Coronaviridae

VMC 321

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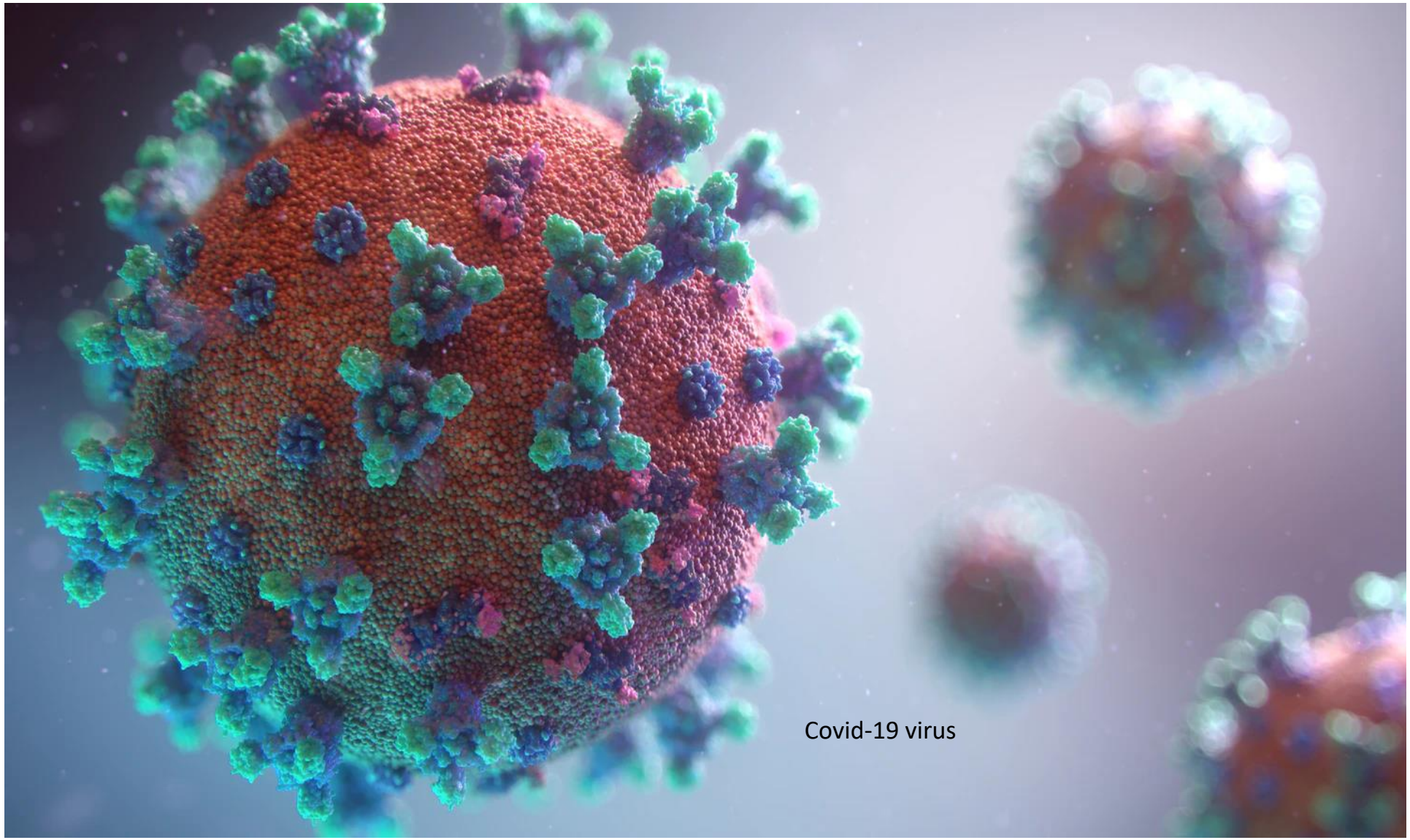


Covid-19 virus

Important diseases caused by *Coronaviruses*

Infectious bronchitis virus (IBV)	Chicken
Transmissible Gastroenteritis (TGE)	Porcines
Feline Infectious Peritonitis (FIP)	Cats
Severe Acute Respiratory Syndrome(SARS)	Humans, porcine
Winter-dysenteries	Cattle

Family: *Coronaviridae*

Virus Taxonomy: 2019 Release

EC 51, Berlin, Germany, July 2019
Email ratification March 2020 (MSL #35)
Coronaviridae: 2 subfamilies, 5 genera, 26 subgenera, 46 species

Virus species	Host
Canine coronavirus type I	Dog
Canine coronavirus type II	Dog
Feline coronavirus type I	Cat
Feline coronavirus type II	Cat
Transmissible gastroenteritis virus	Pig
Bovine coronavirus	Cow
Equine coronavirus	Horse
Porcine hemagglutinating encephalomyelitis virus	Pig

