



# Coronaviridae

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**WHO**

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Coronaviruses are **enveloped RNA viruses** that are broadly distributed among **humans, other mammals,** and **birds**, causing acute and persistent infections. Members of this family were isolated as early as the 1930s as the causative agents of infectious **bronchitis** in chickens, transmissible **gastroenteritis** in pigs, and severe **hepatitis** and **neurologic** disease in mice. ■

# History

- Common cause of respiratory and enteric diseases of humans and domestic animals
- Corona = Crown and Avian infectious bronchitis virus (IBV) was first observed by EM with an structure similar to crown
- First identified by this morphology in 1965 by Tyrrel and Bynoe
- **club-shaped spikes**  
Mouse hepatitis virus (MHV), human respiratory coronaviruses (HCoV), and coronaviruses of domestic animals were found to share the same structure and, later, the same genome organization and replication strategy



- **Human Corona Virus (HCV)**- two types have been identified. **HCV-229E is in serotype I** and causes **respiratory infections**. **HCV-OC43 is in serotype II and causes respiratory and enteric infections**.
- In 2002 the worldwide spread of a new human disease, severe acute respiratory syndrome (SARS)
- **In 2012** another zoonotic coronavirus crossed species from camels to cause the often-fatal human disease, Middle East respiratory syndrome (MERS)
- **Avian Infectious Bronchitis Virus (IBV)**- IBV is in **serotype III and causes respiratory and liver infections in chickens** and may lead to other disease states.
- **Mouse Hepatitis Virus (MHV)**- leads to a broad range of pathology in mouse infections.
- **Turkey Blue Comb Disease Virus**- one of the great names in virology



# Clasification

The coronaviruses are the largest group within the *Nidovirales* order comprises the families: *Coronaviridae*, *Arteriviridae*, *Roniviridae*, and *Mesoniviridae*.

The arteriviruses consist of five genera of mammalian pathogens. The roniviruses, which infect shrimp, invertebrate hosts *Coronaviridae* consists **two subfamily** : *Coronavirinae* and *Torovirinae* *Torovirinae* contains the Toroviruses, which are pathogens of **cattle, horses, swine** and **fish**.

Coronaviruses contains four genera: **alpha-, beta-, gamma- delta** It is based on phylogenetic clustering and pair-wise evolutionary distances in **seven key domains** of the replicase–transcriptase polyprotein.

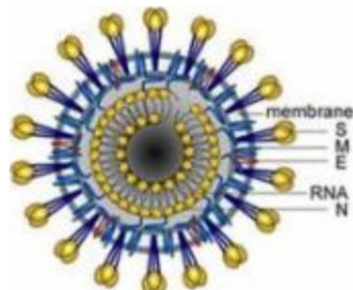
Within a genus, viruses are classified if they share more than **90%** amino acid sequence identity in the conserved replicase domains.

# Diseases in mammals and birds

## Relatively important till 2002

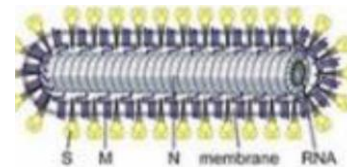
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TOROVIRUS

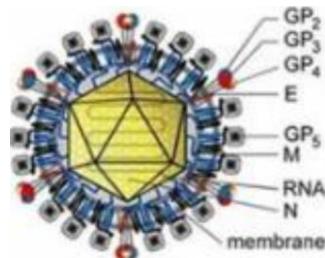


CORONAVIRUS

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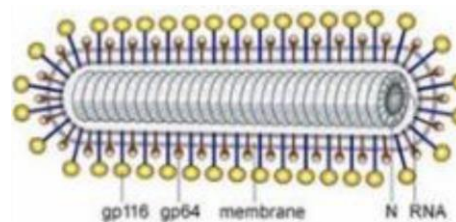


ARTERIVIRUS



RO IVIRUS

*Ronivirus*



# Classification of coronaviruses 1

Species <sup>a</sup>	GenBank Accession <sup>b</sup>	Viruses Included Within Species
<b>Genus <i>Alphacoronavirus</i></b>		
Alphacoronavirus 1	AJ271965	Transmissible gastroenteritis virus (TGEV)
	EU186072	Feline coronavirus type I (FCoV-I)
	AY994055	Feline infectious peritonitis virus (FIPV)
	GQ477367	Canine coronavirus (CCoV)
Human coronavirus 229E (HCoV-229E)	AF304460	
Human coronavirus NL63 (HCoV-NL63)	AY567487	
Porcine epidemic diarrhea virus (PEDV)	AF353511	
Mink coronavirus 1 (MCoV 1)	HM245925	
Wénchéng shrew virus (WESV)	KY967735	
Lucheng Rn rat coronavirus (LRNV)	KF294380	
Rhinolophus bat coronavirus HKU2 (Rh-BatCoV HKU2)	EF203067	
Scotophilus bat coronavirus 512 (Sc-BatCoV 512)	DQ648858	
Miniopterus bat coronavirus 1 (Mi-BatCoV 1)	EU420138	
Miniopterus bat coronavirus HKU8 (Mi-BatCoV HKU8)	EU420139	
Bat coronavirus HKU10 (BatCoV HKU10)	JQ989266	
Bat coronavirus CDPHE15 (BatCoV CDPHE15)	KF430219	



# Classification of coronaviruses 2

Species <sup>a</sup>	GenBank Accession <sup>b</sup>	Viruses Included Within Species
<b>Genus Betacoronavirus</b>		
Betacoronavirus 1	U00735	Bovine coronavirus (BCoV)
	EF446615	Equine coronavirus (EqCoV)
	AY903460	Human coronavirus OC43 (HCoV-OC43)
	DQ011855	Porcine hemagglutinating encephalomyelitis virus (PHEV)
	KF906249	Dromedary camel coronavirus HKU23 (DcCoV HKU23)
Murine coronavirus	AY700211	Mouse hepatitis virus (MHV)
	FJ938068	Rat coronavirus (RCoV)
Human coronavirus HKU1 (HCoV-HKU1)	AY597011	
Severe acute respiratory syndrome–related coronavirus (SARSr-CoV)	AY278741	Human SARS coronavirus (SARS-CoV)
	DQ071615	SARS-related <i>Rhinolophus</i> bat coronavirus Rp3 (SARSr-Rh-BatCoV Rp3)
Middle East respiratory syndrome–related coronavirus (MERS-CoV)	JX869059	
Hedgehog coronavirus (EriCoV)	KC545383	
<i>Tylonycteris</i> bat coronavirus HKU4 (Ty-BatCoV HKU4)	EF065505	
<i>Pipistrellus</i> bat coronavirus HKU5 (Pi-BatCoV HKU5)	EF065509	
<i>Rousettus</i> bat coronavirus HKU9 (Ro-BatCoV HKU9)	EF065513	
<b>Genus Gammacoronavirus</b>		
Avian coronavirus	AJ311317	Infectious bronchitis virus (IBV)
	EU022526	Turkey coronavirus (TuCoV)
Cetacean coronavirus	EU111742	Beluga whale coronavirus SW1 (BWCoV SW1)
	KF793824	Bottlenose dolphin coronavirus HKU22 (BdCoV HKU22)
<b>Genus Deltacoronavirus</b>		
Porcine deltacoronavirus (PDCoV)	JQ065042	
Munia coronavirus HKU13 (MuCoV HKU13)	FJ376622	
Bulbul coronavirus HKU11 (BuCoV HKU11)	FJ376620	
Thrush coronavirus HKU12 (ThCoV HKU12)	FJ376621	
White-eye coronavirus HKU16 (WECov HKU16)	JQ065044	
Common-moorhen coronavirus HKU21 (CMCoV HKU21)	JQ065049	
Widgeon coronavirus HKU20 (WiCoV HKU20)	JQ065048	
Night heron coronavirus HKU19 (NHCov HKU19)	JQ065047	

(ABLE Serof, a natural host, and diseases of coronavirus

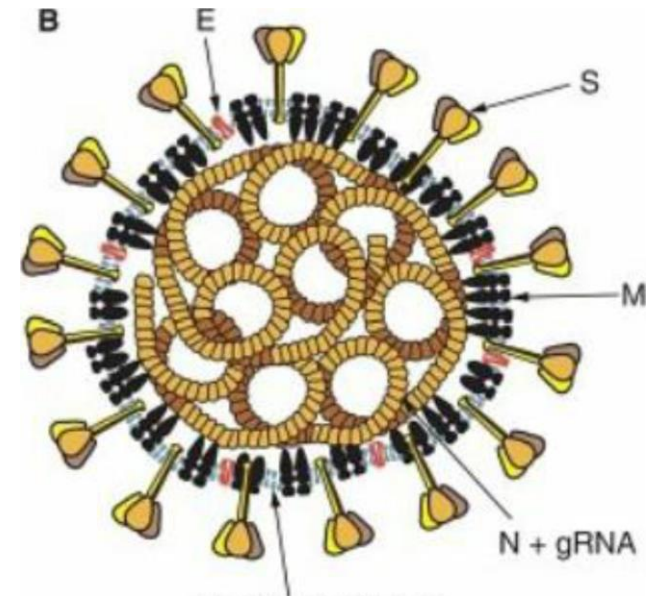
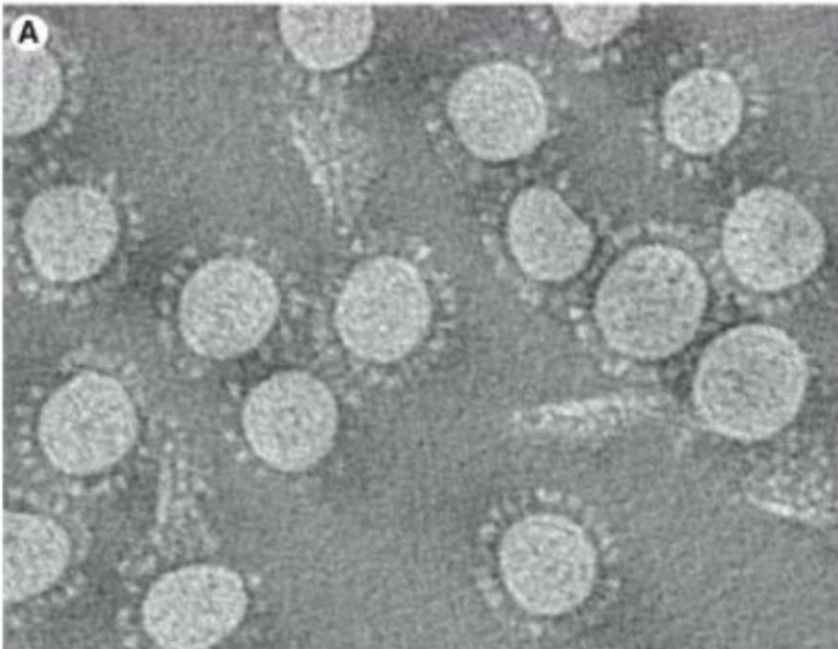
Agent	Host	Reservoir	Enteric infection	Hepatitis	Immunologic infection	Other
Human	Human	X			?	
Porcine	Pig	X	X			X
Canine	Dog	X	X			
Feline	Cat	X	X			
Canine	Cat	X	X	X	X	X
Rabbit	Rabbit	X	X			X
Human	Human	X	?		?	
Mouse	Mouse	X	X	X	X	
Rat	Rat					X
Pig	Pig	X	X		X	
Cow	Cow	X	X			
Turkey	Turkey	X	X			
Chicken	Chicken	X		X		X
Turkey	Turkey	X	X			

