Coronaviridae

Chapter 10 Fields Virology, Emerging Viruses, Seventh Edition, 2021 - Chapters 35 and 36 of Fields Virology

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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.

Clasification

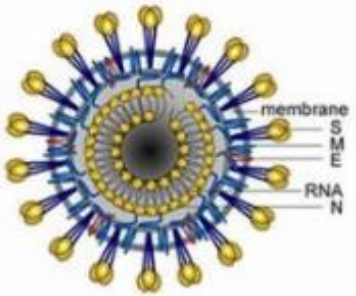
The coronaviruses are the largest group within the ***Nidovirales***
Nidovirales order comprises the families: ***Coronaviridae, Arteriviridae, Roniviridae,* and *Mesoniviridae***.
The arteriviruses consist of five genera of mammalian pathogens.
The roniviruses, which infect shrimp, invertebrate hosts
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 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

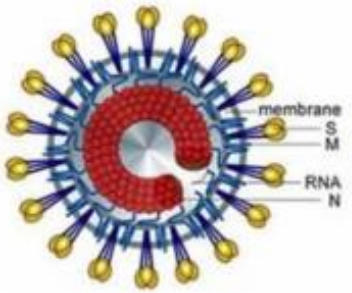
Relatively important till 2002

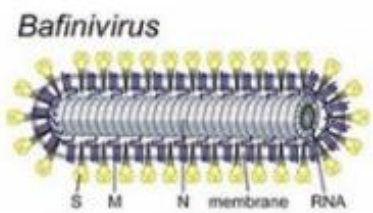
Nidoviruses

CORONAVIRUS

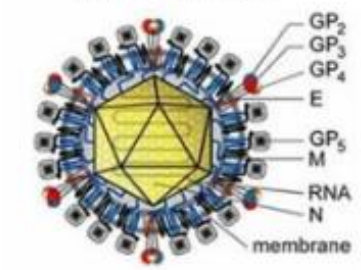


TOROVIRUS

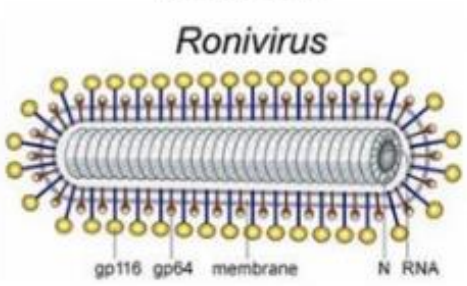




ARTERIVIRUS



RONIVIRUS



Classification of coronaviruses 1

Species[a]	GenBank Accession[b]	Viruses Included Within Species
Genus *Alphacoronavirus*		
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[a]	GenBank Accession[b]	Viruses Included Within Species
Genus *Betacoronavirus*		
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 *Rhinolophus* bat coronavirus Rp3 (SARSr-*Rh*-BatCoV Rp3)
Middle East respiratory syndrome–related coronavirus (MERS-CoV)	JX869059	
Hedgehog coronavirus (EriCoV)	KC545383	
Tylonycteris bat coronavirus HKU4 (*Ty*-BatCoV HKU4)	EF065505	
Pipistrellus bat coronavirus HKU5 (*Pi*-BatCoV HKU5)	EF065509	
Rousettus bat coronavirus HKU9 (*Ro*-BatCoV HKU9)	EF065513	
Genus *Gammacoronavirus*		
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)
Genus *Deltacoronavirus*		
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	

TABLE 1. *Serotypes, natural hosts, and diseases of coronavirus*

Antigenic group	Virus	Host	Respiratory infection	Enteric infection	Hepatitis	Neurologic infection	Other[a]
I	HCoV-229E	Human	X			?	
	TGEV, PRCoV	Pig	X	X			X
	CCoV	Dog		X			
	FECoV	Cat		X			
	FIPV	Cat	X	X	X	X	X
	RbCoV	Rabbit		X			X
II	HCoV-OC43	Human	X	?		?	
	MHV	Mouse	X	X	X	X	
	SDAV	Rat					X
	HEV	Pig	X	X		X	
	BCoV	Cow	X	X			
	TCoV	Turkey	X	X			
III	IBV	Chicken	X		X		X
	TCoV	Turkey	X	X			

[a]Other diseases caused by coronaviruses include infectious peritonitis, immunologic disorders, runting, nephritis, pancreatitis, parotitis, myocarditis, and sialodacryoadenitis.

HCoV-229E, human respiratory coronavirus; TGEV, porcine transmissible gastroenteritis virus; PRCoV, porcine respiratory coronavirus; CCoV, canine coronavirus; FECoV, feline enteric coronavirus; FIPV, feline infectious peritonitis virus; RbCoV, rabbit coronavirus; HCoV-OC43, human respiratory coronavirus; MHV, murine hepatitis virus; SDAV, sialodacryoadenitis virus; HEV, porcine hemagglutinating encephalomyelitis virus; BCoV, bovine coronavirus; IBV, avian infectious bronchitis virus; TCoV, turkey coronavirus.

Diverse alpha- and betacoronaviruses have been described in **bats.** These viruses include the likely immediate predecessors of SARS CoV.

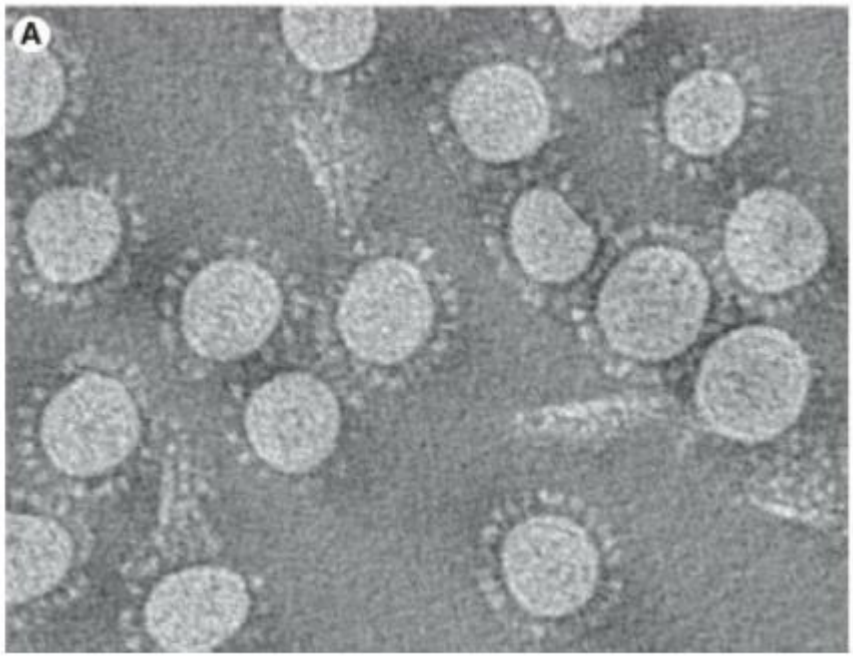
Birds have also proven to be a rich source of novel coronaviruses that are so highly divergent as to constitute a separate genus— the delta coronaviruses.

