

VMC 321 GENERAL AND SYSTEMATIC VIROLOGY**COURSE MATERIAL**

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INSTRUCTORS

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VMC 321 LECTURE # 1**GENERAL PROPERTIES OF VIRUSES**

HISTORY: Generally the information regarding viruses are only a century old. Most of the information regarding viruses were obtained after the development of electron microscope (EM) and the techniques of negative staining in EM and x-ray crystallography. However, without any idea of the nature of virus infection, certain information were found in old literatures. Few classical examples are as follows;

- The Greek poet Homer characterizes one of his characters Hector as rabid in *The Iliad*.
- The Mesopotamian law before 1000 B.C describes the dog owner's responsibility pertaining to rabid dogs.
- In Egyptian hieroglyphs, one of the characteristic squeal of polio virus infection – withered leg has been depicted.
- The spread of small pox, which was common in Ganges river basin into continental Europe has been mentioned in old records of 5 century B.C.
- Other common viral infections prevalent in those days include yellow fever, influenza and mumps.
- The control measures of small pox (variolation) that include irrational approaches of Indians and Chinese, which was fine tuned by Lady Mary Wortley Montague in Asia Minor during 18th century.
- In 1790, Edward Jenner standardized the protection method against small pox using cowpox virus, which was termed as vaccination.

FIRSTS

Animal virus – Foot and mouth disease by **Loeffler and Paul Frosch**

Human virus – Yellow fever by **Reeds Commission**

Plant virus – Tobacco mosaic disease by **Dimitrii Ivanowsky**

- In 1885, Louis Pasteur developed an inactivated vaccine against rabies, which was found successful in post exposure treatment of rabies.
- **First report of viruses:** In 1892, Russian scientist **Dimitrii Ivanowsky** attributed the causative agent of tobacco mosaic disease to an agent that is filterable in porcelain filters. **Martinus Beijerinck** also made the same observation seven year later. He termed the causative agent of tobacco mosaic disease as *Contagium vivium fluidum* with clear idea about its distinct physical properties. This term evolved to **virus**,

which in Latin means poison.

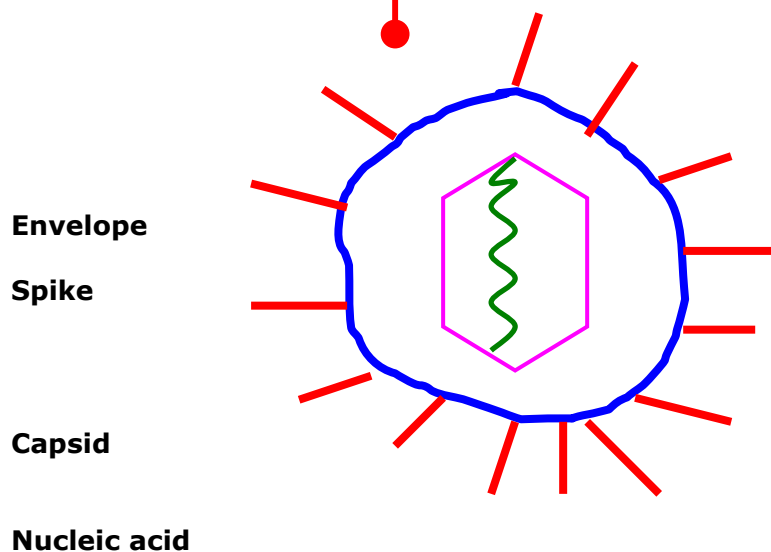
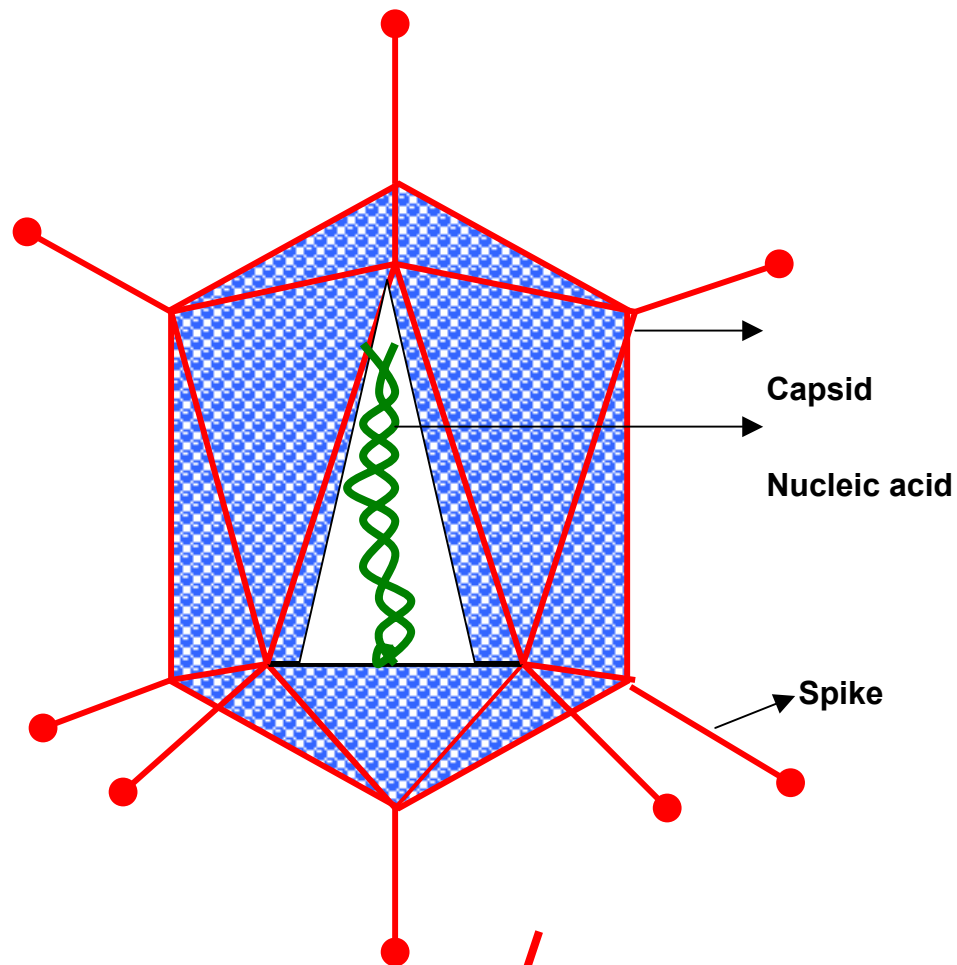
- In 1898, Friedrich **Loeffler and Paul Frosch** attributed the causative agent of foot and mouth disease to an agent that is filterable
- In 1915 and 1917 **Frederick Twort and Felix d'Herelle** respectively described viruses affecting bacteria, which were described as bacteriophages.
- In 1901, the **Reeds Commission** gave details of yellow fever virus, which was the first human virus to be identified.

DEFINITION: Viruses are defined as small ultra-microscopic obligate parasites, which have either DNA or RNA as genetic material. They replicate using host cell machinery and produce progeny virion by *de novo* synthesis. The spread of virus infection from one host to another is through progeny virions.

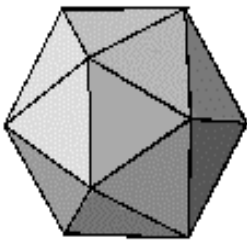
The term virion refers to whole acellular viral particles. A virion possesses nucleic acid contained in protective coat called capsid. The complete infective virus particle is also referred as virion.

TYPES OF VIRUSES: The viruses known are broadly grouped under animal viruses, plant viruses, insect viruses and bacterial. The correct classification of viruses will be dealt separately.

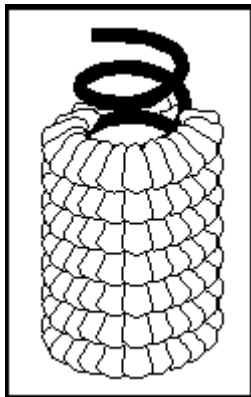
STRUCTURE OF VIRUSES: Viruses are extremely small particles comprises of just proteins and nucleic acid. The largest viruses are about 300nm in size, whereas the smallest known viruses are about 30nm. Viruses are comprised of two important components a protein **capsid** covering the **nucleic acid**. Some viruses also possess a lipid **envelope** but the envelope is not a virus- mediated structure.



Outer coat or capsid: The outer structure of virus is referred as capsid. It is made up of proteins. It is a protein shell that encloses the nucleic acid. It is built of **structure units**, which are the smallest functional equivalent building units of the capsid. **Capsomeres** are morphological units seen on the surface of particles and represent clusters of structural units. Capsomeres are also referred as the basic structural subunit of capsid. The capsid together with its enclosed nucleic acid is called the **nucleocapsid**. The nucleocapsid may be enclosed in an **envelope**, which may contain material of host cell as well as viral origin. The important function of the capsid of virus particle is to protect the fragile nucleic acid genome from physical, chemical and enzymatic damages. The protein subunits in a virus capsid are **multiply redundant**, i.e. present in many copies per particle. The viral nucleocapsid have the following three different morphological forms, which is also referred as symmetry. There are basic symmetries – cubical and helical.



complete a shell. A virus with icosahedral symmetry (5:3:2 symmetry) requires a multiple of 60 subunits to cover the surface completely.



Cubical symmetry: It is also referred as icosahedral symmetry. In this arrangement, the nucleic acids are arranged inside a shell, which is in the shape of an icosahedron. Icosahedron is a geometrical figure with 12 vertices (corners) and 20 identical facets (faces). Each facet is an equilateral triangle. There are six 5-fold axes of symmetry passing through the vertices, ten 3-fold axes extending through each face and fifteen 2-fold axes passing through the edges of an icosahedron. Icosahedral symmetry requires definite numbers of structure units to

Helical symmetry: In this arrangement, the nucleic acid and capsomeres are helically coiled together. The length of the helical viral nucleocapsid is determined by the length of the nucleic acid. In this symmetry the identical protein subunits are arranged in the form of a circle to form a disc shaped appearance. The cylinder or helical structure is formed as a result of stacking of multiple discs, with the virus genome coated by the protein shell or contained in the hollow centre of the cylinder.

Complex symmetry: It is also referred as undefined symmetry. This arrangement does not fit into either helical or cubical symmetries. It has the feature of both cubical and helical symmetries.

VIRAL NUCLEIC ACID: The genetic material of viruses are made up of only one type of nucleic acid i.e. either DNA or RNA but never composed of both. The nucleic acid both DNA and RNA may be present either as single stranded or double stranded. The nucleic acid (RNA) may also present either as a single molecule or in segments. The DNA may present either as linear or circular molecule. The RNA may present either as positive sense or negative sense. The molecular weight ranges from 2 million to 200 million. In some viruses the nucleic acids are just packed inside the capsid, whereas in some viruses, they are integrated with the nucleocapsid.

ENVELOPE: The capsid of some viruses are surrounded by a membrane called envelope. Viral envelopes are derived from cellular membranes of host, which are acquired during the release of virus from host cell. The envelope also contain proteins that are referred as matrix proteins and are specified by viral genes. The envelope proteins appear as spikes and are also referred as spike proteins. The spike proteins are responsible for attachment of virus to cells.

The viral structures are studied by electron microscopic techniques like negative staining, freeze etching and shadowing. The method that is more commonly used is X ray crystallography. Of late nuclear magnetic resonance (NMR) imaging is used to study structural details.

CLASSIFICATION OF VIRUSES

The classification of viruses is difficult due to its simple structure and presence of only few components.

Classical system of classification: In 1962, viruses were classified under classical system of classification by Lwoff, Horne and Tournier under hierarchical system consisting of phylum, class, order, family, genus and species. In this system the viruses were grouped based on the properties they share among themselves than the properties of the cells or organisms they infect. The other important features included in classical system are nature of nucleic acid, symmetry of the capsid, presence or absence of envelope and dimensions of virion.

Baltimore classification system: This system was proposed by David Baltimore. This classification is based on genetic system of each virus and the obligatory relationship between viral genome and its mRNA. In Baltimore classification, viruses are grouped under six different groups. They are as follows;

- Group I** – Double stranded DNA viruses
- Group II** – Single stranded DNA viruses
- Group III** – Double stranded RNA viruses
- Group IV** – Positive sense RNA viruses
- Group V** – Negative sense RNA viruses
- Group VI** – Reverse transcribing RNA viruses
- Unclassified viruses.

ICTV classification: As for classification of bacteria, a separate committee called **International Committee on Taxonomy of Viruses** decides the grouping and regrouping of viruses. In this system certain aspects of classical system of classification like grouping under family, genera and Baltimore system of classification are used. The committee has published its latest report on classification (7th Report). At present there are more than 1,550 virus species belonging to 3 orders, 56 families, 9 subfamilies and 233 genera are recognized in this report. Only the viruses that affect vertebrates are included in the following table. Baculo virus is also included since it is commonly used in genetic engineering as vectors.

Group I: dsDNA Viruses			
Family (Subfamily)	Genus	Type Species	Hosts
<u>Adenoviridae</u>	<i>Mastadenovirus</i>	<i>Human adenovirus C</i>	Vertebrates
	<i>Aviadenovirus</i>	<i>Fowl adenovirus A</i>	Vertebrates
<u>Asfarviridae</u>	<i>Asfivirus</i>	<i>African swine fever virus</i>	Vertebrates
<u>Herpesviridae:</u> <i>Alphaherpesvirinae</i>	<i>Simplexvirus</i>	<i>Human herpesvirus 1</i>	Vertebrates
	<i>Varicellovirus</i>	<i>Human herpesvirus 3</i>	Vertebrates

<i>Betaherpesvirinae</i>	<i>Cytomegalovirus</i>	<i>Human herpesvirus 5</i>	Vertebrates
	<i>Muromegalovirus</i>	<i>Murine herpesvirus 1</i>	Vertebrates
	<i>Roseolovirus</i>	<i>Human herpesvirus 6</i>	Vertebrates
<i>Gammaherpesvirinae</i>	<i>Lymphocryptovirus</i>	<i>Human herpesvirus 4</i>	Vertebrates
	<i>Rhadinovirus</i>	<i>Simian herpesvirus 2</i>	Vertebrates
<u><i>Iridoviridae</i></u>	<i>Ranavirus</i>	<i>Frog virus 3</i>	Vertebrates
	<i>Lymphocystivirus</i>	<i>Lymphocystis disease virus 1</i>	Vertebrates
<u><i>Polyomaviridae</i></u>	<i>Polyomavirus</i>	<i>Simian virus 40</i>	Vertebrates
<u><i>Papillomaviridae</i></u>	<i>Papillomavirus</i>	<i>Cottontail rabbit papillomavirus</i>	Vertebrates
<i>Poxviridae:</i> <u><i>Chordopoxvirinae</i></u>	<i>Orthopoxvirus</i>	<i>Vaccinia virus</i>	Vertebrates
	<i>Parapoxvirus</i>	<i>Orf virus</i>	Vertebrates
	<i>Avipoxvirus</i>	<i>Fowlpox virus</i>	Vertebrates
	<i>Capripoxvirus</i>	<i>Sheeppox virus</i>	Vertebrates
	<i>Leporipoxvirus</i>	<i>Myxoma virus</i>	Vertebrates
	<i>Suipoxvirus</i>	<i>Swinepox virus</i>	Vertebrates
	<i>Molluscipoxvirus</i>	<i>Molluscum contagiosum virus</i>	Vertebrates
	<i>Yatapoxvirus</i>	<i>Yaba monkey tumor virus</i>	Vertebrates
<u><i>Baculoviridae</i></u>	<i>Nucleopolyhedrovirus</i>	<i>Autographa californica nucleopolyhedrovirus</i>	Invertebrates
Group II: ssDNA Viruses			
<u><i>Circoviridae</i></u>	<i>Circovirus</i>	<i>Porcine circovirus</i>	Vertebrates
	<i>Gyrovirus</i>	<i>Chicken anemia virus</i>	Vertebrates
<i>Parvoviridae:</i> <u><i>Parvovirinae</i></u>	<i>Parvovirus</i>	<i>Mice minute virus</i>	Vertebrates
	<i>Erythrovirus</i>	<i>B19 virus</i>	Vertebrates
	<i>Dependovirus</i>	<i>Adeno-associated virus 2</i>	Vertebrates
Group III: dsRNA Viruses			
<u><i>Birnaviridae</i></u>	<i>Aquabirnavirus</i>	<i>Infectious pancreatic necrosis virus</i>	Vertebrates
	<i>Avibirnavirus</i>	<i>Infectious bursal disease virus</i>	Vertebrates
<i>Reoviridae</i>	<u><i>Orthoreovirus</i></u>	<i>Mammalian orthoreovirus</i>	Vertebrates
	<u><i>Orbivirus</i></u>	<i>Bluetongue virus</i>	Vertebrates
	<u><i>Rotavirus</i></u>	<i>Rotavirus A</i>	Vertebrates