Other diseases caused by coronavirus include infectious peritonitis, immunologic disorders, and diarrhoea in pigs, pancreatitis in parrots, myocarditis in humans, and diarrhoea in calves. HCoV-229E human respiratory coronavirus; TGEV porcine transmissible gastroenteritis virus; PRCOV<sub>1</sub> porcine non-respiratory coronavirus; CECoV<sub>1</sub> canine coronavirus; FECOV<sub>1</sub> feline coronavirus; FIPV feline infectious peritonitis virus; RbCoV<sub>1</sub> rabbit coronavirus; HCoV-OC43 human non-respiratory coronavirus; HMV murine hepatitis virus; SDAV<sub>1</sub> swine dysentery virus; HEV porcine haemagglutinating encephalomyelitis virus; BCoV<sub>1</sub> bovine coronavirus; IBV<sub>1</sub> avian infectious bronchitis virus; TCoV<sub>1</sub> turkey coronavirus.

**Bats and birds** are ideally suited as reservoirs for the incubation, spread, and evolution of coronaviruses.

Six of human Coronaviruses: SARS-CoV and MERS-CoV.

The four HCoVs, the alphacoronaviruses HCoV-229E and HCoV-NL63 and the betacoronaviruses HCoV-OC43 and HCoV-HKU1, typically cause common colds.



# Lethal diseases by CoVs



Murine hepatitis virus (MHV)

(IBV)infectious bronchitis virus



Feline infectious peritonitis virus (alfa1) (FIPV)



SARS-CoV



MERS-CoV

SARS-CoV-2

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# Coronavirus Structure

Viral particles have average diameters of **80 to 120 nm**.

With **club-shaped spikes**

They have **helically symmetric nucleocapsids**.

Coronaviruses contain **four major structural proteins**: the spike (S), membrane (M), and envelope (E) proteins, all of which are located in the membrane envelope, and the nucleocapsid (N) protein, which is found in the ribonucleoprotein core.

The surface spikes of coronaviruses are composed of **trimers of S molecules** that bind to host cell receptors and mediate the earliest steps of infection. **S protein** can also induce cell–cell fusion in infection.

Two subunits: **S1 & S2**

**S1 subunits** low sequence homology across the four genera and often **diverge** considerably among different isolates of a single species.

**S2 subunits** are more highly conserved.

alpha- & deltacoronavirus S proteins are more closely related to each other





In many coronaviruses, the S protein is partially or completely cleaved by a **furin-like host cell protease**, separating the **S1** and **S2** subunits.

This cleavage is a **late event in virion assembly and release** from infected cells.

The most abundant structural protein is **M protein** (M monomer; 25-30 kDa). The M structures are likely to be **dimers**.

**E protein** is a small polypeptide, (8 to 12 kDa), in the **virion envelope**.  
E protein is **critical for coronavirus infectivity** (*Assembly and Release of Virions*)

E is **an integral membrane protein**, without cleavable signal peptide and is not glycosylated.

E protein have been assembled into **homo-oligomers**, from **dimers** - **hexamers**

Oligomerization is consistent with the **ion channel activity** of this protein.

**N protein** is the sole protein constituent of the **helical nucleocapsid** (38-52 kDa). **This protein bind along the RNA genome.**

**N protein** provides **no protection** for its genome against ribonucleases. The N protein is **a phosphoprotein**.

The role of phosphorylation is not clear, may be modulate RNA-binding affinity, transcription elongation, or N oligomerization.

**Hemagglutinin–esterase (HE) protein**, is found only in lineage A **betacoronaviruses**; MHV, BCoV, HCoV-OC43, and HCoV-HKU1 , (48-kDa HE monomer)

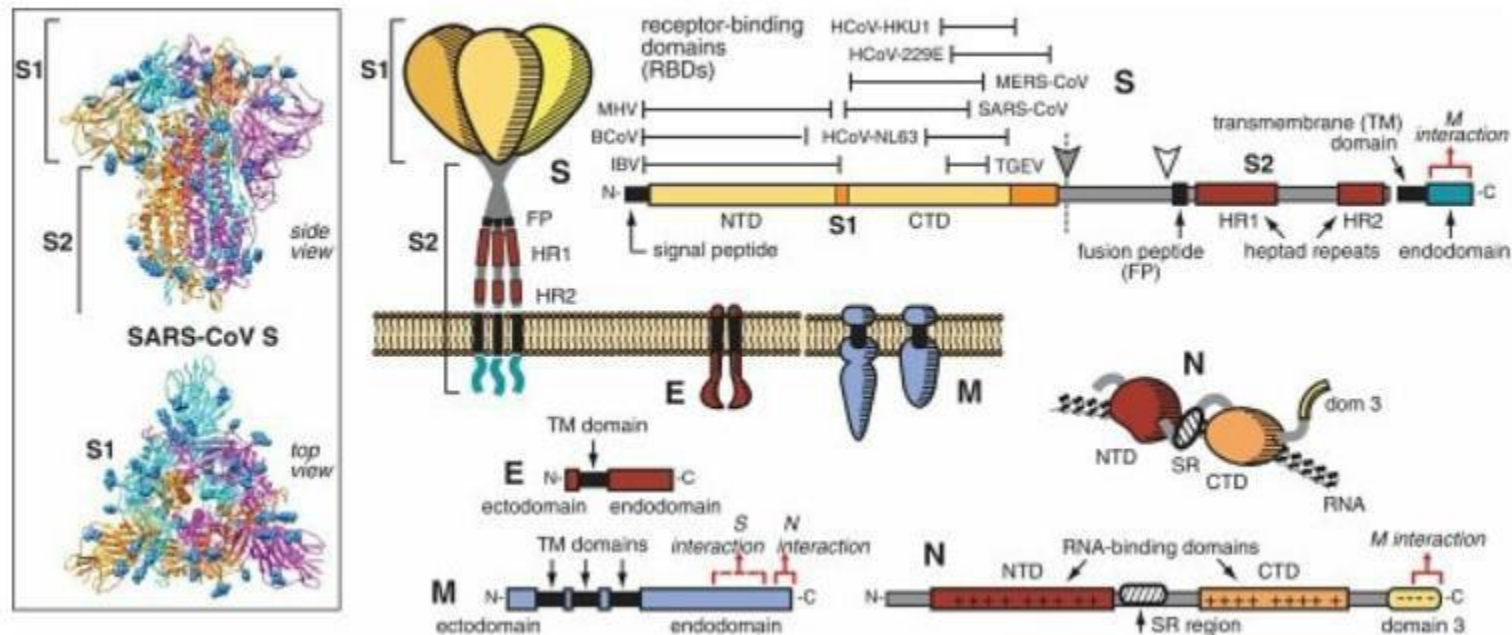
The mature protein is **a disulfide-linked homodimer**.

HE protein is a **hemagglutinin**; it is able to bind to **sialic acid** moieties found on cell-surface glycoproteins and glycolipids.

**HE** possesses **acetylsterase activity**.

These characteristics allow HE as **a cofactor for S protein**, assisting **attachment** of virus to host cells

## Virion structural proteins



## TABLE 2\ Properties and functions of coronavirus structural proteins

<p>Membrane glycoprotein N</p>	<p>Binds to viral RNA Nucleocapsid formation</p>	
<p>Membrane glycoprotein M (formerly E1)</p>	<p>1. non-cell-mediated immunity Membrane protein on Utricular Golgi apparatus Determines virus budding site Triggers virus particle assembly Has viral nucleocapsid interaction</p>	<p>Forms the shell of internal viral core (of TGEV and MHV) Induces apoptosis</p>
<p>Envelope (Small membrane) protein E (formerly S2)</p>	<p>Triggers virus particle assembly Associated with viral envelope</p>	<p>May cause apoptosis Forms large spikes on virion surface Induces cell-cell fusion</p>
<p>Spikes glycoprotein S (formerly E2)</p>	<p>Induces fusion of viral envelope with cell membranes (plasma membrane or endosome membrane) May induce cell-cell fusion</p>	<p>Binds to Fc fragment of immunoglobulin (e.g. TGEV) Binds to 90-acylneuraminic acid or N-glycosylated neuraminic acid Induces neutralizing antibody Elicits cell-mediated immunity</p>
<p>Membrane glycoprotein HE (formerly 13)</p>	<p>Forms small spikes on the virion surface Binds to 90-acylneuraminic acid Causes hemagglutination May cause hemadsorption Esterase cleaves acyl groups from 90-acylneuraminic acid</p>	

TGEV, porcine transmissible gastroenteritis virus; MHV, mouse hepatitis virus.