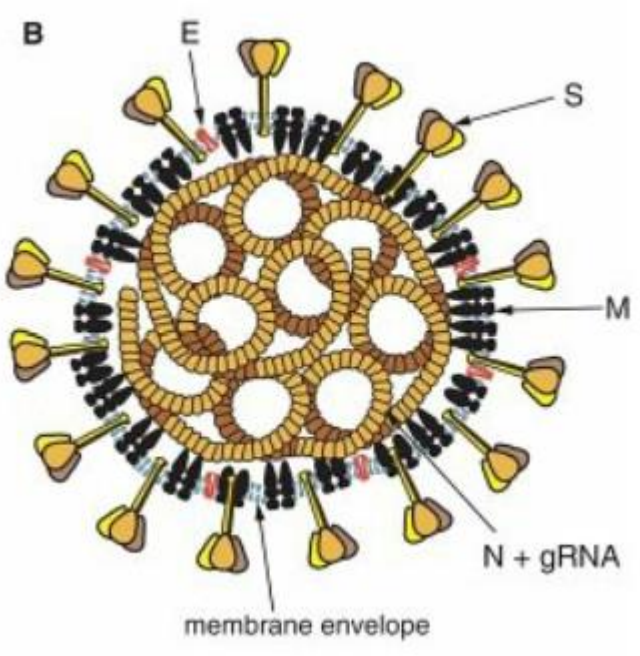
Bats and birds are ideally suited as reservoirs for the incubation, spread, and evolution of coronaviruses.

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.

Corona virus





Lethal diseases by CoVs

Murine hepatitis virus (MHV)

Infectious bronchitis virus (IBV)

Feline infectious peritonitis virus (alfa1) (FIPV)

SARS-CoV

MERS-CoV

SARS-CoV-2

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 subunites: **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.** M dimers appear to adopt two different conformations: a compact form that promotes greater membrane curvature and a more elongated form that contacts the nucleocapsid.

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)
protein bind along the RNA genome
common to other helical viral nucleocapsids.
N protein provides **no protection** for its genome against ribonucleases.
The N protein is **a phosphoprotein**, (with serine and threonine residues).
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 **acetylesterase activity**, specific for either 9-*O*- or 4-*O*-acetylated sialic acids. These characteristics allow HE as **a cofactor for S protein**, assisting attachment of virus to host cells

Virion structural proteins

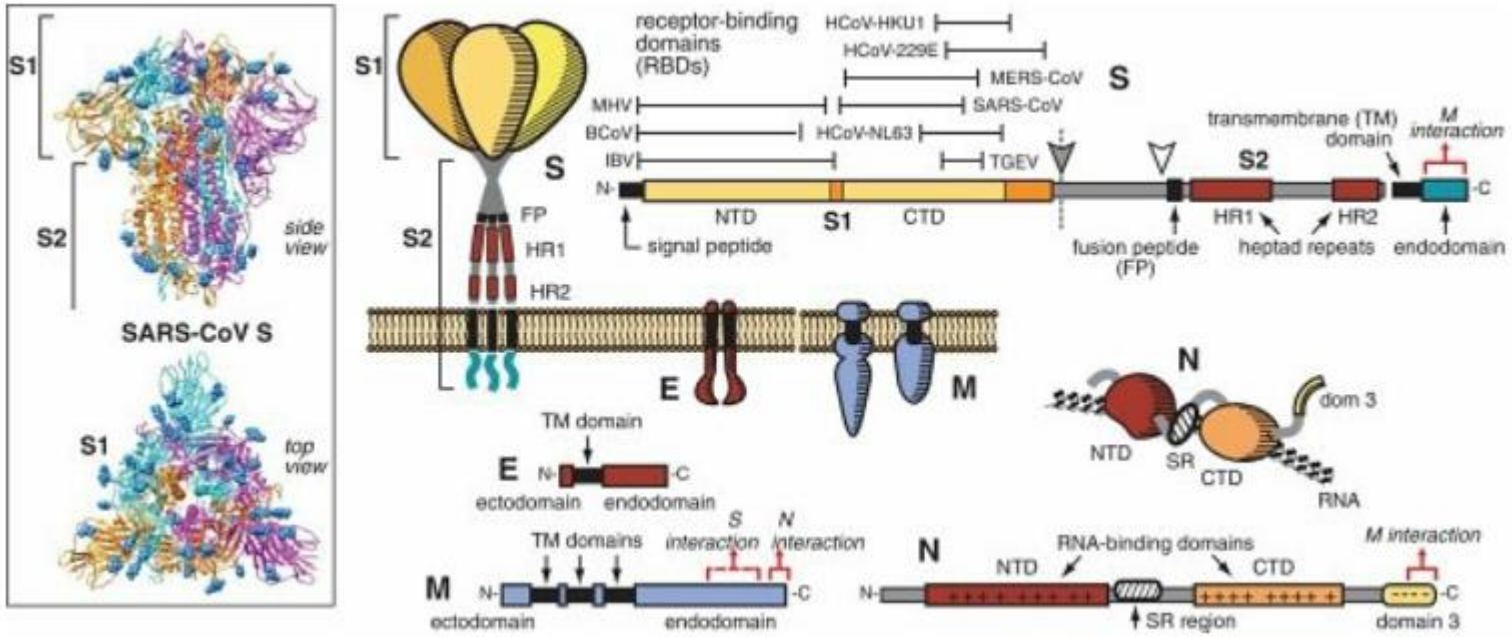


TABLE 2. *Properties and functions of coronavirus structural proteins*

Nucleocapsid phosphoprotein **N**	Binds to viral RNA Forms nucleocapsid Elicits cell-mediated immunity
Membrane glycoprotein **M** (formerly **E1**)	An integral membrane protein on the Golgi Determines virus-budding site Triggers virus particle assembly Interacts with viral nucleocapsid Forms the shell of internal viral core (of TGEV and MHV) Induces α-interferon
Envelope (Small membrane) protein **E** (formerly **sM**)	Triggers virus particle assembly Associated with viral envelope May cause apoptosis
Spike glycoprotein **S** (formerly **E2**)	Forms large spikes on virion surfaces Binds to specific cellular receptors Induces fusion of viral envelope with cell membranes (plasma membrane or endosomal membrane) May induce cell–cell fusion Binds Fc fragment of immunoglobulin (MHV and TGEV) Binds 9-O-acetylated neuraminic acid or *N*-glycolylneuraminic acid Induces neutralizing antibody Elicits cell-mediated immunity
Hemagglutinin-esterase glycoprotein **HE** (formerly **E3**)	Forms small spikes on the virion surface of some coronaviruses Binds to 9-O-acetylated neuraminic acid Causes hemagglutination May cause hemadsorption Esterase cleaves acetyl groups from 9-O-acetyl neuraminic acid

