Coronaviridae

VMC 321
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Covid-19 virus
## Important diseases caused by **Coronaviruses**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Hosts</th>
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</thead>
<tbody>
<tr>
<td>Infectious bronchitis virus (IBV)</td>
<td>Chicken</td>
</tr>
<tr>
<td>Transmissible Gastroenteritis (TGE)</td>
<td>Porcines</td>
</tr>
<tr>
<td>Feline Infectious Peritonitis (FIP)</td>
<td>Cats</td>
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<tr>
<td>Severe Acute Respiratory Syndrome (SARS)</td>
<td>Humans, porcine</td>
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<tr>
<td>Winter-dysenteries</td>
<td>Cattle</td>
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<tr>
<td>Virus species</td>
<td>Host</td>
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<tr>
<td>--------------------------------------------------</td>
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</tr>
<tr>
<td>Canine coronavirus type I</td>
<td>Dog</td>
</tr>
<tr>
<td>Canine coronavirus type II</td>
<td>Dog</td>
</tr>
<tr>
<td>Feline coronavirus type I</td>
<td>Cat</td>
</tr>
<tr>
<td>Feline coronavirus type II</td>
<td>Cat</td>
</tr>
<tr>
<td>Transmissible gastroenteritis virus</td>
<td>Pig</td>
</tr>
<tr>
<td>Bovine coronavirus</td>
<td>Cow</td>
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<tr>
<td>Equine coronavirus</td>
<td>Horse</td>
</tr>
<tr>
<td>Porcine hemagglutinating encephalomyelitis virus</td>
<td>Pig</td>
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</tbody>
</table>
Taxonomy

• Order – *Nidovirales*
• Family – *Coronaviridae*
• The family contains two subfamilies
  • *Subfamily* - *Coronavirinae*
  • *Subfamily* - *Letovirinae*
• Members of the family *Coronaviridae* classified into three groups on the basis of
  ✓ Antigenicity,
  ✓ Genome organization
  ✓ Sequence similarity
  • *Alphacoronavirus* (Group I) - *Porcine epidemic diarrhea virus*
  • *Betacoronavirus* (Group II) - *Severe acute respiratory syndrome*
  • *Gammacoronavirus* (Group III) - *Avian coronavirus*
  • *Deltacoronavirus* (Group III) - *Bulbul coronavirus HKU11*
Subfamily: *Orthocoronavirinae*

- Family – *Coronaviridae*
- Subfamily – *Coronavirinae*
- Members of the subfamily *Coronavirinae* classified into three genus on the basis of
  - Antigenicity,
  - Genome organization
  - Sequence similarity

**Genus:**
- *Alphacoronavirus* (Group I) - *Porcine epidemic diarrhea virus*
- *Betacoronavirus* (Group II) - *Severe acute respiratory syndrome*
- *Gammacoronavirus* (Group III) - *Avian coronavirus*
- *Deltacoronavirus* (Group III) - *Bulbul coronavirus HKU11*
Genus: *Alphacoronavirus*

**Species:**
- Canine coronavirus
- Feline coronavirus
- Porcine respiratory coronavirus
- Transmissible gastroenteritis virus
- *Porcine epidemic diarrhea virus*
Genus: *Betacoronavirus*

**Species:**
- Bovine coronavirus
- Equine coronavirus
- *Severe acute respiratory syndrome-related coronavirus*
Genus: *Gammacoronavirus*

**Species:**
- Infectious bronchitis virus
- Turkey coronavirus
Properties of *Coronaviridae*

All members of the *Coronaviridae* family share the following characteristics:

- **Virions**: enveloped and decorated with large (15–20 nm) surface projections.
- **Nucleocapsid**: helical, comprised of genome and multiple copies of a single basic phosphoprotein species (N).
- **Envelope**: contains a variable number of viral membrane protein species:
  - integral membrane protein M
  - fusion protein S which forms peplomers.
- **Genome**: positive sense RNA, linear, unimolecular, infectious, 26–32 kb in length
- **Morphogenesis**: virion assembly through budding of preformed nucleocapsids at smooth intracellular membranes of endoplasmic reticulum/early Golgi compartments.
Coronavirus: Structure

