 27 of the 50 Orders of the class. Almost all birds are susceptible to infection. ND viruses have also been reported to infect animals other than birds, ranging from reptiles to man.
- The pathogenesis and production of clinical symptoms depend on the virus, the host species, age of host, infection with other organisms, environmental stress and immune status.

NEWCASTLE DISEASE
PATHOTYPES

- Infection with the extremely virulent viruses may result in sudden, high mortality with comparatively few clinical signs. The virus is grouped into five "pathotypes" on the basis of the predominant signs in affected chickens. They are as follows;
    - *Viscerotropic velogenic (Doyles form):* viruses responsible for disease characterised by acute lethal infections, usually with haemorrhagic lesions in the intestines of dead birds. The infection caused by this pathotype is also called as Asiatic or exotic ND.
    - *Neurotropic velogenic (Beach's form):* viruses causing disease characterised by high mortality, which follows respiratory and neurological disease, but where gut lesions are usually absent.
    - *Mesogenic (Beaudettes form):* viruses causing clinical signs consisting of respiratory and neurological signs, with low mortality.
    - *Lentogenic (Hitchner's form):* viruses causing mild infections of the respiratory tract.
    - *Asymptomatic enteric:* viruses causing avirulent infections in which replication appears to be primarily in the gut.
- Some workers differentiate NDV into three pathotypes:
    - Lentogenic,
    - Mesogenic, and
    - Velogenic, reflecting increasing levels of virulence. The most virulent (velogenic) isolates are further subdivided into neurotropic and viscerotropic types.

NEWCASTLE DISEASE
TRANSMISSION

- The infection is transmitted via aerosols, direct contact with infected birds, fomites, contaminated meat and water, through visitors and imported psittacines. Village chicken and birds like crows and sparrow can also spread the infection to chickens.
- The infection is not usually vertical but chicks may become infected in hatcheries from contaminated shells.

NEWCASTLE DISEASE
SYMPTOMS

- Signs are predominantly seen in nervous, respiratory or reproductive systems. Morbidity is usually high and mortality varies from 0–100%. Higher mortality is seen in velogenic disease in unvaccinated stock.

- The clinical signs are highly variable and depend on the nature of the infecting virus (pathotype), the infective dose and the degree of immunity birds possess from previous infection or vaccination.
- The first clinical sign in laying chickens is usually a marked drop in egg production followed within 24 to 43 hours by high death losses. At the onset, 10-15 percent of a flock may be lost in 24 hours. After 7 to 10 days, deaths usually subside, and birds surviving 12 to 14 days generally do not die but may display permanent paralysis and other neurologic signs.
- The reproductive system may be permanently impaired, and thus egg production does not return to previous levels. In vaccinated chickens, or chicks protected by parental antibodies, the clinical signs are less severe and are proportional to the level of protective antibodies.
- With viscerotropic strains (VVND), edema of the head, especially around the eyes may become apparent after birds have been sick for 2 or 3 days. This edema usually does not involve the comb and wattle as in case of highly pathogenic avian influenza (HPAI).
- A dark ring sometimes forms around the eye, probably due to cyanosis and poor blood circulation in the edematous tissue. This "black eye" appearance is especially visible in white chickens. Bile-stained, greenish-dark diarrhea may be noted 2 to 3 days after onset of illness. Some birds in an affected flock usually have diarrhea throughout the course of the disease.
- The most noteworthy clinical sign in unvaccinated flocks is sudden death without prior indications of illness. Neurotropic strains cause respiratory signs followed by neurologic signs, including muscular tremors, paralysis of legs or wings, torticollis, and opisthotonos.
- The peracute onset often causes the owner to suspect poisoning. Fall in egg production leading to complete cessation of egg laying and deaths.

NEWCASTLE DISEASE
LESIONS

- As with clinical signs, no gross or microscopic lesions can be considered pathognomonic for any form of ND. Carcasses of birds dying as a result of virulent ND usually have a fevered, dehydrated appearance. Gross lesions vary with the infecting virus.
- Virulent ND viruses cause haemorrhagic lesions of the intestinal tract. These are most easily seen if the intestine is opened and may vary considerably in size.
- Haemorrhages are seen in the proventriculus, duodenum, jejunum and ileum. Haemorrhages and necrotic lesions in proventriculus and ileo-caecal junction are considered as specific for ND.
- Even in birds showing neurological signs prior to death, minimal gross lesions are seen in the central nervous system. Changes in the central nervous system are nonpurulent encephalomyelitis.
- Lesions are usually present in the respiratory tract when respiratory signs are observed. These generally appear as haemorrhagic lesions and congestion in lungs, tracheitis and airsacculitis. Egg peritonitis is seen in laying hens infected with virulent NDV.

NEWCASTLE DISEASE
DIAGNOSIS

- Dealt as
    - Field and

- Laboratory diagnosis.

NEWCASTLE DISEASE
FIELD DIAGNOSIS

- Tentative diagnosis of ND made on the basis of history, clinical signs, and gross lesions.

NEWCASTLE DISEASE
LABORATORY DIAGNOSIS

Virus isolation and identification

- *Clinical materials:* Samples from dead birds should consist of oro-nasal swabs, as well as samples collected from lung, kidneys, intestine (including contents), spleen, brain, liver and heart tissues. These may be collected separately or as a pool.
- *Virus isolation:* virus isolation is attempted by inoculating 9- to 11-day-old embryonating chicken eggs. Amnioallantoic fluid (AAF) is collected from all embryos dying after 24 hours postinoculation and tested for hemagglutination (HA) activity. If positive, the hemagglutination-inhibition (Hl) test is used with known NDV-positive serum to confirm the presence of NDV in the AAF. If NDV is found, it is characterized by inoculating 4- to 6-week-old chickens free of ND antibodies with the suspect AAF by swabbing the cloaca, instilling into the nares or conjuctival sac, or injecting into the thoracic air sac. If VVND virus is present, the inoculated chicks usually die in 3 to 7 days, revealing typical visceral lesions on postmortem examination. Neurotrophic viruses will cause severe neurologic and respiratory signs in inoculated chickens but no visceral lesions. If no bird dies in 10 days, the APMV-1 is not considered to be the velogenic, viscerotropic type but is either a lentogenic or mesogenic.
- *Nucleic acid identification methods:* The virus can also be identified from clinical materials and AAF by RT-PCR (Reverse transcriptase Polymerase chain reaction) using primers for F gene.
- Nucleic acid probes
- *ELISA techniques:* Antigen capture and Antigen competitive ELISA techniques can also be used to identify APMV-1 from clinical materials and AAF.
- Microscopic lesions are not considered to have any diagnostic significance. In most tissues and organs where changes occur, they consist of hyperaemia, necrosis, cellular infiltration and oedema.

NEWCASTLE DISEASE
PATHOTYPING

- Newcastle disease virus are classified into Velogenic, mesogenic and lentogenic based on following three tests:
  - Intracerebral pathogenicity index (ICPI) in day old chickens. This involves the inoculation of virus derived from fresh infective allantoic fluid into the brain of ten day-old chicks from specific pathogen-free parents. Each bird is examined at 24-hour intervals for eight days and graded zero if normal, one if sick and two if

dead. The index is the mean score per bird per observation over the 8-day period. The most virulent viruses give ICPI values approaching the maximum score of 2.0, while lentogenic viruses give values of, or close to, 0.0

- Intravenous pathogenicty index (IVPI) in 6 weeks old specific pathogen free (SPF) chickens. 0.1 ml of the diluted virus is injected intravenously into each of ten 6-week-old SPF chickens. Birds are examined at 24-hour intervals for 10 days and scored at each observation - 0 if normal, 1 if sick, 2 if paralysed or showing other nervous signs, and 3 if dead. The intravenous pathogenicity index (IVPI) is the mean score per bird per observation over the 10-day period. Lentogenic strains and some mesogenic strains will have IVPI values of 0, whereas the indices for virulent strains will approach 3.0.
- Mean death time (MDT) in embryonated eggs. The mean death time (MDT) in eggs means time in hours for the minimum lethal dose to kill all the inoculated embryos. The MDT has been used to classify ND virus strains into velogenic (taking under 60 hours to kill); mesogenic (taking 60 to 90 hours to kill); and lentogenic (taking more than 90 hours to kill).
- Molecular typing by studying the aminoacid between 112-119th position in F gene.
- Panels of MAbs are also used to establish antigenic profiles of ND virus isolates based on whether or not they react with the viruses.

NEWCASTLE DISEASE
SEROLOGY

- A number of tests are performed to identify antibodies against NDV in chickens. The two common tests are
  - Haemagglutination inhibition (HI) test and
  - ELISA.
- HI test is preformed in most of the laboratories. The HI test is based on the principle that the haemagglutinin on the viral envelope can bring about the agglutination of chicken red blood cells and that this can be inhibited by specific antibodies. A titre of $log2^3$ is indicative of protection and a titre of $log2^6$ or more suggests a recent infection by the virus.
- The ELISA works on the principle of recognition of anti-NDV antibodies, attached to a plate coated with viral antigen, by antibodies produced in another species against chicken antibodies. This anti-chicken antibody is conjugated to an enzyme that catalyses a reaction, causing a change of colour which can then be read quantitatively on a photo spectrometer designed to read microtitration plates

Monoclonal antibody based HI

- Mouse monoclonal antibodies (MAbs) directed against strains of ND virus have been used in HI tests to allow rapid identification of ND virus without the possible cross-reactions with other APMV serotypes that may occur with polyclonal sera. MAbs have been produced that give reactions in HI tests that are specific for particular strains or variant ND virus isolates.

NEWCASTLE DISEASE
CONTROL

- Dealt as
  - Vaccination and
  - Eradication.

NEWCASTLE DISEASE
VACCINATION

- There are three types of vaccines used for ND:
  - Live lentogenic,
  - Live mesogenic and
  - Inactivated vaccines.
- Live lentogenic vaccines are usually derived from field viruses that have been shown to have low pathogenicity for poultry but produce an adequate immune response. Typical vaccine strains are HB1, La Sota, F, V4 or Ulster 2C viruses. These vaccines are given to young chicks through eyes are nostrils. They are very safe and only La Sota will produce mild post-vaccinal reaction in certain flocks. This type immunization is also called as priming.
- Mesogenic strains are used only in areas where ND is epidemic and in village chickens. Normally mesogenic vaccines, such as Komarov and Mukteswar are used as secondary vaccines after a primary vaccination with a lentogenic vaccine.
- Inactivated vaccines are produced by growing a ND virus in eggs, and then treating the infective allantoic fluid with an inactivating agent, such as formalin or betapropiolactone. An adjuvant, such as mineral oil, is usually then added to make the inactivated virus more immunogenic. Inactivated vaccines are usually applied after an initial priming vaccination with a live vaccine.
- Thermostable live vaccines: Some asymptomatic enteric viruses have greater heat resistance than more conventional lentogenic viruses. This property has been enhanced by selection and cloning in the laboratory to produce heat tolerant vaccines. These have a distinct advantage in the village situation because it is possible to transport the vaccine without a cold chain.
- Recombinant vaccine: APMV-1 has two surface glycoproteins, fusion [F] and haemagglutinin/neuraminidase [HN]. The genes coding for either of these can be inserted into a different virus like herpes virus of turkeys to produce a vaccine, which gave good protection against virulent NDV.
- Marker vaccine: Recently an immunodominat epitope of NP gene has been identified and replaced with mouse herpes virus gene. The resultant vaccine helps to differentiates between vaccinated and infected birds.

Vaccine application methods

- Mass administration through water or aerosol spray
- Feed based techniques
- As eye and/or nasal drops
- As injection

NEWCASTLE DISEASE
ERADICATION

- Biosecurity is the best method to prevent the occurrence of infection. Farms and flocks should be well separated, hatcheries should be isolated from poultry farms, different species should be reared on different sites, and there should be an adequate fresh water supply, preferably one that does not draw on surface water.

On the farms the following points should be observed

- Regular vaccination and periodical antibody monitoring
- Houses, food stores and water tanks should be bird-proofed.
- Movements on and off the farm should be kept to a minimum.
- All equipment, especially vehicles, should be disinfected before access to the site is permitted.
- Movements between different farms for egg collection, carcass collection, food delivery and the like should be confined to a specified collection and delivery point away from the poultry flocks.
- Visits by personnel such as bleeding or vaccination teams, inseminators and veterinarians are the most likely method of introduction of ND and if such visits are unavoidable, regimens of clothing change, equipment disinfection and other basic hygiene controls must be enforced.

NEWCASTLE DISEASE
PUBLIC HEALTH ASPECT

- People may become infected with APMV-1, the resulting disease is typically limited to a conjunctivitis. Recovery is usually rapid, and the virus is no longer present in eye fluids after 4 to 7 days.
- Infections have occurred mostly in laboratory workers and vaccinating crews with rare cases in poultry handlers. No instance of transmission to humans through handling or consuming of poultry products is known.
- Individuals with conjunctivitis from APMV-1 virus should not enter poultry premises or come in contact with live avian species.

MODULE-3: PARAMYXOVIRUSES - RINDERPEST

Learning objectives

- Group V viruses – Negative sense single stranded RNA viruses
- Morbilli virus
    - Rinderpest virus – RPV
    - About the virus
    - About the disease
    - About the diagnosis
    - About the vaccines
    - About the control.

RINDER PEST
INTRODUCTION

- Rinderpest is an acute, viral disease of domestic cattle, buffaloes and yaks characterised by high morbidity and mortality rates. Sheep, goats, pigs and wild ungulates may also be affected.
- It is characterized by fever, oral erosions, Rinderpest is an acute, viral disease of domestic cattle, buffaloes and yaks characterised by high morbidity and mortality rates. Sheep, goats, pigs and wild ungulates may also be affected.
- It is characterized by fever, oral erosions, diarrhea, lymphoid necrosis, and high mortality.diarrhea, lymphoid necrosis, and high mortality.

RINDER PEST
GENERAL ASPECTS

- Deals about
  - Classification,
  - Morphology,
  - Physicochemical properties,
  - Replication,
  - Strains etc.

RINDER PEST
CLASSIFICATION

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Paramyxoviridae
Subfamily	Parmyxovirinae
Genus	Morbilli virus
Species	Rinderpest virus

- Rinderpest virus shares similarities in structure and antigenicity with measles and canine distemper viruses. These three viruses together form the MEDIPEST group.

RINDER PEST
MORPHOLOGY

- Rinderpest virus is pleomorphic. It is single stranded, negative sense RNA viruses. They are enveloped viruses and are ether sensitive. They possess helical symmetry.
- Virus measures approximately 100 to 300 nm in diameter. The envelopes are covered with minute projections, which are the surface glycoproteins (H and F proteins) responsible for cell attachment and fusion.
- Rinderpest virus is differentiated from PPR virus (PPR is a very important infection resembling rinderpest in small ruminants) through nucleic acid probes for N gene.

- In total The virus has 6 important proteins and two non-structural proteins. They are
  - N - nucleocapsid protein which covers and protects the virion RNA
  - P- polymerase-associated or phosphoprotein
  - M- matrix protein, interacts with cytoplasmically located nucleocapsids and membrane associated glycoproteins in cell envelope to produce virus bud
  - F - fusion protein enables virus and host cell membrane to fuse
  - H - haemagglutinin or attachment protein, enables virus to attach to host cell membrane
  - L - large protein, acts as a virus transcriptase and replicase in association with P protein
  - C/V - two non-structural virus-encoded proteins produced in infected cells, may have function in virus reproduction
- The H and F proteins are very important in establishing the infection. H is responsible for attachment and F is responsible for entry into the cell.

RINDER PEST
SYNONYMS

- Cattle plague

RINDER PEST
OIE LISTING AND RISK GROUP

- *OIE Listing:* List A infection
- *Risk group:* Class II Animal Pathogen

RINDER PEST
RESISTANCE

- Rinderpest virus is not a stable virus. The virus is readily destroyed at relative humidity between 50 and 60 percent. It is sensitive to heat, light and ultrasonic waves.
- High and low p^H also denature the virus. Being enveloped, rinderpest virus is destroyed by lipid solvents and lipophilic disinfectants. In dung material 5 percent sodium hydroxide and 50 percent lysol destroy the virus effectively.
- Molar concentration of magnesium sulphate heptahydrate (25g in 100ml) will slow down inactivation of the virus in water.

RINDER PEST
STRAINS AND SEROTYPES

- There is only one serotype of rinderpest virus, but field strains vary widely in virulence, ease of transmission and host affinity.
- Some of the important strains of virus are Nakumara and Kabate O.

RINDER PEST
CULTIVATION

- Rinderpest virus is readily cultivable in cell culture. Some of the common cell cultures used for cultivation of rinderpest virus are calf kidney cells, African green monkey cells (Vero cells), lymphoblastoid cell line, B95a. Specific cytopathic effects (CPE) develop after three days.
- The CPE are in the form of small foci of rounded retractile cells with long cytoplasmic processes, stellate cells and small multinucleate syncytia with irregular shapes.
- With further incubation, the CPE generalizes to involve large areas of the cell sheet and, by the ninth or tenth day after inoculation cells peal off from the surface. Rarely the syncytia may become highly vacuolated.
- The laboratory animal of choice for virus growth is rabbits. Rinderpest virus also grows in the chorioallantoic membrane of embryonated eggs, but no lesions are produced.

RINDER PEST
PATHOGENESIS

- Dealt as hosts affected, distribution, transmission, symptoms, lesion etc.

RINDER PEST
HOST AFFECTED

- The common host is the domestic ungulates cattle and buffalo. Sheep, goat and pigs are the other important domestic animals affected by rinderpest. The infection has also been reported in a variety of wild animals including bushbuck, bush pig, giraffe, gaur, nilgai and sambar.

RINDER PEST
TRANSMISSION

- The secretions and excretions from infected animals, particularly nasal-ocular discharges and faeces, 1 to 2 days before clinical signs to 8 to 9 days after onset of clinical signs contain large quantities of virus.
- Rinderpest virus spreads from healthy to susceptible animals mostly through this infected droplets, either in the breath of a sick animal or in its virus-rich secretions or excretions.
- The important source of infection for a new herd is arrival of live animal from infected areas. Since the droplets are large and short-lived, the contact between sick and healthy animals must be close for transmission to occur.
- Transmission also occurs through indirect methods like contact with contaminated bedding, fodder or water. In pigs infection also occurs through eating uncooked infected meat scraps.
- There is no vertical transmission. Further the infection is also not transmitted by arthropod vectors. There is no carrier state of infection also.

RINDER PEST
INCUBATION PERIOD

- The incubation period varies with the strain of virus, dosage and route of exposure. Following natural exposure, the incubation period ranges from 3 to 15 days but it is usually 4 to 5 days.

RINDER PEST
SYMPTOMS

Buffaloes and cattle

- Clinical reactions in buffalo and cattle are similar and the infection may be exhibited as
  - Peracute,
  - Acute,
  - Subacute or even inapparent.

RINDER PEST
PERACUTE INFECTION

- The onset of a peracute reaction is sudden and unexpected. It is manifested by inappetence, high fever, depression, deep congestion of visible mucous membranes, severe panting and racing pulse.
- Death occurs within two or three days, even before mucosal erosions develop. The peracute reactions are not common and occur most frequently in young calves and exotic animals.

RINDER PEST
ACUTE INFECTION

- This form is divided into five phases
  - An incubation period,
  - A prodromal fever,
  - An erosive-mucosa phase,
  - A diarrhoeic phase and
  - A convalescent phase in surviving animals.

RINDER PEST
PRODROMAL FEVER

- The onset of the prodromal fever is sudden, it is frequently missed because other clinical signs are minimal, except in lactating cows where there is drop in milk yield.
- Typical illness is clearly evident 24 to 48 hours later, when the animal becomes restless and then stands depressed, apart and alone. Respirations are shallow and rapid.
- The coat hairs stand erect, the muzzle dries, tears are wept and the nose runs. Appetite is impaired, rumination is retarded and defecation stops. Visible mucous membranes are congested but intact.

RINDER PEST

EROSIVE MUCOSAL PHASE

- The erosive mucosl phase is observed two to three days after prodormal fever and is characterized by raised pinheads of necrotic epithelium emerging from the surfaces of the mucous membranes lining the mouth, nasal passages and urogenital tracts. These lesions erode exposing a haemorrhagic layer. At this stage salivation is profuse.
- The erosions enlarge and coalesce. Thick yellow patches of necrotic cells begin to coat the nasal passages and mix with the nasal secretions, producing a fetid mucopurulent discharge.
- Secretions from eye also become mucopurulent. This stage is also characterized by intense thirst, poor appetite and voiding of soft faeces.

RINDER PEST
DIARRHOEIC PHASE

- The diarrhoeic phase begins after the fever subsides The dark, fluid faeces contain excess mucus and shreds of epithelium and necrotic debris streaked with blood. The smell is fetid and offensive.
- Affected animals arch their backs and strain frequently, exposing congested and eroded rectal mucosae. The diarrhoea is also referred as shooting diarrhoea. Respirations are laboured and painful, characterized by an audible grunt when exhaling.
- In fatal cases of rinderpest the diarrhoea worsens progressively, causing rapid dehydration. Affected animals waste visibly, have sunken eyes and stand with lowered heads and arched backs. Most animals collapse and die six to 12 days after the onset of the prodromal fever.
- In surviving cases, the diarrhoea stops within a week of its onset. Pregnant animalsl abort during convalescence.

RINDER PEST
CONVALESCENT PHASE

- Most animals collapse and die six to 12 days after the onset of the prodromal fever. In surviving cases, the diarrhoea stops within a week of its onset. Pregnant animalsl abort during convalescence.

RINDER PEST
SUBACUTE INFECTION

- Subacute infections are seen in immature and young adult animals. The incubation period is longer than that of the acute syndrome and may even last 15 days.
- The clinical signs are mild and often one or more of the cardinal features of the classic disease, such as fever, mucosal erosions, mucopurulent nasal and ocular discharges or diarrhoea, are absent. Most affected animals survive the infection.
- In cattle the rinderpest virus selectively destroys T- and B-lymphocytes. Because of this the latent pathogens are commonly activated and supra-infection occurs.

RINDER PEST

SHEEP AND GOAT

- Acute, subacute and inapparent rinderpest reactions occur in goats and sheep. Many of the clinical signs resemble those seen in cattle, but the course of the disease is shorter and pneumonic symptoms are more prominent.
- Acute cases die six to seven days after the onset of illness, whereas survivors show signs of recovery within two weeks.

RINDER PEST
PIGS

- Peracute reactions are characterized by sudden sharp fevers and death before other premonitory signs develop. Acute cases have a similar sudden onset, manifested by fever, inappetence and depression.
- Two days after the onset of fever, affected pigs shiver, vomit and bleeding is seen from the nose. Shallow erosions emerge in the oral mucosa while vesicles erupt in the perineal skin. Diarrhoea follows erosions in oral mucosa. The fluid faeces is fetid and heavily streaked with blood. Pregnant sows abort.

RINDER PEST
LESIONS

- Rinderpest virus has affinity for lymphoid tissues and a secondary affinity for the epithelium of the alimentary, upper respiratory and urogenital tracts.
    - Oral lesions start as small grey foci that may coalesce. The grey (necrotic) epithelium then sloughs off and leaves a red erosion. At mouth lesions occur on the gums, lips, hard and soft palate, cheeks, and base of the tongue. The early lesions are grey, necrotic, pinhead-sized areas that later coalesce and erode and leave red areas.
    - Intestinal lesions are characterized by necrosis or erosion of Peyer's patches in the jejunum, necrosis or erosions over the lymphoid area in the ileum. Lesions are more severe in the upper colon (edema of the wall, erosions in the mucosa, and congestion). Further down the colon, the colonic ridges may be congested; this is referred to as "tiger striping". Severity of intestinal lesions varies between isolates.
    - Lymph nodes are swollen and edematous.
    - Lung lesions are emphysema, congestion, and areas of pneumonia.

RINDER PEST
DIAGNOSIS

- Dealt as
    - Field and
    - Laboratory diagnosis.

RINDER PEST
FIELD DIAGNOSIS

- Based on the symptoms and lesion.

RINDER PEST
LABORATORY DIAGNOSIS

- *Specimens to be collected:* specimens should preferably be collected from animals with a high fever and oral lesions. The following samples should be collected from live animals
- *Live animals*
  - Blood in EDTA or heparin
  - Blood for serum
  - Swabs containing lacrimal fluid
  - Tears
  - Necrotic tissue from the oral cavity
  - Aspiration biopsies of superficial lymph nodes
- *Dead animals:* Sections of
  - Spleen
  - Lymph nodes
  - Tonsil
- Isolation and identification of virus in calf kidney cells and vero cells using specimens specified above.
- *Detection of virus specific antigen by*
  - Agar gel immunodiffusion test
  - Counter immunoelectrophoresis
  - Passive haemagglutination
  - Immunofluorescense and immunoperoxidase techniques
  - Antigen capture ELISA
  - RT-PCR
  - DNA probes for N gene

RINDER PEST
DIFFERENTIAL DIAGNOSIS

- Rinderpest should be differentiated from
  - Bovine virus diarrhea (mucosal disease),
  - Infectious bovine rhinotracheitis,
  - Malignant catarrhal fever,
  - Foot-and-mouth disease,
  - Vesicular stomatitis,
  - Salmonellosis,
  - Paratuberculosis and
  - Arsenic poisoning.

RINDER PEST
TREATMENT

- There is no effective treatment against rinderpest.

RINDER PEST
CONTROL

- Dealt under
  - Vaccination and
  - Eradication

RINDER PEST
VACCINATION

- Since India has been declared provisionally free from rinderpest no vaccination is carried out in cattle.
- However two types of vaccines have been used previously.
  - Goat tissue adapted vaccine (GTV) and
  - Tissue culture adapted rinderpest virus vaccine (TCRV).
- Though GTV produces good immunity in native zebu cattle it caused severe post vaccinal reaction in cross bred animals. Hence, it was discontinued and TCRV was used.
- Since 1996-97, vaccination of cattle and sheep against rinderpest have been discontinued. Apart from GTV and TCRV, lapininsed (rabbit passaged), lapinised and avianised rinderpest virus have also been used as vaccine is many Asian countries.
- Vaccinia-vectored vaccine containing the F and H genes of RPV was also used experimentally.

RINDER PEST
ERADICATION

- Countries and areas free of RP should prohibit unrestricted movement of RP-susceptible animals and uncooked meat products from areas infected by RP or practicing RP vaccination.
- Since recovered animals are not carriers, serological techniques like ELISA can be effectively used to check the status of animals imported.
- Wild ruminants and swine imported should be properly quarantined and tested with serological tests.
- During an outbreak occurs, the area should be quarantined, infected and exposed animals slaughtered and buried or burned, and ring vaccination considered. (Ring vaccination is a technique, wherein the animals in surrounding areas of actual location of infection are vaccinated)
- High-risk countries (those trading with, or geographically close to, infected countries) should protect themselves by having all susceptible animals vaccinated before they enter the country or vaccinating the national herd, or both.
- Endemic countries should vaccinate their national herd. The schedule for vaccination is annual vaccination for at least 4 years, followed by annual vaccination of calves.
- Foci of infection should be quarantined and stamped out. Wildlife, sheep, and goats should be monitored serologically.
- RP vaccine should not be used to protect against peste des petits ruminants since it may interfere with serological monitoring of sheep and goats for rinderpest.

RINDER PEST

GREP

- The Global Rinderpest Eradication Programme (GREP) is a time-bound programme to eliminate rinderpest from the world by the year 2010. This programme is operated by the FAO. It forms part of EMPRES.
- Strategies have been devised and programmes implemented to reduce the clinical incidence of rinderpest to zero. Elimination of disease and infection will be confirmed by statistically valid active disease.

RINDER PEST
OIE PATHWAY

- The OIE has developed a set of Recommended Standards for Epidemiological Surveillance for Rinderpest (the 'OIE Pathway') that governs the actions of Member Countries wishing to demonstrate that they have achieved freedom from infection.
- To this end, both competition and indirect enzyme-linked immunosorbent assays are available that will determine the presence of rinderpest antibodies in animals that have been infected with field virus or with rinderpest vaccine.

RINDER PEST
PUBLIC HEALTH ASPECT

- There is no report of rinderpest infection in a human.

MODULE-4: PARAMYXOVIRUSES - PESTE DES PETITS RUMINANTS

Learning objectives

- Group V viruses – Negative sense single stranded RNA viruses
- Morbilli virus
    - Peste des petit ruminants – PPRV
    - About the virus
    - About the disease
    - About the diagnosis
    - About the vaccines
    - About the control.

PESTE DES PETITS RUMINANTS
INTRODUCTION

- Peste des petits ruminants (PPR), also known as goat plague, is caused by a paramyxovirus of the Morbillivirus genus. It was first described in 1942 in Cote d'Ivoire, West Africa and is closely related to rinderpest virus, canine distemper virus, and human measles virus.

- PPR is an acute or subacute viral disease of goats and sheep characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia. Goats are usually more severely affected than sheep (hence called goat plague).

PESTE DES PETITS RUMINANTS
GENERAL ASPECTS

- Deals about classification, morphology, physicochemical properties, strains etc.

PESTE DES PETITS RUMINANTS
CLASSIFICATION

-

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Paramyxoviridae
Subfamily	Paramyxovirinae
Genus	Morbilli virus
Species	PPR virus

PESTE DES PETITS
MORPHOLOGY

- PPR virus is pleomorphic. It is single stranded, negative sense RNA viruses. They are enveloped viruses and are ether sensitive. They possess helical symmetry.
- Virus measures approximately 150 nm in diameter. The envelopes are covered with minute projections, which are the surface glycoproteins (H and F proteins) responsible for cell attachment and fusion.
- The nucleocapsid is hollow, coiled, rod-shaped with herring-bone pattern of sub-units. PPR virus is differentiated from rinderpest virus (PPR is a very important infection resembling rinderpest in small ruminants) through nucleic acid probes for N gene. In total the virus has 6 six structural proteins:
    - Nucleocapsid protein - Np covers and protects the virion RNA.
    - Phosphoprotein P - polymerase-associated or phosphoprotein.
    - Large protein - L - acts as a virus transcriptase and replicase in association with P protein.
    - Matrix protein – M – acts as a link between nucleocapsid and virus external glycoprotein H.
    - Fusion – F - enables virus and host cell membrane to fuse.
    - Haemagglutinin H - haemagglutinin or attachment protein, enables virus to attach to host cell membrane.
- C / V - two non-structural virus-encoded proteins produced in infected cells. They have function in virus reproduction.
- The matrix protein, intimately associated with the internal face of the viral envelope, makes a link between the nucleocapsid and the virus external glycoproteins H and F,

which are responsible for the attachment and the penetration of the virus into the cell to be infected.

PESTE DES PETITS
OIE LISTING AND RISK GROUP

A infection
II Animal Pathogen

PESTE DES PETITS
STRAINS AND SEROTYPES

- PPR virus is closely related to rinderpest virus. But it different from rinderpest.
- Different PPRV strains are grouped into *four groups*, of which 3 groups are found in Africa and 1 at Asia. One African group is also prevalent in Asia.
- The Arasur strain of PPRV (referred as AR-87) is used as vaccine in Southern India. Other South Indian PPRV isolates are Coimbatore (CBE) and Poondi (92).

PESTE DES PETITS
RESISTANCE

- PPR virus is not a stable virus. PPRV is a heat sensitive virus and destroyed at 56°C. It is stable at a pH between 4.0 to 10.0. However, the virus is killed by alcohol, ether, and detergents as well as by most disinfectants including common disinfectants like phenol, sodium hydroxide). PPRV also survives for a long time in chilled and frozen tissues.

PESTE DES PETITS
HA PROPERTY

- PPRV caused haemagglutination of chicken RBC. However, the p^H of PBS used should be at 6.8.

PESTE DES PETITS
CULTIVATION

- Tissue culture systems are ideal for the cultivation of PPRV. The virus grows well in lamb kidney cells and African green monkey cells (Vero cells).
- The CPE develop within 5 days and consists of cell rounding and aggregation and syncytia formation in lamb kidney cells. In Vero cells syncytia are rare and if present are very small.
- Syncytia are recognised by a circular arrangement of nuclei giving a ‘clock face’ appearance. Other important CPE include intracytoplasmic and intranuclear inclusions and vacuolation of cells.

PESTE DES PETITS

PATHOGENESIS

- Dealt as
  - Hosts infected,
  - Distribution,
  - Transmission,
  - Symptoms and
  - Lesions.

PESTE DES PETITS
HOSTS AFFECTED

- PPR is primarily a disease of sheep and goats. The infection also occurs in wild ungulates. Cattle, buffaloes, camels, and pigs are also susceptible to infection but do not exhibit clinical signs and are unable to transmit the disease to other animals.

PESTE DES PETITS
DISTRIBUTION

- PPR is prevalent in Sub-Saharan Africa, Indian sub continent and Middle East countries.

PESTE DES PETITS
TRANSMISSION

- PPR is not very contagious and transmission requires close contact between animals. Ocular, nasal, and oral secretions and faeces are the sources of virus. Infection occurs mainly through inhalation of aerosols produced by sneezing and coughing. Fomites such as bedding may also contribute to the onset of an outbreak. There is no known carrier state. The spread is not dependant on vectors. The appearance of clinical PPR may be associated with any of the following:
  - History of recent movement or gathering together of sheep and/or goats of different ages
  - Introduction of recently purchased animals; contact in a closed/village flock with sheep and/or goats that had been sent to market but returned unsold;
  - Change in weather such as the onset of the rainy season (hot and humid) or dry, cold periods
  - Contact with trade or nomadic animals
  - A change in husbandry (e.g. towards increased intensification) and trading practices.

PESTE DES PETITS
INCUBATION PERIOD

- The incubation period is from 4 to 5 days.

MORBIDITY AND MORTALITY

of the animals in a flock may be affected in a PPR outbreak with between 20 and 90 percent mortality.

PESTE DES PETITS
SYMPTOMS

- Characterized by sudden onset of fever (40° to 41°C).
- Affected animals are markedly depressed and appear sleepy. Their hair stands erect giving them a bloated appearance.
- Soon after this stage, a clear watery discharge is noticed from the eyes, nose and mouth, which become thick and yellow as a result of secondary bacterial infection. The discharges wet the chin and the hair below the eye; they tend to dry, causing matting together of the eyelids, obstruction of the nose and difficulty in breathing.
- One to two days after fever has set in, the mucous membranes of the mouth and eyes become very reddened.
- This is followed by pin-point greyish areas on the gums, dental pad, palate, lips, inner aspects of the cheeks and upper surface of the tongue. These areas increase in number and size and join together. In severe cases, the normal membrane may be completely covered by a thick cheesy material. Underneath the dead surface cells shallow erosions are noticed.
- Similar changes may also be seen in the mucous membranes of the nose, the vulva and the vagina. The lips tend to swell and crack and become covered with scabs.
- As the disease progresses, a characteristic foul smell exudes from the mouth. Affected animals resist attempts to open their mouths because of the pain.
- Diarrhoea commonly appears about two to three days after the onset of fever. The faeces are initially soft and then watery, foul-smelling and may contain blood streaks and pieces of dead gut tissue.
- Affected animals breathe fast exhibiting rocking movements of both the chest and abdominal walls. Severely affected cases show difficult and noisy breathing marked by extension of the head and neck, dilation of the nostrils, protrusion of the tongue and soft painful coughs.
- A common feature in later stages of the disease is the formation of small nodular lesions in the skin on the outside of the lips around the muzzle.

PESTE DES PETITS
LESIONS

- The carcass of the affected animal is usually emaciated, the hindquarters soiled with soft/watery faeces. The eyes

dried-up discharges. The following changes are also seen:

irty-white, false membranes and erosions are seen on the gums, soft and hard palates, tongue, cheeks and oesophagu

ear Swollen with erosions and scabs or nodules

ity: Congested (reddened) lining with clear or creamy yellow exudates and erosions

ark red or purple areas which are firm to the touch, are mainly seen in the anterior and cardiac lobes. Pleuritis and hy

des (associated with the lungs and the intestines): Appear soft and swollen.

n: Congested (reddened) lining with haemorrhages

stines: Congested (reddened) lining with haemorrhages and erosions are noticed.

stines (caecum, colon and rectum): Small red haemorrhages along the folds of the lining that join together and appe
ck in stale carcasses (Zebra striping).

PESTE DES PETITS
DIAGNOSIS

- Dealt as
  - Field and
  - Laboratory diagnosis.

PESTE DES PETITS
FIELD DIAGNOSIS

- In the field, a presumptive diagnosis is made on the basis of clinical, pathological, and epizootiological findings.

PESTE DES PETITS
LABORATORY DIAGNOSIS

- Clinical materials to be collected
  - *Tears:* Cotton buds or swabs of absorbent cotton wool are inserted into the conjunctival sac and swirled around to collect tears. The bud/swab is broken off into a container and about 150 microlitres of sterile phosphate-buffered saline (PBS pH 7.2 to 7.6)
  - *Gum debris:* This material can be collected by a spatula or finger rubbed across the gum and inside the upper and lower lips. The material collected is then scraped into a container and 150 microlitres of PBS are added
  - *Tissues:* Tissue to be collected are from lymph nodes found around the lungs (mediastinal) and alimentary tract (mesenteric); portions of the spleen and the lungs. Two sets of each tissue are required; one set is chilled but not frozen, and the other is put in 10 percent formalin solution to preserve the samples
  - *Unclotted blood:* This is needed for virus isolation and should be collected in bottles containing anticoagulants (heparin or ethylenediamine tetracetic acid [EDTA]).
  - *Clotted blood or serum:* For identification of antiboies
- Isolation and identification
  - The tissue culture used are lamb kidney cells and Vero cells. The characteristic CPE appear around 5th day and are cell rounding and aggregation and syncytia formation
  - *Identification of virus*
    - Immunocapture enzyme-linked immunosorbent assay (Sandwich ELISA)

- Counterimmunoelectrophoresis (CIEP): Most rapid test for detecting viral antigen
- Agar gel immunodiffusion (AGID): Very simple and inexpensive and gives results within 1 day, but not sensitive to mild forms of PPR
- Immunofluorescence and immunoperoxidase
- Polymerase chain reaction (RT-PCR)
- Nucleic acid probes

PESTE DES PETITS
SEROLOGY

A and
on test (Prescribed test for international trade).

PESTE DES PETITS
DIFFERENTIAL DIAGNOSIS

- *Rinderpest:* Clinically, RP and PPR are similar, but the former should be the prime suspect if the disease involves both cattle and small ruminants.
- *Pasteurellosis:* Enzootic pneumonia or the septicemic form of pasteurellosis is characterized by obvious respiratory signs, infrequent diarrhea, and a fatality rate rarely exceeding 10 percent
- *Contagious caprine pleuropneumonia:* There is no digestive system involvement, and the clinical signs and lesions are confined to the respiratory system and pericardium.
- *Bluetongue:* Swelling of the lips, muzzle, and oral mucosa, together with edema of the head region, helps to differentiate bluetongue from PPR. Coronitis, common in bluetongue, is not a feature of PPR. Also, sheep are more affected than goats.
- *Heartwater:* There is often central nervous system involvement, including convulsions. There is no diarrhea.
- *Contagious ecthyma* (contagious pustular dermatitis, orf): The orf virus causes proliferative, not necrotic lesions, that involve the lips rather than the whole oral cavity. The absence of nasal discharges and diarrhea also distinguish orf from PPR.
- *Foot-and-mouth disease:* This condition is comparatively mild, and the most characteristic clinical sign, lameness, is not a feature of PPR.
- *Nairobi sheep disease:* Sheep are more severely affected than goats.
- Coccidiosis: There is no upper digestive tract and respiratory system involvement.
- *Plant or mineral poisoning:* Several plants and minerals may cause severe intestinal lesions. Case history and absence of fever should distinguish poisoning from PPR.

PESTE DES PETITS
CONTROL

- Dealt as below
  - Vaccination and
  - Eradication

PESTE DES PETITS
VACCINATION

- The tissue culture rinderpest vaccine at a dose of 102.5 TCID50 protects goats for at least 12 months against PPR. The vaccine is currently used in many African countries for vaccination against PPR. This vaccine interferes with rinderpest eradication programmes because it is impossible to determine if seropositive small ruminants have been vaccinated or naturally infected with RPV.
- A homologous attenuated PPR vaccine is being tested and may soon be commercially available. In Southern India, a homologous PPR vaccine using AR-87 strain is used to control PPR in sheep and goat. This vaccine was developed at the Department of Veterinary Microbiology, Madras Veterinary College.

PESTE DES PETITS
ERADICATION

- Methods that are applied for rinderpest eradication can be used for PPR eradication. These should include quarantine, slaughter, and proper disposal of carcasses and contact fomites, decontamination, and restrictions on importation of sheep and goats from affected areas.
- Control of PPR outbreaks relies on movement control (quarantine) combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations.

PESTE DES PETITS
TREATMENT

- There is no treatment for PPR. However, mortality rates may be decreased by the use of drugs that control the bacterial and parasitic complications.
- Specifically, oxytetracycline and chlortetracycline are recommended to prevent secondary pulmonary infections.

PESTE DES PETITS
PUBLIC HEALTH ASPECT

- PPR does not cause infection in humans. Hence, no public health issues are concerned with this infection.

MODULE-5: CANINE DISTEMPER

Learning objectives

- Group V viruses – Negative sense single stranded RNA viruses
- Morbilli virus
    - Peste des petit ruminants – PPRV
    - About the virus
    - About the disease
    - About the diagnosis
    - About the vaccines
    - About the control.

CANINE DISTEMPER
INTRODUCTION

- Canine distemper is a contagious, incurable, often fatal, multi systemic viral disease that affects the respiratory, gastrointestinal, and central nervous systems.
- CDV occurs among domestic dogs and many other carnivores, including raccoons, skunks, and foxes. CDV is fairly common in wildlife.

CANINE DISTEMPER
GENERAL ASPECTS

- Deals about
  - Classification,
  - Morphology,
  - Resistance,
  - Replication,
  - physicochemical properties,
  - strains etc.

CANINE DISTEMPER
CLASSIFICATION

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Paramyxoviridae
Subfamily	Paramyxovirinae
Genus	Morbilli virus
Species	Canine Distemper Virus (CDV)

- Canine distemper virus (CDV) belongs to genus Morbillivirus within the Paramyxoviridae family, which includes other viruses such as: phocine distemper virus, dolphin morbillivirus, rinderpest virus, peste-des-petits-ruminants virus and measles virus.

CANINE DISTEMPER
MORPHOLOGY

- CDV has a spherical shape and diameter ranging from 100 to 250 nm. It contains negative-stranded, non segmented RNA.
- The virus contains six proteins:
  - The nucleocapsid protein (NP),
  - The phosphoprotein (P),

- The matrix protein (M),
- The fusion protein (F),
- The haemaglutinin attachment glycoprotein (H) and
- The large-polymerase protein (L).

