GENERAL AND SYSTEMIC VIROLOGY
(MICRO – 303)

CORONAVIRIDAE

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BORNAVIRIDAE

AND

ARBOVIRUSES

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CORONAVIRIDAE (Corona – Crown shaped)

Classification

Genus	Type	Virus Name
Coronavirus	Group I	Porcine transmissible gastroenteritis virus
		Canine coronavirus
		Feline coronavirus
		Feline infectious peritonitis virus
	Group II	Porcine haemagglutinating encephalitis virus
		Bovine coronavirus
		Turkey coronavirus (Turkey blue comb disease virus)
	Group III	Avian infectious bronchitis virus
Torovirus		Berne virus (enteric disease virus of horse)
		Breda virus (enteric disease virus of cattle)
Arterivirus (Temporarily or tentatively placed in this family but it was the genus of Togaviridae previously.		Equine arterivirus Porcine reproductive virus Lactate dehydrogenase virus Simian hemorrhagic fever virus

General Properties

Shape and Size:

a) Roughly spherical or pleomorphic, medium sized (80-160 nm) particles.
b) *Enveloped*; lipid membrane bears widely spaced glycoprotein peplomers in the form of club-shaped projections (20 nm long) resembling a solar corona. Projections often readily lost resulting in partially or completely bald particles.
c) Has an inner membrane or matrix which may be similar to the inner shell of retroviruses.

Protein		Function	Location
E2	Peplomeric glycoprotein	Fusion protein; binding to host cells	Envelope spike (peplomer)
HI	Hemagglutinating protein	Haemagglutination	Peplomer (strain specific)
N	Nucleoprotein	Ribonucleoprotein	Core
E1	Matrix glycoprotein	Trans-membrane protein	Envelope (group specific)
L	Polymerase	Polymerase activity	Infected cell
E3*	Haemagglutinating – neuraminidase protein		Present in some strains

Nucleocapsid:

a) +ve sense single stranded RNA (27 – 33 Kbp).
The largest genome among RNA viruses.
b) Nucleocapsid symmetry undetermined but some evidence suggests a helix similar to that of 'orthomyxoviruses'.

c) Genome occurs in 1 piece which is capable of dissociation into smaller units.
d) The nucleocapsid is enclosed within a *lipoprotein envelope* which carries large club or petal-shaped projections (widely spread on outer surface of envelope)

Sensitivity: a) Sensitive to detergents, ether, chloroform, and other organic solvents.
b) Heat labile, but many are reasonably acid resistant.
c) Insensitive to *Actinomyecin* D.

Cultivation: *Embryonated Eggs*: Only IBV may be cultivated in embryonated hens' eggs. Replication occurs in the cytoplasm, and envelopment occurs by budding through the endoplasmic reticulum into cytoplasmic vesicles.

Haemagglutination:
Some strains of Infectious Bronchitis Virus (IBV) when treated enzymatically.

1. CORONAVIRUS

AVIAN INFECTIOUS BRONCHITIS VIRUS (AIBV)

Associated disease:

A major pathogen for chicken mainly causing acute respiratory disease in young chicks of 2-3 weeks age, are most affected. Chronic infection affects older birds causing *nephritis*. Lesions in the oviduct in laying hens have been described. Pheasants may be affected. Virus can be recovered from eggs and semen of extremely infected chickens. The virus shed in feces for several months.

The disease (*Infectious bronchitis*) is characterized by depression, coughing, sneezing, tracheal rales, the accumulation of excess mucus in the bronchi, drop in egg production and deterioration of egg quality in laying chicks.

Susceptible Host: Only chickens affected, high mortality in young chicks, older birds usually recover; other species of birds and mammals are not affected.

Pathogenicity:

Avian infectious bronchitis virus: occurs in three forms:

- ► Respiratory form (Sneezing, coughing)
- ► Reproductive form (Misshaped egg, hatchery egg white)
- ► Urinary form (Urates accumulates in kidney tubules and ureter)

As well as the typical symptoms defined above, AIBV has been associated with *nephritis* and *uremia* in chickens. Disease appears to affect many organs especially the upper and lower respiratory tract, genital tract and urinary tract.

Virus is often recovered from lungs, spleen, tonsil and kidney. In isolated chickens infection persists in the trachea for 4 weeks – in flocks under field conditions virus may persists for longer period.