- Hemagglutinin-esterase (HE)
- Spike protein (S)
- Membrane protein (M)
- Nucleocapsid (N) protein
- Envelope (E) protein
FIGURE Virion structure (subfamily *Coronavirinae*) (right). TEM image of MERS-CoV virions in culture (left). From CDC/Cynthia Goldsmith, Azaibi Tamin, Ph.D. Public Health Image Library Image #17280.
• The members of the family *Coronaviridae*, a monophyletic cluster in the order *Nidovirales*, are enveloped, positive stranded RNA viruses of three classes of vertebrates:
  • mammals (corona-and toroviruses),
  • birds (coronaviruses)
  • fish (bafiniviruses).
Morphology

- Coronavirus particles are enveloped with prominent spikes.
- Virions are spherical,
- range in size from 120–160 nm in diameter (*Coronavirinae*),
- flexible or bacilliform
- Virus particles are typically decorated with large, club- or petal-shaped surface projections (the “peplomers” or “spikes”)
- in electron micrographs of spherical particles create an image reminiscent of the *solar corona*
- *Coronaviridae* are the largest RNA viruses identified so far
• All covs encode four structural proteins:
  • Three membrane-associated proteins (S, M, and E)
  • a single nucleocapsid (N) protein.
• Some *betacoronaviruses* have an additional membrane protein with hemagglutinating and esterase activities, called he.
Genomic organisation (1)

The order of the structural genes on the CoV genome is (HE) S, M, E, N

The order of structural genes on the torovirus genome is S, M, HE, and N

FIGURE: Torovirus genome organization.

- Hemagglutinin-esterase (HE)
- Spike protein (S)
- Membrane protein (M)
- Nucleocapsid (N) protein
- Envelope (E) protein
Genomic organisation (2)

Coronaviridae genome/genome length mRNA

Ribosomal frame shift

Translation and cleavage

ORF1a polyprotein

ORF1aB polyprotein

RdRp

16 protein products produced through cleavage

Subgenomic mRNAs
Replication

- Virions attach to dedicated host cell surface receptors via their spikes
- Release their genome into the target cell via fusion of the viral envelope with the plasma membrane and/or the limiting membrane of an endocytic vesicle.
- Replication cycle takes place in the cytoplasm and involves the production of full-length and subgenome-sized (sg) minus-strand RNA intermediates.
- Viral genome serving both as mrna for the replicase polyproteins and as a template for minus-strand synthesis.
- RNA synthesis is catalyzed by replication–transcription complex.
- Double-membrane vesicles derive from endoplasmic reticulum (ER).

**Attachment:** S protein binds with host receptors
**Penetration:** consequence of a membrane fusion
**Amplification:** translation of the viral genome by host cell ribosomes.

**Assembly and Release:**
- Virion assembly takes place on membranes.
- Genomic RNA is bound by N protein, associates with M protein and buds into ER/Golgi membranes.
- M packs tightly into membranes and cause the membrane curvature that drives budding.
- S and E & M are acquired during the budding process.
- Virus particles contained within membrane bound vesicles are released from cells by exocytosis.
Genomic replication

1. The process begins with initiation of cRNA synthesis at the 3' end of the genome RNA strand.

2. The RNA synthesis complex, with its newly synthesized cRNA dissociates from the genome strand and base pairs with the TRS nearest to the 5' end of the genome strand. RNA synthesis continues, to copy the leader sequence.

3. The subgenomic cRNA is the template for transcription of mRNAs.
Infectious Bronchitis Virus (IBV) - Classification

• **SEROTYPE**
  Traditionally, IBV strains classified into different serotypes based largely on
  i.  Cross virus-neutralization tests in embryonated chicken eggs,
  ii. Tracheal organ cultures
  iii. Cell culture

  *Coronavirus genus - more than 26 serotypes*

• **GENOTYPE**
  RT-PCR- RFLP; nucleotide sequencing

• **PROTECTOTYPES**
  IBV strains with different antigenic or genetic features may still cross-protect *in vivo* (in the bird)
  e.g. Massachusetts serotype of IBV
The virion -