CANINE DISTEMPER
SYNONYMS

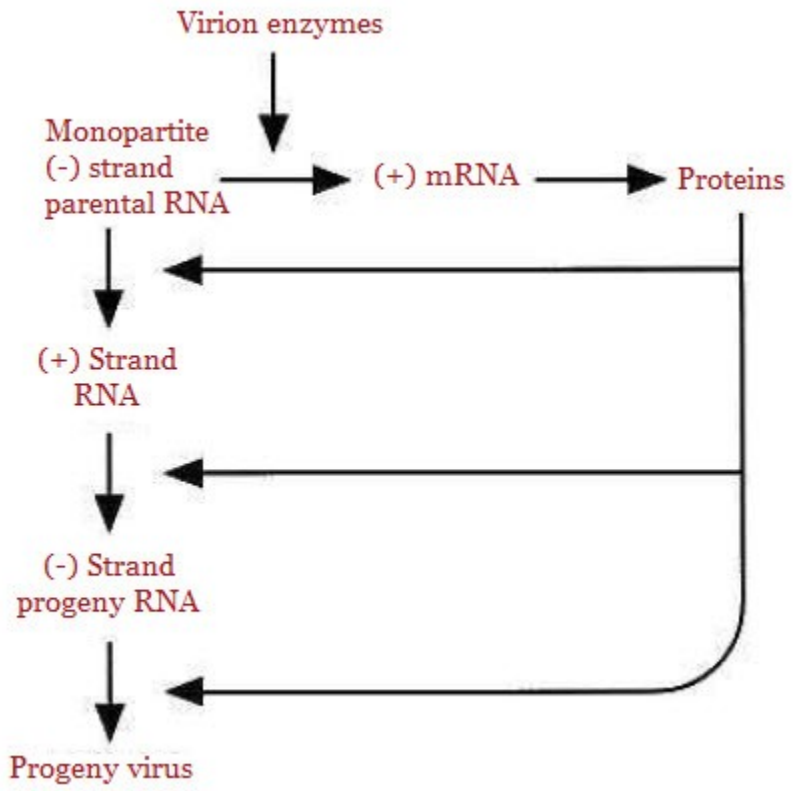
- Carre's disease, Hard pad disease.

CANINE DISTEMPER
RESISTANCE

- The virus is quickly killed by disinfectants and sunlight and heat. However, at normal temperatures the virus is very stable and can stay active in infected material for several weeks, provided the materials are not exposed to sunlight.
- At below zero temperatures the virus can stay active for many months, but at temperatures above 32°C it is rapidly inactivated. Routine disinfection and cleaning readily kills the distemper virus in the kennel setting.

CANINE DISTEMPER
REPLICATION

- Like other paramyxoviruses the CDV rapidly invades cells and uses the cell's reproductive mechanism to reproduce itself.
- Inside the cell the virus is protected and it is very difficult for the immune system to get at it to destroy it. Many thousands of new virus particles are released when the cell dies.



CANINE DISTEMPER
STRAINS AND SEROTYPES

- Rock born strain (Vaccine strain used in India).

CANINE DISTEMPER
CULTIVATION

- *Cell culture:* CDV grows well in dog kidney cells, ferret cells and chick fibroblasts. Formation of cytoplasm and acidophilic intracytoplasmic and intranuclear inclusion bodies are characteristic CPEs.
- *Embryonated eggs:* The virus also grows in the Chorioallantoic membrane. However, no changes are produced upto 10th passage. Subsequent passages show increased thickening and whitish areas in the chorioallantoic membrane.
- *Laboratory animals:* The distemper viruses are cultivated in live dogs or ferrets. Few strains also grown in suckling mouse.

CANINE DISTEMPER
PATHOGENESIS

- Dealt as
  - Hosts affected,
  - Transmission,

- Symptoms and
- Lesions.

CANINE DISTEMPER
HOSTS AFFECTED

- Domestic dogs and many other carnivores, including raccoons, skunks, and foxes are affected by CDV. The infection is more severe in puppies.

CANINE DISTEMPER
TRANSMISSION

- Infected dogs shed the virus through body secretions and excretions, especially respiratory secretions.
- The main mode of transmission is airborne. Normal animals get the infection by breathing the viral particles. Dogs in recovery may continue to shed the virus for several weeks after symptoms disappear and act as source for contamination.
- Once the virus enters the normal host, it is engulfed by macrophages. Viruses are not killed by the macrophages and the virus uses the macrophage as a means of transportation inside host's body.
- Within 24 hours, the virus reaches the lymph nodes of the lung. By the 6th day, the virus migrates to the spleen, stomach, small intestine, and liver and results in production of fever.
- After the host's immune system clears the virus from the different visceral organs, the virus still continues to remain in CNS and skin causing seizures for a long time and hardening of the skin. Though the virus spreads very rapidly inside the body their stability outside the body is questionable.
- The lipid envelope is easily disrupted in the environment, which makes it impossible for infectious virus to persist in the environment. Because an intact fatty envelope is required for infection, virus transmission must involve dog-to-dog contact or at least contact with extremely fresh (less than 30 minutes old) infected body secretions.
- As with other viruses, living virus happily freezes and can survive for years if kept frozen and protected from light. The initial disease in dogs appears quite, but the severity of the symptoms steadily increases despite treatment until the dog is often destroyed on humane grounds.
- CDV infection can take a month to show all of its worst characteristics, and things can still deteriorate from then on. There is no vector involvement in the transmission. In utero infection is rare.

CANINE DISTEMPER
INCUBATION PERIOD

- *Incubation Period:* 5-7 days.

CANINE DISTEMPER
SYMPTOMS AND LESIONS

- The initial symptom is fever (103°F to 106°F), which usually peaks 3 to 6 days after infection. The fever often goes unnoticed and may peak again a few days later. Eye and nose discharge, depression, and loss of appetite (anorexia) are the associated symptoms during the period. After the fever, symptoms vary considerably, depending on the strain of the virus and the dog's immunity.
- At initial stages gastrointestinal and respiratory symptoms are more common, which include conjunctivitis, diarrhoea, pneumonia (cough, labored breathing), rhinitis (runny nose) and vomiting. These symptoms are often aggravated by secondary bacterial infections.
- Dogs develop encephalomyelitis (an inflammation of the brain and spinal cord), the symptoms of which are variable and progressive. Most dogs that die from distemper, die from neurological complications such as the following, ataxia (muscle incoordination), depression, hyperesthesia (increased sensitivity to sensory stimuli, such as pain or touch), myoclonus (muscle twitching or spasm), which can become disabling, paralysis, paresis (partial or incomplete paralysis), progressive deterioration of mental abilities, progressive deterioration of motor skills and seizures that can affect any part of the body (One type of seizure that affects the head, and is unique to distemper, is sometimes referred to as a "chewing gum fit" because the dog appears to be chewing gum).
- A variety of symptoms on the eye are also characteristic, which include keratoconjunctivitis, inflammation of the cornea and conjunctiva, or chorioretinitis, inflammation of the choroid and retina and optic neuritis (inflammation of the optic nerve which leads to blindness).
- Enamel hypoplasia (unenameled teeth that erode quickly in puppies whose permanent teeth haven't erupted yet) and hyperkeratosis (hardening of the foot pads and nose) are seen in chronic infection. In utero infection of fetuses is rare.
- When it happens, this can lead to spontaneous abortion, persistent infection in newborn puppies, or the birth of normal looking puppies that rapidly develop symptoms and die within 4 to 6 weeks.

CANINE DISTEMPER
DIAGNOSIS

- Dealt as
  - Field and
  - Laboratory diagnosis.

CANINE DISTEMPER
FIELD DIAGNOSIS

- Field diagnosis is based on gastrointestinal, respiratory and nervous symptoms. Biphasic fever curves and low white blood cell level are indicative of distemper infection.

CANINE DISTEMPER
LABORATORY DIAGNOSIS

- *Microscopical identification of inclusion bodies:* Distemper inclusion bodies" are actual clumps of virus that are visible under the microscrope within infected cells.

Post-mortem inclusion bodies are readily visible in the urinary bladder tissues. In the living animals the inclusion bodies are readily visible in WBCs and conjunctival membranes. The inclusion bodies, which red stained (eosinophilic) oval structures are also found in the epithelial cells of the salivary glands, central nervous system, adrenal glands, bile duct, urinary tract, lymph nodes spleen and skin.

- *Immunocytolgy*
  - To enhance the visibilty of inclusion bodies, "immunocytology" is used. In this technique, antibodies against distemper virus are tagged with fluorescent markers. The antibodies bind to virus if it is present effectively dying the inclusion body with glow-in-the-dark fluorescent color.
  - The presence of inclusion bodies confirms distemper infection. The lack of dectectable inclusion bodies does not rule out distemper infection as inclusion bodies ultimately become coated with the host's own antibodies, which in turn block the fluorescent-tagged antibodies used in the test.

- *Isolation and identification:* The virus are identified by AGPT, Antigen capture ELISA and PCR techniques.

CANINE DISTEMPER
SEROLOGY

- Distemper titers of either the "IgM" type (produced in early stages of infection) and the "IgG" type (produced in later phases of infection) can be checked. A high IgM titer indicates recent infection or recent vaccination. It is difficult to differentiate between antibodies initiated by vaccination and infection.
  - *Cerebrospinal fluid antibody levels:* In neurologic distemper cases, cerebrospinal fluid is often tapped and distemper antibody levels checked. Distemper antibodies in cerebrospinal fluid are highly indicative of distemper infection as vaccine-induced antibodies do not cross the blood-brain barrier into the CSF fluid.

CANINE DISTEMPER
TREATMENT

- There are no antiviral drugs to treat canine distemper virus. Because of this, only symptomatic treatment is attempte.
- Antibiotics coupled with antiallergic, antispasmodic and elctrolyte fluids are given. Once dogs develop nervous system signs treatment is of no use.

CANINE DISTEMPER
CONTROL

- Effective vaccines are available to protect dogs from canine distemper virus. These vaccines are manufactured from attenuated viruses to induce long-lasting immunity. These vaccines are produced in bird or dog cell cultures. Immunity lasts for many years.

- Vaccinations must not be given too early to puppies. Vaccination must be given to puppies at a time when the level of circulating antibodies that they have received from their mothers is in decline otherwise the vaccine's effects are neutralized (6-10 weeks).
- Twenty percent of this maternal immunity crosses the walls of the womb into the puppy while eighty percent is absorbed from colostrum milk across the intestine.
- Rarely dogs develop post vaccinal reaction referred as neurodistemper or vaccine distemper, which appear 10-21 days after vaccination.
- Primary vaccines are given at 6-8 weeks of age followed by booster at dose at 12-14 weeks of age with annual revaccination. CD vaccines are given in combination with Leptospira vaccine.
- Human measles virus can also be used to vaccinate dogs against CDV, but measles virus does not produce long lasting immunity. Older vaccines include virus adapted in ferrets and avianised virus.
- Since the virus cannot live without fresh secretions and inactivated in minutes outside the living host's body only minimal disinfection necessary.

CANINE DISTEMPER
PUBLIC HEALTH ASPECT

- There is no human hazard associated with canine distemper virus.

MODULE-6: ORTHOMYXOVIRUSES - AVIAN INFLUENZA

Learning objectives

Introduction about

- Group V viruses
  - Negative sense single stranded RNA viruses
- Orthomyxoviruses
  - Important diseases – Bird flu, Swine flu, Equine flu
  - Segmented genome, genetic shift
  - Replication – cap snatching
  - Zoonotic potential
  - Impacts of bird flu.

AVIAN INFLUENZA
INTRODUCTION

- Avian influenza is caused by type A influenza virus. The symptoms can vary from a mild disease with little or no mortality to a highly fatal, rapidly spreading epidemic (highly pathogenic notifiable avian influenza - HPAI) depending on the infecting virus strain, host factors, and environmental stressors.
- Highly pathogenic avian influenza (HPAI) is also known as fowl plague. The disease, was first identified in Italy more than 100 years ago and now occurs worldwide.

AVIAN INFLUENZA
MORPHOLOGY

- Influenza virus particles are highly pleiomorphic (variable), mostly spherical/ovoid, 80-120 nm diameter, but many forms occur, including long filamentous particles (up to 2000 nm long x 80-120 nm diameter).
- The outer surface of the particle consists of a lipid envelope from which project prominent glycoprotein spikes of two types Haemagglutinin (HA) and Neuraminidase (NA).
- The inner side of the envelope is lined by the matrix protein.

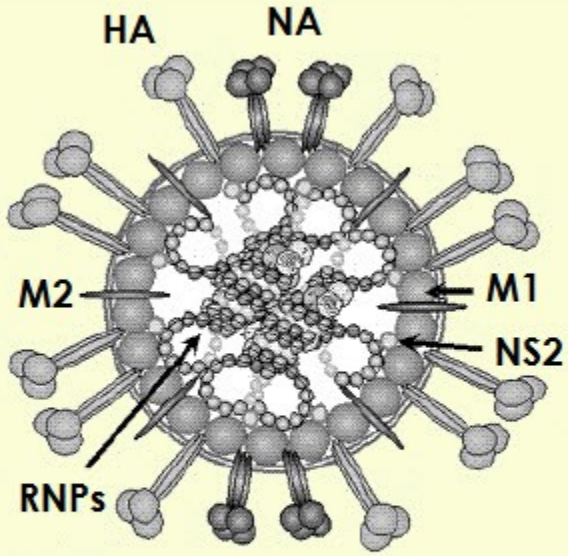


Fig-1 Influenza particle

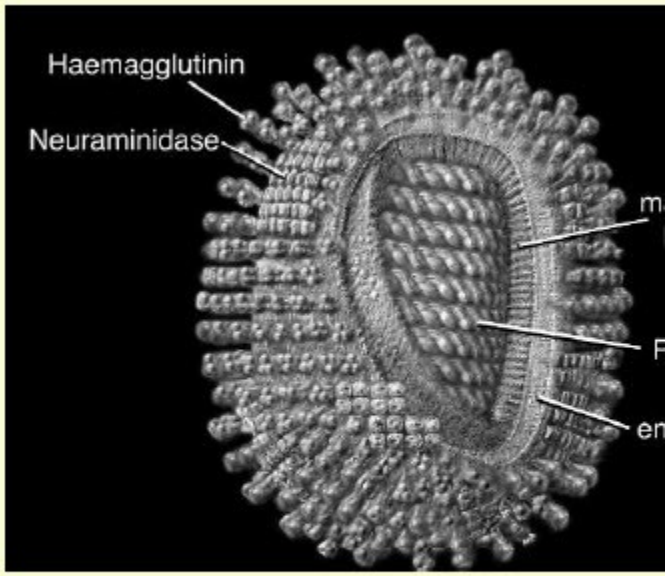


Fig-2 Graphic of influenza particle

- The genome is packed into the core along with nucleoprotein (NP).
- The genome - RNA and nucleoprotein together are called RNP.
- The genome is of s/s (-)sense RNA arranged into 8 segments. The segments 1-3 code for various transcription related activities. Segments 4, 5 and 6 code for HA, NP and NA proteins. Segment 7 codes for matrix protein and 8 codes for non-structural proteins.

AVIAN INFLUENZA
SYNONYMS

- Fowl Plague
- Tamil Name - Paravai Koichal.

AVIAN INFLUENZA
GENERAL POINTS

- OIE Listing: List A disease

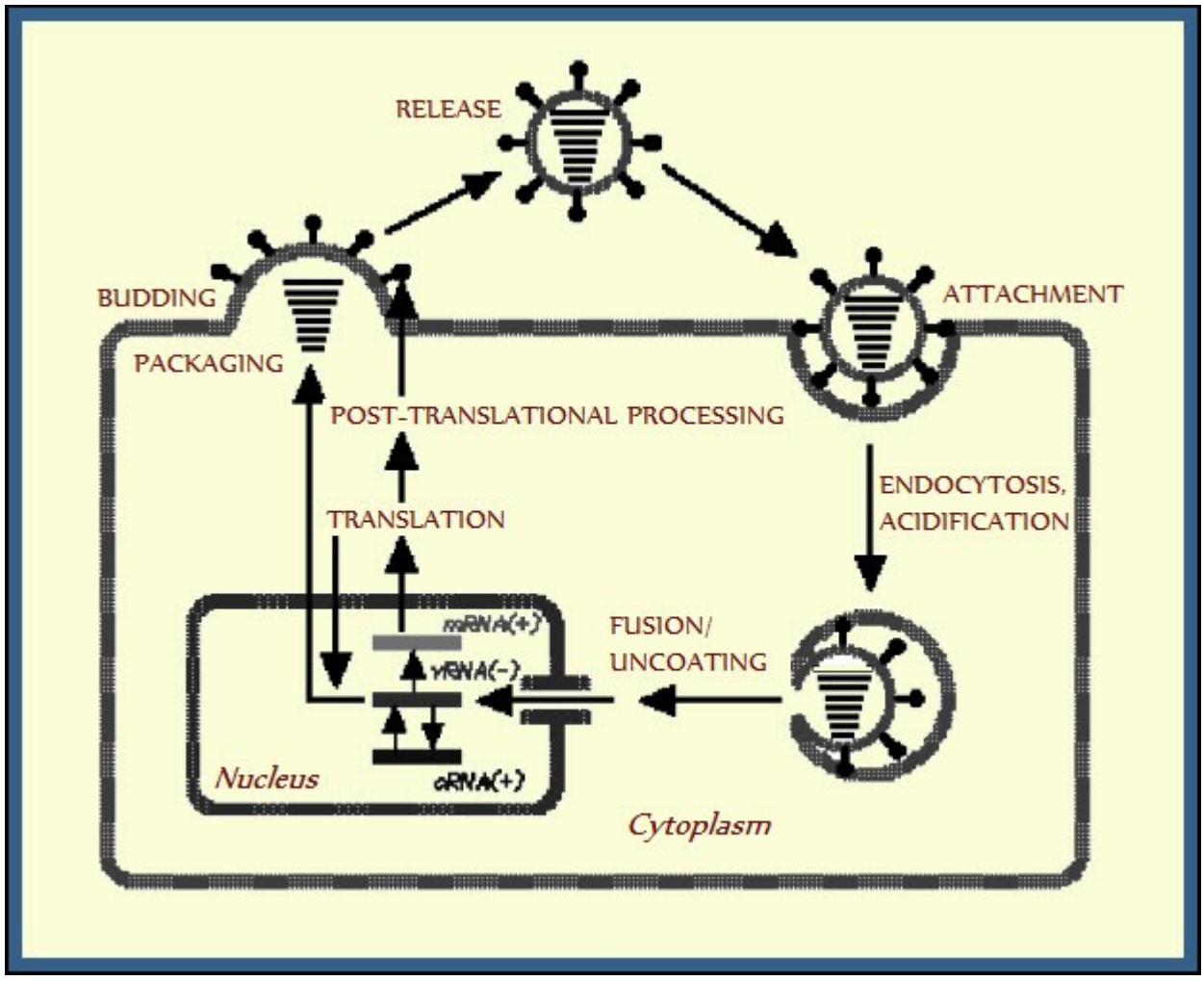
- Risk group (Animal Pathogen): Group III
- Reference Laboratories
    - D.J. Alexander VLA WeybridgeNew Haw, Addlestone, Surrey KT15 3NB UNITED KINGDOM. Email: d.j.alexander@vla.defra.gsi.gov.uk
    - Dr B. Panigrahy National Veterinary Services LaboratoriesP.O. Box 844, Ames, IA 50010 USA. Email:brundaban.panigrahy@aphis.usda.gov.
- HA Property
    - Avian influenza virus causes agglutination of chicken erythrocytes.

AVIAN INFLUENZA
RESISTANCE

- The particles are not very stable and are killed within few hours on exposure to room temperature and are also not resistant to drying and disinfectants.

AVIAN INFLUENZA
REPLICATION

- Entry of avian influenza virus into the cell is facilitated by binding of the HA spikes to mucoproteins on the surface of cells. After binding, the virus enters into the cell by endocytosis via coated pits into endocytotic vesicles as endosomes. Uncoating takes place after acidification of the endosomes. During the initial phase of infection (approximately 2h), active host cell DNA synthesis is required. Inside the nucleus the viral RNA uses the methylated cap of host cell mRNA as primers for RNA synthesis (cap snatching). Two classes of (+) sense RNA are made inside the nucleus of infected cells.
    - Incomplete, 3' polyadenylated transcripts which are exported to the cytoplasm and serve as mRNAs.
    - cRNA - complete, (+) sense copies of the (-) sense viral, which serve as template for the synthesis of progeny (-) sense vRNAs.
- Most of the proteins made (e.g. HA, NA) remain in the cytoplasm or become associated with the cell membrane. However, the NP protein migrates back into the nucleus, where it associates with newly-synthesized vRNA to form new nucleocapsids. These migrate back out into the cytoplasm and towards the cell membrane. The exact packaging mechanism by which eight distinct genome segments are packed into each progeny virion particle is not known.



AVIAN INFLUENZA
SUB TYPES

- Influenza A viruses have antigenically related nucleocapsid and antigenically related matrix proteins, but are classified into subtypes on the basis of their haemagglutinin (HA referred as H) and neuraminidase (NA referred as N) antigens.
- At present, 15 H subtypes (H1–H15) and 9 neuraminidase subtypes (N1–N9) are recognised. There are three subtypes of avian influenza virus referred as H5, H7 and H9. H5 and H7 can produce low pathogenic and highly pathogenic avian influenza, where as H9 produces only low pathogenic infection.
- Within these subtypes there are number of strains. These strains are formed by combination of any one of H5, H7 or H9 with 9 different NA proteins. The following table lists the details of subtypes and strains of avian influenza viruses

Subtype	Characters
H5	• Nine different strains. • Produce highly pathogenic or low pathogenic infections.

	• Human infection possible, sometimes causing severe illness and death.
H7	• Nine different strains. • Produce highly pathogenic or low pathogenic infections. • Human infection is rare, but can occur among persons who have close contact with i symptoms may include conjunctivitis and/or upper respiratory symptom.
H9	• Nine different strains. • Produce only low pathogenic infection. • Human infection possible.

AVIAN INFLUENZA
PATHOGENESIS

- Host affected
  - Many avian species are affected by AI. Certain subtypes of virus may infection predominantly in any one of the species of birds. AI has been reported in chickens, turkeys, ducks, waterfowls, gulls, shorebirds etc.
- Transmission
  - Migratory waterfowl, sea birds, or shore birds are considered responsible for introducing the virus into poultry. Waterfowl serve as reservoir for AIV.
  - Virus is found in large quantities in faeces and respiratory secretions of infected birds. The virus spreads to susceptible birds through inhalation of influenza particles in nasal and respiratory secretions and from contact with the faeces of infected birds.
  - Once introduced into a flock, the virus spreads from flock to flock by the movement of infected birds, contaminated equipment, egg flats, feed trucks, and service crews etc. The infection spreads through shared and contaminated drinking water.
  - Airborne transmission may occur if birds are in close proximity and with appropriate air movement. Vertical transmission is not completely established. There is also no vector involvement in the transmission of infection.
- Incubation period
  - The incubation period is usually 3 to 7 days, depending upon the subtype of virus, the species, and age of the bird.
- Morbidity and mortality
  - Morbidity and mortality rates are upto 100 percent within 2 to 12 days after the first signs of illness in HPAI.

AVIAN INFLUENZA
SYMPTOMS

- Dealt as in

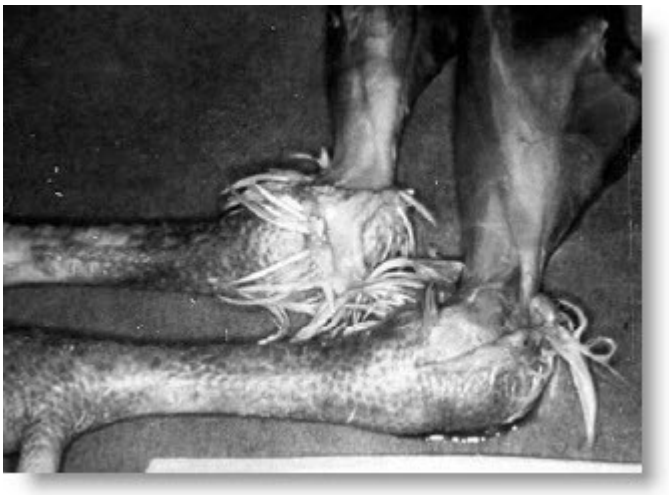
- Chicken (Layers)
- Broilers
- Ducks
- Turkeys

AVIAN INFLUENZA
SYMPTOMS - CHICKENS (LAYERS)

- Marked depression with ruffled feathers, inappetence, excessive thirst, cessation of egg production, and watery diarrhea.
- Mature chickens show swollen combs, wattle, and edema surrounding the eyes. The combs are cyanotic at the tips and may have plasma or blood vesicles on the surface with dark areas of ecchymotic hemorrhage and necrotic foci.
- The last eggs laid, after the onset of illness may not have shells.
- The diarrhea begins as watery bright green and progresses to almost totally white.
- Edema of the head, if present, is often accompanied by edema of the neck.
- The conjunctivae are congested and swollen with occasional hemorrhage.
- The legs, between the hocks and feet, may have areas of diffuse hemorrhage.
- Respiratory signs can be a significant feature of the disease, depending on the extent of tracheal involvement and mucus accumulation.
- Death may occur within 24 hours of first signs of disease, frequently within 48 hours, or be delayed for as long as a week. Some severely affected hens may occasionally recover.

AVIAN INFLUENZA
SYMPTOMS - BROILERS

- The signs of disease are frequently less obvious with severe depression, inappetence, and a marked increase in mortality being the first abnormalities observed.
- Edema of the face and neck and neurologic signs such as torticollis and ataxia may also be seen.



AVIAN INFLUENZA
SYMPTOMS - TURKEYS

- The disease in turkeys is similar to that seen in layers, but it lasts 2 or 3 days longer and is occasionally accompanied by swollen sinuses.

AVIAN INFLUENZA
SYMPTOMS - DUCKS

- In domestic ducks and geese the signs of depression, inappetence, and diarrhea are similar to those in layers, though frequently with swollen sinuses. Younger birds may exhibit neurologic signs.

AVIAN INFLUENZA
LESIONS

- Birds that die with the peracute disease and young birds may not show significant gross lesions other than severe congestion of the musculature and dehydration.
- In the less acute form, and in mature birds, significant gross lesions are frequently observed, which may consist of subcutaneous edema of the head and neck area, exudation of fluid from nares and oral cavity, congested conjuctiva with petechiation, tracheal lumen filled with excessive mucous exudates, hemorrhagic tracheitis, pinpoint petechial hemorrhages on the keel, very small petechia over the abdominal fat, serosal surfaces, and peritoneum and congestion of kidneys with urate deposition in the tubules.
- In layers, the ovary may be hemorrhagic or degenerated with darkened areas of necrosis and the peritoneal cavity is frequently filled with yolk from ruptured ova, causing severe airsacculitis and peritonitis.
- Hemorrhages may be present on the mucosal surface of the proventriculus — particularly at the juncture with the gizzard. The lining of the gizzard peels easily and frequently reveals hemorrhages and erosions underneath. The intestinal muscosa may have hemorrhagic areas — especially in the lymphoid foci such as the cecal tonsils.
- The lesions in turkeys and domestic ducks are similar to those in chickens but may not be as marked.

AVIAN INFLUENZA
DIAGNOSIS

- Dealt as
  - Field diagnosis
  - Laboratory diagnosis
  - Serological test.

AVIAN INFLUENZA
FIELD DIAGNOSIS

- The field diagnosis is based on sudden deaths followed with severe depression, inappetence, a drastic decline in egg production, presence of facial edema, swollen and cyanotic combs and wattles, and petechial hemorrhages on internal membrane are suggestive of HPAI.

AVIAN INFLUENZA
LABORATORY DIAGNOSIS

Isolation and identification

- *Samples from dead birds:* Intestinal contents (faeces) or cloacal swabs, oro-nasal swabs, trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart are the ideal material.
- Samples from tracheal and cloacal swabs.
- The samples should be placed in isotonic phosphate buffered saline (PBS), p^H 7.0–7.4, containing antibiotics, processed, centrifuged and the supernatant is inoculated in 9-11 day old embryonated eggs. The eggs should be incubated for 4 days at 35-37°C. The presence of virus in allantoic fluid are confirmed by
  - Detection of HA activity
  - Agar gel immunodiffusion (AGID) test - by demonstrating the presence of the nucleocapsid or matrix antigens
  - RT-PCR
  - Antigen capture ELISA
- RT-PCR can also be used to identify viral RNA directly in the clinical samples without passaging the materials in embryonated eggs.

AVIAN INFLUENZA
SEROLOGICAL TESTS

- AGID test
- HA / HI test.

AVIAN INFLUENZA
DIFFERENTIAL DIAGNOSIS

- Highly pathogenic avian influenza should be differentiated from

  - VVND,
  - Infectious laryngotracheitis and
  - Acute bacterial diseases such as fowl cholera and Escherichia coli.
- However, in an area where AI is prevalent, such as during an outbreak, sound presumptive diagnosis can be made by flock history, signs, and gross lesions.

AVIAN INFLUENZA
TREATMENT

- Amantadine hydrochloride provide relief to birds when administered in drinking water. However, it results in emergency of strains that are resistant to Amantadine hydrochloride.

AVIAN INFLUENZA
CONTROL

- Vaccination
  - Inactivated oil-emulsion vaccines are found to be effective in reducing mortality, preventing disease, or both, in chickens and turkeys.
  - Naturally avirulent or attenuated strains can also be used as vaccines. The problem with live vaccine is emergence of new strains due to genetic reassortment. A recombinant fowl pox virus vaccine containing the gene that codes for the production of the H_5 antigen.
  - A recombinant fowl pox virus vaccine containing the gene that codes for the production of the H_5 antigen is also used. A recombinant insect virus containing the gene for either the H_5 or H_7 antigen is also used to control AI.
- Biosecurity procedures including control of human traffic, quarantine of birds of unknown disease status into the flock and proper cleaning and disinfection of fomites.
- Free range rearing should not be done in areas where, waterfowls frequent.

AVIAN INFLUENZA
PUBLIC HEALTH

- Severe human infection occurs as a result of close contact with live infected poultry was the source of human infection. Studies at the genetic level determined that the virus had jumped directly from birds to humans. Limited transmission to health care workers occurred, but did not cause severe disease.
- Some of the strains that caused human infection are H_5N_1, H_7N_7, H_9N_2 etc.
- Avian influenza viruses may be transmitted to humans in *two main ways:*
  - Directly from birds or from avian virus-contaminated environments to people and
  - Through an intermediate host, such as a pig.
- Influenza viruses have eight separate gene segments. The segmented genome allows viruses from different species to mix and create a new influenza A virus if viruses from two different species infect the same person or animal. For example, if a pig were infected with a human influenza virus and an avian influenza virus at the same time, the viruses could reassort and produce a new virus that had most of the genes from the human virus, but a hemagglutinin and/or neuraminidase from the avian virus.

- The resulting new virus might then be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or neuraminidase) not previously seen in influenza viruses that infect humans. This type of major change in the influenza A viruses is known as antigenic shift.
- Antigenic shift results when a new influenza A subtype to which most people have little or no immune protection infects humans. If this new virus causes illness in people and can be transmitted easily from person to person, an influenza pandemic can occur. It also is possible that the process of reassortment could occur in a human. For example, a person could be infected with avian influenza and a human strain of influenza at the same time. These viruses could reassort to create a new virus that had a hemagglutinin from the avian virus and other genes from the human virus.
- Theoretically, influenza A viruses with a hemagglutinin against which humans have little or no immunity that have reassorted with a human influenza virus are more likely to result in sustained human-to-human transmission and pandemic influenza. Thus, careful evaluation of influenza viruses recovered from humans who are infected with avian influenza is very important to identify reassortment if it occurs.

MODULE-7: ORTHOMYXOVIRUSES - EQUINE INFLUENZA

Learning objectives

- Equine flu
  - About the virus
  - About the disease
  - About the diagnosis
  - About the vaccines
  - About the control.

EQUINE INFLUENZA
INTRODUCTION

- Equine influenza is an acute, contagious respiratory disease caused by two distinct subtypes (H_7N_7 - formerly equi-1, and H_3N_8 - formerly equi-2) of influenza A viruses within the genus Influenzavirus A of the family Orthomyxoviridae.
- The infection in horses are chracterised by pyrexia, and a harsh dry cough followed by a mucopurulent nasal discharge.

Synonyms

- Horse flu, Epizootic Cellulitis, Pinkeye, Stable Pneumonia, Shipping Fever.

EQUINE INFLUENZA
CLASSIFICATION

-

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales

Family	Orthomyxoviridae
Genus	Type A Influenza virus
Species	Equine influenza virus (EIV)

EQUINE INFLUENZA
MORPHOLOGY

- The morphology of EIV is typical of Influenza virus A. They are pleomorphic enveloped viruses containing 8 segments of single stranded, negative sense, RNA.
- The virion consists of the viral ribonucleoprotein (RNA + NP) and polymerase complex (PA, PB1 and PB2) surrounded by the matrix proteins (M1 and M2) and a cellular lipid envelope containing 2 viral glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA).
- The HA and NA contain the major neutralizing antigenic determinants of influenza viruses.

EQUINE INFLUENZA
SYNONYMS

- Swine Flu, Flu, Influenza.

EQUINE INFLUENZA
GENERAL POINTS

- OIE listing: List B disease
- Risk group: Risk group II

Reference Laboratories

- Dr W. Eichhorn Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Ludwig-Maximilians-UniversityVeterinärstrasse 13, 80539 MünchenGERMANYTel: (49.89) 21.80.25.31 Fax: (49.89) 21.80.25.97 Email: <font color="#0000ff" face="georgia,times new roman,times,serif">werner.eichhorn@micro.vetmed.uni-uenchen.dejenny.mumford@aht.org.uk
- Web http://www.equiflunet.org.uk/ jenny.mumford@aht.org.uk
- Dr Jennifer A. Mumford Animal Health TrustLanwades Park, Kentford, Newmarket, Suffolk CB8 7 UNITED KINGDOM Tel: (44.8700) 50.24.60 Fax: (44.8700) 50.24.61.

EQUINE INFLUENZA
RESISTANCE

- The particles are not very stable and are killed within few hours on exposure to room temperature and are also not resistant to drying and disinfectants.

EQUINE INFLUENZA
CULTIVATION

- EIV are cultivated in embryonated eggs and cell culture systems. 10-11 days old embryonated eggs are used for cultivation of EIV. The routes are allantoic or amniotic routes and the infected eggs are incubated for 2-3 days.
- The eggs are chilled after 2-3 days and the presence of EIV is confirmed by demonstration HA property using chicken or guinea pig RBCs. The cell culture system commonly used is MDCK (Madin–Darby canine kidney cell line).
- The cells are incubated for 7 days. The presence of EIV is confirmed by demonstration of HA property in tissue culture fluid using chicken or guinea pig RBCs and haemadsorption.

EQUINE INFLUENZA
PATHOGENESIS

- Hosts affected
  - Horses and other equines are the normal hosts. Humans are also infected by H7N7 and H3N8 viruses. The infection is common in young horses, especially at racetracks, horse shows, and during transport (planes, etc.). The infection is seasonally more prevalent in the summer and autumn.
- Distribution
  - The infection is worldwide except in Pacific regions.
- Transmission
  - The disease is highly contagious in nature and can occur as epidemics. Infection spreads by direct contact, aerosol from coughing (up to 30 m), wind (up to 8 km) and indirect contact with infected material.
  - Infected or recovered animals do not act as chronic carriers. There is no vertical transmission or involvement of vectors in spreading the infection. The HA glycoprotein (hemagglutinin) of EIV attaches to respiratory epithelial cell receptors, and enters the cell via endocytosis.
  - Viral replication occurs and new viral particles are released into the airway to infect other cells or become aerosolized. In 1-3 days this invasion causes necrosis and desquamation of respiratory epithelial cells, exudation of protein-rich fluid into airways, and clumping of cilia, impairing the mucociliary apparatus for up to 4 weeks.
  - Exudate accumulates and predisposes to secondary bacterial infections. Affected animals shed the virus for 8 days and these animals are important source of infection for healthy stock.
- Incubation period
  - The incubation period is very short ranging from 1-5 days.

EQUINE INFLUENZA
SYMPTOMS

- Influenza in horses is similar to "flu" in humans. The first symptoms are poor performance, letharginess, depression followed by a harsh cough, which usually lasts around 10 days.

- Other symptoms include a high temperature (38.9°C) lack of appetite and nasal discharge, which later turns into mucopurulent due to secondary bacterial infection.
- Watery eyes, enlarged lymph nodes between the mandibles, oedema and stiffness in the legs and breathing difficulty are also observed in affected horses. Horses with relatively mild cases of equine influenza usually recover in a week or so, but severely affected horses may require weeks to months to recover fully, especially if they are not allowed complete rest.
- Affected animals under stress, or ones not allowed to rest, may develop secondary pneumonia, a chronic cough, or inflammation of the heart muscle, sometimes resulting in death.

EQUINE INFLUENZA
LESIONS

ions are bronchopneumonia, bronchitis, pharyngitis, pleuritis, guttural pouch infections and viral myocarditis. abundant bilateral serous discharge is the most important post-mortem lesion.

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EQUINE INFLUENZA
DIAGNOSIS

- Dealt as field diagnosis, serology etc.

EQUINE INFLUENZA
FIELD DIAGNOSIS

- Based on sudden occurrence with characteristic respiratory symptoms and high fever.

EQUINE INFLUENZA
LABORATORY DIAGNOSIS

Isolation and identification

- Clinical materials: Nasopharyngeal swabs and nasal or tracheal washings are the ideal clinical materials for isolation of viruses.
- Isolation systems: Embryonated eggs (allantoic or amniotic routes) and MDCK cell line.
- Identification of antigen:
    - Demonstration of HA activity using chicken or guinea pig RBCs.
    - Neuraminidase typing
    - Antigen capture ELISA using monoclonal antibody against nucleoprotein
    - RT-PCR.

EQUINE INFLUENZA
SEROLOGY

- Infections are detected by performing serological tests on paired sera to show a rise in antibody titre. Three tests that are commonly used include
- Haemagglutination inhibition test
- Single radial haemolysis: In this test, viral antigens are coupled to fixed RBCs that are suspended in agarose containing guinea-pig complement (C'). Wells are punched in the agarose and filled with test sera. Influenza antibodies and C' lyse the antigen-coated RBCs, resulting in a clear, haemolytic zone around the well; the size of this zone is directly proportional to the level of strain-specific antibody in the serum sample
- Complement fixation test: Not commonly used.

EQUINE INFLUENZA
DIFFERENTIAL DIAGNOSIS

- The infection has to be differentiated from
  - Equine rhinopneumonitis
  - Equine viral arteritis
  - Equine rhinovirus and adenovirus infection
  - Pasteurellosis
  - Strangles.

EQUINE INFLUENZA
CONTROL

nza vaccines are used in competition horses in countries where the infection is reported. In free countries vaccinatio
participating to international races. Both live and inactivated equine influenza vaccines are available.
es of the killed-virus vaccine are administered 3-6 weeks apart starting at 6 months of age for younger horses. Revac
tervals of 3 to12 months depending on age and risk of exposure
d live equine AE-2 influenza vaccine, when administered intranasally offers protection for 6 months.
sures: Since the infection is highly contagious the infected horses should be immediately separated and isolated.

EQUINE INFLUENZA
PUBLIC HEALTH

- The equine influenza strains are not known to cause illness in humans. However, rare infection in humans is possible.

MODULE-8: ORTHOMYXOVIRUSES - SWINE INFLUENZA

of the virus
e and its pathogenesis
sis and various lab tests

al

flu

SWINE INFLUENZA
INTRODUCTION

- Swine influenza is a highly contagious respiratory viral infection of pigs characterised by coughing, sneezing, nasal discharge, elevated rectal temperatures, lethargy, difficult breathing, depressed appetite and rarely associated with reproductive disorders such as abortion.

SWINE INFLUENZA
CLASSIFICATION

-

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Orthomyxoviridae
Genus	Type A Influenza virus
Species	Swine influenza virus (SIV)

SWINE INFLUENZA
MORPHOLOGY

- Influenza virus particles are highly pleiomorphic (variable). Mostly they appear as spherical/ovoid (80-120nm diameter). The outer surface of the particle consists of a lipid envelope. Hence these viruses are ether sensitive.
- Glycoprotein spikes project from these envelopes and these spikes are of two types haemagglutinin (HA) and neuraminidase (NA). The inner side of the envelope is lined by the matrix protein. The genome comprises of s/s (-) sense RNA in 8 segments.
- Each segment of RNA codes for one or two proteins. The fourth and sixth segment codes for haemagglutin (HA) and neuraminidase (NA) respectively. Variations observed in these two proteins are used to classify the virus specied into many strains (subtypes). There are 13 H and 9 N types. Common strains of SIV belong to H1N1, H3N2 and H1N2.

SWINE INFLUENZA
GENERAL POINTS

- *OIE listing:* Listed but not under A or B.
- *Risk Group:* Human infection with SIV possible. It is an WHO surveyed. infection. It is also a SPC List D disease. (SPC – Southern Pacific Commission).

SWINE INFLUENZA

HA PROPERTY

- *Haemagglutination property:* The virus causes agglutination of chicken and turkey RBCs.

SWINE INFLUENZA RESISTANCE

- The particles are not very stable and are killed within few hours on exposure to room temperature and are also not resistant to drying and disinfectants.

SWINE INFLUENZA CULTIVATION

- The preferred cell culture systems for virus growth are Madin–Darby Canine Kidney (MDCK), swine kidney, swine testicle or swine lung epithelial cell lines. Specific cytopathic effects appear 7 days of incubation. The virus also grows well in embryonated eggs (9-11 days old).
- The eggs are incubated for four days. The presence of virus is confirmed by HA test using chicken or turkey RBCs.

SWINE INFLUENZA PATHOGENESIS

It is described under different headings.

- Hosts affected
  - Pig is the main host. The virus also affects avians and humans.
- Transmission
  - This infection is transmitted by direct contact between pigs. The important source for spread is the nasal secretions. SIV can also be transmitted by aerosols over a short distance. The virus can be shed for 30 days after infection and has been recovered from clinically normal animals.
  - The disease is spread to new areas and farms by the movement of infected pigs or carrier people. The virus is easily carried and spread by avian species, particularly waterfowl and turkeys. In endemic areas virus is present throughout the year.
  - However in most other areas, outbreaks are seasonal occuring in late autumn and early winter. The epidemics are very severe, with outbreaks occurring on most pig farms in a locality over a short period.
  - The stress factors, which speed up the infection are transport, mixing of pigs, poor isolation facilities, marked diurnal (day and night) temperature fluctuations and overstocking.
- Incubation Period
  - The incubation period is very short 1 to 3 days. In very severe epidemics it can be as short as 4 hours.
- Morbidity and Mortality
  - Morbidity – upto 100%; mortality – very minimal 1%.

SWINE INFLUENZA
SYMPTOMS

- The first clinical signs are fever (40.5-41.5°C), puffy eyes, anorexia leading to loss of weight, depression, prostration and huddling leading to weakness. These signs are followed by sudden onset of acute respiratory signs, which include paroxysmal coughing, sneezing, irregular abdominal breathing and ocular and nasal discharges.
- Most pigs recover about six days after the onset of the disease. The mortality rate is generally about 1%, but may be higher in young piglets. SIV can precipitate outbreak of more serious respiratory disease causing mortality.
- One of the important problem associated with SIV infection is the high rectal temperature, which in breeding stock result in abortions, infertility, production of small weak litters and increased stillbirths.

SWINE INFLUENZA
LESIONS

- Only few lesions are observed in SIV infection. The lesions are firm lobular lung lesions with interlobular oedema with enlargement of associated lymph nodes.
- The trachea may be filled with froth with severe damage to the lining of the trachea destroying the mucociliary escalator.

SWINE INFLUENZA
DIAGNOSIS

- Explained under different headings as field diagnosis, laboratory diagnosis etc.

SWINE INFLUENZA
FIELD DIAGNOSIS

- It is based on high rectal temperature, respiratory symptoms with very high morbidity rate and occurrence of the infection in autumn and winter months.