	<u><i>Coltivirus</i></u>	<i>Colorado tick fever virus</i>	Vertebrates
	<u><i>Aquareovirus</i></u>	<i>Golden shiner virus</i>	Vertebrates
Group IV: (+)sense RNA Viruses			
Order <i>Nidovirales</i> - "Nested" Viruses			
<u><i>Arteriviridae</i></u>	<i>Arterivirus</i>	<i>Equine arteritis virus</i>	Vertebrates
<u><i>Coronaviridae</i></u>	<i>Coronavirus</i>	<i>Infectious bronchitis virus</i>	Vertebrates
	<i>Torovirus</i>	<i>Equine totovirus</i>	Vertebrates
<u><i>Astroviridae</i></u>	<i>Astrovirus</i>	<i>Human astrovirus 1</i>	Vertebrates
<u><i>Caliciviridae</i></u>	<i>Lagovirus</i>	<i>Rabbit haemorrhagic disease virus</i>	Vertebrates
	Norwalk-like viruses	<i>Norwalk virus</i>	Vertebrates
	Sapporo-like viruses	<i>Sapporo virus</i>	Vertebrates
	<i>Vesivirus</i>	<i>Swine vesicular exanthema virus</i>	Vertebrates
<u><i>Flaviviridae</i></u>	<i>Flavivirus</i>	<i>Yellow fever virus</i>	Vertebrates
	<i>Pestivirus</i>	<i>Bovine diarrhea virus 1</i>	Vertebrates
	<i>Hepacivirus</i>	<i>Hepatitis C virus</i>	Vertebrates
<u><i>Picornaviridae</i></u>	<i>Enterovirus</i>	<i>Poliovirus</i>	Vertebrates
	<i>Rhinovirus</i>	<i>Human rhinovirus A</i>	Vertebrates
	<i>Hepatovirus</i>	<i>Hepatitis A virus</i>	Vertebrates
	<i>Cardiovirus</i>	<i>Encephalomyocarditis virus</i>	Vertebrates
	<i>Aphthovirus</i>	<i>Foot-and-mouth disease virus O</i>	Vertebrates
	<i>Parechovirus</i>	<i>Human parechovirus</i>	Vertebrates
	<i>Erbovirus</i>	<i>Equine rhinitis B virus</i>	Vertebrates
	<i>Kobuvirus</i>	<i>Aichi virus</i>	Vertebrates
	<i>Teschovirus</i>	<i>Porcine teschovirus</i>	Vertebrates
<u><i>Togaviridae</i></u>	<i>Alphavirus</i>	<i>Sindbis virus</i>	Vertebrates
	<i>Rubivirus</i>	<i>Rubella virus</i>	Vertebrates
Group V: (-)sense RNA Viruses			
Order <i>Mononegavirales</i>			
<u><i>Bornaviridae</i></u>	<i>Bornavirus</i>	<i>Borna disease virus</i>	Vertebrates
<u><i>Filoviridae</i></u>	Marburg-like viruses	<i>Marburg virus</i>	Vertebrates
	Ebola-like viruses	<i>Ebola virus</i>	Vertebrates

<u>Paramyxoviridae:</u>	<i>Respirovirus</i>	<i>Sendai virus</i>	Vertebrates
<i>Paramyxovirinae</i>	<i>Morbillivirus</i>	<i>Measles virus</i>	Vertebrates
	<i>Rubulavirus</i>	<i>Mumps virus</i>	Vertebrates
<i>Pneumovirinae</i>	<i>Pneumovirus</i>	<i>Human respiratory syncytial virus</i>	Vertebrates
	<i>Metapneumovirus</i>	<i>Turkey rhinotracheitis virus</i>	Vertebrates
<i>Rhabdoviridae</i>	<u><i>Vesiculovirus</i></u>	<i>Vesicular stomatitis Indiana virus</i>	Vertebrates
	<u><i>Lyssavirus</i></u>	<i>Rabies virus</i>	Vertebrates
	<u><i>Ephemerovirus</i></u>	<i>Bovine ephemeral fever virus</i>	Vertebrates
	<u><i>Novirhabdovirus</i></u>	<i>Infectious haematopoietic necrosis virus</i>	Vertebrates
<u><i>Arenaviridae</i></u>	<i>Arenavirus</i>	<i>Lymphocytic choriomeningitis virus</i>	Vertebrates
<i>Bunyaviridae</i>	<u><i>Bunyavirus</i></u>	<i>Bunyamwera virus</i>	Vertebrates
	<u><i>Hantavirus</i></u>	<i>Hantaan virus</i>	Vertebrates
	<u><i>Nairovirus</i></u>	<i>Nairobi sheep disease virus</i>	Vertebrates
	<u><i>Phlebovirus</i></u>	<i>Sandfly fever Sicilian virus</i>	Vertebrates
<u><i>Orthomyxoviridae</i></u>	<i>Influenza A virus</i>	<i>Influenza A virus</i>	Vertebrates
	<i>Influenza B virus</i>	<i>Influenza B virus</i>	Vertebrates
	<i>Influenza C virus</i>	<i>Influenza C virus</i>	Vertebrates
	<i>Thogotovirus</i>	<i>Thogoto virus</i>	Vertebrates
Group VI: RNA Reverse Transcribing Viruses			
<u><i>Retroviridae</i></u>	<i>Alpharetrovirus</i>	<i>Avian leukosis virus</i>	Vertebrates
	<i>Betaretrovirus</i>	<i>Mouse mammary tumor virus</i>	Vertebrates
	<i>Gammaretrovirus</i>	<i>Murine leukemia virus</i>	Vertebrates
	<i>Deltaretrovirus</i>	<i>Bovine leukemia virus</i>	Vertebrates
	<i>Epsilonretrovirus</i>	<i>Walley dermal sarcoma virus</i>	Vertebrates
	<i>Lentivirus</i>	<i>Human immunodeficiency virus 1</i>	Vertebrates
	<i>Spumavirus</i>	<i>Human spumavirus</i>	Vertebrates
Group VII: DNA Reverse Transcribing Viruses			
<u><i>Hepadnaviridae</i></u>	<i>Orthohepadnavirus</i>	<i>Hepatitis B virus</i>	Vertebrates

	<i>Avihepadnavirus</i>	<i>Duck hepatitis B virus</i>	Vertebrates
Subviral Agents: Satellites and Prions			
Prions			Vertebrates

VMC 321 LECTURE # 2**REPLICATION OF VIRUSES****Introduction:**

Viruses are obligate intracellular parasites. Hence, they need the help of a host cell for their replication. All viruses have to penetrate, replicate and come out of a cell. The exact way by which different viruses do this varies widely. Infection of a cell by virus may result in any one of the following types of infection.

Productive infection: The cells allow viruses to replicate and the progeny virions are released from the infected cell.

Abortive infection: The cells do not allow viruses to replicate and as a result of this daughter virions are not produced.

Restrictive infection: The cells allow minimal replication of viruses and as a result only few daughters alone are produced. However, the viral genome persists and can lead to serious consequence for the host. Eg. Epstein Barr and herpes simplex

Stages of virus replication

Virus replication can be divided into following phases and each phase may have number of stages:

Phase – I Initiation:

- Attachment
- Penetration
- Uncoating

This stage is characterized by introduction of genetic material of the virus into the cell

Phase – II Replication:

- Genome synthesis
- RNA production
- Protein synthesis

This stage is characterized by the genome size, composition and organization of viruses. There is no single pattern of replication. But all make proteins with 3 sets of functions to:

- Ensure replication of the genome
- Package the genome into virus particles
- Alter the metabolism of the infected cell so that viruses are produced.

Phase – III Assembly, Release, Maturation:**Phase I - Initiation**

1. **Attachment:** Virus attaches to the cell surface. Attachment is via ionic interactions. Viral attachment proteins referred as **ligands** are present on the surface of viruses, which recognizes specific receptors on the cell surface. The ligands in viruses are usually the fibres and spikes in the virus structures. The receptors on cells are protein or carbohydrate or lipid components of the cell surface. The most common cellular receptors are glycoproteins. Some of the important cell receptors for viruses are CD4, ICAM etc. Cells without the appropriate receptors are not susceptible to the virus. Hence, a virus cannot produce infection in a host, which does not contain receptors for virus attachment. The joining of ligand to receptor on cell is also facilitated by co-receptors (Eg. gp120 of HIV) In some cases attachment leads to irreversible changes in the structure of the virion.
2. **Penetration:** It is a process by which a virus enters into the cell. It is an energy dependant reaction and occurs quickly. It occurs as fusion, endocytosis or translocation. It is different for enveloped and non-enveloped viruses.

Enveloped viruses – There are two methods for enveloped viruses.

(A) *Entry by fusing with the plasma membrane* – Some enveloped viruses fuse directly with the plasma membrane. Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell

(B) *Entry via endosomes at the cell surface* – Some enveloped viruses require an acid pH for fusion to occur and are unable to fuse directly with the plasma membrane. These viruses are taken up by invagination of clathrin coated pits into endosomes. As the endosomes become acidified, the fusion activity of the virus proteins becomes activated by the fall in pH and the virion membrane fuses with the endosome membrane. This results in delivery of the internal components of the virus to the cytoplasm of the cell. This endocytosis is also called **viropexis**.

Non-enveloped viruses - Non-enveloped viruses may cross the plasma membrane directly or may be taken up via clathrin-coated pits into endosomes. They then cross the endosomal membrane.

3. **Uncoating:** This is the general term applied to events after penetration, which allow the virus to express its genome. For successful viral infection, nucleic acid has to be sufficiently uncoated. The lysosomal enzymes play a major role in uncoating.

Phase II - Replication of viral nucleic acid and protein synthesis

Once uncoating has taken place, synthesis of viral nucleic acid starts. This occurs as three different stages with differences between different families of the viruses.

1. Early transcription and translation – The proteins derived from this stage is mostly the enzymes required for virus replication.
2. Replication of Nucleic acid
3. Late transcription and translation – The proteins produced this stage are structural proteins.

The site of production of nucleic acid also varies between viruses. Most of the DNA viruses except Pox and Herpes replicate in nucleus. All RNA viruses replicate in cytoplasm except orthomyxo, borna and retro, which for certain stages of replication get into the nucleus of the cell.

RNA virus replication

RNA viruses have a relatively small genome. Because of this RNA viruses code for only a few proteins. These normally include the enzyme Polymerase, which can copy RNA into a complementary nucleic acid and a viral attachment protein.

Problems associated with RNA virus replication

1. Prokaryotic and eukaryotic cells do not carry RNA dependant RNA Polymerase enzyme, which is needed for nascent RNA strand synthesis from a RNA template. Hence, the negative strand RNA viruses carry this enzyme and positive strand RNA viruses have it as a gene.
2. Eucaryotic host cell translation protein synthesis machinery in general uses monocistronic mRNAs and so there is a problem in making more than one type of protein from a single mRNA. RNA viruses have several solutions to this problem:
 - The virus makes multiple monocistronic mRNAs
 - The virus makes primary transcripts, which are processed by the host splicing machinery to give more than one monocistronic RNA
 - The viral mRNA acts as a monocistronic transcript. A large polypeptide (called a polyprotein) is made which is then cleaved into separate proteins - Thus, one initial translation product is

processed to give rise to multiple proteins. This happens, for example, in picornaviruses

The RNA virus replication is described here as different strategies

1. RNA VIRUSES THAT DO NOT COPY THEIR RNA INTO DNA:

As described earlier, these viruses need an RNA-dependent RNA-polymerase to replicate their RNA.

a. Plus-strand RNA viruses - In these viruses, the virion (genomic) RNA is the same sense as mRNA and so functions as mRNA. This mRNA can be translated immediately upon infection of the host cell. Eg. FMD virus (picornavirus); togaviruses; flaviviruses

b. Negative-strand RNA viruses - The virion RNA is negative sense (complementary to mRNA) and must therefore be copied into the complementary plus-sense mRNA before proteins can be made. Eg. Newcastle disease virus (paramyxoviruses) rabies virus (rhabdovirus)

c. Double-strand RNA viruses - The virion (genomic) RNA is double stranded and so cannot function as mRNA. These viruses also carry the enzyme RNA polymerase as negative strand RNA viruses.

PLUS STRAND RNA VIRUSES

The plus strand viruses are also known as positive strand viruses and in this category of viruses the genomic RNA can act as mRNA. Eg. Picornaviruses; togaviruses; flaviviruses

Transcription and Translation: Poliovirus virion RNA functions as an mRNA and it is translated into a single polypeptide (polyprotein) immediately after entering into a cell. These polyprotein is later cleaved into number of small proteins. The cleavages are carried out by virus-coded proteases. The products of cleavage include RNA polymerase (replicase) needed for RNA synthesis, structural components of the virion and proteases.

RNA replication: RNA replication is the process by, which new copies of genome-length RNAs are made. The products of cleavage of protein support the replication of genomic RNA. The Viral RNA polymerase copies plus-sense genomic RNA into complementary minus-sense RNA. The new minus sense strands serve as template for new plus sense strands. The new plus strand RNA serve as a template for more minus strands.

NEGATIVE STRAND RNA VIRUSES – NON-SEGMENTED

The negative strand viruses those viruses wherein the genomic RNA cannot act as mRNA and mRNA has to be synthesized using genomic RNA as template. Hence, these viruses always carry the enzyme RNA dependant RNA polymerase. Eg. Rhabdoviruses, Paramyxoviruses

Transcription and translation: The RNA polymerase enzyme copy virion RNA into a mRNA. Messenger RNAs are translated on host ribosomes and viral proteins are made. There is no distinction between early and late functions.

RNA replication: RNA replication occurs in the cytoplasm and is carried out by the viral RNA polymerase. The new positive strand (mRNA) is copied into full length minus strand. New negative strands may be used as templates for the synthesis of more full length plus strands (mRNA).

SEGMENTED NEGATIVE STRAND VIRUSES

The important virus is the Influenza virus. They have negative-sense, single stranded segmented genome. Virions contain RNA polymerase packaged within the virus particle.

Transcription and translation: mRNA synthesis and replication of viral RNA occurs in the nucleus. This is very unusual for an RNA virus. Influenza virus has an unusual mechanism for acquiring a methylated capped 5' end to its mRNAs referred as **cap snatching** in which, the virion mRNA go to nucleus to obtain the cap from host mRNA. The viral RNA polymerase (transcriptase) copies the template into complementary plus sense mRNA. mRNAs are translated in the cytoplasm.

RNA replication: RNA replication occurs in the nucleus using a virus-coded enzyme. A full length, exact complementary copy of virion RNA is made. Full length plus strand RNA is then used as a template for full-length minus strand synthesis. New minus strands can be used as templates for replication, mRNA synthesis, or packaged.

DOUBLE STRANDED RNA VIRUSES

The members under this category are peculiar in that the RNA exists in the form of double strands

Transcription and translation: Double stranded RNA does not function as an mRNA and so the initial step is to make mRNA (transcription). The mRNAs are made by virion-packaged RNA polymerase.

2. RNA VIRUSES, WHICH COPY THEIR RNA INTO DNA – REVERSE TRANSCRIPTION:

These are the retroviruses. In this case, their virion RNA, although plus-sense, is not released in the cytoplasm, and so does not function as mRNA immediately upon infection. Instead, it serves as a template for reverse transcriptase and is immediately copied into DNA. Reverse transcriptase is not available in the cell, and so these viruses need to code for this enzyme and package it in virions.

DNA VIRUS REPLICATION

Unlike RNA viruses, the DNA virus replication is a straight-forward one since the mechanism available for viral DNA replication is readily available in the cell. DNA viruses are able to use the host cell's replication machinery to transcribe their genome into mRNA immediately. DNA viruses generally replicate within the host cell nucleus. These viruses include the papovaviruses, papillomaviruses, polyomaviruses, adenoviruses and herpesviruses. Poxviruses are the exception. Members of the family *Poxviridae* replicate in the cytoplasm of the host cell and also carry their own RNA polymerase (transcriptase). A replicating DNA virus has to satisfy the following;

- The virus needs to make mRNAs that can be translated into protein by the host cell translation machinery.
- The virus needs to replicate its genome.
- Host enzymes for mRNA synthesis and DNA replication are available in nucleus hence, it needs to enter the nucleus.

Transcription and translation: The gene expression is divided into early and late phases. The early genes encode enzymes and regulatory proteins needed to start viral replication processes and late genes encode structural proteins, proteins needed for assembly of the mature virus.

DNA Replication

Semi-conservative method: DNA replication uses host cell DNA polymerase. Replication is bi-directional. (There are two replication forks per circular DNA genome and replication involves leading/lagging strands, Okazaki fragments, DNA ligase, etc.). This process of DNA replication is very similar to that which occurs in the host cell (Eg. Papova viruses).

Strand displacement method: There are no Okazaki fragments, both strands are synthesized in a continuous fashion.

Phase III

Assembly: This stage involves the assembly of all the components necessary for the formation of the mature virion at a particular site in the cell. During this process, the basic structure of the virus is formed.

The site of assembly varies for different viruses, e.g:

- Picornaviruses, Poxviruses, Reoviruses - In the cytoplasm.
- Adenoviruses, Papovaviruses, Parvoviruses - In the nucleus.
- Retroviruses - On the inner surface of the cell membrane.

Release: For lytic viruses (most non-enveloped viruses), release is a simple process - the cell breaks open and releases the virus.

Enveloped viruses acquire the lipid membrane as the virus buds out through the cell membrane. Virion envelope proteins are picked up during this process as the virus is extruded. Budding may or may not kill the cell

Maturation: At this stage of the lifecycle normally the virus becomes infectious. Usually it involves structural changes in the particle, often resulting from specific cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid, or the condensation of nucleoproteins with the genome. For some viruses, assembly and maturation are inseparable, whereas for others, maturation may occur after the virus particle has left the cell.

VMC 321 LECTURE # 2 (A)**VETERINARY EPIDEMIOLOGY – AN INTRODUCTION**

Now veterinarians often have to deal with herds or regions remaining diseased after lengthy disease control campaigns. In addition, it is considered necessary to take into account the economic aspects of disease control through the use of benefit/cost analyses of disease control campaigns. Costly multi-factorial *disease complexes* such as mastitis or cattle lameness have become quite common. In addition, old, emerging or new *diseases* with complex aetiologies pose a difficult challenge for the profession. Veterinarians have to respond to the challenges posed by problems such as BSE, FMD and antimicrobial resistance. All these problems require identification, quantification and intensive examination of multiple, directly or indirectly causal, and often interacting, disease determinants. Veterinary epidemiology and evidence-based veterinary medicine provide overlapping sets of tools which can be used to approach these new challenges.

Definition: Veterinary epidemiology deals with the investigation of diseases, productivity and animal welfare in populations. It is used to describe the frequency of disease occurrence and how disease, productivity and welfare are affected by the interaction of different factors or determinants. This information is then used to manipulate such determinants in order to reduce the frequency of disease occurrence. Veterinary epidemiology is a holistic approach aimed at co-ordinating the use of different scientific disciplines and techniques during an investigation of disease or impaired productivity or welfare. Veterinary Epidemiology is broadly divided into descriptive epidemiology and analytical epidemiology.