# GENOME STRUCTURE 1

The coronavirus genome, (25- 32 kb), is among the largest of all RNA viruses.

*Coronaviridae*, are **large, enveloped, positive-stranded RNA viruses** (27-32Kb), diameter (100-120)

RNA genome associates with the N (nucleocapsid) phosphoprotein to form a long, flexible, helical nucleocapsid

This exceptional RNA molecule acts in **at least three capacities**:

- as the **initial mRNA** of the infectious cycle (*Expression of the Replicase–Transcriptase Complex*),
- as the **template for RNA replication** and transcription (*Viral RNA Synthesis*),
- as the **substrate for packaging** into progeny viruses (*Assembly & Release*).

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# GENOME STRUCTURE 2

At the 5' end of the genome and subgenomic mRNAs are a sequence of 65 to 98 nucleotides, termed the *leader RNA*. An untranslated region (UTR) of 200 to 400 nucleotides follows this leader sequence.

At the 3' end of the RNA genome is another UTR of 200 to 500 nucleotides, followed by poly(A) of variable length.

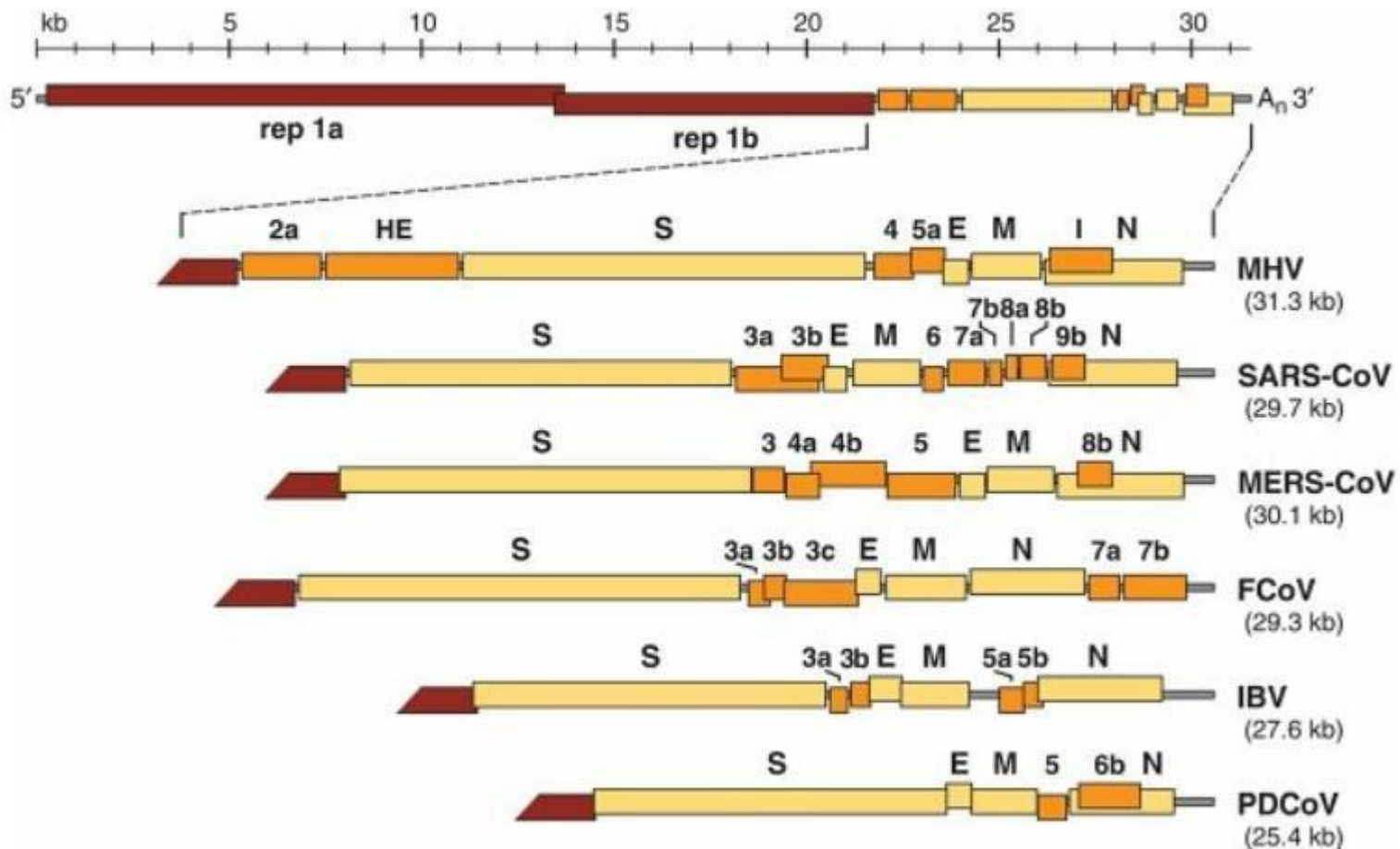
The sequences of both the 3'- and 5'-UTR are important for RNA replication and transcription.

The genomic sequence includes 7 to 10 ORFs.

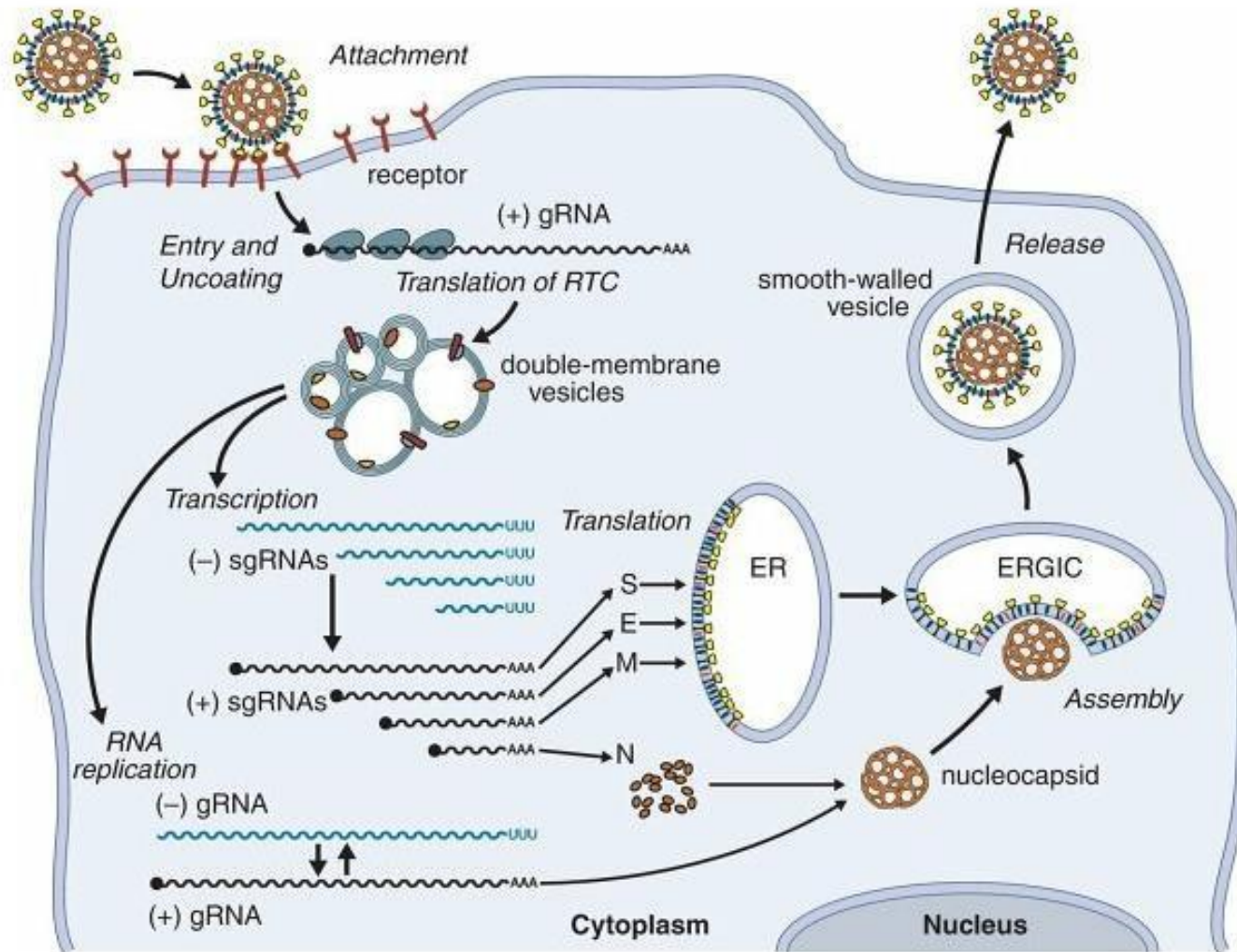
Gene 1 comprises 2/3 of the genome from the 5' end (20 to 22 kb). It consists of two overlapping ORFs (1a and 1b). These ORFs are translated into a polypeptide, which is the precursor of viral polymerase.

The order of the genes encoding the polymerase (*Pol*) and the four structural proteins that are present in all coronaviruses is 5'-*Pol-S-E-M-N*-3'.

# Coronaviruses Genome Organization



# Coronavirus Replication Cycle



# Coronavirus Replication Cycle

Coronavirus infection is initiated by virion binding to a cellular receptor. The more variable subunit, **S1**, is the part of the spike protein that binds to receptor.

Binding leads to **large conformational changes**, mediated by the more conserved **S2 subunit**, that result in the **fusion of virion and cell membranes**.

**The region of S1 that contacts the receptor, the receptor-binding domain (RBD)**, has been mapped to the **CTD** of S1 for SARS-CoV, MERSCoV, HCoV-HKU1, HCoV-NL63, TGEV, and HCoV-229E.