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

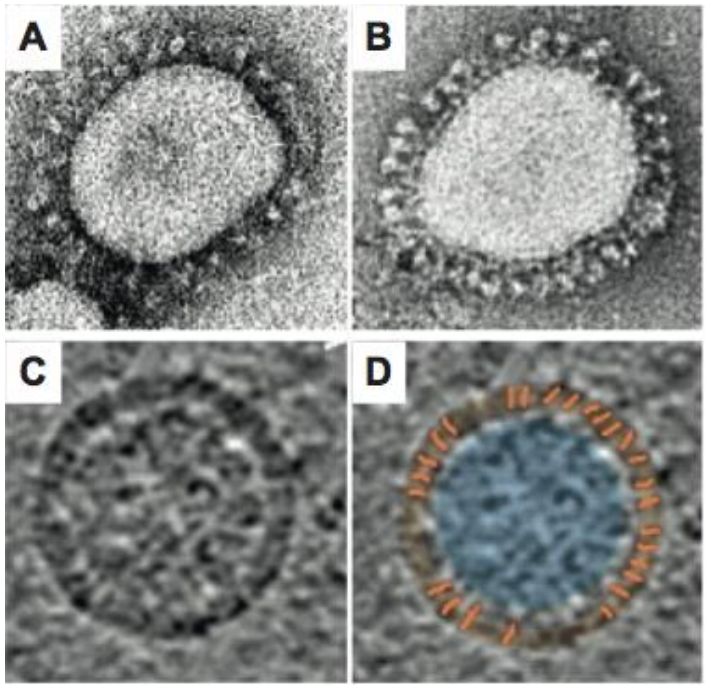
Properties of *Coronaviridae*

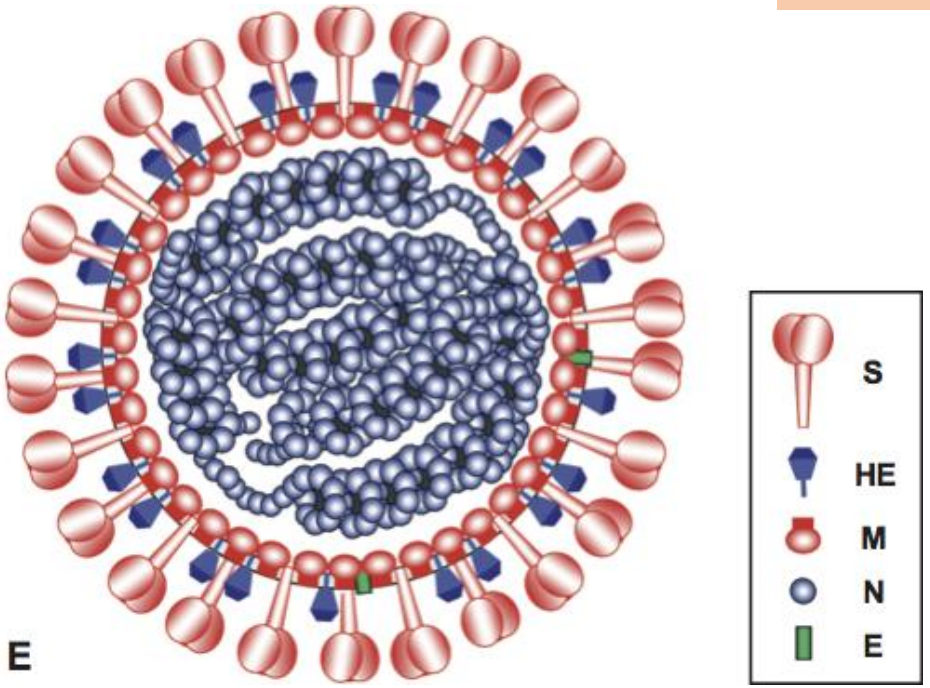
All members of the *Coronaviridae* family share the following characteristics:

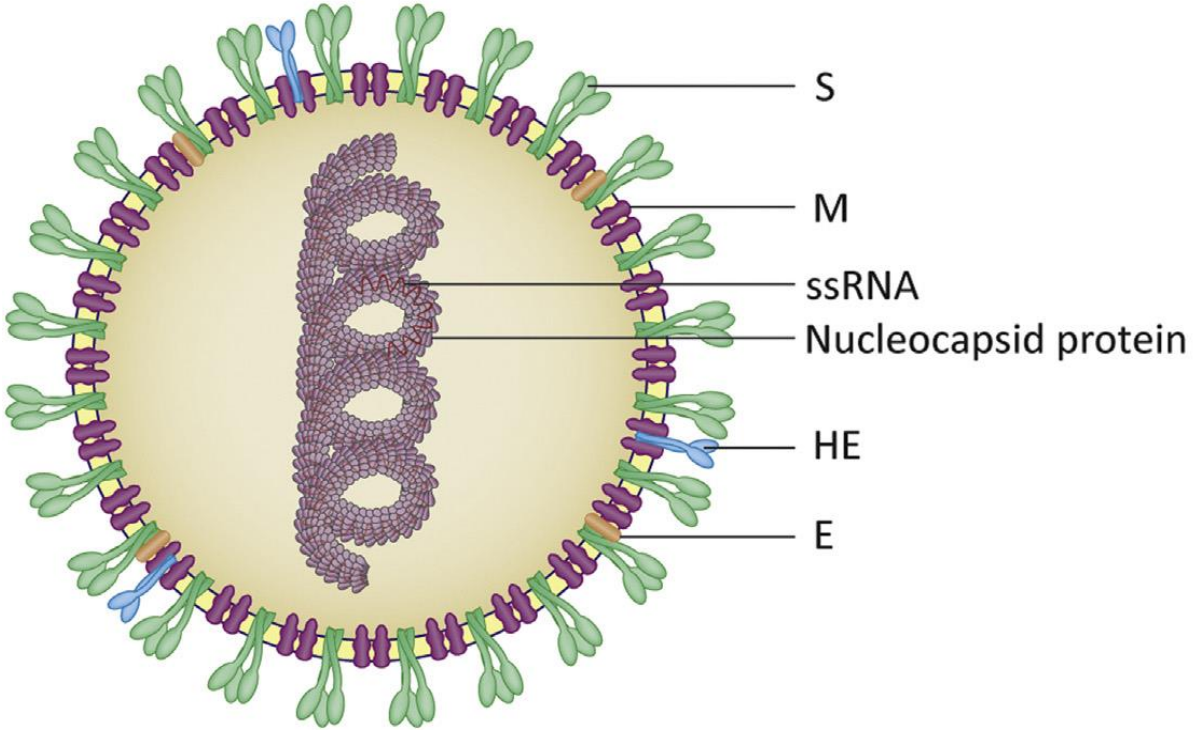
- **Virions:** enveloped and decorated with large (15–20 nm) surface projections.
- **Nucleocapsid:** helical, comprised of genome and multiple copies of a single basic phosphoprotein species (N).
- **Envelope:** contains a variable number of viral membrane protein species:
  - integral membrane protein 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