Components of Veterinary Epidemiology: Veterinary epidemiology can be divided into different components. It involves collection of data, which then has to be analysed using qualitative or quantitative approaches in order to formulate causal hypotheses. As part of the quantitative approach to epidemiological analysis, epidemiological investigations involving field studies or surveys are being conducted and models of epidemiological problems can be developed. The ultimate goal is to control a disease problem, reduce productivity losses and improve animal welfare. The components are provided here in brief

- Qualitative evaluation
- Quantitative evaluation
- Simulation
- Modelling
- Surveillance
- Causal hypothesis testing
- Economic evaluation

Disease triad: The most important aspect in veterinary epidemiology is the disease triad contributed by the agent, host and environment. The various factors under each of these agents are listed below;

Agent

- Infectivity
- Pathogenicity
- Virulence
- Immunogenicity
- Antigenic Stability
- Survival

Host

- Species
- Age

Sex
Breed
Conformation
Genotype
Nutritional Status
Physiologic Condition
Pathologic Status

Environment

Weather
Housing
Geography
Geology
Management
Noise
Air Quality
Food
Chemical

VMC 321 LECTURE # 3**NEWCASTLE DISEASE**

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.

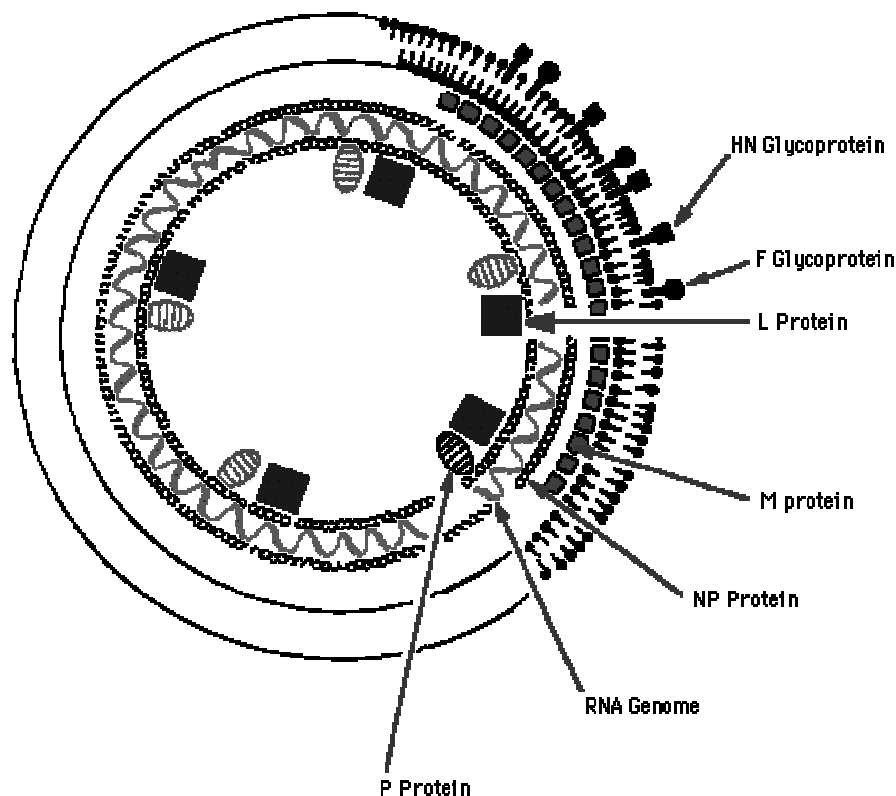
General characters:**1. Classification:**

Baltimore group	-	Group V – Negative sense RNA viruses
Order	-	Mononegavirales
Family	-	Paramyxoviridae
Subfamily	-	Paramyxovirinae
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.

2. Synonyms: Pseudo fowlpest, Ranikhet disease, Vellaikalichal (vernacular)

3. 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.

4. OIE Listing: List A infection (*ND and highly pathogenic avian influenza are the only two poultry diseases specified as List A.*

5. Risk group(Animal Pathogen): Group II

6. Reference Laboratories:

Dr D.J. Alexander

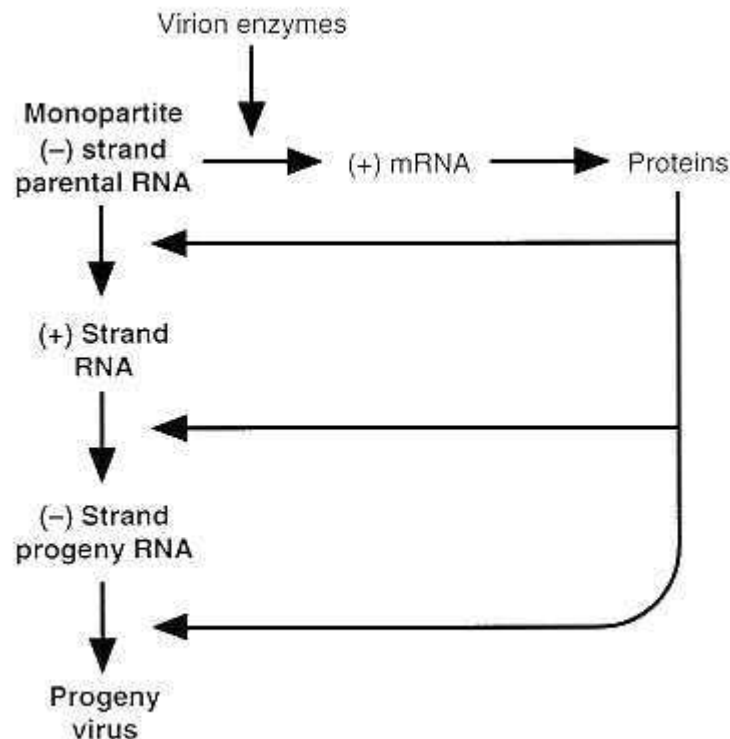
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7. Resistance: The virus is comparatively a stable virus and survives for long periods at ambient temperature, especially in faeces and can persist in houses (in faeces, dust etc.) for up to 12 months. However, it is sensitive to disinfectants, fumigants and sunlight. The virus is inactivated by temperatures of 56°C for 3 hours or 60°C for 30 min, acid pH, formalin and phenol, and is ether sensitive.

8. Replication:



9. Haemagglutination 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.

10.Virus strains: 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

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 throughout the body particularly in occipital region. Lentogenic strains do not cause death. Presence 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.

Pathogenesis

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. 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;

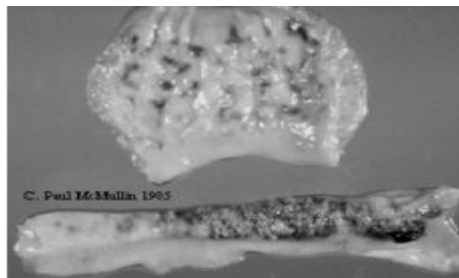
- **Viscerotropic velogenic (Doyle's form):** viruses responsible for disease characterised by acute lethal infections, usually with haemorrhagic lesions in the intestines of dead birds. The infection caused by this pathotype is also called as **Asiatic** or **exotic ND**.
- **Neurotropic velogenic (Beach's 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.

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. The incubation period varies from 2 to 15 days. For velogenic ND in chickens, an incubation period of just 2 to 6 days is common. The incubation period in other species of birds may be longer.

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.



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. Ovaries may be edematous, hemorrhagic, or degenerated. 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.

Diagnosis:

1. **Field diagnosis:** Tentative diagnosis of ND made on the basis of history, clinical signs, and gross lesions
2. **Virus isolation and identification:**
 - a. **Clinical materials:** Samples from dead birds should consist of oronasal 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.
 - b. **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 (HI) 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 conjunctival 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.

- c. **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.
- d. **Nucleic acid probes:**
- e. **ELISA techniques:** Antigen capture and Antigen competitive ELISA techniques can also be used to identify APMV-1 from clinical materials and AAF.

3. Pathotyping: Newcastle disease virus are classified into Velogenic, mesogenic and lentogenic based on following three tests

- a. **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
- b. **Intravenous pathogenicity 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.
- c. **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).
- d. **Molecular typing by studying the aminoacid between 112-119th position in F gene.**
- e. **Panels of MAbs** are also used to establish antigenic profiles of ND virus isolates based on whether or not they react with the viruses.

4. 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 log₂3 is indicative of protection and a titre of log₂6 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

- 5. 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.

Control

Vaccination: These different approaches to vaccination have been applied to ND. There are three types of vaccines used for ND: live lentogenic, live mesogenic and inactivated vaccines.

1. 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 or 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. Marker vaccine: Recently an immunodominant epitope of NP gene has been identified and replaced with mouse herpes virus gene. The resultant vaccine helps to differentiate between vaccinated and infected birds.

Vaccine application methods:

1. Mass administration through water or aerosol spray
2. Feed based techniques
3. As eye and/or nasal drops
4. As injection

Prevention:

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:

1. Regular vaccination and periodical antibody monitoring
2. Houses, food stores and water tanks should be bird-proofed.
3. Movements on and off the farm should be kept to a minimum.
4. All equipment, especially vehicles, should be disinfected before access to the site is permitted.
5. 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.
6. 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.

Public health hazards: 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.

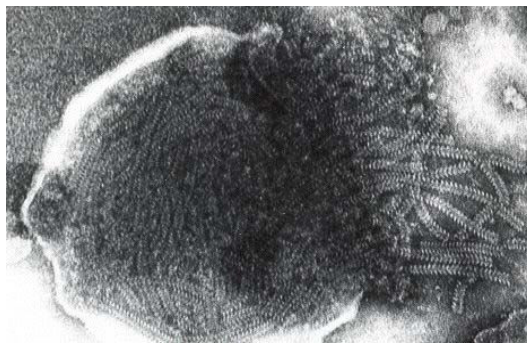
VMC 321 LECTURE # 4**RINDERPEST**

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.

General characters**1. Classification:**

Baltimore group	-	Group V – Negative sense RNA viruses
Order	-	Mononegavirales
Family	-	Paramyxoviridae
Subfamily	-	Paramyxovirinae
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.

2. Synonyms: Cattle plague**3. 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

- a. N - nucleocapsid protein which covers and protects the virion RNA
- b. P- polymerase-associated or phosphoprotein
- c. M- matrix protein, interacts with cytoplasmically located nucleocapsids and membrane associated glycoproteins in cell envelope to produce virus bud
- d. F - fusion protein enables virus and host cell membrane to fuse
- e. H - haemagglutinin or attachment protein, enables virus to attach to host cell membrane
- f. L - large protein, acts as a virus transcriptase and replicase in association with P protein
- g. 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.

4. OIE Listing: List A infection**5. Risk group:** Class II Animal Pathogen

6. Reference laboratories:**Dr J. Anderson**

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- 7. 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 pH 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 (25 g in 100 ml) will slow down inactivation of the virus in water.
- 8. Virus strains:** 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.

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 peel 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.

Pathogenesis:

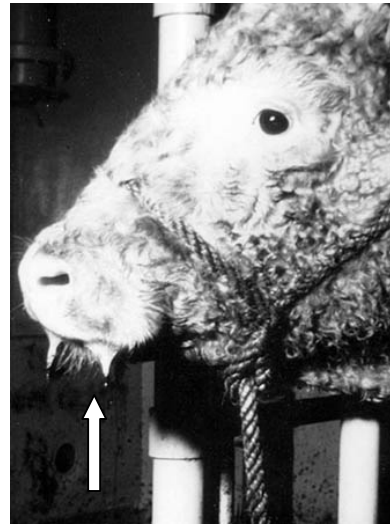
- 1. Host affected:** The common host is the domestic ungulates cattle and buffalo. Sheep, goat and pigs are the other important domestic animals affected by rinderpest. The infection has also been reported in a variety of wild animals including bushbuck, bush pig, giraffe, gaur, nilgai and sambar.
- 2. 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.
- 3. 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.

4. Symptoms:

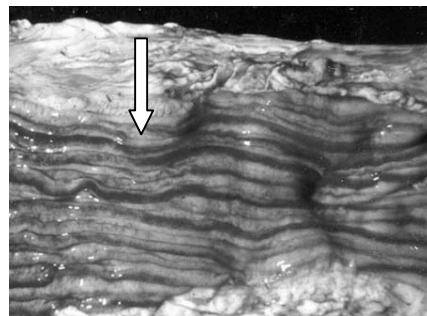
- a. **Buffaloes and cattle:** Clinical reactions in buffalo and cattle are similar and the infection may be exhibited as peracute, acute, subacute or even inapparent.

i. **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.

- ii. **Acute infection:** This form is divided into five phases 1) an incubation period, 2) a prodromal fever, 3) an erosive-mucosa phase, 4) a diarrhoeic phase and 5) a convalescent phase in surviving animals. 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. The **erosive mucosal phase** is observed two to three days after prodromal 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. 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 animals abort during convalescence.



- iii. **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.
 - b. **Sheep and goats:** 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.
 - c. **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.
- 5. Lesions:** Rinderpest virus has affinity for lymphoid tissues and a secondary affinity for the epithelium of the alimentary, upper respiratory and urogenital tracts.
- a. 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. The esophagus 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.
 - b. 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.
 - c. Lymph nodes are swollen and edematous.
 - d. Lung lesions are emphysema, congestion, and areas of pneumonia.



Diagnosis:

- 1. **Field diagnosis**
- 2. **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:
 - a. **Live animals:**
 - i. Blood in EDTA or heparin
 - ii. Blood for serum
 - iii. Swabs containing lacrimal fluid
 - iv. Tears

- v. Necrotic tissue from the oral cavity
 - vi. Aspiration biopsies of superficial lymph nodes
 - b. **Dead animals:** Sections of
 - i. Spleen
 - ii. Lymph nodes
 - iii. Tonsil
 - 3. **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.
 - 4. **Isolation and identification of virus** in calf kidney cells and vero cells using specimens specified above.
 - 5. **Detection of virus specific antigen by**
 - a. Agar gel immunodiffusion test
 - b. Counter immunoelectrophoresis
 - c. Passive haemagglutination
 - d. Immunofluorescence and immunoperoxidase techniques
 - e. Antigen capture ELISA
 - f. RT-PCR
 - g. DNA probes for N gene
 - 6. **Serological tests**
 - i. Competitive ELISA (test prescribed for International Trade)
 - ii. Virus neutralization test (Gold Standard)
 - iii. Measles haemagglutination inhibition test (MHI test)

Control and Eradication:

Treatment: There is no effective treatment against rinderpest.

Vaccines: 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 a 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, lapinised (rabbit passaged), lapinised and avianised rinderpest virus have also been used as vaccine in many Asian countries. Vaccinia-vectored vaccine containing the F and H genes of RPV was also used experimentally.

Eradication:

- a. 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.
- b. Since recovered animals are not carriers, serological techniques like ELISA can be effectively used to check the status of animals imported.
- c. Wild ruminants and swine imported should be properly quarantined and tested with serological tests.
- d. 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*)
- e. 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.

- f. 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.
- g. Foci of infection should be quarantined and stamped out. Wildlife, sheep, and goats should be monitored serologically.
- h. 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.
- i. **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
- j. **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.

Public Health

There is no report of rinderpest infection in a human.

VMC 321 LECTURE # 5**PESTE DES PETITS RUMINANTS**

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*).

General characters:**1. General characters****1. Classification:**

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

2. Synonyms: Goat plague**3. 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 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.

4. OIE Listing: List A infection**5. Risk group:** Class II Animal Pathogen

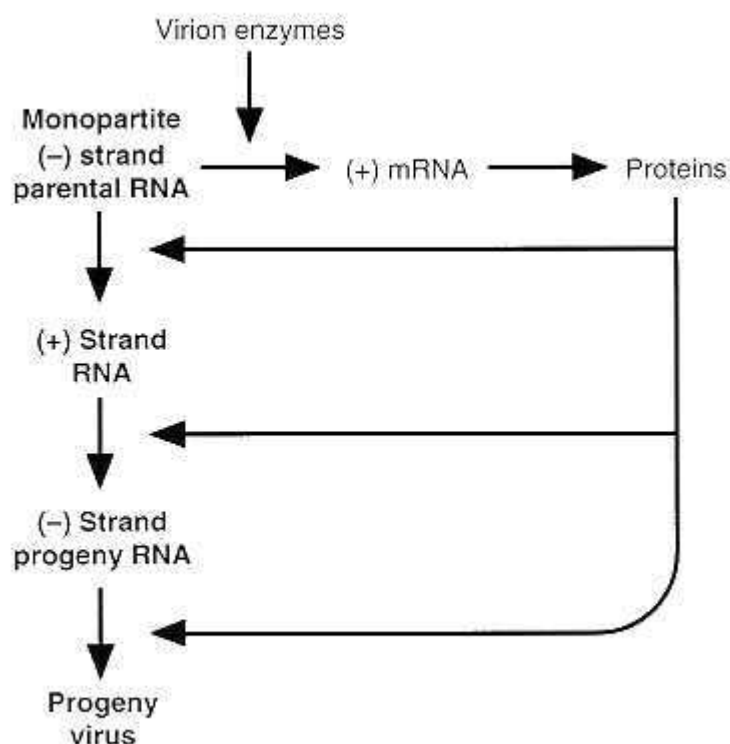
6. Reference laboratories:**Dr J. Anderson**

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34398 Montpellier Cedex 5 FRANCE

- 7. 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

8. Replication:

- 9. Haemagglutination:** PPRV caused haemagglutination of chicken RBC. However, the pH of PBS used should be at 6.8.

- 10. Virus strains:** PPR virus is closely related to rinderpest virus. But it is 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 to as AR-87) is used as vaccine in Southern India. Other South Indian PPRV isolates are Coimbatore (CBE) and Poondi (92).

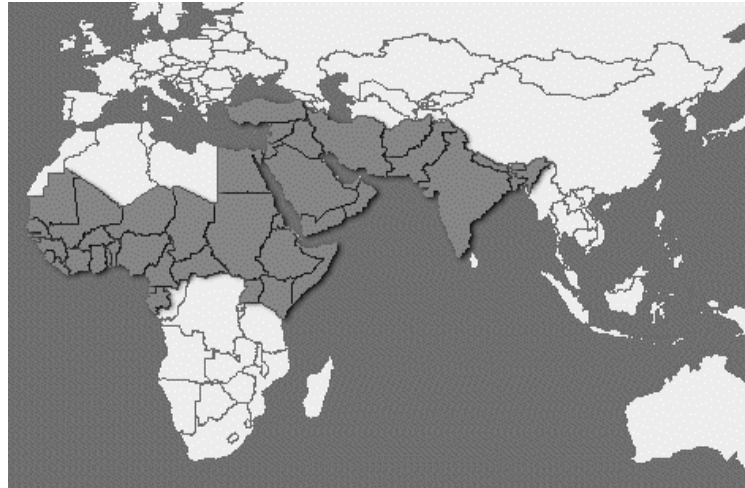
Cultivation:

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.