By contrast, the RBD for MHV maps to the **NTD** of its S1 subunit.

Many alpha-CoV and one deltacoronavirus use **aminopeptidase N (APN)** of their respective hosts as a receptor.

APN (CD13) is a cell-surface, zinc-binding protease that is resident in respiratory and enteric epithelia and in neural tissue.

# Coronaviruses Receptors

Virus <sup>a</sup>	Receptor	References
<b>Alphacoronaviruses</b>		
TGEV	Porcine aminopeptidase N <sup>b</sup> (pAPN)	117
PRCoV	Porcine aminopeptidase N (pAPN)	118
PEDV	(Unknown, but not pAPN)	386,257
FCoV-II, FIPV	Feline aminopeptidase N (fAPN)	420
FCoV-I	(Unknown, but not fAPN)	131
CCoV	Canine aminopeptidase N (cAPN)	35
HCoV-229E	Human aminopeptidase N (hAPN)	470
HCoV-NL63	Angiotensin-converting enzyme 2 (ACE2)	172
<b>Betacoronaviruses</b>		
MHV	Murine carcinoembryonic antigen-related adhesion molecule 1 (mCEACAM1)	450
BCoV	N-Acetyl-9-O-acetylneuraminic acid <sup>e</sup>	374
HCoV-OC43	N-Acetyl-9-O-acetylneuraminic acid <sup>e</sup>	226
SARS-CoV	Angiotensin-converting enzyme 2 (ACE2) <sup>c</sup>	258
MERS-CoV	Dipeptidyl peptidase 4 (DPP4) <sup>d</sup>	355
<b>Gammacoronaviruses</b>		
IBV	Alpha-2,3-linked sialic acid <sup>e</sup>	348
<b>Deltacoronaviruses</b>		
PDCoV	Porcine aminopeptidase N (pAPN)	253,442

b: Mammalian aminopeptidase N (APN) is also known as **CD13**

c: Human **CD209L** (L-SIGN), a **lectin family** member, can also act as a receptor for SARS-CoV but with much lower efficiency than **ACE2**

d: DPP4 is also known as **CD26** (MERS-Cov)



# Coronaviruses Receptors

The receptor for SARS-CoV, **angiotensin-converting enzyme 2 (ACE2)** is a cell surface, zinc-binding carboxypeptidase involved in regulation of cardiac function and blood pressure.

It is expressed in **epithelial cells of the lung and the small intestine**, the primary targets of SARS-CoV, as well as in **heart, kidney, and other tissues**.

The cryo-EM structure of the complete S trimer bound to **ACE2** indicates that receptor binding induces a conformational change in the **RBD** and may trigger the release of S1 subunits from S2 subunits .

The mutation of merely **two key RBD residues** facilitated civet-to human transmission and subsequent human-to-human transmission.

The receptor for MERS-CoV is dipeptidyl peptidase 4 (**DPP4 or CD26**)

DPP4 is a membrane bound **exoprotease** with a wide tissue distribution.

The critical residues in DPP4 are well conserved between DPP4 of humans and camels.

# Viral entry & un-coating

The entry of virions into cells results from large-scale **rearrangements of the S protein** that lead to the **fusion of viral and cellular membranes**

In most tissue culture cell types, **entry of Sars Cov is dependent upon cathepsins**, which are acid-activated endosomal proteases.

The infectivity of SARS-CoV, as well as many other coronaviruses, is thus suppressed by **cathepsin inhibitors**.

During endocytosis, an acid-dependent proteolytic cleavage of the S1 protein by **cellular proteases, like cathepsin, TMPRRS2**, and trypsin, exposes the S2 subunit, a fusion peptide that allows the fusion of the viral envelope with the endosome membrane, and **releases the capsid** into the cell cytoplasm.

# Expression of the Replicase–Transcriptase Complex

Following delivery of the viral nucleocapsid to the cytoplasm, the next event is the translation of **the replicase gene** from genomic RNA.

This gene consists of two large ORFs, rep 1a and rep 1b, which share a small region of overlap.

Rep 1a was translated to **pp 1a** that is cleaved in 11 nsps.

Rep 1b was translated to **pp1ab** which is cleaved into 15 nsps.

One or two **papain-like proteases (PLpro)**, which are situated within **nsp3**, carry out the separations of nsp1, nsp2, and nsp3.

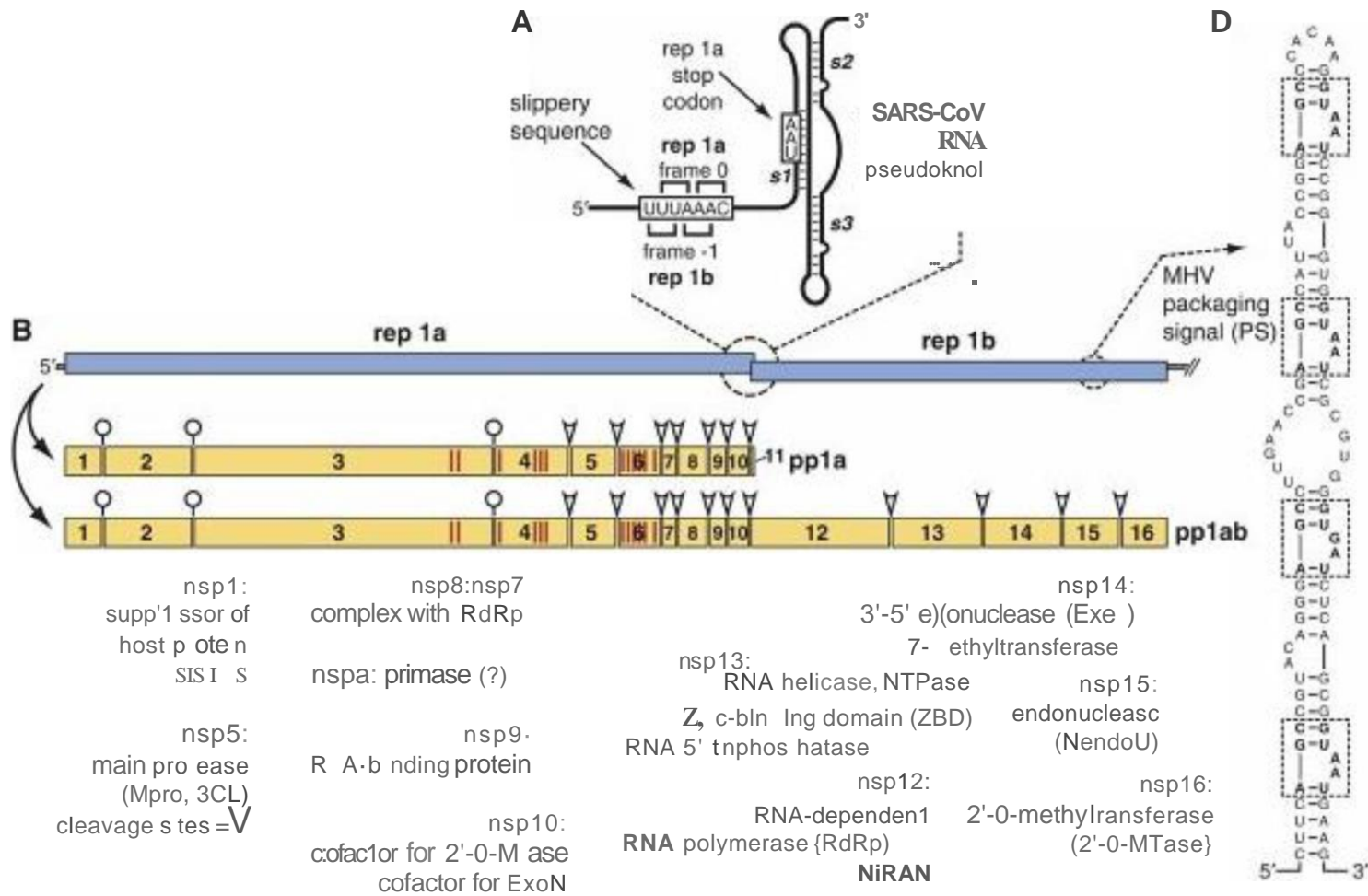
The **main protease (Mpro)**, **nsp5**, performs the remaining 11 cleavage events.

Because of the critical roles in infection, **PLpro** and **Mpro** present attractive **targets for antiviral drug design**.

The nsps assemble to form a **replicase-transcriptase complex (RTC)** responsible for RNA synthesis, replication, and transcription of nine subgenomic RNAs (sgRNAs).

The coronavirus **RdRp is nsp12**.

