**OXYMETAZOLINE HYDROCHOLORIDE IS COMMON NAME OF DEMETTHPHENOL HYDROCHOLORIDE[C16H25CIN2O] BUT PERCENTAGE OF LOW,IT IS DIRECT ACTING WITH BENGALKONIUM CHOLORIDE APPLICATION FOR MEDICINE OF NASAL DROPS.BUT PERCENTAGE OF HIGH MORE THAN .01% IS NOT SAFE FOR NASAL DROPS AS BECAUSE IT ACTS LIKE ACID TO CLEAR THE MUCUS AND GROBULES INSIDE NOSETRILS FOR CLEANING THE BLOCKAGE.**

**BUT FROM MOLECULAR BIOLOGY IT IS NOT SAFE WHEN VIRUSES LIKE ENTERS IN THROAT FOR NEXT ENTRY IN LUNGS AND STARTS ITS ACTION SO WHY I HAVE ADOPTED CHEMISTRY SOFTWARE LIKE INB RXN AND OTHERS HOW TO STOP BREATHING FOR CONTROLLING WITH OTHER LIKE AZIOTHROMYCIN IN FIRST OR SECOND STAGE OF CORONAVIDE ATTACK WHEN AMONIA CHOLORIDE CnH2n +1 WHERE n=8,10,12,14.16,18 etc .**

**But we will use Benzakonium with very low percentage of organic salt as quaternary ammonia choloride.Benzamin choloride also poses as surfactant properties when it is playing transfer positive catalysis,an important technology,the synthesis of organic compounds,may be mixture solution liquid chemotheraphy activity to kill the viruses.**

**Benzakonium choloride is used in very low percentage for ear and eye application but if it is more than standard percentage it will condemn eye or irritant for eyes and skin.if any one swallows it then it will be like poision,also Benzakonium choloride[BAC] is major non-alcohol based active ingredient for food products and domestic bioside**

**purposes which is chemical agent that may be applied tyopically in so living tissue as antiseptic or disinfectant to kill the microorganism.BAC with an quaternary ammonia choloride as catrionic surface agent whem microorganism bacteria spreaded over surface with its positive catalystic nature and to kill bacteria viruses over surface then goes to inner side gradually to kill cell to cell and to kill microorganism bacteria or viruses.**

**N CH3+NCH3-CnH2n+1 likely 8,10,12,14,16,18**

**N=8 is least we use,Europian medical for its BAC 28.**

**BAC With others like [99,100] ipcats upon human microbiolism.**

**Aziothromycin Is best for throat infection with fever along with hydroxycholoroquin it is most effective in the treatment of chronic obstructive of pulmonary infection and it has no side effects for pregnant woman and it is used to produce keep immersive response so that patient cannot loss immune power and easily control inflammation throat infection which may cause endanger at next stage of lungs with pulmonary infection.**



*Coronavirinae*: As seen in conventional electron micrographs, coronaviruses are roughly spherical enveloped particles, 120–160 nm in diameter, with a characteristic fringe of 15–20 nm petal-shaped surface projections (peplomers). In a subset of betacoronaviruses a second, inner fringe of 5–7 nm surface projections is also seen. Coronavirus (CoV) particles as studied by cryo-electron tomography

[Hydroxychloroquine is a 4-aminoquinoline with immunosuppressive, ant autophagy, and antimalarial activities.]

**----------**

## Nidovirales: evolving the largest RNA virus genome

## WRITTEN BY Dr skkhan

# Sources adopted as there needed to establish CORONAVIRUS SHELL STRUCTURE [DEMON OF DAY]

**Corticosteroids** were the most commonly reported and used medicine in this review, however, they are not recommended in any of the mentioned guidelines. The World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC), in the absence of conclusive scientific evidence, recommended that Corticosteroids should not be routinely used in patients with COVID-19 for treatment of viral pneumonia or acute respiratory distress syndrome (ARDS) unless indicated for other conditions, such as asthma or chronic obstructive pulmonary disease (COPD)

https://en.wikipedia.org/wiki/File:Umifenovir_ball-and-stick_model.png

**9958103**

| | |
|---|---|
| **Molecular Formula**: | $C_{22}H_{26}BrClN_2O_3S$ |
| Synonyms: | **Arbidol Hydrochloride** 131707-23-8 **Arbidol HCl** Umifenovir **hydrochloride Arbidol** (**hydrochloride**) More... |
| **Molecular Weight**: | 513.9 g/mol |
| Parent Compound: | CID 131411 (**Arbidol**) |



Hydroxychloroquine is a 4-aminoquinoline with immunosuppressive, antiautophagy, and antimalarial activities. Although the precise mechanism of action is unknown, hydroxychloroquine may suppress immune function by interfering with the processing and presentation of antigens and the production of cytokines. As a lysosomotropic agent, hydroxychloroquine raises intralysosomal pH, impairing autophagic protein degradation; hydroxychloroquine-mediated accumulation of ineffective autophagosomes may result in cell death in tumor cells reliant on autophagy for survival. In addition, this agent is highly active against the erythrocytic forms of P. vivax and malariae and most strains of P. falciparum but not the gametocytes of P. falciparum.

Hydroxychloroquine has not been associated with significant serum enzyme elevations during therapy of rheumatologic diseases. Furthermore, clinically apparent liver injury from hydroxychloroquine is rare. A single case series (two cases) of acute liver failure attributed to hydroxychloroquine was published twenty years ago, but case reports of clinically apparent liver injury have not appeared subsequently. Thus, acute liver injury with jaundice due to hydroxychloroquine must be very rare, if it occurs at all.

An exception to this is the use of hydroxychloroquine in patients with porphyria cutanea tarda. When used in relatively high doses, hydroxychloroquine can trigger an acute hepatic injury with sudden onset of fever and marked serum enzyme elevations with increased excretion of porphyrins. This reaction appears to be caused by the sudden mobilization of porphyrins and is often followed by an improvement in porphyric symptoms. The reaction is uncommon if therapy is started with lower doses of hydroxychloroquine and is less severe than similar reactions that occur with chloroquine. Indeed, chronic low doses of hydroxychloroquine (100 to 200 mg twice weekly) have been used to alleviate symptoms in patients with prophyria cutanea tarda who are resistant or intolerant of phlebotomy, the usual therapy of this condition.
Likelihood score: C (probable rare cause of idiosyncratic, clinically apparent liver injury, but capable of causing acute hepatoxicity with high doses in patients with porphyria).
Hydroxychloroquine has not been associated with significant serum enzyme elevations during therapy of rheumatologic diseases. Furthermore, clinically apparent liver injury from hydroxychloroquine is rare. https://pubchem.ncbi.nlm.nih.gov/compound/66553 A single case series (two cases) of acute liver failure attributed to hydroxychloroquine was published twenty years ago, but case reports of clinically apparent liver injury have not appeared subsequently. Thus, acute liver injury with jaundice due to hydroxychloroquine must be very rare, if it occurs at all.
An exception to this is the use of hydroxychloroquine in patients with porphyria cutanea tarda. When used in relatively high doses, hydroxychloroquine can trigger an acute hepatic injury with sudden onset of fever and marked serum enzyme elevations with increased excretion of porphyrins. This reaction appears to be caused by the sudden mobilization of porphyrins and is often followed by an improvement in porphyric symptoms. The reaction is uncommon if therapy is started with lower doses of hydroxychloroquine and is less severe than similar reactions that occur with chloroquine. Indeed, chronic low doses of hydroxychloroquine (100 to 200 mg twice weekly) have been used to alleviate symptoms in patients with prophyria cutanea tarda who are resistant or intolerant of phlebotomy, the usual therapy of this condition.
Likelihood score: C (probable rare cause of idiosyncratic, clinically apparent liver injury, but capable of causing acute hepatoxicity with high doses in patients with porphyria).

Molecular biology is the study of living things at the level of the molecules which control them and make them up. While traditional biology concentrated on studying whole living organisms and how they interact within populations (a “top down” approach), molecular biology strives to understand living things by examining the components that make them up (a “bottom up” approach). Both approaches to biology are equally valid, although improvements to technology have permitted scientists to concentrate more on the molecules of life in recent years.

Molecular biology is a specialised branch of biochemistry, the study of the chemistry of molecules which are specifically connected to living processes. Of particular importance to molecular biology are the nucleic acids (DNA and RNA) and the proteins which are constructed using the genetic instructions encoded in those molecules. Other biomolecules, such as carbohydrates and lipids may also be studied for the interactions they have with nucleic acids and proteins. Molecular biology is often separated from the field of cell biology, which concentrates on cellular structures (organelles and the like), molecular pathways within cells and cell life cycles.

Gorbalenya AE[1], Enjuanes L, Ziebuhr J, Snijder EJ.
**Author information**

**Abstract**

This chapter focuses on the Nidovirales order whose member families include Arteriviridae, Coronaviridae, and Roniviridae. The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated. Based on the genome size, they are divided into two groups large and small nidoviruses. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range. Based on the genome size, they can be distinguished into two groups, large and small nidoviruses. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range. Although the structural proteins of the nidoviruses are generally functionally equivalent, there is no firm indication that any single protein species is evolutionary conserved across all of the families. Members of the family Coronaviridae generally possess three or four envelope proteins. The most abundant one is the membrane (M) protein. Though different in sequence, the M proteins of corona-, toro-, and bafiniviruses are alike in size, structure, and presumably also in function. Nidoviruses have lipid envelopes, which are commonly acquired by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi complex. In coronaviruses, the S protein is an important target for T cell responses and is the major inducer of virus-neutralizing antibodies, which are elicited by epitopes located mostly in the N-terminal half of the moleculeThis review focuses on the monophyletic group of animal RNA viruses united in the order Nidovirales. The order includes the distantly related coronaviruses, toroviruses, and roniviruses, which possess the largest known RNA genomes (from 26 to 32kb) and will therefore be called "large" nidoviruses in this review. They are compared with their arterivirus cousins, which also belong to the Nidovirales despite having a much smaller genome (13-16kb). Common and unique features that have been identified for either large or

all nidoviruses are outlined. These include the nidovirus genetic plan and genome diversity, the composition of the replicase machinery and virus particles, virus-specific accessory genes, the mechanisms of RNA and protein synthesis, and the origin and evolution of nidoviruses with small and large genomes. Nidoviruses employ single-stranded, polycistronic RNA genomes of positive polarity that direct the synthesis of the subunits of the replicative complex, including the RNA-dependent RNA polymerase and helicase. Replicase gene expression is under the principal control of a ribosomal frameshifting signal and a chymotrypsin-like protease, which is assisted by one or more papain-like proteases. A nested set of subgenomic RNAs is synthesized to express the 3'-proximal ORFs that encode most conserved structural proteins and, in some large nidoviruses, also diverse accessory proteins that may promote virus adaptation to specific hosts. The replicase machinery includes a set of RNA-processing enzymes some of which are unique for either all or large nidoviruses. The acquisition of these enzymes may have improved the low fidelity of RNA replication to allow genome expansion and give rise to the ancestors of small and, subsequently, large nidoviruses

https://ars.els-cdn.com/content/image/3-s2.0-B9780123846846000665-f66-01-9780123846846.jpg



The members of the order *Nidovirales* are enveloped, positive-strand RNA viruses of widely different architecture. Depending on whether the external appearance of the virion or the nucleocapsid is considered, similarities and differences can be discerned (Figure 1).

*Coronavirinae*: As seen in conventional electron micrographs, coronaviruses are roughly spherical enveloped particles, 120–160 nm in diameter, with a characteristic fringe of 15–20 nm petal-shaped surface projections (peplomers). In a subset of betacoronaviruses a second, inner fringe of 5–7 nm surface

projections is also seen. Coronavirus (CoV) particles as studied by cryo-electron tomography are homogeneous in size and distinctively spherical (envelope outer diameter 85±5 nm). The envelope exhibits an unusual thickness (7.8±0.7 nm), almost twice that of a typical biological membrane. The nucleocapsid is helical and tightly folded to form a compact structure that tends to closely follow the envelope.

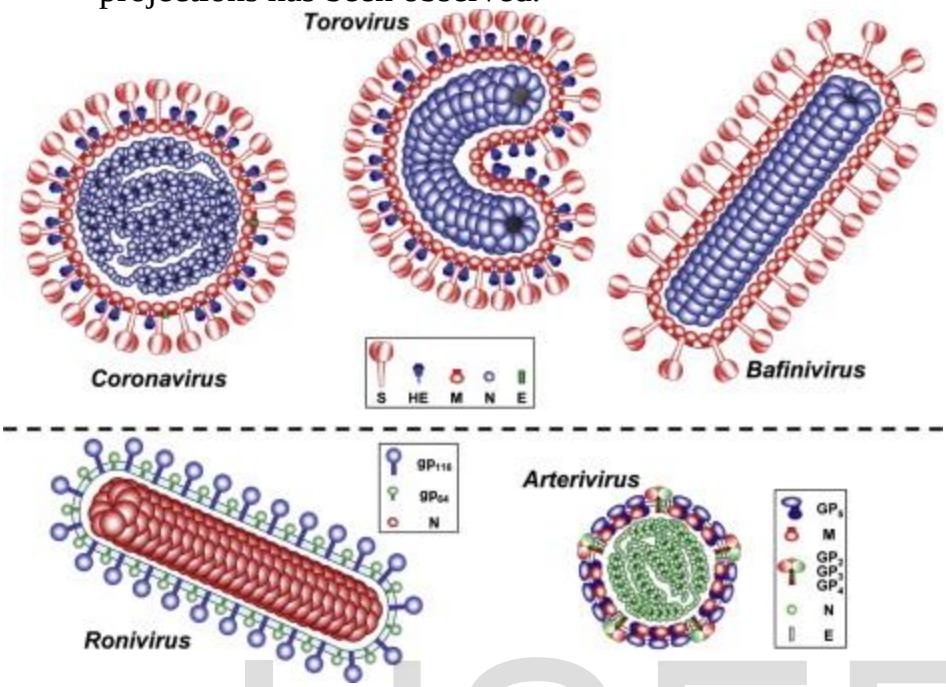
*Torovirinae*, ***Torovirus*:** Toroviruses appear as a mixture of rod-shaped, kidney-shaped and spherical particles. This is, however, most likely an EM artifact. Native torovirus particles are presumably bacilliform with rounded ends, measuring 100–140 nm in length and 35–42 nm in width (envelope outer dimensions). Virions carry two types of surface projections that in size and shape closely resemble those of (beta)coronaviruses. The most distinctive virion element, the core, is a flexible and seemingly hollow tube of helical symmetry (periodicity ca. 4.5 nm), about 100 nm in length and about 23 nm across with a central channel of about 10 nm in diameter.

***Torovirinae, Bafinivirus*:** Bafiniviruses (*ba*cilliform *fi*sh *ni*doviruses) are 130–160×37–45 nm in dimension (excluding spikes) with a rod-like nucleocapsid in the form of a rigid cylinder (120–150×19–22 nm with a central channel of 2–5 nm). The virion envelope is studded with 20–25 nm coronavirus-like peplomers.

***Roniviridae*:** Roniviruses (*ro*d-shaped *ni*doviruses) are also bacilliform in shape, 150–200 nm in length and about 45 nm in diameter, and contain a tightly coiled nucleocapsid with a diameter of about 25 nm and a 5–7 nm helical periodicity. The ronivirus envelope bears spikes, but smaller in size than those of coronaviruses, projecting approximately 11 nm from the surface.

*Arteriviridae*: Arterivirus virions are significantly smaller than those of the other nidoviruses, spherical or egg-shaped and with a seemingly isometric core that contains the genome. Complete particles and nucleocapsids, as measured by cryo-EM, average 54 nm and 39 nm in diameter, respectively, with core and envelope separated by a 2–3 nm gap. Three-dimensional reconstructions, based on cryo-EM tomography, suggests that the core might consist of a helical nucleocapsid wrapped into a hollow ball. No spikes are obvious on

the arterivirus surface, but a surface pattern of relatively small and indistinct projections has been observed.




1. Download : Download full-size image

Figure 1. Schematic structure of particles of members of the order *Nidovirales*.



## Physicochemical and physical properties

The coronavirus virion Mr is $400\times10^6$, the buoyant density in sucrose is 1.15–1.20 g $cm^{-3}$, the density in CsCl is 1.23–1.24 g $cm^{-3}$, and the virion $S_{20,W}$ is 300–500S. Torovirus and bafinivirus virions have buoyant densities in sucrose of 1.14–1.18 and 1.17–1.19 g $cm^{-3}$, respectively. Arterivirus virion buoyant density is 1.13–1.17 g $cm^{-3}$ in sucrose and 1.17–1.20 g $cm^{-3}$ in CsCl; virion $S_{20,W}$ is 200 to 300S. Ronivirus virion buoyant density in sucrose is 1.18–1.20 g $cm^{-3}$. Nidovirus virions are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation.