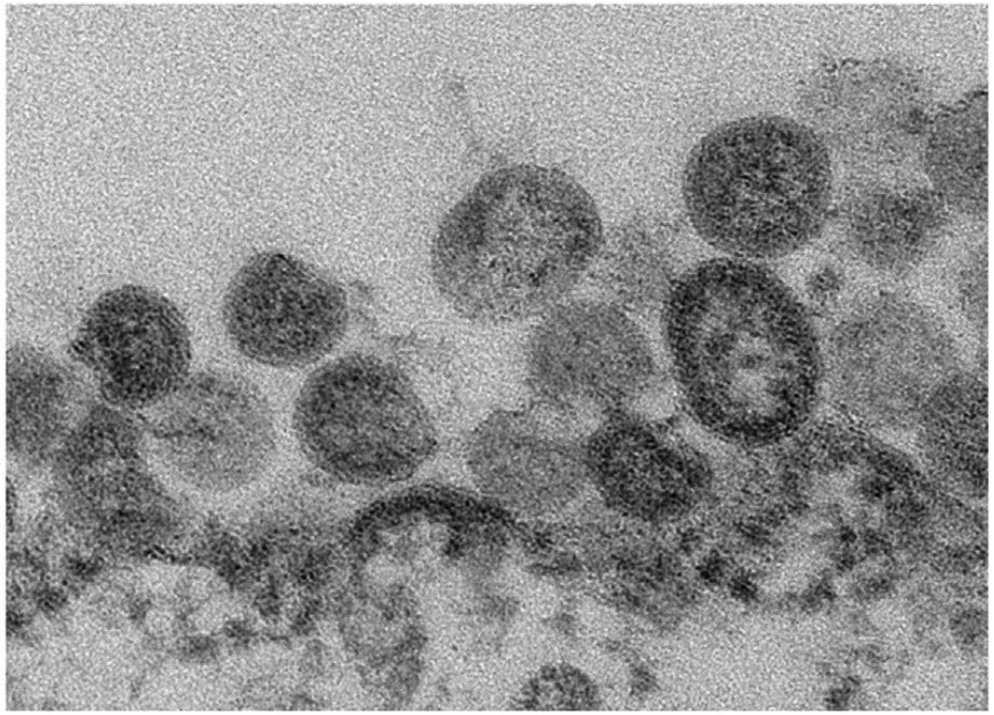


FIGURE Virion structure (subfamily *Coronavirinae*) (right). TEM image of MERS-CoV virions in culture (left). From CDC/Cynthia
Goldsmith, Azaibi Tamin, Ph.D. Public Health Image Library Image #17280.