**nsp13** has RNA-stimulated NTPase (**helicase**) activity and unwinds RNA duplexes in the 5'-to-3' direction.



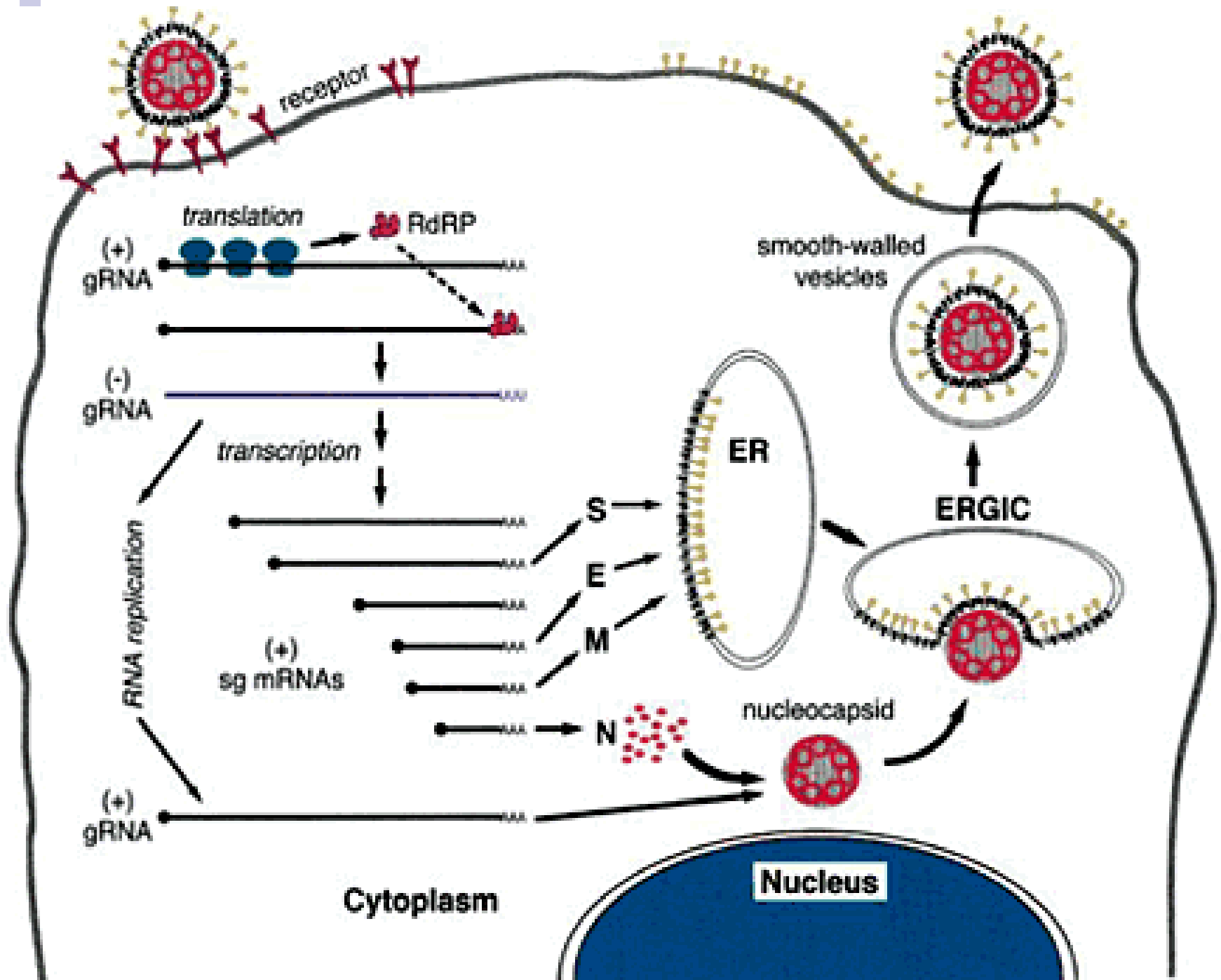
## Viral RNA Synthesis

Expression and assembly of the RTC sets the stage for viral RNA synthesis, a process resulting in the **replication of genomic RNA** and the transcription of **subgenomic RNAs** (sgRNAs).

The Pol products use the genomic RNA as a template to synthesize negative-stranded RNAs, which are used to synthesize genomic RNA and subgenomic mRNAs

**The high transmissibility of SARS-CoV-2** may be related to its entry into host cells. Although both SARS-CoV and SARS-CoV-2 glycoprotein S attach to ACE2 to enter the host cells, the **binding affinity of SARS-CoV-2 is higher**, thus enhancing its infectivity.





# Assembly and Release of Virions

The structural proteins S, E, and M are translated from the sgRNAs and forwarded to the endoplasmic reticulum (ER) and are subsequently inserted into an intermediate compartment of **ER with Golgi** (ERGIC). There, viral genomes are encapsulated by N proteins and assembled with the structural proteins to form virions.

The M proteins bind to E protein and nucleocapsid, and then, the S protein is incorporated, forming a complete virion.

Finally, the virions are transported to the cell surface in vesicles and released in a pathway mediated by exocytosis.

**S and HE** proteins that are not incorporated into virions are transported to the plasma membrane, where they may participate in **cell–cell fusion or hemadsorption**, respectively.

Virions are apparently released by **exocytosis-like fusion** of smooth-walled, virion-containing **vesicles with the plasma membrane**.

The entire cycle of coronavirus replication takes place in the cytoplasm.

# **PATHOGENESIS AND PATHOLOGY OF CORONAVIRUS INFECTIONS**

Most coronaviruses spread to susceptible hosts by **respiratory or fecal–oral** routes of infection, with replication first occurring in epithelial cells.

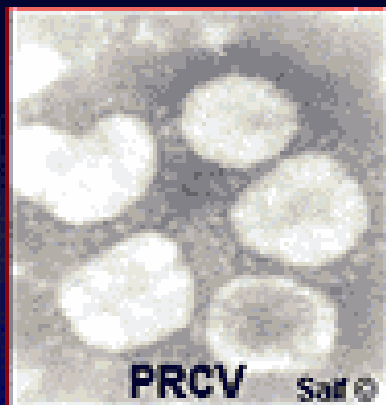
HCoV-OC43, HCoV-229E, HCoV-NL63, HCoVHKU1, and PRCoV, replicate principally in respiratory epithelial cells.

Other coronaviruses, including TGEV, BCoV, PHEV, CCoV, FCoV, and enteric strains of MHV, infect epithelial cells of the enteric tract.

Some of these viruses, such as TGEV, PEDV, and porcine deltacoronavirus (PDCoV), cause severe diarrhea, and sometimes fatal, in young animals.

SARS-CoV and MERSCoV spread from the upper airway to cause a **severe lower respiratory tract infection**.

**Feline infectious peritonitis (FIP)** spreads systemically to cause a generalized lethal disease in felines.



## Porcine Coronaviruses Group I



### Enteric Infections

**TGEV** : Transmissible Gastroenteritis Virus (1965)

Infect all age groups but highest mortality in baby pigs

**PEDV** : Porcine Epidemic Diarrrhea Virus (1978, Europe; 1980's, Asia)

Older pigs (>3 month) more commonly infected

### Respiratory Infections

**PRCV** : Porcine Respiratory Coronavirus (1986, Europe; 1989, USA)  
S gene deletion mutant of TGEV (621 – 682 bp near the N-terminus)

Infect all age groups - 1- 3-month-old, most disease



# Human Coronavirus Infections

## Human Coronaviruses, Other Than SARS-CoV and MERS-CoV, Associated with Respiratory and Enteric Disease

Prior to 2003, HCoV-229E were primarily considered to be agents of URT disease and to cause little mortality.

HCoV-229E and HCoV-OC43 were isolated from patients with URT infections in the 1960s.

In contrast, HCoV-229E **does not cross species** and does not infect mice.

HCoV-NL63, which causes **mild respiratory disease**, displays homology with HCoV-229E.

HCoV-HKU1, isolated from an adult patient in Hong Kong with pneumonia and causes **mild respiratory disease**.

# Coronaviruses diseases

Virus*	Host Species	Sites of Infection	Clinical Disease
<b>Alphacoronaviruses</b>			
CCoV	Canine	GI tract	Gastroenteritis
FeCoV	Felidae	GI tract, respiratory	Gastroenteritis
FIPV	Felidae	Systemic disease	Peritonitis, wasting disease
HCoV-229E	Human	Respiratory	Upper respiratory tract infection
HCoV-NL63	Human	Respiratory	Upper respiratory tract infection, croup
PEDV	Swine	GI tract	Gastroenteritis
TGEV	Swine	GI tract, respiratory	Gastroenteritis
BatCoV	Bat	GI tract, respiratory	Unknown
Rabbit CoV	Rabbit	Heart, GI tract, respiratory	Enteritis, myocarditis
SADS-CoV	Swine	GI tract	Gastroenteritis
<b>Betacoronaviruses</b>			
BCoV	Bovine, ruminants	GI tract, respiratory	Enteritis, upper and lower respiratory tract infection
HCoV-OC43	Human	Respiratory	Upper respiratory tract infection
HCoV-HKU1	Human	Respiratory	Upper and lower respiratory tract infection
MHV	Mouse, rat	GI tract, liver, brains, lung	Gastroenteritis, hepatitis, encephalitis, chronic demyelination
PHEV	Swine	Respiratory, brain	Vomiting, wasting, encephalomyelitis
RCoV	Rat	Respiratory, salivary and lachrymal glands, urogenital tract	Respiratory tract infection, metritis, sialodacryoadenitis
SARS-CoV	Human	Respiratory, GI tract	Pneumonia (SARS)
MERS-CoV	Camel, human	Respiratory tract	Pneumonia (MERS)
BatCoV	Bat	GI tract, respiratory tract	Unknown
<b>Gammacoronaviruses</b>			
IBV	Chicken	Respiratory, kidney	Bronchitis, nephritis
TuCoV	Turkey	GI tract	Gastroenteritis
BWCoV	Beluga whale	Respiratory tract	Pneumonia, hepatitis
<b>Deltacoronaviruses</b>			
ThCoV	Thrush	Respiratory, GI tract	Unknown
PDCoV	Swine	GI tract	Gastroenteritis



# SARS-CoV Infections

SARS-CoV causes severe respiratory disease, infects both upper airway and alveolar epithelial cells, resulting in **mild to severe lung injury**.

Virus or viral products are also detected **in kidney, liver, and small intestine**, and in **stool**.

Pathological findings in patients who died from SARS were nonspecific.

Cells in the upper airway were initially infected, virus rapidly spread to the alveoli, causing diffuse alveolar damage, alveolar edema, inflammatory cell infiltration, and **hyaline membrane** formation.

Alveolar damage progressed, eventually resulting in pathological signs of **ALI** (acute lung injury) and, in the most severe cases, **ARDS** (acute respiratory distress syndrome).

**Multinucleated giant cells**, originating either from macrophages or respiratory epithelial cells, were detected in autopsy specimens.

MHV and FIPV, SARS-CoV infects **macrophages and dendritic cells**.

# SARS-CoV Infections

Several **proinflammatory cytokines and chemokines**: IP-10 (CXCL10), MCP-1 (CCL2), MIP-1a (CCL3), RANTES (CCL5), MCP-2 (CCL8), TNF, and IL-6, are expressed by **infected dendritic cells**.