## Nucleic acid

The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated. Based on the genome size, two groups – large and small nidoviruses – can be distinguished. The genomes of the large nidoviruses are

well over 25 kb in length with size differences in the 5 kb range: 26.4–31.7 kb (*Coronavirus*), 28–28.5 kb (*Torovirus*), about 26.6 (*Bafinivirus*), and 26.2– 26.6 kb (*Okavirus*). The small nidoviruses include a single family (*Arteriviridae*) with genomes from 12.7– 15.7 kb in length. Members of the families *Corona-* and *Roniviridae* are the largest RNA viruses known to date. Complete genome sequences are available for representatives of all seven nidovirus genera.

## Proteins

Although the structural proteins of the nidoviruses are generally functionally equivalent, there is no firm indication that any single protein species is evolutionary conserved across all of the families. The virion proteins typical for each of the five main nidovirus taxa are listed in Table 1.

Table 1. Structural proteins of nidoviruses: acronyms and sizes (in amino acid residues). Boxed proteins are believed to be evolutionarily related

| **Protein**[a] | | ***Coronavirus*** | ***Torovirus*** | ***Bafinivirus*** | ***Okavirus*** | ***Arterivirus*** |
|---|---|---|---|---|---|---|
| Spike glycoprotein | S | 1128–1472 | 1562–1584 | 1220 | - | - |
| Large spike glycoprotein | gp116 | - | - | - | 873[c]–899 | - |
| Small spike glycoprotein | gp64 | - | - | - | 539 | - |
| Minor surface glycoprotein | GP2 | - | - | - | - | 227–249 |
| | GP3 | - | - | - | - | 163–256 |
| | GP4 | - | - | - | - | 152–183 |
| Major surface glycoprotein | GP5 | - | - | - | - | 199–278 |
| Membrane protein | M | 218–263 | 233 | 227 | - | 162–174 |
| Nucleocapsid protein | N | 349–470 | 159–167 | 161 | 144–146 | 110–128 |
| Envelope protein | E | 74–109 | - | - | - | 67–80 |
| Hemagglutinin-esterase protein | HE | 386–440[b] | 416–430 | - | - | - |

a Only proteins typical for each lineage are listed; for some CoVs additional, virus species-specific accessory envelope proteins have been described

b

Only found in a cluster of betacoronaviruses ("phylogroup A", *Betacoronavirus 1*, *Murine coronavirus*, *Human coronavirus HKU-1*).

c

Size predicted for gill-associated virus gp116 protein.

Members of the family *Coronaviridae* generally possess three or four envelope proteins. The most abundant one (at least in corona- and toroviruses) is the membrane (M) protein. Though different in sequence, the M proteins of corona-, toro- and bafiniviruses are alike in size, structure and presumably also in function. They have a similar triple-spanning membrane topology with a short amino terminus located on the outside of the virion, and a long C-terminal endodomain, comprising an amphiphilic region and a hydrophilic tail. The amphiphilic segment is believed to associate with the inner leaflet of the membrane to form a matrix-like lattice, which would explain the remarkable thickness of the coronavirus envelope as observed by cryo-electron tomography. Of note, in transmissible gasteroenteritis virus of swine (*Alphacoronavirus 1*), a second population of M proteins adopting an $N^{exo}$-$C^{exo}$ topology in the viral envelope has been described.

The spike (S) proteins of corona-, toro- and bafiniviruses are exceptionally large type I membrane glycoproteins (1200–1600 aa residues), heavily *N*-glycosylated and with features characteristic of class I fusion proteins. Remote but significant sequence similarity among S proteins of toro-, bafini- and (to lesser extent) coronaviruses suggests common ancestry and similarity in structure and function, i.e. receptor-binding and membrane fusion. With few exceptions, the S proteins become proteolytically cleaved during virion biogenesis into subunits S1 and S2 that remain associated. Coronavirus S proteins assemble into homotrimers and this most likely also occurs with the S proteins of toro- and bafiniviruses. The bulbous membrane-distal part of the peplomers, comprising the receptor-binding domains, are largely composed of S1 subunits, whereas the C-terminal S2 subunits form a membrane-anchored stalk. Heptad repeat regions in S2 are assumed to drive membrane fusion during entry by undergoing a series of conformational changes culminating in a six-helical bundle. In the primary structure, the N-terminal repeat, HR1, is located immediately downstream of a predicted internal fusion peptide and the other repeat, HR2, immediately upstream of the transmembrane domain.

Coronaviruses code for a small envelope protein (E), a pentameric integral membrane protein exhibiting ion channel and/or membrane permeabilizing (viroporin) activities. With around 20 copies per particle, the E protein is only a minor structural component. Although its precise function remains to be defined, the E protein has been implicated in virion morphogenesis and identified as a virulence factor for Severe Acute Respiratory Syndrome (SARS)-CoV. So far, no E homologs have been identied in toro- and bafiniviruses.

A subset of betacoronaviruses (*Betacoronavirus* 1, *Murine coronavirus* and *Human coronavirus HKU1*), and all toroviruses known to date, code for an additional homodimeric type I membrane glycoprotein, the hemagglutinin-esterase (HE), that mediates reversible virion attachment to *O*-acetylated sialic acids by acting both as a lectin and as a sialate-*O*-acetylesterase. Corona- and torovirus HEs share 30% sequence identity and thus are far more closely related to each other than are the S and M proteins of these nidovirus lineages. While the latter two protein species might have been encoded in the last common ancestor of the *Corona-* and *Torovirinae* lineages, the HE proteins must have been acquired relative recently. Originating from a hemagglutinin-esterase fusion protein resembling that of influenza C virus, the HEs appear to have been introduced into the betacorona- and torovirus proteomes independently (i.e. through two separate horizontal gene transfer events) well after the *Corona–Torovirinae* split, and in the case of the coronaviruses even after their separation into alpha-, beta- and gammacoronaviruses. The nucleocapsid (N) proteins of corona- and toroviruses are highly basic, RNA-binding phosphoproteins, involved in encapsidation and packaging of the genome. However, as demonstrated for coronaviruses, N proteins might also play essential roles in RNA synthesis and translation, exhibit RNA chaperone activity and act as antagonists of interferon type I. With molecular masses of about 18 kDa, the torovirus N and proposed bafinivirus N proteins are less than half the size of their coronavirus equivalents. The structural, functional and evolutionary relationships between these protein species remain to be established.

The structural proteins of arteriviruses are apparently unrelated to those of the other members of the order *Nidovirales*. The nucleocapsid contains a single protein species, N. In equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV), six envelope proteins have been identified, each essential for virion

infectivity. The non-glycosylated membrane protein (M) is thought to span the membrane three times and thus to structurally resemble the M protein of corona- and toroviruses. It forms a disulfide-linked heterodimer with the major glycoprotein (GP5 for EAV, PRRSV and lactate dehydrogenase-elevating virus, LDV; GP7 in simian hemorrhagic fever virus, SHFV), which is also a putative triple-spanning membrane protein. Viral glycoproteins GP2, GP3 and GP4 are minor virion components and form heterotrimers. The remaining envelope protein, E (for envelope), is small, hydrophobic and non-glycosylated, and believed to function as an ion-channel protein. LDV virion composition has been studied in less detail, but is likely similar to that of EAV and PRRSV. Remarkably, SHFV may possess up to three additional envelope proteins.

Ronivirus structural proteins have been studied only for yellow head virus (YHV). Virions contain a highly basic nucleoprotein species (p20) and two envelope glycoprotein species (gp116 and gp64) that form the prominent peplomers on the virion surface. Both gp116 and gp64 are encoded by the ORF3 gene and generated from a long (1640–1666 aa residues) precursor glycopolyprotein (pp3) by post-translational processing at two internal signal peptidase type-1 sites (Figure 2). They are not linked by intramolecular disulfide bonds and are anchored in the envelope by either one (gp64) or two (gp116) hydrophobic C-terminal transmembrane domains. Processing of pp3 would also yield an N-terminal product of about 25 kDa, a putative triple-spanning membrane protein, the fate and function of which are not known.



## Abstract

A chromosome is the packaged form of a single linear double-helical DNA molecule. The genome of a eukaryotic organism consists of from 1 to over 200 chromosomes. Chromosomes are located in the nucleus of the cell and exist in the form of chromatin, a complex between DNA, histones (small, highly basic proteins), nonhistone chromosomal proteins (both enzymatic complexes and structural components), and a small amount of RNA (both nascent transcripts and structural components). The numbers and sizes of chromosomes vary considerably among species (*Drosophila melanogaster* has four chromosomes per haploid cell, *Homo sapiens* (human) has 23, etc.). As cells enter mitosis, the chromatin is condensed into readily observed, rod-

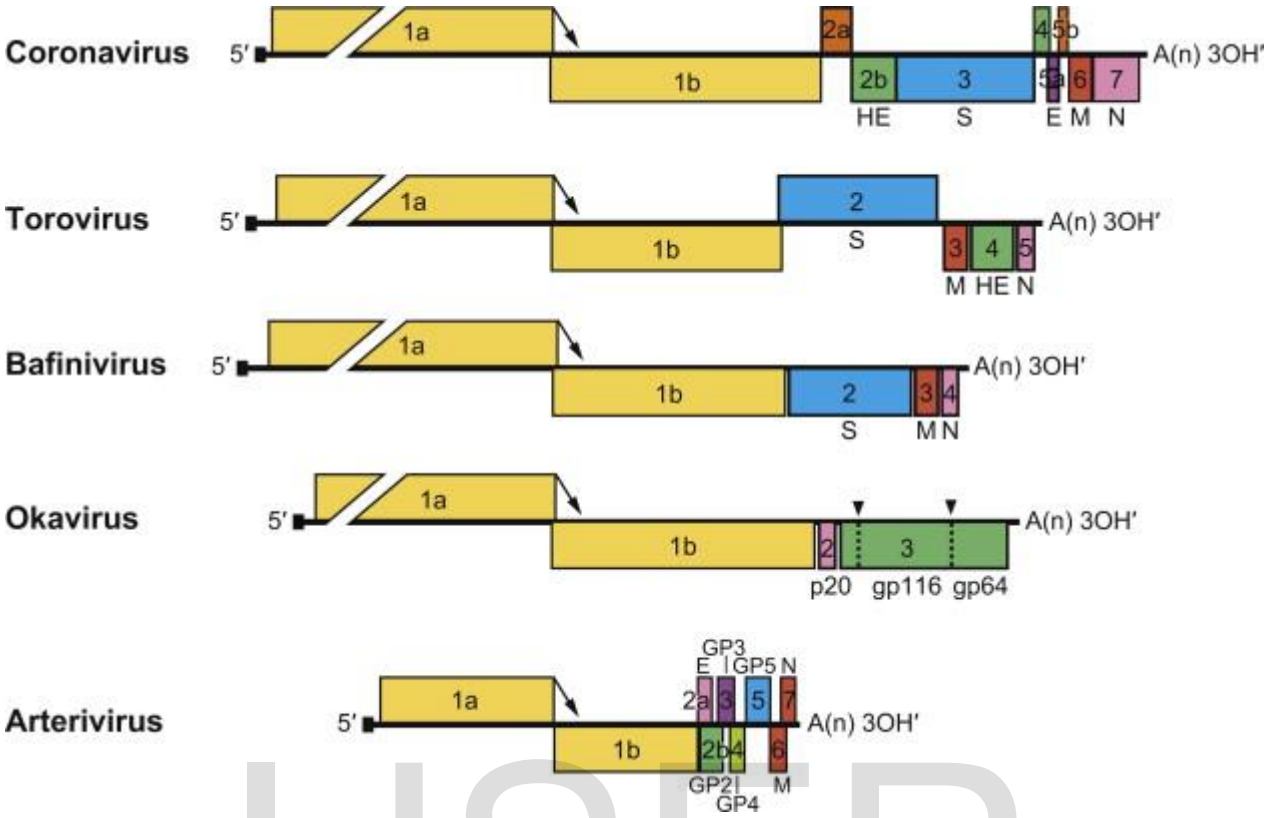
shaped structures, the metaphase chromosomes; a complex process, as described below, is required to package the linear DNA molecule in this form, typically achieving 10 000-fold compactness. Metaphase chromosomes were one of the first subcellular structures observed by cell biologists of the nineteenth century. Chromosomes were subsequently identified as the vehicle for transmission of genetic information during cell division. As the daughter cells return to interphase, the nucleus reforms, and the chromosomes are DE condensed and distributed within the nucleus. The level of DNA packing is much lower during interphase, but is of critical importance in the regulation of gene expression.

All genetic information – genes, regulatory elements, and signals for structural organization – is arranged linearly along chromosomes. The chromosomes provide not only the means for inheritance, but also the template for transcription, the process of reading out the information required by the cell. Different and specific expression profiles are needed for each cell type and stage of development. A full understanding of the mechanisms used in the global regulation of gene expression will not be achieved until the relationships between the linear content of each chromosome, its local and higher-order packaging, or chromatin structure, and the three-dimensional arrangement of the chromosomes in the nucleus have been defined.



## Keywords

In general, there are three **levels** of chromatin organization: **DNA** wraps around histone proteins, forming nucleosomes and the so-called "beads on a string" structure (euchromatin). Multiple histones wrap into a 30-nanometer fiber consisting of nucleosome arrays in their most compact form (heterochromatin)




1. Download : Download full-size image

Figure 2. Schematic representation of the genome structure of members of the order *Nidovirales* (from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). Note that, in coronaviruses, the 3′ genome organization and the complement of accessory genes can differ even among members of the same genus. ORFs are represented by boxes. Untranslated sequences are indicated by solid lines. The ribosomal frameshift sites in ORF1 (located at the 1a-1b junction and indicated by arrows) are aligned. Numbers refer to the mRNA species from which the ORFs are expressed. The proteins encoded by the ORFs are indicated above or below. Signal peptidase type-1 cleavage sites in okavirus precursor glycopolyprotein pp3 are indicated by dashed lines and arrowheads. The 5′ leader sequences are depicted by a small black box. Poly(A) tails are indicated by A(n). S, spike protein; M, membrane protein; E, envelope protein; N, nucleocapsid protein; HE, hemagglutinin-esterase protein.

## Lipids

**Lipids** are also used for cell structure and hormones. **Proteins** are used in a variety of ways: Energy, carrier molecules, enzymes, some hormones, and water retention (albumin). **Nucleic acids** are used to synthesize DNA. **Carbs** and **Proteins** can also be used for cell markers

Nidoviruses have lipid envelopes, which are commonly acquired by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi complex. Coronavirus S and E proteins are palmitoylated; the arterivirus E protein is myristoylated.

### Carbohydrates

Coronavirus S and HE proteins are heavily glycosylated and contain multiple *N*-linked glycans (20–35 and 5–11, respectively). The M protein of coronaviruses contains a small number of either *N*- or *O*-linked glycans, depending on the virus species, located near the amino-terminus. Coronavirus E proteins are not glycosylated. Torovirus S and HE proteins are also heavily *N*-glycosylated (19–25 and 7–13 glycans, respectively); the M protein is not glycosylated, however. Bafinivirus structural proteins have not been characterized in great detail. The S and M proteins appear to be glycosylated. The S protein binds lectins and likely contains α-mannose. The gp116 and gp64 proteins of ronivirus YHV contain 6 and 3 *N*-linked glycans, respectively. In arteriviruses, GP2, GP3, GP4 and GP5 contain *N*-linked glycans. GP5 of EAV, LDV, and PRRSV are modified by heterogeneous *N*-acetyl lactosamine addition. Due to extensive and heterogeneous glycosylation, GP5 is of highly variable size (between 26 and 42 kDa). The M and E proteins are not glycosylated.



## Genome organization and replication

Nidovirus replication takes place in the cytoplasm of infected cells and proceeds through the synthesis of minus-strand intermediates. RNA synthesis is catalyzed by an as yet poorly characterized replication–transcription complex, composed of viral and host proteins and presumably associated (at least in corona- and arteriviruses) with a network of modified intracellular membranes, which is derived from the ER and includes unusual double-membrane vesicles.