- 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

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

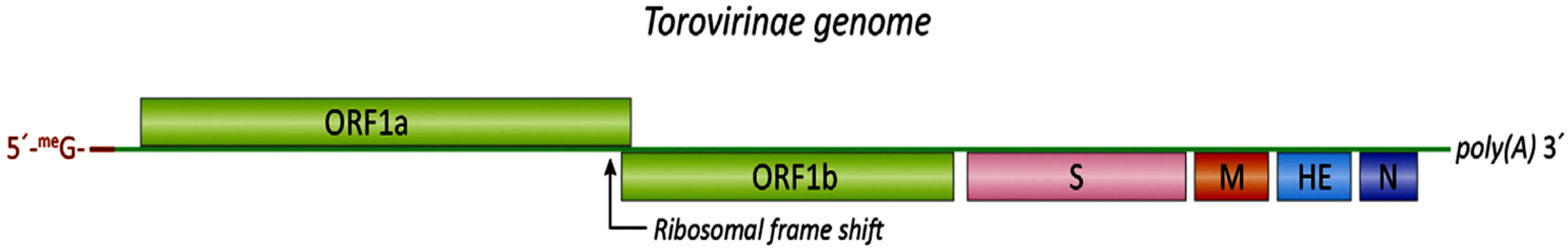
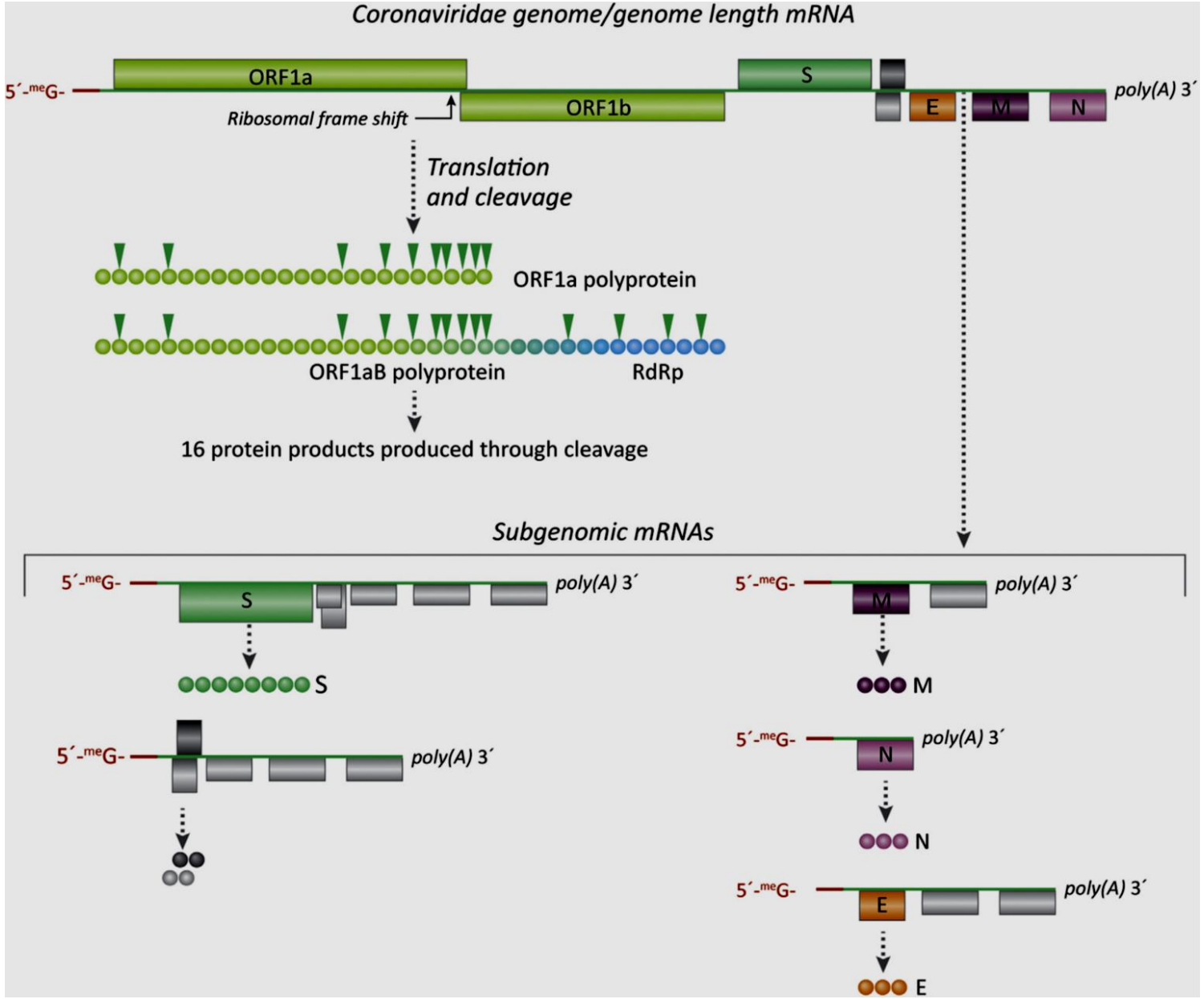


FIGURE : Torovirus genome organization.

Genomic organisation (2)