SWINE INFLUENZA
LABORATORY DIAGNOSIS

Isolation and identification

- Lung tissues are the ideal material for isolation of virus. The supernatant from the lung tissue homogenates are used for infecting the cell culture system or embryonated eggs. The preferred cell culture systems for virus growth are Madin–Darby canine kidney (MDCK), swine kidney, swine testicle or swine lung epithelial cell lines. Specific cytopathic effects appear 7 days of incubation. The virus can also be isolated in embryonated eggs (9-11 days old). The presence of the virus are confirmed by
    - *Haemagglutination test:* This test is performed using 0.5% chicken or male turkey red blood cells. The HA activity is confirmed by HI test using specific serum (8HA units of virus are used unlike HA test for NDV)

- Neuraminidase inhibition test
- Fluorescent antibody test
- Immunoperoxidase test
- Antigen capture ELISA
- Polymerase chain reaction

SWINE INFLUENZA
SEROLOGICAL TESTS

- *Haemagglutination inhibition test:* The primary serological test for detection of SIV antibodies is the HI test and it is subtype specific. It should be conducted on paired sera collected 10–21 days apart. A four-fold or greater increase in titre between the first and second sample is suggestive of a recent SIV infection.
- *Virus neutralization test:* Not commonly performed.
- *ELISA:* Not commonly performed.

SWINE INFLUENZA
DIFFERENTIAL DIAGNOSIS

- The following diseases should be differentiated
  - Aujeszky's disease
  - Atrophic rhinitis
  - Enzootic (mycoplasmal) pneumonia
  - Bacterial pneumonia due to Pasteurella or Haemophilus spp.

SWINE INFLUENZA
TREATMENT

- There is no specific treatment for SIV infection. Only supportive treatment is suggested for infected animals.

SWINE INFLUENZA
CONTROL

- The best way to control swine influenza is to prevent the occurrence and spread of the disease.
- Good husbandry practices including All-in/All-out to limit the spread of the disease, provision of fresh clean drinking water, avoiding ducks and turkey contamination's/contact including staff and proper use of disinfectants to clean infected buildings.
- Commercial vaccines are available in Europe and North America.

SWINE INFLUENZA
PUBLIC HEALTH HAZARDS

- Swine Influenza may rarely affect human beings. Hence, all work with infectious tissues, swabs, embryonated eggs, and cell cultures should be done in a class II biological safety cabinet.

MODULE-9: RHABDOVIRUSES - RABIES

Learning objectives

- Group V – Negative sense RNA viruses
  - About the family Rhabdoviridae and its member viruses including rabies
  - About the morphology and the nature of the virus
  - About the disease and its pathogenesis
  - About the diagnosis and various lab tests
  - About vaccines
  - Public health hazard
  - Control of Rabies

RABIES
INTRODUCTION

- Rabies is caused by a neurotropic virus of the genus Lyssavirus of the family Rhabdoviridae, and is transmissible to all mammals. It is considered as one of the very serious diseases.

RABIES
CLASSIFICATION

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Rhabdoviridae
Genus	Lyssa virus
Species	Classical Rabies virus (CRBV)

- There are seven distinct genetic lineages under lyssa viruses. They are
  - Classical rabies virus itself (RABV)
  - Lagos bat virus (LBV)
  - Mokola virus (MOKV)
  - Duvenhage virus (DUUV)
  - European bat lyssaviruses (EBLV) – 2 biotypes
  - Australian bat lyssavirus (ABLV)

RABIES
MORPHOLOGY

- Rabies virus is approximately 180 nm long and 75 nm wide and is bullet shaped. It is an enveloped virus and hence is ether sensitive. The nucleic acid is single stranded and negative sense RNA. The genome encodes 5 proteins designated as N, P, M, G, and L.
- The RNA is arranged as ribonucleoprotein core (RNP) along with nuceleoprotein (N), polymerase (L) and phosphoprotein (P). Inside the core the RNA is tightly encased by the nucleoprotein. The surface proteins are matrix protein (M) and glycoprotein (G).
- The glycoprotein forms approximately 400 trimeric spikes, which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP.

RABIES
SYNONYMS

- Hydrophobia, mad dog disease.

RABIES
GENERAL POINTS

List A disease
Risk Group III
on property: Virus does not haemagglutinate.

La

RABIES
RESISTANCE

- The virus is killed on exposure to 70°C for 15 minutes. Virus can be preserved for more than one year in freezing temperatures. UV rays and disinfectants like phenol, chloroform destroys the virus rapidly.

RABIES
REPLICATION

- Rabies virus attaches to a cell by interaction between G protein and receptors on the surface of cells. After adsorption, the virus penetrates the host cell and enters the cytoplasm by pinocytosis (via clathrin-coated pits).
- The virions are present in the cytoplasm as large endosomes (cytoplasmic vesicles). The viral membranes fuse to the endosomal membranes, causing the release of viral RNP into the cytoplasm (uncoating).
- Since rabies virus have a linear single-negative-stranded ribonucleic acid (RNA) genome, messenger RNAs (mRNAs) must be transcribed to permit virus replication, which is facilitated by polymerase (L) present in the core.
- The polymerase (L) transcribes the genomic strand of rabies RNA into leader RNA and five capped and polyadenylated mRNAs, which are translated into proteins.

RABIES
VIRUS STRAINS

- Flury, Street-Alabama-Dufferin [SAD], Vnukovo and Kelev. Rabies viruses are also referred as Street viruses and fixed viruses.
- Street viruses refer to virulent viruses obtained from rabid animals and fixed viruses are those, which are adapted in laboratories by passaging them in animals such as rabbits.
- Fixed viruses are characterised by a shortened incubation period and multiplies faster. Fixed virus also loses their ability to form Negri bodies, less pathogenic, stable and are used for vaccine production.

RABIES
CULTIVATION

- Rabies viruses are normally cultivated in tissue culture lines, such as WI-38, BHK-21, or CER. Since rabiesvirus induce minimal CPE, presence of rabiesvirus in tissue culture system is confirmed by antigen detection systems.
- The other more commonly used method for virus isolation is by the inoculation of saliva, salivary gland tissue and brain tissue intracerebrally into infant mice. The mice should develop paralysis and death within 28 days. Upon death, the brains are examined for the presence of the virus by immunofluorescence.
- The virus can be cultivated in chicken embryos but the lesions are irregular and not characteristic.

RABIES
PATHOGENESIS

ted,
period,
on,
and mortality etc.

RABIES
HOSTS AFFECTED

- All mammals are affected by rabies.

RABIES
TRANSMISSION

- Rabies virus is more commonly transmitted to a new host only through an open wound or, less likely, through the mouth, the eyes, or the mucous membranes of the nose. Since the virus is present in the saliva and brain material of infected individuals, most transmission events occur through bite wounds.
- The respiratory transmission has been reported in very rare circumstances. The incubation period vary from as little as a few days to many years in rare cases. However, in most cases, incubation occurs within one to three months. Once an individual is infected with the rabies virus, it replicates within the cytoplasm of muscle cells and pass from cell to cell.

- Finally, it reaches nerve receptors and enters the nervous system. The virus passes along the nerve network, traveling to the central nervous system, where it concentrates in the brain and upper spinal cord. As the disease progresses, the virus continues to multiply and spreads back through the peripheral nervous system to the salivary glands.
- Not all animals or humans exposed to the virus contract the disease. However, once symptoms become evident, the disease usually is fatal. Many animals like dogs, bats, skunks act as reservoirs.
- When dogs act as reservoirs for spread of rabies to other animals including dogs it is referred as canine rabies. In Asia, Africa and Europe canine rabies are common. However, in US bat rabies pose major problem.

RABIES
INCUBATION PERIOD

- The incubation period is both prolonged and variable. Most cases in dogs occur within 21-80 days after exposure, but the incubation period may be shorter or considerably longer.

RABIES
MORBIDITY AND MORTALITY

- Rabies is a fatal infection and once symptoms are exhibited animals will certainly die.

RABIES
SYMPTOMS

- Clinical signs of rabies are not very definitive. Rabid animals of all species exhibit typical signs of CNS disturbance, with minor variations among species. The most reliable signs, regardless of species, are behavioral changes and unexplained paralysis.
- Behavioral changes include anorexia, signs of apprehension or nervousness, irritability, and hyperexcitability. The animal may prefer to be in isolation. Ataxia, altered phonation (sound), and changes in temperament are apparent. Uncharacteristic aggressiveness may develop—a normally docile animal may suddenly become vicious. Commonly, rabid wild animals lose their fear of man, and species that are normally nocturnal may be seen wandering about during the daytime.
- The clinical course is divided into three phases—prodromal, excitative, and paralytic. During the prodromal period, which lasts 1-3 days, animals show only vague CNS signs, which intensify rapidly. The disease progresses rapidly after the onset of paralysis, and death is virtually certain within 10 days after the initial onset of signs. Some animals die rapidly without marked clinical signs.
- Normally rabies is exhibited as
    - “Furious rabies” refers to animals in which aggression (the excitative phase) is pronounced and
    - “Dumb or paralytic rabies” in which the behavioral changes are minimal or absent, and the disease is manifest principally by paralysis.

RABIES
FURIOUS FORM

- This is referred as “mad-dog syndrome,” although it is seen in all species. There is rarely any evidence of paralysis during this stage. The animal becomes irrational and, with the slightest provocation, may viciously and aggressively use its teeth, claws, horns, or hooves. The posture and expression is of alertness and anxiety, with pupils dilated.
- Noise invites attack. Such animals lose all caution and fear of natural enemies. Carnivores with this form of rabies frequently roam extensively, attacking other animals, including people, and any moving object. They commonly swallow foreign objects, eg, feces, straw, sticks, and stones.
- Rabid dogs chew the wire and frame of their cages, breaking their teeth, and will follow a hand moved in front of the cage, attempting to bite.
- Young pups apparently seek human companionship and are overly playful, but bite even when petted, usually becoming vicious in a few hours. Rabid skunks appear to seek out and attack litters of puppies or kittens.
- Rabid domestic cats and bobcats attack suddenly, biting and scratching viciously. As the disease progresses, muscular incoordination and seizures are common. Death is the result of progressive paralysis.

RABIES
DUMB FORM

- This is first manifested by paralysis of the throat and masseter muscles, often with profuse salivation and inability to swallow. Dropping of the lower jaw is common in dogs.
- Owners frequently examine the mouth of dogs and livestock searching for a foreign body or administer medication with their bare hands, thereby exposing themselves to rabies. These animals are not vicious and rarely attempt to bite. The paralysis progresses rapidly to all parts of the body, and coma and death follow in a few hours.
- Cattle with furious rabies are dangerous, attacking and pursuing man and other animals. Lactation ceases abruptly in dairy cattle. Instead of the usual placid expression, there is one of alertness. The eyes and ears follow sounds and movement. A common clinical sign is a characteristic abnormal bellowing, which may continue intermittently until shortly before death.
- Horses and mules frequently show evidence of distress and extreme agitation. These signs, especially when accompanied by rolling, may be interpreted as evidence of colic. As with other species, horses may bite or strike viciously and, because of size and strength, become unmanageable in a few hours. Such animals frequently suffer self-inflicted wounds.

RABIES
LESIONS

- The most important lesions in rabies is encephalomyelitis (inflammation) in brain tissue and meninges with mononuclear infiltration, perivascular cuffing of lymphocytes or polymorphonuclear cells, Babes nodules consisting of glial cells and Negri bodies.
- Negri bodies appear as round or oval inclusions within the cytoplasm of nerve cells of animals infected with rabies. Negri bodies may vary in size from 0.25 to 27 μm. They are found most frequently in the pyramidal cells of Ammon's horn, and the Purkinje cells of the cerebellum. They are also found in the cells of the medulla and various other ganglia. Negri bodies can also be found in the neurons of the salivary glands, tongue, or other organs.

- Staining with Mann's, giemsa, or Sellers stains can permit differentiation of rabies inclusions from other intracellular inclusions. With these stains, Negri bodies appear magenta in color and have small (0.2 µm to 0.5 µm), dark-blue interior basophilic granules.

RABIES
DIAGNOSIS

- Described as
  - Field and
  - Laboratory diagnosis.

RABIES
FIELD DIAGNOSIS

- Is based on characteristic symptoms of rabies coupled with presence of Negri bodies in the brain.

RABIES
LABORATORY DIAGNOSIS - DIRECT DETECTION OF VIRUS ANTIGEN IN TISSUE MATERIALS

- The ideal clinical materials for rabies virus identification are brain, salivary gland and saliva. In the brain, rabies virus is particularly abundant in the thalamus, pons and medulla.
  - *Histological identification of characteristic lesions:* Demonstration of Negri bodies by Sellers method (for unfixed sections) or Mann's method (for fixed sections).
  - *Fluorescent antibody test:* This is the most widely used test for rabies diagnosis and is recommended by both WHO and OIE.
  - Immunoperoxidase test
  - *ELISA:* Referred as rapid rabies enzyme immunodiagnosis test (RREID)
  - PCR and DNA sequencing
  - DNA probe
  - Electron microscopy
  - Monoclonal antibody based typing

RABIES
ISOLATION AND IDENTIFICATION

- Is attempted FAT gives an uncertain result or when the FAT is negative in the case of known human exposure.
  - *Mouse inoculation test:* The infected mice develop paralysis and death occurs within 28 days. Upon death, the brain should be examined for the presence of the virus by immunofluorescence.

- *Cell culture system:* Neuroblastoma cell lines, e.g. CCL-131 is commonly used for isolation of rabies virus. Presence of rabies virus in the cells is revealed by the FAT.

RABIES
SEROLOGICAL TESTS

- Serological tests are rarely used due to late seroconversion and the low percentage of animals surviving the disease having post-infection antibodies. The tests that are normally performed are
  - *Virus neutralisation test in cell culture:* Fuorescent antibody virus neutralisation test – FAVN (accepted for international trade). The principle of the fluorescent antibody virus neutralisation (FAVN) test is the neutralisation in vitro of a constant amount of rabies virus before inoculating cells susceptible to rabies virus in BHK-21 C13 cells.
  - Rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralising antibody (accepted test for international trade).
  - Virus neutralization in mice.
  - *ELISA:* Indirect ELISA is used to determine the level of rabies antibodies in individual dog and cat serum samples following vaccination.

RABIES
CONTROL

- Dealt under
  - Control of rabies in dogs and
  - Vaccines.

RABIES
CONTROL OF RABIES IN DOGS

uidelines for control in dogs have been prepared by the World Health Organization and include the following:
n of suspected cases, and destruction of dogs with clinical signs and dogs bitten by a suspected rabid animal.
of contact rates between susceptible dogs by leash laws, dog movement control, and quarantine.
unization of dogs by campaigns and by continuing vaccination of young dogs.
control and destruction of unvaccinated dogs with low levels of dependency on, or restriction by man.
ration.

RABIES
VACCINES

- *Pasteur type vaccine:* One of the oldest vaccines against rabies. Prepared by injecting the fixed virus into rabbits. The brain cords are collected and dried by dessication and treating with KOH. Not in use at present.
- *Nervous tissue preparation and phenolized (Fermi, Semple and Umeno-doi vaccines):* This consisted of a 5% suspension of infected animal nervous tissue which had been inactivated (eg. the Semple vaccine was derived from phenol-inactivated

infected rabbit brain), These preparations are now out of date as they were associated with the rare complication of demyelinating allergic encephalitis. This appears to be related to myelin basic protein in the vaccine. This complication was shown to occur in 4.6 case for 1000 persons vaccinated by the Semple vaccine. The case-fatality proportion was 3.13%. The Semple vaccine is still used in some developing countries. A suckling mouse brain vaccine is used in some Central and S.American countries.

- *UV treated vaccines (Webster):* Inactivated vaccines prepared from brain of infected animals, which were exposed to UV rays. UV rays readily kill the rabies virus.
- Duck Embryo Vaccine (Peck) - this vaccine strain is grown in embryonated duck eggs and is inactivated with B-propriolactone. This vaccine has a lower risk of allergic encephalitis. However, it is considerably less immunogenic and does have minor side effects. Almost all vaccinees experience local reactions, 33% have constitutional symptoms such as fever, malaise, myalgia, and generalized lymphadenopathy.
- *Live vaccines – (Hoegyes):* Live fixed viruses are given at a very low level (below the infective dose).
- *Avianized vaccines (Koprowsky and Cox):* The Flurry strain of rabies virus grows in chicken embryos. It loses its pathogenicity after 40-50 passages in embryos for rabbits and dogs (referred as low egg passage vaccines – LEP) and loses the pathogenicity completely except for mice after 180 passages (referred as high egg passage vaccines – HEP). HEP were used for humans and LEP were used only for dogs and not for any other animals.
- *Human Diploid Cell Vaccine (HDCV):* HDCV was introduced in 1978. It is a grown on WI-38 (U.S.) or MRC-5 (Europe) cells. The vaccine is highly effective, in several studies, antibodies have been demonstrated in 100% of all recipients. Serious adverse reactions to HDCV are extremely rare. However, the vaccine is very expensive, as human cell cultures are more difficult to handle than other animal cell culture systems.
- Efforts are being made to use other inexpensive cell culture systems such as VERO cells.

RABIES
PUBLIC HEALTH HAZARDS

- Rabies virus causes highly fatal disease in humanbeings. The incubation period is highly variable, ranging from 7 days to several years. It depends on several factors such as dose of inoculum, severity of the wound, length of the neural path from the wound to the brain e.g. wounds on the face have a shorter incubation period than wounds in the leg.
- The illness begins with a non-specific prodrome period, comprising of fever, malaise, anorexia, sore throat, myalgia and headache. The patient nay exhibit irritability and abnormal sensations around the wound. The prodrome is followed by one of two basic clinical patterns: the more common "furious" form characterized by hyperexcitability, spasms and hydrophobia; or "dumb" rabies featuring an ascending paralysis. Survival tends to be longer for patients with "dumb" rabies than those with "furious" rabies.
- Complications involving the Cardiovacular System, CNS, and the respiratory systems eventually develop and contribute to death. Cardiac dysrhythmias of all types occur and respiratory disturbances occur in all cases. Raised intracranial pressure contributes to the decreased level of consciousness and to focal convulsions. Other CNS complications include disturbances of thermoregulation, diabetes insipidus, autonomic dysfunction and convulsions.
- Once rabies is established, it is always fatal (except in one case in US).

RABIES

PRE EXPOSURE PROPHYLAXIS

- Persons who are regularly at high risk of exposure, such as vets, laboratory workers, animal handlers and wildlife officers should be considered for preexposure prophylaxis by active immunization with the cell culture vaccine.
- Immunization normally consists of 3 doses of vaccine. Antibody can be demonstrated in the sera of virtually 100% of those vaccinated if the diploid cell culture vaccine is used.
- Booster doses should be offered to persons at continuing risk every one to three years. Local treatment of wounds should always be carried out in exposed persons who have been vaccinated previously.

RABIES
POST EXPOSURE PROPHYLAXIS

- In cases of animal bites, dogs and cats in a rabies endemic area should be held for 10 days for observation. If signs develop, they should be killed and their tissue examined in the laboratory. Wild animals are not observed but if captured, the animal should be killed and examined. The essential components of postexposure prophylaxis are the local treatment of wounds and active and passive immunization.
    - *Wound treatment:* Surgical debridement should be carried out. The wound should not be sutured up.
    - *Passive immunization:* Human rabies immunoglobulin should be applied around the area of the wound; to be supplemented with an i.m. dose to confer short term protection. There is convincing evidence that combined treatment with rabies immunoglobulin and active immunization is much more effective than active immunization alone. Equine rabies immunoglobulin (ERIG) is available in many countries and is considerably cheaper than HRIG.
    - *Active immunization:* The human diploid cell vaccine is the best preparation available. The vaccine is usually administered into the deltoid region, and 5 doses are usually given.

MODULE-10: RHABDOVIRUS - BOVINE EPHEMERAL FEVER

Learning objectives

- Group V – Negative sense RNA viruses
    - About the morphology and the nature of the virus
    - About the disease and its pathogenesis
    - About the diagnosis and various lab tests
    - About vaccines
    - Control.

BOVINE EPHEMERAL FEVER
INTRODUCTION

- **Classification**

Baltimore group	Group V – Negative sense RNA viruses
Order	Mononegavirales
Family	Rhabdoviridae
Genus	Ephemero virus
Species	Bovine ephemeral fever virus (BEFV)

BOVINE EPHEMERAL FEVER
MORPHOLOGY

- Typical of other rhabdoviridae. The BEF virus is an enveloped virus and hence ether sensitive.
- The genome is single-stranded, negative sense linear RNA. The genome has five genes coding for five proteins.

BOVINE EPHEMERAL FEVER
SYNONYMS

- Three-day sickness, Bovine epizootic fever, Three-day stiff sickness, Dragon boat disease.

BOVINE EPHEMERAL FEVER
OIE LISTING AND RISK GROUP

- *OIE listing:* No listing; SPC List D disease
- *Risk group:* Group I

BOVINE EPHEMERAL FEVER
RESISTANCE

- Typical of other rhabdoviruses. The virus is rapidly inactivated at pH levels below 5.

BOVINE EPHEMERAL FEVER
REPLICATION

- *Replication:* Typical of other rhabdoviruses.

BOVINE EPHEMERAL FEVER
HA PROPERTY

- *HA property:* The virus has no HA property.

BOVINE EPHEMERAL FEVER

VIRUS STRAINS

- This virus is antigenically related to at least three other viruses non-pathogenic for cattle:
  - Kimberley virus,
  - Berrimah virus, and
  - Adelaide River virus and
  - two that produce an ephemeral fever-like disease in cattle, Kotonkan and Puchong viruses.

BOVINE EPHEMERAL FEVER
CULTIVATION

- BEFV are cultivated in embryonated eggs, suckling mice, vero cells and cattle. BEFV are cultivated through intravenous inoculation into chicken embryos.
- Lesions are not characteristic and infected embryos die. BEFV are identified by immunofluoresence in heart, brain, lung and liver of chicken embryos at 1-5 days and in lung and liver of one-day-old chickens at 1-2 days.

BOVINE EPHEMERAL FEVER
PATHOGENESIS

- Dealt as host affected, distribution, transmission etc.

BOVINE EPHEMERAL FEVER
HOSTS AFFECTED

- Cattle and buffaloes are the main host.

BOVINE EPHEMERAL FEVER
DISTRIBUTION

- BEF occurs widely across Africa and Asia, and in areas of northern and eastern Australia. It does not occur in Europe or the Americas.

BOVINE EPHEMERAL FEVER
TRANSMISSION

- The BEF virus spreads in nature only by an insect bite. The disease will not spread from cow to cow by close contact, droplet infection, bodily excretions including semen, or by the transfer or injection of exudates. Strong winds can transport vectors long distances, over land and water.
- Epizootics of BEF are associated with recent rainfall. Mosquitoes are the major vectors in spreading BEF. The BEF virus has been isolated from Culicine and Anopheline mosquitoes and biting midges.

- However, establishment of the disease depends on suitable environmental conditions for the vector to increase and spread. There is also no carrier stage as BEFV is only present in the blood for short periods during the acute stage of the diseases.

BOVINE EPHEMERAL FEVER
INCUBATION PERIOD

- The incubation period is between 2 and 4 days, and 9 days in rare cases.

BOVINE EPHEMERAL FEVER
MORBIDITY AND MORTALITY

- Morbidity and morality are highly variable. Morbidity is very high and mortality is very low.

BOVINE EPHEMERAL FEVER
SYMPTOMS

- The clinical signs are very characteristic and can be very severe. Fever is most important symptom. The fever of ephemeral fever is generally biphasic, sometimes triphasic, with peaks of 40-41.5°C (104-107°F) spaced 12-18 hours apart.
- The physical signs during the first febrile phase tend to be mild except for the dramatic fall in milk production of lactating cows. The characteristic signs associated with BEF are those of the second febrile phase. These signs include accelerated heart and respiratory rates, anorexia, ruminal atony, depression, serous or mucoid nasal and ocular discharges, salivation, muscle twitching or waves of shivering, a generalized stiffness or a shifting lameness.
- There may be submandibular edema or patchy edema on the head. Nasal and ocular discharges, drooling of saliva and periorbital swelling and increased excitability and agitation are other signs. With the onset of fever, there is a rapid fall in circulating lymphocytes with eosinopenia.
- Many animals become recumbent for 12-24 hours. Animals may be unable to rise and remain in sternal recumbency for hours or days with the head turned to the flank, or in lateral recumbency with or without loss of most reflexes.
- Recovery begins 1-2 days after the clinical signs are first noticed and is usually complete and without sequelae in a further 1- 2 days after the overt clinical signs are first noticed. By day three the affected animal is usually standing again and will begin to eat.
- However, lameness and weakness may last for another two or three day. Milk yield will return to normal after three weeks. Death can occur suddenly in the febrile or in the recovery phase.

BOVINE EPHEMERAL FEVER
LESIONS

- The most important gross lesions are accumulation of fibrin-rich fluid in the pleural, peritoneal, and pericardial cavities and in the joint capsules.
- The lungs may show patchy edema. Lymphadenitis with petechial hemorrhages and focal necrosis are also observed.

BOVINE EPHEMERAL FEVER
DIAGNOSIS

- Dealt as field diagnosis, laboratory diagnosis etc.

BOVINE EPHEMERAL FEVER
FIELD DIAGNOSIS

- Field diagnosis is made from clinical observations (presence of lameness, muscular stiffness, pain, rapid spread of the disease through herds and short fever) and the history of the outbreak.

BOVINE EPHEMERAL FEVER
ISOLATION AND IDENTIFICATION

material and should be taken during the period of fever and another sample 1-2 weeks later. Samples should be take
the disease to facilitate a rapid laboratory confirmation
ample of blood is allowed to clot, and another portion is mixed with anticoagulant. From the uncoagulated blood, a s
lowed to dry in air. The balance is used for virus isolation. When blood taken during illness is allowed to clot, it usua
er several days. It may be streaked with fibrin.
r isolation of the virus. Blood from infected cattle is used as inoculum for infecting cattle. Exhibition of characteristi
EFV.

BOVINE EPHEMERAL FEVER
SEROLOGY

- Virus neutralization test is commonly done to identify antibodies against BEFV.
- ELISA is also done.

BOVINE EPHEMERAL FEVER
DIFFERENTIAL DIAGNOSIS

- BEF confuse with infections like early Rift Valley fever, FMD, heartwater, bluetongue, botulism, babesiosis and blackleg.

BOVINE EPHEMERAL FEVER
TREATMENT

- Ephemeral fever is one of the rare virus diseases for which treatment is effective.
- Since the infection is inflammatory in nature, antiinflmmatory drugs are useful in controlling the infection.
- During fever, the paresis or paralysis responds to injected calcium borogluconate in the same manner as parturient paresis (milk fever).

BOVINE EPHEMERAL FEVER
VACCINES

- Once cattle have been infected with the disease, most are resistant to infection for many years or for life. However, live attenuated vaccines, killed vaccine and a sub-unit vaccine has been used in certain part of the world.
- The live vaccine gives at least 12 months protection after two doses. The inactivated vaccine gives protection for about six months protection. Animals can be vaccinated from six months of age and should then be revaccinated each year to ensure continued protection.

BOVINE EPHEMERAL FEVER
PREVENTION

- Vaccination in endemic areas.
- Vector control: Useful steps are to place the cattle in an insect-proof area, spray with insecticides, or suppress insects in the local environment.

BOVINE EPHEMERAL FEVER
PUBLIC HEALTH HAZARD

- There is no evidence of human infection.

MODULE-11: PICORNOVIRUSES

Learning objectives

- Group IV – Positive sense RNA viruses
    - About the family picornaviridae and its member viruses - FMDV
    - About the morphology and the nature of the virus
    - Cultivation systems for FMDV
    - About the disease and its pathogenesis
    - About the diagnosis and various lab tests
    - About vaccination strategies and vaccines available for field use
    - Control

FOOT AND MOUTH DISEASE
INTRODUCTION

- Members of the family Picornaviridae are very small. The work pico in Greek means small. This family contains important viruses like
    - Foot and mouth disease virus (first animal viruses to be known),
    - Poliomyelitis,
    - Duck viral hepatitis etc.
- Foot and mouth disease (FMD) is the most contagious disease of mammals and cause severe economic loss in susceptible cloven-hoofed animals (cattle, pigs, sheep, goats, and water buffalo).

- The disease is characterised by the formation of vesicles (fluid-filled blisters) and erosions in the mouth, nose, teats and feet. The infection is not very lethal in adult animals. But it causes serious production losses and is considered as a major problem in international trade.

FOOT AND MOUTH DISEASE
GENERAL ASPECTS

- Deals about
  - Classification,
  - Morphology,
  - Physicochemical properties,
  - Replication,
  - Resistance,
  - Strains etc.

FOOT AND MOUTH DISEASE
CLASSIFICATION

-

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Picornaviridae
Genus	Aphthovirus
Species	Foot and mouth disease virus
Type species	FMDV O strain – FMDV O

FOOT AND MOUTH DISEASE
MORPHOLOGY

- The virions are non-enveloped and hence is not ether sensitive. The virion has icosahedral symmetry. The virion comprises of four polypeptides, VP1, 2, 3 and 4 and all these proteins are derived from cleavage of a single polypeptide VP0.
- The virion contains one molecule of linear positive-sense single stranded RNA. The Genomic RNA is infectious. Both ends of the genome are modified, the 5' end by a covalently attached small, basic protein VPg and the 3' end by polyadenylation.
- The genome contains approximately 7000-8500 nucleotides. 2,500nm and it is tightly packed into the capsid.

FOOT AND MOUTH DISEASE
SYNONYMS

- Afta epizootica, Bek-en-klouseer, Fiebra aftosa, Fievre aphteuse, Maul-und-Klauenseuche, Komari (vernacular), Kal voi noi (vernacular).

FOOT AND MOUTH DISEASE
OIE LISTING AND RISK GROUP

- *OIE Listing:* List A infection.
- *Risk group:* The virus can affect human beings. Since the infection is uncommon, FMD is not considered as a public health problem.

FOOT AND MOUTH DISEASE
RESISTANCE

- Under in vitro conditions virions relatively stable. The FMD virus is pH sensitive and is inactivated at pH below 6.5 or above 11. In milk and milk products, the virion can survive at 70°C for 15 seconds and pH 4.6. The virus in cell culture medium will remain viable for a year at 4°C.
- The virus in serum or other organic material will survive drying and can be carried on inanimate objects. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes.
- The virus inactivated by sodium hydroxide (2%), sodium carbonate (4%), and citric acid (0.2%) and is Resistant to iodophores, quaternary ammonium compounds, hypoclorite and phenol, especially in the presence of organic matter.

FOOT AND MOUTH DISEASE
REPLICATION

- Replication occurs in the cytoplasm and is rapid. The replication cycle occurs very fast and is completed between 5-10 hours. Protein synthesis in the virus infected cell declines sharply almost to nil level and this is called Shut off.
- Since the genome is positive sense, it is immediately translated into a polyprotein, which is cleaved into enzymes required for genome replication and into structural protein by protease enzymes.
- One such product is the RNA-dependent RNA polymerase enzyme, which copies the genomic RNA to produce a (-) sense strand. This (-) sense strand replicative intermediate and from which (+) sense RNAs produced. These (+) sense RNAs are packed into the capsid. RNA is packed into preformed capsids. Hence, empty capsids (defective) are common in all Picornavirus infections. Release (in most cases) of the virus from the cytoplasm occurs when the cell lyses.
- This is a 'preprogrammed' event, which occurs a set time after the cessation of 'housekeeping' macromolecular synthesis at shutoff. The sequence of events that take place in a cell infected with FMDV is as below:

Time after infection	Event
1-2h	Sharp decrease in host cell macromolecular synthesis
2.5-3h	Start of viral protein synthesis (Positive sense RNA hence translated immediately)

3-4h	Permeabilization of plasma membrane
4-6h	Virus assembly in cytoplasm
6-10h	Lysis of cell and release of virus particles

FOOT AND MOUTH DISEASE
HA PROPERTY

- The virus has not HA property.

FOOT AND MOUTH DISEASE
STRAINS AND SEROTYPES

- There are seven serotypes of FMDV:
  - A, O, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. Within these serotypes, over 60 subtypes have been described, and new subtypes occasionally arise spontaneously.
  - The distribution of serotypes and subtypes globally is given below.

Geographic location	Serotypes and subtypes
Europe (historically)	A (5), O (1), C (1)
Asia	
Near East	A (22), O (1)
Middle East	A (22), O (1), C, Asia (1)
Far East	A, O (1), C, Asia (1)
Africa	
Central East to West	A, O
Northeast Central and South	SAT 1, and 2
South	SAT 3 Serotype C is uncommon in Africa
South America	A (24), A (27), O (1), C (3)

FOOT AND MOUTH DISEASE
CULTIVATION

- *Cell cultures:* The virus grows well in cell cultures. The cell cultures that are commonly used are primary bovine thyroid cells, primary pig, calf or lamb kidney cells and cell lines, such as BHK-21 (baby hamster kidney) and IB-RS-2 cells. Cytopathic effects are produced in 24-48 hours.

- *Laboratory animals:* Unweaned mice are used for cultivation FMDV. Mice of 2-7 days old are commonly used.

FOOT AND MOUTH DISEASE
PATHOGENESIS

- Deals about
  - Hosts infected,
  - Distribution,
  - Transmission,
  - Symptoms,
  - Incubation period,
  - Mortality and morbidity,
  - Lesions etc.

FOOT AND MOUTH DISEASE
HOSTS AFFECTED

- Cloven-footed domestic and wild animals are primarily affected. Bovines (cattle, zebus, domestic buffaloes, yaks), sheep, goats, swine and all wild ruminants are severely affected.
- Members of the family Camelidae have low susceptibility. Other susceptible species are hedgehogs, armadillos, nutrias, elephants, capybaras, rats, and mice.

FOOT AND MOUTH DISEASE
DISTRIBUTION

- Foot-and-mouth disease is widely distributed throughout the world. Endemic areas were Asia, Africa, and parts of South America.
- Most European countries have are generally free from FMD barring few outbreaks.
- Countries belonging to the European Union have stopped FMD vaccination. North and Central America, Australia, New Zealand, Japan, and the British Isles are free from FMD.

FOOT AND MOUTH DISEASE
TRANSMISSION

- FMDV spread by following methods:
  - Direct or indirect contact with infected animals.
  - Through aerosol from infected animals
  - Feeding contaminated garbage (meat, milk, blood, glands, bones, cheese, etc.)
  - Contact with contaminated objects (hands, footwear, clothing).
  - Artificial insemination.
  - Contaminated biological such as hormones
  - Through animate objects (A person in contact with infected animals can have sufficient FMDV in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals)

    - As airborne, especially in temperate zones for a distance of (up to 60 km overland and 300 km by sea.
- In an outbreak of FMD, the roles of the three primary hosts in transmission are as follows:
    - Sheep act as maintenance hosts,
    - Pigs act as amplifiers,
    - Cattle act as indicators.
- When sheep or goats become infected with FMDV, the disease may not be diagnosed for a long time because signs and lesions are very mild. But during this time, the animals will be producing infectious aerosols, contaminating fomites, and spreading the virus by contact.
- Foot-and-mouth disease in pigs spreads very rapidly, for they produce 30 to 100 times more virus in aerosols than sheep or cattle. (An infected pig can produce a hundred million infectious doses per day).
- When cattle are infected with FMDV, signs and lesions usually develop more rapidly and are more severe than in pigs, sheep, or goats. If cattle, sheep, and pigs are exposed together, cattle will usually get sick first.
- Some animals particulary recovered cattle can be carriers of FMDV. Most ruminant species harbour the virus in their pharyngeal tissues for a long period.

FOOT AND MOUTH DISEASE
INCUBATION PERIOD

- In experimentally infected cattle the incubation period is 24-48 hours. In other conditions the incubation period is 2-14 days.
- In pigs the incubation period is as short as 1-3 days.

FOOT AND MOUTH DISEASE
MORBIDITY AND MORTALITY

- The morbidity is 100 percent and mortality is usually less than 1 percent, but in young animals and with certain isolates mortality can be high.

FOOT AND MOUTH DISEASE
SYMPTOMS

- Dealt separately for
    - Cattle,
    - Sheep and goat and
    - Pigs.

FOOT AND MOUTH DISEASE
SYMPTOMS - CATTLE

- Initial signs are pyrexia (39.4-40.6°C), dullness, anorexia, and fall in milk production. These signs are followed by excessive salivation; drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation.

- Sites of predilection for vesicles are the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats. After vesicle formation, drooling may be more marked, and nasal discharge, lameness or both may increase.
- Pregnant cows may abort, and young calves may die without developing any vesicle. The course of an FMD infection is 2 to 3 weeks. Secondary infection may delay recovery.
- A lactating animal may not recover to preinfection production because of damage to the secretory tissue.
- Complications include tongue erosions, superinfection of lesions, hoof deformation, mastitis and permanent impairment of milk production, myocarditis, abortion, death of young animals, permanent loss of weight, loss of heat control ('such animals are called as panters').

FOOT AND MOUTH DISEASE
SYMPTOMS - SHEEP AND GOAT

- Clinical signs, if they occur, tend to be very mild, and may include dullness; fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign.
- In lame animals there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort. Nursing lambs may die without showing any clinical sign.

FOOT AND MOUTH DISEASE
SYMPTOMS - PIGS

- Initial signs are fever (40-40.6°C), anorexia, reluctance to move, and scream when forced to move. These signs are followed by vesicles on the coronary band, heals, interdigital space and on the snout.
- Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-type lesion. There is no drooling. Sows may abort. Piglets may die without showing any clinical sign.

FOOT AND MOUTH DISEASE
LESIONS

- Dealt separately for
  - Cattle,
  - Sheep and goat and
  - Pigs.

LESIONS - CATTLE

- The diagnostic lesions are single or multiple vesicles ranging from 2 mm to 10 cm. These can occur at all sites of predilection.

Gross lesions on the tongue

- The lesions usually progress in the following manner

    - A small blanched whitish area develops in the epithelium.
    - Fluid fills the area, and a vesicle (blister) is formed.
    - Vesicle enlarges and may coalesce with adjacent ones.
    - Vesicle ruptures.
    - Vesicular covering sloughs leaving an eroded (red) area
    - Gray fibrinous coating forms over the eroded area.
    - Coating becomes yellow, brown or green.
    - Epithelium is restored, but line of demarcation remains; line then gradually fades.
- Occasionally "dry" FMD lesions develop. Instead of forming a vesicle, the fluid is apparently lost as it forms and the upper layers of the epithelium become necrotic and discolored. The lesion therefore appears necrotic rather than vesicular.

Gross Lesions on the Feet

- The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by movement and weight. The lesion at the coronary band at first appears blanched; then there is separation of the skin and horn. When healing occurs, new horn is formed, but a line resulting from the coronitis is seen on the wall of the hoof.

Gross Cardiac and Skeletal Lesions

- Animals that die have grayish or yellowish streaking in the myocardium - degeneration and necrosis. These findings are known as "tiger heart" .

FOOT AND MOUTH DISEASE
LESIONS - SHEEP AND GOAT

- Lesions in the mouth and vesicles on the coronary band may be few, small, and difficult to find.
- Animals that die may have grayish or yellowish streaking in the myocardium with degeneration and necrosis ("tiger heart").

FOOT AND MOUTH DISEASE
LESIONS - PIGS

- Vesicles on the snout can be large and filled with clear or bloody fluid. Mouth lesions are usually the "dry" type and appear as necrotic epithelium.
- Feet lesions are usually severe, and the hoof can become detached. Animals that die may have grayish or yellowish streaking in the myocardium with degeneration and necrosis ("tiger heart").

FOOT AND MOUTH DISEASE
DIAGNOSIS

- Dealt as
    - Field and
    - Laboratory diagnosis .

FOOT AND MOUTH DISEASE
FIELD DIAGNOSIS

- Field diagnosis is based on characteristic symptoms in cattle, sheep and pigs.
- FMD should be considered whenever salivation and lameness occur simultaneously and a vesicular lesion is seen or suspected.
- In pigs, sheep, and goats, FMD should be considered when animals have sore feet, vesicular lesion is suspected, or both.

FOOT AND MOUTH DISEASE
LABORATORY DIAGNOSIS

- Isolation and identification: The following are preferred clinical materials.
    - Vesicular fluid
    - Epithelium covering a vesicle
    - Flaps of epithelial tissue still attached
    - 5 ml of blood with anticoagulant during viraemic phase
    - Esophageal—pharyngeal (OP) fluid from convalescent cattle, sheep, or goats.
    - Serum
    - From dead animals - samples of epithelial lesions, lymph nodes, thyroid, adrenal gland, kidney, and heart
    - Specimens placed in ice should be delivered to a laboratory within 24 hours.
    - When delivery may take longer, samples may be quick-frozen and should not thaw during transit.
    - Isolation should be attempted in primary bovine thyroid cells, primary pig, calf or lamb kidney cells and cell lines, such as BHK-21 (baby hamster kidney) and IB-RS-2 cells. The CPE appears after 24-48 hours and the presence of virus should be confirmed by ELISA or PCR.
- Immunological methods of identification of virus
    - *ELISA:* The preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the ELISA as per the World Referral Laboratory for FMD (Refer General Characters Point 6). It is an indirect sandwich test in which different rows in multiwell plates are coated with rabbit antisera to each of the seven serotypes of FMD virus. These are the ‘capture’ sera. Test sample suspensions are added to each of the rows, and appropriate controls are also included. Guinea-pig antisera to each of the serotypes of FMD virus are added next, followed by rabbit anti-guinea-pig serum conjugated to an enzyme. Extensive washing is carried out between each stage to remove unbound reagents. A colour reaction on the addition of enzyme substrate, indicates a positive reaction. The ELISA is preferable to the complement fixation (CF) test because it is more sensitive and it is not affected by pro- or anti-complementary factors.
    - *Complement fixation test (CFT):* Antisera to each of the seven types of FMD virus are diluted in veronal buffer diluent (VBD) in 1.5-fold dilution steps from an initial 1/16 dilution to leave 25 µl of successive antiserum dilutions in U-shaped wells across a microtitre plate. To these are added 50 µl of 3 units of complement, followed by 25 µl of test sample suspension(s). The test system is incubated at 37°C for 1 hour prior to the addition of 25 µl of 1.4% standardised sheep red blood cells (SRBC) in VBD sensitised with 5 units of rabbit anti-SRBC. The reagents are

incubated at 37°C for a further 30 minutes and the plates are subsequently centrifuged and read. Appropriate controls for the test suspension(s), antisera, cells and complement are included. CF titres are expressed as the reciprocal of the serum dilution producing 50% haemolysis. A CF titre greater than or equal to 36 is considered to be a positive reaction. Titre values of 24 should be confirmed by retesting an antigen that has been amplified through tissue culture passage.

- Nucleic acid recognition methods
  - *PCR:* The polymerase chain reaction is commonly performed to identify virus in diagnostic materials. Specific primers have been designed to distinguish between each of the seven serotypes.
  - *In situ hybridisation*: This technique is used to identify viral RNA in tissue samples.

FOOT AND MOUTH DISEASE
SEROLOGY

- FMD virus infection can be diagnosed by the detection of a specific antibody response. The tests generally used are virus neutralisation (VN) and ELISA. These are also the prescribed tests for international trade.
  - *Virus neutralization test*: The VN test is serotype specific, requires cell culture facilities and takes 2–3 days to provide results.
  - *ELISA:* ELISA techniques are quantitative, serotype specific, sensitive and has the advantage of being quicker to perform, less variable, and are not dependent on tissue culture systems. The following types of ELISA are performed.
    - Solid-phase competitive enzyme-linked immunosorbent assay (Test for International trade).
    - Liquid-phase blocking enzyme-linked immunosorbent assay.
    - Indirect ELISA using recombinant non-structural proteins.
    - Enzyme linked immunosorbant blot assay.

FOOT AND MOUTH DISEASE
DIFFERENTIAL DIAGNOSIS

- FMD should be differentiated from vesicular stomatitis, swine vesicular disease, vesicular exanthema of swine, foot rot, and chemical and thermal burns.
  - In cattle, oral lesions caused by rinderpest, infectious bovine rhinopneumonitis, bovine virus diarrhea, malignant catarrhal fever, and bluetongue can be similar to the later lesions in FMD.
  - In sheep, lesions caused by bluetongue, contagious ecthyma, and lip and leg ulceration can be similar to the later lesions of FMD.