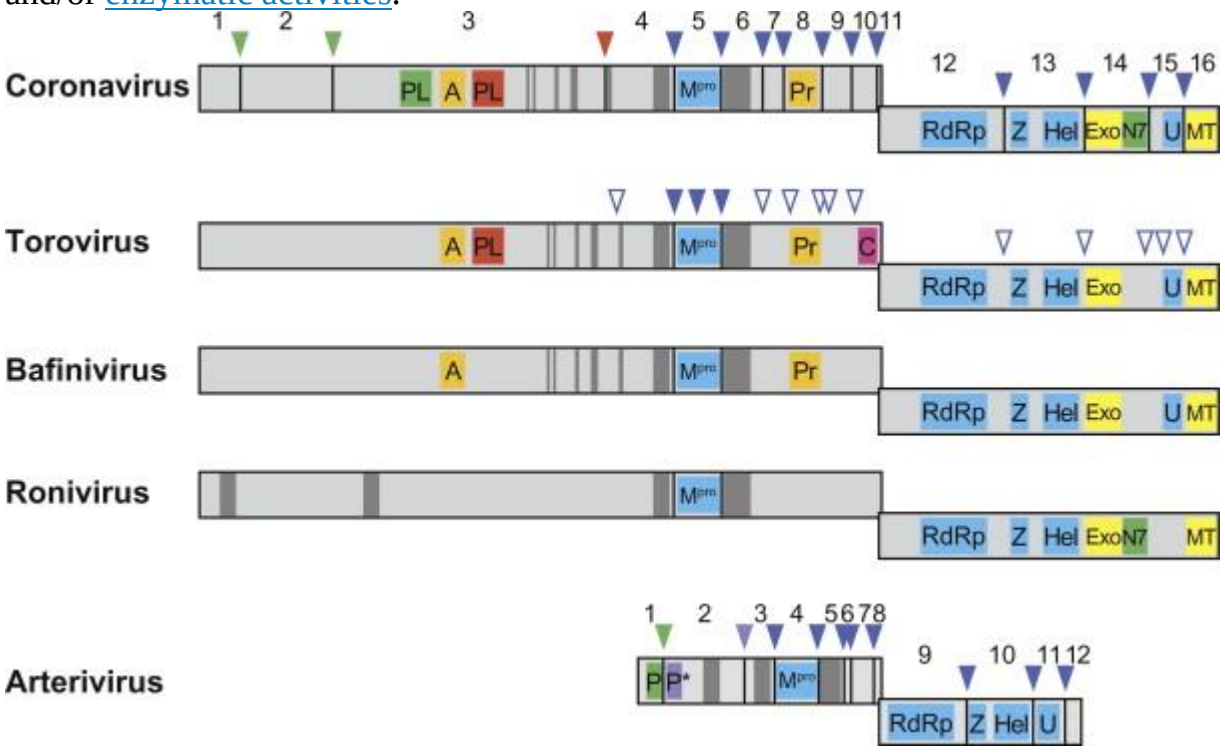
### Genome organization

Despite considerable differences in genome size and gene composition, nidoviruses are remarkably similar in their genome organization (Figure 2). The 5′-most two-

thirds of the genome characteristically comprises two large, partially overlapping ORFs, designated 1a and 1b, that constitute the replicase gene and together encode a collection of enzymes that are part of the replication complex *per se* or control its composition and functioning (see section on *replicase*). The virion RNA functions as mRNA (mRNA1) for ORFs 1a and 1b, but the expression of the latter requires a programmed ribosomal frameshift. Translation of ORF1a yields polyprotein pp1a. In 20–30% of the cases, ribosomes do not reach the ORF1a termination codon, but slip at the ORF1a/1b overlap and shift register to the 21 reading frame to continue translation into ORF1b. The ribosomal frameshift occurs within a specific seven-nucleotide "slippery" sequence, upstream of a pseudoknot structure, and gives rise to a 3′-extended fusion polyprotein, pp1ab. The replicase polyproteins are processed by several virus-encoded proteases to more than a dozen mature products, including the key replicative enzymes/proteins of the virus (further detailed below).
Downstream of the replicase gene there are from three (*Okavirus*) to up to 12 (*Coronavirinae*) ORFs that encode a set of structural proteins typical for the subfamily and/or genus, and, at least for coronaviruses, a variety of "accessory" proteins that may be virus species or even subspecies-specific. These 3′-proximal ORFs are expressed from a 3′-coterminal nested set of dedicated subgenomic (sg) mRNAs, the number of which ranges from two in okaviruses to up to at least eight in certain coronaviruses. All mRNA species, except the smallest ones, are structurally polycistronic. As a rule, however, translation is restricted to the 5′-most ORF(s) not present in the next smaller mRNA of the set; downstream ORF(s) remain translationally silent.
Nidovirus transcription units (i.e one or more ORFs expressed from a single mRNA species) are generally preceded in the genome by short conserved sequence elements commonly termed "transcription-regulating sequences" (TRSs) in corona-, arteri- and bafiniviruses and putative terminator/promoter elements (TPs) in toroviruses. As toro- and roniviruses differ in their transcription mechanism from the other nidoviruses (see below), TRSs and TPs are not functionally equivalent.

Expression of the replicase ORF1a/1b gene yields two huge polyproteins, pp1a and pp1ab. These giant proteins (ranging in size from the approximately 2000-aa pp1a of arteriviruses to the >7000-aa pp1ab of coronaviruses) have not been observed in infected cells. Their processing by viral proteinases is believed to occur both cotranslationally and posttranslationally, yielding more than a dozen mature proteins (13 in arteriviruses and 15 or 16 in coronaviruses) and an as-yet unknown number of functional intermediates. In arteriviruses and coronaviruses, from one to three (and possibly four) papain-like cysteine proteases ($PL^{pro}$ in coronaviruses, PCP and CP in arteriviruses) control the proteolytic processing of the N-terminal part of pp1a/pp1ab at 2-4 sites. A protease with a chymotrypsin-like fold (known as $3CL^{pro}$ or "main" protease, $M^{pro}$; also designated as serine protease SP in arteriviruses) is responsible for the processing of the remaining largest part of pp1a/pp1ab at 8-11 conserved cleavage sites. Nidovirus pp1a/pp1ab processing products, generally referred to as the nonstructural proteins (nsp's), are numbered according to their position (from N- to C-terminus) in the viral polyproteins (nsp1 to nsp12 in arteriviruses and nsp1 to 16 in coronaviruses; Figure 3). In some cases, alternative names are used to refer to functional domain(s) present in these nsp's, especially in cases where the domains are conserved across nidovirus (sub)families and mediate specific functions and/or enzymatic activities.





1. Download : Download full-size image



Figure 3. Schematic representation of the domain organization of the replicase polyproteins pp1a and pp1ab of representative viruses from the five main nidovirus taxa (from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). The position of the ribosomal frameshift site was used to align the polyprotein representations. Cleavage sites in pp1a and pp1ab of papain-like proteinases (PL, P) or of the 3C-like main protease ($M^{pro}$) are indicated by color-coded arrowheads; open arrowheads indicate predicted $M^{pro}$ cleavage sites in the torovirus replicase polyproteins. The processing end-products (nonstructural proteins) of corona- and arterivirus polyproteins are numbered; conserved domains are highlighted as follows: PL, coronavirus papain-like proteinase; P and P*, arterivirus papain-like cysteine proteinases PCP and CP, respectively; A, ADP-ribose-1″-phosphatase (macrodomain); $M^{pro}$, 3C-like main protease; Pr, noncanonical RNA-dependent RNA polymerase, putative primase; C, cyclic nucleotide phosphodiesterase domain; RdRp, RNA-dependent RNA polymerase; Z, zinc-binding domain; Hel, helicase domain; Exo, 3′-to 5′ exoribonuclease domain; N7, guanine-N7-methyltransferase; U, nidoviral uridylate-specific endoribonuclease (NendoU); MT, ribose-2′-*O*-methyltransferase domain.



Despite a more than two-fold difference in size between the replicase genes of arteriviruses and other nidoviruses, a common backbone of conserved domains can be discerned. Sequence alignments and phylogenetic analyses suggest that the conservation of functional domains in the replicase polyproteins is the result of a continuous evolution from a common nidovirus ancestor. Activities and functions have been identified for many of the conserved replicase domains and the corresponding cleavage products. Replicase subunits conserved across all nidoviruses include (from N- to C-terminus): (i) a chymotrypsin-like protease (3C-like or main protease; $3CL^{pro}$, $M^{pro}$) that is flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*) in the viral polyprotein and has a substrate specificity resembling that of picornavirus 3C proteases, (ii) a large RdRp, (iii) a 5′-to-3′ helicase domain containing a putative multinuclear Zn-finger-like domain at its N-terminus (Zn-HEL). Some replicase subunits are present only in a subset of nidoviruses.

Ribonucleic acid (**RNA**) is a linear molecule **composed of** four types of smaller molecules called ribonucleotide bases: adenine (A), cytosine (C), guanine (G), and uracil (U).

A *n*idoviral *endo*ribonuclease specific for *u*ridylate (NendoU) was long considered to be shared by all nidoviruses and to represent a unique diagnostic molecular marker that would distinguish the members of this order from all other RNA viruses known to date. However, a very recent study shows roniviruses to lack this domain. A 3'-to-5' exoribonuclease (ExoN) and ribose-2'-*O*-methyltransferase (O-MT) are conserved in the large nidoviruses, but not in arteriviruses. An ADP-ribose-1"-phosphatase (ADRP, also called macrodomain) and a noncanonical "secondary" RdRp with possible primase activity (coronavirus nsp8), have been (tentatively) mapped only in members of the family *Coronaviridae*. A guanine N7 methyltransferase, recently identified in coronaviruses, appears to be conserved in roniviruses. The conservation of key proteolytic and RNA-processing enzymes in the main nidovirus lineages is summarized in Figure 3.

The N-terminal half of pp1ab is quite variable among nidoviruses, even among members of the same genus. This variability contributes significantly to the major size differences between the genomes of large and small nidoviruses. A comparison between the coronavirus and arterivirus N-terminal pp1a/pp1ab sequences does not yield significant sequence similarities beyond the conservation of active sites of papain-like proteases.



## Synthesis of genomic and subgenomic rnas

Genome replication and sg mRNA synthesis ("transcription") proceed through minus-strand intermediates. The genome serves as a template for the synthesis of full-length minus-strand RNA, from which in turn new genome copies are produced, but it is also believed to be the template for the synthesis of sg minus-strand RNA species (*vide infra*). The synthesis of viral RNAs is highly asymmetrical as plus-strand RNAs are produced in fast excess.

A hallmark of nidovirus transcription is the production of a 3′-coterminal nested set of sg mRNAs. The sg mRNAs of corona-, arteri- and bafiniviruses are chimeric, that is, comprised of sequences that are non-contiguous in the viral genome. Each carries a short 5′ leader sequence of 55–92, 170–210 nt, and 42 nucleotides, respectively, which is identical to the 5′ end of the viral genome. It was established early on that leader and "body" sequences are not joined through splicing, but via a process of

discontinuous RNA synthesis. A key observation was the presence of mirror-copy nested sets of sg minus-strand RNAs in corona- and arterivirus-infected cells. Combined experimental evidence from biochemical and reverse genetics analyses indicates that these sg minus-strand RNAs are in fact the templates for sg mRNA synthesis. Replicative intermediates (RI)/replicative forms with sizes corresponding to the different sg mRNAs were shown to be actively involved in transcription. According to the prevailing 3′-discontinuous extension model, the discontinuous step occurs during the production of sg minus-strand RNAs and entails attenuation of RNA synthesis at the TRSs, followed by a similarity-assisted copy choice RNA recombination event. In corona-, arteri- and bafiniviruses, a TRS is present immediately downstream of the genomic leader sequence. It is believed that, during minus-strand RNA synthesis, the replicase complex upon encounter of an internal TRS dissociates from the template and is transferred to the 5′ end of the genome, guided by sequence complementarity between the anti-TRS on the nascent strand and the genomic TRS. Reinitiation and completion of RNA synthesis would then result in a chimeric minus-strand that in turn would serve as a template for uninterrupted (continuous) synthesis of 5′ leader-containing sg mRNAs.

Discontinuous sg RNA synthesis is not a trait of all nidoviruses. Ronivirus sg mRNAs lack a common 5′ leader and thus apparently arise from non-discontinuous RNA synthesis. Toroviruses employ a mixed transcription strategy; of the four sg RNAs, only RNA 2 carries a 15–18 nt 5′ leader derived from the 5′ end of the genome, whereas the others do not. It is likely that sg mRNAs are transcribed from sg minus-strand templates also in toro- and in roniviruses. Here, the conserved sequence elements (TPs) preceding the 3′-proximal genes might serve dual roles as signals for premature termination of minus-strand synthesis and as promoters for plus-strand production. The torovirus S gene, expressed from mRNA 2, lacks a TP. Apparently, transcription-competent minus-strand sg RNAs are produced by inclusion of a complementary copy of the 5′-terminal genomic TP via a similarity-assisted RNA recombination process analogous to that seen in corona- and arteriviruses.

## Antigenic properties

In coronaviruses, the S protein is an important target for T cell responses and is the major inducer of virus-neutralizing antibodies, which are elicited by epitopes located mostly in the N-terminal half of the molecule. The surface-exposed N-terminus of the M protein induces antibodies that neutralize virus infectivity in the presence of complement. The N protein is a dominant antigen during the natural infection and, like the S protein, might evoke protective T cell responses. HE induces antibodies that prevent binding to *O*-acetylated sialic acids or inhibit sialate-*O*-acetylesterase activity. The ectodomains of the S and HE proteins are highly variable, suggestive of extensive antigenic drift. In addition, there are several examples of intergenotypic exchange of coding sequences of S (for *Avian coronavirus*, *Murine coronavirus* and for the feline and canine coronaviruses belonging to *Alphacoronavirus 1*) and HE (*Murine coronavirus*) ectodomains through homologous RNA recombination, consistent with the occurrence of antigenic shifts.

All toroviruses described so far are serologically related. During natural infection, antibodies are raised against each of the four structural proteins (S, HE, M and N). The spike (S) protein induces virus-neutralizing antibodies; sera from BToV- or PToV-infected animals cross-neutralize EToV. Comparative sequence analysis of bovine and porcine torovirus field variants revealed several instances in which coding sequences for the HE ectodomain had been exchanged through intergenotypic homologous RNA recombination. Bovine torovirus variants currently prevalent in the field (genotypes II and III) have apparently arisen from a recombination event during which the ancestral BToV (genotype I) swapped its N gene for that of porcine torovirus.

Antibodies against the known arteriviruses (EAV, LDV, PRRSV, SHFV) do not cross-react and there is considerable antigenic variation among different strains of EAV, LDV and PRRSV. Major glycoprotein GP5 (designated GP7 in SHFV) is the main determinant of virus-neutralization. In some arteriviruses (PRRSV type I), minor glycoprotein GP4 also induces neutralizing antibodies. A number of arterivirus proteins have been reported to evoke T cell responses, including GP5 and M.

At present, there are no data available about the antigenic properties of bafinivirus proteins or about the innate defense responses mounted against roniviruses in their invertebrate hosts. Serological interfamily or intergenus cross-reactivity has not been demonstrated.

## Biological properties

Coronaviruses infect birds and mammals, including humans, livestock and companion animals. Bats are believed to play a pivotal role in CoV ecology and evolution as they appear to harbor an exceptionally wide diversity of CoVs. It has even been proposed that bats may be the original hosts from which many if not all alpha- and betacoronavirus lineages are derived.

CoVs predominantly target the epithelia and, consequently, infections are mostly associated with respiratory and gastrointestinal disease. Biological vectors are not known. Depending on the virus species, coronaviruses are transmitted via aerosols, fomites or the fecal–oral route. In many instances a persistent chronic infection develops with prolonged shedding of virus from the enteric tract. Coronavirus infections are often mild. However, in 2002–2003, a novel coronavirus, SARS-CoV, caused an epidemic in human populations of a severe pulmonary disease with a mortality rate of 10%. For other CoVs, hepatitis and infection of the central nervous system (MHV), heart and eye (RbCoV) have been described. Variants of *Alphacoronavirus 1* (feline, canine and ferret coronaviruses) may infect cells of the monocyte/macrophage lineage and cause fatal systemic infections characterized by wide-spread granulomatous lesions in multiple organs.

Toroviruses infect ungulates: horses (EToV, Berne virus), bovines (BToV, Breda virus) and swine (PToV). Humans (HToV) and probably carnivores (mustellids) have also been proposed as hosts for toroviruses. Transmission is probably by the fecal–oral route.

The bafinivirus white bream virus is the only known teleost nidovirus and so far was isolated from one species of fresh water fish (*Blicca bjoerkna L.*). At present no further information is available on its ecology, biology and pathogenic properties.