Pathogenesis:

1. **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.
2. **Distribution:** PPR is prevalent in Sub-Saharan Africa, Indian sub continent and Middle East countries.



3. **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:
 - a. History of recent movement or gathering together of sheep and/or goats of different ages
 - b. 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;
 - c. Change in weather such as the onset of the rainy season (hot and humid) or dry, cold periods
 - d. Contact with trade or nomadic animals
 - e. A change in husbandry (e.g. towards increased intensification) and trading practices
4. **Incubation period:** The incubation period is from 4 to 5 days.
5. **Morbidity and mortality:** Up to 100 percent of the animals in a flock may be affected in a PPR outbreak with between 20 and 90 percent mortality.
6. **Symptoms:**
 - a. Characterized by sudden onset of fever (40° to 41°C).
 - b. Affected animals are markedly depressed and appear sleepy. Their hair stands erect giving them a bloated appearance.
 - c. 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.

- d. One to two days after fever has set in, the mucous membranes of the mouth and eyes become very reddened.
- e.



Mouth lesions showing areas of dead cells

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.



Swollen, eroded lips

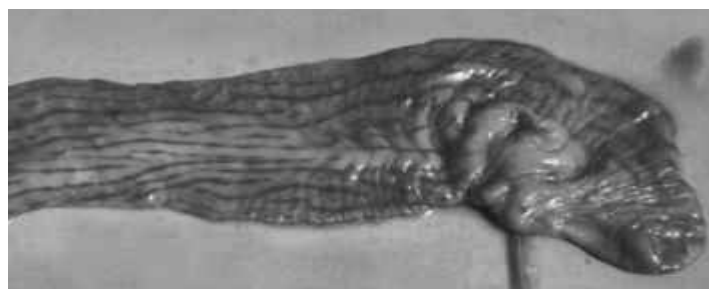
- f. Similar changes may also be seen in the mucous membranes of the nose, the vulva and the vagina. The lips tend to swell and crack and become covered with scabs.
- g. As the disease progresses, a characteristic foul smell exudes from the mouth. Affected animals resist attempts to open their mouths because of the pain.
- h. 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.

- i. 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.
 - j. 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.
- 7. Lesions:** The carcass of the affected animal is usually emaciated, the hindquarters soiled with soft/watery faeces. The eyes and nose contain dried-up discharges. The following changes are also seen:
- a. **Mouth** - Dirty-white, false membranes and erosions are seen on the gums, soft and hard palates, tongue, cheeks and oesophagus.
 - b. **Lips** - Appear Swollen with erosions and scabs or nodules
 - c. **Nasal cavity** - Congested (reddened) lining with clear or creamy yellow exudates and erosions
 - d. **Lungs** -Dark red or purple areas which are firm to the touch, are mainly seen in the anterior and cardiac lobes. Pleuritis and hydrothorax are also observed



Lesions of pneumonia

- e. **Lymph nodes** (associated with the lungs and the intestines) - Appear soft and swollen.
- f. **Abomasum** - Congested (reddened) lining with haemorrhages
- g. **Small intestines** - Congested (reddened) lining with haemorrhages and erosions are noticed.
- h. **Large intestines** (caecum, colon and rectum) - Small red haemorrhages along the folds of the lining that join together and appear darker or green/black in stale carcasses (**Zebra striping**)



Zebra striping

Diagnosis:

1. **Field diagnosis:** In the field, a presumptive diagnosis is made on the basis of clinical, pathological, and epizootiological findings.
2. **Clinical materials to be collected**
 - a. 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)
 - b. 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
 - c. 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
 - d. Unclotted blood - This is needed for virus isolation and should be collected in bottles containing anticoagulants (heparin or ethylenediamine tetracetic acid [EDTA]).
 - e. Clotted blood or serum - For identification of antibodies
3. **Isolation and identification:**
 - a. 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
 - b. Identification of virus:
 - i. Immunocapture enzyme-linked immunosorbent assay (Sandwich ELISA)
 - ii. Counterimmunoelectrophoresis (CIEP): Most rapid test for detecting viral antigen
 - iii. Agar gel immunodiffusion (AGID): Very simple and inexpensive and gives results within 1 day, but not sensitive to mild forms of PPR
 - iv. Immunofluorescence and immunoperoxidase
 - v. Polymerase chain reaction (RT-PCR)
 - vi. Nucleic acid probes
4. **Serology:**
 - a. Competitive ELISA
 - b. Virus neutralization test (Prescribed test for international trade)
5. **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
Bluetongue**

There is no digestive system involvement, and the clinical signs and lesions are confined to the respiratory system and pericardium.

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,

**Contagious
ecthyma
(contagious
pustular
dermatitis, orf)**

including convulsions. There is no diarrhea. 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.

Control:

1. The tissue culture rinderpest vaccine at a dose of $10^{2.5}$ 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.
2. 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.

Eradication:

1. 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.
2. Control of PPR outbreaks relies on movement control (quarantine) combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations.

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

Public Health

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

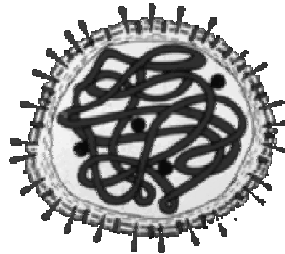
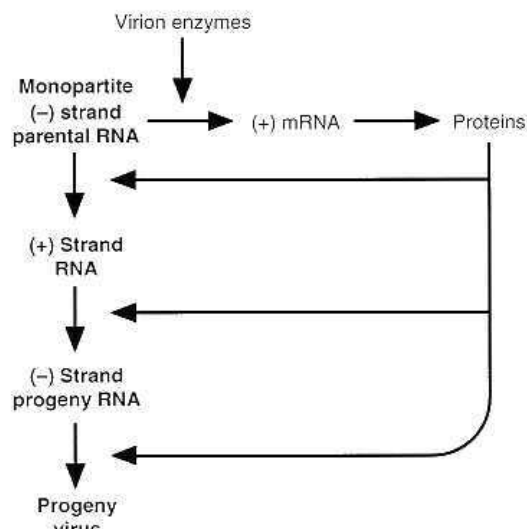
VMC 321 LECTURE # 6**CANINE DISTEMPER**

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

General characters:**1. 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.

2. Synonyms: Carre's disease, Hard pad disease**3. 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 haemagglutinin attachment glycoprotein (H) and the large-polymerase protein (L).**4. 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.**5. 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

6. Virus strains: Rock born strain (Vaccine strain used in India)**Cultivation:**

1. **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.
2. **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.
3. **Laboratory animals:** The distemper viruses are cultivated in live dogs or ferrets. Few strains also grown in suckling mouse.

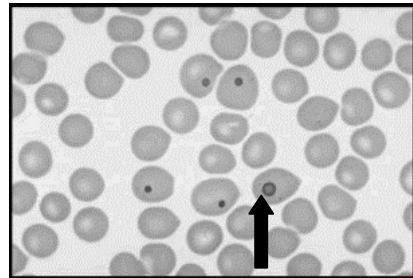
Pathogenesis:

1. **Hosts affected:** Domestic dogs and many other carnivores, including raccoons, skunks, and foxes are affected by CDV. The infection is more severe in puppies.
2. **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.
3. **Incubation period** – 5-7 days
4. **Symptoms & 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.

Diagnosis:

1. **Field diagnosis:** Is based on gastrointestinal, respiratory and nervous symptoms. Biphasic fever curves and low white blood cell level are indicative of distemper infection.
2. **Microscopical identification of inclusion bodies:** Distemper inclusion bodies" are actual clumps of virus that are visible under the microscope 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.
 - a. **Immunocytology:** To enhance the visibility 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 dyeing the inclusion body with glow-in-the-dark fluorescent color. The presence of inclusion bodies confirms distemper infection. The lack of detectable 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.
3. **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.
 - a. **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.
4. **Isolation and identification:** The virus are identified by AGPT, Antigen capture ELISA and PCR techniques.



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

Prevention and 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.

Public health: There is no human hazard associated with canine distemper virus.

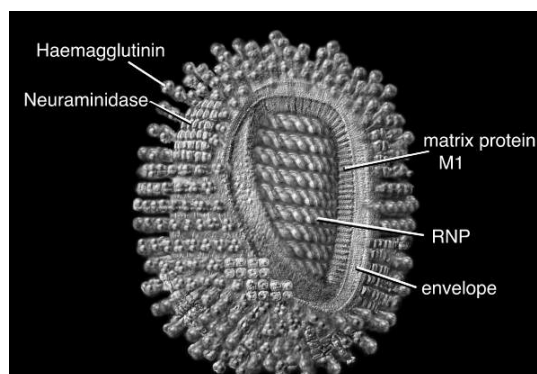
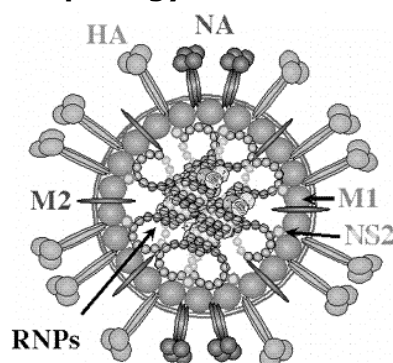
VMC 321 LECTURE # 7**FAMILY - ORTHOMYXOVIRIDAE****AVIAN INFLUENZA**

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 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.

General characters:**1. Classification:**

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

Members of the family Orthomyxoviridae are classified into Influenza virus A, B and C. The type designation (A, B, or C) is based upon the antigenic character of the matrix (M) protein of the virus envelope and the nucleoprotein (NP) within the virus particle. All influenza viruses affecting domestic animals (equine, swine, avian) belong to Type A, and Type A influenza virus is the most common type producing serious epidemics in humans. Types B and C do not affect domestic animals.

2. Synonyms: Fowl plague**3. Morphology:** Influenza virus particles are highly pleiomorphic (variable),

ical/ovoid, 80-120nm diameter, but many forms occur, including long filamentous particles (up to 2000nm long x 80-120nm 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**. 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.

4. OIE Listing: List A disease**5. Risk group (Animal Pathogen):** Group II**6. Reference Laboratories:****Dr D.J. Alexander**

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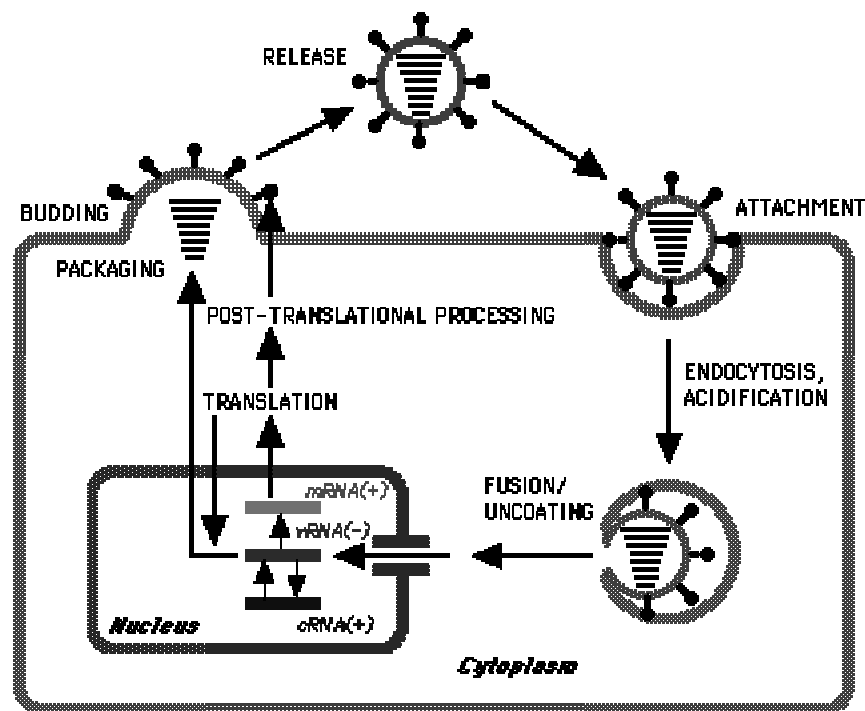
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7. **Resistance:** The particles are not very stable and are killed within few hours on exposure to room temperature and are also not resistant to drying and disinfectants.
8. **Replication:** Entry of avian influenza virus into the cell is facilitated 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.

a. Incomplete, 3' polyadenylated transcripts which are exported to the cytoplasm and serve as **mRNAs**

b. **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.



9. **Haemagglutination property:** Avian influenza virus causes agglutination of chicken erythrocytes.
10. **Viral subtypes:** 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	Example
H5	1. Nine different strains. 2. Produce highly pathogenic or low pathogenic infections 3. Human infection possible, sometimes causing severe illness and death	H5N1 (Hong Kong)
H7	1. Nine different strains 2. Produce highly pathogenic or low pathogenic infections. 3. Human infection is rare, but can occur among persons who have close contact with infected birds; symptoms may include conjunctivitis and/or upper respiratory symptom	H7N7 (Netherlands)
H9	1. Nine different strains 2. Produce only low pathogenic infection 3. Human infection possible	H9N2

Cultivation: The virus grows well in embryonated eggs by allantoic route (Nine to 11-day-old embryonated chicken eggs). The eggs used for isolation of AIV should be either from SPF birds or from SAN (Specific antibody negative birds). The eggs should be incubated at 35–37°C for 4–7 days. Avian influenza virus will usually kill embryos within 48-72 hours. Presence virus in allantoic fluid is confirmed by demonstration of HA activity or by AGID or by RT-PCR.

Pathogenesis:

- 1. 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.
- 2. 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.
- 3. Incubation period:** The incubation period is usually 3 to 7 days, depending upon the subtype of virus, the species, and age of the bird.

4. Morbidity and mortality: Morbidity and mortality rates are upto 100 percent within 2 to 12 days after the first signs of illness in HPAI.

5. Symptoms (HPAI):

Chickens (Layers)

- a. Marked depression with ruffled feathers, inappetence, excessive thirst, cessation of egg production, and watery diarrhea.
- b. 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.
- c. The last eggs laid, after the onset of illness may not have shells.
- d. The diarrhea begins as watery bright green and progresses to almost totally white.
- e. Edema of the head, if present, is often accompanied by edema of the neck.
- f. The conjunctivae are congested and swollen with occasional hemorrhage.
- g. The legs, between the hocks and feet, may have areas of diffuse hemorrhage.
- h. Respiratory signs can be a significant feature of the disease, depending on the extent of tracheal involvement and mucus accumulation.
- i. 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.



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.



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.

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.

6. Lesions:

- a. 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.
- b. 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 conjunctiva 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.

- c. 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.
- d. 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 mucosa may have hemorrhagic areas — especially in the lymphoid foci such as the cecal tonsils.
- e. The lesions in turkeys and domestic ducks are similar to those in chickens but may not be as marked.

Diagnosis:

1. **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.
2. **Isolation and identification:**
 - a. 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
 - b. Samples from - tracheal and cloacal swabs
 - c. The samples should be placed in isotonic phosphate buffered saline (PBS), pH 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 35-37C. The presence of virus in allantoic fluid are confirmed by
 - i. Detection of HA activity
 - ii. Agar gel immunodiffusion (AGID) test - by demonstrating the presence of the nucleocapsid or matrix antigens
 - iii. RT-PCR
 - iv. Antigen capture ELISA
 - d. RT-PCR can also be used to identify viral RNA directly in the clinical samples without passaging the materials in embryonated eggs.
3. **Serological tests:**
 - a. AGID test
 - b. HA / HI test
4. **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.

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.

Control and eradication:

1. Vaccination:

- a. Inactivated oil-emulsion vaccines are found to be effective in reducing mortality, preventing disease, or both, in chickens and turkeys.
- b. 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 H5 antigen

- c. A recombinant fowl pox virus vaccine containing the gene that codes for the production of the H5 antigen is also used. A recombinant insect virus containing the gene for either the H5 or H7 antigen is also used to control AI.

2. **Biosecurity procedures** including control of human traffic, quarantine of birds of unknown disease status into the flock and proper cleaning and disinfection of fomites
3. Free range rearing should not be done in areas where, waterfowls frequent

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 H5N1, H7N7, H9N2 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.

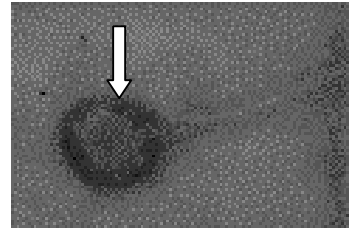
(Influenza viruses have eight separate gene segments. The segmented genome allows viruses from different species to mix and create a new influenza A virus if viruses from two different species infect the same person or animal. For example, if a pig were infected with a human influenza virus and an avian 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).

VMC 321 LECTURE # 8**FAMILY - ORTHOMYXOVIRIDAE
SWINE INFLUENZA**

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.

General characters:**1. Classification:**

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

2. Synonyms: Swine Flu, Flu, Influenza**3. 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 haemagglutinin (HA) and neuraminidase (NA) respectively. Variations observed in these two proteins are used to classify the virus species into many strains (subtypes). There are 13 H and 9 N types. Common strains of SIV belong to H1N1, H3N2 and H1N2.**4. 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.**5. OIE listing:** Listed but not under A or B**6. 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)**7. Haemagglutination property:** The virus causes agglutination of chicken and turkey RBCs.

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.

Pathogenesis:**1. Hosts affected:** Pig is the main host. The virus also affects avians and humans.**2. 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 occurring 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.