FOOT AND MOUTH DISEASE
TREATMENT

- There is no effective treatment and only supportive treatment is given.

CONTROL

on and eradication.

FOOT AND MOUTH DISEASE
VACCINATION

have been used to control FMD for a long time.
thod of vaccine production is a preferred method. Normal tongue epithelium was removed, minced, placed in a nutr
with FMDV. After replication of FMDV, the virus was inactivated with formalin, and aluminum hydroxide was adde
instead of tongue epithelium BHK21 cell line is used. These vaccines are prepared using binary-ethyleneimine (BEI)
um hydroxide-saponin or oil as an adjuvant. According to prevalence of serotypes in a geographical area vaccine co
is used. Aluminum hydroxide vaccine offers immunity for a duration of 4-6 months after two initial vaccinations, 1-
n vaccine offers protection for 1 year.
engineered vaccines are not as effective or as economical as the cell culture vaccines
inating animals, it is important that the vaccine contain the same subtype of virus as is in the area. This necessitates
d subtype during an outbreak because FMD virus frequently changes during natural passage through various specie
cinated animals that are not completely protected can be a source of infection. The virus may replicate and be shed,
linical sign of infection.

FOOT AND MOUTH DISEASE
ERADICATION

- To eradicate the disease a stamping out policy can be best applied. This involves quarantine, movement restrictions and slaughter and disposal of all affected an in-contact livestock on affected premises followed by cleaning and disinfection.
- The following are the essential features of a control and eradication program:
  - Prevention of movement of animals and animal products in the area affected.
  - Slaughter of infected animals and known contact animals
  - Destroy carcasses
  - Disinfect vehicles leaving the infected area.
  - Perform vaccination.
- Global eradication programmes have also been initiated by the FAO to control FMD under EMPRES. The following two programmes are aimed at controlling FMD at continental Europe and South East Asia respectively.
  - EUFMD – European Union FMD control programme.
  - SEAFMD – South East Asia FMD control programme.

MODULE-12: PICORNAVIRUS - DUCK VIRAL HEPATITIS

Learning objectives

- Group IV – Positive sense RNA viruses
  - About the morphology and the nature of the Duck viral Hepatitis virus
  - Cultivation systems for DVH virus
  - About the disease and its pathogenesis
  - About the diagnosis and various lab tests
  - About vaccination strategies and vaccines available for field use

- Control

DUCK VIRAL HEPATITIS
INTRODUCTION

- Duck hepatitis is caused by at least three different viruses, namely duck hepatitis virus (DHV) types I, II and III.
  - The most common is DHV type I is an enterovirus.
  - DHV type II is an astrovirus, and
  - DHV type III is considered to be a picornavirus.
- The more prevalent and internationally mentioned duck viral hepatitis is the duck hepatitis virus (DHV) type I, which is an enterovirus that causes a highly lethal, acute, contagious infection in ducklings upto 6 weeks of age. This infection is not seen in older birds.
- DHV type II is caused by an astrovirus. The infection occurs in ducklings from 10 days to 6 weeks of age, and caused pathological changes similar to those of DHV type I
- DHV type III is reported only in the United States of America. It causes similar liver lesions in young ducklings, but is less virulent than DHV type I. It is believed to be a picornavirus, serologically unrelated to type I virus.
- The viruses that cause hepatitis in ducklings should not be confused with duck hepatitis B virus, a hepadnavirus infection of older ducks.

DUCK VIRAL HEPATITIS
GENERAL ASPECTS

ion,
gy,
emical properties,
e and replication,
.

DUCK VIRAL HEPATITIS
CLASSIFICATION

For DHV I

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Picornaviridae
Genus	Enterovirus
Species	Duck viral hepatitis virus

For DHV II

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Astroviridae
Genus	Avastrovirus
Species	Duck viral hepatitis virus II

DUCK VIRAL HEPATITIS
MORPHOLOGY

- DHV I (Enterovirus of Pircornaviridae)
  - The virions not enveloped and hence are not ether sensitive. The capsids are 28-30 nm in diameter and the symmetry is icosahedral. Nucleocapsids appear to be round.
  - Incomplete virus particles (empty capsids) are often observed. The virions contain one molecule of linear positive-sense single stranded RNA. Total genome contains 7400 nt. The 5' end of the genome has a protein (VPg). 3' end has a poly (A) tract.
- DHV II (Astrovirus)
  - The virions are not enveloped and hence are not ether sensitive. The capsid is round and exhibits polyhedral symmetry. The capsid surface structure reveals a regular pattern with distinctive features (star-like with six points).
  - The surface projections appear as small spikes protruding from the 12 vertices. The genome is not segmented and consists of a single molecule of linear positive-sense single-stranded RNA. The complete genome is about 6800-7900 nucleotides long. There is no poly A tail.

DUCK VIRAL HEPATITIS
OIE LISTING AND RISK GROUP

- *OIE Listing:* List B infection.
- *Risk group:* No known infection to human beings.

DUCK VIRAL HEPATITIS
STRAINS AND SEROTYPES

- Under DHV Type Ia serological variant named DHV Type Ia has been identified

DUCK VIRAL HEPATITIS
CULTIVATION

- DHV I grows well in ducklings, embryonated eggs and in cell cultures.
  - *Ducklings:* Ducklings between 1 and 7 days of age are highly susceptible to DHV type I. The suspected materials are injected subcutaneously or intramuscularly.

The characteristic clinical signs are followed by death of ducklings within 18–48 hours of inoculation, often in under 24 hours.

- Duck eggs (10–14 days) and chicken eggs (8–10 days) are used for culturing DHV I. The route of inoculation is allantoic sac route. Duck embryos die between 24 and 72 hours later, whereas chicken embryos are more variable and die between 5 and 8 days. Gross pathological changes in the embryos include stunting and subcutaneous haemorrhages over the whole body, with oedema particularly of the abdominal and hind limb regions. The embryo livers may be red and yellowish, swollen and may show some necrotic foci. In embryos that take longer to die, the greenish colour of the allantois is more pronounced, and both the liver lesions and stunting become more prominent.
- Primary cell cultures like duck embryo liver (DEL) cells are commonly used. DHV type I cause a cytopathic effect (CPE), which is characterised by cell rounding and necrosis. When overlaid with a maintenance medium containing 1% agarose (w/v), the CPE gives rise to plaques approximately 1 mm in diameter.
- DHV II (Astrovirus) are difficult to cultivate under laboratory conditions.
- DHV III (Pirconavirus) can be cultivated in the laboratory. The virus will not grow in embryonated chicken eggs.

DUCK VIRAL HEPATITIS
PATHOGENESIS

- *Hosts affected: Ducks and gooses* (young birds) are more commonly affected. The infection is seen in ducklings.
- *Distribution*: DHV I is present in Northern America, Europe and Asia, DHV II is restricted to the United Kingdom and III is restricted to the United States of America. It has not been reported in Pacific region.
- *Transmission*: The disease is very contagious and the virus excreted by faeces is transmitted by direct contact between birds or through fomites such as brooders, waterer, feeder and such other equipment. Recovered birds can shed the virus for up to 8 weeks. DHV can also be through import live ducks from an infected country. Introduction of virus into susceptible population is also possible through duck meat or duck product. Rats have also been described as a reservoir for DHV viruses.
- *Incubation period*: Incubation period is 18-48 hours.
- *Morbidity and mortality*: Morbidity is 100% and mortality is 80-95%.

DUCK VIRAL HEPATITIS
SYMPTOMS

- Clinical signs include letharginess, lose of balance, spasmodic paddling, anorexia and sudden death with opisthotonos within a few days. At death the head is usually drawn back in the opisthotonos position.
- The whole disease sequence is rapid and can take as little as 1–2 hours.
- The clinical course of DHV Type II infection is similar to that of Type I and occur in ducklings immune to Type I infection.
- DHV Type III infections occur in ducklings despite immunity to Type I virus. The clinical course of Type III infection is less severe, and mortality is rarely >30%.

DUCK VIRAL HEPATITIS

LESIONS

- DHV I infection
  - The liver is enlarged and covered with hemorrhagic foci up to 1 cm in diameter.
  - The spleen may be enlarged and mottled.
  - Kidneys may be swollen with congested blood vessels.
  - Microscopic changes in the liver are characterised by extensive hepatocyte necrosis and bile duct hyperplasia, together with varying degrees of inflammatory cell response and haemorrhage.
- DHV II infection
  - Lesions include multiple haemorrhages, both punctate and confluent bands in the liver, swollen pale kidneys with congested blood vessels, and enlarged spleens.
  - The alimentary tract is often empty although the small intestine may contain mucus, and haemorrhagic areas are occasionally seen.
  - Petechial haemorrhages are also occasionally seen on the heart.
  - Histologically, changes in the liver are similar to those seen in DHV type I infections.
- DHV III infection
  - The gross pathology is also similar to type I infection. The liver surface is pale and mottled with many red bands and some petechial haemorrhages.
  - The spleen is paler, but not noticeably enlarged, and the kidneys may show patchy congestion.

DUCK VIRAL HEPATITIS
DIAGNOSIS

- Dealt as
  - Field diagnosis,
  - Laboratory diagnosis etc.

DUCK VIRAL HEPATITIS
FIELD DIAGNOSIS

- Field diagnosis is based on symptoms and lesions.
- Sudden onset, rapid spread and typical short course together with characteristic liver lesions are highly suggestive of duck viral hepatitis.

DUCK VIRAL HEPATITIS
LABORATORY DIAGNOSIS

- Isolation and identification
  - *Clinical materials:* Liver is ideal clinical material for isolation of virus.
  - *Systems for isolation:* Ducklings, Duck or chicken embryonated eggs and primary cell culture like Duck embryo liver.
  - Changes / CPE / Lesions: As mentioned under cultivation.

- Immunological tests
  - The virus can be identified by neutralization with specific antisera or by inoculation into both susceptible and immune ducklings.
  - Types II and III viruses are not neutralized by classic Type I antiserum.

DUCK VIRAL HEPATITIS
DIFFERENTIAL DIAGNOSIS

- The infection has to be differentiated from
  - Duck virus enteritis (caused by Herpes virus),
  - Coccidiosis,
  - Mycotoxicosis and
  - New duck disease (caused by Riemerella anatipestifer)

DUCK VIRAL HEPATITIS
SEROLOGY

- These tests are not applicable since the infection sets in rapidly and affected birds die within short time.
- However, virus neutralization assays have been developed.

DUCK VIRAL HEPATITIS
TREATMENT

- Not effective. Antibody against Type I virus, prepared from the eggs of hyperimmunized chickens, administered SC in the neck, at the time of initial loss, is an effective flock treatment.

DUCK VIRAL HEPATITIS
CONTROL

- Dealt as vaccination and eradication.

DUCK VIRAL HEPATITIS
VACCINATION

- Immunization of breeder ducks with modified live virus vaccines, using Types I, II, and III viruses, provides parenteral immunity that effectively prevents high losses in young ducklings. The Type I virus vaccine is administered SC in the neck to breeder ducks at 16, 20, and 24 wk of age and every 12 wk thereafter throughout the laying period. The three immunizations are advisable for passive protection of ducklings.
- Inactivated vaccines against DHV I can also be used in primed ducks, which give high antibody titre.
- The chick-embryo origin, modified live Type I virus vaccine also can be used for early vaccination of ducklings susceptible to Type I (progeny of nonimmune breeders).

This vaccine is administered SC or by foot web stab in a single dose to day-old ducklings. These ducklings rapidly develop an active immunity over 3-4 days.

DUCK VIRAL HEPATITIS
ERADICATION

- Prevention is focussed on strict isolation, particularly during the first 5 wk of age and during this period contact with wild waterfowl should be avoided.
- Since rats are reservoir host for DHV, rats should be controlled.

MODULE-13: TOGAVIRUSES - EQUINE ENCEPHALOMYELITIS

Learning objectives

- Group IV – Positive sense RNA viruses
    - About the morphology and the nature of the Togaviruses
    - Cultivation
    - About the disease and its pathogenesis
        - Eastern Equine Encephalomyelitis virus (EEEV)
        - Western Equine Encephalomyelitis virus (WEEV)
        - Venezuelan Equine Encephalomyelitis virus (VEEV)
    - About the diagnosis and various lab tests
    - About vaccination strategies and vaccines available for field use
    - Control.

EQUINE ENCEPHALOMYELITIS
INTRODUCTION

- The equine encephalitides are clinically similar and are characterized by signs of CNS dysfunction and moderate to high mortality.
- Arboviruses (Arthropod borne viruses) are the most common cause of equine encephalitis.
- Arboviruses are transmitted by mosquitoes or other hematophagous insects and infect a variety of vertebrate hosts, sometimes including man, and may cause serious disease.
- Four different species of the genus Alphavirus of the family
- Togaviridae cause equine encephalitides, which include
    - Eastern (Eastern equine encephalomyelitis) (EEE)
    - Western (Western equine encephalomyelitis),
    - Highlands J and
    - Venezuelan (Venezuelan equine encephalomyelitis).

EQUINE ENCEPHALOMYELITIS
GENERAL ASPECTS

- Dealt as classification, morphology etc.

EQUINE ENCEPHALOMYELITIS

CLASSIFICATION

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Togaviridae
Genus	Alphavirus
Species	• Eastern Equine Encephalomyelitis virus (EEEV) • Western Equine Encephalomyelitis virus (WEEV) • Venezuelan Equine Encephalomyelitis virus (VEEV)

EQUINE ENCEPHALOMYELITIS
MORPHOLOGY

- The virions are enveloped; spherical to pleomorphic with 65-70 nm in diameter. The surface projections are prominent with distinctive spikes.
- The genetic material comprises of a single molecule non-segmented linear positive-sense single-stranded RNA. The complete genome is 11,700 nucleotides long.
- The 5'-end of the genome has a cap, and the 3'-terminus has poly (A) tail. The viral RNA resembles cellular mRNA in structure.

EQUINE ENCEPHALOMYELITIS
OIE LISTING AND RISK GROUP

OIE Listing

- All three infections EEE, WEE and VEE are listed as List B infections.

Risk group

- All three viruses can affect human beings and hence classified under Risk Group III.

EQUINE ENCEPHALOMYELITIS
HA PROPERTY

- These viruses agglutinate RBCs from geese. Male geese are preferred over female.

EQUINE ENCEPHALOMYELITIS
REPLICATION

- Replication occurs in the cytoplasm and is rapid. The glycoprotein spikes over the envelope of the virus are responsible for binding of the virus to cellular receptors.
- Upon entry into the cell the virus gets uncoated and (+) sense genomic RNA acts as mRNA and is partially translated (5' end) to produce NS proteins. These NS proteins are responsible for replication, forming a complementary (-) strand (replicative intermediate), which acts as the template for further (+)strand synthesis.

- Two species of (+) RNA are synthesized, full length genomic RNA and sub-genomic mRNAs. Translation of the newly synthesized sub-genomic RNA results in production of structural proteins. Assembly occurs at the cell surface, and the envelope is acquired as the virus buds from the cell.

EQUINE ENCEPHALOMYELITIS
CULTIVATION

- Newborn mouse, chicken embryos and cell lines like African green monkey kidney (Vero), rabbit kidney (RK-13) and baby hamster kidney (BHK-21) cell lines are used for cultivation of viruses.
- In newborn mice the virus are injected intracranially. The inoculation site is just lateral to the midline into the midportion of one lateral hemisphere. Mice are observed for 10 days; dead mice are collected daily and frozen at –70°C. Mouse brains are harvested for virus identification.
- The chicken embryo is less sensitive than newborn mice. Tissue suspensions are inoculated by the yolk-sac route into 6–8-day-old embryonated chicken eggs. There are no diagnostic signs or lesions in the embryos infected with these viruses. Inoculated embryos are incubated for 7 days, but deaths usually occur between 2 and 4 days post-inoculation.
- The most commonly used cell cultures are primary chicken or duck embryo fibroblasts, continuous cell lines of African green monkey kidney (Vero), rabbit kidney (RK-13), or baby hamster kidney (BHK-21). Cultures are incubated for 7 days. EEE and WEE viruses produce a cytopathic change in cell culture. The fluid from the thawed cultures is used for virus identification.

EQUINE ENCEPHALOMYELITIS
VIRUS STRAINS

EEEV

- Strain VA33, Ten Broeck.

VEV

- VEE viruses are divided into six antigenic subtypes (I–VI).
- Within subtype I there are five antigenic variants (variants AB–F).
- Antigenic variants I-AB and I-C are associated with epizootic activity in equids.
- The other three variants of subtype I (I-D, I-E, I-F) and the other five subtypes of VEE have been associated with natural enzootic cycles. These variants and subtypes have been considered to be nonpathogenic for equids, although they can cause clinical disease in humans.

EQUINE ENCEPHALOMYELITIS
PATHOGENESIS

- Dealt as hosts affected, distribution, incubation period, transmission etc.

EQUINE ENCEPHALOMYELITIS
HOSTS AFFECTED

- These viruses cycle between birds and mosquitoes. Horses and humans are dead-end hosts.

EQUINE ENCEPHALOMYELITIS
DISTRIBUTION

- Canada, States east of Mississippi river in US, Caribbean Islands, Mexico, and Central and South America.

EQUINE ENCEPHALOMYELITIS
INCUBATION PERIOD

- The incubation period from the inoculation of the virus until the febrile response generally is 0.5 to 2 days but may be as long as 5 days, depending on the virus strain or quantity of virus in the inoculum.

EQUINE ENCEPHALOMYELITIS
MORBIDITY AND MORTALITY

- Dealt under EEE, WEE and VEE separately.

EQUINE ENCEPHALOMYELITIS
MORBIDITY AND MORTALITY - EEE

- Both are highly variable and mortality is between 50-90%.

EQUINE ENCEPHALOMYELITIS
MORBIDITY AND MORTALITY - WEE

- Morbidity highly variable and mortality upto 30%.

EQUINE ENCEPHALOMYELITIS
MORBIDITY AND MORTALITY - VEE

- Morbidity rates vary from 50 to 100 percent and mortality rates vary from 50 to 90 percent.

EQUINE ENCEPHALOMYELITIS
TRANSMISSION

- Dealt separately for

- Eastern Equine Encephalitis (EEE),
- Western Equine Encephalitis (WEE) and
- Venezuelan Equine Encephalitis (VEE).

TRANSMISSION - EASTERN EQUINE ENCEPHALITIS

- The main mode of transmission and amplification of EEE is a mosquito-vertebrate-mosquito cycle.
- The primary mosquito vector for the EEE is the Culiseta melanura. Large numbers of these insects are found in swamp areas.
- During the late summer and early fall, mosquitoes leave the swamp breeding sites and move to drier, upland forested habitats.
- Aedes vexans and A canadensis mosquitoes (which breed in containers) are also believed to be responsible for bird to mammal transmission.
- The identification of vectors in epidemics is difficult because no single species is consistently associated with the transmission of the virus to horses and people.

TRANSMISSION - WESTERN EQUINE ENCEPHALITIS

- WEE is transmitted by mosquito vectors (primarily C tarsalis) that breed in sunlit marshes and in pools of irrigation water in pastures and by *Dermacentor andersoni*.
- Epizootics of WEE are associated with increased rainfall in early spring followed by warmer than normal temperatures.

TRANSMISSION - VENEZUELAN EQUINE ENCEPHALITIS

- VEE occurs as an epizootics in horses. VEE virus variants I-D to I-F and subtypes II through VI of VEE virus are associated with a rodent-mosquito transmission, in which human beings and horses are only incidentally get affected.
- Many species of mosquitoes under the genera Aedes, Anopheles, Culex, Deinocerites, Mansonia, and Psorophora and other hematophagous insects are involved in the explosive outbreaks.
- Horses are the most important amplifiers of VEE virus during epizootics due to the extremely high viraemias that they develop and the large numbers of hematophagous insects that can feed on an animal of such size. Human beings do not have a significant role in the maintenance and amplification of VEE virus.
- After inoculation by the vector, the virus travels via the lymphatics to lymph nodes and replicates in macrophages and neutrophils, resulting in lymphopenia, leukopenia, and fever. Subsequent replication occurs in other organs and is associated with viremia.

EQUINE ENCEPHALOMYELITIS
SYMPTOMS

- Dealt separately for
  - EEE,
  - WEE and
  - VEE.

SYMPTOMS - EASTERN AND WESTERN EQUINE ENCEPHALITIS

- The clinical signs of EEE and WEE can be identical. Following an incubation period of 5–14 days, clinical signs include fever, anorexia, and depression.
- In severe cases, the disease in horses progresses to hyperexcitability, blindness, ataxia, severe mental depression, recumbency, convulsions, and death.
- Horses infected with WEE do not have a significant viremia, where as significant viraemia is characteristic for EEE.

SYMPTOMS - VENEZUELAN EQUINE ENCEPHALITIS

- VEE virus infection may be expressed as
    - Subclinical with no signs.
    - Moderate and characterized primarily by anorexia, high fever, and depression.
    - Severe but non-fatal, and characterized by anorexia, high fever, stupor, weakness, staggering, blindness, and, occasionally, permanent neurologic sequelae or
    - Fatal, with the same clinical signs. In general, two forms of the disease exist:
        - The *fulminating form* in which signs of generalized, acute, febrile disease predominate and
        - The *encephalitic form* in which the more impressive signs of central nervous system (CNS) involvement usually dominate.
- An incubation period of 0.5 to 5 days precedes a rise in body temperature to 39-41°C (103-105°F), which is accompanied by a hard, rapid pulse and depression.
    - The onset of VEE virus infection is characterised with fever, inappetence, and mild excitability, which progresses to depression, weakness, and ataxia followed by signs of encephalitis such as muscle spasms, chewing movements, incoordination, and convulsions.
    - Early encephalitic signs include loss of both cutaneous neck reflexes and visual responsiveness; diarrhoea and colic may also develop.
    - Some animals may stand quietly in their surroundings whereas others may wander aimlessly or press their heads against solid objects. A braced stance or circling may occur late in the disease.
    - A characteristic paddling motion of the limbs may be observed with lateral recumbency. The course of the disease may be rapid with death ensuing within hours after the observation of the first clinical manifestations of encephalitis.

EQUINE ENCEPHALOMYELITIS LESIONS

- Dealt separately for
    - EEE,
    - WEE and
    - VEE.

EQUINE ENCEPHALOMYELITIS LESIONS - EEE & WEE

- The extent of the lesions depends on the severity of the infection and the duration of the neurological involvement.
- Gross pathological lesions are rarely observed in horses and, if present, consist only of the congestion of the brain and meninges.
- Ecchymotic haemorrhages of traumatic origin may also be observed. Microscopic lesions are usually found throughout the central nervous system, which include inflammatory response involving the grey matter, neuronal degeneration with infiltration by polymorphonuclear leukocytes, diffuse and focal gliosis, perivascular cuffing with lymphocytes and neutrophils, neuronophagia and liquefaction of the neutrophils.

EQUINE ENCEPHALOMYELITIS
LESIONS - VEE

- The macroscopic appearance of the CNS of horses with VEE virus varies from no visible lesion to extensive necrosis and hemorrhages.
- Lesions reported in other tissues have been too variable to be of any diagnostic significance.

EQUINE ENCEPHALOMYELITIS
DIAGNOSIS

- Dealt as field and laboratory diagnosis.

EQUINE ENCEPHALOMYELITIS
FIELD DIAGNOSIS

- Very difficult. It is possible only when the infection occurs as an epidemic. Seasonality of the disease and association with large populations of mosquitoes help in field diagnosis.
- The signs of EEE, WEE and VEE are difficult to differentiate. Histopathologic lesions from tissues of dead animals will also help in field diagnosis.

EQUINE ENCEPHALOMYELITIS
LABORATORY DIAGNSOSIS

- Isolation and identification
    - Clinical materials: Specimens for diagnosis are heparinized blood, serum (paired [acute and convalescent] sera if animal survives), and half the brain and piece of pancreas unfixed and a completed set of tissues in 10 percent formalin.
    - Isolation systems: Newborn mice, embryonted eggs and cell lines like Vero, BHK21, RK13.
    - Antigen identification:
        - Complement fixation test
        - RT-PCR
        - Antigen capture ELISA
        - Immunofluorescence
- Identification of viral nucleic acid in mosquitoes by RT-PCR.

EQUINE ENCEPHALOMYELITIS
SEROLOGICAL TESTS

- Serological confirmation of EEE, WEE and VEE virus infection is confirmed by a four-fold or greater increase or decrease in antibody titre in paired serum samples collected 10–14 days apart. The common serological tests performed are as below:
- Complement fixation test
- Haemagglutination inhibition test using male geese RBCs
- ELISA
- Plaque reduction neutralization test (PRN test) - The PRN test is very specific and can be used to differentiate between EEE and WEE virus infections. The PRN test is performed in duck embryo fibroblast, Vero, or BHK-21 cell cultures.

EQUINE ENCEPHALOMYELITIS
CONTROL

- Dealt under vaccination and eradication.

EQUINE ENCEPHALOMYELITIS
VACCINATION

- Formalin-inactivated viral vaccines for EEE, WEE, and VEE. Vaccines in mono-, bi-, or trivalent forms are available. The viral strain of VEE in vaccines is TC-83, which was originally developed as a modified live inoculation to protect laboratory workers investigating VEE.
- The vaccination protocol consists of two injections 30 days apart, followed by an annual or biannual booster dependent on the geographic location of the horses. An attenuated VEE virus vaccine is also used in certain parts of America.

EQUINE ENCEPHALOMYELITIS
ERADICATION

- Eradication is aimed at restriction of the movement of infected horses and insect control programmes coupled with large scale mass immunization programmes in horses.
- Restriction of horse movement between the epizootic zone and noninfected areas is important to control the spread.
- Mosquito control measures such as aerial spraying with ultralow volumes of insecticides.

EQUINE ENCEPHALOMYELITIS
PUBLIC HEALTH HAZARD

- EEE, WEE and VEE can cause human infections. EEE virus causes severe disease in humans with a mortality rate of 30–70% and a high frequency of permanent sequelae in patients who survive.
- WEE is usually mild in adult humans, but can be a severe disease in children. Human infections occur from bites of infected mosquitoes.
- Transmission can also occur by exposure to aerosolized infective material.

- In human beings, a flu-like syndrome predominates accompanied by high fever and frontal headache. Human deaths may occur in the young or the aged.

MODULE-14: FLAVIVIRUS - CLASSICAL SWINE FEVER

Learning objectives

- Group IV – Positive sense RNA viruses
  - About the morphology and the nature of the flaviviruses
  - Classical swine fever – the disease
  - About the pathogenesis
  - Cultivation of swine fever virus
  - About the diagnosis and various lab tests
  - About the vaccines
  - Control

CLASSICAL SWINE FEVER
INTRODUCTION

- Classical swine fever (CSF) is a contagious viral disease of pigs. CSF is caused by a virus belonging to the family Flaviviridae.
- The infection can occur as acute, subacute, chronic or in a persistent form.
- The infection is characterized by high fever, severe depression, multiple superficial and internal hemorrhages, and high morbidity and mortality.

CLASSICAL SWINE FEVER
GENERAL ASPECTS

- Deals about Classification, Morphology etc.

CLASSICAL SWINE FEVER
CLASSIFICATION

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Flaviviridae
Genus	Pestivirus
Species	Classical swine fever virus

- The family Flaviviridae is classified into three genus:
  - Flavivirus,
  - Pestivirus and
  - Hepacivirus.
- Most of the members under the genus flavivirus are arthropod transmiteed and cause very severe infection in humanbeings. The important infections are

    - Dengue Fever,
    - Yellow Fever,
    - Kyasanur Forest Disease etc.
- Members under the genus pestivirus are generally not transmitted by arthropods. The important infections caused by the pestivirus include as
    - Classical Swine Fever (CSF) and
    - Bovine Viral Diarrhoea (BVD).

CLASSICAL SWINE FEVER
MORPHOLOGY

- The virions are enveloped; spherical to pleomorphic with 40-60 nm in diameter.
- The surface projections are prominent with distinctive spikes surrounded by a prominent fringe. Surface projections also form ring-like subunits.
- The virions consist of structural protein (s), nucleocapsid (C), matrix (M) glycoprotein (E) and a non-structural (NS) protein.
- The nucleocapsid is round and exhibits polyhedral symmetry. The genetic material comprises of a single molecule non-segmented linear positive-sense single-stranded RNA.
- The complete genome is 12,500 nucleotides long. The 5'-end of the genome has a cap, and the 3'-terminus has no poly (A) tail.

CLASSICAL SWINE FEVER
SYNONYMS

- English swine fever, hog cholera, peste du porc, colera porcina.

CLASSICAL SWINE FEVER
LISTING AND RISK GROUP

- *OIE Listing*: List A infection.
- *Risk group:* No infection to human beings.

CLASSICAL SWINE FEVER
RESISTANCE

- CSFV are resistant to temperature upto 56C and survive at wide range of pH between 3 and 11.
- They are susceptible to ether, chloroform and ß-propiolactone and are inactivated by cresol, sodium hydroxide (2%), formalin (1%), sodium carbonate (4% anhydrous or 10% crystalline, with 0.1% detergent), ionic and non-ionic detergents and strong iodophors (1%) in phosphoric acid.
- The virus is also sensitive to drying (desiccation). The virus also survives well in cold conditions and can survive some forms of meat processing (curing and smoking).
- In a protein-rich environment, the virus is very stable and can survive for months in refrigerated meat and for years in frozen meat.

CLASSICAL SWINE FEVER
REPLICATION

- The glycoprotein spikes are responsible for attaching to receptors on the cell. The entire replication of the virus takes place in the cytoplasm.
- The (+) sense genomic RNA acts directly as mRNA. The entire genome is translated as a single polyprotein, which is then cleaved into the mature proteins.
- The NS protein synthesizes complementary (-)strand, which used as a template for genomic progeny RNA synthesis.
- Assembly occurs during budding into cytoplasmic vacuoles than at the cell surface and release of progeny virion occurs when cell lyses.

CLASSICAL SWINE FEVER
VIRUS STRAINS

- The organism has a close antigenic relationship with the bovine viral diarrhea virus (BVDV) and the border disease virus (BDV).
- There are many strains - some are very virulent and some less pathogenic.
- Some of the important virus strains are Alfort/187, Alfort-Tübingen, Brescia and C strain.

CLASSICAL SWINE FEVER
CULTIVATION

- The virus grows slowly in cell culture. The viruses are cultivated in PK-15 cell lines.
- Presence of the virus in cell lines are confirmed by FAT or RT-PCR.

CLASSICAL SWINE FEVER
PATHOGENESIS

- Hosts affected
  - Pigs and wild boar are the only natural host for CSF.
- Distribution
  - The disease is present in Asia, Central and South America, and parts of Europe and Africa.
- Incubation period
  - The incubation period is usually 3 to 4 days but can range from 2 to 14 days.
- Morbidity and Mortality
  - In acute CSF, the morbidity and mortality are high and go upto 100%.

CLASSICAL SWINE FEVER
SYMPTOMS

rs as acute, subacute, chronic or congenital forms. The infection also appears in a milder form in sows.

CLASSICAL SWINE FEVER
SYMPTOMS - ACUTE FORM

- Peracute infection
  - Observed in piglets, where young pigs may be found dead without any prior sign of illness.
- Acute infection
  - In acute form the pigs appear sick, inactive and drowsy with arched back. Some pigs stand with droopy head and straight tail.
  - They may huddle to a corner for warmth. Some pigs also vomit a yellow fluid containing bile.
  - The most important symptom in acute infection is the high fever that may reach 108°F (42.2°C) with an average of 106°F (41.1°C) accompanied by anorexia and constipation.
  - Conjunctivitis with encrustation of the eyelids and the presence of dirty streaks below the eyes is the other important symptom. Sick pigs will have a staggering gait and posterior weakness.
  - In last stage of the infection, pigs will become recumbent, and convulsions may occur shortly before death. Sever diarrhoea will also occur during last stages.
  - There may be purple discoloration of abdominal skin, or necrosis of the tips of extremities (ears, tail, vulva). Neurological signs may occur, which include incoordination, tremors, convulsions and circling. Death usually occurs 5-15 days after the onset of illness.

CLASSICAL SWINE FEVER
SYMPTOMS - CHRONIC FORM

- The chronic form is characterised by dullness, capricious appetite, pyrexia and diarrhoea for up to 1 month. Weight loss, hair loss, dermatitis and discoloration of abdomen or ears are the other symptoms.
- A chronically infected pig may have a disproportionately large head relative to the small trunk.
- The affected pigs may stand with arched backs and their hind legs placed under the body. The animals may recover followed by relapse and death.
- Chronic form occurs in vaccinated herds or can also be caused by less virulent strains of CSFV.

CLASSICAL SWINE FEVER
SYMPTOMS - CONGENITAL FORM

- Congenital CSF infection by virulent strains result in abortions or in the birth of diseased pigs, which will die shortly after birth.
- Transplacental transmission with low-virulent CSF strains result in mummification, stillbirth, or the birth of weak and "shaker" pigs.
- Malformation of the visceral organs and of the central nervous system occurs frequently. Some pigs may be born virtually healthy but persistently infected with CSF. Such infection usually follows exposure of fetuses to CSF of low virulence in the first trimester of fetal life.
- Pigs thus infected do not produce neutralizing antibodies to CSF and have a lifelong viremia. The pigs may be free of disease for several months before developing mild anorexia, depression, conjunctivitis, dermatitis, diarrhoea, runting, and locomotive disturbance leading to paresis and death.

CLASSICAL SWINE FEVER
LESIONS

- Described as
  - Acute,
  - Chronic and
  - Congenital.

CLASSICAL SWINE FEVER
LESIONS - ACUTE FORM

- *Peracute infection:* No gross lesions.
- *Acute infection:* Acute infection is characterised by following lesions:
  - Haemorrhages and purplish discoloration of the skin.
  - Necrotic foci in the tonsils.
  - Swollen and oedematous submandibular and pharyngeal lymphnodes with peripheral haemorrhages.
  - Spleen with raised edges and dark wedge shaped areas.
  - Pinpoint to ecchymotic hemorrhages on the surface of the kidneys (turkey egg appearance). The haemorrhages are most marked under the kidney capsule.
  - Hemorrhages on the surface of the small and large intestine, larynx, heart, epiglottis, and the fascia lata of the back muscles.
  - Accumulation of straw-colored fluids in the peritoneal and thoracic cavities and in the pericardial sac.
  - Congestion of the lung and bronchopneumonia.
  - In brain the lesions are perivascular cuffing, endothelial proliferation, and microgliosis.

CLASSICAL SWINE FEVER
LESIONS - CHRONIC FORM

- Lesions are less severe and are often complicated by secondary bacterial infections.
- Button ulcers are an expression of such a secondary bacterial infection.

CLASSICAL SWINE FEVER
LESIONS - CONGENITAL FORM

- Hypoplasia of the cerebellum, thymus atrophy, ascites and deformities of the head and of the limbs.
- Edema and petechial hemorrhages of the skin and of the internal organs.

CLASSICAL SWINE FEVER
DIAGNOSIS

- Dealt as
  - Field and

- Laboratory Diagnosis.

CLASSICAL SWINE FEVER
FIELD DIAGNOSIS

- Based on high morbidity and mortality, high fever, diarrhoea. Kidney and lymph node lesions will help in field diagnosis.

Clinical materials

- Tonsils, lymph nodes (pharyngeal, mesenteric), spleen, kidney, distal part of ileum and blood in EDTA (live cases) are the ideal materials for isolation and identification of viruses. These materials should be kept under refrigeration and shipped to laboratory as quickly as possible.

CLASSICAL SWINE FEVER
LABORATORY DIAGNOSIS

- Laboratory diagnosis dealt under direct identification of CSF from tissues etc.

CLASSICAL SWINE FEVER
LABORATORY DIAGNOSIS - DIRECT IDENTIFICATION OF CSF FROM TISSUES

- Immunological methods
  - *Fluorescent antibody test (FAT):* Also known as fluorescent antibody tissue section test (FATST). It a rapid test that can be used to detect CSFV antigen in cryostat sections of tonsils, spleen, kidney, lymph nodes or distal portions of the ileum. Cryostat sections are stained directly with anti-CSF immunoglobulin conjugated to fluorescein isothiocyanate (FITC) or indirectly using a secondary FITC conjugate and examined by fluorescence microscopy.
  - *Immunoperoxidase test (IPT):* This test is used to differentiate between field strains of CSFV and vaccine strain of CSFV. A panel of three monoclonal antibodies (Mabs) are used to detect all field strains of CSFV, vaccine strains of CSFV and ruminant pestiviruses.
  - *Antigen capture ELISA (Double antibody sandwich ELISA):* Blood leukocyte fraction or anticoagulated whole blood or clarified tissue homogenate are used as antigen for ELISA. Monoclonal and polyclonal antibodies are used as detector and capture antibodies respectively. The technique is relatively simple to perform, does not require tissue culture facilities, is suitable for automation and can provide results within half a day. The disadvantage is less sensitive than virus isolation, especially in adult pigs and in mild or subclinical cases.
- RT-PCR
  - This method is rapid and more sensitive than antigen-capture ELISAs or virus isolation.

CLASSICAL SWINE FEVER

LABORATORY DIAGNOSIS - ISOLATION AND IDENTIFICATION

- Isolation of virus in cell cultures is a more sensitive but slower method for diagnosis of CSF.
- Isolation is best performed in rapidly dividing PK-15 cells. The cultures are examined for fluorescent foci by FAT after 24–72 hours.

CLASSICAL SWINE FEVER
LABORATORY DIAGNOSIS - SEROLOGICAL TESTS

- Detection of virus-specific antibodies is particularly useful on premises suspected of having infections with CSF strains of low virulence.
- Due to the immunosuppressive effect of CSFV, antibodies cannot be detected with certainty until 21 days post-infection.
- Commonly performed serological tests are as below. All the three tests are specified tests for international trade as
    - Fluorescent antibody virus neutralisation test (FAVN)
    - Neutralising peroxidase-linked assay (NPLA)
    - ELISA

CLASSICAL SWINE FEVER
DIFFERENTIAL DIAGNOSIS

- Classical Swine Fever (CSF) should be differentiated from following infections:
    - African swine fever (indistinguishable clinico-pathologically. It is essential to send samples for laboratory examination)
    - Infection with bovine viral diarrhoea virus
    - Salmonellosis
    - Erysipelas
    - Acute pasteurellosis
    - Other viral encephalomyelitis
    - Streptococcosis
    - Leptospirosis
    - Salt poisoning.

CLASSICAL SWINE FEVER
CONTROL

- Dealt under vaccination and eradication.

CLASSICAL SWINE FEVER
VACCINATION

- Modified live vaccines (MLV) are used to control CSF. The lapinized Chinese (C) strain, the Japanese guinea pig cell culture-adapted strain, and the French Thiverval strain have been widely used.
- All three strains are considered safe for pregnant sows and piglets over 2 weeks old.

CLASSICAL SWINE FEVER
ERADICATION

- Eradication is possible with strict vaccination of pigs, garbage cooking laws and serological surveys targeted primarily to breeding sows to detect subclinical infections.
- In an outbreak, slaughter of all pigs in affected farms, proper disposal of carcasses, bedding, etc., thorough disinfection, designation of infected zone, with control of pig movements, detailed epidemiological investigation, with tracing of possible sources (up-stream) and possible spread (down-stream) of infection and surveillance of infected zone, and surrounding area will prevent the further spread. Following good husbandry practices in the farm will also prevent the spread of infection.

MODULE-15: BOVINE PESTIVIRUS INFECTIONS

Learning objectives

- Group IV – Positive sense RNA viruses
  - About the morphology and the nature of the pestiviruses
  - Bovine viral diarrhea and Mucosal disease and its pathogenesis
  - Cultivation of BVD virus
  - About the diagnosis and various lab tests
  - About the vaccines
  - Treatment and Control

BOVINE PESTIVIRUS INFECTIONS
INTRODUCTION

- *Bovine virus diarrhoea / mucosal disease* is observed as variety of clinical syndromes in cattle. This disease complex can cause significant economic losses in susceptible herds.
- Losses are due to abortions, congenital defects, poor growth, impaired reproductive performance and death due to mucosal disease and acute BVD infection.

BOVINE PESTIVIRUS INFECTIONS
GENERAL ASPECTS

- Describes the morphology of the virus and other general points.

BOVINE PESTIVIRUS INFECTIONS
CLASSIFICATION

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Flaviviridae
Genus	Pestivirus

Species	Bovine viral diarrhoea virus (BVDV)

BOVINE PESTIVIRUS INFECTIONS
MORPHOLOGY

- Is typical of other pestivirues. The virions are enveloped; spherical to pleomorphic with 40-60 nm in diameter.
- The genetic material comprises of a single molecule non-segmented linear positive-sense single-stranded RNA, which is about 12,300 nucleotides long.
- The BVDV genome encodes for both structural and nonstructural proteins.
- The structural proteins include the capsid protein C and three glycoproteins Erns, E_1, and E_2.
- The capsid protein functions to package the genomic RNA and to provide structure for the formation of the virion envelope. The three glycoproteins are associated with the lipid envelope.

BOVINE PESTIVIRUS INFECTIONS
OIE LISTING AND RISK GROUP

- Listed infection but not under List A or B.
- *Risk group:* No infection to human beings.

BOVINE PESTIVIRUS INFECTIONS
RESISTANCE

- As other pestiviruses, they are mildly thermo-tolerant. They can survive under wide range of p^H. They are sensitive to lipid soluble detergents and common disinfectants.

BOVINE PESTIVIRUS INFECTIONS
REPLICATION

- Replication of BVDV begins with receptor-mediated endocytosis into a cell.
- The E_2 glycoprotein mediates this step. Once inside the cell, the viral RNA is released and RNA translation begins immediately.
- Viral proteins can be observed as early as three hours after cell infection. Following gene translation, the large polyprotein product is processed by both cellular and viral enzymes into mature proteins.
- One of the proteins is the RNA-dependent RNA polymerase. New genomic RNA is produced to be packaged into virus packages.
- Viral packaging occurs in either the Golgi apparatus or endoplasmic reticulum where they acquire their lipid envelope through budding into the vesicle lumen.
- Mature virus packages are then released from the cell exocytosis. New virus can be released as early as 10 hours post cell infection.

BOVINE PESTIVIRUS INFECTIONS
HA PROPERTY

- The virus has no HA property.

BOVINE PESTIVIRUS INFECTIONS
ANTIGENICITY & STRAINS

- BVDV is closely related to classical swine fever and ovine Border disease viruses.
- BVDV occurs in two forms
  - Non-cytopathogenic and
  - Cytopathogenic.
- There are two antigenically distinct genotypes (types 1 and 2), and virus isolates within these groups exhibit considerable biological and antigenic diversity.
- BVD-I (genotype 1) comprises the classic BVD virus isolates including commonly used vaccine strains.
- BVD-II (genotype 2) comprises newly described BVD viruses strains but have been isolated from as early as 1981.
- Each genotype contains cytopathic and non-cytopathic biotypes and both genotypes are capable of producing clinically similar syndromes in cattle. However, it is the non-cytopathogenic biotype that circulates in cattle populations.
- The two genotypes may be differentiated from each other, and from other pestiviruses, by monoclonal antibodies (MAbs) directed against the E2 and ERNS major glycoproteins, or by genetic analysis.

BOVINE PESTIVIRUS INFECTIONS
CULTIVATION

- The virus is cultivated in a number of bovine monolayer cell cultures (e.g. kidney, lung, testis or turbinate). Both biotypes grow well in cell cultures.
- Noncytopathogenic BVDV is a common contaminant of fresh bovine tissue. Hence, the cell cultures from bovine origin used for BVDV cultivation should be checked for residual contamination.
- Now, such problems are overcome by the use of continuous cell lines, which can be obtained as BVD-free cell lines.
- The foetal bovine serum used in cell culture should also BVDV and antibodies against BVDV. The presence of virus in the cell culture is confirmed by immunoperoxidase technique, ELISA and nucleic acid identification methods.