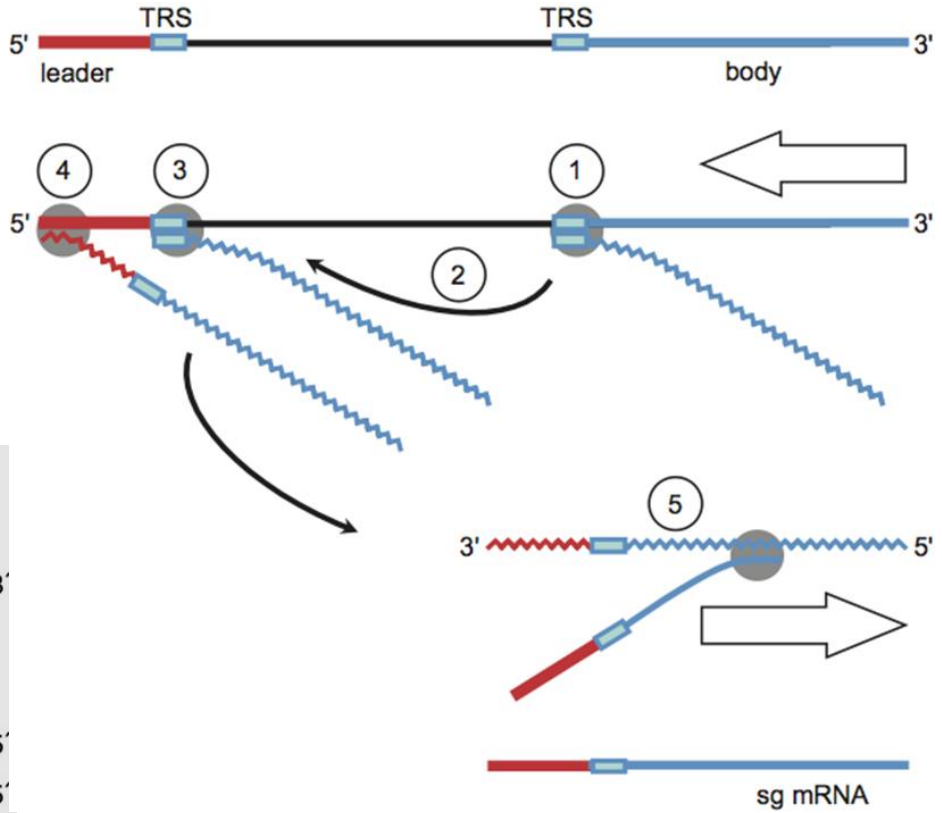
Replication

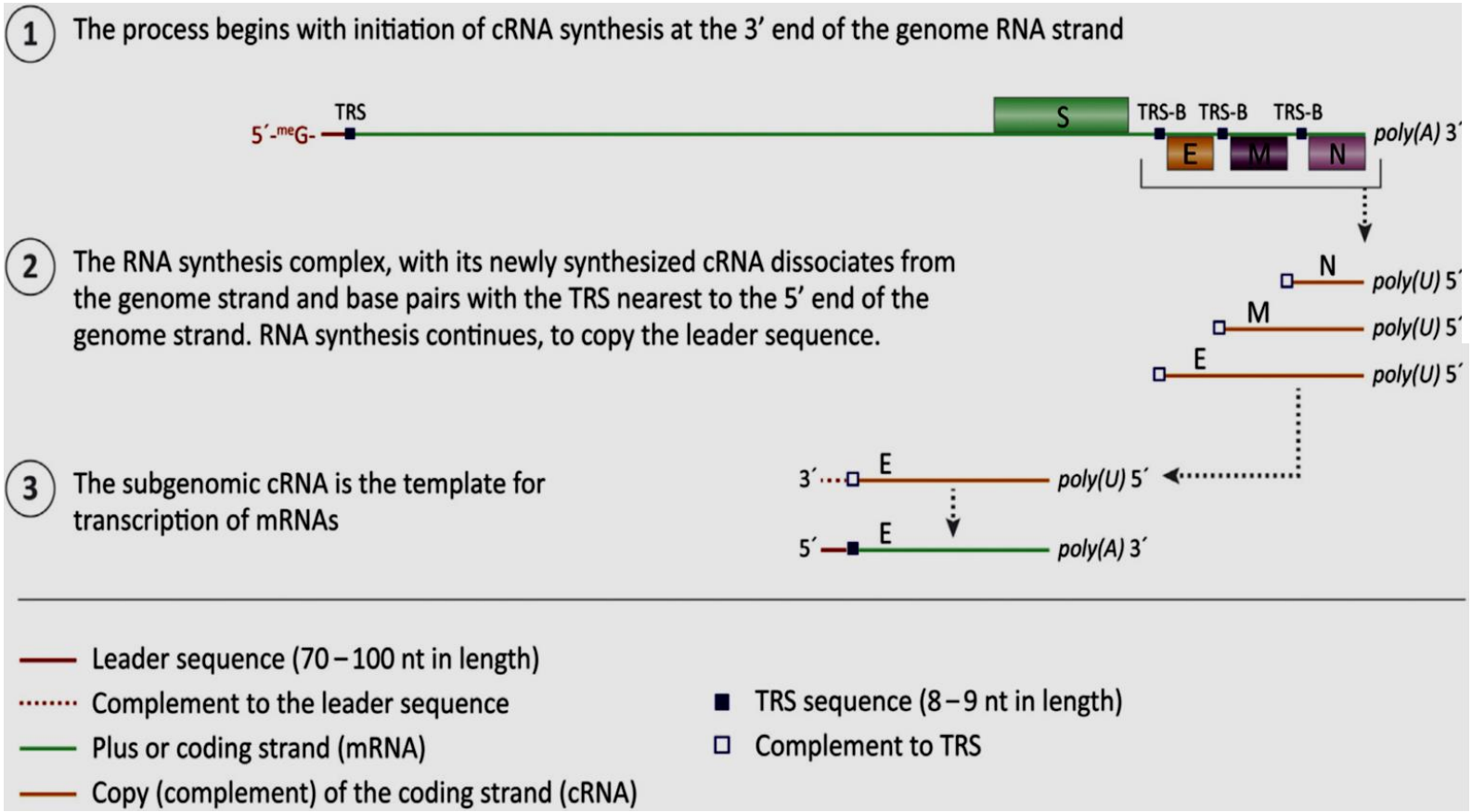
- Virions attach to dedicated host cell surface receptors via their spikes
- Release their genome into the target cell via fusion of the viral envelope with the plasma membrane and/or the limiting membrane of an endocytic vesicle.
- Replication cycle takes place in the cytoplasm and involves the production 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