Cultivation: *Embryonated Eggs*: allantoic cavity. Infected embryos are often stunted, curled tightly into balls; the amount of amniotic fluid is decreased. Necrotic foci are seen in liver and urate deposits found in kidneys and ureters.

Mortality and occurrence of stunting of embryos with freshly isolated virus is low, but some egg-adapted strains are lethal to embryos.

Cell Culture: Field isolates do not grow in cell cultures but some egg-adapted strains e.g. *Beaudette* produce multinucleate Syncytia and *CPE:* in chick kidney cell culture.

Haemagglutination:

Some strains agglutinate RBCs from a variety of sources, provided that the virus is concentrated and treated with the enzyme *phospholipase* C.

Inactivation: Maximum stability at pH 6.0 – 6.5. Readily inactivated by formalin, other commonly used antiseptics and detergents.

Strains: There are several antigenic variants; e.g.
Connecticut, Massachusetts, JMK, Iowa97 etc.
The egg-adapted *Beaudette* strain is antigenically related to *Massachusetts* serotype and is useful reference for neutralization tests.

Diagnosis: Serological diagnosis may be affected by *serum neutralization*, *precipitation tests* or by *haemagglutination inhibition* (HI) test.

Control: Only achieved by careful isolation of flocks or by vaccination.
Several live (attenuated) vaccines are available. However, the existence of multiple serotypes is the main factor obstructing adequate prophylaxis.
All the vaccines currently available in UK are of Massachusetts serotype which gives poor immunity against some other serotypes.

CANINE CORONAVIRUS

Associated disease:

Canine coronavirus infection may be characterized by a contagious, fetal gastroenteritis followed by anorexia, lethargy, vomiting, and diarrhea and ultimately leading to death.

Susceptible Hosts:

CCV infects domestic and wild canine species.

Pathogenesis:

Inapparent or persistent infection (or both) contributes to viral maintenance. Infected dogs secrete virus in feces for about 2 weeks. Virus is stable under low temperature. Thus, disease is more prevalent in winter months.

Mode of transmission – feces contaminated food.
Incubation period: 1-4 days.

Virus infects cells of upper duodenum and then proceeds throughout small intestine. Diarrhea occurs 1-7 days postinfection. Then, virus spreads to regional mesenteric LN and occasionally to liver and spleen.

Viral replication in the intestinal epithelium results in desquamation and shortening of villi. There may be loss of digestive enzymes which may lead to diarrhea. Consequently, excessive fluid loss may leads to death.

Intestinal healing occurs with in a week. Neutralizing Abs are generally low – because viremia stage does not occur. Mucosal immunity is protective as dogs infected orally rather than parenteral route.

Isolation: Virus can be isolated from the feces.

Cultivation: Several primary and continuous canine cell lines are susceptible for virus growth.

Diagnosis: Virus or viral antigen can be visualized by EM, FAT in feces or intestinal necropsy tissues.

Vaccination: Antigenic diversity of CCV makes vaccine development difficult.
Parenteral vaccines - are available but their use is questionable.

FELINE CORONAVIRUS

Feline Infection Peritonitis Virus (FIPV)
Feline Enteric Corona Virus (FECV)

Susceptible Host:

Domestic and wild cats. Distribution is world wide.

Transmission:

Ingestion and Transplacental

Pathogenesis: Uptake or ingestion of virus contaminated food → Digestive system has been infected → Virus goes to local lymph nodes and *phagocyte mediated*

transmission occur to various target organs such as peritoneum, omentum, pleura, kidneys, meninges etc. → Abdominal enlargement caused by fibrinous peritonitis, pleurisy and some neurological signs may appear.

Immunity: *Immunodeficient cats* are mostly infected.
Virus + Abs + Complement → play a role in lesion development.

Cultivation: *Cell Culture:* Feline cells
Lab Animal: Suckling mice

Diagnosis: Serological diagnosis includes techniques such as FAT, PCR, and ELISA.

Control: Quarantine and decontaminating premises.
Vaccines are also available.

BOVINE CORONAVIRUS
(Neonatal Calf Diarrhea Virus)

Pathogenesis: Followed by the virus-contaminated food ingestion, diarrhea occur and lasts 24 – 30 hours. 4 days after diarrhea – virus can be detected in SI. Proteolytic enzymes of SI facilitate infection. Mesenteric lymph node and villious atrophy may occur.