**Lymphopenia and neutrophilia** were detected in infected patients.

SARS-CoV infects several species of animals, including mice, ferrets, hamsters, cats, and monkeys, but most of these animals develop either mild or no clinical disease, making them not useful for studies of lethal SARS.

Rodent-adapted strains that cause severe disease in some strains of mice and rats were used for studying.

Mice infected with a lethal dose of SARS-CoV were protected if IFN-I expression was genetically deleted or if IFN signaling was blocked with antibody, suggesting that **IFN-I contributed to severe disease**.



# MERS-CoV Infections

MERS-CoV was first isolated in 2012 from a patient in Saudi Arabia with **severe human respiratory disease**.

MERS-CoV introduced into human from **infected camels**.

Similar to SARS, the infection begins in the URT with spread to the lower airway. Patients are most contagious after developing pneumonia.

Infection of **macrophages & dendritic cells** results in a strong inflammatory response.

MERS-CoV actively replicates in activated **human T cells**.

MERS-CoV causes mild disease in experimentally infected camels, macaques, and rabbits but does not infect mice or rats.

# Immune Responses

As in most viral infections, both **the innate and adaptive** arms of the immune response are required for successful virus clearance.

One of the first steps in the host immune response to a coronavirus infection is the production of type 1 IFN (IFN- $\alpha/\beta$ ).

DCs & macrophages are the source for production of IFN- $\alpha/\beta$  in infected hosts.

Once the initial IFN response is induced, virus clearance requires expression of proinflammatory cytokines and chemokines and their receptors, such as CCL2, CXCL9, CXCL10, and CCL3.

In the absence of neutrophils or of neutrophil chemoattractants, such as CXCL1 and CXCL2, breakdown does not occur, resulting in more severe disease.

A robust T-cell response is required for destruction of infected cells and clearance of infectious virus.

Virus is not cleared in MHV- or SARS-CoV–infected mice that lack T cells.

The majority of CD4 and CD8 T-cell epitopes are located on the N, M, and S proteins.

Once virus has been cleared, the proinflammatory response must be controlled to prevent immunopathology.

# Immune Responses

IL-10, anti-inflammatory factor important for minimizing immunopathological changes.

**T cells** are responsible for initial virus clearance, but an effective **antivirus antibody response** is required to prevent virus recrudescence.

**Neutralizing antibody response** was detected in survivors during the 2002– 2003 SARS outbreak and in MERS survivors who had severe disease.

Coronaviruses use several approaches, to evade the host IFN response.

Coronaviruses replicate in double-membraned vesicles (DMVs) which may shield viral RNA from recognition by intracellular sensor molecules , such as RIG-I, MDA5, and TLR3. Thus, in fibroblasts or DCs infected with MHV or SARS-CoV, no IFN is induced .

**Nsp3**, NSP15, NSP16 prevent IFN induction.

**Nsp1** enhances host cell mRNA degradation and inhibits host cell protein synthesis, with specific effects on IFN signaling.

# Host Immune Response Against SARS-CoV-2

After entry to host cells, viral antigens get presented APCs to virus-specific cytotoxic T lymphocytes (CTL).

SARSCoV-2 infected patients showing the **reduction in CD4+ and CD8+ T cell counts**.

SARS-CoV-2 patients have been found to present with **acute respiratory distress syndrome (ARDS)**.

ARDS is a **cytokine storm syndrome (CSS)** which is a **lethal uncontrollable inflammatory response** resulting from the release of large pro-inflammatory cytokines (IL-1 $\beta$ , IFN- $\alpha$ , IFN- $\gamma$ , IL-12, IL-6, IL-18, TNF- $\alpha$ , IL-33, TGF $\beta$ , etc.) and chemokines (CCL3, CCL2, CXCL8, CCL5, CXCL9, CXCL10, etc.) by immune cells.



# Development of Antibodies and Immunity in Sars Cov-2

All immunocompetent persons develop an **adaptive immune response** following SARS-CoV-2 infection, including **B and T cell-mediated immunity** due to antiviral **humoral and cellular immune responses**.

The humoral response includes **antibodies directed against S and N proteins**.

**RBD** is the main target for neutralizing antibodies.

Antibodies – including IgM, IgG, and IgA – against S and its subunits can be detected within **1-3 weeks after infection**.

How long anti-SARS-CoV-2 antibodies persist after infection remains unknown.

IgG antibodies, including IgG against the S and N proteins, persist for at least several months in most persons.

Persons with **more severe disease** appear to develop **a more robust antibody response** with IgM, IgG, and IgA all achieving **higher titers** and exhibiting **longer persistence**.

# Development of Antibodies and Immunity in Sars Cov-2

Both IgM and IgG may be detected **around the same time after infection**. While **IgM** is most useful for determining **recent infection** as it usually becomes undetectable weeks to months following infection, **IgG** may remain detectable for **longer periods**.

IgA is important for **mucosal immunity** and can be detected in mucous secretions like saliva in addition to blood.

Tests that detect binding antibodies:

- **Point-of-care (POC)** tests that detect IgG and IgM, or total antibody in fingerstick whole blood.
- **Laboratory tests** use ELISA or chemiluminescent immunoassay (CIA) methods for antibody detection in serum, plasma, whole blood.
- **Neutralizing antibody detection tests** determine the functional ability of antibodies to prevent infection by virus *in vitro*.

# Development of Antibodies and Immunity in Sars Cov-2

SARS-CoV-2 neutralizing antibodies that inhibit viral replication *in vitro* mainly target the **RBD**.

**SARS-CoV-2 reinfection** has been documented; however, studies indicate that persons with anti-SARS-CoV-2 antibodies are less likely to develop subsequent infection than persons without such antibodies.

Another British cohort study found **an 83% reduction** in SARS-CoV-2 infection incidence **over a five-month period** among persons who had tested **antibody positive** for SARS-CoV-2 or had prior infection documented by RT-PCR.

A large study in the United States of laboratory results found a **90% reduction** in infection among persons with antibody compared to persons without.

While life-long immunity has not been observed with endemic seasonal coronaviruses, studies of persons infected with the SARS-CoV-1 and MERS-CoV coronaviruses demonstrated **measurable antibody for 18–24 months** following infection, and **neutralizing antibody was present for 34 months**.

# Genetic Diversity of Coronaviruses

The SARS and MERS outbreaks demonstrated the ability of coronaviruses to cross species.

Porcine hemagglutinating encephalomyelitis virus PHEV diverged from HCoV-OC43 and BCoV 100 to 200 years ago.

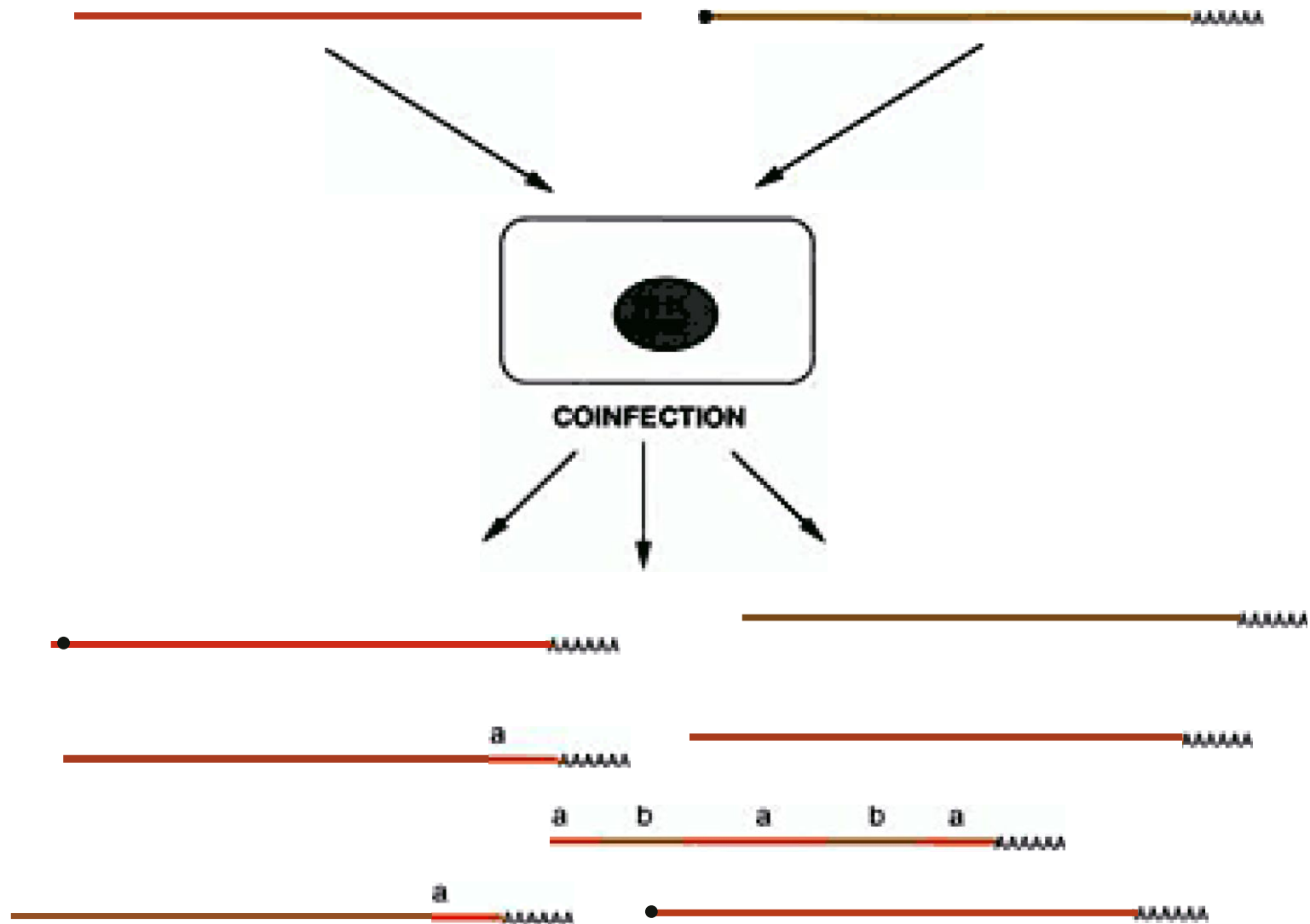
BCoV has crossed species to infect many ruminants.