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 (50–60 kd) 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*).
-

GENOME STRUCTURE 2

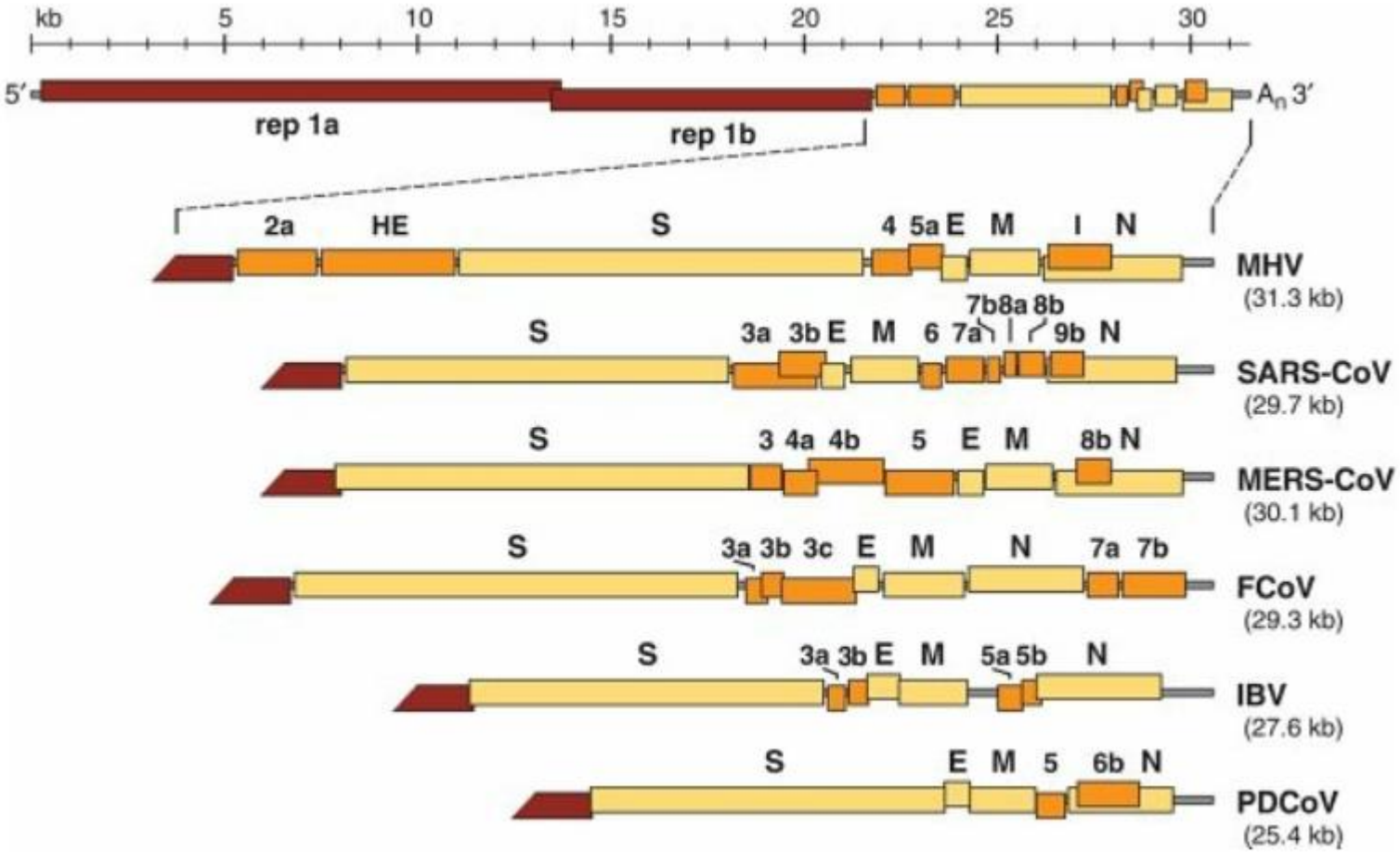
At the 5′ end of the genome is a sequence of 65 to 98 nucleotides, termed the *leader RNA*, that is also present at the 5′ ends of all subgenomic mRNAs.
An untranslated region (UTR) of 200 to 400 nucleotides follows this leader sequence.
At the 3′ end of the RNA genome is another UTR of 200 to 500 nucleotides, followed by poly(A) of variable length.
The sequences of both the 3′- and 5′-UTR are important for RNA replication and transcription.

Coronavirus Genome-2

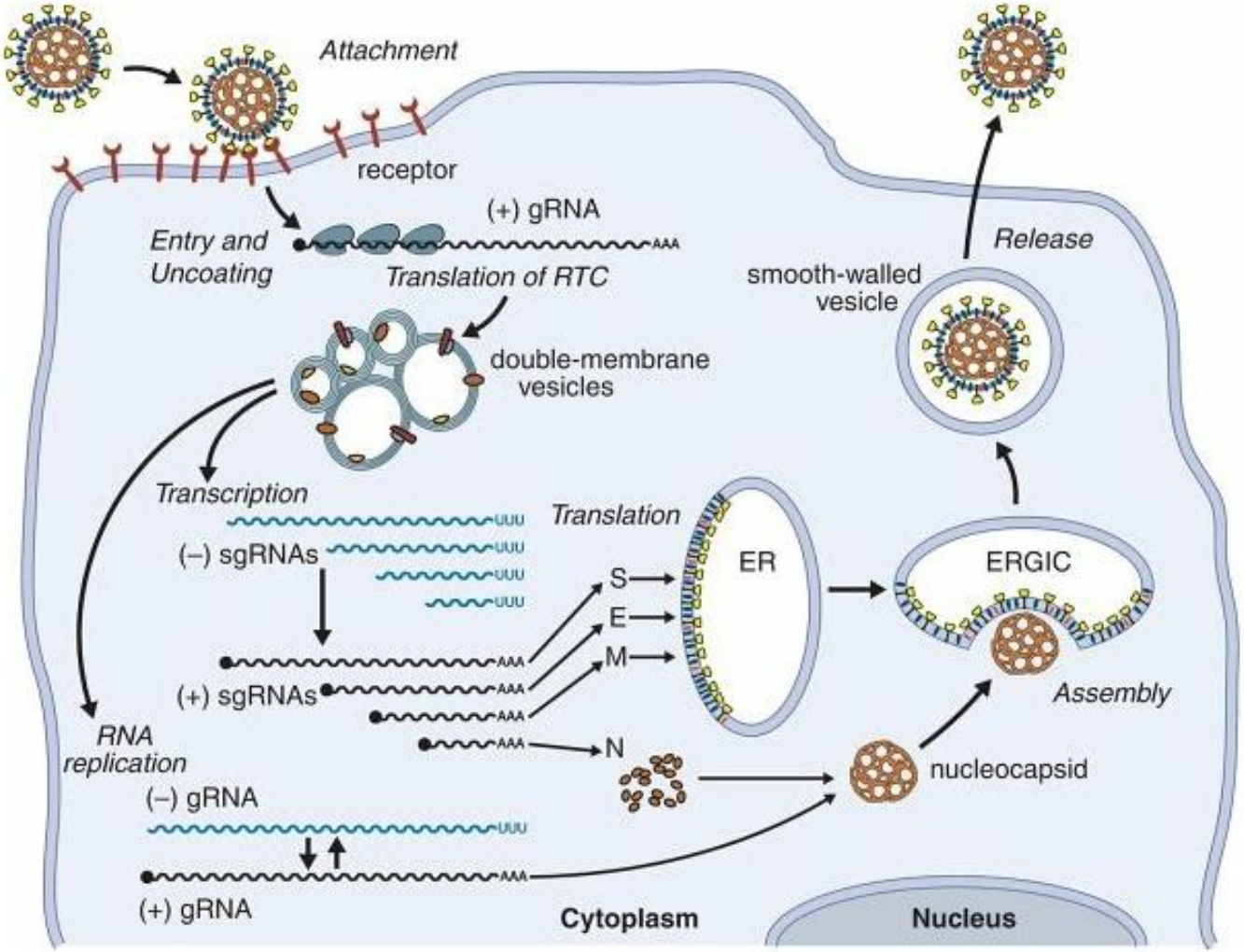
- The remaining genomic sequence includes 7 to 10 open reading frames (ORFs). Gene 1, which comprises two thirds of the genome from the 5′ end, is about 20 to 22 kb in length. It consists of two overlapping ORFs (1a and 1b). These ORFs are translated into a polyprotein, which is the precursor of viral 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′**. These genes are interspersed with several ORFs encoding various nonstructural proteins and the HE glycoprotein, which differ markedly among coronaviruses in number, nucleotide sequence, gene order, and method of expression