Immunity: The virus is antigenically related to Human Coronavirus.
Humeral response:
Local immune response: - but not circulating abs -
Colostrum antibodies: - it is very important - protects claves 1-20 days of age

Cultivation: Cell culture: Bovine foetal brain, Bovine foetal thyroid, and Bovine kidney cells. VERO cells. Trypsin treatment enhances the growth of virus on cell culture.

Haemagglutination:
RBCs of hamsters, mice, rats

Sensitivity: Stable at acid pH i.e. 3.
Sensitive to ether, chloroform, sodium deoxycholate & high temperature.

Diagnosis: EM, FAT, Counter immuno-electrophoresis (modification of AGPT)

Coronavirus

Avian infectious bronchitis virus does not agglutinate RBCs unless treated with trypsin due to some mucigenious substances cover HA of the virus.

PORCINE CORONAVIRUSES

The viruses are associated with;

i. Transmissible Gastroenteritis of Swine
ii. Haemagglutinating encephalitis of Swine.

Transmissible Gastroenteritis of Swine

Associated disease:
Disease is characterized into high infectivity, diarrhea, vomiting, dehydration and a high mortality (approaching 100%) in piglets less than 2 weeks of age. Symptoms are less severe in adult pigs and disease occurs mostly in winter months. Virus is first described in 1946; having short incubation period (18-24 hours).

Susceptible Hosts: Pigs are natural host. Distribution is world wide.

Pathogenicity: Although virus has been recovered from many organs, but the clinical disease is confined primarily to the GI tract. Characteristic pathological lesion is being severe blunting of jejunal and ileal villi. Virus is shed from GI tract for as long as 8 weeks after infection.

Cultivation: *Cell Culture*: virus grows fairly readily in various primary pig cells such as Pig kidney and thyroid cells.

Haemagglutination:
None

Diagnosis: Virus can be detected/identified by EM, FAT, serum neutralization test and by other immunofluorescence techniques.

Control: Only control measures available at present are careful management and strict hygiene measures to prevent spread of virus.

Vaccines: Some *attenuated live vaccines* have been tested recently, though are not generally available.

Haemagglutinating Encephalitis of Swine

(Synonym: vomiting and wasting disease of piglets)

Associated disease:
Disease is characterized by squealing, vomiting, constipation, occasional diarrhea, progressive paralysis (often accompanied by peddling movements of legs). Almost 100% mortality in piglets less than 1 week. Adult pigs may develop vomiting and anorexia but usually recover. Virus first described in Canada 1958

Susceptible Hosts: Only pigs are affected.
Originally isolated in Canada, subsequently found in USA and Great Britain

Cultivation: *Cell culture*: Pig kidney cells and CPE will be Syncytial formation.

Haemagglutination:
Agglutination occurs with RBCs from rats, chickens, turkeys, mice and hamsters but ------ does not agglutinate RBCs of guinea pig, calf, sheep, goose, horse, rabbit and human.

2. ARTERIVIRUS

EQUINE ARTERIVIRUS

General Properties

- +ve sense ssRNA, enveloped
- Inactivated by lipid solvent, sodium deoxycholate and other disinfectants.
- Stable at low temperature (-70 C), killed by heating at 56 C for 30 min.

Associated disease:
The disease is also termed as “Pink eye disease”, which is characterized by fever, stiff gait and respiratory distress.

Susceptible Host:
Equines (horses) are natural host. Virus develops persistent infection and can be transmitted by aerosols and through semen of infected stallions.

Pathogenesis:
Incubation period of virus: 2 – 15 days
Virus replicates in endothelial cells of small arteries. Veins are distended (No inflammation/necrosis). → Necrosis of muscles, edema, dehydration and abortion.
Neutralizing antibodies develop within 20 weeks postinfection.

Diagnosis: Histopathology. Virus isolation from edema fluid and blood. PCR, ELISA.

Cultivation: *Cell culture*: Horse kidney, Rabbit kidney, hamster kidney cell culture. Cell lines such as BHK – 21, RK – 13 and VERO.

Vaccine: Attenuated cell culture vaccine is available.