Other phylogenetic studies suggest that the porcine alphacoronavirus TGEV resulted from cross species transmission of a canine coronavirus.

A bat coronavirus, HKU2, was shown to cross species to cause gastroenteritis in pigs (swine acute diarrheal syndrome [SADS]).

PDCoV, a cause of swine diarrhea, is classified as a deltacoronavirus, a genus of avian viruses. PDCoV appeared to cross from avian to mammalian species, using APN as a receptor. In addition to their ability to cross species, coronaviruses readily undergo recombination.

# RNA recombination in coronaviruses



# CLINICAL FEATURES

Clinical features of respiratory infections in humans follow two distinct patterns, one for non-SARS-CoV/MERS-CoV (i.e., HCoV-229E, HCoVNL63, HCoV-OC43, HCoV-HKU1) and one for the zoonotic coronaviruses, SARS-CoV and MERS-CoV.

Studies PCR to detect viral RNA in **middle ear fluids** suggest that coronaviruses, like other respiratory viruses, can cause **otitis**.

HCoV-NL63 and HCoV-HKU1 have been detected in persons with **acute upper and lower respiratory tract illness**.

HCoV-NL63 is associated with **croup** in children under the age of 3.

**Reinfection** with coronaviruses is **common**, indicating that infection **does not induce stable protective immunity**.



# CLINICAL FEATURES- SARS-CoV Infections

SARS-CoV always resulted in a serious lower respiratory tract illness that required hospitalization.

In 2002-2003 epidemic, 8,000 individuals were infected, with a mortality rate of 10%. Disease severity increased proportionally to age.

About 50% of infected individuals greater than 60 years.

Illness had an incubation period of 10 to 14 days.

Virus particles and RNA were detected in several organs including the gastrointestinal tract, kidneys, and brain.

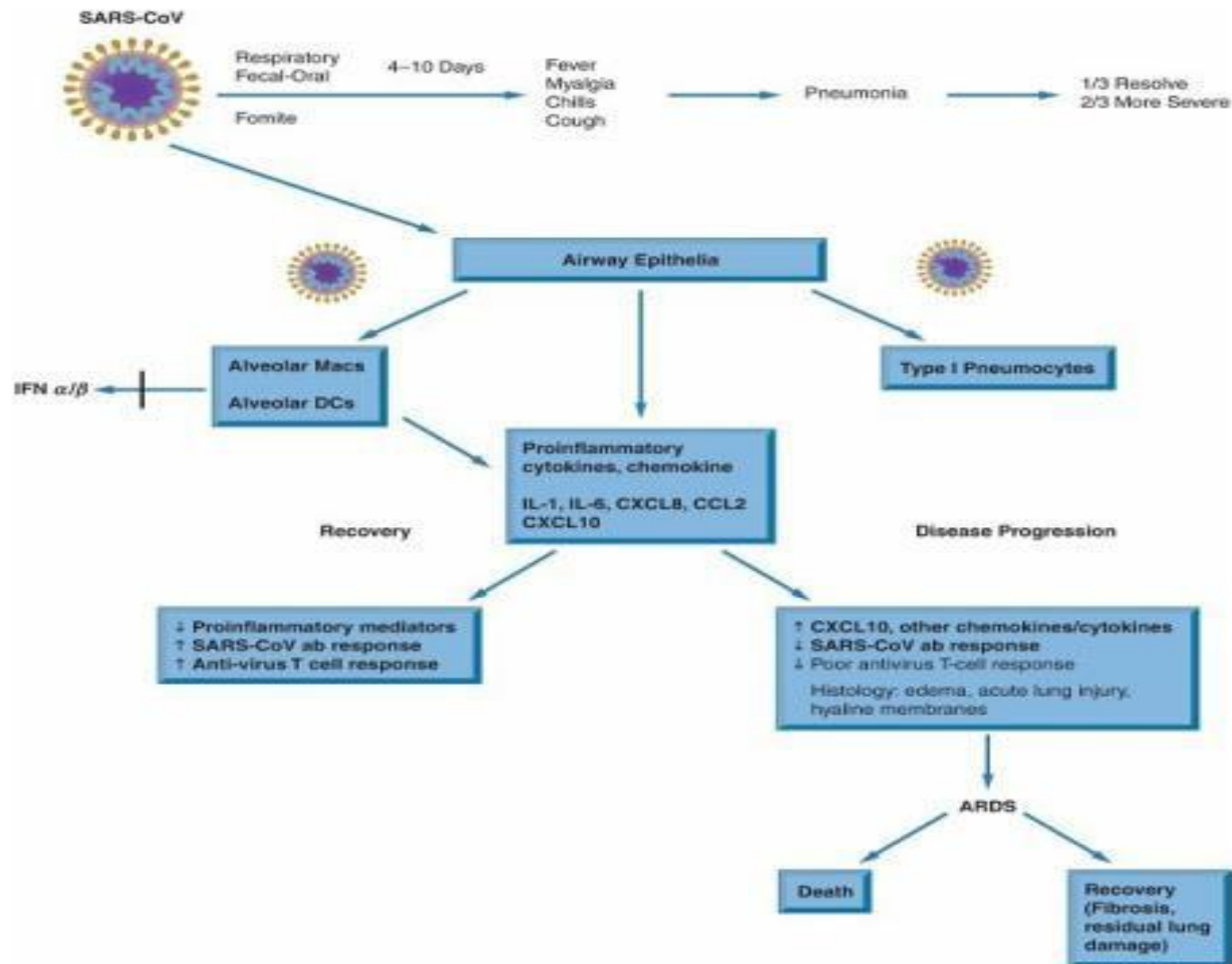
Diarrhea occurred at disease onset in 25% of patients, up to 70% developed gastrointestinal disease during the illness.

Most patients developed abnormal liver function tests and lymphopenia.

Lymphocyte and platelet counts remained abnormally low.

Elevated proinflammatory cytokines for prolonged periods of time.

# Clinical disease in patients infected with SARSCoV which spread to susceptible individuals via respiratory and fecal–oral routes



# CLINICAL FEATURES- SARS-CoV Infections

In patients who **recovered**, expression of proinflammatory cytokines diminished, and **robust antiviral antibody responses** were detected.

In patients who **developed progressively more severe disease**, cytokine production continued, and patients remained lymphopenic without developing an effective anti-SARS-CoV antibody response.

**MERS-CoV Infections causes** ranges from asymptomatic to severe, with an overall mortality of approximately 36%.

Fever, cough, difficult breathing, and diarrhea, Renal damage is common in MERS patients.

**Kidney damage** result from direct infection, because levels of **renal DPP4** are **high**.

# DIAGNOSIS

Most human coronavirus infections, other than SARS and MERS, are not diagnosed because they cause mild, self-limited upper respiratory disease, and no specific therapy is available.

Coronavirus infections in animals and humans were initially diagnosed by isolation of infectious virus, by EM and using serologic assays.

HCoV-229E, HCoV-OC43, and HCoV-NL63 can be grown in tissue culture cells, but HCoV-HKU1 has been grown only in primary human airway epithelial cells.

**RT-PCR** methods and immunofluorescence assays (**IFAs**) for virus antigen are the gold standards for diagnosis of respiratory coronavirus infections.

A variety of serologic assays have been used to detect URT-associated coronavirus infections, including **complement fixation**, **hemagglutination inhibition (HI)** for viruses with an HE protein (i.e., some betacoronaviruses), **neutralization** and **immunofluorescence (IFA)** assays, and **ELISA**.

# DIAGNOSIS

SARS-CoV was initially isolated in tissue culture cells, but during 2003 epidemic, a **combination of serologic and RT-PCR assays**, not virus culture, was used to detect SARS-CoV infection.

SARS-CoV N protein ELISA were positive in 50% to 80% of serum specimens collected during the 1st week of illness and in more than 50% of respiratory and stool specimens collected during the 2nd and 3<sup>rd</sup> weeks of illness.

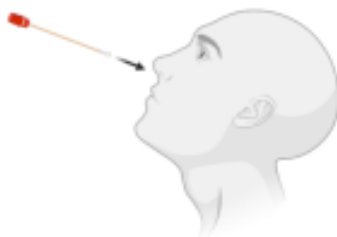
SARS-CoV–specific **antibodies** were usually detected **4 weeks after infection**. A **quantitative RT-PCR** assay is used for diagnosis, with confirmation by sequencing of PCR products.

Isolation of infectious MERS-CoV requires a BSL3 laboratory. Even in patients with more severe disease, **antibody responses rapidly wane**.

# COVID-19 Molecular Diagnostic Test through RT-PCR

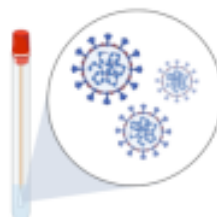
## 1 Nasopharyngeal (NP) or Oropharyngeal (OP) swab

Cotton swab is inserted into nostril to absorb secretions. <15 min



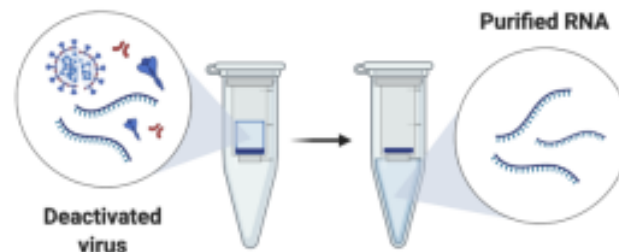
## 2 Collected specimen 0-72 h

Specimen is stored at 2-8°C for up to 72 hours or proceed to RNA extraction.