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[a]	Receptor	References
Alphacoronaviruses		
TGEV	Porcine aminopeptidase N[b] (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
Betacoronaviruses		
MHV	Murine carcinoembryonic antigen–related adhesion molecule 1 (mCEACAM1)	450
BCoV	*N*-Acetyl-9-*O*-acetylneuraminic acid[e]	374
HCoV-OC43	*N*-Acetyl-9-*O*-acetylneuraminic acid[e]	226
SARS-CoV	Angiotensin-converting enzyme 2 (ACE2)[c]	258
MERS-CoV	Dipeptidyl peptidase 4 (DPP4)[d]	355
Gammacoronaviruses		
IBV	Alpha-2,3-linked sialic acid[e]	348
Deltacoronaviruses		
PDCoV	Porcine aminopeptidase N (pAPN)	253,442

b: Mammalian aminopeptidase N 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

Coronaviruses can enter cells either by an "early" pathway of fusion at the plasma membrane or by a "late" pathway of receptor-mediated endocytosis followed by fusion with membranes of acidified endosomes
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.

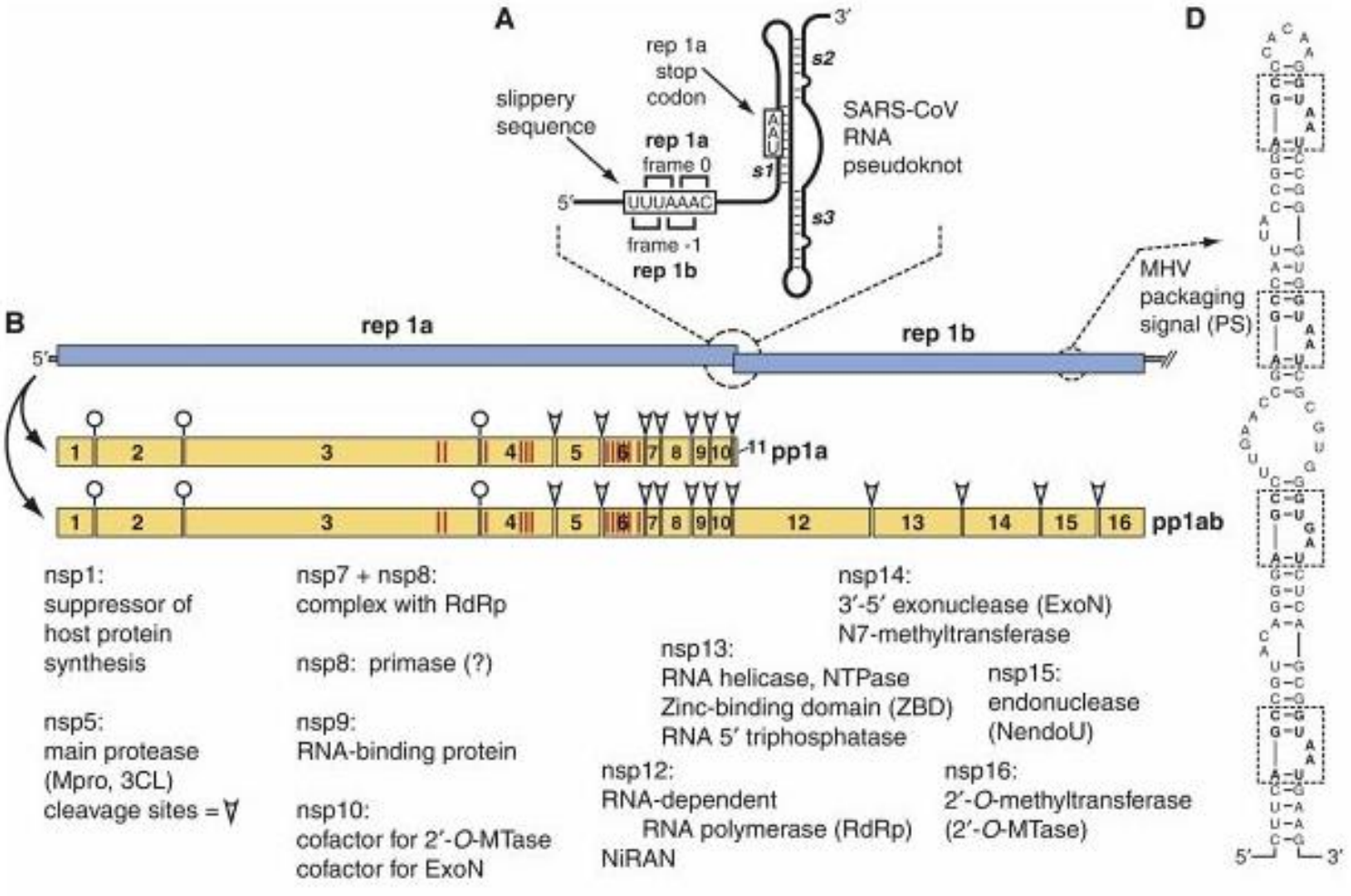
The Viral fusion proteins, the coronavirus S2 moiety contains two separated heptad repeats, HR1 and HR2, with the fusion peptide upstream of HR1 and the transmembrane domain immediately downstream of HR2