Arteriviruses infect horses (EAV), mice (LDV), monkeys (SHFV) and swine (PRRSV). Primary host cells for all arteriviruses are macrophages. EAV causes inflammation of small arteries and EAV infection can lead to a wide range of clinical manifestations. A fatal outcome of the disease has been reported in both natural and experimental infections, but most natural infections are either mild or subclinical. In pregnant animals, arteriviruses can cause abortions (PRRSV and EAV) or *in utero* fetal death (PRRSV). Persistent infections – lifelong in the case of LDV – are

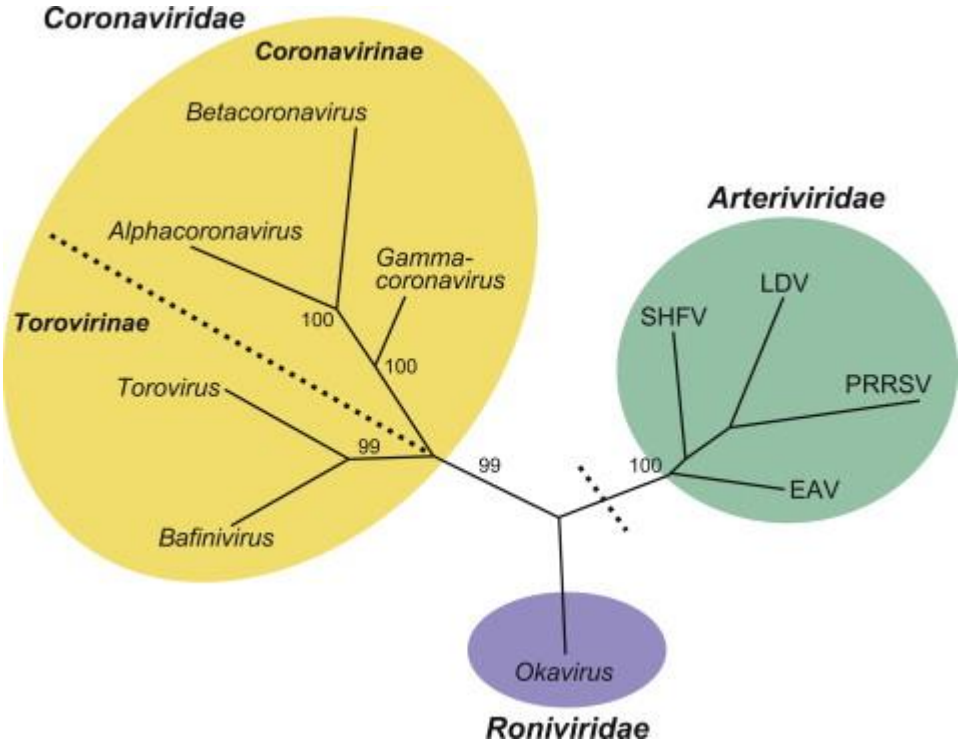
frequently established. Virus may be shed in saliva and respiratory secretions, feces, urine and milk. Persistently-infected males may shed virus in the semen (EAV, PRRSV). Spread is in general horizontal, via direct contact, aerogenic, fecal–oral and/or venereal transmission routes.

Roniviruses are the only known invertebrate nidoviruses and have been detected exclusively in crustaceans. The black tiger prawn (*Penaeus monodon*) appears to be the natural host of YHV and gill-associated virus (GAV), but other prawn species are susceptible to experimental infection. Infections may be chronic or acute and transmission can occur horizontally and vertically. During acute infections, mortality is usually high and virus occurs in most tissues of ectodermal and mesodermal origin, and particularly in the "Oka" or lymphoid organ. Necrotic cells display intensely basophilic cytoplasmic inclusions. The geographic range of infection encompasses the natural Indo-Pacific distribution of *P. monodon*, in which the prevalence of subclinical infection is commonly high, and there is recent evidence of infection occurring in shrimp species farmed in the Americas.



## Phylogenetic relationships within the order

In rooted and unrooted phylogenetic trees constructed for the main replicative enzymes, members of the families *Corona*-, Arteri- and *Roniviridae* consistently form distinct, well-separated monophyletic clusters. Viruses in the subfamily *Torovirinae* (genera *Bafini*- and *Torovirus*) are phylogenetically more related to each other than to those in the subfamily *Coronavirinae*. The evolutionary relationships between nidovirus (sub)families and genera are illustrated in Figure 4.




1. Download : Download full-size image

Figure 4. Nidovirus phylogeny. The evolutionary relationships between the five major nidovirus lineages are depicted by an unrooted maximum parsimonious tree, inferred by using multiple nucleotide sequence alignments of the RdRp-Hel region of representative members of nidovirus (sub)families and genera. For the main bifurcations, support from 100 bootstraps is given. (Sub)families and genera are highlighted and/or labeled. For arteriviruses, the four main clusters, prototyped by EAV, SHFV, LDV and PRRSV (only one of two currently recognized genotypes shown), are indicated. The divisions between large (*Coronaviridae*, *Roniviridae*) and small (*Arteriviridae*) nidoviruses and the one between *Corona-* and *Torovirinae* are indicated by black dotted lines.

(Modified from Gorbalenya, A.E. (2008). Genomics and evolution of the Nidovirales. In: Perlman, Gallagher and Snijder (Eds.), *Nidovirales.* ASM Press, Washington DC, pp.15–28.)



## Similarity with other taxa

Nidoviruses can be uniquely distinguished from other RNA viruses on the basis of their replicase polyproteins that comprise a number of characteristic domains arranged in a conserved order. Key diagnostic molecular markers are:

- An ORF1a-encoded protease with a chymotrypsin-like fold and substrate specificity resembling that of picornavirus 3C protease (3C-like protease, also

called main protease, $M^{pro}$), flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*).

- An ORF1b-encoded putative multinuclear Zn-finger-like domain associated with a nucleoside triphosphate (NTP)-binding/5′-to-3′-helicase domain (Zn-HEL).

- The replicase gene constellation separated by a ribosomal frameshifting signal (*fs*): *tm*-$M^{pro}$-*tm*_*fs*_RdRp_Zn-HEL.

Homologs of several (putative) enzymes encoded by viruses of the order *Nidovirales* have been found in non-nidoviruses. The proteolytic enzymes and RdRps cluster together with homologs of viruses of the "Picornavirus-like" supergroup, and RdRps also with homologs in double stranded RNA *Birnaviridae* family members and a subset of members of the family *Tetraviridae*. The nidovirus helicase and ADRP have counterparts in viruses of the "Alphavirus-like" supergroup. The organization of the replicase ORFs, including the $M^{pro}$_FS_RdRp constellation, is also conserved in the family *Astroviridae* and in some viruses in the Sobemo-like supergroup. Parallels in the genome organization and expression strategy are also evident between members of the order *Nidovirales* and the family *Closteroviridae*.



## Derivation of names

*Nido*: from Latin *nidus*, "nest", refers to the synthesis of a 3′-coterminal, nested set of mRNAs, hallmark of nidovirus transcription.
*Arteri*: from equine *arteri*tis, the disease caused by the reference virus.
*Corona*: from Latin *corona*, "halo"; refers to the characteristic appearance of surface projections that create an image reminiscent of the solar corona.
*Toro*: from Latin *torus*, a term used in architecture for the convex molding at the base of a column and in geometry for a three-dimensional structure in the shape of a hollow donut; refers to the nucleocapsid morphology in a subset of particles.

*Bafini*: from *ba*cilliform *fi*sh *ni*doviruses, refers to the virion morphology and host tropism.

*Roni*: from *ro*d-shaped *ni*doviruses, refers to the virion morphology.

## Note added in proof

During the completion of this manuscript, two papers appeared reporting the discovery of insect nidoviruses. These mosquito-associated nidoviruses are likely representatives of a novel family within the order *Nidovirales*.

## Further reading

### Journals and books

Bárcena et al., 2009

Bárcena M., Oostergetel G.T., Bartelink W., Faas F.G., VerkleijA., Rottier P.J., Koster A.J., Bosch B.J.**Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion**

Proc. Natl Acad. Sci., U S A, 106 (2009), pp. 582-587

CrossRefView Record in ScopusGoogle Scholar

Enjuanes et al., 2006

Enjuanes L., Almazan F., Sola I., Zuñiga S.**Biochemical aspects of coronavirus replication and virus host-interaction**

Ann. Rev. Microbiol., 60 (2006), pp. 211-230

CrossRefView Record in ScopusGoogle Scholar

Gorbalenya et al., 2006

Gorbalenya A.E., Enjuanes L., Ziebuhr J., Snijder E.J.**Nidovirales: evolving the largest RNA virus genome**

Virus Res., 117 (2006), pp. 17-37

ArticleDownload PDFView Record in ScopusGoogle Scholar

Nga et al., 2011

Nga P.T., Parquet M.D.C., Lauber C., Parida M., Nabeshima T., YuF., Thuy N. T., Inoue S., Ito T., Okamoto K., Ichinose A., Snijder E.J., Morita K., Gorbaleny a A.E.**Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes**

PLoS Pathogens (2011), p. 7
in press
Google Scholar

Pasternak et al., 2006
Pasternak A.O., Spaan W.J., Snijder E.J.**Nidovirus transcription: how to make sense …?**
J. Gen. Virol., 87 (2006), pp. 1403-1421
CrossRefView Record in ScopusGoogle Scholar

Perlman et al., 2008
Perlman S., Gallagher T, Snijder E.J. (Eds.), Nidoviruses, ASM Press, Washington, DC (2008)

Google Scholar

Perlman and Netland, 2009
Perlman S., Netland J.**Coronaviruses post-SARS: update on replication and pathogenesis**
Nat. Rev. Microbiol., 7 (2009), pp. 439-450
CrossRefView Record in ScopusGoogle Scholar

Sawicki et al., 2007
Sawicki S.G., Sawicki D.L., Siddell S.G.**A contemporary view of coronavirus transcription**
J. Virol., 81 (2007), pp. 20-29
View Record in ScopusGoogle Scholar

Schütze et al., 2006
Schütze H., Ulferts R., Schelle B., Bayer S., Granzow H., Hoffmann B., Mettenl eiter T.C., Ziebuhr J.**Characterization of White bream virus reveals a novel genetic cluster of nidoviruses**
J. Virol., 80 (2006), pp. 11598-11609
View Record in ScopusGoogle Scholar

Spilman et al., 2009
Spilman M.S., Welbon C., Nelson E., Dokland T.**Cryo-electron tomography of porcine reproductive and respiratory syndrome virus: organization of the nucleocapsid**

J. Gen. Virol., 90 (2009), pp. 527-535
CrossRefView Record in ScopusGoogle Scholar

Thiel, 2007

Thiel V.

Coronaviruses: Molecular and Cellular Biology, Caister Academic Press, Norfolk (2007)

Google Scholar

Zirkel et al., 2011

Zirkel F., Kurth A., Quan P-L., Briese T., Ellerbrok H., Pauli G., Leendertz F.H., Lipkin W.I., Ziebuhr J., Drosten C., Junglen S.

An insect nidovirus emerging from a primary tropical rainforest. mBio, 2 (2011), pp. E00077-11

View Record in Scopus



Websites

VIPR Virus Pathogen Resource: http://www.viprbrc.org/brc/

Contributed by

de Groot, R.J., Cowley, J.A, Enjuanes, L., Faaberg, K.S., Perlman, S., Rottier, P.J.M., Snijder, E.J., Ziebuhr, J. and Gorbalenya, A.E.



Covid-19 Resources

Health information and medical research on Novel Coronavirus (2019-nCoV) are available at Elsevier's Novel Coronavirus Information Center. This free site is updated frequently.

## Recommended articles

- Arteriviridae

Virus Taxonomy, 2012, pp. 796-805
Download PDFView details

- Alphaflexiviridae

  Virus Taxonomy, 2012, pp. 904-919
  Download PDFView details

- Betaflexiviridae

  Virus Taxonomy, 2012, pp. 920-941
  Download PDFView details

12Next

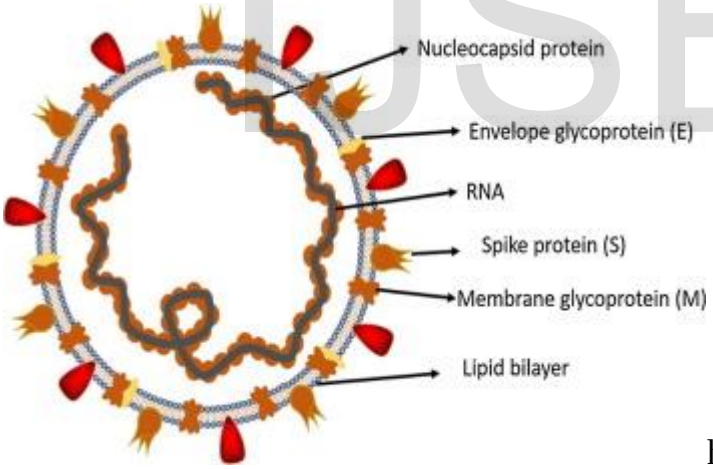
## Citing articles (0)

## Article Metrics

Captures

- Readers:2






https://www.mdpi.com/482034

RESEARCH ARTICLE

s chapter focuses on the Nidovirales order whose member families include Arteriviridae, Coronaviridae, and Roniviridae. The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated. Based on the genome size, they are divided into two groups large and small nidoviruses. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range. Based on the genome size, they can be distinguished into two groups, large and small

nidoviruses. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range. Although the structural proteins of the nidoviruses are generally functionally equivalent, there is no firm indication that any single protein species is evolutionary conserved across all of the families. Members of the family Coronaviridae generally possess three or four envelope proteins. The most abundant one is the membrane (M) protein. Though different in sequence, the M proteins of corona-, toro-, and bafiniviruses are alike in size, structure, and presumably also in function. Nidoviruses have lipid envelopes, which are commonly acquired by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi complex. In coronaviruses, the S protein is an important target for T cell responses and is the major inducer of virus-neutralizing antibodies, which are elicited by epitopes located mostly in the N-terminal half of the molecule.

INTENSITY OF CORONA

---



## Virion properties

### Morphology

The members of the order *Nidovirales* are enveloped, positive-strand RNA viruses of widely different architecture. Depending on whether the external appearance of the virion or the nucleocapsid is considered, similarities and differences can be discerned (Figure 1).

*Coronavirinae*: As seen in conventional electron micrographs, coronaviruses are roughly spherical enveloped particles, 120–160 nm in diameter, with a characteristic fringe of 15–20 nm petal-shaped surface projections (peplomers). In a subset of betacoronaviruses a second, inner fringe of 5–7 nm surface projections is also seen. Coronavirus (CoV) particles as studied by cryo-electron tomography are homogeneous in size and distinctively spherical (envelope outer diameter 85±5 nm). The envelope exhibits an unusual thickness (7.8±0.7 nm), almost twice that of a typical biological membrane.

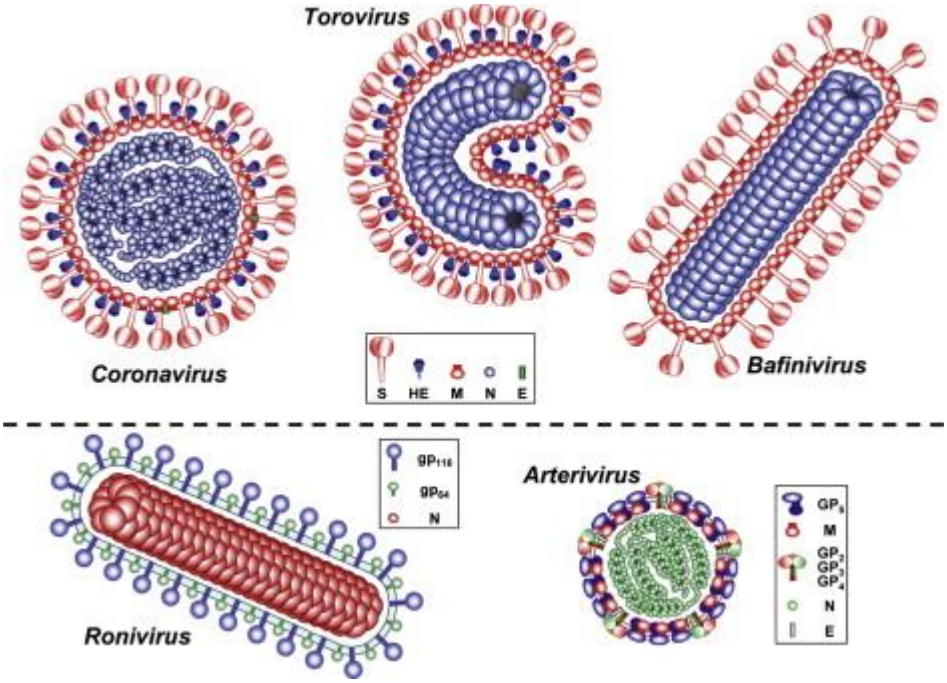
The nucleocapsid is helical and tightly folded to form a compact structure that tends to closely follow the envelope.

*Torovirinae*, ***Torovirus*:** Toroviruses appear as a mixture of rod-shaped, kidney-shaped and spherical particles. This is, however, most likely an EM artifact. Native torovirus particles are presumably bacilliform with rounded ends, measuring 100–140 nm in length and 35–42 nm in width (envelope outer dimensions). Virions carry two types of surface projections that in size and shape closely resemble those of (beta)coronaviruses. The most distinctive virion element, the core, is a flexible and seemingly hollow tube of helical symmetry (periodicity ca. 4.5 nm), about 100 nm in length and about 23 nm across with a central channel of about 10 nm in diameter.

***Torovirinae, Bafinivirus*:** Bafiniviruses (*ba*cilliform *fi*sh *ni*doviruses) are 130–160×37–45 nm in dimension (excluding spikes) with a rod-like nucleocapsid in the form of a rigid cylinder (120–150×19–22 nm with a central channel of 2–5 nm). The virion envelope is studded with 20–25 nm coronavirus-like peplomers.