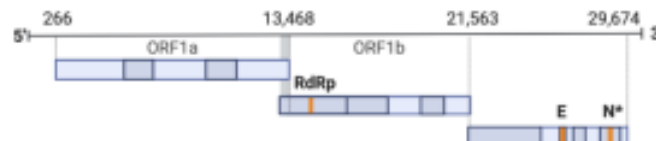
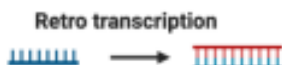
## 3 RNA extraction ~45 min

Purified RNA is extracted from deactivated virus.



## 4 RT-qPCR ~1 h per primer set

Purified RNA is reverse transcribed to cDNA and amplified by qPCR.



### Example primers and probes for screening

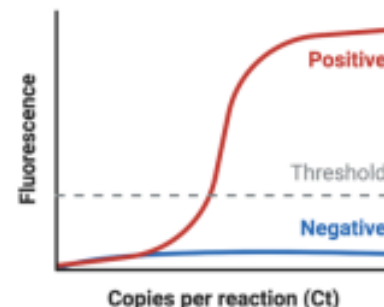
E\_Foward: ACAGGTACGTTAATAGTTAATAGCGT  
E\_Probe1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ  
E\_Reverse: ATATTGCAGCAGTACGCACACA  
  
RdRp\_Foward: GTGARATGGTCATGTGTGGCGG  
RdRp\_Probe1: FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ  
RdRp\_Probe2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ  
RdRp\_Reverse: CARATGTTAAASACACTATTAGCATA

E gene  
First-line  
screening tool  
  
RdRp gene  
Confirmatory  
testing

Primer sequences are for illustrative purposes only.

## 5 Test results real-time

Positive SARS-CoV2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).

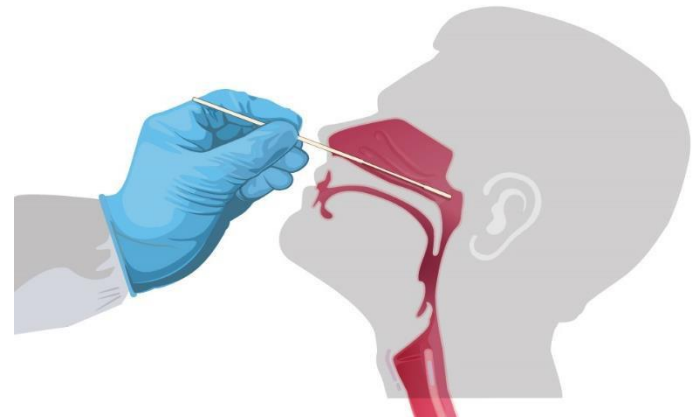






# Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing

- Use recommended personal protective equipment (PPE), which includes an N95 or higher-level respirator (or facemask if a respirator is not available), eye protection, gloves, and a gown.
- Nasopharyngeal specimen (NP) collection /Oropharyngeal (OP) (throat) specimen collection (performed by a trained healthcare provider, only)
- Use only **synthetic fiber swabs** with thin **plastic or wire shafts** that have been designed for sampling the nasopharyngeal mucosa. **Do not use calcium alginate swabs or swabs with wooden shafts**, as they may contain substances that inactivate some viruses and may inhibit molecular tests.



## Collecting an NP specimen:

Patient's head back 70 degrees.  
Gently and slowly insert a minitip swab with a flexible shaft (wire or plastic) through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient.  
Gently rub and roll the swab.  
Leave swab in place for several seconds to absorb secretions.  
Slowly remove swab while rotating it.  
Place swab, tip first, into the transport tube provided.

### NASOPHARYNGEAL (NP) SPECIMEN COLLECTION STEPS

**GENERAL GUIDANCE:**

- Ensure that recommended personal protective equipment (PPE) is worn when collecting specimens. This includes gloves, a gown, eye protection (face shield or goggles), and an N-95 or higher-level respirator (or surgical mask if a respirator is not available).
- Gloves must be changed to a new pair for each patient; properly remove old pair and discard into a biohazard waste container.

**ANATOMICAL REFERENCE:**

**PROPER PPE:**

**PROPER SWAB:**

**PROPER SWAB PLACEMENT:**

**IMPROPER SWAB PLACEMENT:**

**Nasopharyngeal (NP) swab specimen collection:**

**STEP 1:** Tilt patient's head back 70 degrees. Gently and slowly insert a minitip swab with a flexible shaft through the nostril parallel to the palate until resistance is encountered. The distance is equivalent to that from the nostril to the ear of the patient, indicating contact with the nasopharynx.

**STEP 2:** Gently rub and roll the swab, leaving it in place for several seconds to absorb secretions. If a deviated septum or blockage creates difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.

**STEP 3:** Slowly remove swab while rotating it. Specimens can be collected from both nostrils, but it is not necessary if the minitip swab is saturated with fluid from the first nostril.

**STEP 4:** Place swab, tip first, into the transport tube provided. Once the tip is near the bottom, break the swab handle at the swab breakpoint by bending back and forth or cut it off with sterile scissors. The swab should fit in the tube comfortably so that the cap can be screwed on tightly to prevent leakage and contamination.

Find additional testing guidance, resources and training by visiting [www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-dispose-specimens.html](http://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-dispose-specimens.html)



## Collecting an OP specimen:

- Insert swab into the posterior pharynx and tonsillar areas.
- Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.
- Place swab, tip first, into the transport tube provided.

## Nasal mid-turbinate (NMT) specimen

### NASAL MID-TURBINATE (NMT) SPECIMEN COLLECTION STEPS

**GENERAL GUIDANCE:**

- Ensure that recommended personal protective equipment (PPE) is worn when collecting specimens. This includes gloves, a gown, eye protection (face shield or goggles), and an N-95 or higher-level respirator (or surgical mask if a respirator is not available).
- Gloves must be changed to a new pair for each patient; properly remove old pair and discard into a biohazard waste container.

**PROPER PPE**

**ANATOMICAL REFERENCE**

**NMT SWAB**

**PROPER SWAB PLACEMENT**

**IMPROPER SWAB PLACEMENT**

Find additional testing guidance, resources and training by visiting [www.cdc.gov/coronavirus/2019-ncov/lab/guidelines/collection-specimens.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines/collection-specimens.html)

**Nasal mid-turbinate (NMT) swab specimen collection**

**STEP 1**

Tilt patient's head back 70 degrees.

Use a flocked tapered swab.

Some swabs come with a stopper, which indicates proper nasal insertion depth.

**STEP 2**

While gently rotating the swab, insert it less than one inch (about 2 cm) into nostril parallel to the palate until resistance is met at turbinate.

**STEP 3**

Rotate the swab several times against nasal wall.

Remove swab, insert it into the other nostril and repeat the process.

**STEP 4**

Place swab, tip first, into the transport tube provided.

Once the tip is near the bottom, break the swab handle at the swab breakpoint by bending back and forth or cut it off with sterile scissors.

The swab should fit in the tube comfortably so that the cap can be screwed on tightly to prevent leakage and contamination.

Find additional testing guidance, resources and training by visiting [www.cdc.gov/coronavirus/2019-ncov/lab/guidelines/collection-specimens.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines/collection-specimens.html)

# TREATMENT

There are **no approved antiviral drugs for human coronavirus** infections and therapy is **supportive**. During the SARS epidemic, most patients were treated with **ribavirin and high-dose steroids**.

Late in the SARS-epidemic, **IFN- $\alpha$ , anti SARS immunoglobulin, and lopinavir plus ritonavir**, two proteases licensed for the treatment of HIV, were used to treat patients.

Review of all of these therapies concluded that while **some showed efficacy in inhibiting SARS-CoV replication in tissue culture cells**, none showed a beneficial effect in patients.

Similarly, convalescent sera and various combinations of ribavirin, IFN- $\alpha$ , corticosteroids, and lopinavir–ritonavir **did not improve** outcomes in MERS patients.

The mechanism of coronavirus replication involves several proteins that are potential targets for antiviral drugs, including the **viral RdRp, virus-encoded proteases, host cell receptors** used by the virus for entry, and the **viral S protein**.

# TREATMENT

Several antiviral drugs targeting these viral proteins or processes have been developed and evaluated for their ability to inhibit SARS-CoV and MERS-CoV replication *in vitro* and in experimentally infected animals.

These include **protease inhibitors**, **monoclonal antibodies** that inhibit binding to cells, peptides from the heptad repeat regions of the **S protein** that inhibit receptor binding or fusion, **small interfering RNAs**, and **polymerase and helicase inhibitors**.

# PREVENTION

No vaccines had been available to prevent human coronavirus infection, **but vaccines against domestic animal coronaviruses**, such as IBV, PEDV, TGEV, and BCoV, are routinely used to prevent serious disease in young animals.

Several SARS-CoV vaccines have been developed, including **inactivated whole virus, live virus vectors expressing single viral proteins, and recombinant proteins and DNA vaccines**.

All of these vaccines express the **S glycoprotein** and are designed to induce SARS-CoV neutralizing antibodies.

Large stocks of anti-SARS-CoV **neutralizing antibody** have been prepared and are available for **passive immunization** of health care workers and other high-risk personnel.

Similar to SARS-CoV, **several MERS-CoV-specific vaccines** are developed, including **subunit, measles, adenovirus, and vaccinia vaccines expressing the S glycoprotein, recombinant proteins, nanoparticles, VLPs, and DNA vaccines**.



# PREVENTION

Vaccination with a camel poxvirus expressing the MERS S glycoprotein conferred immunity against both MERS-CoV and camelpox, representing a vaccine candidate with dual efficacy.

Use of a poxvirus has the advantage of inducing potent antiviral T-cell as well as antibody responses.

The genetic and antigenic variability of coronaviruses makes a vaccine may not provide equal protection to all antigenic variants.

In the absence of effective vaccines and antiviral drugs, the most important ways to prevent human coronavirus infections are a highly active public health surveillance system.