- Enveloped viruses
- Spherical; diameter between 60 and 220 nm.
- Single stranded; +ve sense RNA genome;
- Largest of all groups of RNA viruses - 27.6 kb
- Lollipop-shaped projections sticking out from the surface like a crown
- Replicates in the cytoplasm and the RNA works directly as a messenger RNA
- For the RNA-polymerase
• Four structural proteins,
  I. Spike (S) glycoprotein,
  II. Envelope (E) glycoprotein,
  III. Membrane (M) glycoprotein
  IV. Nucleocapsid (N) protein
Morphology

• IBV is a pleomorphic, enveloped virus with club-shaped surface projections (spikes)
• single-stranded, positive-sense RNA genome of >27 kb in length
Coronaviridae

- Petal-shaped surface, like a solar corona
- More human coronaviruses cause upper respiratory infections
- Severe acute respiratory syndrome (SARS)

- Genome: ssRNA (plus strand)
- Capsid symmetry: helical
- Enveloped virion with a corona of glycoproteins
- Includes SARS coronavirus
S (Spicule)
M' (Protéine Membranaire Intégrée) autre conformation
M (Protéine Membranaire Intégrée)
N (Nucléocapside)
HE (Hémaglutinine Estérase)
E (Petite Protéine de Membrane)
Infectious Bronchitis Virus

• Causes Infectious bronchitis in chicken

• **Susceptibility:** Chicken

• **Transmission:** Aerosolization of nasal secretion & faeces; equipment; contaminating personnel

• **Pathogenicity:** The IBV replicates in tissues of respiratory tract, kidney, intestinal tract & oviduct.

• **Incubation period:** 18-36 hrs.
Cultivation

- Inoculated into the allantoic cavity of 9- to 11-day-old
  - dwarfing,
  - stunting,
  - curling
  - embryo
  - Death
  - embryo stunting,
  - curling,
  - Clubbing down of the,
  - urate deposits in the mesonephros of the kidney
Cell culture

Chorioallantoic cells

CPE
Replication
Misshapen eggs
Pathogenicity

- Incubation period can be as short as 24 to 72 hours
- Disease course - less than two weeks
- Morbidity = 100%
- Mortality = below 30%
- Young animals are more seriously affected
- Predilection site:
  - Trachea is the primary target
  - Other: Epithelium in kidneys, Intestines & Oviducts
Clinical Signs

• Divided into:
  ✓ Respiratory,
  ✓ Reproductive
  ✓ Subclinical
Clinical Signs - Respiratory

- Depression
- Ruffled feathers
- Reduced weight gain
- Swollen face
- Rales
- Cough
- Dyspnea
- Gasping
- Sneezing
- Watery nasal & ocular discharge

- Mucosal thickening and catarrhal exudate in the respiratory tract.
- Pneumonia,
- Sinusitis,
- Conjunctivitis
- Pulmonary stasis
- Oedema
- Whitish green watery diarrhoea
Clinical Signs- Reproductive

• Two distinct syndromes of reproductive disease

  Young birds
  • Deformed oviduct
  • Eggs deposited in the body cavity

  Adult birds
  • Production and quality of the eggs impaired in adult birds
  • Egg peritonitis

Misshaped eggs:
  - Small
  - Lack shell
  - Bad quality shell
  - Albumen
Clinical Signs - Reproductive
Large abdomen
Young chicken showing respiratory signs
Comparison of normal eggs (above, left) with shell-less eggs (above, right), rough-shelled egg (centre), and misshapen eggs (bottom) laid by hens during an outbreak of IB
Clinical Signs - Reproductive

- Histologically (Oviduct):
  - degeneration of the epithelium
  - infiltration of lymphocytes
  - hypoplasia and cystic lesions
Diagnosis