***Roniviridae*:** Roniviruses (*ro*d-shaped *ni*doviruses) are also bacilliform in shape, 150–200 nm in length and about 45 nm in diameter, and contain a tightly coiled nucleocapsid with a diameter of about 25 nm and a 5–7 nm helical periodicity. The ronivirus envelope bears spikes, but smaller in size than those of coronaviruses, projecting approximately 11 nm from the surface.

*Arteriviridae*: Arterivirus virions are significantly smaller than those of the other nidoviruses, spherical or egg-shaped and with a seemingly isometric core that contains the genome. Complete particles and nucleocapsids, as measured by cryo-EM, average 54 nm and 39 nm in diameter, respectively, with core and envelope separated by a 2–3 nm gap. Three-dimensional reconstructions, based on cryo-EM tomography, suggests that the core might consist of a helical nucleocapsid wrapped into a hollow ball. No spikes are obvious on the arterivirus surface, but a surface pattern of relatively small and indistinct projections has been observed.




1. Download : Download full-size image

Figure 1. Schematic structure of particles of members of the order *Nidovirales*.



## Physicochemical and physical properties

The coronavirus virion Mr is $400\times10^6$, the buoyant density in sucrose is 1.15–1.20 g $cm^{-3}$, the density in CsCl is 1.23–1.24 g $cm^{-3}$, and the virion $S_{20,W}$ is 300–500S. Torovirus and bafinivirus virions have buoyant densities in sucrose of 1.14–1.18 and 1.17–1.19 g $cm^{-3}$, respectively. Arterivirus virion buoyant density is 1.13–1.17 g $cm^{-3}$ in sucrose and 1.17–1.20 g $cm^{-3}$ in CsCl; virion $S_{20,W}$ is 200 to 300S. Ronivirus virion buoyant density in sucrose is 1.18–1.20 g $cm^{-3}$. Nidovirus virions are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation.

## Nucleic acid

The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated. Based on the genome size, two groups – large and small nidoviruses – can be distinguished. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range: 26.4–31.7 kb (*Coronavirus*), 28–28.5 kb (*Torovirus*), about 26.6 (*Bafinivirus*), and 26.2– 26.6 kb

(*Okavirus*). The small nidoviruses include a single family (*Arteriviridae*) with genomes from 12.7– 15.7 kb in length. Members of the families *Corona-* and *Roniviridae* are the largest RNA viruses known to date. Complete genome sequences are available for representatives of all seven nidovirus genera.

## Proteins

Although the structural proteins of the nidoviruses are generally functionally equivalent, there is no firm indication that any single protein species is evolutionary conserved across all of the families. The virion proteins typical for each of the five main nidovirus taxa are listed in Table 1.

Table 1. Structural proteins of nidoviruses: acronyms and sizes (in amino acid residues). Boxed proteins are believed to be evolutionarily related

| **Protein**[a] | | ***Coronavirus*** | ***Torovirus*** | ***Bafinivirus*** | ***Okavirus*** | ***Arterivirus*** |
|---|---|---|---|---|---|---|
| Spike glycoprotein | S | 1128–1472 | 1562–1584 | 1220 | - | - |
| Large spike glycoprotein | gp116 | - | - | - | 873[c]–899 | - |
| Small spike glycoprotein | gp64 | - | - | - | 539 | - |
| Minor surface glycoprotein | GP2 | - | - | - | - | 227–249 |
| | GP3 | - | - | - | - | 163–256 |
| | GP4 | - | - | - | - | 152–183 |
| Major surface glycoprotein | GP5 | - | - | - | - | 199–278 |
| Membrane protein | M | 218–263 | 233 | 227 | - | 162–174 |
| Nucleocapsid protein | N | 349–470 | 159–167 | 161 | 144–146 | 110–128 |
| Envelope protein | E | 74–109 | - | - | - | 67–80 |
| Hemagglutinin-esterase protein | HE | 386–440[b] | 416–430 | - | - | - |

a

Only proteins typical for each lineage are listed; for some CoVs additional, virus species-specific accessory envelope proteins have been described

b

Only found in a cluster of betacoronaviruses ("phylogroup A", *Betacoronavirus 1*, *Murine coronavirus*, *Human coronavirus HKU-1*).

c

Size predicted for gill-associated virus gp116 protein.

Members of the family *Coronaviridae* generally possess three or four envelope proteins. The most abundant one (at least in corona- and toroviruses) is the membrane (M) protein. Though different in sequence, the M proteins of corona-, toro- and bafiniviruses are alike in size, structure and presumably also in function. They have a similar triple-spanning membrane topology with a short amino terminus located on the outside of the virion, and a long C-terminal endodomain, comprising an amphiphilic region and a hydrophilic tail. The amphiphilic segment is believed to associate with the inner leaflet of the membrane to form a matrix-like lattice, which would explain the remarkable thickness of the coronavirus envelope as observed by cryo-electron tomography. Of note, in transmissible gasteroenteritis virus of swine (*Alphacoronavirus 1*), a second population of M proteins adopting an $N^{exo}$-$C^{exo}$ topology in the viral envelope has been described.

The spike (S) proteins of corona-, toro- and bafiniviruses are exceptionally large type I membrane glycoproteins (1200–1600 aa residues), heavily *N*-glycosylated and with features characteristic of class I fusion proteins. Remote but significant sequence similarity among S proteins of toro-, bafini- and (to lesser extent) coronaviruses suggests common ancestry and similarity in structure and function, i.e. receptor-binding and membrane fusion. With few exceptions, the S proteins become proteolytically cleaved during virion biogenesis into subunits S1 and S2 that remain associated. Coronavirus S proteins assemble into homotrimers and this most likely also occurs with the S proteins of toro- and bafiniviruses. The bulbous membrane-distal part of the peplomers, comprising the receptor-binding domains, are largely composed of S1 subunits, whereas the C-terminal S2 subunits form a membrane-anchored stalk. Heptad repeat regions in S2 are assumed to drive membrane fusion during entry by undergoing a series of conformational changes culminating in a six-helical bundle. In the primary structure, the N-terminal repeat, HR1, is located immediately downstream of a predicted internal fusion peptide and the other repeat, HR2, immediately upstream of the transmembrane domain.

Coronaviruses code for a small envelope protein (E), a pentameric integral membrane protein exhibiting ion channel and/or membrane permeabilizing (viroporin) activities. With around 20 copies per particle, the E protein is only a minor structural

component. Although its precise function remains to be defined, the E protein has been implicated in virion morphogenesis and identified as a virulence factor for Severe Acute Respiratory Syndrome (SARS)-CoV. So far, no E homologs have been identied in toro- and bafiniviruses.

A subset of betacoronaviruses (*Betacoronavirus* 1, *Murine coronavirus* and *Human coronavirus HKU1*), and all toroviruses known to date, code for an additional homodimeric type I membrane glycoprotein, the hemagglutinin-esterase (HE), that mediates reversible virion attachment to *O*-acetylated sialic acids by acting both as a lectin and as a sialate-*O*-acetylesterase. Corona- and torovirus HEs share 30% sequence identity and thus are far more closely related to each other than are the S and M proteins of these nidovirus lineages. While the latter two protein species might have been encoded in the last common ancestor of the *Corona-* and *Torovirinae* lineages, the HE proteins must have been acquired relative recently. Originating from a hemagglutinin-esterase fusion protein resembling that of influenza C virus, the HEs appear to have been introduced into the betacorona- and torovirus proteomes independently (i.e. through two separate horizontal gene transfer events) well after the *Corona–Torovirinae* split, and in the case of the coronaviruses even after their separation into alpha-, beta- and gammacoronaviruses. The nucleocapsid (N) proteins of corona- and toroviruses are highly basic, RNA-binding phosphoproteins, involved in encapsulation and packaging of the genome. However, as demonstrated for coronaviruses, N pro

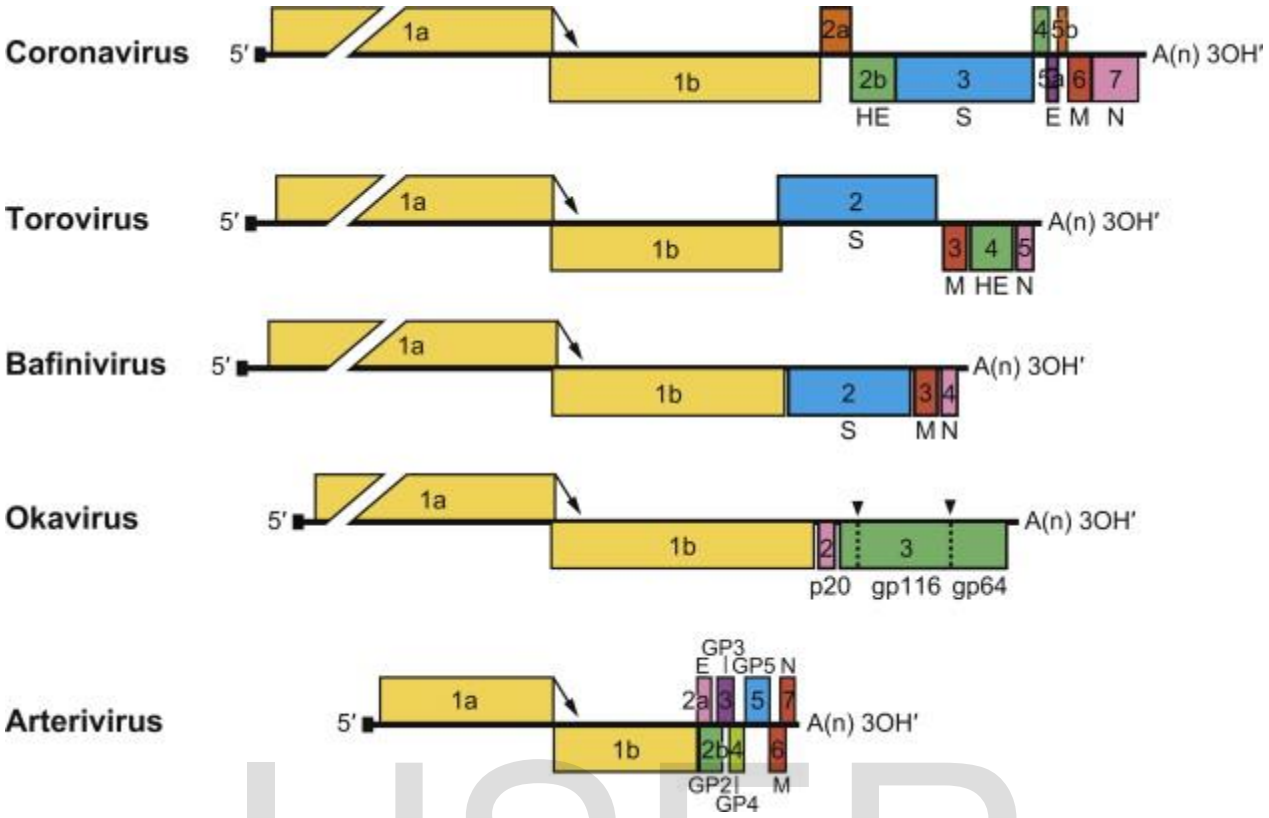


teins might also play essential roles in RNA synthesis and translation, exhibit RNA chaperone activity and act as antagonists of interferon type I. With molecular masses of about 18 kDa, the torovirus N and proposed bafinivirus N proteins are less than half the size of their coronavirus equivalents. The structural, functional and evolutionary relationships between these protein species remain to be established.

The structural proteins of arteriviruses are apparently unrelated to those of the other members of the order *Nidovirales*. The nucleocapsid contains a single protein species, N. In equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV), six envelope proteins have been identified, each essential for virion infectivity. The non-glycosylated membrane protein (M) is thought to span the

membrane three times and thus to structurally resemble the M protein of corona- and toroviruses. It forms a disulfide-linked heterodimer with the major glycoprotein (GP5 for EAV, PRRSV and lactate dehydrogenase-elevating virus, LDV; GP7 in simian hemorrhagic fever virus, SHFV), which is also a putative triple-spanning membrane protein. Viral glycoproteins GP2, GP3 and GP4 are minor virion components and form heterotrimers. The remaining envelope protein, E (for envelope), is small, hydrophobic and non-glycosylated, and believed to function as an ion-channel protein. LDV virion composition has been studied in less detail, but is likely similar to that of EAV and PRRSV. Remarkably, SHFV may possess up to three additional envelope proteins.

Ronivirus structural proteins have been studied only for yellow head virus (YHV). Virions contain a highly basic nucleoprotein species (p20) and two envelope glycoprotein species (gp116 and gp64) that form the prominent peplomers on the virion surface. Both gp116 and gp64 are encoded by the ORF3 gene and generated from a long (1640–1666 aa residues) precursor glycopolyprotein (pp3) by post-translational processing at two internal signal peptidase type-1 sites (Figure 2). They are not linked by intramolecular disulfide bonds and are anchored in the envelope by either one (gp64) or two (gp116) hydrophobic C-terminal transmembrane domains. Processing of pp3 would also yield an N-terminal product of about 25 kDa, a putative triple-spanning membrane protein, the fate and function of which are not known.




1. Download : Download full-size image

Figure 2. Schematic representation of the genome structure of members of the order *Nidovirales* (from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). Note that, in coronaviruses, the 3′ genome organization and the complement of accessory genes can differ even among members of the same genus. ORFs are represented by boxes. Untranslated sequences are indicated by solid lines. The ribosomal frameshift sites in ORF1 (located at the 1a-1b junction and indicated by arrows) are aligned. Numbers refer to the mRNA species from which the ORFs are expressed. The proteins encoded by the ORFs are indicated above or below. Signal peptidase type-1 cleavage sites in okavirus precursor glycopolyprotein pp3 are indicated by dashed lines and arrowheads. The 5′ leader sequences are depicted by a small black box. Poly(A) tails are indicated by A(n). S, spike protein; M, membrane protein; E, envelope protein; N, nucleocapsid protein; HE, hemagglutinin-esterase protein.

## Lipids

Nidoviruses have lipid envelopes, which are commonly acquired by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi

complex. Coronavirus S and E proteins are palmitoylated; the arterivirus E protein is myristoylated.

### Carbohydrates

Coronavirus S and HE proteins are heavily glycosylated and contain multiple *N*-linked glycans (20–35 and 5–11, respectively). The M protein of coronaviruses contains a small number of either *N*- or *O*-linked glycans, depending on the virus species, located near the amino-terminus. Coronavirus E proteins are not glycosylated. Torovirus S and HE proteins are also heavily *N*-glycosylated (19–25 and 7–13 glycans, respectively); the M protein is not glycosylated, however. Bafinivirus structural proteins have not been characterized in great detail. The S and M proteins appear to be glycosylated. The S protein binds lectins and likely contains α-mannose. The gp116 and gp64 proteins of ronivirus YHV contain 6 and 3 *N*-linked glycans, respectively. In arteriviruses, GP2, GP3, GP4 and GP5 contain *N*-linked glycans. GP5 of EAV, LDV, and PRRSV are modified by heterogeneous *N*-acetyl lactosamine addition. Due to extensive and heterogeneous glycosylation, GP5 is of highly variable size (between 26 and 42 kDa). The M and E proteins are not glycosylated.



## Genome organization and replication

Nidovirus replication takes place in the cytoplasm of infected cells and proceeds through the synthesis of minus-strand intermediates. RNA synthesis is catalyzed by an as yet poorly characterized replication–transcription complex, composed of viral and host proteins and presumably associated (at least in corona- and arteriviruses) with a network of modified intracellular membranes, which is derived from the ER and includes unusual double-membrane vesicles.