BOVINE PESTIVIRUS INFECTIONS
PATHOGENESIS

- Describes under
  - Host affected,
  - Method of transmission,
  - Incubation period,
  - Moribidity and mortality,
  - Symptoms and lesions.

BOVINE PESTIVIRUS INFECTIONS

HOSTS AFFECTED

- Cattle is the main host for the infection. The infection is also observed in even-toed ungulates.
- All ages of cattle are susceptible, but infection usually occurs between 6 and 24 months of age. Colostral antibody appears to protect most calves for 3-6 months after birth.

BOVINE PESTIVIRUS INFECTIONS
TRANSMISSION

- The natural reservoir for BVDV is persistently infected cattle. Noncytopathic viruses cause the majority of acute BVDV infections.
- Transmission dealt under acute and intra-uterine infection.

BOVINE PESTIVIRUS INFECTIONS
ACUTE INFECTION

- The virus spreads mainly by contact between cattle. Large numbers of BVDV are shed in the secretions and excretions of persistently infected cattle.
- Infected animals shed virus in nasal and oral secretions, feces and urine. The primary virus entry route is through oral and nasal.
- Other less important routes of entry may include infected semen, biting insects, and contaminated instruments. Vertical transmission plays an important role in its epidemiology and pathogenesis.
- Noncytopathic BVDV is transmitted transplacentally during the first 4 months of foetal development; therefore, infection is present at birth and lasts for life.
- Clinical disease and reproductive failure are reported in healthy cattle after contact with a persistently infected animal. Following entry and contact with the mucosal lining of the mouth or nose, initial replication occurs in epithelial cells with a predilection for the palatine tonsils.
- From here, the virus is able to spread systemically through the blood stream. Spread can occur through both free virus in the serum and virus infected leucocytes, particularly lymphocytes and monocytes.
- During systemic spread, the virus is able to gain entry to most tissues with a preference for lymphoid tissues. However, the tissues infected may vary between different virus strains.

BOVINE PESTIVIRUS INFECTIONS
INTRA-UTERINE INFECTION

- BVDV can cross the placenta and infect foetuses of all ages. The outcome of these infections are largely dependent on the stage of gestation when infection occurs. Fetal calves develop immunocompetency at about 180 days of gestation
- If the cow is infected in the first month of pregnancy, the pregnancy is terminated either by abortion or resorption of the foetus by the dam.
- If the foetus is infected in the second to the sixth month of pregnancy, a variety of different syndromes are observed. The foetus may still be aborted or the foetus survives full term with the resultant offspring born malformed, weak, "dwarfed", stillborn, or

clinically healthy but "persistently infected" with pestivirus. Persistently infected calves may grow well, but generally are unthrifty compared to others the same age and they often suffer from chronic scours or pneumonia.
- If infection occurs around three to five months of pregnancy, the virus affects the developing nervous system of the foetus. Calves may not be able to stand or suck or may develop convulsions after birth because they are missing parts of their brain. These calves may also have eye abnormalities such as blindness and cataracts or bent up front legs. Some calves will survive but have a wobbly gait and may have a permanent head tremor.
- If exposure to a noncytopathic virus occurs between 42 and 125 days gestation, the fetus accepts the invading virus, becomes immunotolerant to that particular virus and will never mount an immune response to eliminate it. These calves are born persistently infected (PI).

BOVINE PESTIVIRUS INFECTIONS
INCUBATION PERIOD

- Highly variable (7-14 days).

BOVINE PESTIVIRUS INFECTIONS
MORBIDITY AND MORTALITY

- Variable (High morbidity and low mortality).

BOVINE PESTIVIRUS INFECTIONS
SYMPTOMS

- There are several clinical manifestations
    - BVD,
    - Acute and chronic mucosal disease,
    - Unthrifty persistently viremic calves,
    - Calves with congenital defects, and
    - Abortions.

BOVINE PESTIVIRUS INFECTIONS
SYMPTOMS - BVD

- It is most often a subclinical to mild infection in immunocompetent but naive cattle.
- Occasionally, in naive animals that are infected by virulent strains of BVD, death can occur.
- Biphasic fever (approximately 104°F (40°C)), depression, decreased milk production, and inappetence are typical signs of acute BVD.
- An increased respiratory rate, diarrhoea, and excessive lacrimation may be seen.
- Disease lasts for 1-3 days, and is followed by rapid recovery with production of viral neutralizing antibody. There may be some loss of productivity.
- BVDV can be immunosuppressive and subclinical disease can predispose cattle to other infections such as pneumonia.

- Some noncytopathic BVDV induce clinically severe disease with a high fever (approximately 107°F (41-42°C)), oral ulcerations, eruptive lesions of the coronary band and interdigital cleft, diarrhoea, dehydration, leukopenia, and thrombocytopenia.

BOVINE PESTIVIRUS INFECTIONS
MUCOSAL DISEASE

- Acute and chronic mucosal disease are highly fatal forms of BVD seen in persistently infected cattle. These diseases occur when persistently infected cattle become superinfected with cytopathic BVDV.
- The origin of the cytopathic BVDV is usually internal, resulting from mutation of the persistent noncytopathic BVDV.
- Clinical signs of chronic mucosal disease may last several weeks to months. This form is characterized by fever, depression, anorexia and excessive salivation followed by profuse, watery diarrhoea 2-4 days later.
- Affected animals have erosive lesions in their mouths, mainly on the front of the hard palate, the corners of the mouth and the dental pad (gums).
- Other signs include a mucopurulent discharge from the nostrils, tearing of the eyes, corneal edema and lameness.
- Affected animals have a low white blood cell count, up to 50% below normal. Affected animals progressively become weaker and dehydrated and die in 5-7 days.
- Some animals do not die after developing mucosal disease but become chronically ill.
- They become progressively more emaciated with bouts of diarrhoea and bloat. Hoof deformities and erosive lesions on the skin that do not heal.

BOVINE PESTIVIRUS INFECTIONS
UNTHRIFTY PERSISTENTLY VIRAEMIC

- These calves are stunted and have very poor growth rates.
- They do not have detectable mucosal disease lesions and are seronegative to BVDV. The life span of persistently infected cattle often is ≤2 yr.

BOVINE PESTIVIRUS INFECTIONS
CONGENITAL DEFECTS

- These calves have defects relating to lesions in the brain (cerebellum) and the eyes. Signs range from inability to get up and incoordination to blindness.

BOVINE PESTIVIRUS INFECTIONS
ABORTIONS

- Abortions occur in dams exposed during the first trimester of gestation.
- Early embryonic death can occur if the virus is present at breeding in either the dam or semen.

BOVINE PESTIVIRUS INFECTIONS
LESIONS

Dealt under

- Bovine Viral Diarrhoea and
- Mucosal disease.

BOVINE PESTIVIRUS INFECTIONS
BOVINE VIRAL DIARRHOEA

- In acute infection petechial hemorrhages on conjunctiva, sclera, nictitating membrane and mouth are seen.
- Swollen lymph nodes, erosions and ulcerations of the GI tract, petechial and ecchymotic hemorrhages on the serosal surfaces of the viscera, and lymphoid depletion are other important lesions. These lesions are not observed in persistently infected cattle.

BOVINE PESTIVIRUS INFECTIONS
MUCOSAL DISEASE

- Is characterised by erosions and ulcerations throughout the GI tract. The mucosa over Peyer's patches may be hemorrhagic and necrotic.
- Extensive necrosis of lymphoid tissues, especially those associated with the intestines, is seen on microscopical examination.

BOVINE PESTIVIRUS INFECTIONS
DIAGNOSIS

nosis, laboratory diagnosis etc.

BOVINE PESTIVIRUS INFECTIONS
FIELD DIAGNOSIS

- Is based on history, clinical signs, and gross and microscopic lesions.
- Diagnostic laboratory support is required when clinical signs and gross lesions are minimal and is essential in some outbreaks of mucosal disease or clinically severe acute bovine viral diarrhoea.

BOVINE PESTIVIRUS INFECTIONS
LABORATORY DIAGNOSIS

- Isolation and identification (Accepted for International trade)
  - *Clinical materials:* Whole blood, swabs from mucosal or nasal surfaces, semen and lymphnodes.
  - *Isolation systems*: Bovine monolayer cell cultures from kidney, lung, testis or turbinate.
  - *Antigen identification*
    - Microplate immunoperoxidase method
    - Double antibody sandwich ELISA
    - RT-PCR

- Direct identification of BVDV from tissue sections
  - *Clinical materials*: Tissues from lymph nodes, thyroid gland, skin, brain, abomasum and placenta are ideal for direct identification of BVDV.
  - *Techniques*
    - Immunoperoxidase method using monoclonal antibodies
    - RT-PCR
    - In situ hybridisation with enzyme-linked riboprobes

BOVINE PESTIVIRUS INFECTIONS
SEROLOGY

- To properly assess serology, paired acute and convalescent samples should be collected 30 days apart to identify four fold increases in serum antibody titers following convalescence. Serological techniques that are commonly used are
  - Virus neutralization test
  - ELISA: Both indirect and blocking types of test can be used.

BOVINE PESTIVIRUS INFECTIONS
HERD SCREENING

- Infection status of a herd are established by methods such as testing bulk milk for anti-BVDV antibody or the testing of the bulk milk sample for BVDV by RT-PCR amplification.
- Other screening methods such as serology on groups of non-vaccinated calves at 6 months of age also are useful.

BOVINE PESTIVIRUS INFECTIONS
TREATMENT

- Not effective. Supportive therapy and administration of antiserum may be of benefit in outbreaks of acute disease.

BOVINE PESTIVIRUS INFECTIONS
CONTROL

- Dealt under vaccination and eradication.

BOVINE PESTIVIRUS INFECTIONS
VACCINATION

- The use of killed or modified-live vaccines can provide protection by decreasing the consequences of acute infections.
- However, it is questionable whether killed or modified-live vaccines provide complete foetal protection from the development of in utero fetal infections.
- In a breeding herd all breeding females 3-6 weeks prior to breeding should be vaccinated. Both modified-live (MLV) and killed vaccines are available commercially, generally as a component in multivalent vaccines.

- Modified live virus (MLV) vaccines can potentially infect the fetus so should not be given to pregnant cows. The inactivated vaccines are safe but must be given twice, two to four weeks apart.

BOVINE PESTIVIRUS INFECTIONS
ERADICATION

- In herds that are free of BVD, control is aimed at appropriate quarantine to keep replacement stock away from pregnant females by adopting strict biosecurity measures.
- Control is based on sound management practices, elimination of persistently infected cattle, and vaccination.
- Cattle should be tested for persistent infection and antibody against BVDV before entry into a herd, as should embryo donor and embryo recipients.
- Cattle selected as embryo recipients or herd replacements should be vaccinated if they are negative for antibody against BVDV. Cattle that are persistently infected should be sold for slaughter.
- Bull semen may transmit BVDV and should be tested for virus before use. All breeding bulls should be blood tested and shown to be free of persistent infection because the semen of persistently infected bulls contains high levels of virus.

BOVINE PESTIVIRUS INFECTIONS
PUBLIC HEALTH ASPECT

- No human infection is reported.

MODULE-16: CORONAVIRUS - AVIAN INFECTIOUS BRONCHITIS

ut Group IV viruses
ense single stranded RNA viruses
idae
e and its pathogenesis
an infectious bronchitis
ality, antigenic pleurality
n countries, where ND is not present
vaccines
nd eradication

AVIAN INFECTIOUS BRONCHITIS
INTRODUCTION

- Avian Infectious Bronchitis (IB) is a contagious disease of birds due to a Coronavirus and causing important economic loss in chicken operations.
- The infection was first described in 1931 in the USA as a respiratory disease of chicks.
- The virus – IBV is a corona virus and is prevalent in all countries with an intensive poultry industry including India.

- The infection has a significant economic impact; in broilers, production losses are due to poor weight gains, condemnation at processing and mortality.
- In laying birds, losses are due to suboptimal egg production and downgrading of eggs.

AVIAN INFECTIOUS BRONCHITIS
GENERAL ASPECTS

- Deals about classification, morphology, replication, resistance etc.

AVIAN INFECTIOUS BRONCHITIS
CLASSIFICATION

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Coronaviridae
Genus	coronavirus
Species	Avian infectious bronchitis virus (IBV)

- The surface projections are distinctive club-shaped peplomers that evenly cover the surface. They are densely dispersed. Capsid/nucleocapsid is elongated and exhibits helical symmetry.
- The nucleocapsid is cylindrical; 2 nm in diameter. The genome is not segmented and consists of a single molecule of linear positive-sense single-stranded RNA.
- The viral genome encodes structural proteins and non-structural proteins.
- Characters of the order Nidovirales: Virions have a complex construction and consist of an envelope and a nucleocapsid. Virions are enveloped; spherical, or kidney-shaped, or pleomorphic.

AVIAN INFECTIOUS BRONCHITIS
SYNOYMS

AVIAN INFECTIOUS BRONCHITIS
MORPHOLOGY

- The virions are enveloped and slightly pleomorphic or spherical in shape with 60-220 nm in diameter. The surface of the virus has surface projections, which are distinct and club-shaped. These projections are spaced widely apart and dispersed evenly over all over the surface providing a crown like appearance and hence these viruses are known as corona viruses.
- The nucleocapsid is filamentous with helical symmetry. The virions contain one molecule of linear positive-sense single stranded RNA, which also act as mRNA.

- The total genome length is 20000-33000 nt. The 5' end of the genome has a cap and 3' end has a poly (A) tail. The virus has five structural proteins – S, M, N, HE and E.
    - Surface glycoprotein (or spike, S), which S protein is responsible for attachment to cells, hemagglutination and membrane fusion.
    - Integral membrane protein (M) which spans the virus envelope three times
    - Nucleocapsid protein (N)
    - Hemagglutinine-esterase protein (HE), which forms short surface projections, and have receptor binding, hemagglutination and receptor destroying activities
    - Envelope protein (E), small, envelope-associated protein
    - Unlike (-)sense RNA viruses polymerase enzymes are not present in the nucleocapsid

AVIAN INFECTIOUS BRONCHITIS
OIE LISTING AND RISK GROUP

- *OIE Listing*: List B infection
- *Risk group*: Group I pathogen. No risk to humanbeings.

AVIAN INFECTIOUS BRONCHITIS
RESISTANCE

- The viruses are not very stable and are destroyed by heat and common disinfectants used. Following good biosecurity measures at the poultry house will greatly minimise the loss of birds due to this infection.

AVIAN INFECTIOUS BRONCHITIS
REPLICATION

- The replication of corona viruses is slow compared to other enveloped viruses and the viruses replicate in the cytoplasm.
    - The (+) sense genome is first translated to produce a viral polymerase (RNA dep RNA Polymerase), which then produces a full-length (-) sense strand.
    - This (-) sense stand is used as a template to produce mRNA as a 'nested set' of transcripts, all with an identical 5' non-translated leader sequence and coincident 3' polyadenylated ends.
    - Each mRNA produced is monocistronic, the genes at the 5' end being translated from the longest mRNA and so on.
    - These unusual cytoplasmic structures are produced not by splicing (post-transcriptional modification) but by the polymerase during transcription. Between each of the genes there is a repeated intergenic sequence - UCUAAAC - which interacts with the transcriptase plus cellular factors to 'splice' the leader sequence onto the start of each ORF
    - Assembly occurs by budding into the golgi apparatus, particles being transported to the surface of the cell by the secretory nature of this organelle & released

AVIAN INFECTIOUS BRONCHITIS

HA PROPERTY

- Many IBV cause agglutination of chicken RBC after treatment with an enzyme neuraminidase (Previously it was thought as Phospholipase C).

AVIAN INFECTIOUS BRONCHITIS
STRAINS AND SEROTYPES

- The virus elicits distinct antigen determinants on envelope and spikes. Variations within the virus are due to differences in the S and HE proteins. As a result of this, these are many serotypes and within each serotype there are many strains. These serotypes are immunologically distinct (seropleurality; antigenic pleurality) and antibodies against one serotype may not protect the birds against the infection caused by other serotypes.
- Some of the common serotypes are Massachusetts, Connecticut, Arkansas etc. Some of the strains are M5, M41, H151, Holte, Gray, Florida etc.

AVIAN INFECTIOUS BRONCHITIS
CULTIVATION

- IBV are cultivated in embryoated eggs and tracheal organ culture (TOC) system. Cell cultures are not used cultivation as it is usually necessary to adapt IBV isolates to grow in chicken embryos before cytopathic effects (CPE) of virus infection are seen in cell culture. The virus is inoculated into the allantoic cavity of 9–11-day-old embryos.
- The allantoic fluids of all eggs are pooled after harvesting 3–7 days after infection; this pool is diluted 1/5 or 1/10 in antibiotic broth and further passaged into another set of eggs. This is repeated as desired.
- A field strain will induce teratological changes in the embryo at the second or third passage consisting of stunted and curled embryos with feather dystrophy and urate deposits in the embryonic mesonephros. The allantoic fluid should not agglutinate red blood cells.
- TOCs prepared from 20-day-old embryos can be used to isolate IBV directly from field material. Tracheal rings of 0.5–1.0 mm thick are prepared using automatic tissue chopper. Infection of tracheal organ cultures produce ciliostasis within 24–48 hours.

AVIAN INFECTIOUS BRONCHITIS
PATHOGENESIS

- Deals about hosts affected, distribution, transmission, symptoms and lesions

AVIAN INFECTIOUS BRONCHITIS
HOSTS AFFECTED

- Chickens and commercially reared pheasants are the only natural hosts for IBV. Other species are not considered as reservoirs of IBV.. Young birds are more prone than adult birds.

AVIAN INFECTIOUS BRONCHITIS
DISTRIBUTION

orldwide in distribution.

AVIAN INFECTIOUS BRONCHITIS
TRANSMISSION

shed the virus in respiratory secretion and faeces. The virus spreads rapidly among chickens in a flock through inha
zing) produced by infected chickens. Infection is also transmitted by aerosols, contaminated feed and water, contact

birds is considered as a potential source for the introduction of IBV. Affected birds recover within fourteen days. Ho
me latently infected with erratic shedding of virus for a prolonged period of time via both faeces and aerosol. There
ion. No vectors are also involved in the spread of infection.

AVIAN INFECTIOUS BRONCHITIS
MORBIDITY AND MORTALITY

- Morbidity goes upto 100% and mortality is variable (upto 50%).

AVIAN INFECTIOUS BRONCHITIS
INCUBATION PERIOD

- The incubation period is 18 to 36 hours.

AVIAN INFECTIOUS BRONCHITIS
SYMPTOMS

- The infection in young chickens is characterised by gasping, coughing and nasal discharge with wet eyes and swollen sinuses. The chicks appear depressed and may huddle near the heat source. Food consumption and weight gain are also reduced.
- In adult laying flocks, the respiratory symptoms of gasping and coughing are usually followed by a drop in production and the birds may molt. Pullets in good condition may suffer only a slight drop in production and regain normal production within few weeks.
- Following the decline, production of misshapen soft-shelled eggs with inferior internal quality is often observed. This change in shell and internal quality may be permanent. Secondary bacterial infection due to E.coli complicates the disease scenario and lead increased condemnation of birds.

AVIAN INFECTIOUS BRONCHITIS
LESIONS

- The infection is exhibited as respiratory, renal and reproductive forms.
    - Respiratory forms of the disease are usually observed in poorly vaccinated flocks. Secondary bacteria such as E.coli, as explained, can complicate the production parameters minimizing profit returns. Lesions include inflammation and

accumulation of mucous in the trachea, nasal passages and sinuses. Air sacs may be cloudy and thickened.
    - Renal form of the infections is characterised by swollen and inflamed kidneys, distension of the ureters with build up of urate deposits. Holte and Gray are considered as nephrogenic strains and they have a high affinity (attraction) for the kidneys and the ureters. Mortality in renal form may go upto 60%.
    - Reproductive form characterised by damage to ovaries and shortened reproductive tract

AVIAN INFECTIOUS BRONCHITIS
DIAGNOSIS

- Dealt as field and laboratory diagnosis

AVIAN INFECTIOUS BRONCHITIS
FIELD DIAGNOSIS

- Sudden appearance of respiratory infection with high morbidity and characteristic respiratory symptoms help in field diagnosis. In adult birds, the causes for malformed egg should be analysed in details before confirming it as IB.

AVIAN INFECTIOUS BRONCHITIS
LABORATORY DIAGNOSIS

Isolation and identification

- Clinical materials
    - *Live birds:* Tracheal mucus from young chicks
    - *Dead birds:* Tissue samples from the trachea, kidney, oviduct and caecel tonsils in sterile transport media with antibiotics and dry swabs from the respiratory tract or cloaca
- Isolation systems
    - Embryonated eggs or tracheal organ culture (TOC). In eggs characteristic lesions include teratological changes in the embryo at the second or third passage consisting of stunted and curled embryos with feather dystrophy and urate deposits in the embryonic mesonephros. The infection in TOCs is characterised by ciliastasis. The lesions have to confirmed by any one of the following antigen identification system. In outbreaks from affected birds, the virus can be isolated using sentinel birds.
- Antigen identification system
    - Fluorescent antibody test (FAT)
    - Immunohistochemistry methods like IPT
    - Antigen competitive ELISA
    - RT-PCR for S gene
    - CDNA probe
- Serotype identification: Since IBV are antigenically distinct identification of correct serotype is essential. Serotype identification is carriedout by
    - Virus neutralization test in eggs, TOCs

- HI test
- Monoclonal antibody (serotype) based ELISA
- Monoclonal antibody (serotype) based immunohistochemistry
- RT-PCR using serotype specific primers

AVIAN INFECTIOUS BRONCHITIS
SEROLOGY

- The serological tests are normally performed to identify antibodies produced after vaccination. During infection, paired sera sample with a time interval of three weeks will yield clues about infection. Some of the commonly performed serological tests are
  - Virus neutralization test
  - HI test
  - AGID
  - ELISA

AVIAN INFECTIOUS BRONCHITIS
DIFFERENTIAL DIAGNOSIS

- Newcastle disease, Infectious Laryngeal Tracheitis and Infectious Coryza

AVIAN INFECTIOUS BRONCHITIS
TREATMENT

- Not effective and only supportive treatment is provided.

AVIAN INFECTIOUS BRONCHITIS
CONTROL AND ERADICATION

- *Vaccines:* At present both live and inactivated vaccines are used. Live vaccines based on the Massachusetts serotype alone are used now. Live vaccines confer a better local immunity on the respiratory tract and may protect against a wider antigenic spectrum of field strains. While single dose of live vaccine may protect broilers, it may not protect for the life of the layer flock as variant serotype challenge is very high on farms. Further, the use of some live vaccines carries the risk of residual pathogenicity associated with vaccine back-passage in flocks. Inactivated oil emulsion vaccines preceded by a live virus vaccine produce persistent antibody response.
- Strict biosecurity measures and good husbandry practices will minimize the spread of IBV.
- During outbreaks, the virus should be serotyped and vaccine should be altered.
- In areas where there is no IB, use of live vaccine should be minimized.

AVIAN INFECTIOUS BRONCHITIS
PUBLIC AND HEALTH

- No human infection is reported.

MODULE-17: CORONAVIRUS - TRANSMISSIBLE GASTROENTERITIS

Learning objectives

- Group IV viruses Positive sense single stranded RNA viruses
  - The disease and its pathogenesis
    - Transmissible gasteroenteritis of Pigs
  - About the diagnosis
  - About the vaccines
  - Treatment, control and eradication of TGE

TRANSMISSIBLE GASTROENTERITIS INTRODUCTION

- Transmissible gastro-enteritis (TGE) is an acute highly contagious disease of pigs characterised by profuse diarrhoea and vomiting with high morbidity and mortality in piglets. Of late, a respiratory variant of the TGE virus (TGEV), known as the porcine respitratory coronavirus (PRCV) has emerged in Europe and is causing interference in the diagnosis of TGE.
- PRCV is probably a deletion mutant of TGEV. PRCV does not appear to be an important primary pathogen, but it has greatly complicated the diagnosis of TGE, particularly by serological means.

TRANSMISSIBLE GASTROENTERITIS GENERAL ASPECTS

- Deals about morphology, replication, resistance etc.

TRANSMISSIBLE GASTROENTERITIS CLASSIFICATION

Baltimore group	Group IV – Positive sense RNA viruses
Order	Nidovirales
Family	Coronaviridae
Genus	Coronavirus
Species	• Transmissible gastroenteritis virus (TGEV); • PRCV Porcine Respiratory Coronavirus is a mutant of TGE

TRANSMISSIBLE GASTROENTERITIS MORPHOLOGY

- Morphological features are typical of other members of the family coronaviridae. The virions are enveloped and slightly pleomorphic or spherical in shape. The surface of the virus has surface projections, which are distinct and club-shaped. The nucleocapsid is filamentous with helical symmetry. The virions contain one molecule of linear positive-sense single stranded RNA, which also act as mRNA. The virus has five structural proteins – S, M, N, HE and E

TRANSMISSIBLE GASTROENTERITIS
OIE LISTING AND RISK GROUPING

- *OIE Listing:* List B infection
- *Risk group:* Risk group I

TRANSMISSIBLE GASTROENTERITIS
RESISTANCE

- The viruses are not very stable, higly heat sensitive and common disinfectants used. The virus has some resistance for cold temperature. Following good biosecurity measures at the pig stall will greatly minimise the loss of piglets due to this infection.

TRANSMISSIBLE GASTROENTERITIS
CULTIVATION

- TGEV does not grow well in cell culture. However, they grow in 3–4-day-old primary or secondary pig kidney monolayers and low passage porcine cultures (such as thyroid or testis). The cytopathic effect (CPE) may be observed after 3–7 days, characterised by cell rounding, enlargement and formation of syncytia.
- Wild-type TGEV (virus obtained from an outbreak in field is referred as wild type) does not grow readily in tissue culture, so several subpassages may be necessary before distinctive CPE become apparent.
- The cytopathic isolates must be confirmed as TGEV by FAT or by in-vitro neutralisation tests or by nucleic acid identification methods. Young piglets are more susceptible to TEGV, Hence, they are commonly used to cultivate TEGV in laboratories.

TRANSMISSIBLE GASTROENTERITIS
PATHOGENESIS

- Deals about hosts affected, distribution, transmission, symptoms, lesions etc.

TRANSMISSIBLE GASTROENTERITIS
HOSTS AFFECTED

- The clinical disease only occurs in pigs. However, cats, dogs and foxes are potential carriers of the virus without clinical signs of the disease.

TRANSMISSIBLE GASTROENTERITIS

DISTRIBUTION

- TGE is present in Europe, Americas (North, South and Central), Asia (including China, Japan and Korea), and parts of West Africa.

TRANSMISSIBLE GASTROENTERITIS
TRANSMISSION

- Outbreaks of TGE are seen commonly following the introduction of infected pigs into susceptible herd. Sub-clinically infected animals can also be a source of infection and recovered pigs often become carriers and can shed the virus for 2-3 weeks in their faeces. Large amounts of virus are excreted in the faeces of infected animals.
- The exact mechanisms of transmission of the disease are still unclear. However, the proposed means are, faeco-oral contamination, through milk from infected sows, contaminated litter, through carriers like dogs cats, foxes, infected swills and through infected husbandry material.
- The virus is relatively fragile and susceptible to disinfectants and drying. However, the virus can survive for few days in the cold temperature, hence the disease is more commonly seen during the winter months. There is no vertical transmission and no vectors are involved in the transmission.

TRANSMISSIBLE GASTROENTERITIS
MORBIDITY AND MORTALITY

- Morbidity and mortality are nearly 100% in piglets

TRANSMISSIBLE GASTROENTERITIS
INCUBATION PERIOD

- Incubation period is relatively short – just 18 hours to 3 days

TRANSMISSIBLE GASTROENTERITIS
SYMPTOMS

- The disease spreads rapidly around the farm. Piglets less than 21 days of age are all affected and generally die. Watery diarrhea (foul smelling yellowish-green often containing flecks of undigested milk particles in the piglet), vomiting, weight loss, dehydration and loss of appetite are the common symptoms.
- The weaned piglets become unthrifty. The growers, finishers and adults are generally mildly affected and will survive if their water supplies are adequate (prevention of dehydration). In adult pigs agalactia is a common symptom.

TRANSMISSIBLE GASTROENTERITIS
LEISONS

- Gross lesions are confined to the gastrointestinal tract, except for the dehydration. The common lesions are distended stomach with curdled milk, congestion with small area of haemorrhage on diaphragmatic surface, distended small intestine with yellow foamy fluid containing curdled milk, enteritis and thinning of intestinal due to severe atrophy of intestinal villi.

TRANSMISSIBLE GASTROENTERITIS
DIAGNOSIS

- Dealt as field and laboratory diagnosis

TRANSMISSIBLE GASTROENTERITIS
FIELD DIAGNOSIS

rrence of infection more severely in piglets in winter months with characteristic symptoms.

TRANSMISSIBLE GASTROENTERITIS
LABORATORY DIAGNOSIS

Isolation and identification

- Clinical materials
  - i. Faeces from affected animals or from dead animals is the material of choice.
  - ii. Loops of affected small intestine, ligated at each end to retain the contents are also the preferred specimens for isolation of virus.
- Isolation systems: TGEV does not grow well in cell culture. However, they grow in 3–4-day-old primary or secondary pig kidney monolayers and low passage porcine cultures (such as thyroid or testis).
  - The sample material is homogenised in cell culture medium or phosphate buffered saline (PBS), pH 7.2, containing antibiotics.
  - This homogenised sample is allowed to stand out off direct sunlight for 30 minutes at room temperature.
  - The suspension is then sonicated and clarified by low-speed centrifugation.
  - The supernatant fluid is mixed with an equal volume of heat-inactivated bovine serum in order to reduce the cytotoxic effect of the material and it is then used to inoculate susceptible tissue cultures.
  - The cytopathic effect (CPE) may be observed after 3–7 days, characterised by cell rounding, enlargement and formation of syncytia. Wild-type TGEV (virus obtained from an outbreak in field is referred as wild type) does not grow readily in tissue culture, so several subpassages may be necessary before distinctive CPE become apparent.
- Antigen identification tests: Since CPEs are not produced immediately and require number of blind passages, following antigen identification tests are performed.
  - Immunofluorescence
  - Reversed passive hemagglutination
  - Enzyme-linked immunosorbent assays (ELISAs)
  - Radioimmunoassay (RIA)
  - DNA probes

    - Electron microscopy
    - RT-PCR
- Identification of antigen directly in tissue sections/clinical materials
    - Fluorescent antibody test: Used to identify viral antigens in sections of small intestine
    - Double antibody sandwich ELISA: This test is based in the capture of the viral antigen from the faecal sample by three MAb, two specific for the S protein (site A and D) and one for the nucleoprotein N.
    - In situ hybridisation (ISH) and RT-PCR: This technique is used for the direct detection of TGEV in clinical samples and for differentiation from PRCV.

TRANSMISSIBLE GASTROENTERITIS
SEROLOGY

- Serological tests are performed on paired serum samples. A rise in antibody titre is an indication of infection. The tests that are commonly performed include
    - Virus neutralization test
    - Indirect ELISA
    - Competitive ELISA
    - Blocking ELISA using monoclonal antibodies also allows the differentiation of TGEV and PCRV

TRANSMISSIBLE GASTROENTERITIS
DIFFERENTIAL DIAGNOSIS

- Haemagglutinating encephalomyelitis (vomiting and wasting disease)
- Classical swine fever (Hog cholera)
- Porcine rotavirus infection
- Swine dysentery
- Colibacillosis,
- Arsenic poisoning

TRANSMISSIBLE GASTROENTERITIS
TREATMENT

- No effective treatment is available. Treatment of TGE should be aimed towards treating dehydration and starvation

TRANSMISSIBLE GASTROENTERITIS
CONTROL AND ERADICATION

- Vaccines: Vaccination of pregnant sows is being tried but commercial vaccines are not yet available.
- To reduce risk of spread, animals from serologically negative herds alone should be introduced and the swill should always be sterilized.
- If an outbreak occurs, good hygiene practices can help reducing the impact of the disease. The spread can be prevented by isolating the newborn piglets.

- The other approach aims to minimize the duration of the disease by exposing all the pregnant sows to the disease to develop transplacental immunity of farrows.

TRANSMISSIBLE GASTROENTERITIS
MORPHOLOGY

tures are typical of other members of the family coronaviridae. The virions are enveloped and slightly pleomorphic c
virus has surface projections, which are distinct and club-shaped.
is filamentous with helical symmetry. The virions contain one molecule of linear positive-sense single stranded RNA
has five structural proteins – S, M, N, HE and E

MODULE-18: REOVIRUS - BLUE TONGUE

Learning objectives

- Introduction about Group III viruses
  - Double stranded RNA viruses
  - About the nature of the orbivirus
  - Cultivation systems for bluetongue virus
  - Pathogenesis of Bluetongue
  - About the diagnosis and various lab tests
  - About vaccines
  - About control of bluetongue and the biological vector

BLUE TONGUE
INTRODUCTION

- The term Reo refers to Respiratory Enteric Orphan viruses, i.e. infect the human respiratory and intestinal tracts, usually without disease symptoms. Bluetongue (BT) is a non-contagious, athropod-borne viral disease of sheep and other domestic and wild ruminants, such as goats, cattle and deer.
- Camelids are also affected by BT. The infection in animals is transmitted through the bite of Culicoides insects. Bluetongue virus (BTV) is endemic in some areas with cattle and wild ruminants serving as reservoirs for the virus.

BLUE TONGUE
GENERAL ASPECTS

- Deals with morphology, classification, physicochemical properties, resistance, replication, strains etc.

BLUE TONGUE
CLASSIFICATION

Baltimore group	Group III double stranded RNA viruses

Family	Reoviridae
Genus	Orbivirus
Species	Bluetongue virus - BTV

BLUE TONGUE MORPHOLOGY

- The virus measures approximately 70-85nm in diameter. The virions not enveloped and hence are not ether sensitive. The capsid shell of virion is composed of two layers.
- The virus has icosahedral symmetry. The nucleocapsids appear to be round without any surface projections or distinct spikes. The outer layer contains two proteins, VP2 and VP5. VP2 is the major neutralising antigen and determinant of serotype specificity. This is followed by a core comprising of VP7, VP3 and three minor proteins and RNA. VP7 is a major determinant of serogroup specificity. VP7 can also mediate attachment of BTV to insect cells.
- The virions contain 10 segments of double stranded RNA. The total genomic length is 19200 nucelotides and the largest segment L1 has got approximately 2800-3900 nucelotides. The viral RNA has methylated cap structure, but no poly A tail.

BLUE TONGUE SYNONYMS

- Sore muzzle, pseudo foot-and-mouth disease, muzzle disease.

BLUE TONGUE OIE LISTING AND RISK GROUP

- *OIE listing*: List A infection
- *Risk group*: No known human infection are associated with BTV

BLUE TONGUE RESISTANCE

- The viruses are resistant to lipid. However, the viruses are relatively acid-labile, and slow freezing at -10 to -200C (14 - 40 F) is harmful to the virus. The viruses are inactivated by 50°C/3 hours and 60°C/15 minutes. ß-propiolactone, iodophores and phenolic compounds also inactivate the viruses. The viruses are very stable in the presence of protein and can survive for years in blood stored at 20°C.

BLUE TONGUE REPLICATION

- The virus replicates in the cytoplasm. The virus enters into the cell through endocytosis. This is followed by partial uncoating. BTV is inactivated if complete uncoating occurs.

Early transcription of the d/s RNA genome by viral polymerase occurs inside this sub-viral particle. The various genome segments are transcribed/translated at different frequencies.

- Transcription occurs in two stages, primary and secondary. Only (-) sense strands are transcribed, resulting in synthesis of (+)sense mRNAs, which are capped inside the core. These mRNAs leave the core and are translated in the cytoplasm.
- The genome is replicated in the cytoplasm in a conservative fashion. An excess of (+)sense strands are produced, which serve as late mRNAs and as template for (-)sense strand synthesis (i.e. each (-) strand leads to many (+) strands - not one-for-one as semi-conservative replication). The mechanism responsible for segregation of the various genome segments into developing particles is not known.

BLUE TONGUE
HA PROPERTY

- BTV agglutinates mammalian RBCs.

BLUE TONGUE
STRAINS AND SEROTYPES

- There are 14 serogoups and these serogroups in total contain 24 serotypes. The serogroups are differentiated by immunological tests that identify viral proteins, which are unique for each serogroup.
- Most serogroups are immunologically distinct, but there is considerable cross-reaction between members of the BT and EHD serogroups. (EHD - epizootic haemorrhagic disease is a viral infection caused by a orbivirus and has clinical symptoms same as BT)

BLUE TONGUE
CULTIVATION

- The virus grows well in embryonated chicken eggs and cell culture systems.
    - In embryonted chickens the preferred route of inoculation is yolk sac. The eggs should be incubated in a humid chamber at 33.5C. The embryos die between 2 and 7 days. Infected haemorrhages have cherry red appearance due to extensive haemorrhages. The presence of virus can also be confirmed by immunofluorescense, immunoperoxidase or antigen capture ELISA tests.
    - BTV also grow in cell culture systems like baby hamster kidney (BHK)-21, African green monkey kidney (Vero) and Aedes albopictus (AA) cells. The cytopathic effects should be confirmed by antigen-capture ELISA, immunofluorescence, immunoperoxidase, or virus neutralisation (VN) tests.

BLUE TONGUE
PATHOGENESIS

- Dealt as hosts affected, distribution, transmission, symptoms and lesions

BLUE TONGUE

HOSTS AFFECTED

- The host range of BTV is very broad and includes all ruminants. However, the infection is well pronounced in sheep followed by other domestic and wild ruminants, such as goats, cattle and deer. Cattle act as reservoir for BTV.

BLUE TONGUE DISTRIBUTION

- The bluetongue virus is present in regions where the Culicoides vector is present (e.g. Africa, the Americas, Australia and some countries of southern Asia and Oceania).

BLUE TONGUE TRANSMISSION

- BTV infection is transmitted in both wild and domestic ruminants/camelids from the bite of the vector midge of the genus Culicoides. Culicoides act as biological vectors for BTV.
- Experimental studies have also demonstrated that ticks are also capable of mechanically or biologically transmitting BTV. The virus can also be transmitted sexually in infected semen, embryos (in embryo technology) and transplacentally from dam to offspring.
- Mechanical transmission also occurs in BT through use for contaminated needles and equipment contaminated with blood of infected animals. Transmission by Culicodes is the most common method in the spread of BT. The Culicoides vector infects animals mostly during mid-summer and rainy seasons, when it is most active.

BLUE TONGUE INCUBATION PERIOD

- The incubation period of BT in sheep is usually 7-10 days. However, viraemia can occur within 4 days in sheep and cattle. The incubation period of BTV infection in deer is 7 to 12 days.

BLUE TONGUE MORBIDITY AND MORTALITY

- In sheep morbidity is 100 percent, whereas mortality is between 0 and 50 percent. Many affected animals usually recover within a few days to 2 weeks

BLUE TONGUE SYMPTOMS

- Bluetongue is clinically manifested as two syndromes:
    - vascular affection of several organ systems particularly oral and buccal mucosa and
    - a reproductive syndrome.

- Sheep are commonly seen with clinical disease, but other domestic ruminants such as cattle and goats only rarely show clinical signs
- Sheep
    - The clinical signs of BT are variable and not all strains of BTV that infect sheep cause clinical disease. The first clinical sign is the rise in temperature to 41.6-41.70 C. Within 24 hours of the initial rise in temperature, excessive salivation and frothing at the mouth develop and are associated with hyperemia and swelling of the buccal and nasal mucosa followed by erosions and ulcerations. By 4 to 7 days in severe cases, extensive ulcerations may be covered by gray necrotic tissue on the dental pad and dorsal surface of the tongue. Hyperemia is also observed around the coronary bands of the hooves. The hooves are tender and varying degrees of lameness are also observed. In more severe cases, the animals stand with an arched back. Sloughing of hooves is common at this stage. Animals that recover may have a dark line in the wall of the hoof. The lesions in the mouth, the reluctance to move, and the necrosis of striated musculature lead to weakness, depression, and rapid weight loss. These can result in prostration and eventual death in severely affected animals. Sheep that recover from severe infections may have a break in the wool 3 to 4 weeks after the fever has subsided. This can lead to partial or complete shedding of wool. The reproductive form of the disease varies greatly. Signs include abortions, stillbirths, and weak "dummy lamb" live births.
- Cattle: Bluetongue virus infection in cattle usually does not cause any clinical sign of disease. Clinical signs consist of
    - mild hyperemia in the buccal cavity and around the coronary band;
    - vesicular lesions, which lead to ulcerations, in the buccal mucosa;
    - erect hair over the cervical and dorsal thoracic areas; and
    - hyperesthesia.
    - In addition, the dermis becomes thickened with prominent folds apparent in the cervical areas, and a dry crusty exudate leads to matting of hair in affected areas. These lesions may persist for 10 to 20 days. Similar lesions have been reported on teats of cattle with clinical BT. Hoof lesions may be associated with lameness.
    - BTV is both abortigenic and teratogenic in cattle experimentally, but not observed commonly in field conditions. Early embryonic loss and decreased reproductive efficiency are the more frequently seen manifestations of the disease in cattle and can severely affect calf/milk production.
- Goats
    - Bluetongue infection of goats is an inapparent infection similar to that described for cattle.

BLUE TONGUE
LESIONS

- The lesions of BT in sheep vary greatly depending on
    - the strain of virus,
    - individual animal and breed susceptibility and
    - environmental (stress) factors. Prominent lesions include facial edema, edematous ears, and dry, crusty exudate over the nostrils.
- *Oral cavity and digestive system*: Lesions in the oral cavity include focal petechial hemorrhages that progress to gray necrotized debris over erosions and ulcerations on the lips; on the dorsal, lateral, and ventral surfaces of the tongue; and on the dental

pad. The buccal mucosa may be cyanotic. Hyperemia and occasional erosions can occur on papillae and laminae in the reticulum and omasum.

- *Respiratory System:* Lesions include cyanosis and edema of the nasal mucosa and pharynx.
- *Vascular system*: Lesions include hyperemia, edema, and hemorrhages. A characteristic lesion is hemorrhage at the base of the pulmonary artery. Petechial and ecchymotic (larger than pinhead-size) hemorrhages may be observed at times in the endocardium. Focal gray-white areas of necrosis are often found in the papillary muscles and less frequently in other areas of the myocardium
- *Skin*: Dermal and subcutaneous edema of the head and ears and an irregular rash (exanthematous eruptions) with serous and crusty exudates on the skin are the common lesions.
- *Muscle*: A yellow gelatinous exudate is present in the fascia (connective tissue) along and between skeletal muscles. On the cut surface of the heavy muscle there may be focal hemorrhages and areas that appear dry and gray-white.
- *Newborn lambs*: Newborn lambs with congenital BT have hydranencephaly or porencephaly. Abnormal development of cerebellum and spinal cord are the other lesions.

BLUE TONGUE
DIAGNOSIS

- Dealt as field and laboratory diagnosis

BLUE TONGUE
FIELD DIAGNOSIS

- Field diagnosis is based on appearance of clinical signs in susceptible population, coincidence of occurrence of symptoms with prevalence of insect vectors, characteristic gross lesions, and a flock history of recent wasting (loss of weight) and pododermatitis (foot rot).