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**
Rep 1b was translated to **pp1ab**

Polyproteins pp1a (402–502 kDa) and pp1ab (700–811 kDa) are auto-proteolytically processed into mature products: **nsp1 to nsp16** (except in the gamma- and delta coronaviruses, which do not have nsp1)

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 processed nsps assemble to form **replicase–transcriptase complex (RTC)**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s3
frame -1
rep 1b
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rep 1b
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10
11
pp1a
12
13
14
15
16
pp1ab
nsp1:
suppressor of
host protein
synthesis
nsp5:
main protease
(Mpro, 3CL)
cleavage sites = ∀
nsp7 + nsp8:
complex with RdRp
nsp8: primase (?)
nsp9:
RNA-binding protein
nsp10:
cofactor for 2′-O-MTase
cofactor for ExoN
nsp14:
3′-5′ exonuclease (ExoN)
N7-methyltransferase
nsp13:
RNA helicase, NTPase
Zinc-binding domain (ZBD)
RNA 5′ triphosphatase
nsp15:
endonuclease
(NendoU)
nsp12:
RNA-dependent
RNA polymerase (RdRp)
NiRAN
nsp16:
2′-O-methyltransferase
(2′-O-MTase)
5′
3′

The coronavirus RdRp is nsp12.
Nsp7 & nsp8 has been shown to greatly enhance **RNA binding** by nsp12
nsp13 has RNA-stimulated NTPase (**helicase**) activity and unwinds RNA duplexes in the 5′-to-3′ direction.

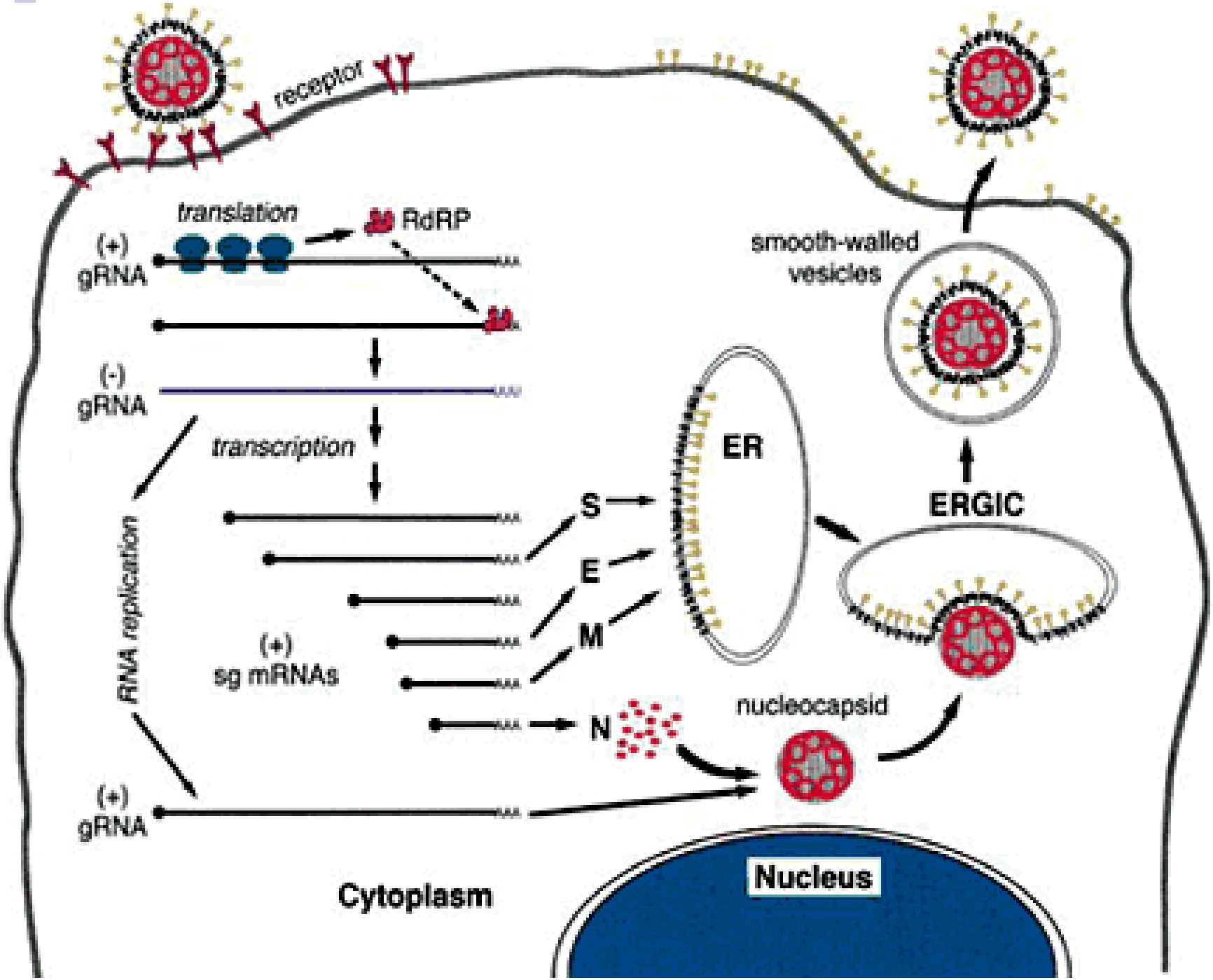
Viral RNA Synthesis

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 multiple subgenomic RNAs (sgRNAs).

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

- The mRNAs in infected cells consist of an overlapping nested set of 3′ coterminal RNAs containing a common leader RNA sequence at the 5′ end.

- The mechanism of subgenomic mRNA synthesis has not been established with a few exceptions, each mRNA is translated to yield only the protein encoded by the 5′ ORF of the mRNA. These proteins include structural proteins N, M, E, S, and HE and several nonstructural proteins.


receptor
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RNA replication
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N
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ERGIC
smooth-walled
vesicles
nucleocapsid
(+)
gRNA
Cytoplasm
Nucleus

Assembly and Release of Virions

The membrane-bound proteins M, S, and E are initially inserted into the ER, and from there, they transit to the site of virion assembly, the endoplasmic reticulum–Golgi intermediate compartment (ERGIC)

The nucleocapsids composed of progeny genomes encapsidated by N protein with the envelope components to form virions, which bud into the ERGIC.

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 diarrhea that is particularly severe, and sometimes fatal, in young animals.
SARS-CoV and MERSCoV spread from the upper airway to cause a severe lower respiratory tract infection.

Animal Coronavirus Infections

Mouse Hepatitis Virus

These viruses spread within infected colonies to young, uninfected animals. They do not generally cause symptomatic disease but may subtly impair the host immune response to other pathogens.