3. **Incubation period:** The incubation period is very short 1 to 3 days. In very severe epidemics it can be as short as 4 hours.
4. **Morbidity and mortality:** Morbidity – upto 100%; mortality – very minimal 1%.
5. **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.
6. **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



Diagnosis:

1. **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.
2. **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
 - a. 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).
 - b. Neuraminidase inhibition test
 - c. Fluorescent antibody test
 - d. Immunoperoxidase test
 - e. Antigen capture ELISA
 - f. Polymerase chain reaction

3. Serological tests:

- a. 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.
- b. Virus neutralization test: Not commonly performed
- c. ELISA: Not commonly performed

4. Differential diagnosis: The following diseases should be differentiated - Aujeszky's disease, atrophic rhinitis, enzootic (mycoplasmal) pneumonia and bacterial pneumonia due to *Pasteurella* or *Haemophilus* spp.

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

Control:

1. The best way to control swine influenza is to prevent the occurrence and spread of the disease.
2. 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.
3. Commercial vaccines are available in Europe and North America

Public health:

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.

VMC 321 LECTURE # 9**FAMILY - ORTHOMYXOVIRIDAE
EQUINE INFLUENZA**

Equine influenza is an acute, contagious respiratory disease caused by two distinct subtypes (H7N7 - formerly equi-1, and H3N8 - formerly equi-2) of influenza A viruses within the genus Influenzavirus A of the family Orthomyxoviridae. The infection in horses are characterised by pyrexia, and a harsh dry cough followed by a mucopurulent nasal discharge.

General characters:**1. Classification:**

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

2. Synonyms: Horse flu, Epizootic Cellulitis, Pinkeye, Stable Pneumonia, Shipping Fever**3. 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.**4. OIE listing:** List B disease**5. Risk group:** Risk Group II**6. Reference Laboratories:****Dr W. Eichhorn**

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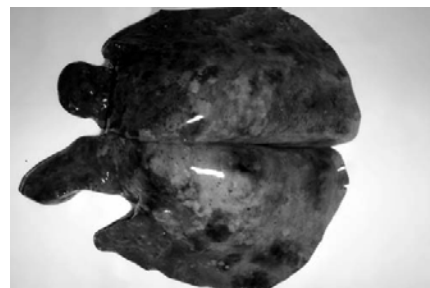
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7. Resistance: The particles are not very stable and are killed within few hours on exposure to room temperature and are also not resistant to drying and disinfectants**8. Replication:** The replication strategy of EIV is typical of other members of the family orthomyxoviridae**9. Haemagglutination property:** EIV agglutinates chicken and guinea pig RBCs.**10. Virus subtypes:** The H7N7 (Equine-1) and H3N8 (Equine-2 + new avian strain) subtypes have been isolated in horses

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.

Pathogenesis:

1. **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.
2. **Distribution:** The infection is worldwide except in Pacific regions.
3. **Transmission:** The disease is highly contagious in nature and can occur as epidemics. Infection spreads by direct contact, aerosol from coughing (up to 30m), wind (up to 8km) 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.
4. **Morbidity and mortality:** Morbidity very high (90-100%). However, mortality is almost nil.
5. **Incubation period:** The incubation period is very short ranging from 1-5 days.
6. **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 (38.9C) 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.
7. **Lesions:** The important lesions are bronchopneumonia, bronchitis, pharyngitis, pleuritis, guttural pouch infections and viral myocarditis. Bronchiolitis with abundant bilateral serous discharge is the most important post-mortem lesion.

**Diagnosis:**

1. **Field diagnosis:** Based on sudden occurrence with characteristic respiratory symptoms and high fever.
2. **Isolation and identification:**
 - a. Clinical materials: Nasopharyngeal swabs and nasal or tracheal washings are the ideal clinical materials for isolation of viruses.
 - b. Isolation systems: Embryonated eggs (allantoic or amniotic routes) and MDCK cell line.
 - c. Identification of antigen:

- i. Demonstration of HA activity using chicken or guinea pig RBCs.
- ii. Neuraminidase typing
- iii. Antigen capture ELISA using monoclonal antibody against nucleoprotein
- iv. RT-PCR

3. 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

- a. Haemagglutination inhibition test
- b. 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
- c. Complement fixation test: Not commonly used.

4. Differential diagnosis: The infection has to be differentiated from

- a. Equine rhinopneumonitis
- b. Equine viral arteritis
- c. Equine rhinovirus and adenovirus infection
- d. Pasteurellosis
- e. Strangles

Treatment:

Symptomatic treatment is given, as the disease usually subsides in 7-14 days on its own. Complete rest for 3-4 weeks (guideline 1 week of rest for every day of fever) and good ventilation are very important to prevent systemic disease. Antibiotics are given only if secondary bacterial infection is observed.

Control:

1. Vaccines: Influenza vaccines are used in competition horses in countries where the infection is reported. In free countries vaccination is prohibited except for sport horses participating to international races. Both live and inactivated equine influenza vaccines are available.

- a. Three doses of the killed-virus vaccine are administered 3-6 weeks apart starting at 6 months of age for younger horses. Revaccination should be done at intervals of 3 to 12 months depending on age and risk of exposure
- b. A modified live equine AE-2 influenza vaccine, when administered intranasally offers protection for 6 months.

2. Biosecurity measures: Since the infection is highly contagious the infected horses should be immediately separated and isolated.

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

VMC 321 LECTURE # 10**FAMILY - RHABDOVIRIDAE
RABIES**

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.

General characters:**1. Classification:**

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

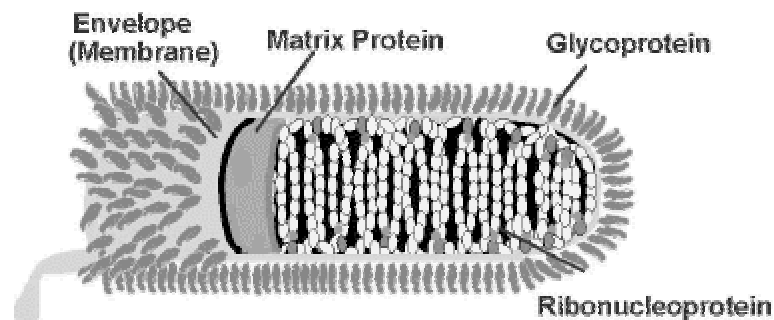
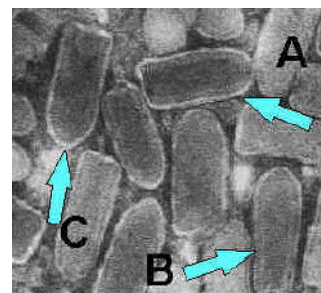
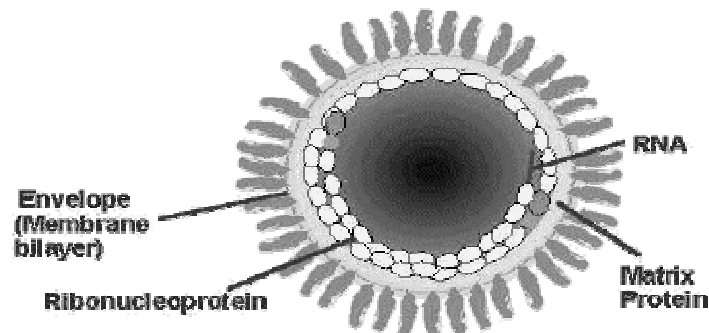
There are seven distinct genetic lineages under lyssa viruses. They are

- a. Classical rabies virus itself (RABV)
- b. Lagos bat virus (LBV)
- c. Mokola virus (MOKV)
- d. Duvenhage virus (DUUV)
- e. European bat lyssaviruses (EBLV) – 2 biotypes
- f. Australian bat lyssavirus (ABLV)

2. Synonyms: hydrophobia, mad dog disease**3. 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 in core (RNP)

along with nucleoprotein (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.

**Cross Sectional****4. OIE listing:** List A disease

5. Risk group: Risk Group III**6. Reference Laboratories:****Dr A. Wandeler**

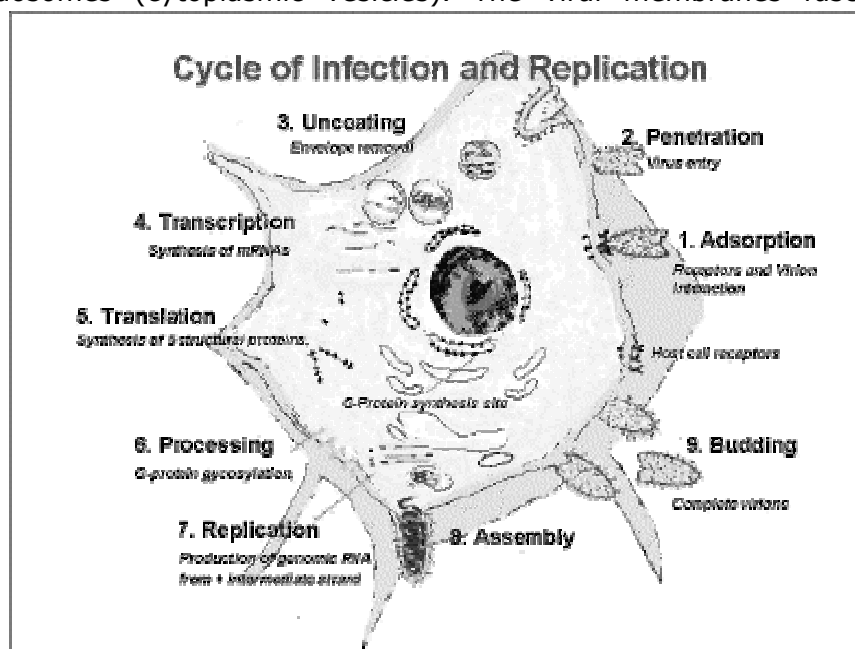
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7. Resistance: The virus is killed on exposure to 70C for 15 minutes. Virus can be preserved for more than one year in freezing temperatures. UV rays and disinfectants like phenol, chloroform destroys the virus rapidly.

8. 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.

9. Haemagglutination property: Virus does not haemagglutinate.

10. 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.

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.

Pathogenesis:

1. **Hosts affected:** All mammals are affected by rabies.
2. **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.
3. **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.
4. **Mortality:** Rabies is a fatal infection and once symptoms are exhibited animals will certainly die.
5. **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

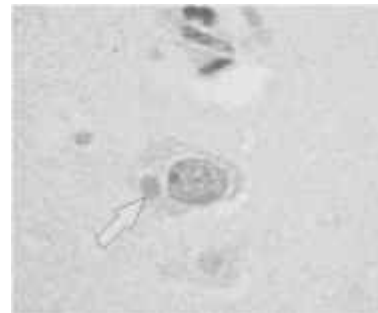
Furious form: This is referred as "mad-dog syndrome," although it is seen in all species. There is rarely any evidence of paralysis during this stage. The animal becomes irrational and, with the slightest provocation, may viciously and aggressively use its teeth, claws, horns, or hooves. The posture 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

Dumb form: This is first manifested by paralysis of the throat and masseter muscles, often with profuse salivation and inability to swallow. Drooping 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

- 6. 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.

Diagnosis:

1. **Field diagnosis:** Is based on characteristic symptoms of rabies coupled with presence of Negri bodies in the brain.
2. **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.
 - a. **Histological identification of characteristic lesions** – Demonstration of Negri bodies by Sellers method (for unfixed sections) or Mann's method (for fixed sections).
 - b. **Fluorescent antibody test** – This is the most widely used test for rabies diagnosis and is recommended by both WHO and OIE.
 - c. **Immunoperoxidase test**
 - d. **ELISA** – Referred as rapid rabies enzyme immunodiagnosis test (RREID)
 - e. **PCR and DNA sequencing**
 - f. **DNA probe**
 - g. **Electron microscopy**
 - h. **Monoclonal antibody based typing**
3. **Isolation and identification:** Is attempted FAT gives an uncertain result or when the FAT is negative in the case of known human exposure.
 - a. **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.
 - b. **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.
4. **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
 - a. **Virus neutralisation test** in cell culture: **fluorescent 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
 - b. **Rapid fluorescent focus inhibition test (RFFIT)** for determining rabies virus-neutralising antibody (accepted test for international trade)
 - c. **Virus neutralization in mice**
 - d. **ELISA** - Indirect ELISA is used to determine the level of rabies antibodies in individual dog and cat serum samples following vaccination

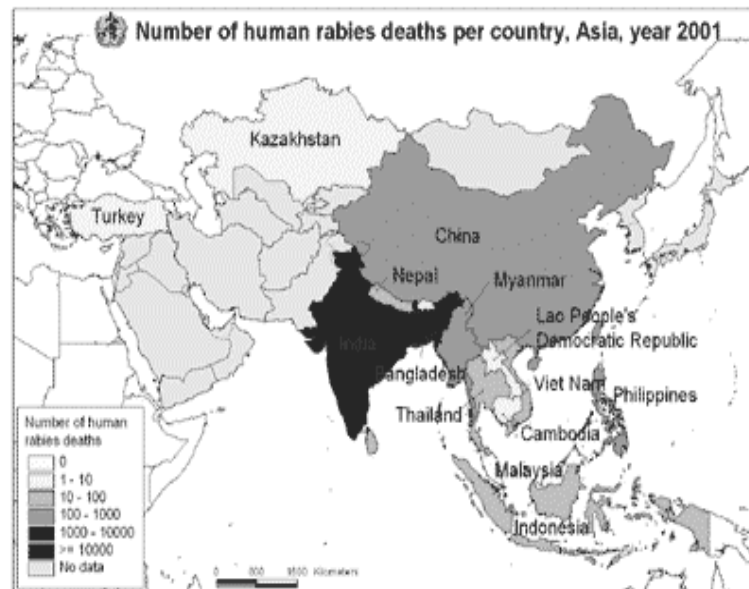
Control: Comprehensive guidelines for control in dogs have been prepared by the World Health Organization and include the following:

1. Notification of suspected cases, and destruction of dogs with clinical signs and dogs bitten by a suspected rabid animal;
2. Reduction of contact rates between susceptible dogs by leash laws, dog movement control, and quarantine;
3. Mass immunization of dogs by campaigns and by continuing vaccination of young dogs;
4. Stray dog control and destruction of unvaccinated dogs with low levels of dependency on, or restriction by, man
5. Dog registration
6. **Vaccines:**

- a. **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.
- b. **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.
- c. **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.
- d. **Duck Embryo Vaccine (Peck)** - this vaccine strain is grown in embryonated duck eggs and is inactivated with B-propiolactone. 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.
- e. **Live vaccines – (Hoegyes):** Live fixed viruses are given at a very low level (below the infective dose).
- f. **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.
- g. **Human Diploid Cell Vaccine (HDCV)** - HDCV was introduced in 1978. It is 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.
- h. Efforts are being made to use other inexpensive cell culture systems such as VERO cells.

Public health: Rabies virus causes highly fatal disease in human beings. 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 may 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 Cardiovascular 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).

1. **Postexposure 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.
 - i. **Wound treatment** - surgical debridement should be carried out. The wound should not be sutured up.
 - ii. **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.
 - iii. **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.
2. **Preexposure 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.

VMC 321 LECTURE # 11**FAMILY - RHABDOVIRIDAE
BOVINE EPHEMERAL FEVER**

Bovine ephemeral fever (BEF or Bovine Epizootic Fever) is a non-contagious arthropod-borne disease (arbovirus from the Rhabdoviridae family) of cattle, characterised by an acute fever of short duration, with high morbidity and low mortality. Typically, affected animals are only sick for a few days, and they recover and hence the infection is also known as **three Day Sicknes**.

General characters**1. Classification:**

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

2. Synonyms: Three-day sickness, Bovine epizootic fever, Three-day stiff sickness, Dragon boat disease**3. 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.**4. OIE listing:** No listing; SPC List D disease**5. Risk group:** Group I**6. Resistance:** Typical of other rhabdoviruses. The virus is rapidly inactivated at pH levels below 5**7. Replication:** Typical of other rhabdoviruses**8. HA property:** The virus has no HA property**9. Virus strains:** This virus is antigenically related to at least three other viruses nonpathogenic 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.

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 immunofluorescence 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.

Pathogenesis:**1. Hosts affected:** Cattle and buffaloes are the main host.**2. 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**3. 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.**4. Incubation period:** The incubation period is between 2 and 4 days, and 9 days in rare cases.

5. **Morbidity and mortality:** Morbidity and mortality are highly variable. Morbidity is very high and mortality is very low.
6. **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.
7. **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.

Diagnosis:

1. **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.
2. **Isolation and identification:** Blood is the ideal material and should be taken during the period of fever and another sample 1-2 weeks later. Samples should be taken from animals in various stages of the disease to facilitate a rapid laboratory confirmation
 - a. Part of the first sample of blood is allowed to clot, and another portion is mixed with anticoagulant. From the uncoagulated blood, a smear is made on a glass slide and allowed to dry in air. The balance is used for virus isolation. When blood taken during illness is allowed to clot, it usually fails to contract on standing, even over several days. It may be streaked with fibrin.
 - b. Cattle are used for isolation of the virus. Blood from infected cattle is used as inoculum for infecting cattle. Exhibition of characteristic clinical symptoms are used to identify BEFV.
 - c. PCR
3. **Serology**
 - a. Virus neutralization test is commonly done to identify antibodies against BEFV.
 - b. ELISA is also done
4. **Differential diagnosis:** BEF confuse with infections like early Rift Valley fever, FMD, heartwater, bluetongue, botulism, babesiosis and blackleg.

Treatment: Ephemeral fever is one of the rare virus diseases for which treatment is effective. Since the infection is inflammatory in nature, antiinflammatory 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).

Control:

- 1. 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
- 2. Prevention:**
 - a. Vaccination in endaeamic areas
 - b. Vector control - Useful steps are to place the cattle in an insect-proof area, spray with insecticides, or suppress insects in the local environment

Public health: There is no evidence of human infection.