### Genome organization

Despite considerable differences in genome size and gene composition, nidoviruses are remarkably similar in their genome organization (Figure 2). The 5′-most two-thirds of the genome characteristically comprises two large, partially overlapping ORFs, designated 1a and 1b, that constitute the replicase gene and together encode a

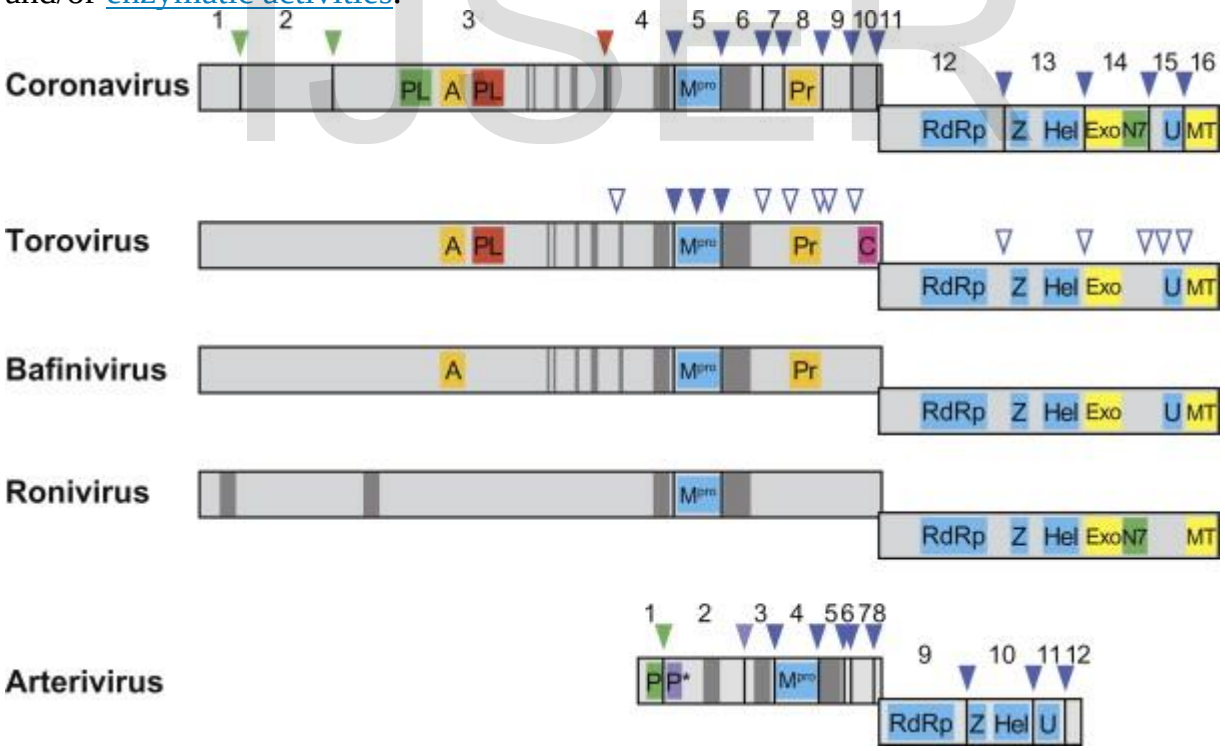
collection of enzymes that are part of the replication complex *per se* or control its composition and functioning (see section on *replicase*). The virion RNA functions as mRNA (mRNA1) for ORFs 1a and 1b, but the expression of the latter requires a programmed ribosomal frameshift. Translation of ORF1a yields polyprotein pp1a. In 20–30% of the cases, ribosomes do not reach the ORF1a termination codon, but slip at the ORF1a/1b overlap and shift register to the 21 reading frame to continue translation into ORF1b. The ribosomal frameshift occurs within a specific seven-nucleotide "slippery" sequence, upstream of a pseudoknot structure, and gives rise to a 3′-extended fusion polyprotein, pp1ab. The replicase polyproteins are processed by several virus-encoded proteases to more than a dozen mature products, including the key replicative enzymes/proteins of the virus (further detailed below).
Downstream of the replicase gene there are from three (*Okavirus*) to up to 12 (*Coronavirinae*) ORFs that encode a set of structural proteins typical for the subfamily and/or genus, and, at least for coronaviruses, a variety of "accessory" proteins that may be virus species or even subspecies-specific. These 3′-proximal ORFs are expressed from a 3′-coterminal nested set of dedicated subgenomic (sg) mRNAs, the number of which ranges from two in okaviruses to up to at least eight in certain coronaviruses. All mRNA species, except the smallest ones, are structurally polycistronic. As a rule, however, translation is restricted to the 5′-most ORF(s) not present in the next smaller mRNA of the set; downstream ORF(s) remain translationally silent.
Nidovirus transcription units (i.e one or more ORFs expressed from a single mRNA species) are generally preceded in the genome by short conserved sequence elements commonly termed "transcription-regulating sequences" (TRSs) in corona-, arteri- and bafiniviruses and putative terminator/promoter elements (TPs) in toroviruses. As toro- and roniviruses differ in their transcription mechanism from the other nidoviruses (see below), TRSs and TPs are not functionally equivalent.

## The replicase gene

Expression of the replicase ORF1a/1b gene yields two huge polyproteins, pp1a and pp1ab. These giant proteins (ranging in size from the approximately 2000-aa pp1a of arteriviruses to the >7000-aa pp1ab of coronaviruses) have not been observed in

infected cells. Their processing by viral proteinases is believed to occur both cotranslationally and posttranslationally, yielding more than a dozen mature proteins (13 in arteriviruses and 15 or 16 in coronaviruses) and an as-yet unknown number of functional intermediates. In arteriviruses and coronaviruses, from one to three (and possibly four) papain-like cysteine proteases ($PL^{pro}$ in coronaviruses, PCP and CP in arteriviruses) control the proteolytic processing of the N-terminal part of pp1a/pp1ab at 2-4 sites. A protease with a chymotrypsin-like fold (known as $3CL^{pro}$ or "main" protease, $M^{pro}$; also designated as serine protease SP in arteriviruses) is responsible for the processing of the remaining largest part of pp1a/pp1ab at 8-11 conserved cleavage sites. Nidovirus pp1a/pp1ab processing products, generally referred to as the nonstructural proteins (nsp's), are numbered according to their position (from N- to C-terminus) in the viral polyproteins (nsp1 to nsp12 in arteriviruses and nsp1 to 16 in coronaviruses; Figure 3). In some cases, alternative names are used to refer to functional domain(s) present in these nsp's, especially in cases where the domains are conserved across nidovirus (sub)families and mediate specific functions and/or enzymatic activities.






1. Download : Download full-size image

Figure 3. Schematic representation of the domain organization of the replicase polyproteins pp1a and pp1ab of representative viruses from the five main nidovirus taxa

(from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). The position of the ribosomal frameshift site was used to align the polyprotein representations. Cleavage sites in pp1a and pp1ab of papain-like proteinases (PL, P) or of the 3C-like main protease ($M^{pro}$) are indicated by color-coded arrowheads; open arrowheads indicate predicted $M^{pro}$ cleavage sites in the torovirus replicase polyproteins. The processing end-products (nonstructural proteins) of corona- and arterivirus polyproteins are numbered; conserved domains are highlighted as follows: PL, coronavirus papain-like proteinase; P and P*, arterivirus papain-like cysteine proteinases PCP and CP, respectively; A, ADP-ribose-1″-phosphatase (macrodomain); $M^{pro}$, 3C-like main protease; Pr, noncanonical RNA-dependent RNA polymerase, putative primase; C, cyclic nucleotide phosphodiesterase domain; RdRp, RNA-dependent RNA polymerase; Z, zinc-binding domain; Hel, helicase domain; Exo, 3′-to 5′ exoribonuclease domain; N7, guanine-N7-methyltransferase; U, nidoviral uridylate-specific endoribonuclease (NendoU); MT, ribose-2′-*O*-methyltransferase domain.

Despite a more than two-fold difference in size between the replicase genes of arteriviruses and other nidoviruses, a common backbone of conserved domains can be discerned. Sequence alignments and phylogenetic analyses suggest that the conservation of functional domains in the replicase polyproteins is the result of a continuous evolution from a common nidovirus ancestor. Activities and functions have been identified for many of the conserved replicase domains and the corresponding cleavage products. Replicase subunits conserved across all nidoviruses include (from N- to C-terminus): (i) a chymotrypsin-like protease (3C-like or main protease; $3CL^{pro}$, $M^{pro}$) that is flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*) in the viral polyprotein and has a substrate specificity resembling that of picornavirus 3C proteases, (ii) a large RdRp, (iii) a 5′-to-3′ helicase domain containing a putative multinuclear Zn-finger-like domain at its N-terminus (Zn-HEL). Some replicase subunits are present only in a subset of nidoviruses. A *n*idoviral *endo*ribonuclease specific for *u*ridylate (NendoU) was long considered to be shared by all nidoviruses and to represent a unique diagnostic molecular marker that would distinguish the members of this order from all other RNA viruses known to date. However, a very recent study shows roniviruses to lack this domain. A 3'-to-5' exoribonuclease (ExoN) and ribose-2'-*O*-methyltransferase (O-MT) are conserved in the large nidoviruses, but not in arteriviruses. An ADP-ribose-1"-phosphatase (ADRP, also called macrodomain) and a

noncanonical “secondary” RdRp with possible primase activity (coronavirus nsp8), have been (tentatively) mapped only in members of the family *Coronaviridae*.
A guanine N7 methyltransferase, recently identified in coronaviruses, appears to be conserved in roniviruses. The conservation of key proteolytic and RNA-processing enzymes in the main nidovirus lineages is summarized in Figure 3.
The N-terminal half of pp1ab is quite variable among nidoviruses, even among members of the same genus. This variability contributes significantly to the major size differences between the genomes of large and small nidoviruses. A comparison between the coronavirus and arterivirus N-terminal pp1a/pp1ab sequences does not yield significant sequence similarities beyond the conservation of active sites of papain-like proteases.

## Synthesis of genomic and subgenomic rnas

Genome replication and sg mRNA synthesis (“transcription”) proceed through minus-strand intermediates. The genome serves as a template for the synthesis of full-length minus-strand RNA, from which in turn new genome copies are produced, but it is also believed to be the template for the synthesis of sg minus-strand RNA species (*vide infra*). The synthesis of viral RNAs is highly asymmetrical as plus-strand RNAs are produced in fast excess.
A hallmark of nidovirus transcription is the production of a 3′-coterminal nested set of sg mRNAs. The sg mRNAs of corona-, arteri- and bafiniviruses are chimeric, that is, comprised of sequences that are non-contiguous in the viral genome. Each carries a short 5′ leader sequence of 55–92, 170–210 nt, and 42 nucleotides, respectively, which is identical to the 5′ end of the viral genome. It was established early on that leader and “body” sequences are not joined through splicing, but via a process of discontinuous RNA synthesis. A key observation was the presence of mirror-copy nested sets of sg minus-strand RNAs in corona- and arterivirus-infected cells. Combined experimental evidence from biochemical and reverse genetics analyses indicates that these sg minus-strand RNAs are in fact the templates for sg mRNA synthesis. Replicative intermediates (RI)/replicative forms with sizes corresponding to the different sg mRNAs were shown to be actively involved in transcription.
According to the prevailing 3′-discontinuous extension model, the discontinuous step

occurs during the production of sg minus-strand RNAs and entails attenuation of RNA synthesis at the TRSs, followed by a similarity-assisted copy choice RNA recombination event. In corona-, arteri- and bafiniviruses, a TRS is present immediately downstream of the genomic leader sequence. It is believed that, during minus-strand RNA synthesis, the replicase complex upon encounter of an internal TRS dissociates from the template and is transferred to the 5′ end of the genome, guided by sequence complementarity between the anti-TRS on the nascent strand and the genomic TRS. Reinitiation and completion of RNA synthesis would then result in a chimeric minus-strand that in turn would serve as a template for uninterrupted (continuous) synthesis of 5′ leader-containing sg mRNAs.

Discontinuous sg RNA synthesis is not a trait of all nidoviruses. Ronivirus sg mRNAs lack a common 5′ leader and thus apparently arise from non-discontinuous RNA synthesis. Toroviruses employ a mixed transcription strategy; of the four sg RNAs, only RNA 2 carries a 15–18 nt 5′ leader derived from the 5′ end of the genome, whereas the others do not. It is likely that sg mRNAs are transcribed from sg minus-strand templates also in toro- and in roniviruses. Here, the conserved sequence elements (TPs) preceding the 3′-proximal genes might serve dual roles as signals for premature termination of minus-strand synthesis and as promoters for plus-strand production. The torovirus S gene, expressed from mRNA 2, lacks a TP. Apparently, transcription-competent minus-strand sg RNAs are produced by inclusion of a complementary copy of the 5′-terminal genomic TP via a similarity-assisted RNA recombination process analogous to that seen in corona- and arteriviruses.



## Antigenic properties

In coronaviruses, the S protein is an important target for T cell responses and is the major inducer of virus-neutralizing antibodies, which are elicited by epitopes located mostly in the N-terminal half of the molecule. The surface-exposed N-terminus of the M protein induces antibodies that neutralize virus infectivity in the presence of complement. The N protein is a dominant antigen during the natural infection and, like the S protein, might evoke protective T cell responses. HE induces antibodies that prevent binding to *O*-acetylated sialic acids or inhibit sialate-*O*-acetylesterase activity. The ectodomains of the S and HE proteins are highly variable, suggestive of extensive

antigenic drift. In addition, there are several examples of intergenotypic exchange of coding sequences of S (for *Avian coronavirus*, *Murine coronavirus* and for the feline and canine coronaviruses belonging to *Alphacoronavirus 1*) and HE (*Murine coronavirus*) ectodomains through homologous RNA recombination, consistent with the occurrence of antigenic shifts.

All toroviruses described so far are serologically related. During natural infection, antibodies are raised against each of the four structural proteins (S, HE, M and N). The spike (S) protein induces virus-neutralizing antibodies; sera from BToV- or PToV-infected animals cross-neutralize EToV. Comparative sequence analysis of bovine and porcine torovirus field variants revealed several instances in which coding sequences for the HE ectodomain had been exchanged through intergenotypic homologous RNA recombination. Bovine torovirus variants currently prevalent in the field (genotypes II and III) have apparently arisen from a recombination event during which the ancestral BToV (genotype I) swapped its N gene for that of porcine torovirus.

Antibodies against the known arteriviruses (EAV, LDV, PRRSV, SHFV) do not cross-react and there is considerable antigenic variation among different strains of EAV, LDV and PRRSV. Major glycoprotein GP5 (designated GP7 in SHFV) is the main determinant of virus-neutralization. In some arteriviruses (PRRSV type I), minor glycoprotein GP4 also induces neutralizing antibodies. A number of arterivirus proteins have been reported to evoke T cell responses, including GP5 and M.

At present, there are no data available about the antigenic properties of bafinivirus proteins or about the innate defense responses mounted against roniviruses in their invertebrate hosts. Serological interfamily or intergenus cross-reactivity has not been demonstrated.

## Biological properties

Coronaviruses infect birds and mammals, including humans, livestock and companion animals. Bats are believed to play a pivotal role in CoV ecology and evolution as they appear to harbor an exceptionally wide diversity of CoVs. It has even been proposed that bats may be the original hosts from which many if not all alpha- and betacoronavirus lineages are derived.

CoVs predominantly target the epithelia and, consequently, infections are mostly associated with respiratory and gastrointestinal disease. Biological vectors are not known. Depending on the virus species, coronaviruses are transmitted via aerosols, fomites or the fecal–oral route. In many instances a persistent chronic infection develops with prolonged shedding of virus from the enteric tract. Coronavirus infections are often mild. However, in 2002–2003, a novel coronavirus, SARS-CoV, caused an epidemic in human populations of a severe pulmonary disease with a mortality rate of 10%. For other CoVs, hepatitis and infection of the central nervous system (MHV), heart and eye (RbCoV) have been described. Variants of *Alphacoronavirus 1* (feline, canine and ferret coronaviruses) may infect cells of the monocyte/macrophage lineage and cause fatal systemic infections characterized by wide-spread granulomatous lesions in multiple organs.

Toroviruses infect ungulates: horses (EToV, Berne virus), bovines (BToV, Breda virus) and swine (PToV). Humans (HToV) and probably carnivores (mustellids) have also been proposed as hosts for toroviruses. Transmission is probably by the fecal–oral route.

The bafinivirus white bream virus is the only known teleost nidovirus and so far was isolated from one species of fresh water fish (*Blicca bjoerkna L.*). At present no further information is available on its ecology, biology and pathogenic properties.

Arteriviruses infect horses (EAV), mice (LDV), monkeys (SHFV) and swine (PRRSV). Primary host cells for all arteriviruses are macrophages. EAV causes inflammation of small arteries and EAV infection can lead to a wide range of clinical manifestations. A fatal outcome of the disease has been reported in both natural and experimental infections, but most natural infections are either mild or subclinical. In pregnant animals, arteriviruses can cause abortions (PRRSV and EAV) or *in utero* fetal death (PRRSV). Persistent infections – lifelong in the case of LDV – are frequently established. Virus may be shed in saliva and respiratory secretions, feces, urine and milk. Persistently-infected males may shed virus in the semen (EAV, PRRSV). Spread is in general horizontal, via direct contact, aerogenic, fecal–oral and/or venereal transmission routes.