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-

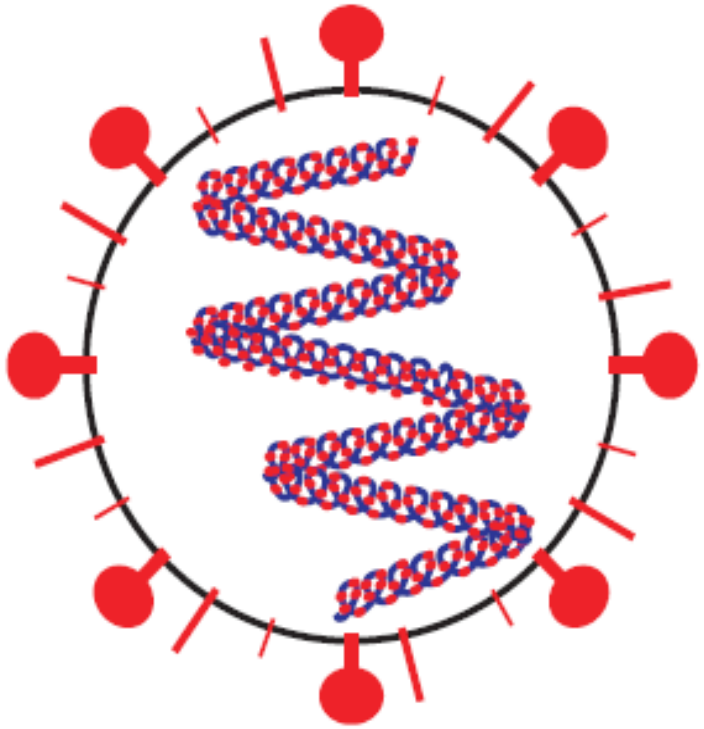
- Enveloped viruses
- Spherical ; diameter between 60 and 220 nm.
- Single stranded ; +ve sense RNA genome;
- largest of all groups of RNA viruses - 27.6 kb
- Lollipop-shaped projections sticking out from the surface like a crown
- Replicates in the cytoplasm and the RNA works directly as a messenger 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

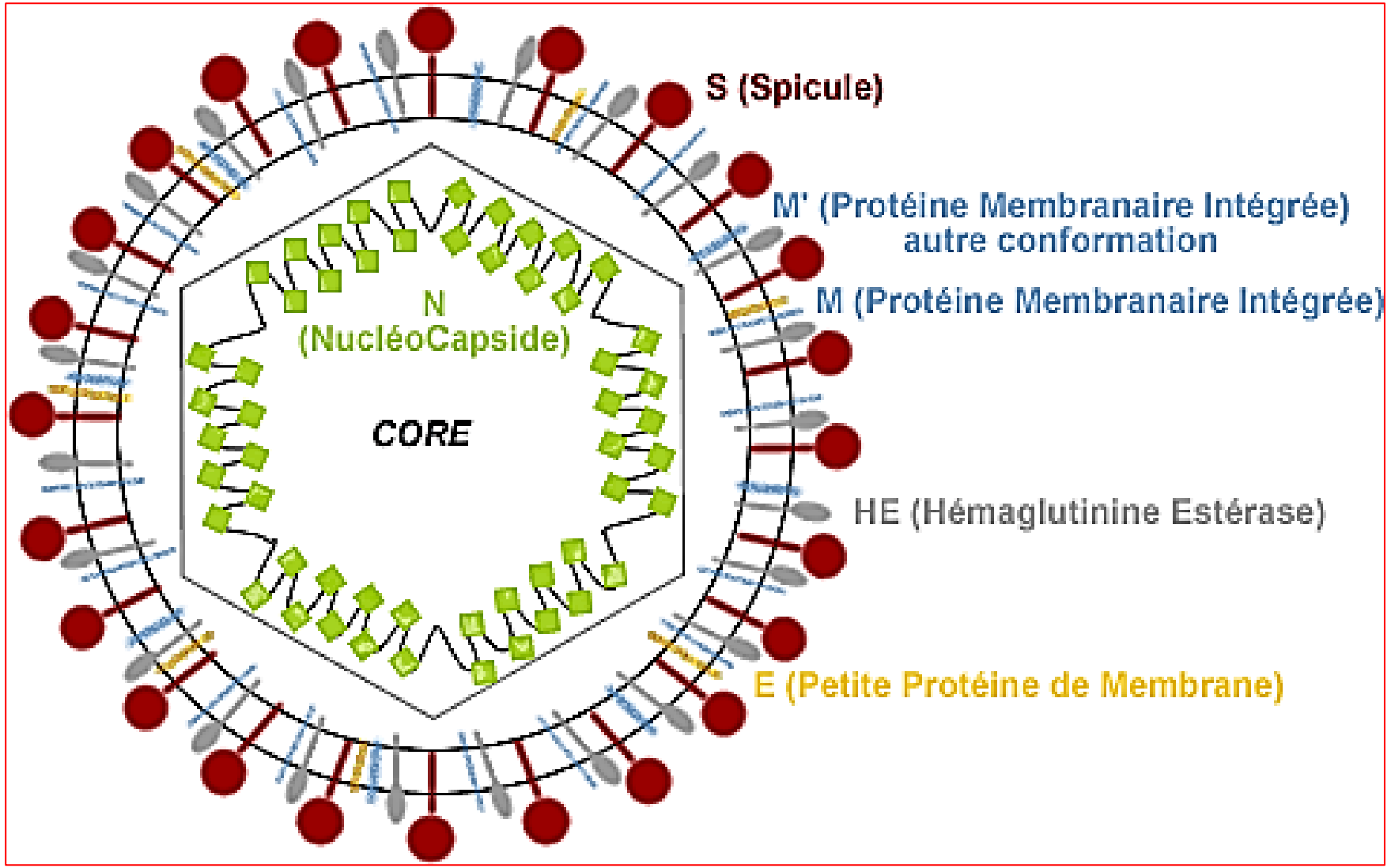
- IBV is a pleomorphic,
- enveloped
- virus with club-shaped surface projections (spikes)
- single-stranded, positive-sense RNA genome of >27 kb in length