VMC 321 LECTURE # 12**FAMILY - CORONAVIRIDAE****AVIAN INFECTIOUS BRONCHITIS**

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

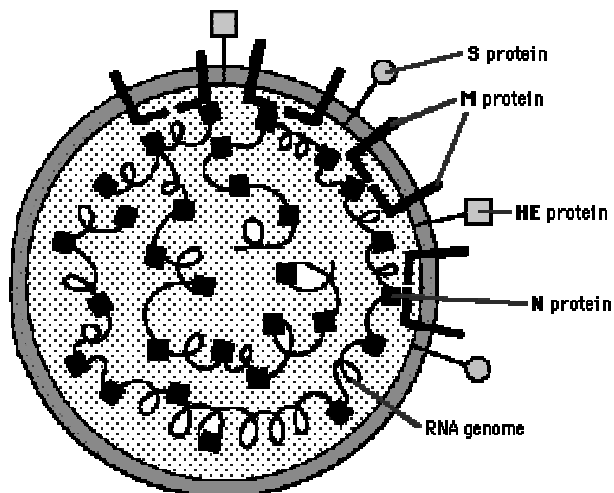
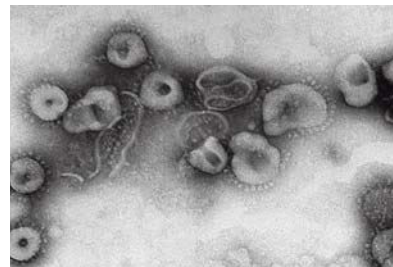
General characters:**1. Classification:**

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

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. 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.

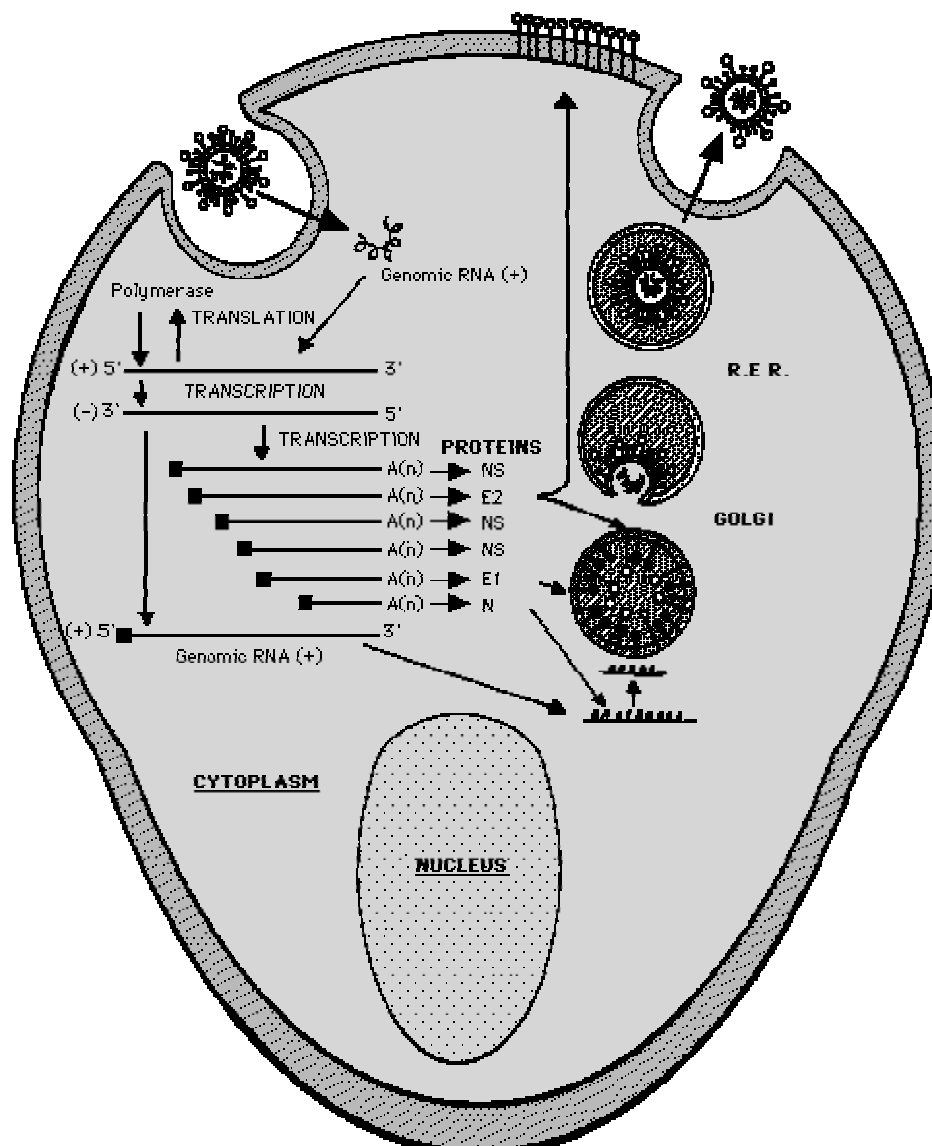
2. Synonyms:

- 3. 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.



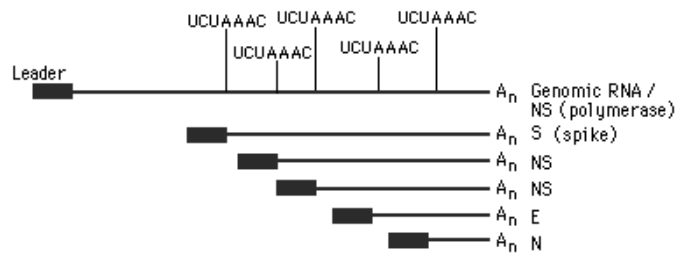
- a. Surface glycoprotein (or spike, S), which S protein is responsible for attachment to cells, hemagglutination and membrane fusion.

- b. Integral membrane protein (M) which spans the virus envelope three times
 - c. Nucleocapsid protein (N)
 - d. Hemagglutinine-esterase protein (HE), which forms short surface projections, and have receptor binding, hemagglutination and receptor destroying activities
 - e. Envelope protein (E), small, envelope-associated protein
 - f. unlike (-)sense RNA viruses polymerase enzymes are not present in the nucleocapsid
4. **OIE Listing:** List **B** infection
 5. **Risk group:** Group I pathogen. No risk to human beings.
 6. **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
 7. **Replication:** The replication of corona viruses is slow compared to other enveloped viruses and the viruses replicate in the cytoplasm.



- a. The (+) sense genome is first translated to produce a viral polymerase (RNA dep RNA Polymerase), which then produces a full-length (-) sense strand.

- b. 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.

- c. Each mRNA produced is monocistronic, the genes at the 5' end being translated from the longest mRNA and so on.
- d. 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
- e. **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
8. **HA property:** Many IBV cause agglutination of chicken RBC after treatment with an enzyme neuraminidase (*Previously it was thought as Phospholipase C*).
9. **Virus strains:** 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, there 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.

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.

Pathogenesis:

1. **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.
2. **Distribution:** The infection is worldwide in distribution.
3. **Transmission:** The affected birds shed the virus in respiratory secretion and faeces. The virus spreads rapidly among chickens in a flock through

inhalation of virus droplets (coughing or sneezing) produced by infected chickens. Infection is also transmitted by aerosols, contaminated feed and water, contact with animals or material. Movement of live birds is considered as a potential source for the introduction of IBV. Affected birds recover within fourteen days. However, a small number of chicks may become latently infected with erratic shedding of virus for a prolonged period of time via both faeces and aerosol. There is no carrier state and vertical transmission. No vectors are also involved in the spread of infection.

4. **Incubation period:** The incubation period is 18 to 36 hours
5. **Morbidity and mortality:** Morbidity goes upto 100% and mortality is variable (upto 50%).
6. **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* complicate the disease scenario and lead increased condemnation of birds.
7. **Lesions:** The infection is exhibited as respiratory, renal and reproductive forms.
 - a. **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.
 - b. **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%.
 - c. **Reproductive form** characterised by damage to ovaries and shortened reproductive tract

Diagnosis

1. **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.
2. **Isolation and identification:**
 - a. **Clinical materials:**
 - i. Live birds: Tracheal mucus from young chicks
 - ii. Dead birds: Tissue samples from the trachea, kidney, oviduct and caecal tonsils in sterile transport media with antibiotics and dry swabs from the respiratory tract or cloaca
 - b. **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.

c. **Antigen identification system:**

- i. Fluorescent antibody test (FAT)
- ii. Immunohistochemistry methods like IPT
- iii. Antigen competitive ELISA
- iv. RT-PCR for S gene
- v. CDNA probe

d. **Serotype identification:** Since IBV are antigenically distinct identification of correct serotype is essential. Serotype identification is carried out by

- i. Virus neutralization test in eggs, TOCs
- ii. HI test
- iii. Monoclonal antibody (serotype) based ELISA
- iv. Monoclonal antibody (serotype) based immunohistochemistry
- v. RT-PCR using serotype specific primers

3. Serological tests: 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

- a. Virus neutralization test
- b. HI test
- c. AGID
- d. ELISA

4. Differential diagnosis: IB should be differentiated from Newcastle disease, Infectious Laryngeal Tracheitis and Infectious Coryza

Treatment: Not effective and only supportive treatment is provided.

Control

- 1. 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.
- 2.** Strict biosecurity measures and good husbandry practices will minimize the spread of IBV.
- 3.** During outbreaks, the virus should be serotyped and vaccine should be altered.
- 4.** In areas where there is no IB, use of live vaccine should be minimized.

Public health: No human infection is reported.

VMC 321 LECTURE # 13**FAMILY - CORONAVIRIDAE
TRANSMISSIBLE GASTROENTERITIS**

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 respiratory 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

General characters:**1. 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

2. Synonyms:

3. 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

4. OIE Listing: List **B** infection

5. Risk group: Risk group I

6. Reference laboratory:

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7. Resistance: The viruses are not very stable, highly 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

8. Replication: Typical of members of the family Coronaviridae

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 TGEV, Hence, they are commonly used to cultivate TGEV in laboratories.

Pathogenesis:

1. **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.
2. **Distribution:** TGE is present in Europe, Americas (North, South and Central), Asia (including China, Japan and Korea), and parts of West Africa.
3. **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.
4. **Incubation period:** Incubation period is relatively short – just 18 hours to 3 days
5. **Morbidity and mortality:** Morbidity and mortality are nearly 100% in piglets
6. **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.
7. **Lesions:** 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.

Diagnosis:

1. **Field diagnosis:** Is based on occurrence of infection more severely in piglets in winter months with characteristic symptoms.
2. **Isolation and identification:**
 - a. 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.
 - b. 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).
 - i. The sample material is homogenised in cell culture medium or phosphate buffered saline (PBS), pH 7.2, containing antibiotics.

- ii. This homogenised sample is allowed to stand out off direct sunlight for 30 minutes at room temperature.
- iii. The suspension is then sonicated and clarified by low-speed centrifugation.
- iv. 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.
- v. 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.
- c. Antigen identification tests: Since CPEs are not produced immediately and require number of blind passages, following antigen identification tests are performed.
 - i. Immunofluorescence
 - ii. Reversed passive hemagglutination
 - iii. Enzyme-linked immunosorbent assays (ELISAs)
 - iv. Radioimmunoassay (RIA)
 - v. DNA probes
 - vi. Electron microscopy
 - vii. RT-PCR

3. Identification of antigen directly in tissue sections/clinical materials:

- a. **Fluorescent antibody test**: Used to identify viral antigens in sections of small intestine
- b. **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.
- c. **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.

4. Serological tests: 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

- a. Virus neutralization test
- b. Indirect ELISA
- c. Competitive ELISA
- d. Blocking ELISA using monoclonal antibodies also allows the differentiation of TGEV and PRCV

5. TEGV specific tests (test accepted for international trade): TGEV-specific tests are ELISAs that use an MAb that recognises TGEV but not PRCV and are the tests of choice for qualifying animals for export.

- a. Haemagglutinating encephalomyelitis (vomiting and wasting disease)
- b. Classical swine fever (Hog cholera)
- c. Porcine rotavirus infection
- d. Swine dysentery
- e. Colibacillosis,
- f. Arsenic poisoning

Treatment:

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

Control:

1. **Vaccines:** Vaccination of pregnant sows is being tried but commercial vaccines are not yet available.
2. To reduce risk of spread, animals from serologically negative herds alone should be introduced and the swill should always be sterilized.
3. 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.
4. 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.

VMC 321 LECTURE # 14**FAMILY - FLAVIVIRIDAE
CLASSICAL SWINE FEVER**

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.

General characters:**1. Classification**

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

The family Flaviviridae is classified into three genus – flavivirus, pestivirus and hepacivirus. Most of the members under the genus flavivirus are arthropod transmitted and cause very severe infection in human beings. 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 CSF and bovine viral diarrhoea (BVD)

2. Synonyms: English swine fever, hog cholera, *peste du porc*, *colera porcina*

3. 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.

4. OIE Listing: List A infection

5. Risk group: No infection to human beings

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7. 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 β -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.

- 8. 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.
- 9. HA property:** The virus do not agglutinate erythrocytes.
- 10. 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.

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.

Pathogenesis:

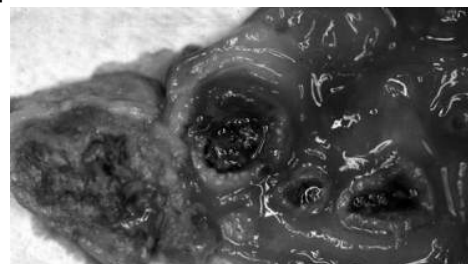
- 1. Hosts affected:** Pigs and wild boar are the only natural host for CSF.
- 2. Distribution:** The disease is present in Asia, Central and South America, and parts of Europe and Africa.
- 3. Transmission:** Introduction of infected pigs is the principal source of infection in CSF free herds. Blood, tissues, secretions and excretions from an infected animal contain viruses. Transmission occurs mostly by direct contact between animals through secretions, excretions, semen and blood, through oral route, through the conjunctiva, mucous membrane, skin abrasion, insemination, and percutaneous blood transfer (e.g., common needle, contaminated instruments). Infection also spreads by farm visitors, veterinarians and pig traders. Insufficiently cooked waste food fed to pigs are also a source of spread for the infection. Airborne transmission can occur in mechanically ventilated units when animal within close proximity to each other. During the warm season, CSF may be carried mechanically by insect vectors that are common to the farm environment. However, CSF doesn't replicate in invertebrate vectors. Transplacental infection also occurs in CSF. Congenitally infected piglets may shed the virus for months.
- 4. Incubation period:** The incubation period is usually 3 to 4 days but can range from 2 to 14 days.
- 5. Morbidity and mortality:** In acute CSF, the morbidity and mortality are high and go upto 100%.
- 6. Symptoms:** The infection occurs as acute, subacute, chronic or congenital forms. The infection also appears in a milder form in sows.
 - a. Peracute infection:** Observed in piglets, where young pigs may be found dead without any prior sign of illness.
 - b. 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. Severe 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.

- c. **Chronic infection:** 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.
- d. **Congenital infection:** 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.

7. Lesions:

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



and are often complicated by secondary bacterial infections

- ii. Button ulcers are an expression of such a secondary bacterial infection

d. Congenital infection:

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

Diagnosis:

1. **Field diagnosis:** Based on high morbidity and mortality, high fever, diarrhoea kidney and lymph node lesions will help in field diagnosis.
2. **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
3. **Direct identification of CSF from tissues:**
 - a. Immunological methods:
 - i. 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.
 - ii. 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,
 - iii. 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.

- iv. RT-PCR - This method is rapid and more sensitive than antigen-capture ELISAs or virus isolation.

- 4. 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.
- 5. 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
 - a. Fluorescent antibody virus neutralisation test (FAVN)
 - b. Neutralising peroxidase-linked assay (NPLA)
 - c. ELISA
- 6. Differential diagnosis:** CSF should be differentiated from following infections;
 - a. African swine fever (indistinguishable clinico-pathologically. It is essential to send samples for laboratory examination)
 - b. Infection with bovine viral diarrhoea virus
 - c. Salmonellosis
 - d. Erysipelas
 - e. Acute pasteurellosis
 - f. Other viral encephalomyelitis
 - g. Streptococcosis
 - h. Leptospirosis
 - i. Salt poisoning

Treatment: Not effective. Affected pigs must be slaughtered and the carcasses buried or incinerated

Control:

- 1. 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.
- 2. 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.

VMC 321 LECTURE # 15**FAMILY - FLAVIVIRIDAE****BOVINE VIRAL DIARRHOEA / MUCOSAL DISEASE**

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.

General characters:**1. Classification:**

Baltimore group	-	Group IV – Positive sense RNA viruses
Order	-	Nidovirales
Family	-	Flaviviridae
Genus	-	Pestivirus
Species	-	Bovine viral diarrhoea virus (BVDV)

2. Synonyms:

- 3. Morphology:** Is typical of other pestiviruses. 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 E_{ns}, E1, and E2. 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.

- 4. OIE Listing:** Listed infection but not under List A or B

- 5. Risk group:** No infection to human beings

6. Reference laboratories:**Dr D. Deregt**

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- 7. Resistance:** As other pestiviruses. They are mildly thermo tolerant. They can survive under wide range of pH. They are sensitive to lipid soluble detergents and common disinfectants

- 8. Replication:** Replication of BVDV begins with receptor-mediated endocytosis into a cell. The E2 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.

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

- 10. Antigenicity and Strains:** BVDV is closely related to classical swine fever and ovine border disease viruses. BVDV occurs in two forms: noncytopathogenic 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 noncytopathogenic 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 E^{RNS} major glycoproteins, or by genetic analysis.

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 be BVDV and antibodies against BVDV. The presence of virus in the cell culture is confirmed by immunoperoxidase technique, ELISA and nucleic acid identification methods.

Pathogenesis:

1. **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 mo of age. Colostral antibody appears to protect most calves for 3-6 mo after birth.
2. **Distribution:** The infection is worldwide in distribution.
3. **Transmission:** The natural reservoir for BVDV is persistently infected cattle. Noncytopathic viruses cause the majority of acute BVDV infections.
 - a. **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.
 - b. **Intrauterine 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

- i. 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.
- ii. 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.
- iii. 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.
- iv. 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).

4. Incubation period: Highly variable (7-14 days).

5. Morbidity and mortality: Variable (High morbidity and low mortality)

6. Symptoms: There are several clinical manifestations: BVD, acute and chronic mucosal disease, unthrifty persistently viremic calves, calves with congenital defects, and abortions.

a. **Bovine viral diarrhoea:** It is most often a subclinical to mild infection in immunocompetent but naïve cattle. Occasionally, in naïve animals that are infected by virulent strains of BVD, death can occur. Biphasic fever ($\sim 104^{\circ}\text{F}$ [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 ($\sim 107^{\circ}\text{F}$ [$41\text{-}42^{\circ}\text{C}$]), oral ulcerations, eruptive lesions of the coronary band and interdigital cleft, diarrhoea, dehydration, leukopenia, and thrombocytopenia.

b. **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.