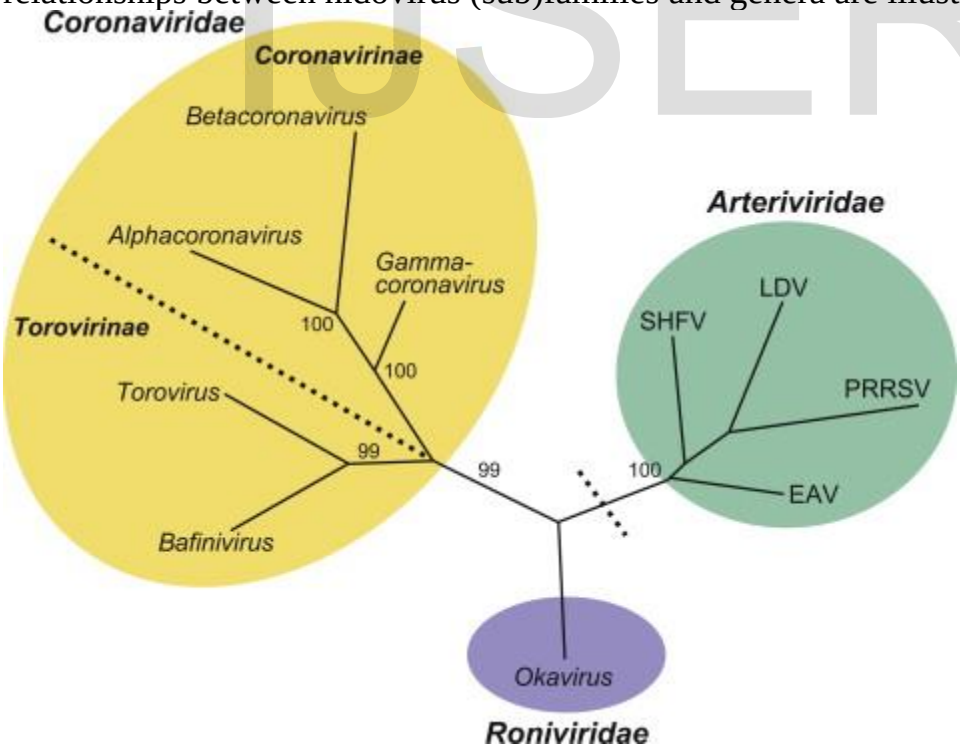
Roniviruses are the only known invertebrate nidoviruses and have been detected exclusively in crustaceans. The black tiger prawn (*Penaeus monodon*) appears to be the natural host of YHV and gill-associated virus (GAV), but other prawn species are

susceptible to experimental infection. Infections may be chronic or acute and transmission can occur horizontally and vertically. During acute infections, mortality is usually high and virus occurs in most tissues of ectodermal and mesodermal origin, and particularly in the "Oka" or lymphoid organ. Necrotic cells display intensely basophilic cytoplasmic inclusions. The geographic range of infection encompasses the natural Indo-Pacific distribution of *P. monodon*, in which the prevalence of subclinical infection is commonly high, and there is recent evidence of infection occurring in shrimp species farmed in the Americas.

## Phylogenetic relationships within the order

In rooted and unrooted phylogenetic trees constructed for the main replicative enzymes, members of the families *Corona*-, Arteri- and *Roniviridae* consistently form distinct, well-separated monophyletic clusters. Viruses in the subfamily *Torovirinae* (genera *Bafini*- and *Torovirus*) are phylogenetically more related to each other than to those in the subfamily *Coronavirinae*. The evolutionary relationships between nidovirus (sub)families and genera are illustrated in Figure 4.






1. Download : Download full-size image

Figure 4. Nidovirus phylogeny. The evolutionary relationships between the five major nidovirus lineages are depicted by an unrooted maximum parsimonious tree, inferred by using multiple nucleotide sequence alignments of the RdRp-Hel region of representative members of nidovirus (sub)families and genera. For the main bifurcations, support from 100 bootstraps is given. (Sub)families and genera are highlighted and/or labeled. For arteriviruses, the four main clusters, prototyped by EAV, SHFV, LDV and PRRSV (only one of two currently recognized genotypes shown), are indicated. The divisions between large (*Coronaviridae*, *Roniviridae*) and small (*Arteriviridae*) nidoviruses and the one between *Corona*- and *Torovirinae* are indicated by black dotted lines.

(Modified from Gorbalenya, A.E. (2008). Genomics and evolution of the Nidovirales. In: Perlman, Gallagher and Snijder (Eds.), *Nidovirales.* ASM Press, Washington DC, pp.15–28.)

## Similarity with other taxa

Nidoviruses can be uniquely distinguished from other RNA viruses on the basis of their replicase polyproteins that comprise a number of characteristic domains arranged in a conserved order. Key diagnostic molecular markers are:

- An ORF1a-encoded protease with a chymotrypsin-like fold and substrate specificity resembling that of picornavirus 3C protease (3C-like protease, also called main protease, $M^{pro}$), flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*).
- An ORF1b-encoded putative multinuclear Zn-finger-like domain associated with a nucleoside triphosphate (NTP)-binding/5′-to-3′-helicase domain (Zn-HEL).
- The replicase gene constellation separated by a ribosomal frameshifting signal (*fs*): *tm*-$M^{pro}$-*tm*_*fs*_RdRp_Zn-HEL.

Homologs of several (putative) enzymes encoded by viruses of the order *Nidovirales* have been found in non-nidoviruses. The proteolytic enzymes and RdRps cluster together with homologs of viruses of the “Picornavirus-like” supergroup, and RdRps also with homologs in double stranded RNA *Birnaviridae* family members and a subset of members of the

family *Tetraviridae*. The nidovirus helicase and ADRP have counterparts in viruses of the "Alphavirus-like" supergroup. The organization of the replicase ORFs, including the $M^{pro}$_FS_RdRp constellation, is also conserved in the family *Astroviridae* and in some viruses in the Sobemo-like supergroup. Parallels in the genome organization and expression strategy are also evident between members of the order *Nidovirales* and the family *Closteroviridae*.

## Derivation of names

*Nido*: from Latin *nidus*, "nest", refers to the synthesis of a 3′-coterminal, nested set of mRNAs, hallmark of nidovirus transcription.
*Arteri*: from equine *arteri*tis, the disease caused by the reference virus.
*Corona*: from Latin *corona*, "halo"; refers to the characteristic appearance of surface projections that create an image reminiscent of the solar corona.
*Toro*: from Latin *torus*, a term used in architecture for the convex molding at the base of a column and in geometry for a three-dimensional structure in the shape of a hollow donut; refers to the nucleocapsid morphology in a subset of particles.
*Bafini*: from *ba*cilliform *fi*sh *ni*doviruses, refers to the virion morphology and host tropism.
*Roni*: from *ro*d-shaped *ni*doviruses, refers to the virion morphology.



## Note added in proof

During the completion of this manuscript, two papers appeared reporting the discovery of insect nidoviruses. These mosquito-associated nidoviruses are likely representatives of a novel family within the order *Nidovirales*.

## Further reading

### Journals and books

Bárcena et al., 2009

Bárcena M., Oostergetel G.T., Bartelink W., Faas F.G., VerkleijA., Rottier P.J., Koster A.J., Bosch B.J.**Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion**

Proc. Natl Acad. Sci., U S A, 106 (2009), pp. 582-587
CrossRefView Record in ScopusGoogle Scholar

Enjuanes et al., 2006

Enjuanes L., Almazan F., Sola I., Zuñiga S.**Biochemical aspects of coronavirus replication and virus host-interaction**
Ann. Rev. Microbiol., 60 (2006), pp. 211-230
CrossRefView Record in ScopusGoogle Scholar

Gorbalenya et al., 2006

Gorbalenya A.E., Enjuanes L., Ziebuhr J., Snijder E.J.**Nidovirales: evolving the largest RNA virus genome**
Virus Res., 117 (2006), pp. 17-37
ArticleDownload PDFView Record in ScopusGoogle Scholar

Nga et al., 2011

Nga P.T., Parquet M.D.C., Lauber C., Parida M., Nabeshima T., YuF., Thuy N. T., Inoue S., Ito T., Okamoto K., Ichinose A., Snijder E.J., Morita K., Gorbalen ya A.E.**Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes**
PLoS Pathogens (2011), p. 7
in press
Google Scholar

Pasternak et al., 2006

Pasternak A.O., Spaan W.J., Snijder E.J.**Nidovirus transcription: how to make sense …?**
J. Gen. Virol., 87 (2006), pp. 1403-1421
CrossRefView Record in ScopusGoogle Scholar

Perlman et al., 2008

Perlman S., Gallagher T, Snijder E.J. (Eds.), Nidoviruses, ASM Press, Washington, DC (2008)

Google Scholar

Perlman and Netland, 2009

Perlman S., Netland J.**Coronaviruses post-SARS: update on replication and pathogenesis**

Nat. Rev. Microbiol., 7 (2009), pp. 439-450
CrossRefView Record in ScopusGoogle Scholar

Sawicki et al., 2007

Sawicki S.G., Sawicki D.L., Siddell S.G.**A contemporary view of coronavirus transcription**
J. Virol., 81 (2007), pp. 20-29
View Record in ScopusGoogle Scholar

Schütze et al., 2006

Schütze H., Ulferts R., Schelle B., Bayer S., Granzow H., Hoffmann B., Metten leiter T.C., Ziebuhr J.**Characterization of White bream virus reveals a novel genetic cluster of nidoviruses**
J. Virol., 80 (2006), pp. 11598-11609
View Record in ScopusGoogle Scholar

Spilman et al., 2009

Spilman M.S., Welbon C., Nelson E., Dokland T.**Cryo-electron tomography of porcine reproductive and respiratory syndrome virus: organization of the nucleocapsid**
J. Gen. Virol., 90 (2009), pp. 527-535
CrossRefView Record in ScopusGoogle Scholar

Thiel, 2007

Thiel V.

https://images.app.goo.gl/hE8RCJw3ZuLfnkQJ8

Coronaviruses: Molecular and Cellular Biology, Caister Academic Press, Norfolk (2007)

Google Scholar

Zirkel et al., 2011

Zirkel F., Kurth A., Quan P-
L., Briese T., Ellerbrok H., Pauli G., Leendertz F.H., Lipkin W.I., Ziebuhr J., D rosten C., Junglen S.

An insect nidovirus emerging from a primary tropical rainforest.
mBio, 2 (2011), pp. E00077-11

View Record in Scopus

Websites

VIPR Virus Pathogen Resource: http://www.viprbrc.org/brc/

Contributed by

de Groot, R.J., Cowley, J.A, Enjuanes, L., Faaberg, K.S., Perlman, S., Rottier, P.J.M., Snijder, E.J., Ziebuhr, J. and Gorbalenya, A.E.



Covid-19 Resources

Health information and medical research on Novel Coronavirus (2019-nCoV) are available at Elsevier's Novel Coronavirus Information Center. This free site is updated frequently.



**(0)**

## Article Metrics

Captures

- Readers:2

Paper number (e.g., I054334) (only one paper per form) _____

Institution: _____RESEARCH FELLOW- [IRED_]__[RESERCH SCHOLAR OPJS UNIVERSITY _____

Street Address: _____ KOLKATA GARIA STATION ROAD_____

City: __KOLKATA_____ State:WESTBENGAL _____ Zip: 700084_____ Country: __india_____

Phone: ____918240837053_____ Fax: ____skkhankolkata@hotmail.com_____ Email: _____

Registration Fees

Standard Paper (IJSER Format) € 65

Research Scholar / Student

(provide id proof for association with academic

institution) € 45

TOTAL: € __45_____trying by paypal [send other way]

Date: _16.04.2020_____ Author's Signature: _saroj kumar khan_____

**Topic……COVID 19 AND ITS REMEADY,AUTHOR Dr saroj kumar khan……**

**ASSIGNMENT-1**

**SUBMITTED FOR THE PARTIAL FULLFIL FOR AWARD OF DEGREE**

**OF**

**DOCTOR OF PHILOSOPHY**

**IN**

**ENGLISH**

**Supervised by:**

**Name: Dr. Saroj khan**

**Designation: Assoc. Professor**

**Submitted by:**

**Name: ----------**

**Reg. No……**

**Faculty of English**

**OPJS University, Churu, Rajasthan**

**2017**

## Part – A

**Q.1 What is Research and describe the type of Research? Write about your research topic and explain?**

Ans. Research is a systematic inquiry that investigates hypotheses, suggests new interpretations of data or texts, and poses new questions for future research to explore.

Research consists of:

- Asking a question that nobody has asked before;
- Doing the necessary work to find the answer; and
- Communicating the knowledge you have acquired to a larger audience.

In practice, research methods vary widely, depending upon the academic discipline's accepted standards, the individual researcher's preferences, or a particular study's needs. Research in science and engineering often involves conducting experiments in the lab or in the field. Research in the arts, humanities, and social sciences may include archival work in the library or on the internet, conducting surveys or in-depth interviews, and a wide range of creative and artistic projects- from costume design to playwriting to curating a fine arts exhibit. Research is not a solitary activity –but an act of community. As a member of the research community, you are building on the knowledge that others have acquired before you and providing a road map for those who come after you. You are adding to a body of work that will never be complete. Research is an ongoing, collaborative process with no finish line in sight.

**Types of research**

**1. Quantitative research**

Quantitative research is generally associated with the positivist/post positivist paradigm. It usually involves collecting and converting data into numerical form so that statistical calculations can be made and conclusions drawn.

Researchers will have one or more **hypotheses.** These are the questions that they want to address which include predictions about possible relationships between the things they want to investigate **(variables)**. In order to find answers to these questions, the researchers will also have various instruments and materials (e.g. paper or computer tests, observation check lists etc.) and a clearly defined plan of action. Data is collected by various means following a strict procedure and prepared for **statistical analysis**. Nowadays, this is carried out with the aid of sophisticated statistical computer packages. The analysis enables the researchers to determine to what extent there is a relationship between two or more variables.

**2. Qualitative research**

Qualitative research is the approach usually associated with the social constructivist paradigm which emphasizes the socially constructed nature of reality. It is about recording, analyzing and attempting to uncover the deeper meaning and significance of human behavior and experience, including contradictory beliefs, behaviors and emotions. Researchers are interested in gaining a rich and complex understanding of people's experience and not in obtaining information which can be generalized to other larger groups. The approach adopted by qualitative researchers tends to be inductive which means that they develop a theory or look for a pattern of meaning on the basis of the data that they have collected. This involves a move from the specific to the general and is sometimes called a bottom-up approach. However, most research projects also involve a certain degree of deductive.

Qualitative researchers do not base their research on pre-determined hypotheses. Nevertheless, they clearly identify a problem or topic that they want to explore and may be guided by a theoretical lens - a kind of overarching theory which provides a framework for their investigation.

**Review of Topic**

As my research topic is **"An Analytical Study on wireless network management tools aided troubleshooting Wi-Fi problems: Assessing benefits for IT Infrastructure"**, we would focus on analyzing the wireless network management tools.

To enhance the precision of the calculation, WiSlow additionally utilizes a heuristic strategy that considers the historical backdrop of impedance scenes and matches it to the regular utilization qualities of different gadgets (e.g., microwave broilers are frequently utilized discontinuously for times of 5– 30 minutes, though infant screens are utilized

constantly) to learn the wellspring of the problem. Given our exploratory outcomes and heuristic techniques, we have built up a calculation that effectively recognizes channel dispute from non-Wi-Fi obstruction and gathers the item sort of the culpable gadget.

We trust that this innovation will be helpful to end clients since it can advise them of what should be done with a specific end goal to enhance the execution of their networks whether to redesign their Internet data transmission or expel a gadget that is radiating the impedance. In non-Wi-Fi impedance situations, another objective is to recognize the area of the wellspring of obstruction.

Despite the fact that it is hard to pinpoint the correct physical area of the source without the help of equipment or APs, we can surmise the relative area of the problem source by working together with flip side clients associated with a similar wireless network. WiSlow gathers examples of factors from peers and decides if others watch the obstruction in the meantime. If every one of the machines watches it, it is exceptionally likely that the problematic source is near the wireless AP. Be that as it may, if just a single of the associates watches the obstruction, the source is probably going to be found near that companion. Our test comes about unmistakably demonstrate that this approach is practical.

**Q.2 Explain the Component of research problem and step of scientific research.**

Ans. A research problem is the statement which defines the research area and also gives a quick synopsis of how the hypothesis was arrived at. There are a number of things which you should consider before creating a research problem like first outline the general context of the problem. Then highlight the key theories, concepts and ideas. Underline the main assumptions of the area and highlight why these issues are identified important. After that determine what needs to be solved and also take into consideration the unanswered questions and controversies. By keeping all these things in mind, develop a research problem which should be precise, simple and clear. The problem statement is the guiding theme of the proposal. This section should include a statement of the purpose of the study and should specify its objectives.



**Purpose of the Study**

This section should explain why the research is being conducted. It should establish the importance of the problem addressed by the research and explain why the research is needed. For example, it might establish the seriousness of juvenile antisocial behavior nationally and describe the gaps that exist in the knowledge about this behavior. It might also explain why the specific knowledge gap chosen is of particular importance.

**Objectives**

This section should describe what the investigator hopes to accomplish with the research. After reading this section, the reader should be clear about the questions to be asked, the kinds of answers expected, and the nature of the information to be provided by the proposed research. For example, one might propose to test a drug abuse treatment approach to determine the intervention characteristics that contribute differentially to the

success of adolescent boys and girls who participate in the program. Expected outcomes might also include the provision of descriptive information not currently available. An example of this might be a comparison of arrest rates for participants in the years prior to and following participation in the program.