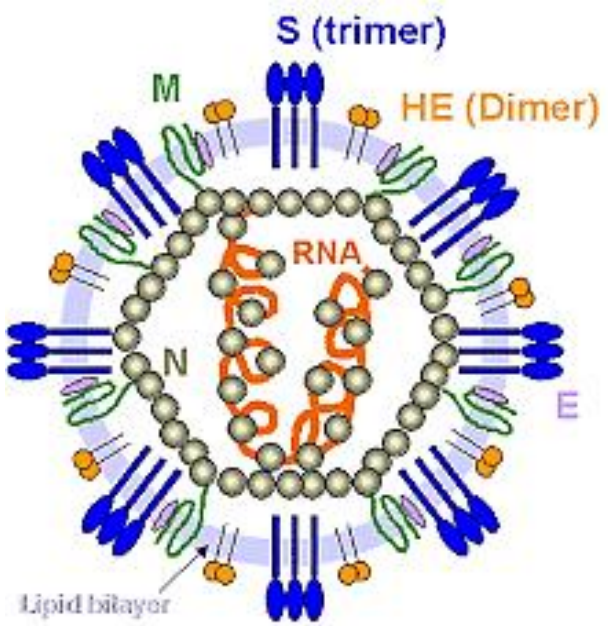
Coronaviridae



- Genome: ssRNA (plus strand)
- Capsid symmetry: helical
- Enveloped virion with a corona of glycoproteins
- Includes SARS coronavirus

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


S (Spicule)
M' (Protéine Membranaire Intégrée)
autre conformation
M (Protéine Membranaire Intégrée)
HE (Hémaglutinine Estérase)
E (Petite Protéine de Membrane)
N
(NucléoCapside)
CORE

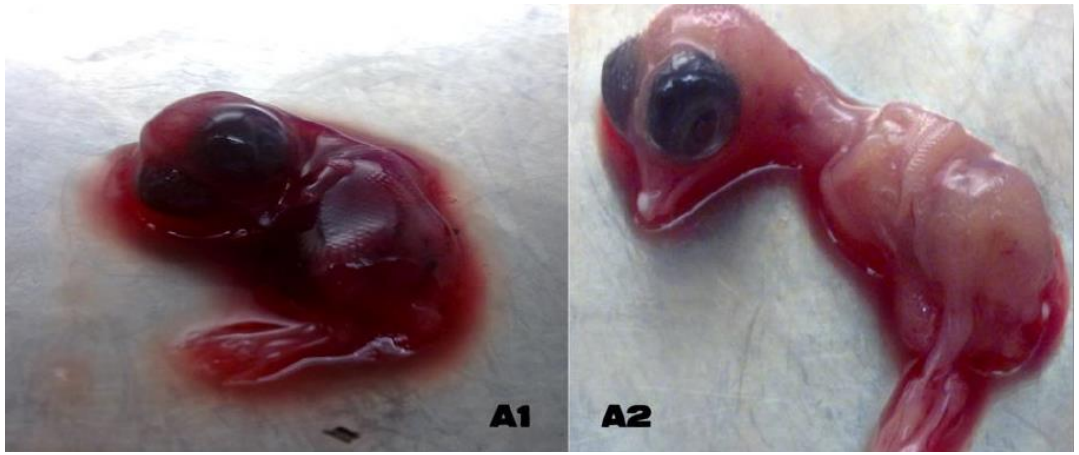

S (trimer)
M
HE (Dimer)
RNA
N
E
Lipid bilayer

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.