BLUE TONGUE
LABORATORY DIAGNOSIS

ification

s

parinized blood samples from animals with clinical signs or spleen or bone marrow, or both, from dead animals are t
mples from aborted and congenitally infected newborn animals should include heparinized blood and, if possible, sp
ecimens should be shipped refrigerated, not frozen. Freezing makes virus isolation difficult.

s

mbryos and cell cultures like baby hamster kidney (BHK)-21, African green monkey kidney (Vero) and Aedes albopic

ests

ping: BTV are serogrouped on the basis of their reactivity with specific standard antisera that detect proteins, such as
h serogroup. Monoclonal antibodies specific for VP7 are used in the assay. The immunological assays that are perfor
s against VP7 protein are immunofluorescense, antigen capture ELISA and immunospot test (dot ELISA).

g: Serotyping of BTV is carried out using serum specific for each of 24 serotypes. Virus neutralization assays are perf
different types neutralization assays are performed in BHK21, Vero and L929 cells. These tests are plaque reductio
titre neutralization and fluorescent inhibition test.
id detection methods: Polymerase chain reaction (PCR) is an accepted test for international trade. Serotyping is also

BLUE TONGUE
SEROLOGY

- These tests are performed to identify antibodies in the serum. The tests that are performed include
  - Complement fixation test
  - *Agar gel immunodiffusion test:* It is an accepted test for international trade.
  - *Competitive ELISA:* It is also an accepted test for international trade.

BLUE TONGUE
DIFFERENTIAL DIAGNOSIS

- Contagious ecthyma, foot and mouth disease, photosensitisation, pneumonia, polyarthritis, footrot, foot abscesses, plant poisonings, peste des petits ruminants, coenurosis and epizootic haemorrhagic disease of deer

BLUE TONGUE
TREATMENT

- Not very effective and only supportive. The only applicable treatment available is to minimize animal stress and administer broad-spectrum antibiotics to combat secondary infection.

BLUE TONGUE
CONTROL AND ERADICATION

- Control is mainly aimed at vector control. Certain measures that have potential effectiveness against Culicoides include water management (reduction of Culicoides breeding sites), use of insecticides and larvacides (spraying breeding areas), and insect repellents in which animals are dipped.
- Vaccination should be practiced only in endaemic areas. In such areas serotyping of available BTV should be carried out followed by incorporation of all serotypes into the vaccine. Because of the multiplicity of BTV serotypes and variable cross-protection between serotypes, vaccination has resulted in varying degrees of success. No inactivated or subunit vaccines are currently available.

PUBLIC HEALTH ASPECTS

- There is no human infection associated with blue tongue virus.

MODULE-19: REOVIRUS - AFRICAN HORSE SICKNESS

Learning objectives

- Group III viruses double stranded RNA viruses
  - About the nature of the AHS virus
  - Cultivation systems for AHSV
  - Pathogenesis of African horse sickness
  - About the diagnosis and various lab tests
  - About vaccines.

AFRICAN HORSE SICKNESS
INTRODUCTION

- African horsesickness (AHS) is a non-contagious, highly fatal, viscerotropic, insect-borne viral disease of horses, mules and donkeys. The infection is caused by an orbivirus under the family reoviridae. AHS occurs in Equidaes in areas endemic for certain Culicoides midges or gnats. The infection is often fatal and is characterised by fever and oedema in the lungs and subcutaneous tissues. Nine different serotypes have been described as on date.

AFRICAN HORSE SICKNESS
GENERAL ASPECTS

- Deals with morphology, classification, physicochemical properties, resistance, replication, strains etc.

AFRICAN HORSE SICKNESS
CLASSIFICATION

Baltimore group	Group III double stranded RNA viruses
Family	Reoviridae
Genus	Orbivirus
Species	African horse sickness virus - AHSV

AFRICAN HORSE SICKNESS
MORPHOLOGY

- The virus measures approximately 55-70nm in diameter. The virions not enveloped and hence are not ether sensitive. The capsid shell of virion is composed of three layers (outer, middle and inner capsids). The virus has icosahedral symmetry.
- The genome of AHS virus (AHSV) is composed of ten double-stranded RNA segments comprising 18000 nucleotides, which encode seven structural proteins (VP1-7) and four nonstructural proteins (NS1, NS2, NS3, NS3A). Proteins VP2 and VP5 form the outer

capsid of the virion, and proteins VP3 and VP7 are the major inner capsid (middle) proteins. Proteins VP1, VP4 and VP6 constitute minor inner capsid proteins. VP2 protein is highly variable and is responsible for serotype variations.

AFRICAN HORSE SICKNESS
SYNONYMS

- Peste equina africana, Peste equine, perdesiekte.

AFRICAN HORSE SICKNESS
OIE LISTING AND RISK GROUP

- *OIE listing*: List A infection
- *Risk group*: No known human infection is associated with AHSV. However, occasional intranasal exposure to some vaccine strains has been reported.

AFRICAN HORSE SICKNESS
RESISTANCE

- AHSV is comparatively a stable virus. The virus can survive at room temperature (37°C for 37 days) for nearly a month. However the viruses are inactivated by temperatures >50°C for 3 hr, 60°C for 15 min, 70°C for 5 min.
- The viruses are also stable for up to 20 yr at 4°C; however, inactivated by repeated freezing and thawing. The viruses are stable at basic pH range of 6.3-12.0. The viruses are inactivated by ether, Beta-propiolactone , phenol, iodophores, acetic acid, 2% formalin and chlorine dioxide.

AFRICAN HORSE SICKNESS
REPLICATION

- Typical of other orbiviruses under reoviridae. The virus replicates in the cytoplasm. The virus enters into the cell through endocytosis. This is followed by partial uncoating.
- AHSV is inactivated if complete uncoating occurs. Early transcription of the d/s RNA genome by viral polymerase occurs inside this sub-viral particle. The various genome segments are transcribed/translated at different frequencies.
- Transcription occurs in two stages, primary and secondary. Only (-) sense strands are transcribed, resulting in synthesis of (+)sense mRNAs, which are capped inside the core. These mRNAs leave the core and are translated in the cytoplasm.
- The genome is replicated in the cytoplasm in a conservative fashion. An excess of (+)sense strands are produced, which serve as late mRNAs and as template for (-)sense strand synthesis (i.e. each (-) strand leads to many (+) strands - not one-for-one as semi-conservative replication).
- The mechanism responsible for segregation of the various genome segments into developing particles is not known.

AFRICAN HORSE SICKNESS
HA PROPERTY

- The virus has HA property

AFRICAN HORSE SICKNESS
STRAINS AND SEROTYPES

- There are 9 serotypes of AHSV and all these serotypes are immunologically distinct. These serotypes are indicated as Serotype 1, 2, 3, 4, 5, 6. 7, 8 and 9. Infection with serotype 9 has been reported in India.

AFRICAN HORSE SICKNESS
CULTIVATION

- *Cell culture*: AHSV can be cultivated in baby hamster kidney-21 (BHK-21), monkey stable (MS) and African green monkey kidney (Vero).
- *Embryonated chicken eggs (ECE)*: The virus grows in chicken embryos but no lesions or death of embryo take place. The virus can also be cultivated in chicken embryo fibroblast, but no CPE are produced.
- *Lab animals*: The virus can be cultivated in newborn mice and guinea pigs. In newborn mice the viruses are inoculated through intracerebral route.

AFRICAN HORSE SICKNESS
PATHOGENESIS

- Dealt as hosts affected, distribution, transmission, symptoms and lesions

AFRICAN HORSE SICKNESS
HOSTS AFFECTED

- Members of the family Equidae are affected by AHS. The important hosts are horses, mules and donkeys. Several other species in wild population like zebra, elephant and camels harbor the virus without showing clinical disease. Dogs can be infected by eating contaminated meat or animal products, and show severe clinical disease

AFRICAN HORSE SICKNESS
DISTRIBUTION

- occurs in the tropical area of central Africa where it is endemic. The disease spreads from there to the Southern Africa and occasionally to Northern Africa. Infection has also been reported in Asia (from Middle East to India) and in Europe (Spain and Portugal). AHS has never occurred in the Pacific region.

AFRICAN HORSE SICKNESS
TRANSMISSION

- AHS is not transmitted directly between horses. The infection is transmitted by via the biting Culicoides. C imicola, C obsoletus and C pulicaris are the common biological

vectors for AHSV. The virus is also transmitted biologically by midges, and these insects are most active just after sunset and at about sunrise.

- Other insects such as mosquitoes also act as biological vectors, and large biting flies (e.g., Stomoxys, Tabanus) transmit AHS virus mechanically. Wind-borne spread of midges may assist the short-distance spread of the disease but that long-distance jumps of the infection are invariably the result of movement of infected Equidae.
- The spread of disease is influenced by climatic conditions, which favour the spread of carrier insects (vectors) including warm, moist weather and high rainfall, as well as spread by wind dispersal.
- In order for AHSV to become infective, it must be taken up in a blood meal from an infected host.
- After the virus matures within the arthropod vector, it can be introduced into a susceptible host at the following blood meal. Once inside the new host, the virus replicates in the local lymph nodes and disseminated through viraemia.

AFRICAN HORSE SICKNESS
MORBIDITY AND MORTALITY

- Morbidity depends on the number of infected Culicoides insects as well as the duration of exposure. Mortality varies between 70% and 95% horses, 50% in mules and 5-10% in donkeys.

AFRICAN HORSE SICKNESS
INCUBATION PERIOD

- Incubation period is usually 7-14 days, but may be as short as 2 days.

AFRICAN HORSE SICKNESS
SYMPTOMS

- AHSV fever is a seasonal, viscerotropic disease characterized by subcutaneous/pleural edema and fever. The virus is manifested in several forms: peracute, subacute, acute, and horse sickness. Often, the associated respiratory and circulatory distress results in severe disease and death. Severity of disease varies among species, with horses being the most affected, followed in order by mules, donkeys, and zebras.

AFRICAN HORSE SICKNESS
HORSES

- The per-acute pulmonary form (also referred as dunkob) has a short incubation period of 3-5 days with 95 per cent mortality. The course of the infection is 3 days. The infection is characterized by fever (40-41°C) lasting 1-2 days, congested ocular, nasal, and oral mucous membranes, increased respiratory rate, wide abnormal stance with head and neck extension, flared nostrils, forced expiration resulting in heave lines, profuse sweating, spasmadic cough and appearance of frothy and serofibrinous blood-tinged fluid from nostrils. The death of animals is due to anoxia.
- The sub-acute cardiac form (also referred as dikkob) has an incubation period of from 7-14 days and with a mortality rate of around 60 per cent. This form of the disease is

characterised by fever (39-41°C) lasting 3-6 days, oedema of the supraorbital fossa, oedematous swellings over the head and eyelids, lips, cheeks and under the jaw, petechial hemorrhage of conjunctiva and ventral surface of tongue, depression and appearance of colic signs. Death results from cardiac failure.

- The acute or mixed form is a combination of the previous two types with an incubation period of from 5-7 days and the disease shows itself initially by mild pulmonary symptoms followed by the typical oedernatous swellings of the cardiac form.
- Horse sickness fever. This is the mildest form, characterised by a febrile (very active and nervous) reaction with low temperatures in the morning rising to a high peak in the afternoon. Other symptoms include slight congestion of conjunctivae, increased pulse rate, mild anorexia and mild depression

AFRICAN HORSE SICKNESS
OTHER ANIMALS

- The clinical disease in mules resembles that in horses. Donkeys and zebras, usually manifest the horse sickness form of the disease and mortality is significantly lower than for horses. Affected dogs exhibit the pulmonary form of the disease

AFRICAN HORSE SICKNESS
LEISONS

- In peracute form the most characteristic changes are edema of the lungs or hydrothorax. In very peracute cases, extensive alveolar edema and mottled hyperemia of the lungs are seen. Other less commonly observed lesions are periaortic and peritracheal edematous infiltration, diffuse or patchy hyperemia of the glandular fundus of the stomach, hyperemia and petechial hemorrhages in the mucosa and serosa of the small and large intestines. Most of the lymph nodes are enlarged and edematous, especially those in the thoracic and abdominal cavities.
- In cardiac form the prominent lesion is a yellow gelatinous infiltration in the subcutaneous and intermuscular fascia primarily of the head, neck, and shoulders. Extensive petechial and ecchymotic hemorrhages on the epicardium and endocardium, particularly of the left ventricle are also observed.
- In mixed form the lesions represent a combination of those found in the pulmonary and cardiac forms

AFRICAN HORSE SICKNESS
DIAGNOSIS

- Dealt as field and laboratory diagnosis

AFRICAN HORSE SICKNESS
FIELD DIAGNOSIS

- In early febrile phase field diagnosis is very difficult. It is based on season, prevalence of Culicoides insects, movement of horses from affected areas and characteristic symptoms and lesions.

AFRICAN HORSE SICKNESS
LABORATORY DIAGNOSIS

Isolation and identification

- *Clinical materials*: Blood collected from early febrile period, spleen, lung, and lymph nodes. Specimens for virus isolation should be shipped to the laboratory refrigerated, NOT FROZEN.
- *Isolation systems*: The virus can be isolated in baby hamster kidney-21 (BHK-21), monkey stable (MS), African green monkey kidney (Vero), newborn mice (intracerebral inoculation) and ECE (intravenous route).
- *Lesions / CPE*: Not very characteristic and dependant on confirmation of antigen. In mice inoculatin, in positive cases, animals develop nervous signs between 3 and 15 days post-inoculation.
- *Identification of antigen*: The presence of antigen in isolation systems are confirmed by ELISA, virus neutralization test or by PCR.

AFRICAN HORSE SICKNESS
SEROLOGY

- The following serological tests are used to identify antibodies against AHSV. They are
  - Indirect ELISA (Accepted for international trade) - VP7 protein isn used as antigen for AHSV antibody determination. This test has high degree of sensitivity and specificity.
  - Complement fixation test (Accepted for international trade)
  - Immunoblot test: The binding of antibodies to viral proteins separated by electrophoresis and transferred to nitrocellulose paper is used for the determination of anti-AHSV antibodies.
  - NS3 ELISA: This test distinguish between infected horses and horses vaccinated with the inactivated purified AHSV serotype 4 vaccine.
  - Haemagglutination inhibition test
  - Virus neutralization test: Done to serotype AHSV

AFRICAN HORSE SICKNESS
DIFFERENTIAL DIAGNOSIS

- Clinical signs associated with AHSV are clearly not pathognomic. Hence, the infection has to be differentiated from
  - Viral
    - Equine infectious anemia (Retroviridae)
    - Equine viral arteritis (Arteriviridae)
    - Equine encephalosis (Reoviridae) (often occurs concurrently with AHSV; differentiable by absence of edema and lower mortality rates)
  - Bacterial
    - Anthrax (Bacillus anthracis)
    - Protozoan
    - Trypanosomiasis (Trypanosoma evansi)
    - Piroplasmosis (Babesia caballi and Babesia equi)

- Other
  - Purpura haemorrhagica (lesions on necropsy tend to be more widely distributed in this condition than in AHS)

AFRICAN HORSE SICKNESS
TREATMENT

- No specific treatment is available for AHS. Recovered animals demonstrate life-long immunity to that serotype and partial immunity to similar serotypes. Passive immunity from maternal antibodies lasts about 6 months.

AFRICAN HORSE SICKNESS
CONTROL - VACCINATION

- Since the serotypes are immunologically distinct monovalent, quadrivalent and polyvalent vaccines are used to control AHSV. The problem with modified live attenuated vaccines are that they do not allow differentiation between vaccinated and infected animals and may aid in persistence of the virus
  - A live attenuated vaccine was developed by adapting the virus to the brains of adult mice. This vaccine though offers protection, occasionally caused encephalitis in horses mules and particularly in donkeys.
  - An alternate and safe vaccine was developed by attenuation of the virus in Vero cell cultures. This vaccine is currently used in South Africa, which consists of two quadrivalent vaccines that are administered 3 weeks apart.

AFRICAN HORSE SICKNESS
ERADICATION

- Aimed at following aspects
  - Vaccination of susceptible animals
  - Vector control (use of insecticides, repellents and protective screens)
  - Reduction of exposure to arthropod vector by keeping horses in stables or barns from dusk till dawn, because Culicoides species are most active at sunrise and sunset

AFRICAN HORSE SICKNESS
PUBLIC HEALTH ASPECTS

- There is no evidence that man can become infected with field strains of AHS virus, either through contact with infected animals or from working in laboratories. However, it has been shown that certain neurotropic vaccine strains may cause encephalitis and retinitis in humans following transnasal infection.

MODULE-20: BIRNAVIRUS - INFECTIOUS BURSAL DISEASE

Learning objectives

- Group III viruses double stranded RNA viruses
    - About the nature of the IBD virus
    - Cultivation of IBDV
    - Pathogenesis of infectious bursal disease
    - About the diagnosis and various lab tests
    - About vaccines
    - About control

INFECTIOUS BURSAL DISEASE
INTRODUCTION

- Infectious bursal disease (IBD, Gumboro disease) is an immuno-suppressive disease of domestic poultry caused by a birnavirus. The disease was discovered in 1962 in Gumboro, Delaware, USA. Hence, it is also known as Gumboro disease.
- Chickens between two and three weeks of age were affected and showed signs of disease and a transient immunosuppression. However, in the 1980s the disease changed character. And the birds infected within the first week of life, developed bursal atrophy leading to a subclinical immunosuppression with increasing incidence of diseases of the skin, gastrointestinal and respiratory systems.
- Later a new strain appeared, causing a more rapid bursal atrophy, immunosuppression, diarrhoea and weight loss. Even vaccinated birds were affected and the natural immunity declined faster than after infection with the old type of virus.
- The mortality in Leghorns reached up to 90% and the morbidity almost 100%. Three different syndromes occur and these are referred to as “classical”, “very virulent” (vvIBD), and “variant” infectious bursal disease. This classification is based on pathogenicity.

INFECTIOUS BURSAL DISEASE
GENERAL ASPECTS

- Deals with classification, morphology, replication, resistance etc

INFECTIOUS BURSAL DISEASE
CLASSIFICATION

- Baltimore group – Group III double stranded RNA viruses
- Family – Birnaviridae (Birnavirus means bisegmented dsRNA virus)
- Genus – Avibirnavirus
- Species – Infectious bursal disease virus – IBDV.

INFECTIOUS BURSAL DISEASE
MORPHOLOGY

- The virions are non-enveloped and hence are not ether sensitive. The capsid is composed of a single layer and appear hexagonal in outline with icosahedra symmetry.
- The capsid consists of 132 capsomers and surface projections are not present. The virions measure 60 nm in diameter. Virions contain two segments of linear double stranded RNA.

- The genome contains 5850-6050 nucleotides. The largest segment is termed as A and is comprised of 3100-3200. The second largest segment is termed as B and is comprised of 2750-2850.
- The 5' end of the genome has on both segments a genome-linked protein (VPg) and the 3' end has no poly (A) tract. Segment A codes for four proteins VP2, VP3, VP4 and VP5. VP2 and VP3 are capsid proteins and VP4 and VP5 are non-structural proteins.
- The antigenic determinants are located in the VP2 protein. VP5 is cytotoxic in nature and responsible for lysis of cell during release of virus. Segment B contains only one gene, VP1, which codes for the virus polymerase.

INFECTIOUS BURSAL DISEASE
SYNONYMS

- Gumboro disease

INFECTIOUS BURSAL DISEASE
OIE LISTING AND RISK GROUP

- *OIE listing:* List B infection
- *Risk group:* No known human infection.

INFECTIOUS BURSAL DISEASE
RESISTANCE

- The virions are stable in acid environment of pH 3-7; stable in alkaline environment of pH 7-9.
- Virions are not sensitive to treatment with heat (60C, 1 hour), or ether (and 1% SDS at 20C, pH 7.5 for 20 min.)

INFECTIOUS BURSAL DISEASE
REPLICATION

- Virus replication occurs in the cytoplasm and does not affect the cells own synthesis of RNA and proteins (There is no complet shut off). The two double stranded DNA-segments, A and B, code for different viral proteins (VP).
- The replication is characterized by presence of subgenomic RNAs - a mRNA, a transcription of RNA-A and RNA-B.
- The non-structural protein VP5 has been shown to be important since it causes lysis of the host cells and release of the virus.
- The protein accumulates in the plasma membrane and causes cell deformation prior to cell lysis.

INFECTIOUS BURSAL DISEASE
HA PROPERTY

- The virus has no HA property.

INFECTIOUS BURSAL DISEASE
STRAINS AND SEROTYPES

- There are two distinct serotypes of the virus named as Serotype 1 and Serotype 2, which are both capable of infecting chicken as well as some other species of domestic poultry like turkey and ducks.
- However, only serotype 1 cause clinical disease in chickens younger than 10 weeks.
- Serotype 2 does not cause any clinical disease in chickens.
- Using molecular techniques, serotype 1 has been further subdivided into six distinct groups

INFECTIOUS BURSAL DISEASE
CULTIVATION

- The virus is easily cultivable in chickens, ECE and chicken embryo fibroblasts.
    - *Chickens*
        - This method is not recommended due to animal welfare concerns. The virus is inoculated as eyedrop.
        - The virus kills the chickens 72–80 hours after inoculation. The bursae of chickens infected with virulent serotype 1 IBDV appear yellowish (sometimes haemorrhagic) and turgid, with prominent striations.
        - Peribursal oedema is sometimes present, and plugs of caseous material are occasionally found. The plicae are petechiated.
        - The bursae of chickens infected with serotype 2 IBDV do not exhibit any gross lesions.
    - *Embryonated chicken eggs*
        - The virus is inoculated via yolk sac of five 6–8-day-old specific antibody negative (SAN) chicken embryos and on to the chorioallantoic membrane of five 9–11-day-old SAN chicken embryos.
        - Serotype 1 IBD produces dwarfing of the embryo, subcutaneous oedema, congestion and subcutaneous or intracranial haemorrhages. The liver is usually swollen, with patchy congestion producing a mottled effect. In later deaths, the liver may be swollen and greenish, with areas of necrosis.
        - The spleen is enlarged and the kidneys are swollen and congested, with a mottled effect. Serotype 2 IBDV does not induce subcutaneous oedema or haemorrhages in the infected embryos, but embryos are of a smaller size with a pale yellowish discolouration.
    - *Cell culture*
        - Chicken embryo fibroblasts are normally used for cultivation: The CPE is characterised by small round refractive cells.

INFECTIOUS BURSAL DISEASE
PATHOGENESIS

- Deals about hosts affected, distribution, transmission, symptoms and lesions

INFECTIOUS BURSAL DISEASE
HOSTS AFFECTED

- The domestic fowl is the natural host. Sub-clinical infection may occur in turkeys.
- Disease is most common in 3 to 6 weeks old birds, however severe infection occurs in Leghorn up to 18 weeks.

INFECTIOUS BURSAL DISEASE
DISTRIBUTION

- Very virulent IBD have been reported in Europe, Americas and South East Asia.

INFECTIOUS BURSAL DISEASE
TRANSMISSION

- The main route of infection is through ingestion.
- Affected birds serve as source of infection.
- Chickens infected with the IBD virus shed the virus in their feces.
- Feed, water, and poultry house litter become contaminated with faecal materials and they serve as source of infection. Other chickens in the house become infected by ingesting the virus.
- The lesser mealworm (Alphitobus diaperinus) has also been shown to carry the virus. Because of the resistant nature of the IBD virus, it is easily transmitted mechanically among the farms by people, equipment and vehicles.

INFECTIOUS BURSAL DISEASE
MORBIDITY AND MORTALITY

- Morbidity rates are high (upto 100%).
- Mortality rates are variable and may go up to 25% in broilers and 60% in layers.

INFECTIOUS BURSAL DISEASE
INCUBATION PERIOD

- Incubation period is 2-3 days.

INFECTIOUS BURSAL DISEASE
SYMPTOMS

- IBD is a disease only affecting chickens, and mainly those around 3-4 weeks old when the bursa is well developed. In younger birds the disease usually has a subclinical course.
- Diseased chickens are pale, depressed, dehydrated, anorectic, shivering, have an insecure, atactic pace and ruffled feathers. They often develop transient diarrhoea that can be white with red or green staining.
- Further there is self-inflicted vent picking. When the infection occurs earlier in life the immunosuppression becomes permanent. In other cases, it usually is transient.
- Some birds may also show growth retardation after recovery. No disease can occur after 15-16 weeks of age when the bursa is regressed.
- The immunodeficiency caused by the virus depends on viral strains, age of the chicken and co-infection with other pathogens.

INFECTIOUS BURSAL DISEASE
LESIONS

- The following lesions are observed in dead birds
  - Enlareged cloacal bursa which is swollen and haemorrhagic in birds dead of the disease and is atrophied in recovered birds
  - Dehydrated carcass
  - Dark skeletal muscles with haemorrhages (especially thigh and pectoral muscles)
  - Opaque thymus with thickened gelatinous capsule
  - Fatty and yellow or pink bone marrow
  - Swollen liver
  - Swollen kidneys
  - Intestines with increased mucus
  - Changes in bursa:
    - Within three to four days post infection there is an inflammatory hypertrophy of the bursa and apoptosis of B-lymphocytes.
    - Within two days of infection there is complete depletion of cells in the follicular cortex and after a week the follicular structure may not be visible at all. At four to six days post infection the bursa is swollen, hemorrhagic and covered by a gelatinous exudate.
    - The bursa will reach five times its normal size during acute infection before atrophy begins. Ten days post infection the bursa will be one eighth of its original size and after two months a repopulation of the bursa occurs.
    - At necropsy an atrophied, grey bursa is seen with no Blymphocytes in the bursal follicles or in other lymphoid tissues. (Recently it has been shown that T-cells also are affected by IBDV infection. This might be explained by a macrophage-mediated inhibition of the T-cells ability to respond to mitogenic stimulation or an activation of T-suppressor cells)

INFECTIOUS BURSAL DISEASE
DIAGNOSIS

- Dealt as field and laboratory diagnosis.

INFECTIOUS BURSAL DISEASE
FIELD DIAGNOSIS

- Based on clinical symptoms and lesions, vaccination history, prevalence of the infection in the area and age of the birds affected by the infection.
- A flock will show very high morbidity with severe depression in most birds lasting for 5–7 days.
- Mortality rises sharply for 2 days then declines rapidly over the next 2–3 days.
- Usually between 5% and 10% of birds die, but mortality can reach 30–40%.

INFECTIOUS BURSAL DISEASE
LABORATORY DIAGNOSIS

- *Isolation and identification*
  - *Clinical materials*: Samples should be collected from sick or freshly dead birds.
    - Fresh bursa and spleen for virus isolation.
    - Samples of bursa, spleen, intestines, caecal tonsil, liver and kidney should be collected in neutral buffered formalin for histopathology
    - Blood samples for serology.
  - *Isolation systems*: Chickens, ECE and Chicken embryo fibroblast (follow as given under cultivation).
- *Direct identification of antigen from bursa*
  - Agar gel immunodiffusion test
  - Immunofluorescense test
  - Antigen capture ELISA
  - RT-PCR
- *Strain differentiation*
  - IBDV serotypes are differentiated by cross neutralization virus neutralization test or by serological tests using serotype specific monoclonal antibodies.
- *Pathotype identification*
  - The terms 'variant', 'classical' and 'very virulent' have been used to qualify IBDV strains that exhibit a different pathogenicity. Based on the signs and lesions observed in White Leghorn SPF chickens during acute experimental IBD
    - 'Variant' IBDVs induce little if any clinical signs and no mortality but marked bursal lesions,
    - 'Classical' IBDVs induce approximately 10–50% mortality with typical signs and lesions
    - 'Very virulent' IBDVs induce approximately 50–100% mortality with typical signs and lesions

INFECTIOUS BURSAL DISEASE
SEROLOGY

- *AGID:* The AGID test is the most useful of the serological tests for the detection of specific antibodies in serum
- *Quantitative AGID test:* The AGID test is used to measure antibody levels by using dilutions of serum in the test wells and taking the titre as the highest dilution to produce a precipitin line. This can be very useful for measuring maternal or vaccinal antibodies and for deciding on the best time for vaccination.
- *Virus neutralization test:* VN tests are carried out in cell culture. The test is more laborious and expensive than the AGID test, but is more sensitive in detecting antibody. This test is also useful for evaluating vaccine responses or for differentiating between IBDV 1 and 2 serotypes
- *ELISA:* This is the preferred test for identification and quantification of antibodies against IBD.

INFECTIOUS BURSAL DISEASE
DIFFERENTIAL DIAGNOSIS

- IBD should be differentiated from Marek's disease Mycotoxicosis, Coccidiosis, Haemorrhagic syndrome, Avian adenovirus infection and Infectious bronchitis.

INFECTIOUS BURSAL DISEASE
TREATMENT

- Treatment is not effective

INFECTIOUS BURSAL DISEASE
CONTROL

- Two types of vaccine are used for the control of IBD. These are live attenuated vaccines, or inactivated oil-emulsion adjuvanted vaccines.
- Of late a live recombinant vaccine expressing IBDV antigens is also being used.
- To date, IBD vaccines are made with serotype 1 IBDV only.

INFECTIOUS BURSAL DISEASE
LIVE VACCINES

- Live IBD vaccines are produced from fully or partially attenuated strains of virus, known as ‘mild’, ‘intermediate’, or ‘intermediate plus’ (‘hot’), respectively.
- Mild or intermediate vaccines are used in parent chickens to produce a primary response prior to vaccination near to point-of-lay using inactivated vaccine.
- They are susceptible to the effect of maternally derived antibodies so should be administered only after all maternally derived antibodies has waned.
- Application is by means of intramuscular injection, spray or in the drinking water, usually at 8 weeks of age. Intermediate or intermediate plus vaccines are used to protect broiler chickens and commercial layer replacements.
- Some of these vaccines are also used in young parent chickens if there is a high risk of natural infection with virulent IBD. Recently, technology has been developed to deliver live vaccine into eggs during the incubation period.
- Live vaccine virus is blended with IBD antibody and the complex is injected in ovo at 18 days of incubation. The eggs go on to hatch and the vaccine virus is released when the chicks are about 7 days of age.
- In this way, the problem of maternally derived IBD antibody is overcome and the chicks are effectively immunized. Live IBD vaccines are generally regarded as compatible with other avian vaccines.
- However, it is possible that IBD vaccines that cause bursal damage could interfere with the response to other vaccines. Only healthy birds should be vaccinated.

INFECTIOUS BURSAL DISEASE
INACTIVATED VACCINES

- Inactivated IBD vaccines are used to produce high, long-lasting and uniform levels of antibodies in breeding hens that have previously been primed by live vaccine or by natural exposure to field virus during rearing.
- Only healthy birds, known to be sensitised by previous exposure to IBDV, should be vaccinated.
- Used in this way the vaccine should produce such a good antibody response that chickens hatched from those parents will have passive protection against IBD for up to about 30 days of age.

INFECTIOUS BURSAL DISEASE
RECOMBINANT VACCINE

- A live recombinant vaccine expressing IBDV VP2 antigen has been licensed recently in Europe.
- There is limited information available on the use of this vaccine.

INFECTIOUS BURSAL DISEASE
ERADICATION

- Recovered and vaccinated birds can carry and shed virus for long periods.
- Control of the disease implies depopulation and rigorous disinfection of contaminated premises. Because of the stability of the virus and its ability to spread readily with infected birds and contaminated fomites, strict quarantine measures and movement restrictions should be used.

INFECTIOUS BURSAL DISEASE
PUBLIC HEALTH ASPECT

- No human infection has been reported with IBDV.

MODULE-21: RETROVIRUS - EQUINE INFECTIOUS ANAEMIA

Learning objectives

- Group VI Reverse transcribing RNA viruses
  - About the nature and morphology of EIAV
  - Pathogenesis of EIAV
  - About the diagnosis and various lab tests
  - About vaccines
  - About the various tests and regulation for international trade
  - About the control and eradication of EIAV

EQUINE INFECTIOUS ANAEMIA
INTRODUCTION

- Equine infectious anemia (EIA) is an infectious and potentially fatal viral disease of members of the horse family. EIA is significant historically because it is the first disease of horses proven to be caused by a “filterable virus”. EIAV is the first lentivirus–induced disease proven to be transmitted by insects. And EIA is the first persistent virus for which antigenic drift was defined. EIA is also the first lentivirus–induced disease for which a diagnostic test was approved.

EQUINE INFECTIOUS ANAEMIA
GENERAL ASPECTS

- Explains classification, morphology, replication etc.

EQUINE INFECTIOUS ANAEMIA
CLASSIFICATION

Baltimore group	Group VI Reverse transcribing RNA viruses
Family	Retroviridae
Sub-family	Orthoretrovirinae
Genus	Lentivirus
Species	Equine infectious anaemia virus (EIAV)

EQUINE INFECTIOUS ANAEMIA
SYNONYMS

- Swamp fever.

EQUINE INFECTIOUS ANAEMIA
MORPHOLOGY

- The virions have a complex construction and consist of an envelope, a nucleocapsid, a nucleoid, and a matrix protein. Virions are enveloped, spherical to pleomorphic in shape and measure 80-100 nm in diameter. The surface has numerous glycoprotein spikes, which are 8nm in length. The core is rod-shaped and the nucleoid is concentric. The genome is dimeric (diploid) comprises of two monomers. The genome consists of non-segmented two molecules of linear positive-sense, single-stranded RNA joined by hydrogen bonds. Minor species of non-genomic nucleic acid are also found in virions. The genome is composed of 9200 nucleotides. The 5'-end of the genome has a methylated cap and the 3'-terminus has a poly (A) tract. The genome of retroviruses is unique in the following aspects.
    - They are the only viruses, which are truly diploid.
    - They are the only RNA viruses whose genome is produced by cellular transcriptional machinery
    - They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.
    - They are the only (+) sense RNA viruses whose genome does not serve directly as mRNA immediately after infection. Hence, the nucleic acid is not infectious
- The genome is comprised of four genes gag, pro, pol and env. Each gene codes for a single or more than one protein. The important proteins are listed below.

Name	Protein	Function
MA	Matrix matrix protein (gag gene)	lines envelope
CA	Capsid capsid protein (gag gene)	• protects the core

		• most abundant protein in virus particle
NC	Nucleocapsid capsid protein (gag gene)	protects the genome; forms the core
PR	Protease	Essential for gag protein cleavage during maturation
RT	Reverse transcriptase (RNA dependant DNA polymerase)	Reverse transcribes the RNA genome
IN	Integrase Encoded by the pol gene	needed for integration of the provirus
SU	Surface glycoprotein	The outer envelope glycoprotein; major virus antigen
TM	Transmembrane protein	The inner component of the mature envelope glycoprotein

EQUINE INFECTIOUS ANAEMIA
OIE LISTING AND RISK GROUP

- *OIE Classification*: List B infection
- *Risk group*: No known human infection.

EQUINE INFECTIOUS ANAEMIA
RESISTANCE

- EIAV are fragile. They are sensitive to treatment with heat, detergents, and formaldehyde. Their infectivity is not affected by irradiation. The virus lasts for several months at room temperature, in urine, faeces, and dried blood etc.

EQUINE INFECTIOUS ANAEMIA
REPLICATION

rs inside the cytoplasm and it is the only RNA virus that depends on host cell transcriptionary mechanisms. The viru
a host cell receptor. The SU and TM proteins help in this stage. The virus enters by fusion. Only partial uncoating oc
gh the RNA is positive sense they are not translated immediately inside the cytoplasm.
A is synthesized inside the cell with a virus coded enzyme RNA dependant RNA polymerase (reverse transcriptase).
action is known as the provirus. Three forms of provirus DNA are found in all infected cells. This DNA later migrate
ost cell DNA with the help of the enzyme integrase. Retroviruses use the cellular transcriptional machinery for expre
e cellular genes.
at the cytoplasm and the mature virions later bud out through the cell membrane, acquiring the envelope during th
on steps, the replication processes of retroviruses are error prone. Further changes in the retro viral genome occurs
bination and exchange of genes with host genome.

EQUINE INFECTIOUS ANAEMIA

HA PROPERTY

- The virus has no HA property.

EQUINE INFECTIOUS ANAEMIA STRAINS AND SEROTYPES

- No distinct serotypes are present.

EQUINE INFECTIOUS ANAEMIA CULTIVATION

- The virus is cultivated in susceptible horses or in leukocyte cultures. The presence of virus in theses systems is confirmed by antigen confirmation tests.

EQUINE INFECTIOUS ANAEMIA PATHOGENESIS

- Describes host affected, transmission, symptoms and lesions

EQUINE INFECTIOUS ANAEMIA HOSTS AFFECTED

- The infection is limited to members of the family equidae. Donkeys are less severely affected than horses.

EQUINE INFECTIOUS ANAEMIA DISTRIBUTION

- The infection is worldwide. Few countries like Australia and New Zealand have claimed freedom.

EQUINE INFECTIOUS ANAEMIA TRANSMISSION

- EIA is considered a classic bloodborne infection. The infection occurs in summer and autumn since the insects appear in large numbers in these seasons. The spread of EIA is usually slow although it can be quite fast where large groups of horses are gathered in a small area. EIA is very easily spread by the injection of small quantities of infected blood.
- The EIAV most frequently is transmitted between horses in close proximity by large biting insects, such as horseflies and deerflies. The bites from these flies stimulate defensive movement by the horse, which often results in an interruption of the blood–feeding. When interrupted, the fly is motivated to complete the feeding as soon as possible. It then attacks the same or a second host and feeds to repletion.

- In this manner, any infective material from the blood of the first host that is present on the mouthparts of the insect can be mechanically transmitted to the second host. The virus can survive only less than 4 hours in the insect.
- Insect transmission of EIAV is dependent on the number and habits of the insects, the density of the horse population, the number of times the insect bites the same and other horses, the amount of blood transferred between horses, and the level of virus in the blood of the infected horse from which the initial blood meal was obtained.
- Iatraogenic transmission is also possible during any manipulation that may involve the transfer of infected blood or saliva, for example through the use of unsterilised instruments or equipment. Vertical transmission (in utero or through the milk) is also possible

EQUINE INFECTIOUS ANAEMIA
SYMPTOMS

- The first attack is usually the most acute with the horse suffering from depression, profound weakness, and loss of condition. Intermittent fever with large and rapid swings in temperature may also occur. Jaundice and bleeding around the mouth and nasal areas may also occur. This form of the disease is the most damaging and the most difficult to diagnose because the signs appear rapidly, and often only an elevated body temperature is noted. One–fifth of a teaspoon of blood from a horse with acute EIA contains enough virus to infect 1 million horses.
- Animals may show a temporary recovery and appear normal for 2 - 3 weeks and then relapse with similar, but less sever symptoms. It is also referred as chronic form. The symptoms include fever, petechial hemorrhages on the mucous membranes, depression, weight loss, dependent edema under the skin in the legs and under the chest and other underbody surfaces and anemia. The animal may also have an irregular heartbeat, and a jugular pulse. One fifth of a tea spoon of blood from a chronic case during a feverish episode contains enough virus to infect 10,000 horses.
- Most horses also remain as inapparent carriers. These horses show no obvious clinical signsas a result of infection. They survive as reservoirs of the infection for extended periods. Inapparent carriers have dramatically lower concentrations of EIAV in their blood than horses with active clinical signs of the disease. Only 1 horsefly out of 6 million is likely to pick up and transmit EIAV from this horse. All horses infected with EIAV are thought to remain virus carriers for life. The inapparent form may become chronic or acute due to severe stress, hard work, or the presence of other diseases.

EQUINE INFECTIOUS ANAEMIA
LESIONS

- In horses dying of the disease there is haemorrhages in the liver, spleen, kidneys, on serous membranes and in the mucosa of the intestines. The liver is enlarged and often yellowish-brown in colour. The spleen is also enlarged with soft pulp.The heart muscle appear pale, flabby and friable

EQUINE INFECTIOUS ANAEMIA
DIAGNOSIS

- Dealt as Field and Laboratory diagnosis

EQUINE INFECTIOUS ANAEMIA
FIELD DIAGNOSIS

- Field diagnosis is based on the Coggins test. Since, no vaccines are available against EIAV, presence of antibodies against EIA in the serum of horses is an indication of infection. Clinical signs and lesions also provide clue about EIA.

EQUINE INFECTIOUS ANAEMIA
LABORATORY DIAGNOSIS

- *Isolation and identification*: Rarely attempted since it is of no practical value. Blood samples should be collected to confirm the diagnosis using the Coggins (agar gel diffusion) test or the more recent ELISA test. Isolation of the virus from suspect horses may be made by inoculating their blood on to leukocyte cultures prepared from horses free of infection. Virus production in cultures can be confirmed by detection of specific EIA antigen by ELISA, or imunofluorescence assay.
- *Nucleic acid identification method*: Recently a RT-PCR method has been developed to detect EIA proviral DNA from the peripheral blood of horses

EQUINE INFECTIOUS ANAEMIA
SEROLOGY

- Due to the persistence of EIA virus in infected equids, detection of serum antibody to EIA virus confirms the diagnosis of EIA virus infection.
    - *Coggins test – (Agar gel immunodiffusion test):* It is a test prescribed for International trade. Precipitating antibody is rapidly produced as a result of EIA infection, and can be detected by the AGID test. Specific reactions are indicated by precipitin lines between the EIA antigen and the test serum and confirmed by their identity with the reaction between the antigen and the positive standard serum.
    - *ELISA*: Competitive and non-competitive ELISAs are also used to identify antibodies against EIAV.

EQUINE INFECTIOUS ANAEMIA
DIFFERENTIAL DIAGNOSIS

- EIA should be differentiated from purpura haemorrhagica, babesiosis, leptospirosis, severe strongyliasis or fascioliasis, phenothiazine toxicity and auto-immune haemolytic anaemia

EQUINE INFECTIOUS ANAEMIA
TREATMENT

- There is no effective treatment available against EIA. Supportive treatment, including blood transfusions can be considered. However, because recovered horses can become carriers, if the disease occurs in a free area it is generally better to destroy affected horses.

EQUINE INFECTIOUS ANAEMIA
CONTROL AND ERADICATION

- Vaccines
  - There are no vaccines against EIA
- Eradication
  - Eradication is aimed in minimizing or eliminating contact of horses with the secretions, excretions, and blood of EIAV–infected horses. This has to be carried out by testing and segregating test–positive horses from those with negative test results. Once the reservoirs of EIAV are identified, separated, and maintained a safe distance from the other horses.
  - As recovered horses continue to carry the virus, the most likely source of introduction of EIA is through importation of horses. OIE guidelines suggest that, when importing horses, as well as showing no clinical signs, they should be tested negative during the 30 days prior to shipment.
  - In endemic areas, risk of infection can be reduced by protecting horses from insect vectors by
    - Keeping horses away from low lying areas
    - Providing draining swamps
    - Through use of insecticides and by providing insect-proof stabling

EQUINE INFECTIOUS ANAEMIA
ADDITIONAL READING

- By following precautions, horse owners can get rid of the infection. These precautions are specified by the USDA, APHIS, Veterinary Services, National Center for Animal Health Programs, Certification and Control Team
  - Use disposable syringes and needles. Follow the rule of one horse—one needle.
  - Clean and sterilize all instruments thoroughly after each use.
  - Keep stables and immediate facilities clean and sanitary. Remove manure and debris promptly, and ensure that the area is well drained.
  - Implement insect controls. The local veterinarian or animal health official can provide information about approved insecticides and other insect–control measures. Avoid habitats favorable to insect survival.
  - Do not intermingle infected and healthy animals. Do not breed EIAV–positive horses.
  - Isolate all new horses, mules, and asses brought to the premises until they have been tested for EIA.
  - Obtain the required certification of negative EIA test status for horse shows, county fairs, race tracks, and other places where many animals are brought together.
  - Abide by State laws that govern EIA.