- c. **Unthrifty persistently viraemic calves:** 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.
- d. **Congenital defects in calves:** 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.
- e. **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.

7. Lesions:

- a. **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.
- b. **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.

Diagnosis:

- 1. **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.
- 2. **Isolation and identification (*Accepted for International trade*):**
 - a. **Clinical materials:** Whole blood, swabs from mucosal or nasal surfaces, semen and lymphnodes
 - b. **Isolation systems:** Bovine monolayer cell cultures from kidney, lung, testis or turbinate
 - c. **Antigen identification:**
 - i. Microplate immunoperoxidase method
 - ii. Double antibody sandwich ELISA
 - iii. RT-PCR
- 3. **Direct identification of BVDV from tissue sections**
 - a. **Clinical materials:** Tissues from lymph nodes, thyroid gland, skin, brain, abomasum and placenta are ideal for direct identification of BVDV.
 - b. **Techniques**
 - i. Immunoperoxidase method using monoclonal antibodies
 - ii. RT-PCR
 - iii. *in situ* hybridisation with enzyme-linked riboprobes
- 4. **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
 - a. Virus neutralization test
 - b. ELISA - Both indirect and blocking types of test can be used.

- 5. 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 nonvaccinated calves at 6 months of age also are useful.

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

Control and eradication:

1. **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.
2. **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.

Public Health: No human infection is reported.

VMC 321 LECTURE # 16**FAMILY - TOGAVIRIDAE****EQUINE ENCEPHALOMYELITIS (EASTERN, WESTERN AND VENEZUELAN)**

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*).

General characters**1. 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)

2. Synonyms:

3. 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.

4. OIE Listing: All three infections EEE, WEE and VEE are listed as List B infections.

5. Risk group: All three viruses can affect human beings and hence classified under Risk Group III.

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7. Resistance:**8. 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



Arbovirus genomes

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.



Togavirus replication

9. HA Property: These viruses agglutinate RBCs from geese. Male geese are preferred over female.

10.Virus strains:

Species	Strains
EEEV	Strain VA33 Ten Broeck
WEEV	
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

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.

- a. 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.
- b. 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.
- c. 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.

Pathogenesis

1. **Hosts affected:** . These viruses cycle between birds and mosquitoes. Horses and humans are dead-end hosts.
2. **Distribution:** Canada, States east of Mississippi river in US, Caribbean Islands, Mexico, and Central and South America.
3. **Transmission:**
 - a. **EEE:** 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.
 - b. **WEE:** 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.
 - c. **VEE:** 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

4. **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.

5. **Morbidity and mortality:**

- a. **EEE** – Both are highly variable and mortality is between 50-90%
- b. **WEE** – Morbidity highly variable and mortality upto 30%
- c. **VEE** - Morbidity rates vary from 50 to 100 percent and mortality rates vary from 50 to 90 percent

6. **Symptoms:**

- a. **EEE & WEE:** 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.



- b. **VEE:** VEE virus infection may be expressed as (a) subclinical with no signs; (b) moderate and characterized primarily by anorexia, high fever, and depression; (c) severe, but nonfatal, and characterized by anorexia, high fever, stupor, weakness, staggering, blindness, and, occasionally, permanent neurologic sequelae; or (d) fatal, with the same clinical signs. In general, two forms of the disease exist: (a) the fulminating form in which signs of generalized, acute, febrile disease predominate, and (b) 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.

7. Lesions:

- a. **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 neuropil.
- b. **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.

Diagnosis:

1. **Field diagnosis:** Very difficult. 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
2. **Isolation and identification:**
 - a. **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.
 - b. **Isolation systems:** Newborn mice, embryonated eggs and cell lines like Vero, BHK21, RK13.
 - c. **Antigen identification:**
 - i. Complement fixation test
 - ii. RT-PCR
 - iii. Antigen capture ELISA
 - iv. Immunofluorescence
3. **Identification of viral nucleic acid in mosquitoes by RT-PCR**
4. **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;
 - a. Complement fixation test
 - b. Haemagglutination inhibition test using male geese RBCs
 - c. ELISA
 - d. 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.

Treatment: Not very effective and only supportive treatment is given

Control:

1. **Vaccines:** 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 Americas

2. 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.

- a. Restriction of horse movement between the epizootic zone and noninfected areas is important to control the spread.
- b. Mosquito control measures such as aerial spraying with ultralow volumes of insecticides

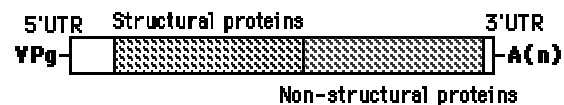
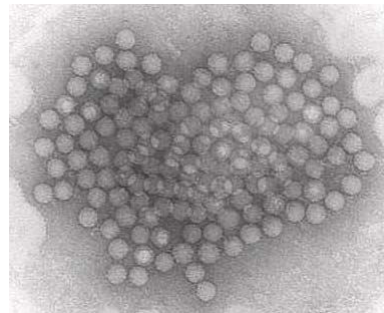
Public health: EEE, WEE and VEE can cause human infections. EEE virus causes severe disease in humans with a mortality rate of 30–70% and a high frequency of permanent sequelae in patients who survive. WEE is usually mild in adult humans, but can be a severe disease in children. Human infections occur 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.

VMC 321 LECTURE # 17**FAMILY - PICORNAVIRIDAE
FOOT AND MOUTH DISEASE**

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.

General characters**1. 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

2. Synonyms: Afta epizootica, Bek-en-klouseer, Fiebra aftosa, Fievre aphteuse, Maul-und-Klauenseuche, Komari (vernacular), Kal voi noi (vernacular)**3. 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.**4. OIE Listing:** List A infection.**5. Risk group:** The virus can affect human beings. Since the infection is uncommon, FMD is not considered as a public health problem.**6. Reference Laboratories:****Dr D. Paton**

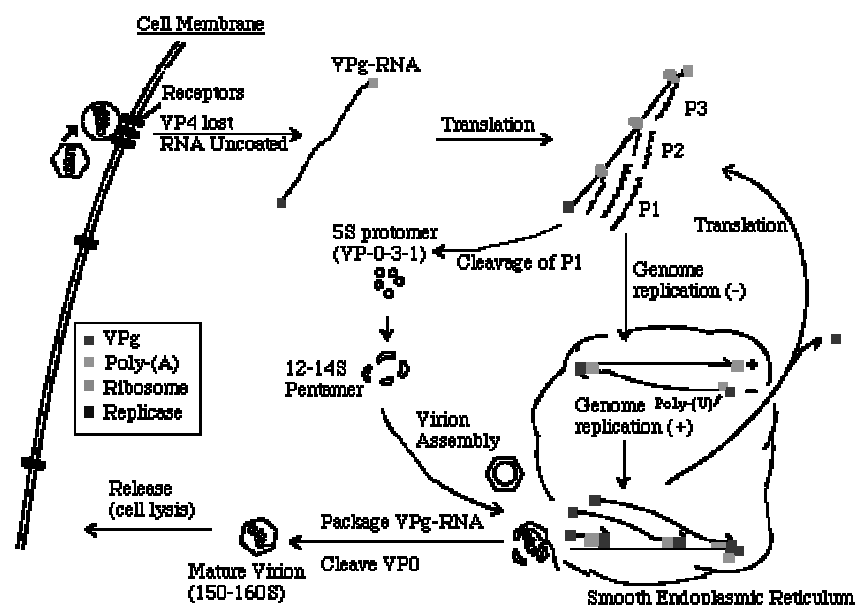
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- 7. Resistance:** Under *in vitro* conditions virions relatively stable. The FMD virus is pH sensitive and is inactivated at pH below 6.5 or above 11. In milk and milk products, the virion can survive at 70° C for 15 seconds and pH 4.6. The virus 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, hypochlorite and phenol, especially in the presence of organic matter
- 8. 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 (<i>Positive sense RNA hence translated immediately</i>)
3-4h	Permeabilization of plasma membrane
4-6h	Virus assembly in cytoplasm
6-10h	Lysis of cell and release of virus particles



9. HA Property: The virus has not HA property.

10. Virus strains: 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)

Cultivation:

- 1. 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.
- 2. Laboratory animals:** Unweaned mice are used for cultivation FMDV. Mice of 2-7 days old are commonly used

Pathogenesis:

- 1. Hosts affected:** Cloven-footed domestic and wild animals are primarily affected. Bovines (cattle, zebu, 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.
- 2. 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.
- 3. Transmission:** FMDV spread by following methods
 - a. Direct or indirect contact with infected animals.
 - b. Through aerosol from infected animals
 - c. Feeding contaminated garbage (meat, milk, blood, glands, bones, cheese, etc.)
 - d. Contact with contaminated objects (hands, footwear, clothing).
 - e. Artificial insemination.

- f. Contaminated biological such as hormones
- g. 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*)
- h. 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 particularly recovered cattle can be carriers of FMDV. Most ruminant species harbour the virus in their pharyngeal tissues for a long period.

4. 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.

5. 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.

6. Symptoms:

- a. **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 panthers')
- b. **Swine:** 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, heels, 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.
- c. **Sheep and Goats:** 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.

7. **Lesions:** The main lesions are the vesicles in buccal and foot area. Another important lesion is the tiger heart (*yellowish streaking due to degeneration and necrosis*)

- a. **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".

- b. **Swine:** 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").
- c. **Sheep:** 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").

Diagnosis

1. **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.
2. **Isolation and identification:** The following are preferred clinical materials.
 - a. Vesicular fluid
 - b. epithelium covering a vesicle
 - c. Flaps of epithelial tissue still attached
 - d. 5 ml of blood with anticoagulant during viraemic phase
 - e. Esophageal—pharyngeal (OP) fluid from convalescent cattle, sheep, or goats.
 - f. Serum
 - g. 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

3. Immunological methods of identification of virus

- a. **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.
- b. **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.

4. Nucleic acid recognition methods:

- a. **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.
- b. ***in situ* hybridisation:** This technique is used to identify viral RNA in tissue samples.

5. Serological tests: 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.

- a. **Virus neutralization test:** The VN test is serotype specific, requires cell culture facilities and takes 2–3 days to provide results.
- b. **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.
 - i. Solid-phase competitive enzyme-linked immunosorbent assay (Test for International trade)
 - ii. Liquid-phase blocking enzyme-linked immunosorbent assay
 - iii. Indirect ELISA using recombinant non-structural proteins
 - iv. Enzyme linked immunosorbant blot assay

6. Differential diagnosis: FMD should be differentiated from vesicular stomatitis, swine vesicular disease, vesicular exanthema of swine, foot rot, and chemical and thermal burns.

- a. 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.
- b. In sheep, lesions caused by bluetongue, contagious ecthyma, and lip and leg ulceration can be similar to the later lesions of FMD.

Treatment: There is no effective treatment and only supportive treatment is given.

Control and eradication:

1. Vaccines: Different vaccines have been used to control FMD for a long time.

- a. **Frenkel method** of vaccine production is a preferred method. Normal tongue epithelium was removed, minced, placed in a nutrient broth, and inoculated with FMDV. After replication of FMDV, the virus was inactivated with formalin, and aluminum hydroxide was added as an adjuvant.
- b. At present instead of tongue epithelium BHK21 cell line is used. These vaccines are prepared using binary-ethyleneimine (BEI) as inactivating agent and aluminum hydroxide-saponin or oil as an adjuvant. According to prevalence of serotypes in a geographical area vaccine containing corresponding serotype(s) is used. Aluminum hydroxide vaccine offers immunity for a duration of 4-6 months after two initial vaccinations, 1-month apart and double oil emulsion vaccine offers protection for 1 year.
- c. Molecular-engineered vaccines are not as effective or as economical as the cell culture vaccines
- d. When vaccinating animals, it is important that the vaccine contain the same subtype of virus as is in the area. This necessitates frequent checking of the serotype and subtype during an outbreak

because FMD virus frequently changes during natural passage through various species

- e. Further Vaccinated animals Vaccinated animals that are not completely protected can be a source of infection. The virus may replicate and be shed, but the animals may not show any clinical sign of infection.
2. To eradicate the disease a stamping out policy can be best applied. This involves quarantine, movement restrictions and slaughter and disposal of all affected and in-contact livestock on affected premises followed by cleaning and disinfection.
 3. The following are the essential features of a control and eradication program:
 - a. Prevention of movement of animals and animal products in the area affected.
 - b. Slaughter of infected animals and known contact animals
 - c. Destroy carcasses
 - d. Disinfect vehicles leaving the infected area.
 - e. Perform vaccination.
 4. 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
 - a. EUFMD – European Union FMD control programme
 - b. SEAFMD – South East Asia FMD control programme

VMC 321 LECTURE # 18**FAMILY - PICORNAVIRIDAE****DUCK VIRAL HEPATITIS**

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.

1. 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.
2. 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
3. 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.

General characters:**1. Classification:**For DHV I

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

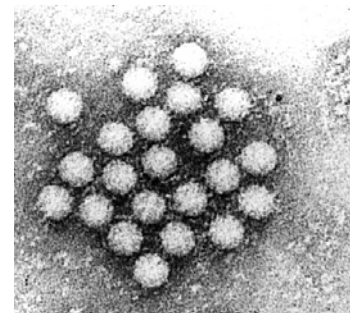
For DHV II

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

2. Synonyms:**3. Morphology**

DHV I (Enterovirus of Picornaviridae) – 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.

**4. OIE Listing:** List B infection**5. Risk group:** No known infection to human beings

- 6. Resistance:** For DHV I as described for FMD
- 7. Replication:** For DHV I and DHV III as described for FMD. The full details of the replication cycle for Astroviruses are not known, but it is suspected that replication occurs primarily in the cytoplasm, with a possible nuclear step
- 8. HA property:** The virus has no HA property
- 9. Serotypes / strains:** Under DHV Type I a serological variant named DHV Type Ia has been identified

Cultivation: DHV I grows well in ducklings, embryonated eggs and in cell cultures.

1. 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.
2. 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.
3. 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.
4. DHV II (Astrovirus) are difficult to cultivate under laboratory conditions.
5. DHV III (Pirconavirus) can be cultivated in the laboratory. The virus will not grow in embryonated chicken eggs

Pathogenesis:

- 1. Hosts affected:** Ducks and geese (young birds) are more commonly affected. The infection is seen in ducklings.
- 2. 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.
- 3. 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.
- 4. Incubation period:** Incubation period is 18–48 hours.
- 5. Morbidity and mortality:** Morbidity is 100% and mortality is 80–95%.
- 6. 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%.

7. Lesions:

- a. **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.
- b. **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;
- c. **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

Diagnosis:

1. **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
2. **Isolation and identification**
 - a. **Clinical materials:** Liver is ideal clinical material for isolation of virus.
 - b. **Systems for isolation:** Ducklings, Duck or chicken embryonated eggs and primary cell culture like Duck embryo liver
 - c. **Changes / CPE / Lesions:** *As mentioned under cultivation.*
3. **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.
4. **Serological tests:** 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.
5. **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*).

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.

Control and eradication:**1. Vaccines:**

- a. 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.
- b. Inactivated vaccines against DHV I can also be used in primed ducks, which give high antibody titre

- c. 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.
- 2. **Control:** 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

VMC 321 LECTURE # 19**REOVIRIDAE
BLUETONGUE**

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, arthropod-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.

General characters**1. Classification**

Baltimore group – Group III double stranded RNA viruses

Family – Reoviridae

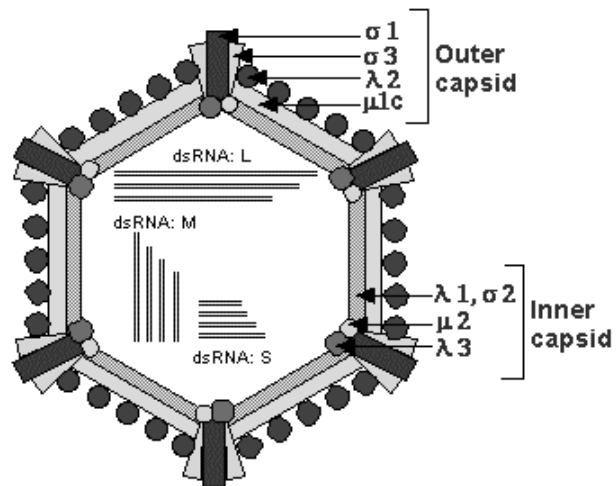
Genus – Orbivirus

Species – Bluetongue virus - BTV

2. 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 nucleotides and the largest segment L1 has got approximately 2800-3900 nucleotides. The viral RNA has methylated cap structure, but no poly A tail.

**3. Synonyms:** Sore muzzle, pseudo foot-and-mouth disease, muzzle disease**4. OIE listing:** List A infection**5. Risk group:** No known human infection are associated with BTV**6. Reference laboratories:****Dr G.H. Gerdes**

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7. Resistance: The viruses are resistant to lipid. However, the viruses are relatively acid-labile, and slow freezing at -10 to -20°C (14 - 40 F) is harmful to the virus. The viruses are inactivated by 50°C/3 hours and 60°C/15 minutes. β-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.

- 8. 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.

- 9. HA property:** BTV agglutinates mammalian RBCs.

- 10. Serotypes / strains:** There are 14 serogroups 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*)

Cultivation: The virus grows well in embryonated chicken eggs and cell culture systems.

1. In embryonated chickens the preferred route of inoculation is yolk sac. The eggs should be incubated in a humid chamber at 33.5°C. 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 immunofluorescence, immunoperoxidase or antigen capture ELISA tests.
2. BTV also grows 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.

Pathogenesis:

1. **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.
2. **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).
3. **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 of contaminated needles and equipment contaminated with blood of infected animals. Transmission by *Culicoides* 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.