Studies of MHV pathogenesis predominantly use the neurotropic JHM and A59 strains of virus (JHMV and MHV-A59), in part because they cause a demyelinating encephalomyelitis with similarities to the human disease multiple sclerosis (MS)

Both CD4 and CD8 T cells are required for virus clearance from the central nervous system (CNS), with CD8 T cells considered most important in this process. CD8 T cells eliminate virus from infected astrocytes and microglia by perforin-dependent pathways, while clearance from oligodendrocytes is IFNγ-dependent.

However, T-cell–mediated virus clearance is not complete, and antivirus antibody is required to prevent virus recrudescence.

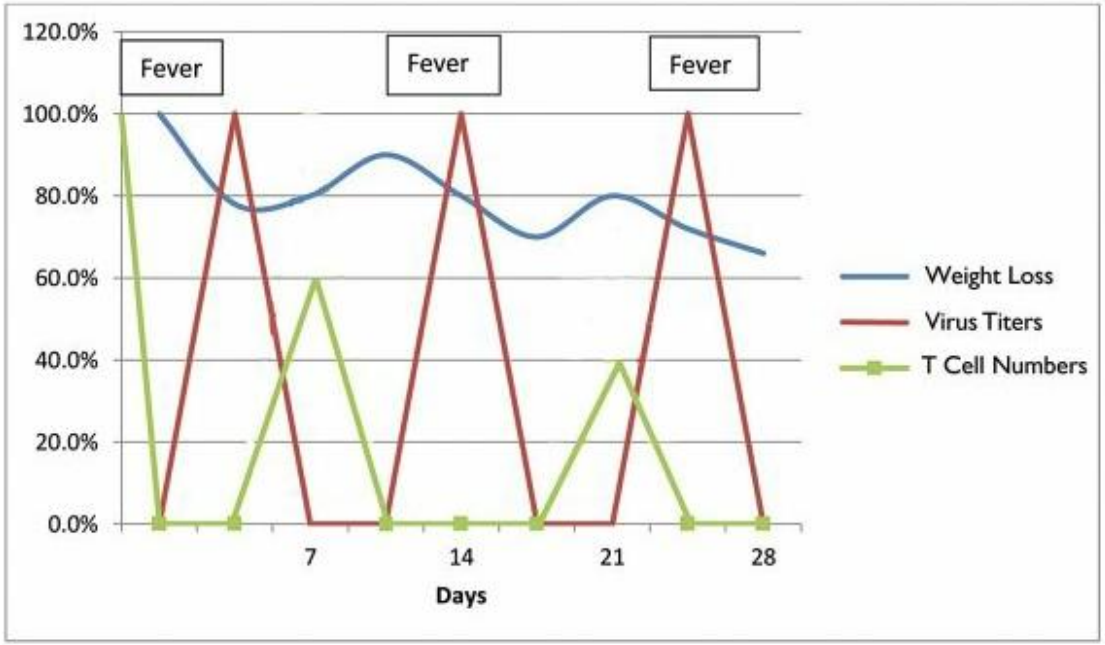
Feline Enteric Coronaviruses (FCoV) and FIPV

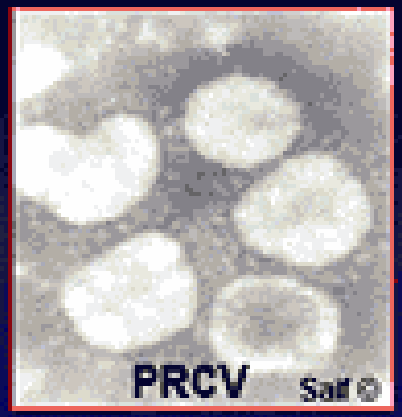
FCoV commonly cause mild or asymptomatic infections in domestic cats and other felines. Two serotypes of FCoV are recognized.

In some cats infected persistently with FCoV, mutations in the virus occur, resulting in the development of a lethal disease called

feline infectious peritonitis (FIP)

Recurrent feline infectious peritonitis (FIP)





Porcine Coronaviruses Group I

Enteric Infections

TGEV : Transmissible Gastroenteritis Virus (1965)
Infect all age groups but highest mortality in baby pigs

PEDV : Porcine Epidemic Diarrhea 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, HCoVs 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.
The ability of HCoV-OC43 to tolerate mutations accounts for its ability to grow in mouse cells and infect the mouse brain as well as its ability to cross species.

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
Alphacoronaviruses			
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
Betacoronaviruses			
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
Gammacoronaviruses			
IBV	Chicken	Respiratory, kidney	Bronchitis, nephritis
TuCoV	Turkey	GI tract	Gastroenteritis
BWCoV	Beluga whale	Respiratory tract	Pneumonia, hepatitis
Deltacoronaviruses			
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.
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.

Infected camels develop rhinitis with high virus titers, consistent with their role in camel–camel and camel–human transmission.

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-*α*/*β*).
DCs & macrophages are the source for production of IFN-*α*/*β* 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 .

To counter IFN induction, all coronaviruses express a 2′-*O*-methyltransferase (**nsp16**).

Nsp3, ADP-ribose-1″-monophosphatase prevents IFN induction.

Nsp15, an endoribonuclease encoded by all coronaviruses, probably has a role in virus replication but also inhibits IFN expression.