OTHER ARTERIVIRUSES

- Porcine reproductive virus
- Lactate dehydrogenase virus
- Simian hemorrhagic fever virus

CALICIVIRIDAE

General Properties

- ► Have the same size as Picornaviruses
- ► They are spherical in shape.
- ► +ve sense single stranded RNA.
- ► Naked capsid consisting of one 60 kDa capsid protein.
- ► These viruses have cup-shaped indentations (spheres) and a 6 pointed star shaped on the surface.
    - o Whereas *Norwalk virus* have rugged appearance.

NORWALK VIRUS

- The virus compromises the functions of intestines; prevent the absorption of water and nutrients.
- Norwalk virus in man causes gastroenteritis, diarrhea, vomiting.
- It has not been grown on cell culture yet.
- The virus gets entry through fecal-oral rout; but self limiting.
- The virus can be isolated from feces or vomit.

HEPATITIS E VIRUS

- ❖ Incubation period is about 2 – 8 weeks. Transmission is through fecal oral routes.
- ❖ The disease is self limiting and does not progress to chronic hepatitis, cirrhosis or cancer.
- ❖ There is no carrier state.
- ❖ Infection possibility can be minimized by improved sanitation and prophylaxis measures.

VESICULAR EXANTHEMA OF SWINE

- Acute disease characterized by vesicle formation on mouth, lips, tongue, oral cavity, inter-digital spaces ad the coronary hard of the feet.
- High morbidity but low mortality
- Great economic importance

Other Resembled Diseases:
Clinically confused with FMD, VS and swine vesicular disease

Inactivation:
Virus can be inactivated through high temperature at 41.5 C for 6 days.

Susceptible Host: Swine is the natural host.

Isolation: Virus can be isolated from vesicles or lymph nodes.

Cultivation: Vero monkey kidney cells, Swine kidney cells

Lab Diagnosis
Electron microscopy, FAT, CFT, VN, ELISA, PCR.

Vaccination: No vaccine is available.

FELINE CALICIVIRUS

- ❖ Causes upper respiratory tract infection in cat.
- ❖ Characterized by high fever (40-49 C), sneezing, nasal + ocular discharge (rhinitis) , conjunctivitis, ocular ulceration and pneumonia.

Cultivation: can be grown on *Feline cell culture*.

RABBIT HEMORRHAGIC DISEASE

- It is an acute infection with a high mortality.
- This disease is characterized by fever, and hemorrhagic lesions in the lung and liver.

Cultivation: No growth on cell culture.

ASTROVIRIDAE

General Properties

- Viruses have a 5 or 6 pointed shape on the surface but no indentations.
- Small spherical virions (diameter of 28 – 30 nm), characteristic 5 to 6 pointed star-shaped outline by negative staining.
- Genome is +ve sense ssRNA (7 kbp).

Genus: ASTROVIRUS

- ► It is the only genus of this family.
- ► 5 – 6 serotypes.
- ► Can be isolated in primary cell cultures of human embryos.
- ► Have been found on feces of humans, calves, lambs suffering from enteritis.
- ► Transmission is through fecal oral route.

Pathogenesis: After incubation period of 1 – 4 days, patient develops watery diarrhea lasting for 1 – 4 days or more; more in young calves.

Diagnosis: The virus can be found in feces via immuno-electron microscopy and ELISA.

FILOVIRIDAE

General Properties

- ○ Filamentous
- ○ Enveloped
- ○ Genome is –ve sense ss RNA
- ○ Nucleopcapsid is helical, enclosed in an envelop containing one glycoprotein.

Replication:

- Virus replicate in the cytoplasm like “Rhabdoviruses”.
- Filovirus replicate and producing large amount of virions causing tissue necrosis in the parenchymal cells, liver, spleen and lungs.
- There is wide spread of hemorrhages that occur in the affected patients, causes oedema and hypovolemic shock.
- Causes sever *fetal hemorrhagic fever* ----- in Africa.

Marburg virus: first seen in lab worker in *Marburg* (Germany).
Ebola virus: causes lethal viral hemorrhagic fever.

- ❖ The virus is endemic in wild monkeys but can be spread from monkeys to human.
  - Can also occur through contaminated food and water.
  - Can be spread through injection.

Marburg virus may grow rapidly in tissue culture (Vero cells), but animal (like guinea pig) inoculation may be necessary to recover the Ebolavirus.

Lab Diagnosis: IF, RtPCR, RIA, ELISA.