• Clinical and Pathological findings
• Electron microscopic (EM) examination
• Virus isolation
  • using tracheal organ cultures/ chorioallantoic cellschicken embryo kidney(CEK) and chicken Kidney(CK) & chicken embryo liver(CEL)- CPE: CK cells show syncytia, vacuolation, rounding & detachment
  • **Embryonated SPF eggs.** (9-11 day old ) via allantoic route – dwarfism & mortality of embryo)
• Indirect immunofluorescent assay (IFA)
• Agar gel Immunodiffusion (AGID) test
• Immunofluorescence test (IFT)
• Immunoperoxidase test (IPT)
• Enzyme linked immunosorbent assay (ELISA)
• Haemagglutination inhibition (HI) test.
• Virus neutralization test (VNT)
• Dot-immunobinding assay
• Histopathology
• RT-PCR with RFLP
Precaution:

1. Disease awareness, early detection, proper disposal of affected birds, timely notification,
2. Strict biosecurity, isolation, zoning, quarantine, control of live bird market are prerequisites for
3. Vaccination

### Vaccination schedule

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Earliest age</th>
<th>Route</th>
<th>Interval</th>
<th>Protection</th>
<th>Secondary vaccination</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live: milder strain</td>
<td>1-3 weeks</td>
<td>Intra occular</td>
<td>1 week</td>
<td>Several weeks</td>
<td>8 weeks</td>
<td>Layer Breeder Broiler</td>
</tr>
<tr>
<td>Inactivated</td>
<td>16-20 weeks</td>
<td>Nasal</td>
<td>1 week</td>
<td>Several weeks</td>
<td>16 weeks</td>
<td>Layer Breeder</td>
</tr>
</tbody>
</table>
two types of vaccines

• Two types of vaccines are available
  • live attenuated –
    - administered in the forms of sprays or aerosols
  • inactivated or “dead”, vaccines
    - administered by injection
•
Transmissible Gastroenteritis Virus (TGEV)
• Transmissible Gastroenteritis Virus (TGEV)

• The Disease

• Transmissible Gastroenteritis

• Susceptibility: Pigs under two weeks of age

• Reservoir: Swine

• Transmission: Ingestion, aerosol
Pathogenicity:

i. Virus replicates in respiratory tract & induces viraemia

ii. Invasion of epithelial cells of intestinal villi & sloughing of infected cell

iii. Migration of undifferentiated cells to fill the gap

iv. Invasion induces diarrhea due to malabsorption & osmotic reversal

v. Dehydration; death occurs due to acidaemia
Clinical sign:

- Incubation period: 18-72 hrs.
  - Vomition
  - profuse yellowish diarrhoea
  - thirst
  - loss of body weight
  - high morbidity & mortality
Diagnosis:

1. Clinical signs and symptoms
2. Isolation and identification of virus
3. Demonstration of viral antigen in clinical specimen
   - Agar Gel Immunodiffusion (AGID) test
   - Immunofluorescence test (IFT)
   - Immunoperoxidase test (IPT) and
   - Enzyme linked immunosorbent assay (ELISA)
4. Demonstration of antibody in convalescent sera
   - Haemagglutination inhibition (HI) test.
   - Agar Gel Immunodiffusion (AGID) test

5. Detection of viral nucleic acid
   - In situ hybridization (with labeled gene probes)
   - Reverse transcriptase – polymerase chain reaction (RT-PCR)
Precaution:

• 1. Vaccination

• 2. Disease awareness, early detection, proper disposal of affected piglet

• 3. Strict biosecurity, isolation, zoning, quarantine
Neonatal calf diarrhoea virus
Neonatal calf diarrhoea virus causes

• Neonatal calf diarrhoea
  • Susceptibility: Neonatal calves
  • Reservoir: infected cow
  • Transmission: Oro – faecal route
  • Incubation period: 17-20 hrs.
  • Clinical sign: Depression, inappetance, yellowish diarrhea, dehydration & death
Diagnosis:

• 1 Clinical signs and symptoms

• 2 Isolation and identification of virus

• 3 Demonstration of viral antigen in clinical specimen
  ▪ Agar Gel Immunodiffusion (AGID) test
  ▪ Immunofluorescence test (IFT)
  ▪ Enzyme linked immunosorbent assay (ELISA)

• 4. Demonstration of antibody in convalescent sera
  ▪ Agar Gel Immunodiffusion (AGID) test
Prevention:

• Vaccination – live attenuated vaccine
• Disease awareness, early detection
• Strict biosecurity, isolation, zoning, quarantine
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