MODULE-22: POXVIRUSES - SHEEP POX AND GOAT POX

Learning objectives

- Group I double stranded DNA viruses

- About the family poxviridae and its member viruses including capripox
- About the morphology and the nature of capripox virus
- Cultivation of sheep pox and goat pox virus
- About the disease and its pathogenesis
- About the diagnosis and various lab tests
- About vaccines available for sheep pox and goat pox
- Control and eradication

SHEEP POX AND GOAT POX
INTRODUCTION

- Poxviruses have been known about for centuries. The characteristic "pocks" produced by variola virus (Smallpox) giving their name to all forms of infectious disease. Smallpox first appeared in China and the Far East at least 2000 years ago. Smallpox has now been eradicated - the last naturally occurring outbreak of smallpox was in Somalia on 26th October 1977.

SHEEP POX AND GOAT POX
MORPHOLOGY

- The virions are enveloped and slightlyovoid or brick-shaped with 140-260 nm in diameter; 220-450 nm long. The extracellular forms contain 2 membranes (EEV - extracellular enveloped virions), intracellular particles only have an inner membrane (IMV - intracellular mature virions).
- Thin sections in E.M. reveal that the outer surface is composed of lipid and protein, which surrounds the core, which is biconcave (dumbbell-shaped), with two "lateral bodies" (function unknown). The core is composed of a tightly compressed nucleoprotein.
- The virions have 10 enzymes, which are essential for genome replication. The genome is linear, d/s DNA having 130-300kbp. There are approximately 250 genes in the genome of which some are essential for virus and some are not essential. The essential genes are located in the central part of the genome, while non-essential (in tissue culture) genes are located at the ends.

SHEEP POX AND GOAT POX
REPLICATION

- Poxvirus replication occurs in the cytoplasm. The virus has all the enzymes necessary for genome replication and hence do not depend on the host cell nucleus. Replication is simple and straightforward. Entry is by endocytosis and there are more than one receptor in the cell for attachment.
- Penetration is complex and involves more than one mechanism. Uncoating occurs in two stages. Removal of the outer membrane occurs as the particle enters the cell and the inner membrane is removed as the core passes into the cytoplasm.
- After uncoating the enzymes required for viral genome are produced (early transcription and translation). This is followed by genome replication. Late transcription and translation occurs following this and proteins produced are mostly structural proteins.

Assembly occurs in the cytoskeleton and the events involved in putting together such a complex particle are not understood.

SHEEP POX AND GOAT POX
GENERAL CHARACTERS

- Describes definition, classification, resistance, HA property and strains and serotypes

SHEEP POX AND GOAT POX
DEFINITION

- Sheep pox and goat pox (collectively known as SGP) are viral diseases of sheep and goats characterised by fever, generalised papules or nodules, vesicles (rarely), internal lesions (particularly in the lungs), and death. Both diseases are caused by strains of the genus Capripoxvirus.

SHEEP POX AND GOAT POX
CLASSIFICATION

Baltimore group	Group I (dsDNA viruses)
Family	Poxviridae
Subfamily	Chordopoxvirinae
Genus	Capripoxvirus
Species	Sheeppox virus (SPV)

SHEEP POX AND GOAT POX
RESISTANCE

- The viruses are susceptible to 56°C/2 hours; 65°C/30 min. They are also susceptible to highly alkaline or acid pH. They are sensitive to ether (20%), chloroform, and formalin (1%) and are inactivated by phenol (2%) in 15 min. They can survive for many years in dried scabs at ambient temperatures. Virus remains viable in wool for 2 months and in premises for as long as 6 months

SHEEP POX AND GOAT POX
HA PROPERTY

- Unlike other poxviruses SPV does not agglutinate RBCs.

SHEEP POX AND GOAT POX

STRAINS AND SEROTYPES

d goatpox are caused by strains of capripoxvirus, all of which can infect sheep and goats, and although most of the st
al disease in either sheep or goats, some strains have been isolated that are equally pathogenic in both species.
sed that the malignant pox diseases of sheep and goats caused by capripoxvirus and including Kenyan sheep and goa
rth African stone pox of sheep and goats be referred to as capripox. Some of the common sheeppox virus strains are

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SHEEP POX AND GOAT POX
CULTIVATION

- Cell culture: Capripoxvirus will grow in tissue culture of bovine, ovine or caprine origin. Primary or secondary cultures of lamb testis (LT) or lamb kidney (LK) cells are considered to be the most susceptible. The CPE include retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. The other characteristic CPE is the eosinophilic intracytoplasmic inclusion bodies, which are variable in size but up to half the size of the nucleus and surrounded by a clear halo. Syncytia formation is not a feature of capripoxvirus infection.
- Animal inoculation: Lambs are used for cultivation of SGPV.

SHEEP POX AND GOAT POX
PATHOGENESIS

- Describes about hosts affected, distribution, transmission,incubation period, morbidity and mortality, symptoms and lesions

SHEEP POX AND GOAT POX
HOSTS AFFECTED

- Sheep pox and goat pox viruses are generally considered host specific, but different strains show different host preferences and it is not unknown for both sheep and goats to be infected by the same strain of virus. Merino and European breeds of sheep are more susceptible than other breeds. Goat breeds also vary in susceptibility.

SHEEP POX AND GOAT POX
DISTRIBUTION

- SGP occurs in Africa (mainly north of the equator), the Middle East, central Asia (including south Russia and western China), and the Indian sub-continent as far east as Myanmar.

SHEEP POX AND GOAT POX
TRANSMISSION

- Transmission is through direct contact. Inhalation of aerosols from acutely affected animals, aerosols generated from dust contaminated from pox scabs in barns and night

holding areas, and contact through skin abrasions either by fomites or by direct contact are the natural means of transmitting SGPV. Mechanical transmission by insects also occurs.

SHEEP POX AND GOAT POX
INCUBATION PERIOD

- The incubation of SGP is between 4 and 8 days.

SHEEP POX AND GOAT POX
MORBIDITY AND MORTALITY

- Morbidity rate in endemic areas is 70-90% and mortality rate in endemic areas is only 5-10%, although it can approach 100%.

SHEEP POX AND GOAT POX
SYMPTOMS

- Symptoms vary in clinical cases from mild to severe.
    - The common symptoms include fever, depression, polypnoea, conjunctivitis, lacrimation, rhinitis, oedema of eyelids and photophobia. The cutaneous eruption begin with erythematous areas especially noticeable in hair or wool-free parts of the body, such as the perineum, inguinal area, scrotum, udder, muzzle, eyelids and axillae which evolve into papules.
    - In papulovesicular form Papules become a white-grey colour, desiccate and form crusts that are easy to remove. Rarely, papules may transform into vesicles. After rupture of vesicles, a thick crust covers the lesions.
    - In nodular form Papules give rise to nodules involving all the layers of the skin and the subcutaneous tissue. Necrosis and sloughing of the nodules leaves a hairless scar.
    - In both forms, nodules develop in the lungs causing bronchopneumonia with cough, abundant nasal discharge, depression, anorexia and emaciation. Animals may recover within 20-30 days. Death is frequent when complications like abortion, secondary infections, fly strike, septicaemia and digestive localisation occur.

SHEEP POX AND GOAT POX
LESIONS

- The skin lesions include congestion, haemorrhage, oedema, vasculitis and necrosis. All the layers of epidermis, dermis and sometimes musculature are involved. The lymph nodes draining infected areas enlarge up to eight times normal size with lymphoid proliferation, oedema, congestion and haemorrhage.
- Typical pox lesions are noticed on mucous membranes of the eyes, mouth, nose, pharynx, epiglottis, trachea, on the rumenal and abomasal mucosae, and on the muzzle, nares, in the vulva, prepuce, testicles, udder, and teats. The lung lesions include severe and extensive pox lesions, focal and uniformly distributed throughout the lungs; congestion, oedema, focal areas of proliferation with necrosis, and lobular atelectasis.

SHEEP POX AND GOAT POX
DIAGNOSIS

- Describes about field diagnosis, laboratory diagnosis, serology and differential diagnosis.

SHEEP POX AND GOAT POX
FIELD DIAGNOSIS

- Based on the characteristic symptoms and pox lesions in the hairless parts of the animal.

SHEEP POX AND GOAT POX
LABORATORY DIAGNOSIS

- Isolation and identification
    - *Clinical materials*: Vesicular fluid, scabs and skin scrapings of lesions and lesions in the respiratory and gastro-intestinal tract.
    - *Isolation system*: Primary or secondary cultures of lamb testis (LT) or lamb kidney (LK) cells.
    - *Identification*: immunofluorescence, staining of intracytoplasmic inclusion bodies, inhibition of cytopathic effect using positive serum, ELISA or by PCR.
- Electron microscopy
- Histology: Formalin-fixed biopsy materials and tissue sections are stained by H&E for characteristic histopathological changes.
    - The most striking aspects of acute-stage skin lesions are a massive cellular infiltrate, vasculitis and oedema.
    - Early lesions are characterised by marked perivascular cuffing. Initially infiltration is by macrophages, neutrophils and occasionally eosinophils, and as the lesion progresses, by more macrophages, lymphocytes and plasma cells.
    - A characteristic feature of all capripoxvirus infections is the presence of variable numbers of ‘sheep pox cells’ in the dermis. These sheep pox cells can also occur in other organs where microscopic lesions of sheep and goat pox are present. These cells are large, stellate cells with eosinophillic, poorly defined intracytoplasmic inclusions and vacuolated nuclei.
- Immunological methods: These methods are used to detect SGPV either on the tissue culture fluid or from the lesions. Some of the common immunological methods are
    - AGID
    - ELISA
    - FAT
- Nucleic acid identification methods: The PCR is be used to detect the capripoxvirus genome in biopsy or tissue culture samples.

SHEEP POX AND GOAT POX
DIFFERENTIAL DIAGNOSIS

- SGP should be differentiated from bluetongue, Peste des petits ruminants, contagious ecthyma, photosensitisation, dermatophilosis, insect bites, parasitic pneumonia, caseous lymphadenitis and mange (scrabies).

- Bluetongue — Animals are depressed and have a nonpurulent conjunctivitis. The muzzle is swollen, congested, and edematous, and there may be a coronitis. Deformed aborted fetuses and deformed newborn sheep and goats may be encountered.
- Peste des Petits Ruminants — Conjunctivitis, rhinitis, and oral lesions that are white, raised, and necrotic are common. Pneumonia, diarrhea, and mortality approaching 90 percent in lambs and kids under 1 month of age are characteristic signs.
- Contagious Ecthyma (contagious pustular dermatitis, ORF) — This disease is most severe in lambs and kids. The proliferative pox lesions are common on the muzzle and eyes of affected neonates; mortality may approach 50 percent. Nursing females may have proliferative pox lesions on the teats and muzzle. This is a zoonotic disease; lesions in attendants are not uncommon.
- Photosensitization — Dry, flaky, inflamed areas are confined to the nonpigmented parts of the skin.
- Insect bites — The trauma from insect bites may cause local inflammation, edema, and pruritus. Insects seldom bite mucous membranes.
- Parasitic pneumonia — Severe signs of respiratory distress may occur with extensive parasitic lesions; in these cases, there is no pox lesion in the skin.
- Caseous lymphadenitis — Focal, raised lesions in the skin represent caseous abscesses; abscesses are not seen in SGP.
- Streptothricosis (Dermatophilus congolensis infection) — Lesions are superficial and often moist. Lesions are common in the skin of the neck, axillary region, inguinal region, and perineum. The organism may be demonstrated by Giemsa staining.
- Mange - Scab-like skin lesions are seen in psoroptic mange. Itching and scratching are not seen in SGP.

SHEEP POX AND GOAT POX
TREATMENT

- There is no effective treatment.

SHEEP POX AND GOAT POX
CONTROL

- *Vaccination:* In endemic areas systematic vaccination programs have provided the most effective control over the disease. Cell-cultured attenuated and inactivated vaccines have been used to prevent disease. Live attenuated virus vaccines delivered by subcutaneous or intradermal route confer immunity lasts up to 2 years. Inactivated vaccines provide about five months protection.
- *Eradication:* Eradication can be achieved through
  - Isolation of infected herds and sick animals for at least 45 days after recovery
  - Slaughtering of infected herd (as far as possible)
  - Proper disposal of cadavers and products
  - Stringent disinfection
  - Quarantine before introduction into herds
  - Animal and vehicle movement controls within infected areas

SHEEP POX AND GOAT POX
PUBLIC HEALTH SIGNIFICANCE

- There is no report of human infection.

MODULE-23: POXVIRUSES - FOWL POX

Learning objectives

- Group I double stranded DNA viruses
  - About the morphology and the nature of fowlpox virus
  - Cultivation of fowlpox virus
    - About the disease and its pathogenesis
    - About the diagnosis and various lab tests
    - About vaccination strategies and vaccines available for field use
    - Control and eradication of fowl pox.

FOWL POX
GENERAL CHARACTERS

- Describes definition, classification, resistance, HA property and strains and serotypes

FOWL POX
DEFINITION

- Fowlpox is a disease of chickens and turkeys caused by a DNA virus of the genus Avipoxvirus of the family Poxviridae. It is slow-spreading and characterised by the formation of proliferative lesions and scabs on the skin, and diphtheritic lesions in the upper parts of the digestive and respiratory tracts.

FOWL POX
CLASSIFICATION

Baltimore group	Group I (dsDNA viruses)
Family	Poxviridae
Subfamily	Chordopoxvirinae
Genus	Avipoxvirus
Species	Fowlpoxvirus (FPV)

-

FOWL POX
OIE LISTING AND HUMAN RISK GROUP

- List B disease
- No risk to human beings.

FOWL POX
CULTIVATION

ECE

- The virus are cultivated in ECE by chorioallantoic membrane route. Appearance of focal white pock lesions or generalised thickening of the CAMs after 5-7 days is the characteristic lesion. Histopathological examination of the CAM lesions will reveal eosinopilic intracytoplasmic inclusion bodies following staining with H& E

Cell culture

- Primary chicken embryo fibroblasts, chicken embryo kidney cells, chicken embryo dermis cells, or the permanent quail cell line QT-35, can also be used to propagate fowlpox virus. Production of pock is the characteristic CPE

FOWL POX
PATHOGENESIS

- Describes about hosts affected, distribution, transmission,incubation period, morbidity and mortality, symptoms and lesions

FOWL POX
HOSTS AFFECTED

- Chickens and turkeys are more commonly affected.

FOWL POX
DISTRIBUTION

- The infection is worldwide in distribution.

FOWL POX
TRANSMISSION

- Direct contact between affected birds and susceptible birds is the common route of spread. Fomites also act as source of infection to susceptible birds. Integration of reticuloendotheliosis virus (REV) sequences with fowlpox virus genome was found to increase the virulence of fowlpox virus.

FOWL POX
MORBIDITY AND MORTALITY

- Direct contact between affected birds and susceptible birds is the common route of spread. Fomites also act as source of infection to susceptible birds. Integration of reticuloendotheliosis virus (REV) sequences with fowlpox virus genome was found to increase the virulence of fowlpox virus.

FOWL POX
INCUBATION PERIOD

- 4-8 days.

FOWL POX
SYMPTOMS AND LESIONS

- There are two forms cutaneous (dry pox) and diphtheritic (wet pox). The cutaneous form is characterised by the development of proliferative lesions, ranging from small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas. In the diphtheritic form (wet pox), slightly elevated white opaque nodules develop on the mucous membranes.
- They rapidly increase in size to become a yellowish diphtheritic membrane. In this form the lesions occur on the mucous membranes of the mouth, oesophagus, larynx or trachea.

FOWL POX
DIAGNOSIS

- Describes about field diagnosis, laboratory diagnosis, serology and differential diagnosis

FOWL POX
FIELD DIAGNOSIS

- It is based on symptoms and lesions.

FOWL POX
LABORATORY DIAGNOSIS

- Identification of the virus from lesions
    - *Inclusion bodies*: Fowlpox virus multiplies in the cytoplasm of epithelial cells with the formation of large intracytoplasmic inclusion bodies (Bollinger bodies) that contain smaller elementary bodies (Borrel bodies). The inclusions can be demonstrated in sections of cutaneous and diphtheritic lesions by the use of haematoxylin and eosin (H& E), acridine orange or Giemsa stains. The elementary bodies can be detected in smears from lesions, for example by the Gimenez method.
    - *Electron microscopy*: Used to demonstrate viral particles of typical poxvirus morphology by negative staining or in ultrathin sections of infected tissues

- Isolation and identification
  - *Clinical materials*: Typical pox lesions, scabs
  - *Isolation system*: ECE, CAM route
  - *Lesion*: Appearance of pock lesions after 5-7 days of incubation. Confirmation of virus by FAT or AGID or PCR
- Molecular methods
  - PCR and restriction endonuclease analysis

FOWL POX
SEROLOGY

- Serological tests, such as virus neutralisation (VN), agar gel immunodiffusion (AGID), passive haemagglutination and fluorescent antibody tests, enzyme-linked immunosorbent assay (ELISA) and Western blots are used to measure specific humoral antibody responses.

FOWL POX
DIFFERENTIAL DIAGNOSIS

- The diphtheritic form should be differentiated from ILT (ILT is caused by herpesvirus and produces intranuclear inclusion body)

FOWL POX
TREATMENT

- There is no effective treatment available.

FOWL POX
CONTROL

ination is indicated in areas where fowlpox is endemic or on premises where infection has been diagnosed previousl
, and also fowlpox vectored vaccines that protect against pox, are available commercially. These vaccines are derived
res. Birds are vaccinated around 6th week of age. Evidence of successful immunisation with vaccine can be determin
ccination for 'takes'. A take consists of a swelling of the skin or a scab at the site where the vaccine was applied and it
unisation.
ned at following good husbandry practices coupled with vaccination in endaemic areas.

FOWL POX
PUBLIC HEALTH SIGNIFICANCE

- No human infections have been reported.

MODULE-24: CONTAGIOUS ECTHYMA (ORF)

Learning objectives

- Group I double stranded DNA viruses
    - About the morphology and the nature of parapoxvirus
    - About the disease and its pathogenesis
    - About the diagnosis
    - About the vaccine
    - Treatment and control

CONTAGIOUS ECTHYMA
DESCRIPTION

- Contagious pustular dermatitis otherwise known as Scabby mouth, sore mouth or orf is a highly contagious, viral disease of sheep, goats and occasionally humans. It is an acute infectious disease of sheep characterized by the formation of vesicles, pustules, and finally thick scabs on the lips, nostrils, face, eyelids, teats, udders, feet, and occasionally inside the mouth. The disease is widespread in the sheep population and affects all breeds. Lambs are generally more susceptible than adults. The scabby mouth virus infects a sheep through abrasions in the skin. Cool, non-woolled areas such as the mouth, legs, feet, teats and poll are the usual sites of infection. Sheep grazing stubble or on harsh feed are most susceptible to scabby mouth infection due to a higher incidence of minor injuries of the mouth and feet. The mouth and feet are most commonly affected. At first small, reddened areas appear and begin to weep a clear fluid. This fluid eventually hardens into a thick brown scab. In the early stages the scab is firmly attached and if forcibly removed, a raw bleeding area is exposed. After two to three weeks the scabs dry up and drop off. The underlying skin then heals rapidly. Spread within the flock is usually rapid. In most cases the scabs appear at the corner of the mouth and muzzle. In severe cases they cover the lips and spread into the mouth and nostrils. Occasionally scabs occur around the eyes. Scabs on the legs usually occur on the fetlock or coronet. Scabs can also develop on the udder and teats of ewes. Affected ewes may not allow lambs to suckle resulting in lamb losses and reduced weight gain, and affected lambs may find it difficult to feed or suckle normally. Mastitis may also be a problem in a proportion of ewes. Animals that recover from scabby mouth develop a life long immunity to the disease. Sheep of all ages may become affected during the first outbreak on the property, but in subsequent years usually only the lambs are affected. The disease in sheep and goats is normally self-limiting, and clears without treatment within three to four weeks. Early manual removal of scabs will delay healing. Antibiotics are not necessary, unless secondary bacterial infection occurs
- A scabby mouth vaccine is readily available and provides good protection from the disease. The vaccine is applied to the bare skin on the inside of the thigh by scratching with a forked needle that has been dipped in vaccine. This causes a local reaction that does not spread. A line of pustules should appear along the scratch a week later - this indicates a good response and immunity from the vaccine. A sample of vaccinated sheep (about 10 per mob) should be checked between ten and fourteen days after vaccination to ensure a good "take".
- Humans occasionally become infected with the scabby mouth virus. The disease in man is called orf. The usual signs in humans are red papules, which are most often on the hands or arms. Shearers are sometimes affected in the armpit as a result of holding affected feet under their arms. Humans may also become infected from accidental inoculation of the scabby mouth vaccine. Care should be taken to cover scratches and cuts before using the vaccine, and to not accidentally scratch fingers or hands with the vaccine applicator.

MODULE-25: OTHER POX DISEASES

Learning objectives

- Group I double stranded DNA viruses
  - About the diseases
    - Cow pox
    - Buffalo pox
    - Lumpy skin disease
    - Milkers nodule and pseudocowpox

COW POX

- Cowpox virus, from the genus Orthpoxvirus, is another poxvirus that causes human disease through zoonosis. Cowpox virus has been found only in Europe and in adjacent parts of the former Soviet Union. Despite its name, the reservoir hosts of cowpox virus are rodents, from which it can occasionally spread to cats, cows, humans, and zoo animals, including large cats and elephants. Transmission to humans has traditionally occurred via contact with the infected teats of milking cows.
- However, currently, infection is seen more commonly among domestic cats, from which it can be transmitted to humans. Milkers and milking machines are the main means of spread. Insects may also serve as mechanical vectors for the virus. Cowpox virus produces what is usually a benign infection of the udder and teats.
- Papules are first seen, followed by vesicles, which rupture leading to scab formation. Scabs drop off in about two weeks. Losses in milk production result from the soreness of affected teats and also from secondary bacterial infection, which may complicate the disease and contribute to development of mastitis.

BUFFALO POX

- This disease of water buffaloes is caused by buffalopox virus an orthopoxvirus that is identical or closely related to vaccinia virus. The disease is analogous to cowpox and severe outbreaks have been reported from Southeast Asia.

MILKERS NODULE AND PSEUDO COWPOX

- Pseudocowpox virus a parapoxvirus closely related to the viruses causing bovine papular stomatitis and sheep contagious ecthyma. Pseudocowpox is characterized by the formation of bright red papules, followed by vesicles, scabs, and nodules on the udder and teats of cows, within a course of several weeks. Although the disease spreads slowly the entire herd is eventually affected.

LYMPY SKIN DISEASE

- LSD is a pox disease of cattle characterised by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death. The disease is of economic importance because it causes reduced production, particularly in dairy herds. It also causes damage to the hide.

- The symptoms appear as fever 104 to 1070 F (40-41.50 C), which lasts up to 4 weeks. Generally within 2 days after the appearance of the fever, swellings or nodules 1 to 5 cm in diameter appear in the skin and generalization occurs. Depression, anorexia, excessive salivation, oculonasal discharge, agalactia, and emaciation are presented. Nodules 1 to 7 cm in diameter may occur anywhere on the body but especially in the skin of the muzzle, nares, back, legs, scrotum, perineum, eyelids, lower ear, nasal and oral mucosa, and tail. The hair stands erect over early skin lesions. The nodules are painful and involve the epidermis, dermis, and subcutaneous tissue and may even involve the musculature. As the disease progresses, the nodules become necrotic, and eventually a deep scab forms; this lesion is called a sitfast. Secondary bacterial infection can complicate healing and recovery. Lesions on the teats can result in severe secondary bacterial infection with loss of the quarter owing to mastitis.
- Skin nodules have congestion, hemorrhage, edema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining affected areas are enlarged up to 10 times normal size with extensive lymphoid proliferation, edema, congestion, and hemorrhage. Mucous membranes of the oral and nasal cavities can have pox lesions that coalesce in severe cases. Pox lesions may occur in the pharynx, epiglottis, and trachea. Synovitis and tendosynovitis with fibrin in the synovial fluid can occur. Pox lesions can be present in the testicles and urinary bladder. Pox lesions are not easily visualized in the lungs but appear as focal areas of atelectasis and edema. In severe cases, pleuritis can occur with enlargement of the mediastinal lymph nodes.

MODULE-26: ASFARVIRUSES - AFRICAN SWINE FEVER

Learning objectives

- Group I double stranded DNA viruses
  - About the family Asfarviridae and its general characters including morphology
  - Cultivation of Asfarvirus
  - About the disease and its pathogenesis
  - About the diagnosis and various lab tests
  - About the vaccines
  - Control

AFRICAN SWINE FEVER
INTRODUCTION

- African swine fever (ASF) is a tickborne and contagious, febrile, systemic viral disease of swine. ASF can cause mortality up to 100% of pigs. ASF can only be distinguished from the similar disease classical swine fever (hog cholera) by laboratory tests. The ASF virus was formerly classified in the family Iridoviridae, later as an unclassified virus and now under a new family Asfaviridae. It was first described in Kenya, East Africa, in 1921, and soon afterwards in South Africa and Angola, as a disease that killed settlers' pigs

AFRICAN SWINE FEVER
GENERAL CHARACTERS

- Describes about Classification, morphology, OIE listing, risk group etc.

AFRICAN SWINE FEVER
MORPHOLOGY

- Virions have a complex construction and consist of an envelope, a capsid, a core, and a nucleoprotein complex. During their life cycle, virions have an extracellular transcription phase. Virions are enveloped and are not ether sensitive.
- Virions are spherical and 175-215 nm in diameter. Capsid/nucleocapsid is round and exhibits icosahedral symmetry. The capsid surface structure does not reveal a regular pattern with distinctive features. The capsid consists of 1892-2172 capsomers; each capsomer measures 13 nm in diameter.
- Capsomers consist of a hexagonal base with a central cavity. The genome is not segmented and consists of a single molecule of linear double-stranded DNA. The complete genome is 170000-190000 nucleotides long.

AFRICAN SWINE FEVER
OIE LISTING AND HUMAN RISK GROUP

- *OIE listing:* List A infection
- *Risk group*: No human infection is associated with ASFV.

AFRICAN SWINE FEVER
RESISTANCE

- The virus are highly resistant to low temperatures but inactivated by 56°C/70 min or by 60°C/20 min. The viruses are inactivated by pH <3.9 or >11.5. The viruses are also inactivated by 8/1,000 sodium hydroxide (30 min), hypochlorites - 2.3% chlorine (30 min), 3/1,000 formalin (30 min), 3% ortho-phenylphenol (30 min) and iodine compounds. They remain viable for long periods in blood, faeces and tissues.

AFRICAN SWINE FEVER
REPLICATION

- The virus enters host via endocytosis and uncoating occurs in the cytoplasm. Viral DNA transported to the cell nucleus and transcription is initiated by the host's RNA polymerase II. Host cell DNA replication strategy is used to produce viral genome. Progeny DNA is transported to the cytoplasm where assembly occurs and the virus exit host by budding or cell lysis

AFRICAN SWINE FEVER
HA PROPERTY

- The virus causes adsorption of RBCs in cell cultures

AFRICAN SWINE FEVER

STRAINS AND SEROTYPES

- There are many strains, which vary in their virulence.

AFRICAN SWINE FEVER
CULTIVATION

- The virus is cultivated in primary cultures of pig monocytes or bone marrow cells. The virus in cell culture is identified by haemadsoption, immunofluorescense or by PCR. The virus are also cultivated in susceptible pigs.

AFRICAN SWINE FEVER
PATHOGENESIS

- Describes about hosts affected, distribution, transmission, symptoms and lesions.

AFRICAN SWINE FEVER
HOSTS AFFECTED

- Pigs, warthogs, bush pigs, European wild boar and American wild pigs are affected by ASFV.

AFRICAN SWINE FEVER
DISTRIBUTION

r is enzootic in most countries of Sub-Saharan Africa. In Europe it has been reported in the Iberian Peninsula and ir an countries have had the disease but since eradicated it. The infection is not reported in India.

AFRICAN SWINE FEVER
TRANSMISSION

- The infection is transmitted by both direct and indirect methods. In direct method of transmission the infection spreads from sick animals to susceptible animals through contact. The indirect methods of transmission include feeding of pigs with garbage containing infected meat, through soft ticks of the genus Ornithodoros, which act as biological vector and through fomites. The virus initially enters into a susceptible herd by indirect methods and later spread within herd by both direct and indirect methods. The transmission cycle of ASF virus is given below for academic interest. Appearance of ASF in animals is dependant on
    - close contact between domestic pigs and warthogs that may be harbouring infected tampans;
    - introduction of new pigs into a herd, for example through purchase, for ceremonies or boar loan;
    - introduction of infected pig meat from neighbouring villages;
    - feeding of squil to pigs that contains raw or insufficiently cooked pork and pig remnants or access to such remnants through scavenging;
    - movement of vehicles and people between herds during an outbreak

AFRICAN SWINE FEVER
INCUBATION PERIOD

- Incubation period is very short and is just 48-72 hours

AFRICAN SWINE FEVER
MORBIDITY AND MORTALITY

- Morbidity in an unexposed herd is 100% and mortality varies depending upon the virulence of the isolate. Highly virulent isolated cause 100% mortality and less virulent isolates cause 60-70% mortality.

AFRICAN SWINE FEVER
SYMPTOMS

- The infection is exhibited as peracute, acute, subacute or chronic forms. Clinical signs appear approximately 5–15 days after natural infection with ASF virus. The first sign is usually the development of a high fever (41-42C°), manifested by depression, loss of appetite, seeking shade, huddling together, rapid breathing and, in white-skinned pigs, flushing of the skin, particularly the extremities and the underparts. Pigs often develop a swaying gait, with the hind legs appearing weak. Thick whitish discharges from the nose and eyes are sometimes seen.
- Difficult breathing is usual and foam, often blood-tinged, may appear at the nostrils. Pigs may show signs of abdominal pain. Vomiting is common. Some pigs become constipated, while others may develop a bloody diarrhoea. Sows may abort at all stages of pregnancy. The flushing of the skin in white-skinned pigs may deepen to a bluish-purple colour and there may be bleeding under the skin. Mucous membranes are red and congested. A coma due either to haemorrhagic shock or to excessive fluid in the lungs may develop before death, which usually occurs from one to seven days after development of clinical signs. Pigs that survive for a few days may develop nervous signs.
- Pigs that survive the acute stage of the disease may progress to the subacute or chronic stage. Subacute disease is characterized by fluctuating fever, accompanied by depression and loss of appetite. Walking may appear painful, with swelling of the joints. There may be signs of pneumonia. Death can be due to heart failure. Before death, signs of heart failure such as swelling of the throat may occur. Chronically sick pigs generally become emaciated, with long, dull hair and may have ulcerative sores over bony points. The pigs may walk stiffly due to arthritis. The survival period of such pigs varies from a few weeks to months.

AFRICAN SWINE FEVER
LESIONS

- The important post-mortem lesions are
    - fluids in the chest and abdominal cavities, which may be blood-stained;
    - widespread bleeding over organ and body surfaces with congestion
    - enlarged spleen and lymph nodes containing a lot of blood which may resemble blood clots;

- heavy and shiny lungs with prominent divisions between lobules and oozing moisture and froth when cut;
- trachea filled with blood stained froth
- pinpoint haemorrhages on the surface of the kidneys
- haemorrhages and ulcers in the stomach lining
- congested intestines with bloody contents

AFRICAN SWINE FEVER
DIAGNOSIS

- Describes about field diagnosis, laboratory diagnosis, serology and differential diagnosis.

AFRICAN SWINE FEVER
FIELD DIAGNOSIS

- Field diagnosis based on symptoms and lesions and its differentiation with classical swine fever (CSF) is very difficult.

AFRICAN SWINE FEVER
LABORATORY DIAGNOSIS

- Isolation and identification
  - *Clinical materials*: Heparinized blood, Clotted blood or serum, Submandibular lymph node, Inguinal lymph node, Tonsil, Spleen, Gastrohepatic lymph node, Lung, Liver, kidney and bone marrow.
  - *Isolation system*: Primary pig cell cultures like pig monocytes and bone marrow cells or susceptible pigs
  - *Identification*: Presence of virus in cell cultures can be demonstrated by adding red blood cells to the culture. These are attracted to the surface of infected cells, to which they cling and form “rosettes”, a phenomenon known as haemadsorption. The viruses are also identified by immunofluorescense or by PCR.
- Virus detection in tissue samples
  - Immunofluorescense
  - Immunoperoxidase staining
  - PCR

AFRICAN SWINE FEVER
SEROLOGY

- The enzyme-linked immunosorbent assay (ELISA) is the test most commonly used to detect antibodies to ASF in serum. Other tests that are sometimes used are indirect immunofluorescence and immunoblotting. Antibodies may not be detected in pigs that have died of acute ASF. The test is used to detect animals that have survived infection with ASF and in surveys to determine whether the disease might be endemic in an area.
- Indiret fluorescent antibody test
- Immunoblotting test

- Counter immunoelectrophoresis

AFRICAN SWINE FEVER
DIFFERENTIAL DIAGNOSIS

- ASF should be differentiated from CSF, Swine erysipelas, Salmonellosis and Pasteurellosis.

AFRICAN SWINE FEVER
TREATMENT

- Treatment is ineffective.

AFRICAN SWINE FEVER
CONTROL

- *Vaccination*: There are no vaccines against ASF
- *Eradication*: The OIE specifies the following strategy for control of ASF
- Free countries
    - Careful import policy for animals and animal products
    - Proper disposal of waste food from aircraft or ships coming from infected countries
    - Efficient sterilisation of garbage
- In outbreaks
    - Rapid slaughtering of all pigs and proper disposal of cadavers and litter is essential
    - Thorough cleaning and disinfection
    - Designation of infected zone, with control of pig movements
    - Detailed epidemiological investigation, with tracing of possible sources (up-stream) and possible spread (down-stream) of infection
    - Surveillance of infected zone, and surrounding area
- Infected countries
    - Avoid contact between pigs and soft tick vectors (Africa) - i.e. prevent pigs from wandering

MODULE-27: HERPESVIRUSES

Learning objectives

- Group I double stranded DNA viruses
    - About the family Herpesviridae
    - General characters of pseudorabies virus and Marek's disease virus and its morphology
    - Cultivation of pseudorabies virus and MDV
    - About the disease and its pathogenesis
    - About the diagnosis and various lab tests
    - About the vaccines

- Control and eradication

HERPESVIRUSES
INTRODUCTION

- The name herpes in Greek means 'herpein' ie. 'to creep'. These viruses cause chronic/latent/recurrent infections in man and animals. The family herpesviridae consists of approximately 100 members and they have been grouped under three sub-families, namely – alphaherpesvirinae, betaherpesvirinae and gammaherpesvirinae. Members of this family cause number of infections in animals, which include Pseudorabies, Marek's disease, infectious bovine rhinotracheitis, infectious laryngotracheitis etc.

HERPESVIRUSES
MORPHOLOGY

- Envelope: The virions are enveloped (hence ether sensitive), slightly pleomorphic, spherical and measure approximately 120-200 nm in diameter.
- Spikes: The surface projections (spikes) over the envelope are dispersed evenly all over the surface.
- Symmetry: The virus has icosahedral symmetry.
- Tegument: The tegument is a protein filled region between envelope and capsid.
    - *Capsid:* The surface of the capsid has 162 capsomers per nucleocapsid. The nucleocapsids are sometimes penetrated by stain (although intact envelope impermeable to stain).
- Genome: The core consists of a fibrillar spool on which the DNA is wrapped. The ends of the fibers are anchored to the underside of the capsid shell. The virions contain one molecule of linear double stranded DNA with genome length of 120000-220000 nt. The genome is consists of unique long (UL), unique short (US) and terminal repeat regions.

HERPESVIRUSES
REPLICATION

- Since herpesviruses have DNA as genome, they replicate in the nucleus. The virions attaches to host cell with the envelope glycoprotein, The virus enters into the cell through fusion of viral envelope with plasma membrane of host cell.
- The capsid travels along the cytoskeleton to a nuclear pore where the viral DNA is released. The linear genome enters the nucleus and circularises. Once in the nucleus, the viral DNA is transcribed into mRNA by cellular RNA polymerase II. After transcription in the nucleus, all mRNA transcripts are translated into protein in the cytoplasm.
- Subsequently, the proteins go to the nucleus, stay in the cytoplasm, or become a part of the membrane bilayer. Capsid proteins assemble in the nucleus to form empty capsids and full-length viral DNA is packaged to form nucleocapsids. The nucleocapsids associate with segments of the nuclear membrane where tegument and glycosylated envelope proteins have bound.

- This association triggers envelopement by budding through the nuclear membrane. Enveloped virions accumulate in the endoplasmic reticulum (ER). Mature virions are released by exocytosis

HERPESVIRUSES
CLASSIFICATION

-

Baltimore group	Group I - Double stranded DNA viruses
Family	Herpesviridae
Subfamilies	Alphaherpesvirinae, Betaherpesvirinae, gammaherpesvirinae

AUJESZKEY'S(PSEUDO RABIES) DISEASE
GENERAL ASPECTS

- *Definition:* Pseudorabies is an acute, frequently fatal disease affecting most species of domestic and wild animals. However, pigs are the natural host and dogs and cattle are also affected. The disease is caused by a herpes virus and is characterized by a variety of clinical signs, which include nervous and respiratory symptoms. The infection is characterized by severe itching and self-mutilation. Since, the symptoms resemble rabies, the disease is also known as pseudorabies.
- *Synonyms*: Pseudo rabies, Mad itch, Infectious bulbar paralysis
- *Classification*

Baltimore group	Group I - Double stranded DNA viruses
Family	Herpesviridae
Subfamily	Alphaherpesvirinae
Genus	Varicellovirus
Species	Pseudo rabies virus (PRV)

- *Morphology*: Typical of herpesviruses as described above
- *OIE Listing*: List B disease (multispecies infection)
- *Risk group*: PRV does not produce any infection in human beings
- *Replication*: As mentioned under Herpesviridae
- *Resistance*: PRV is comparatively a stable virus. It can remain viable in pulmonary fluid and under refrigerated conditions for a long time. It can withstand heating to 55-60C for 30-50 minutes, 70C for 10 minutes and 80C for three minutes. The virus is also sensitive to 5% phenol and 1% NaOH. The virus is also destroyed in putrefied carcass.
- *HA property*: The virus does not cause agglutination of RBC.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
CULTIVATION

- Embryonated eggs
  - PRV produce pock lesions, when inoculated in embryonated eggs by chorioallantoic membrane route. The embryo is also killed after 3-5 days.
- Cell culture
  - The PRV can be cultivated in many cell culture systems. However, the pig kidney cells (PK-15 cell line) are found to be more sensitive. The CPE appears within 24–72 hours, which include accumulations of perfringent cells, followed by complete detachment of the cell sheet. Different shaped and size syncytia also develop. Presence of virus is also confirmed by immunofluorescence, immunoperoxidase, or neutralisation tests using specific antiserum.
- Laboratory animals
  - Rabbit is the preferred lab animals. The virus is injected via skin or by intracerebellar route. Rabbits develop characteristic CNS symptoms and intense pruritis.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
PATHOGENESIS

- Deals about hosts affected, distribution, transmission, symptoms and lesions

AUJESZKEY'S(PSEUDO RABIES) DISEASE
HOSTS AFFECTED

- Pigs are the main host. Sporadic cases occur in cattle, sheep, goats, horses, dogs, cats, foxes and rodents.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
DISTRIBUTION

- Pigs are the main host. Sporadic cases occur in cattle, sheep, goats, horses, dogs, cats, foxes and rodents.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
TRANSMISSION

- Pseudorabies spreads mainly by direct contact between swine. Nasal discharges and saliva from infected pigs contain the virus and the virus enters susceptible pigs though ingestion or inhalation. Contaminated drinking water, bedding, and other objects such as clothing and instruments also act as a source of infection.
- Recovered pigs may remain carriers of the virus and later can infect susceptible pigs or cattle with which they come into contact. The disease has also been introduced to swine farms by introduction of carrier swine. Dogs and cats also become infected through contact with infected pigs. Dogs, cats, and wild animals are potential spreaders of the disease within an endemic area.

- Other uncommon methods of spread include via semen, vaginal secretions, colostrums, contaminated veterinary equipment e.g. needles and syringes and transplacental infection. Dogs can get the infection if fed with meat from infected pigs. In the same ways pigs also can infection, if the feed contains infected meat.
- Wind-borne spread from farm to farm can occur under favourable conditions (Upto 2 Km). Rats and wild pigs act as a reservoir for the virus. Movement of live pigs is considered the most likely method of introducing the disease in a new area.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
MORBIDITY AND MORTALITY

- Morbidity is variable and mortality is 20-100%.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
SYMPTOMS

- *Incubation period*: 3-7 days.
- Clinical signs are most likely seen in newborn piglets and breeding sows. Most severe disease occurs in young pigs.
- Pigs
    - In pigs less than 2 weeks old death losses reach upto 100%. Baby pigs may have become infected before birth and die within 2 days after birth after showing violent shaking and shivering. Piglets infected immediately after birth may show clinical signs within the first 2 days of life and usually die before they are 5 days old.
    - In pigs less than 3 weeks old the disease is characterized by sudden death with few any clinical signs. Death is preceded by fever which may exceed 105F, dullness, loss of appetite, vomiting, weakness, incoordination, and convulsions if vomiting and diarrhoea occur.
    - After 3 weeks of age, pigs usually develop a degree of resistance to the disease, and death losses reduce. Fever is a prominent clinical sign in these growing pigs and usually is followed by loss of appetite, listlessness, laboured breathing, excessive salivation, vomiting, trembling, and marked incoordination, especially of the hind legs (pigs sit like dogs). Death is usually preceded by convulsions. Involvement of the respiratory tract with sneezing, clear to yellowish nasal discharges from nose, rubbing of the nose, and coughing.
    - The disease in adult pigs often is characterized by fever and respiratory signs such as nasal discharges, sneezing, nose rubbing and coughing. Nervous signs such as trembling, incoordination, and itching occasionally occur, and blindness may rarely follow pseudorabies infection. Vomiting and diarrhoea or constipation may also be seen. Sows infected in the early stages of pregnancy may return to heat because of death and resorption of their fetuses. Sows infected in middle pregnancy may eventually abort mummified fetuses, whereas sows infected late in pregnancy often abort or give birth to weak, shaker or stillborn pigs.
- Cattle and sheep
    - In cattle and sheep, the infection is always fatal. The infection is characterized by, intense itchiness of localised area of skin, licking, rubbing, self –mutilation, incoordination, prostration and weakness, clonic convulsions, teeth grinding,

rapid shallow breathing and cardiac irregularities. Death occur in about 2 days after onset of clinical signs

- Dogs and cats
  - In dogs and cats, the clinical signs similar to ruminants, which include intense pruritus and self-mutilation, whimpering and howling, paralysis of the pharynx and intense salivation and clonic convulsions. Death occurs in 1-2 days

AUJESZKEY'S(PSEUDO RABIES) DISEASE
LESIONS

- Gross lesions are often absent or minimal. Congested pneumonic lungs are commonly seen. A serous to fibrinous rhinitis, a necrotic tonsillitis, liver and spleen with yellow-white necrotic foci (2-3 mm), necrotic placentitis and endometritis are also seen. In aborted piglets necrotic lesions in the lungs, liver, spleen and tonsils are also seen.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
DIAGNOSIS

- Field diagnosis: Based on clinical symptoms and lesions. But these are not very characteristic hence, it should be confirmed by laboratory diagnostic tests.
- Isolation and identification
  - *Clinical materials*: Oro-pharyngeal fluid, nasal fluid (swabs) or tonsil biopsies are the ideal clinical materials from live animals. From dead animals samples of brain and tonsil are the preferred specimens. In latently infected pigs, the trigeminal ganglion is the most consistent site for virus isolation, although latent virus is usually difficult to culture.
  - *Systems for isolation*: Cell culture (Pig kidney PK-15 cell line), embryonated eggs (CAM route) and rabbits (subcutaneous or intracerebellar
  - *Lesions / CPE*: As mentioned under cultivation for each system. Since latent viruses are difficult to isolate, negative results in isolation does not rule out freedom from PR.
- Direct identification of PRV from clinical specimens: The polymerase chain reaction (PCR) can be used to identify PRV genomes in secretions or organ samples.
- Serological tests
  - Virus neutralization test and ELISA are routinely done to identify antibodies against PR in pigs. The VN and ELISA tests detect pseudorabies antibodies in serum of pigs that have been infected with the virus. These antibodies appears in the serum about day 7 of infection and may persist for years. The presence of pseudorabies antibodies is evidence that the pig has been infected with the virus in the past or has been vaccinated. Absence of antibodies indicates that the animal has probably not been infected or that it may be in the early stages of the disease. The VN and ELISA are extremely reliable tests. While these tests accurately detect antibodies to pseudorabies, they do not differentiate between antibodies resulting from natural disease and those resulting from vaccination. Both VN and ELISA tests are approved tests for international trade.
- Differential diagnosis: The infection has to be differentiated from following infections
  - Porcine polioencephalomyelitis (Teschen)
  - Rabies
  - Classical swine fever and African Swine Fever

- Haemagglutinating encephalomyelitis virus infection
- Erysipela
- Nipah virus
- Streptococcal meningoencephalitis
- Hypoglycaemia
- Organic arsenic and mercury poisoning
- Salt poisoning
- Other causes of abortion — the presence of signs in young pigs should assist in differentiation
- Swine influenza
- Congenital tremor

AUJESZKEY'S(PSEUDO RABIES) DISEASE
TREATMENT

- Treatment is not effective.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
CONTROL AND ERADICATION

- Vaccines
  - Attenuated and inactivated vaccines have been developed. Vaccines protect pigs from clinical disease, reduce the amount and duration of virus excretion, but do not prevent latent infections. Virus can still be transmitted from vaccinated animals to susceptible contacts. Vaccines have also been made from genetically modified virus. These viruses have a variety of nonessential proteins missing from their genome.
- Eradication
  - Strict biosecurity measures, purchase of pigs from PR free herds, movement control and prevention of entry of dogs, cats and rodents into the pig house will prevent the infection. When pseudorabies occurs on a farm, the premises should be quarantined, and all movement of people and animals should be strictly controlled. If possible, healthy pigs should be separated from the sick and movement between them should be strictly controlled.
  - Dead pigs should be disposed by a deep burial or incineration. Recovered pigs should be sold only for slaughter to prevent spreading infection to other farms by carrier swine. Many herds which are infected may be freed of infection by using either "test and removal" procedures or offspring segregation. In highly concentrated operations, the virus appears to cycle intermittently, and many offspring with isolation and removal of infected animals appears to be an effective herd cleanup strategy.