ARENAVIRIDAE

General Properties

- ► The virions have sand-sprinkled appearance in electron microscopy (EM) due to the incorporation within virions of the ribosomes of the host cell during assembly.
- ► The virions are pleomorphic, 110 – 130 nm in diameter.
- ► They posses host plasma membrane – derived envelop into which are embedded glycoprotein peplomers.
- ► The envelope encloses neocleocapsid segments.
- ► The genome consists of ssRNA of negative sense. It exists as 2 molecules, large (L) 7.2 kbp, and a small (S) 3.4 kbp.

Genus: ARENAVIRUS

- ⊠ Lymphocytic Chorio-meningitis virus (Flue like disease in human)
- ⊠ Lassa virus (Causes… African hemorrhagic fever)
- ⊠ Junin virus (……. Argentina hemorrhagic fever)
- ⊠ Machups virus (…… Bolivia hemorrhagic fever)
- ⊠ Guanarito virus (…… Venezuelian hemorrhagic fever)

Susceptible Host:

Rodents are natural host and virus may persist in them and are zoonotic importance.

Pathogenesis: Arenaviruses are able to infect mΦ and mΦ releases mediators and cause vascular damage or hemorrhages.

Isolation:

Can be isolated from blood, CSF, throat washing, pleural fluid, & urine.

Cultivation: *Animal inoclulation*: Suckling mice, hamsters and guinea pig.
Cell culture: BHK – 21

Arenavirus infection should be differentiated from *malaria, enteric fever, listeriosis, trypnosomiasis, leptospirosis, streptococcal pharyngitis*, and other *viral hemorrhagic fever*.

BORNAVIRIDAE (Borna – a town in Germany)

General Properties

- ► ss RNA, helical
- ► Enveloped, projection/spikes are there.
- ► The virus encodes *RNA dependant RNA polymerase*.

Susceptible Hosts:

Horses, sheep, cattle, rabbits, ostriches are naturally infected.
Exp. Hosts: rat, chicken.

Pathogenesis:

Horses infected through intranasal route – result in encephalomyelitis – excitability, ataxia, and incoordination. Intranuclear eosinophilic inclusion bodies – *Joest Deger bodies* in brain cells may be found.

No protective immune response, but a cell mediated immunopathological reaction may develop.

Vaccination: No vaccine is recommended due to immnuopathological reaction.

Control: Slaughtering and disposal of infected animals. Identification and quarantine of carrier animals.

ARBOVIRUSES

Arthropod – borne Viruses:

---- transmitted by blood sucking insects from one vertebrate host to other.

- In vertebrate host, replication of virus produces viremia. The insects acquire virus through ingestion of blood from the viraemic host. The virus multiplies in the tissues of the insect without any disease or damage. Some viruses are maintained in nature by transovarian transmission in insects in which the virus pass from the female through eggs to the offspring.

- Almost all *arboviruses* are zoonotic. They have at least two hosts;
  i) *Vertebrate host*
  ii) *Blood sucking arthropod host*

  Most of the *arboviruses* are maintained either in the human primary vertebrate host or a primary arthropod vector.

- Human and domestic animals get infection after the virus is brought into them by vectors. These vertebrate hosts frequently develop clinical illness and are called *dead – end hosts*, because they do not contribute to the transmission cycle.

  However a few *arboviruses* cause significant viremia in humans and may be transmitted by human – arthropod – human cycle.

- *Arboviruses* have a global distribution, but majority are found in tropical developing countries such as Africa, South America and Asia.

- Majority of *arboviruses* cause a febrile illness but some may lead to severe hemorrhagic disease or encephalitis, often with a fetal outcome or permanent morphological sequel.

- Mosquitoes are the most important vector, followed by ticks, sand fleas, midges & bed bugs.

- Some arbovirus infection may also be acquired by ingestion of infected milk, inhalation, aerosols, and close contact.

- Arboviruses are placed in;
  i) *Togaviruses* (They are further subdivided into arbo- and non-arbo toga viruses)
  ii) *Flaviviruses*
  iii) *Bunyaviruses*

A few arboviruses are members of family *Reoviridae* i.e. *African horse sickness* and *blue tongue* virus. Almost 50% of virology comprises *arboviruses*.

UPCOMING FAMILIES:

☑ **Togaviridae** ☑ **Flaviviridae** ☑ **Bunyaviridae**