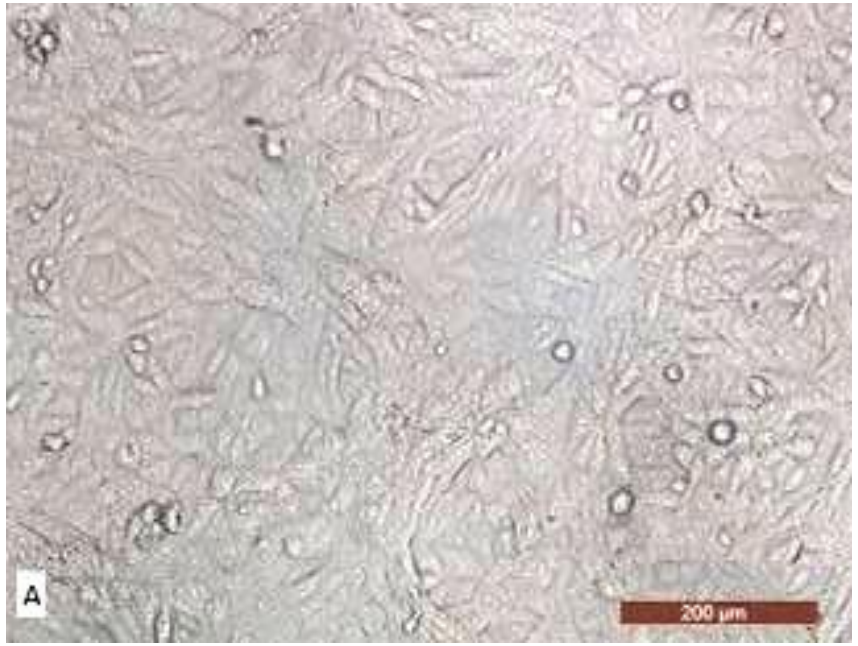
Cultivation

- 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*

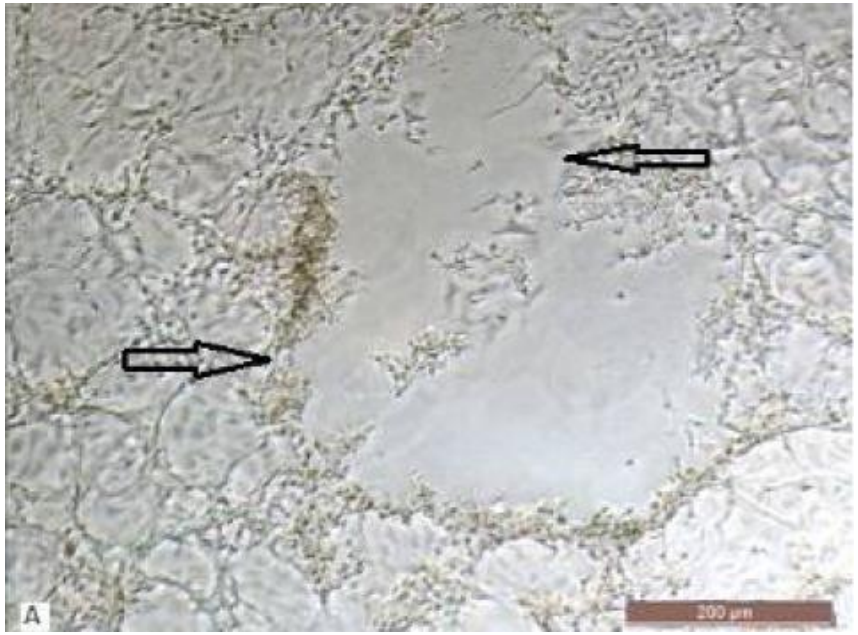


Cell culture

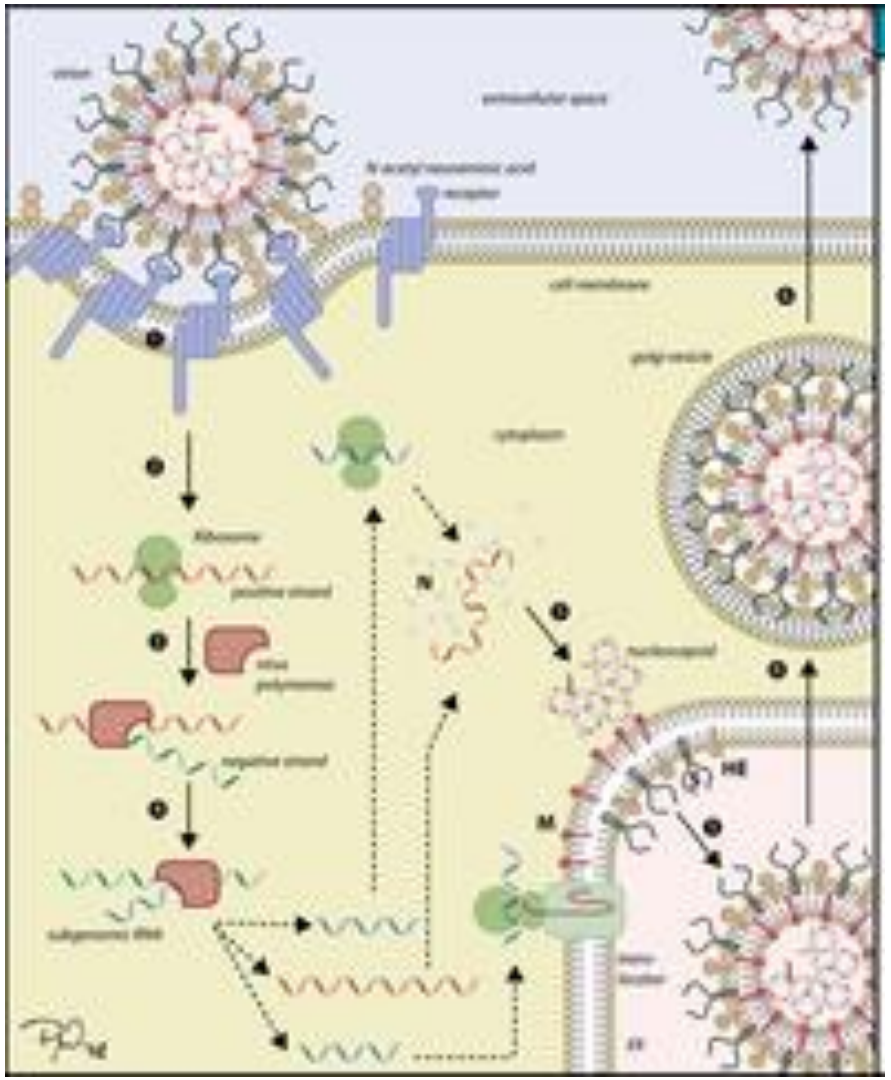
Chorioallantoic cells



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

  or

- bad quality shell

  or

- 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

Two types of vaccines are available

- Live attenuated –
  - administered in the forms of sprays or aerosols
- Inactivated or "dead", vaccines
  - administered by injection

Vaccination schedule

Type of vaccine	Earliest age	Route	Interval	Protection	Secondary vaccination	Species
Live: **milder strain**	1-3 weeks	Intra occular	1 week	Several weeks	8 weeks	Layer Breeder Broiler
Inactivated	16-20 weeks	Nasal	1week	Several weeks	16 weeks	Layer Breeder

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 trancriptase –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

Acknowledgement

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THANKS