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

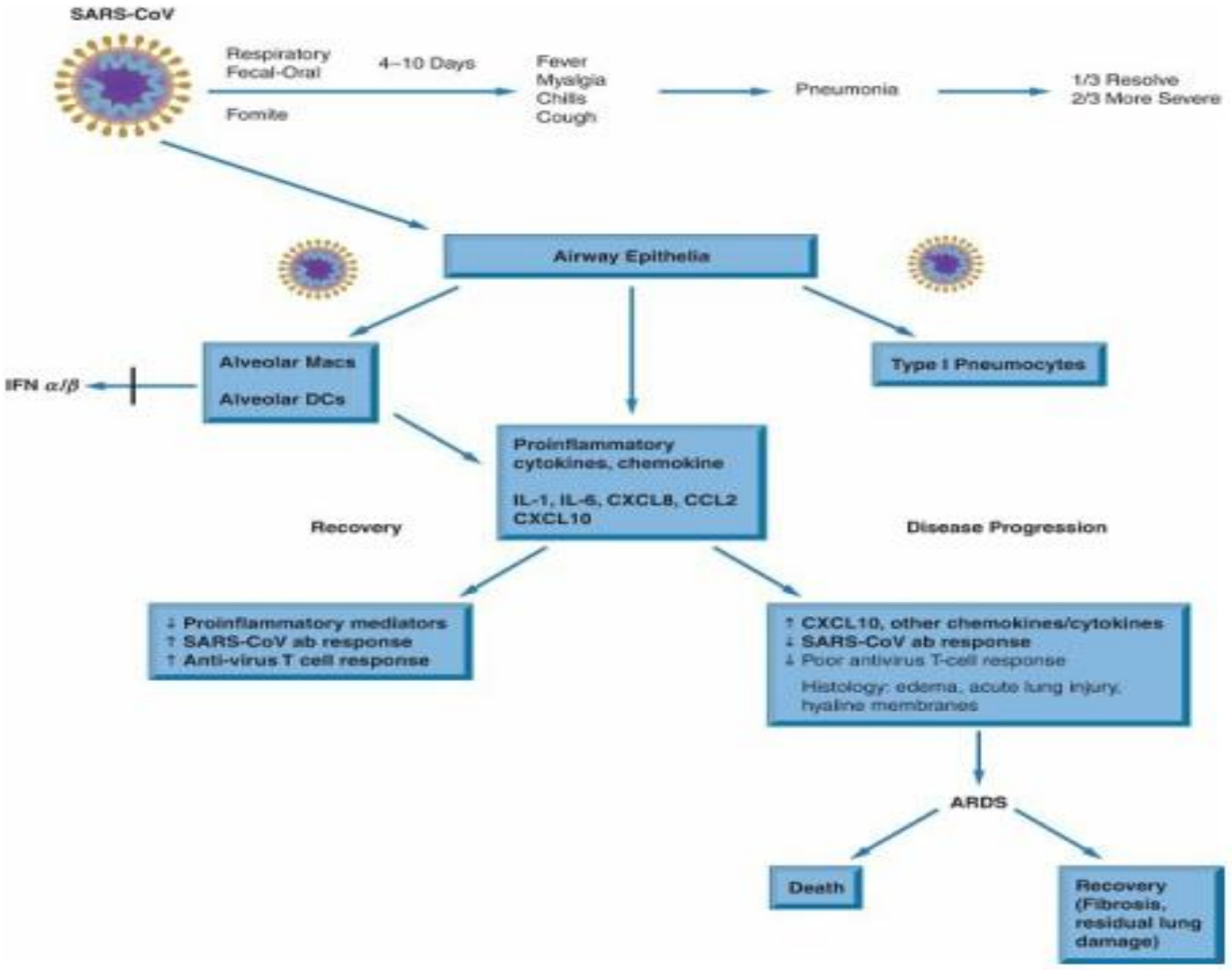
After entry to host cells, viral antigens get presented APCs to virus-specific cytotoxic T lymphocytes (CTL).

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-1β, IFN-α, IFN-γ, IL-12, IL-6, IL-18, TNF-α, IL-33, TGFβ, etc.) and chemokines (CCL3, CCL2, CXCL8, CCL5, CXCL9, CXCL10, etc.) by immune cells.

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



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.

https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

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.

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 (**IFA**s) 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 3rd weeks of illness.

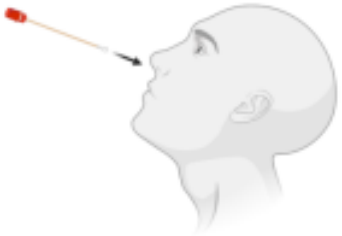
SARS-CoV–specific antibodies were usually detected 4 weeks after infection.
A quantitative RT-PCR assay is used for diagnosis, with confirmation by sequencing of PCR products.

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

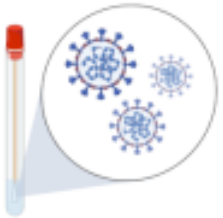
1 Nasopharyngeal (NP) or Oropharyngeal (OP) swab

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



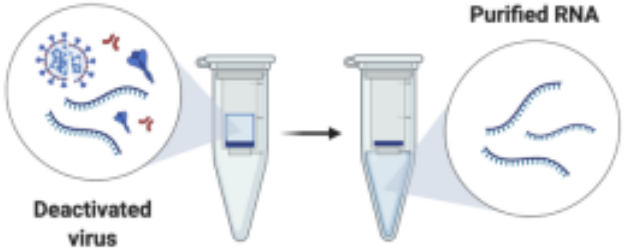
2 Collected specimen 0-72 h

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



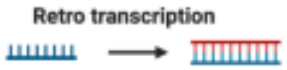
3 RNA extraction ~45 min

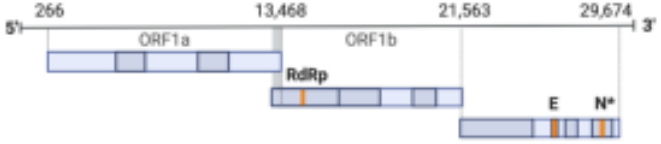
Purified RNA is extracted from deactivated virus.



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_Forward: ACAGGTACGTTAATAGTTAATAGCGT
E_Probe1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E_Reverse: ATATTGCAGCAGTACGCACACA

E gene
First-line screening tool

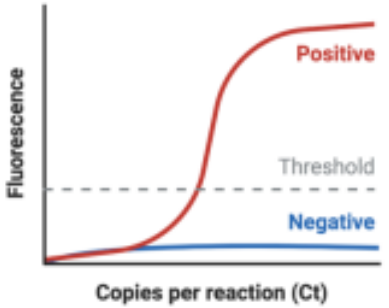
RdRp_Forward: GTGARATGGTCATGTGTGGCGG
RdRp_Probe1: FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRp_Probe2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
RdRp_Reverse: CARATGTTAAASACACTATTAGCATA

RdRp gene
Confirmatory testing

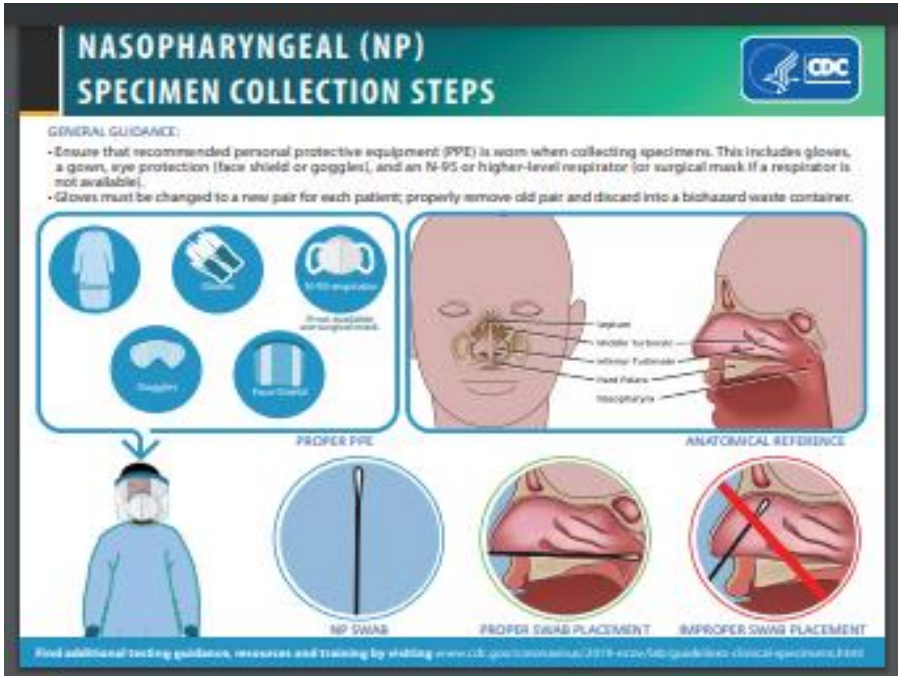
Primer sequences are for *illustrative* purposes only.