AUJESZKEY'S(PSEUDO RABIES) DISEASE
PUBLIC HEALTH SIGNIFICANCE

- No human infection is associated.

MAREK'S DISEASE

GENERAL ASPECTS

- *Definition:* Marek's disease is a lymphomatous and neuropathic disease of domestic fowl caused by a herpesvirus. It causes severe economical losses. It is considered as one of the important viral diseases of poultry.
- *Synonyms:* Neural lymphomatosis, fowl paralysis
- *Classification*

Baltimore group	Group I (ds DNA viruses)
Family	Herpesviridae
Subfamily	Alphaherpesvirinae
Genus	Mardivirus
Species	Gallid herpesvirus 2 (Marek's disease virus)

- *Morphology*: Typical of herpesvirus mentioned above
- OIE Listing: List B disease
- Risk group : No human infection is associated
- Resistance: They are strictly cell associated virus and can be preserved at –60C. Infectivity is retained at pH range 5.5 to 8.4
- HA property: The virus does not cause agglutination of erythrocytes

MAREK'S DISEASE
SEROTYPES / STRAINS

- *Serotype 1 (Gallid Herpesvirus 2)*- contains all pathogenic strains and their derived attenuated variants. Virulence ranges from mild to high. The strains are oncogenic.
- *Serotype 2 (Gallid Herpesvirus 3)*- avirulent and non-oncogenic strains.
- *Serotype 3 (Meleagrid Herpesvirus 1)*- avirulent virus of turkeys.

Serotype 1

- This group includes all oncogenic strains.
  - classical MD
  - V V strains
  - V V+ strains
  - attenuated strains
- Vaccine strains
  - CVI988 (Rispens)
  - 988 clone C
  - 988/C/RC
  - R2/23
  - All can be used as a single vaccine strain or as part of a multivalent combination.

Serotype 2

- non-oncogenic chicken strains
  - Vaccine strains
    - SB1
    - 301B
    - Used in bi-valent or tri-valent vaccines only.

Serotype 3

- non-oncogenic chicken strains
  - Vaccine strains
    - SB1
    - 01B
    - Used in bi-valent or tri-valent vaccines only.

MAREK'S DISEASE
CULTIVATION

- The virus is cultivated in Chicken kidney or or duck embryo fibroblasts. The cytopathic effects, termed plaques, appear within 3–5 days and can be enumerated at about 7–10 days. They also produce intranuclear inclusion bodies. In ECE, the virus produce pock lesions when infected through CAM route. SPF chickens are also used for cultivation of the virus.

MAREK'S DISEASE
PATHOGENESIS

- Deals about hosts affected, distribution, transmission, symptoms and lesions.

MAREK'S DISEASE
HOSTS AFFECTED

- Chicken, quails and turkeys are highly susceptible to the infection. Young birds are the most susceptible to infection Most deaths from Marek’s disease occur between 10 and 24 weeks of age

MAREK'S DISEASE
DISTRIBUTION

- The infection is distributed worldwide.

MAREK'S DISEASE
TRANSMISSION

- The virus is highly infectious and, once it is present in a flock, it spreads rapidly to unvaccinated poultry. Healthy birds can be carriers and infect others. The virus can remain alive in the environment for as long as eight months.

- Virus replication and release occurs in the epithelial cells of feather follicles and copious amounts of infectious virus are shed in dust and dander. Susceptible birds are infected via the respiratory tract through contact with viral contaminated airborne dust particles. Three to five days post-infection B lymphocytes of the bursa of Fabricius, spleen and thymus become infected.
- The virus subsequently infects T lymphocytes of mostly the CD4+ phenotype; infection becomes latent and the virus spreads throughout the host by a cell-associated viremia. There is a secondary cytolytic infection of the feather follicle epithelium from which cell-free virus is produced and shed in feather dander and debris.
- Latently infected T lymphocytes are transformed leading to lesions of lymphomatosis in visceral organs. The main target cells for transformation are CD4+ T cells and probably CD8+ T cells. It is not spread from the hen to the chicken through the egg (No vertical transmission)

MAREK'S DISEASE
MORBIDITY AND MORTALITY

- Morbidity is very high. Mortality varies depending upon the form. Maximum mortality is approximately 15 % over a span of weeks to months, but with outbreaks may reach 70 %.

MAREK'S DISEASE
SYMPTOMS

- Marek's disease occurs in two main forms, depending on which parts of the body are affected by the tumours. The symptoms and lesions of the eye are sometimes referred as occular form (occular lymphomatosis)
    - *Nervous form (Classical form – neural lymphomatosis)* : In this form the nerves, particulary the sciatic nerves (the main nerves to the legs), are affected. The birds are unable to stand, become paralysed and slowly waste away from lack of food and water. In most cases the paralysis comes on quickly. Sometimes the wings or neck are involved. In some cases the iris is involved and this can lead to blindness.
    - *Visceral form (Acute form)*: In this form, greyish-white tumours are found in the ovaries, liver, spleen, kidney, heart and other organs. Sometimes the liver and spleen are swollen without distinct tumours being present. Birds may show signs of depression, paralysis, loss of appetite, loss of weight, anaemia (pale combs), dehydration (shrunken combs), and sometimes diarrhoea. Some birds die without any clinical signs being noticed.

MAREK'S DISEASE
LESIONS

- Classical form
    - The characteristic finding is the enlargement of one or more peripheral nerves. Those most commonly affected and easily seen at post-mortem are the brachial and sciatic plexuses, coeliac plexus, abdominal vagus and intercostal nerves. Affected nerves are often two or three times their normal thickness, the normal cross-striated and glistening appearance is absent, and the nerve may appear greyish or yellowish, and sometimes oedematous. Lymphomas are sometimes

present in the classical form of MD, most frequently as small, soft, grey tumours in the ovary, and sometimes also in the lungs, kidneys, heart, liver and other tissues. 'Grey eye' caused by an iridocyclitis that renders the bird unable to accommodate the iris in response to light and causes a distorted pupil is common in older (16–18 week) birds

- Visceral form
  - Greyish-white tumours are found in the ovaries, liver, spleen, kidney, heart and other organs. Sometimes the liver and spleen are swollen without distinct tumours being present. lymphomas also arise in the skin around the feather follicles and in the skeletal muscles. Affected birds usually have enlarged peripheral nerves, as in the classical form. In younger birds, liver enlargement is usually moderate in extent, but in adult birds the liver may be greatly enlarged and the gross appearance identical to that seen in lymphoid leucosis. In both the classical and acute forms of MD, the disease starts as a proliferation of lymphoid cells, which is progressive in some cases and regressive in others. The peripheral nerves may be affected by proliferative, inflammatory or minor infiltrative changes, which are termed type A, B, and C lesions, respectively.
  - The A-type lesions consist of infiltration by proliferating lymphoblasts, large, medium and small lymphocytes, and macrophages, and appear to be neoplastic in nature.
  - The B-type lesion is characterised by interneuritic oedema, infiltration by mainly small lymphocytes and plasma cells, and Schwann cell proliferation, and appears to be inflammatory.
  - The C-type lesion consists of a light scattering of mainly small lymphocytes, and is often seen in birds that show no gross lesions or clinical signs. It is a regressive, inflammatory lesion. Demyelination frequently occurs in nerves affected by the A- and B-type lesions, and is responsible for the clinical paralysis.
    - Marek's disease tumour associated surface antigen (MATSA): MATSA is defined as activated T cell antigens present on the surface of MD tumour cells. These antigens are characteristic of MD and used to differentiate MD with lymphoid leucosis (LL)

MAREK'S DISEASE
DIAGNOSIS

- Field diagnosis: Field diagnosis is based on clinical symptoms and lesions.
- Isolation and identification
  - *Clinical materials*: Since MD virus is cell associated the virus could be isolated from buffy coat cells from heparinised blood samples, or suspensions of lymphoma cells or spleen cells
  - *Isolation systems*: Chicken kidney cells or duck embryo fibroblasts are normally used for isolation. Chicken embryo fibroblasts are less sensitive for primary isolation. The cytopathic effects, termed plaques, appear within 3–5 days and can be enumerated at about 7–10 days. They also produce intranuclear inclusion bodies. SPF chickens can also be used.
- Direct identification MD virus from clinical materials
  - The cell free MD viruses could be isolated from feather follicles in chicken kidney cells as mentioned above.
  - Polymerase chain reaction (PCR) is also used to identify MD virus from clinical materials. Further in this technique, the seroytpe could also be differentiated.

    - Demonstration of MATSA antigen by indirect fluorescent antibody test.
- Serological tests
    - *Agar gel immunodiffusion test*: This test is employed most commonly to detect antibody. The test is conducted using glass slides coated with 1% agar in phosphate buffered saline containing 8% sodium chloride. Adjacent wells are filled with antigen or serum and these are incubated in a humid atmosphere at 37°C for 24 hours for diffusion to take place. The antigen used in this test is either disrupted MDV-infected tissue culture cells or an extract of feather tips, or skin containing feather tracts obtained from MDV-infected chickens.
    - Indirect fluorescent antibody test
    - Virus neutralization test
    - ELISA
- Differential diagnosis: Marek's disease should be differentiated from lymphoid leucosis and reticuloendotheliosis.

MAREK'S DISEASE
TREATMENT

- Treatment is not effective.

MAREK'S DISEASE
CONTROL AND ERADICATION

- Vaccination
    - Cell associated live virus or cell free Herpes virus turkey are used as vaccine to control MD. These vaccines are injected in ovo at the 17th or 18th day of embryonation or subcutaneously at hatch. The HVT do not spread horizontally; therefore missed birds will not be protected. Missed birds remain fully susceptible, leading to MDV multiplication and spread of virus into the environment in large volumes.
    - Genetically engineered recombinant vaccines are available but they are currently not in commercial use
- Control
    - By appropriate management practices including proper disinfection, all-in all-out policies, separation of young birds from older, genetically resistant stock and vaccination decrease incidence of disease.
    - It is essential that vaccinated chicks be isolated during their first two weeks of life so that their immunity develops before they are subjected to a severe challenge of virus. Chicks reared separately are free from the infected fluff and dust of older birds.
    - Good nutrition and freedom from other diseases and parasites are necessary to maintain the flock's health and to ensure that the birds have optimum resistance against MD infection.

MODULE-28: ADENOVIRUSES

Learning objectives

- Group I double stranded DNA viruses
  - About the family Adenoviridae and it member viruses
  - General characters of EDS virus and ICH virus and its morphology.
  - Cultivation
  - About the disease and its pathogenesis.
  - About the diagnosis and various lab tests
  - About the vaccines
  - Control and eradication.

ADENOVIRUSES
INTRODUCTION

- Adenoviruses are widespread in nature, infecting birds, many mammals and man. They also cause latent infection in lymphoid tissues and some viruses have oncogenic potential. Adenoviruses are a frequent cause of acute upper respiratory tract (URT) infections, i.e. "colds". There are four genera under this family. They are Ataadenovirus, Aviadenovirus, Mastadenovirus and Siadenovirus.

ADENOVIRUSES
MORPHOLOGY

- Adenovirus particles are non-enveloped (hence not ether sensitive) and 60-90nm in diameter. The capsid has icosahedral sy mmetry. The capsid is composed of a single layer. The capsid comprises of 252 capsomers: 240 "hexons" + 12 "pentons" at vertices of icosahedron (2-3-5 symmetry).
- The surface projections are distinct, one or two filaments protruding from the 12 vertices. The pentons have a toxin-like activity (purified pentons cause c.p.e. in the absence of any other virus components (a unique property). A trimeric fibre protein extends from each of the 12 vectices (attached to the penton base proteins) and is responsible for recognition and binding to the cellular receptor.
- Adenoviruses have ten proteins. The genome is linear, non-segmented and d/s DNA . The complete genome is 35800-36200 nucleotides long.

ADENOVIRUSES
REPLICATION

- Adenoviruses replicate in the nucleus. Replication is divided into early and late phases. Entry of adenovirus particle is a two-stage process involving an initial interaction of the fibre protein with cellular receptors followed by interaction between cell proteins and pentamers, which results in the entry of virus into the cell.
- Virus entry also involves phagocytosis of virus particles into phagocytic vacuoles. Due to the toxic activity of the pentons phagocytic membrane ruptures and release the virus into the cytoplasm. The viral core migrates to the nucleus where the DNA enters through nuclear pores. The genome replicates in the nucleus and occurs by a strand-displacement mechanism.
- Virions may provide helper functions to dependent virus during replication. They also act as helper for a satellite virus. Progeny virions assemble in the nucleus and are released from host cell by disintegration.

ADENOVIRUSES
CLASSIFICATION

Baltimore group	Group I ds DNA viruses
Family	Adenoviridae
General	Ataadenovirus, Aviadenovirus, Mastadenovirus, Sidadenovirus

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EGG DROP SYNDROME
GENERAL CHARACTERS

- *Definition*: Egg drop syndrome (EDS) is characterized by production of soft-shelled and shell-less eggs in apparently healthy birds.
- *Classification*

Baltimore group	Group I ds DNA viruses
Family	Adenoviridae
Genus	Ataadenovirus
Species	Duck adenovirus 1

- *Synonyms:*
- *Morphology*: As specified above
- *OIE Listing*: Not listed by OIE
- *Risk group*: No risk to human beings
- *Referral laboratory*: Not provided by OIE
- *Replication*: As provided above
- *Resistance*:
- *HA property*: The virus agglutinates chicken erythrocytes but do not have elution property
- *Serotypes / Strains*: There are three genotypes, one is associated with classical EDS, one with ducks in the UK, and one with EDS in Australia.

EGG DROP SYNDROME
CULTIVATION

- *Embryonated eggs:* The virus grows well in embryonated duck or goose eggs or in cell cultures of duck or goose origin. However, it does not replicate in ECE.
- *Cell culture*: It replicates well in chick kidney or chick-embryo liver cells and to a lesser degree in chick-embryo fibroblasts. The virus also does not grow in mammalian cells.

EGG DROP SYNDROME
PATHOGENESIS

- Describes hosts affected, distribution, symptoms and lesions.

EGG DROP SYNDROME
HOSTS AFFECTED

- The natural hosts for quail. All ages and breeds of chickens are susceptible, although the disease tends to be most severe in heavy broiler-breeders or brown-egg EDS virus are ducks and geese, and the disease has also been described in producers

EGG DROP SYNDROME
DISTRIBUTION

- The infection is worldwide in distribAution except in USA.

EGG DROP SYNDROME
TRANSMISSION

- The virus is transmitted through the egg (vertically) to a few birds in a flock, these birds carry the virus (as latent infection) until the flock comes into lay at which time they begin to excrete virus and infect birds kept in the same house.
- The virus is shed in the faeces and spread can be by contaminated water and fomites. Sporadic outbreaks have also been attributed to wild birds contaminating water. Horizontal spread occur through infected litter. Man and contaminated fomites such as crates or trucks can spread virus, which also can be transmitted by needles when vaccinating and drawing blood.
- Insect transmission is possible but not proved. After horizontal or experimental infection, the virus grows to low titers in the nasal mucosa. This is followed by viremia, virus replication in lymphoid tissue, and then massive replication for ~8 days in the oviduct, especially in the pouch shell gland region.
- Changes in the eggshell occur coincidentally. Both the exterior and interior of eggs produced between 8 and ~18 days after infection contain virus. A copious exudate in the lumen of the oviduct is rich in virus, and this contaminates the droppings.

EGG DROP SYNDROME
MORBIDITY AND MORTALITY

ariable. Mortality is very minimal.

EGG DROP SYNDROME
INCUBATION PERIOD

- 8-18 days in experimental infection.

EGG DROP SYNDROME
SYMPTOMS

- The infection occurs in three forms classical, endaemic and sporadic. EDS affects only layers and breeders at the start of or during their egg production. Affected flocks show a failure to reach peak egg production or a drop in egg production accompanied by an inferior eggshell quality and in the case of brown eggs, a loss of shell color.
- Birds tend to eat the shell-less eggs, which therefore may be missed unless a search is made for the membranes. Affected birds may also appear to be anaemic, may show transient diarrhoea and sometimes the food intake may be reduced.
- No increased mortality or other symptoms are observed. Birds with antibody slow the spread of virus. There is no effect on fertility or hatchability of those eggs suitable for setting. In cage units the virus spread is slow and the clinical signs are overlooked and the problem identified as a small depression (2-4%) of egg yield.

EGG DROP SYNDROME
LESIONS

- The major pathological changes occur in the pouch shell gland. Surface epithelial cells develop intranuclear inclusion bodies and degenerate, and are replaced by squamous, cuboidal, or undifferentiated columnar cells. There is moderate to severe inflammatory infiltration of the mucosa.

EGG DROP SYNDROME
DIAGNOSIS

- Field diagnosis
  - Based on clinical symptoms in correlation with the production performance.
- Isolation and identification
  - *Clinical materials*: Abnormal eggs and pouch shell glands
  - *Isolation system*: Embryonated duck eggs or duck- or chick-embryo liver cell cultures or susceptible antibody free chickens
- Serological tests
  - HI test
  - ELISA
  - Serum neutralization test
  - Double immuno diffusion test
- Nucleic acid identification methods
  - PCR
- Differential diagnosis
  - Newcastle disease
  - Avian Influenza
  - Infectious bronchitis

EGG DROP SYNDROME
TREATMENT

- There is no effective treatment.

EGG DROP SYNDROME
CONTROL

- *Vaccination*: Inactivated vaccines with oil adjuvant are available. They reduce but do not prevent virus shedding. These vaccines are given during the growing phase, usually at 14-18 wk old, and can be combined with other vaccines such as for Newcastle disease.
- *Eradication*: The endemic form can be controlled by washing and disinfecting plastic Keyes trays before reuse. The sporadic form can be prevented by separating chickens from other birds, especially waterfowl. General sanitary precautions are indicated, and potentially contaminated water should be chlorinated before use.

INFECTIOUS CANINE HEPATITIS
GENERAL CHARACTERS

- *Definition:* Infectious canine hepatitis (ICH) is a contagious disease of dogs with signs that vary from a slight fever and congestion of the mucous membranes to severe depression, marked leukopenia, and prolonged bleeding time
- *Classification*

Baltimore group	Group I ds DNA viruses
Family	Adenoviridae
Genus	Mastadenovirus
Species	Canine adenovirus 1 (CAV 1)

- *Synonyms*: Rubarth's disease
- *Morphology*: As specified above
- *OIE Listing*: Not listed by OIE
- *Risk group*: No risk to human beings
- *Referral laboratory*: Not provided by OIE
- *Replication*: As provided above
- *Resistance*: CAV-1 is resistant to lipid solvents and survives outside the host for long durations extending to many months. The virus is sensitive to 1-3% solution of sodium hypochlorite (Bleaching powder).
- *HA property*: The virus has no HA property
- *Strains and serotypes*: CAV 1 is antigenically related to CAV 2, which causes infectious tracheobronchitis in dogs

INFECTIOUS CANINE HEPATITIS
CULTIVATION

- *Cell culture*: The virus can be cultivated in cell culture derived from the kidney tissues of dogs, pigs or ferrets. The CPE is characterised by intranuclear inclusion bodies.

- *Embryonated eggs*: The virus can also be cultivated in ECE.
- *Lab animals*: Dogs and ferrets are used as lab animals for cultivation of the virus.

INFECTIOUS CANINE HEPATITIS
PATHOGENESIS

- Describes about hosts affected, distribution, transmission, symptoms and lesions

INFECTIOUS CANINE HEPATITIS
HOSTS AFFECTED

- The infection is mainly reported in dogs. It is also reported in foxes, wolves, coyotes, and bears. Other carnivores also develop clinical illness.

INFECTIOUS CANINE HEPATITIS
DISTRIBUTION

- The infection is worldwide in distribution.

INFECTIOUS CANINE HEPATITIS
TRANSMISSION

- Dogs recovered from ICH shed the virus in their urine for upto six months. The infection is transmitted from affected animal to normal animals through ingestion of urine, faeces, or saliva from affected animal. Initial infection occurs in the tonsillar crypts and Peyer's patches, followed by viremia and infection of endothelial cells in many tissues. Liver, kidneys, spleen, and lungs are the main target organs for multiplication of virus.

INFECTIOUS CANINE HEPATITIS
INCUBATION PERIOD

- Incubation period is 4-9 days.

INFECTIOUS CANINE HEPATITIS
MORBIDITY AND MORTALITY

- Morbidity and mortality are variable and mortality is very high in young dogs.

INFECTIOUS CANINE HEPATITIS
SYMPTOMS

- Clinical signs start with biphasic fever of >104°F (40°C), which lasts 1-6 days associated with leukopenia, which persists throughout the febrile period. The degree of leukopenia varies and seems to be correlated with the severity of illness.

- Other signs are apathy, anorexia, thirst, conjunctivitis, serous discharge from the eyes and nose, and occasionally abdominal pain and vomiting, intense hyperemia or petechiae of the oral mucosa and enlarged tonsils. Subcutaneous edema of the head, neck, and trunk are also observed.
- The blood clotting time also varies with severe haemorrhage, which is manifested by bleeding around deciduous teeth and by spontaneous hematomas. Severely infected dog may have a terminal convulsion with brain-stem hemorrhages. On recovery, dogs will regain weight slowly.
- Further, seven to 10 days after the acute signs disappear, recovered dogs develop bilateral corneal opacity, which usually disappears spontaneously. In mild cases of ICH, transient corneal opacity may be the only sign of disease. Chronic hepatitis develop in dogs having low levels of passive antibody when exposed. Simultaneous infection with CAV-1 and distemper virus is also seen.

INFECTIOUS CANINE HEPATITIS
LESIONS

- The important lesions are hepatic cell necrosis leading to change in colour of the live, which may be normal in size or swollen. The gallbladder wall may be edematous and thickened. Grayish white foci are also seen in the kidney cortex. Damage to the endothelium of intestine results in “paintbrush” hemorrhages on the gastric serosa, lymph nodes, thymus, pancreas, and subcutaneous tissues.

INFECTIOUS CANINE HEPATITIS
DIAGNOSIS

- *Field diagnosis*: Clinical signs are difficult to differentiate from canine distemper. Sudden onset with severe bleeding is suggestive of ICH. The symptoms should be correlated with blood picture before arriving at final diagnosis.
- Histopathology of liver and demonstration of specific intranuclear inclusion bodies
- *Isolation and identification*: Cell culture systems developed from kidney cells of dogs or ferrets are used for isolation. The presence of virus is confirmed by
    - Demonstration of intranuclear inclusion bodies
    - Immunofluoorescense
    - Polymerase chain reaction
- *Differential diagnosis*: Canine distemper and CAV 2 infection (infectious tracheobronchitis)

INFECTIOUS CANINE HEPATITIS
TREATMENT

- In severely affected dogs blood transfusion and intravenous administration of 5% dextrose saline will provide some remedy.

INFECTIOUS CANINE HEPATITIS
CONTROL

- *Vaccination:* Modified live virus vaccines are available and are often combined with other vaccines. Vaccination against ICH is recommended at the time of canine distemper vaccinations. Attenuated CAV 1 and CAV 2 are used as vaccines. Attenuated CAV-1 vaccines may produce transient unilateral or bilateral opacities of the cornea, and the virus may be shed in urine. However, CAV-2 attenuated live virus strains, which provide cross protection against CAV-1 does not produce corneal opacities or uveitis, and the virus is not shed in urine. Animals should be revaccinated annually.
- *Eradication:* Eradication aimed towards proper vaccination and hygiene at kennels and places where dogs frequent.

MODULE-29: PARVOVIRUSES

Learning objectives

- About the family Adenoviridae and its general characters
- Morphology and properties of CPV
- Cultivation
- About the disease and its pathogenesis
- About the diagnosis and various lab tests
- About the vaccines
- Treatment and control

PARVOVIRUSES
INTRODUCTION

- Parvoviruses are among the smallest and simplest eukaryotic viruses. They are widespread in nature. Parvoviruses are divided into two groups, defective viruses, which are dependent on helper virus for replication and autonomous or replication-competent viruses.
- *Morphology:* Virions are not enveloped and hence are not ether sensitive. The capsid/nucleocapsid is round and exhibits icosahedral symmetry. The virions measure 18-26nm diameter and consist only of protein (50%) + DNA (50%). The capsid is round and consists of 60 capsomers. The surface projections are small and surface appears rough with distinct spikes. There are three capsid proteins, VP1-3. VP2 is essential for virulence. The capsids can be penetrated by stain and some appear dark in the centre. The genome is not segmented and consists of a single molecule of linear negative-sense, or negative-sense and positive-sense single-stranded DNA. The complete genome is 5000 nucleotides long. Palindromic sequences are found at the 3' and 5' end of the genome.
- *Classification:* As per the VIII report of ICTV, the family Parvoviridae is divided into two subfamilies Parvovirinae and Densovirinae. These two subfamilies have been further subdivided into 5 and four genera respectively.
- *Replication:* Replication of parvoviruses occurs at nucleus and they depend entirely on the host cell mechanisms. The replication strategies of parvovirus genome are poorly understood. The virus enters into the cell through endocytosis. Upon uncoating, the DNA is translocated to the nucleus, where is transcribed into mRNA and also serve as template for the synthesis of daughter strands. Host cell DNA polymerase is necesary for genome replication. The mRNA moves to the ribosomes and gets translated into proteins. Capsid assembly takes place in the cytoplasm and DNA is packed into proformed capsid. The virus is released through cell lysis.

PARVOVIRUSES
GENERAL CHARACTERS

Morphology

- Virions are not enveloped and hence are not ether sensitive. The capsid/nucleocapsid is round and exhibits icosahedral symmetry. The virions measure 18-26nm diameter and consist only of protein (50%) + DNA (50%). The capsid is round and consists of 60 capsomers. The surface projections are small and surface appears rough with distinct spikes. There are three capsid proteins, VP1-3. VP2 is essential for virulence.
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Classification

- As per the VIII report of ICTV, the family Parvoviridae is divided into two subfamilies Parvovirinae and Densovirinae. These two subfamilies have been further subdivided into 5 and four genera respectively.

CANINE PARVOVIRUS INFECTION
GENERAL CHARACTERS

- *Classification*

Baltimore group	Group II (ss DNA viruses)
Family	Parvoviridae
Subfamily	Parvovirinae
Genus	Parvovirus
Species	Canine Parvovirus 2 (CPV 2)

- *Synonyms*:
- *Morphology*: As specified above
- *OIE Listing*: Not listed by OIE
- *Risk group*: No risk to human beings
- *Referral laboratory*: Not provided by OIE
- *HA property*: The virus agglutinates porcine, Rhesus monkey RBC or Human O
- *Strains and serotypes*: Canine parvovirus type 2 is closely related to feline panleukopenia virus, mink enteritis virus and raccoon enteritis virus. that canine parvovirus (CPV) differs from the feline virus in two amino acids in the capsid protein VP2. It is thought that CPV originated from FLPV by mutations in these amino acids in the late 1970's. These changes enable CPV to replicate in dogs. The strain Cornell 780916

is commonly used as vaccine. Currently CPV types CPV 2a, 2b and 2c are known to cause the disease world wide.

CANINE PARVOVIRUS INFECTION
RESISTANCE

table in the environment, able to withstand wide pH ranges and high temperatures. It is resistant to a number of co veral months in contaminated areas.

CANINE PARVOVIRUS INFECTION
CULTIVATION

- The virus is cultivated in primary canine cells or in cell lines developed from canines and felines like Canine A72 and Crandell feline kidney cell line. The characteristic CPE is the intranuclear inclusion bodies. The viruses are not cultivated in ECE. The virus has affinity for rapidly dividing cells.

CANINE PARVOVIRUS INFECTION
PATHOGENESIS

- Describes about hosts affected, distribution, transmission, symptoms and lesions

CANINE PARVOVIRUS INFECTION
HOSTS AFFECTED

- Dogs are mainly affected by this infection. Rottweilers, American Pit Bull Terriers, Doberman Pinschers, and German Shepherd Dogs are at increased risk of disease. Toy Poodles and Cocker Spaniels appear at decreased risk.

CANINE PARVOVIRUS INFECTION
DISTRIBUTION

- The infection is worldwide in distribution.

CANINE PARVOVIRUS INFECTION
TRANSMISSION

- The virus is transmitted from direct contact with infected dogs. Indirect transmission, eg, from fecal-contaminated fomites, is also an important source of infection. The virus is shed in the feces of infected dogs for up to 3 wk after infection. Recovered dogs may serve as carriers and shed the virus periodically.
- After ingestion, the virus replicates in lymphoid tissue of the oropharynx; from there, it spreads to the bloodstream. It attacks rapidly dividing cells throughout the body, especially those in the bone marrow, lymphopoietic tissue, and the crypt epithelium of the jejunum and ileum. Replication in the bone marrow and lymphopoietic tissue causes neutropenia and lymphopenia, respectively.

- Replication of the virus in the crypt epithelium of the gut causes collapse of intestinal villi, epithelial necrosis, and hemorrhagic diarrhea. Normal enteric bacteria, eg, Clostridium perfringens and Escherichia coli enter the denuded mucosa leading to secondary bacterial infection

CANINE PARVOVIRUS INFECTION
INCUBATION PERIOD AND MORBIDITY & MORTALITY

- *Incubation period*: 3-8 days.
- *Morbidity and mortality*: Morbidity is variable depending on many environmental factors. Mortality varies from 16-35%

CANINE PARVOVIRUS INFECTION
SYMPTOMS

- Infected dogs are often asymptomatic and infection is triggered by stress, and clinical signs may be intensified by concurrent infection with opportunistic enteric pathogens. Prolonged contact with a dog shedding high levels of virus increases the likelihood of disease. There are two common clinical forms two common clinical forms of the disease —myocarditis and gastroenteritis. Myocarditis is observed in young pups, especially in the early neonatal period.
- Infection lead to myocardial necrosis with either acute cardiopulmonary failure (causing pulmonary edema, cyanosis, and collapse) or scarring of the myocardium and progressive cardiac insufficiency. Gastroenteritis is most common in pups 6-20 wk old. Gastrointesttinal form mostly seen in young male dogs of less than 1 year old.
- The severity of clinical signs varies. Dogs with the enteric form suffer from an acute onset of lethargy, anorexia, fever, vomiting, leukopenia and diarrhea. The feces are loose and may contain mucus or blood.
- Most dogs recover within a few days with appropriate supportive care; others can die within hours of the onset of clinical signs. Other clinical problems that have been associated with CPV include birth defects and infertility. Subclinical infections are common, especially in older dogs.

CANINE PARVOVIRUS INFECTION
LESIONS

- The characteristic lesion in CPV is the damage to the microvilli and Crypts of Lieberkuhn. As a result of damage to the microvilli, the intestinal bacteria enters into the blood stream.

CANINE PARVOVIRUS INFECTION
DIAGNOSIS

- *Field diagnosis*: Based on clinical signs and blood picture (Leukopenia or lymphopenia). However, it has to be confirmed with a diagnostic test.
- Biopsy of the intestinal mucosa – Damage to the microvilli provide conclusive evidence about infection

- *Faecal Parvo ELISA test*: Commercial kits are available to identify the virus particles in faeces. Since the virus is excreted for a long time the results provide conclusive evidence in unvaccinated animals. However, the results are not very conclusive in vaccinated animals
- Detection of CPV particles in the faeces using immunoelectronmicroscopy and HA
- Detection of viral nucleic acid by PCR
- Serological tests like HI and Serum neutralization test. These tests are normally performed to findout the protective status of the animals. A HI titre of 1:80 and SN titre of 1:20 is considered as protective

CANINE PARVOVIRUS INFECTION
TREATMENT

- There is no specific therapy to eliminate the virus. Most dogs recover with appropriate supportive care directed to restoration of fluid balance.

CANINE PARVOVIRUS INFECTION
CONTROL

- *Vaccination*: Vaccines containing live attenuated CPV generally induce more effective immunity than inactivated virus vaccines. The high-titer CPV vaccines now available effectively protect puppies against viral challenge, even during the period when maternal antibody titers remain high enough to interfere with active immunization but have declined enough to predispose pups to infection. Three doses of vaccine are recommended at 6, 9, and 12 wk of age.
- *Eradication*: To eradicate CPV following points should be observed.
    - Isolation of affected dogs from other dogs.
    - Canine parvovirus can survive for weeks in contaminated cages and kennels, and thorough disinfection (e.g., sodium hypochlorite solution) is necessary before uninfected dogs are admitted.
    - Disinfection of hands, clothing, and food and water bowls is recommended.
    - Pups should be kept isolated from adult dogs returning from shows or field trials

MODULE-30: PAPOVAVIRUSES

Learning objectives

Introduction to papilloma and polyoma viruses

- Morphology of papilloma and polyoma viruses
- About the replication
- About the diseases caused by papilloma and polyoma viruses
- Pathogenesis

PAPOVAVIRUSES
INTRODUCTION

taining viruses that are associated with or cause papillomas or polyomas in animals. It comprises of two families.

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PAPILLOMAVIRIDAE
MORPHOLOGY

- Papillomaviruses are small, non-enveloped icosahedral particles ~52-55nm diameter. There are 72 capsomers. The Papillomavirus genome consists of circular, d/s DNA ~8kbp in size, associated with cellular histones to form a chromatin-like substance.

PAPILLOMAVIRIDAE
CLASSIFICATION

- The family comprises of many genus - alpha, beta, gamma, delta, epsilon, zeta, eta, theta, iota, kappa, lambda, mu, nu, xi and pi viruses.
- Important species causing disease in animals
    - Bovine papillomaviruses 3 and 5
    - Equine papillomavirus 1
    - Canine oral papillomavirus

PAPILLOMAVIRIDAE
REPLICATION

- The virus infects the basal cells of the dermal layer, and early gene expression can be detected in these cells. However, late gene expression, expression of structural proteins and vegetative DNA synthesis is restricted to terminally differentiated cells of the epidermis which implies a link between cellular differentiation and viral gene expression.

PAPILLOMAVIRIDAE
PATHOGENESIS

- The viruses are widespread in nature and infect birds and mammals. The usual outcome of infection is the formation of a benign outgrowth of cells, a wart or papilloma. These may occur almost anywhere in or on the body. Skin warts are divided into flat warts (superficial) and plantar warts (deeper). Genital warts (condylomas) occur in the genital tract and are transmitted by sexual intercourse.
- Warts can be treated by topical application of caustic substances or freezing, but surgical removal is more reliable, and is required for internal warts e.g. laryngeal. Warts may persist for many years, but may regress spontaneously due to a CTL response. There may be some enhanced risk of skin warts exposed to U.V. light developing into invasive squamous cell carcinoma.

POLYOMAVIRUSES

- Polyomaviruses infect a wide variety of vertebrates.
- Polyomaviruses virions are non-enveloped. Polyomavirus genomes are d/s, circular DNA molecules, ~5kbp in size.
- Important virus
    - SV40 - Simian vacuolating virus 40.

MODULE-31: CIRCOVIRUSES

Learning objectives

- About the family circoviridae and genus Gyrovirus
- General characters of chicken anemia virus and its morphology
- Cultivation of CAV
- About the disease and its pathogenesis
- About the diagnosis and various lab tests
- About the vaccines
- Control and eradication

CHICKEN INFECTIOUS ANAEMIA
INTRODUCTION

- Chicken Infectious Anaemia is a disease caused by the Chicken Anaemia Virus (CAV). The disease is found worldwide. The virus can infect chickens of all ages but only young chicks may develop clinical signs.

CHICKEN INFECTIOUS ANAEMIA
GENERAL ASPECTS

- Deals about morphology, resistance and other general properties.

CHICKEN INFECTIOUS ANAEMIA
MORPHOLOGY

- Virions are small (19-24 nm in diameter), spherical, single stranded and non enveloped. Virions consist of a capsid. During their life cycle, virions have an extracellular phase. Virus capsid is not enveloped. Virions are not tailed. Capsid/nucleocapsid is round and exhibits icosahedral symmetry.
- The genome is monomeric; not segmented and contains a single molecule of circular, negative-sense, single-stranded DNA. The complete genome is 2298-2319 nucleotides long.The viral genome encodes structural proteins and non-structural proteins. Virions consist of numerous proteins located in the capsid.

CHICKEN INFECTIOUS ANAEMIA
CLASSIFICATION

Family	Circoviridae

Genus	Gyrovirus

CHICKEN INFECTIOUS ANAEMIA
RESISTANCE

- Very resistant virus. Able to withstand pH of 3 and chloroform. Not inactivated by heating at 70°C for one hour or after 5 minutes at 80°C. Resistant to lipid solvents and to treatment for 2 hours at 37°C with 5% solutions of commercial disinfectants (quaternary ammonium compounds, amphoteric soap and orthodichlorobenzene)

CHICKEN INFECTIOUS ANAEMIA
PATHOGENESIS

- The incubation period is long under field conditions. All CAV isolates cause anemia and/or aplastic bone marrow. Eight days after infection the haematocrit levels, thrombocytes and red and white cell counts decrease. Blood takes longer to clot
- Between 28 – 36 days post infection the heamathological parameters in recovered birds return to normal. Under experimental conditions the virus is found present in most organs after one day in brain, liver, spleen, bursa, bone marrow, rectal contents and serum. CAV can be found latent in SPF flocks where the virus was detected in the ovaries, oviduct, testicles and spleen of birds without obvious seroconversion until the birds came into production.

CHICKEN INFECTIOUS ANAEMIA
TRANSMISSION

- CAV is spread vertically and horizontally in chickens.

CHICKEN INFECTIOUS ANAEMIA
SYMPTOMS

- Clinical signs are mainly seen in young birds of less than two weeks of age. Most outbreaks occur in broilers, followed by replacement pullets. Outbreaks in older birds (replacement pullets) have been reported when other immunosuppressive agents like Marek's Disease virus and/or Infectious Bursal Disease virus are involved.
- Young chickens are depressed and huddle under the heat source. The birds appear less developed for their age and anaemic. CAV infection impairs the immune system as it multiplies in most lymphopoietic organs resulting in the depletion of lymphocytes.
- CAV enhances the effect of other immunosuppressive agents such as Marek’s Disease virus and Infectious Bursal Disease virus. The reduction in the development of antibodies after vaccination against Newcastle Disease in CAV infected birds is common.

CHICKEN INFECTIOUS ANAEMIA
LESIONS

- The characteristic lesion is Blue wing disease wherein the focal lesions (mostly in the wings) appear as ecchymotic skin haemorrhages, which may turn blue and may break, releasing serosanguineous exudate which is prone to secondary bacterial infections, leading to gangrenous dermatitis. The mortality peaks 5 -6 days after the appearance of the
- Clinical signs, declining to normal 5 – 6 days later.
- Thymus and bone marrow atrophy are the other lesions.

CHICKEN INFECTIOUS ANAEMIA
DIAGNOSIS

asis of clinical signs and post mortem findings are inconclusive. Testing serum samples at the time of the clinical sig the best basis for serological diagnosis.The retrospective testing of sera from breeders could be done in cases where

CHICKEN INFECTIOUS ANAEMIA
TREATMENT

- Treatment is not effective

CHICKEN INFECTIOUS ANAEMIA
CONTROL AND ERADICATION

- Live vaccine using strain 26P4 is effective against CAV. The vaccine should be given at eight weeks of age or until four weeks before onset of lay. The vaccine should be administered by intramuscular or subcutaneous injection or by the wing web method.
- Control is best achieved by improved biosecurity and vaccination of breeders.

CHICKEN INFECTIOUS ANAEMIA
PUBLIC HEALTH SIGNIFICANCE

- It is not a zoonotic infection.

MODULE-32: PRION DISEASES

Learning objectives

- About the general characters of prion
  - Properties of prions and the prion proteins
- Different diseases caused by prions and its pathogenesis
  - Bovine spongiform encephalopathy
  - Scrapie

PRION DISEASES
INTRODUCTION

- Prions are transmissible particles that are without of nucleic acid and composed of a modified protein (PrPSc). The normal, cellular PrP (PrPC) is converted into PrPSc through a posttranslational process.
- The following are the important prion diseases.
    - Bovine spongiform encephalopathy (BSE)
    - Scrapie of sheep

PRION DISEASES
BOVINE SPONGIFORM ENCEPHALOPATHY

- Commonly known as Mad cow disease. It is a fatal, neurodegenerative disease in cattle, that causes a spongy degeneration in the brain and spinal cord. BSE has a long incubation period, about 4 years, usually affecting adult cattle at a peak age onset of four to five years, all breeds being equally susceptible. It is believed by most scientists that the disease may be transmitted to human beings who eat the brain or spinal cord of infected carcasses. In humans, it is known as Creutzfeldt–Jakob disease

PRION DISEASES
SCRAPIE

- Scrapie is a fatal, degenerative disease that affects the nervous systems of sheep and goats. The name scrapie is derived from one of the symptoms of the condition, wherein affected animals will compulsively scrape off their fleece against rocks, trees or fences. The disease expresses as severe itching sensation in the animals, lip-smacking, strange gaits, and convulsive collapse. Scrapie is infectious and transmissible among similar animals. Scrapie persist in flocks and can arise spontaneously in flocks that have not previously had cases of the disease. The mechanism of transmission between animals and other aspects of the biology of the disease are only poorly understood and these are active areas of research.