4. **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.
5. **Mortality and morbidity:** 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.
6. **Symptoms:** Bluetongue is clinically manifested as two syndromes: 1) vascular affection of several organ systems particularly oral and buccal mucosa and 2) a reproductive syndrome. Sheep are commonly seen with clinical disease, but other domestic ruminants such as cattle and goats only rarely show clinical signs
 - a. **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 41.6-41.7° 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.
 - b. **Cattle:** Bluetongue virus infection in cattle usually does not cause any clinical sign of disease. Clinical signs consist of
 - i. mild hyperemia in the buccal cavity and around the coronary band;
 - ii. vesicular lesions, which lead to ulcerations, in the buccal mucosa;
 - iii. erect hair over the cervical and dorsal thoracic areas; and
 - iv. hyperesthesia.
 - v. 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.
 - vi. 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.
 - c. **Goats:** Bluetongue infection of goats is an inapparent infection similar to that described for cattle.

- 7. Lesions:** The lesions of BT in sheep vary greatly depending on (1) the strain of virus, (2) individual animal and breed susceptibility and (3) environmental (stress) factors. Prominent lesions include facial edema, edematous ears, and dry, crusty exudate over the nostrils.
- a. **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.
 - b. **Respiratory System:** Lesions include cyanosis and edema of the nasal mucosa and pharynx.
 - c. **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.
 - d. **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.
 - e. **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.
 - f. **Newborn lambs:** Newborn lambs with congenital BT have hydranencephaly or porencephaly. Abnormal development of cerebellum and spinal cord are the other lesions.

Diagnosis:

1. **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).
2. **Isolation and identification:**
 - a. **Clinical materials:** Sterile heparinized blood samples from animals with clinical signs or spleen or bone marrow, or both, from dead animals are the clinical materials of choice. Samples from aborted and congenitally infected newborn animals should include heparinized blood and, if possible, spleen, lung, brain, and serum. Specimens should be shipped refrigerated, not frozen. Freezing makes virus isolation difficult.
 - b. **Isolation systems:** Chicken embryos and cell cultures like baby hamster kidney (BHK)-21, African green monkey kidney (Vero) and *Aedes albopictus* (AA) cells.
 - c. **Immunological tests:**
 - i. **Serogrouping:** BTV are serogrouped on the basis of their reactivity with specific standard antisera that detect proteins, such as VP7 that are specific within each serogroup. Monoclonal antibodies specific for VP7 are used in the assay. The immunological assays that are performed using monoclonal antibodies against VP7 protein are immunofluorescence, antigen capture ELISA and immunospot test (dot ELISA).

- ii. **Serotyping:** Serotyping of BTV is carried out using serum specific for each of 24 serotypes. Virus neutralization assays are performed to serotype BTV. Four different types neutralization assays are performed in BHK21, Vero and L929 cells. These tests are plaque reduction test, plaque inhibition test, microtitre neutralization and fluorescent inhibition test.
- d. **Nucleic acid detection methods:** Polymerase chain reaction (PCR) is an accepted test for international trade. Serotyping is also possible in PCR.
- 3. **Serological tests:** These tests are performed to identify antibodies in the serum. The tests that are performed include
 - a. **Complement fixation test**
 - b. **Agar gel immunodiffusion test:** It is an accepted test for international trade.
 - c. **Competitive ELISA:** It is also an accepted test for international trade.
- 4. **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

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.

Control and eradication:

1. 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.
2. Vaccination should be practiced only in endemic 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.

VMC 321 LECTURE # 20**REOVIRIDAE****AFRICAN HORSE SICKNESS**

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 *Equidae* 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.

General characters:**1. Classification:**

Baltimore group – Group III double stranded RNA viruses

Family – Reoviridae

Genus – Orbivirus

Species – African horse sickness virus - AHSV

- 2. 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.

- 3. Synonyms:** *Peste equina africana*, *Peste equine*, *perdesiekte*

- 4. OIE listing:** List A infection

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

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- 7. 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.
- 8. 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.

9. **HA property:** The virus has HA property
10. **Serotypes / strains:** 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.**

Cultivation:

1. **Cell culture:** AHSV can be cultivated in baby hamster kidney-21 (BHK-21), monkey stable (MS) and African green monkey kidney (Vero).
2. **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.
3. **Lab animals:** The virus can be cultivated in newborn mice and guinea pigs. In newborn mice the viruses are inoculated through intracerebral route.

Pathogenesis:

1. **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
2. **Distribution:** AHS 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.
3. **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.
4. **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.
5. **Incubation period:** Incubation period is usually 7-14 days, but may be as short as 2 days.

- 6. 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.

Horses

- a. 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, spasmodic cough and appearance of frothy and serofibrinous blood-tinged fluid from nostrils. The death of animals is due to anoxia.
- b. 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.
- c. 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 oedematous swellings of the cardiac form.
- d. **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

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

7. Lesions:

- a. 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.
- b. 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.
- c. In **mixed form** the lesions represent a combination of those found in the pulmonary and cardiac forms

Diagnosis:

- 1. 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.
- 2. Isolation and identification:**
 - a. **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.
 - b. **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).
 - c. **Lesions / CPE:** Not very characteristic and dependant on confirmation of antigen. In mice inoculation, in positive cases, animals develop nervous signs between 3 and 15 days post-inoculation.
 - d. **Identification of antigen:** The presence of antigen in isolation systems are confirmed by ELISA, virus neutralization test or by PCR.
- 3. Serological tests:** The following serological tests are used to identify antibodies against AHSV. They are
 - a. Indirect ELISA (Accepted for international trade) - VP7 protein is used as antigen for AHSV antibody determination. This test has high degree of sensitivity and specificity.
 - b. Complement fixation test (Accepted for international trade)
 - c. 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.
 - d. NS3 ELISA: This test distinguish between infected horses and horses vaccinated with the inactivated purified AHSV serotype 4 vaccine.
 - e. Haemagglutination inhibition test
 - f. Virus neutralization test: Done to serotype AHSV
- 4. Differential diagnosis:** Clinical signs associated with AHSV are clearly not pathognomonic. Hence, the infection has to be differentiated from
 - a. Viral
 - i. Equine infectious anemia (Retroviridae)
 - ii. Equine viral arteritis (Arteriviridae)
 - iii. Equine encephalosis (Reoviridae) (often occurs concurrently with AHSV; differentiable by absence of edema and lower mortality rates)
 - b. Bacterial
 - i. Anthrax (*Bacillus anthracis*)
 - c. Protozoan
 - i. Trypanosomiasis (*Trypanosoma evansi*)
 - ii. Piroplasmiasis (*Babesia caballi* and *Babesia equi*)
 - d. Other
 - i. Purpura haemorrhagica (lesions on necropsy tend to be more widely distributed in this condition than in AHS)

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.

Control and eradication:

- 1. 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. 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.
- b. 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.

2. Eradication: Aimed at following aspects

- a. Vaccination of susceptible animals
- b. Vector control (use of insecticides, repellents and protective screens)
- c. 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

Public health: 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.

VMC 321 LECTURE # 21**BIRNAVIRIDAE****INFECTIOUS BURSAL DISEASE**

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.

General characters**1. Classification:**

Baltimore group – Group III double stranded RNA viruses

Family – Birnaviridae (*Birnavirus means bisegmented dsRNA virus*)

Genus – Avibirnavirus

Species – Infectious bursal disease virus – IBDV

2. 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

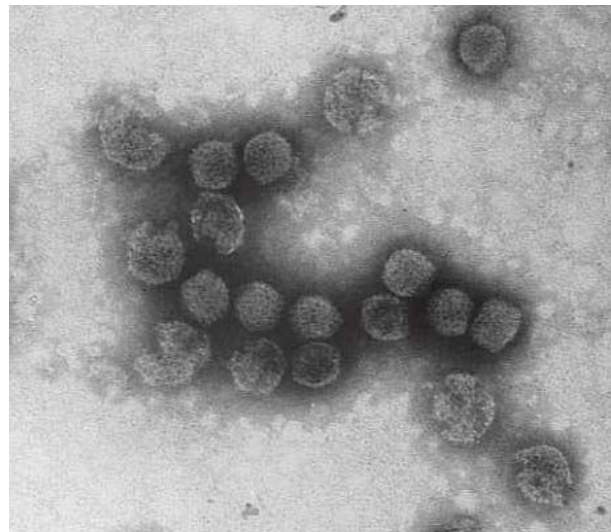
VP5. VP2 and VP3 are capsid proteins and VP4 and VP5 are non-structural

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

**3. Synonyms:** Gumboro disease**4. OIE listing:** List B infection**5. Risk group:** No known human infection

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7. **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.)
8. **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.
9. **HA property:** The virus has no HA property
10. **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.

Cultivation:

The virus is easily cultivable in chickens, ECE and chicken embryo fibroblasts.

1. **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.
2. **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.
3. **Cell culture:** Chicken embryo fibroblasts are normally used for cultivation: The CPE is characterised by small round refractive cells.

Pathogenesis:

- 1. 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.
- 2. Distribution:** Very virulent IBD have been reported in Europe, Americas and South East Asia.
- 3. 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.
- 4. 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.
- 5. Incubation period:** Incubation period is 2-3 days.
- 6. 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
- 7. Lesions:** The following lesions are observed in dead birds
 - a. Enlarged cloacal bursa which is swollen and haemorrhagic in birds dead of the disease and is atrophied in recovered birds
 - b. Dehydrated carcass
 - c. Dark skeletal muscles with haemorrhages (especially thigh and pectoral muscles)
 - d. Opaque thymus with thickened gelatinous capsule
 - e. Fatty and yellow or pink bone marrow
 - f. Swollen liver
 - g. Swollen kidneys
 - h. Intestines with increased mucus
 - i. 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)*

Diagnosis:

- 1. 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%.
- 2. Isolation and identification:**
 - a. Clinical materials:** Samples should be collected from sick or freshly dead birds.
 - i.* Fresh bursa and spleen for virus isolation.
 - ii.* Samples of bursa, spleen, intestines, caecal tonsil, liver and kidney should be collected in neutral buffered formalin for histopathology
 - iii.* Blood samples for serology.
 - b. Isolation systems:** Chickens, ECE and Chicken embryo fibroblast (*follow as given under cultivation*).
- 3. Direct identification of antigen from bursa:**
 - a. Agar gel immunodiffusion test
 - b. Immunofluorescence test
 - c. Antigen capture ELISA
 - d. RT-PCR
- 4. Strain differentiation:** IBDV serotypes are differentiated by cross neutralization virus neutralization test or by serological tests using serotype specific monoclonal antibodies.
- 5. 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
 - a. 'Variant' IBDVs induce little if any clinical signs and no mortality but marked bursal lesions,
 - b. 'Classical' IBDVs induce approximately 10–50% mortality with typical signs and lesions
 - c. 'Very virulent' IBDVs induce approximately 50–100% mortality with typical signs and lesions
- 6. Serological tests:**
 - a. **AGID:** The AGID test is the most useful of the serological tests for the detection of specific antibodies in serum
 - b. **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.
 - c. **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
 - d. **ELISA:** This is the preferred test for identification and quantification of antibodies against IBD.
- 7. Differential diagnosis:** IBD should be differentiated from Marek's disease Mycotoxicosis, Coccidiosis, Haemorrhagic syndrome, Avian adenovirus infection and Infectious bronchitis.

Treatment:

Treatment is not effective.

Control and eradication:

1. Vaccines: 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.

a. 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.

b. 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.

c. Recombinant vaccines: 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.

2. 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.

Public health

No human infection has been reported with IBDV.

VMC 321 LECTURE # 22**RETROVIRIDAE****EQUINE INFECTIOUS ANAEMIA**

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.

General characters**1. Classification:**

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

2. Synonyms: Swamp fever**3. 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

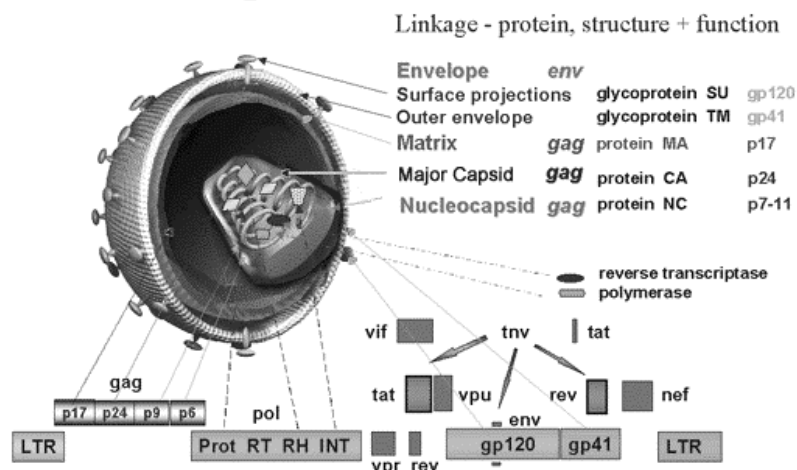
8nm in length. The

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.

1. They are the only viruses, which are truly diploid.
2. They are the only RNA viruses whose genome is produced by cellular transcriptional machinery
3. They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.
4. 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.

Genome map of Lentivirus

Name:	Protein:	Function:
MA	Matrix	matrix protein (<i>gag</i> gene); lines envelope
CA	Capsid	capsid protein (<i>gag</i> gene); protects the core; most abundant protein in virus particle
NC	Nucleocapsid	capsid protein (<i>gag</i> gene); protects the genome; forms the core
PR	Protease	Essential for gag protein cleavage during maturation
RT	Reverse transcriptase (<i>RNA dependant DNA polymerase</i>)	Reverse transcribes the RNA genome
IN	Integrase	Encoded by the <i>pol</i> 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

4. **OIE Classification:** List B infection

5. **Risk group:** No known human infection

6. **Reference laboratories:**

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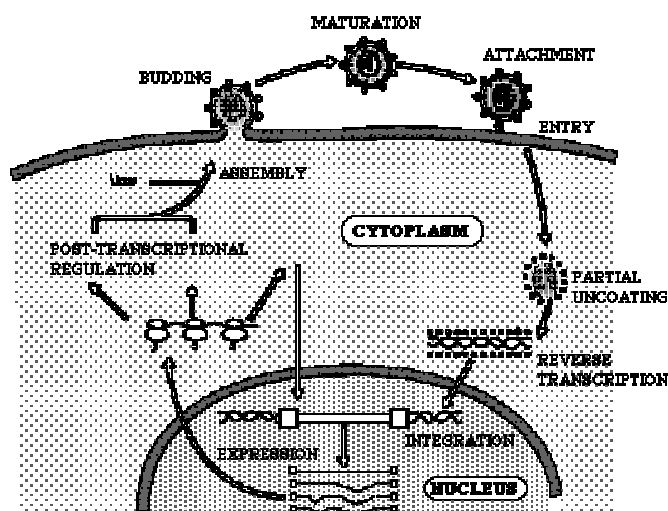
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7. **Resistance:** EIAV are fragile. They are sensitive to treatment with heat, detergents, and formaldehyde. Their infectivity is not affected by irradiation. The virus lasts for several months at room temperature, in urine, faeces, and dried blood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

8. **Replication:** Replication occurs inside the cytoplasm and it is the only RNA virus that depends on host cell transcriptionary mechanisms. The virus enters the cell by attaching it with a host cell receptor. The SU and TM proteins help in this stage. The virus enters by fusion. Only partial uncoating occurs inside the cytoplasm. Though the RNA is positive sense they are not translated immediately



inside the cytoplasm. A copy of the DNA is synthesized inside the cell with a virus coded enzyme RNA dependant RNA polymerase (reverse transcriptase). The d/s DNA product formed by this reaction is known as the **provirus**. Three forms of provirus DNA are found in all infected cells. This DNA later migrates to the nucleus and integrates with host cell DNA with the help of the enzyme integrase. Retroviruses use the cellular transcriptional machinery for expression. Therefore they are expressed like cellular genes. Assembly occurs at the cytoplasm and the mature virions later bud out through the cell membrane, acquiring the envelope during this process. As a result of complex replication steps, the replication processes of retroviruses are error prone. Further changes in the retro viral genome occurs due to high rate of mutation, recombination and exchange of genes with host genome.

9. HA property: The virus has no HA property

10.Serotypes / Strains: No distinct serotypes are present.

Cultivation:

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

Pathogenesis:

- 1. Hosts affected:** The infection is limited to members of the family equidae. Donkeys are less severely affected than horses.
- 2. Distribution:** The infection is worldwide. Few countries like Australia and New Zealand have claimed freedom.
- 3. 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. Iatrogenic 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
- 4. Morbidity and mortality:** Highly variable.
- 5. Incubation period:** Incubation period is 2-4 weeks
- 6. 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 severe symptoms. It is also referred to 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 signs as 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 horse 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.

7. **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 appears pale, flabby and friable.

Diagnosis:

1. **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 a clue about EIA.
2. **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 immunofluorescence assay.
3. **Nucleic acid identification method:** Recently a RT-PCR method has been developed to detect EIA proviral DNA from the peripheral blood of horses.
4. **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.
 - a. **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.
 - b. **ELISA:** Competitive and non-competitive ELISAs are also used to identify antibodies against EIAV.
5. **Differential diagnosis:** EIA should be differentiated from purpura haemorrhagica, babesiosis, leptospirosis, severe strongylosis or fascioliasis, phenothiazine toxicity and auto-immune haemolytic 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.

Control and eradication:

1. Vaccines: There are no vaccines against EIA

2. Eradication:

- a. 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.
- b. 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.
- c. In endemic areas, risk of infection can be reduced by protecting horses from insect vectors by
 - i. Keeping horses away from low lying areas
 - ii. Providing draining swamps
 - iii. Through use of insecticides and by providing insect-proof stabling

Additional Reading:

- d. *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*
 - i. *Use disposable syringes and needles. Follow the rule of one horse—one needle.*
 - ii. *Clean and sterilize all instruments thoroughly after each use.*
 - iii. *Keep stables and immediate facilities clean and sanitary. Remove manure and debris promptly, and ensure that the area is well drained.*
 - iv. *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.*
 - v. *Do not intermingle infected and healthy animals. Do not breed EIAV-positive horses.*
 - vi. *Isolate all new horses, mules, and asses brought to the premises until they have been tested for EIA.*
 - vii. *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.