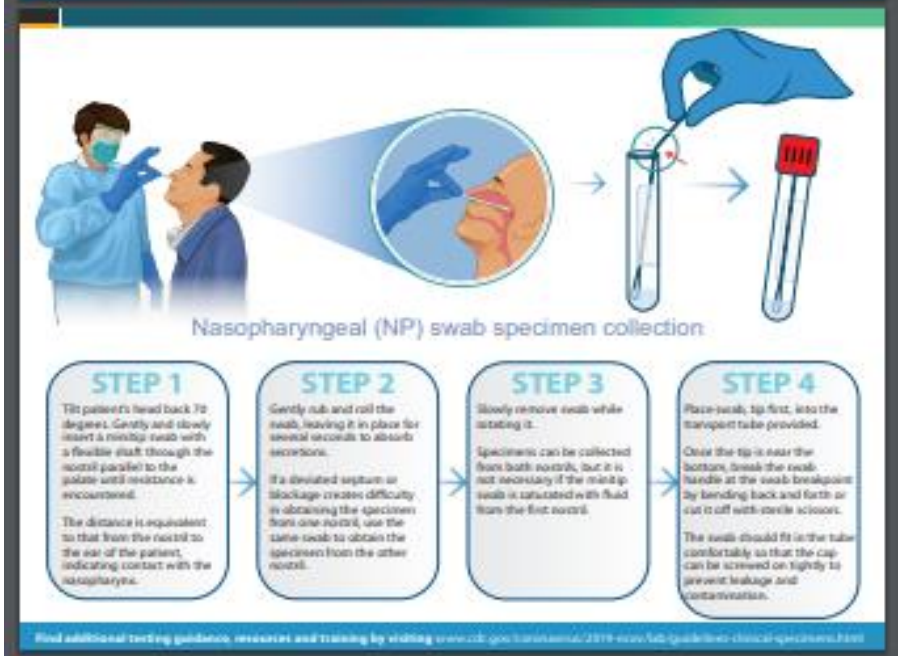
5 Test results real-time

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



- 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.

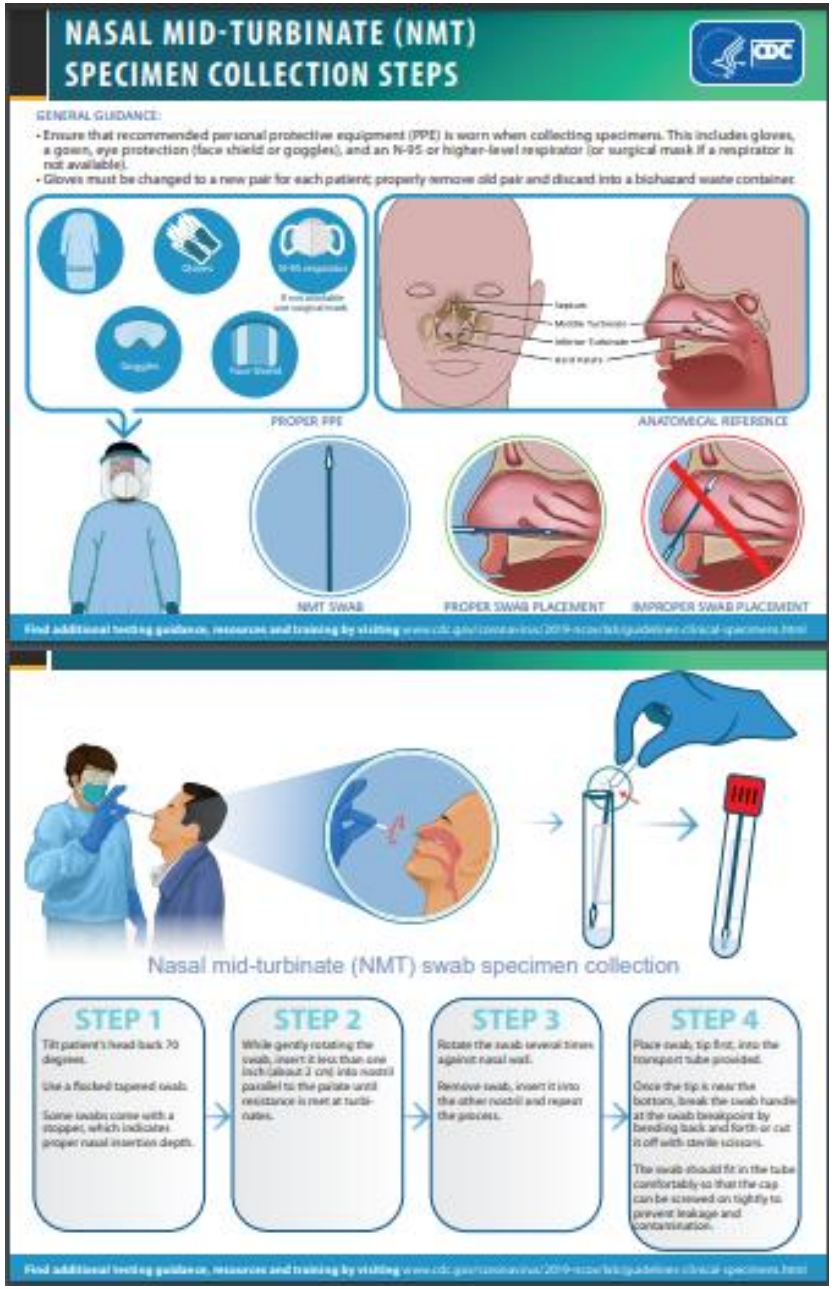




Collecting an OP specimen:

- 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



Cross species transmission of Coronaviruses

The SARS and MERS outbreaks demonstrated the ability of coronaviruses to cross species.
Initially predicted from studies of coronavirus-infected cultured cells.

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 alpha-coronavirus 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 delta-coronavirus, a genus of avian viruses. PDCoV appeared to cross from avian to mammalian species, using APN as a receptor.

CLINICAL FEATURES

HCoVs cause respiratory disease, including SARS and MERS.

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 2003 epidemic, 8,000 individuals were infected, with a mortality rate of 10%.
About 50% of infected individuals greater than 60 years.
Illness had an onset of 4 to 7 days, and 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 FEATURES- SARS-CoV Infections

In patients who recovered, expression of proinflammatory cytokines diminished, and robust antivirus 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.

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-α, 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-α, 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. For some of these vaccines, efficacy has been demonstrated in animal models.

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 if SARS recurs.

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

PREVENTION

Neutralizing antibodies have been detected, which will be useful for passively immunizing health care workers.

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 antivirus 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.

Thanks for taking your time