**Methodology Subjects**

Subjects can be individuals, families, groups, organizations, states, or countries, depending on the unit of analysis. This section will describe how the sample in the study will be selected. For example, will volunteers be solicited? Will every subject who volunteers be included? If not, what criteria will be used to choose those to be included? Will there be a comparison group? How will the subjects in that group be chosen? In addition to describing how subjects will be chosen, this section should provide a rationale for the selection approach taken. This rationale usually includes external validity requirements (i.e., the conditions necessary to generalize the findings to a particular target population). After reading this section, the reader should have a clear understanding of how subjects will be selected for the proposed research and of why they will be selected in that particular manner. The reader should also have a clear idea of the characteristics of the intended subjects, including age, sex, ethnicity, education, SES, and other related variables.

Design, this section will describe the type of research design to be used. Will it be an idiographic, survey, quasi-experimental or experimental design? Will it be cross-sectional or longitudinal? Will it be a retrospective or a prospective design? The design should also describe the sequence of events that will occur in conducting the research. This would include how the subjects will be divided up, what the subjects are expected to experience during the research, and when and how often they will be observed or asked for information. After reading this section, the reader should have a clear understanding of the overall design of the study.

Data Collection: This section will operationalize the variables to be included in the proposed evaluation. It is helpful to divide the variables into dependent variables, independent variables, and covariates. Dependent variables are outcomes (e.g., drug abuse, self-esteem, depression) which are affected directly by other variables. They might also include variables which are affected indirectly (e.g., arrest rates, recidivism, employment record). Independent variables can include intervention approaches, program characteristics, and subject characteristics believed to affect the dependent variables. Covariates are additional independent variables included in the research solely for the purpose of controlling for differences that might exist among subjects. These differences are controlled statistically so that they will not confound conclusions that are drawn about relationships between independent variables and dependent variables.

**Data Analysis**

This section will explain how the data will be analyzed once they are collected. Usually, more than one analysis is conducted. Each analysis that will be used to meet each objective listed above should be described. Also a description of the specific effects to be examined in each analysis, such as main effects, interaction effects. or simple main effects, should be included. The unit of analysis to be used should be specified and the reason for choosing that unit should be explained. After reading this section, the reader should know which effects will guide the data analysis and in exactly what way the data are to be analyzed to meet each objective of the proposed study. Data analyses should be specifically linked to the hypotheses so that it is clear how each hypothesis will be tested.

**Q.3 What is Research Methodology explain it in contest of your research topic?**

Ans. **Research Problem:**



This research entitled "An Analytical Study on wireless network management tools aided troubleshooting Wi-Fi problems: Assessing benefits for IT Infrastructure", will analyze wireless network management tools aided troubleshooting Wi-Fi problems. We don't make any suspicions about the particular network connectors or drivers that end clients may have. Some Atheros chipsets, which are utilized as a part of research think about, bolster an otherworldly output that gives a range investigation of different recurrence ranges. The element to recognize non-Wi-Fi interferers utilizing a product network card without specific equipment. Since our device means to provide the best estimation of the problem source, if the client happens to have this particular network connector, WiSlow can embrace a similar approach. In any case, to the best of our insight, just a couple of chipsets at present give this component. Furthermore, we neglected to find references to this element for any OS other than Linux. Since there are several items that utilization an alternate chipset as well as OS, it is illogical to expect that a general end client has this particular setup. Along these lines, we concentrate rather on dissecting the nature of a connection utilizing client controllable conventions, for example, UDP and 802.11 parcels.

Since the instruments of these conventions are not fundamentally extraordinary for some Wi-Fi devices, we trust WiSlow can help a more extensive scope of end clients. Without original equipment or a specific network connector, it is as yet conceivable to quantify signal quality by observing 802.11 bundles. What's more, signal data from different recurrence groups can be gotten by channel exchanging. It might distinguish the signal mark of each meddling gadget. Be that as it may, without the specific functionalities of

some wireless cards, the AP must be reset at whatever point the channels are exchanged. This isn't viable for general customer machines, not just because it requires a significant stretch of time to check every one of the channels, yet additionally because the frequencies given for the signal specimens are not at an adequately fine-grained determination.

**Methods for Research**

**1. Observational Approach**

It will investigate the wireless network management tools aided troubleshooting Wi-Fi problems: Assessing benefits for IT Infrastructure. This project will improve a search process in unstructured wireless network by reducing flooding configuring uniform nodes using NS-2 simulator to analyze dynamic search and route discovery problems in various networks. To reduce number of transmissions during searching any property by establishing uniform nodes network using simulation methodologies.

**2. Case Studies**

In this type we use the explorative and qualitative studies with taking number of case studies as secondary data sources.

**4. Knowledge – Intensive based Approach**

The knowledge-intensive based approach will focus on R&D and complex problem-solving. They operate in temporary cross-functional teams to integrate their knowledge resources in an effective and flexible way.

**Data Collection Method**

**Primary data collection**

Primary source is a source from where we collect first-hand information or original data on a topic. This research has designed based upon the analytical study it aims to identify the network and computer security threat incidents are on the rise for troubleshooting, and both corporations and governments are continuing to investigate ways to effectively manage the challenges those threats create.

**Secondary data collection**

We will collect secondary data from the published financial statements of the firms, newspaper and articles. This is the minor part of this research but important as well. In this part data would be collected from the internet sites, journals, books, published articles, records of an organization. This type of data have been collected and recorded by another person or organization, sometimes for altogether different purposes.

**Q.4 What is Data, explain type of sources to collect data and information?**

Data is a collection of facts, such as numbers, words, measurements, observations or even just descriptions of things.



Data can be qualitative or quantitative.

- **Qualitative data** is descriptive information (it *describes* something)
- **Quantitative data** is numerical information (numbers).

And **Quantitative data** can also be Discrete or Continuous:

- **Discrete data** can only take certain values (like whole numbers)
- **Continuous data** can take any value (within a range)

Put simply: **Discrete data** is counted, **Continuous data** is measured

The source of data collection is the following:

**DATA COLLECTION:**

**Primary Data Collection:** The researcher decided to meet employee and managers and collected data which are unpublished for tracing workplace diversity challenge in Human resource challenge and making the study more updated with newly financial reforms of banking scenario.

The data is being collected by various means like:

- Experiments
- Questionnaire/schedule

- Meeting
- Observation
- Office records

**Secondary Data Collection:** The study is also based on secondary information, and all the relevant information is collected from various issues of Statistical Tables Relating to the issues, and Database on management published by Organization. In addition to that some information was also collected from different issues of Survey published by the certain important books and journals.

**Q.5 Describe difference between Schedule and Questionnaire?**

Ans. The difference between these two given below:

| S.No | Questionnaire | Schedule |
|---|---|---|
| 1. | Questionnaire is generally sent through mail to informants to be answered as specified in a covering letter, but otherwise without further assistance from the sender. | A schedule is generally filled by the research worker or enumerator, who can interpret the questions when necessary. |
| 2. | Data collection is cheap and economical as the money is spent in preparation of questionnaire and in mailing the same to respondents. | Data collection is more expensive as money is spent on enumerators and in imparting trainings to them. Money is also spent in preparing schedules. |
| 3. | Non response is usually high as many people do not respond and many return the questionnaire without answering all questions. Bias due to non response often remains indeterminate. | Non response is very low because this is filled by enumerators who are able to get answers to all questions. But even in this their remains the danger of interviewer bias and cheating. |
| 4. | It is not clear that who replies. | Identity of respondent is not known. |

| | | |
|---|---|---|
| 5. | The questionnaire method is likely to be very slow since many respondents do not return the questionnaire. | Information is collected well in time as they are filled by enumerators. |
| 6. | No personal contact is possible in case of questionnaire as the questionnaires are sent to respondents by post who also in turn returns the same by post. | Direct personal contact is established |
| 7. | This method can be used only when respondents are literate and cooperative. | The information can be gathered even when the respondents happen to be illiterate. |
| 8. | Wider and more representative distribution of sample is possible. | There remains the difficulty in sending enumerators over a relatively wider area. |
| 9. | Risk of collecting incomplete and wrong information is relatively more under the questionnaire method, when people are unable to understand questions properly. | The information collected is generally complete and accurate as enumerators can remove difficulties if any faced by respondents in correctly understanding the questions. As a result the information collected through schedule is relatively more accurate than that obtained through questionnaires. |
| 10. | The success of questionnaire methods lies more on the quality of the questionnaire itself. | It depends upon the honesty and competence of enumerators |
| 11. | The physical appearance of questionnaire must be quite attractive. | This may not be the case as schedules are to be filled in by enumerators and not by respondents. |
| 12. | This is not possible when collecting data through questionnaire. | Along with schedule observation method can also be used. |

**Q.6 What is sampling explains it in contest of your research?**

Ans. This research is focused on the observation and their analysis methods. A search process in unstructured wireless network generally employee whole network due to this it will generally carried out flooding problem. Existing system contains flooding algorithm to represent search process but this system address lack of search problem and inefficiency factors. The flooding algorithm needs to search on each node on over unstructured network to find out property which consumes an extra time.

**Q.7 What is Computer and explain the use of computer in Research Work?**

Ans. Computer is an electronic device that is designed to work with Information. *The term computer* is *derived from the Latin term* '**computer**', this means to *calculate* or *programmable machine*. **Computer cannot do anything without a Program**. It represents the decimal numbers through a string of binary digits. The Word 'Computer' usually refers to the Center Processor Unit plus Internal memory.

Computers have changed the ways in which scientific research is compiled and analyzed. Scientists, engineers and researchers alike are able to compile vast amounts of data and leave it to the computer to work through the data while focusing on another area of the research project. This creates research results with fewer errors and better-engineered products. Computers used in scientific research have the ability to analyze data in ways and at speeds not possible with the human eye. They are able to analyze percentages of materials present in a variety of compounds from soil samples to chemicals and even the air you breathe. Additionally, computers used in this manner can determine trends in data samples. For example, computer analysis of data in research could determine the temperature at which certain chemical compounds break down or the percentages of improvement patients show when administered a certain medication. Scientific research often requires that complex mathematical equations be solved in order to determine if data is valid or if a certain structure of molecules will remain stable. Computers are integral to this calculation process since scientists can write software programs specifically to provide answers to such questions. This removes the element of human error, which can cost research institutions millions of dollars in fixing a product that was created with even the smallest amount of flawed data.

Scientists and researchers are able to use computer programs to model how data might manifest itself in the future. This ability is useful in predicting climate patterns,

simulating how engineered products might perform in the field, predicting the erosion rate of beaches and anticipating the absorption rate of medications in the body. Scientists and engineers are then able to adjust building strategies or chemical compositions of products to ensure safe operation and consumption.

**Q.8 What is INFLIBNET and how it is useful for Research Scholar?**

Ans. Information and Library Network (INFLIBNET) is an autonomous inter-university centre of the University Grants Commission (UGC) located at Gujarat University Campus, Ahmedabad. It is directed towards modernization of libraries and information centers for information transfer and access, to support scholarship, learning and academic pursuits by establishing a national network of libraries and information centres in universities, institutions of higher learning and R & D institutions in India. It is basically a co-operative endeavor in resource development, sharing and its utilization at national level. Since May 1996 it is an independent autonomous Inter-University Centre under UGC, and it is set out to be a major player in promoting scholarly communication among academicians and researchers in India. The National Commission on Libraries and Information Science (NCLIS), USA in its National Programme Document (1975) defines a network as "two or more libraries and or other organizations engaged in a common pattern of information exchange, through communications, for some functional purpose. A network usually consists of formal arrangement whereby materials, information and services provided by a variety of libraries and other organizations are available to all potential users. Libraries may be in different jurisdictions but agree to serve one another on the same basis as each serves its own constituents. Computer and telecommunication may be among the tools used for facilitating communication among them. The growth of Library and Information Networks in India is now gaining momentum. INFLIBNET has emerged as a front runner, facilitating automation and networking of academic libraries for resource sharing among libraries using networking and access to information. The main objective of this study is to assess the role of the INFLIBNET in higher education in India in modern scenario. The INFLIBNET's beginning was made in 1991 as a major programme of University Grants Commission (UGC) under the Inter-University Centre

for Astronomy and Astrophysics (IUCAA). The INFLIBNET Centre was established in May 1996 as an independent, autonomous Inter-University Centre (IUC) of the University Grants Commission (UGC) with the target to network all the Academic Libraries of Higher Education in India for promotion of scholarly communication among academicians and researchers.

**INFLIBNET's mission and vision is as follows:**

- Leveraging on the latest technology, create a virtual network of people and resources in academic institutions with an aim to provide effective and efficient access to knowledge through perseverance, innovation and collaboration.
- Provide seamless, reliable and ubiquitous access to scholarly, peer-reviewed electronic resources to the academic community in all educational institution with a focus on services and tools, processes and practices that support its effective use and increase value of this information.
- Build and strengthen ICT infrastructure in educational institution with value added services.
- Develop tools, techniques and procedures for secure and convenient access management enabling users to access information in electronic format from anywhere, anytime.
- Develop resource selection guides and online tutorials for effective delivery and usage of e-resources.
- Facilitate creation of open access digital repositories in every educational institution for hosting educational and research content created by these institutions.

Training of manpower working in the universities and colleges in the use of Information Technology (IT) is an important objective of INFLIBNET and has been given due priority. Twenty training courses of four-week duration for operational staff working in the university libraries and seven workshops of one-week duration for senior library staff, focusing on the managing automation and networking, have been conducted so far. INFLIBNET Regional Training Program on Library Automation (IRTPLA), a new series of training programs are conducted at different locations in collaboration with universities across the country to train library professionals from college and university libraries at regional level with emphasis on regional languages. Since as per vision of the INFLIBNET is to provide the library to the scholar for any degree therefore it is most helpful for the research scholar.

**Q.9 What is Internet and use of internet in Research?**

Ans. A means of connecting a computer to any other computer anywhere in the world via dedicated routers and servers. When two computers are connected over the Internet, they can send and receive all kinds of information such as text, graphics, voice, video, and computer programs.

No one owns Internet, although several organizations the world over collaborate in its functioning and development. The high-speed, fiber-optic cables (called backbones) through which the bulk of the Internet data travels are owned by telephone companies in their respective countries.

The Internet grew out of the Advanced Research Projects Agency's Wide Area Network (then called ARPANET) established by the US Department Of Defense in 1960s for collaboration in military research among business and government laboratories. Later universities and other US institutions connected to it. This resulted in ARPANET growing beyond everyone's expectations and acquiring the name 'Internet.'

The development of hypertext based technology (called World Wide web, WWW, or just the Web) provided means of displaying text, graphics, and animations, and easy search and navigation tools that triggered Internet's explosive worldwide growth.

Before the internet, conducting research for school, work or out of curiosity involved a set of encyclopedias and a trip to the library. However, we now live in an age where information is readily accessible from your computer.

On the Web, you can find information about any topic you desire. The World Wide Web is a huge database of user–submitted content where you can access an astronomical number of informative sources, online groups and multi-media.

Because all of the content on the internet is self-submitted, and there are very few regulations as to what a person can and can't publish (depending on local laws), content found on the Web may be inaccurate and opinion based.

The World Wide Web is an extraordinary resource for gaining access to information of all kinds, including historical, and each day a greater number of sources become available online. The advantages that the internet offers students are tremendous; so much so that some may be tempted to bypass the library entirely and conduct all of their research on the web. The History Department wants CU students to pursue knowledge with every tool available, including the internet, so long as they do so judiciously.

It is important to know that the Web is an unregulated resource. Because many unreliable sources exist on the internet, anyone – even people who have no expertise at all in your subject – can post anything at anytime. Many sources on the web have proven to be unreliable, biased, and inaccurate. Too much reliance on the web could do more damage than good. Checking the reliability and accuracy of information taken from random sites could take more time than going to the library. And using information you have not checked from such sources could have a detrimental impact on your final grade.

## Part – B

**Q.1 Explain**

A. Primary Data

Ans. An advantage of using primary data is that researchers are collecting the information for the specific purposes of their study. In essence, the questions the researchers ask are tailored to elicit the data that will help them with their study. Researchers collect the data themselves, using surveys, interviews and direct observations. For this research the researcher has decided to use surveys, interviews and case studies to collect the data.

B. Secondary Data

Ans. Secondary data tends to be readily available and inexpensive to obtain. In addition, secondary data can be examined over a longer period of time. For example, you can look at a company's lost-time rates over several years to see at trends. In the same Institute study mentioned above, the researchers also examined secondary data. They looked at workers' compensation lost-time claims and the amount of time workers were receiving wage replacement benefits. With a combination of these two data sources, the researchers were able to determine which factors predicted a shorter work absence among injured workers. This information was shared with return-to-work professionals to help improve return to work for other injured workers. In this study secondary information and all the relevant information is collected from various issues of Statistical Tables Relating to the issues, and Database on management published by Organization. In addition to that some information was also collected from different issues of Survey published by the certain important books and journals.



C. Sampling Errors and Treatments

Ans. Sampling process error occurs because researchers draw different subjects from the same population but still, the subjects have individual differences. Keep in mind that when you take a sample, it is only a subset of the entire population; therefore, there may be a difference between the sample and population. The most frequent cause of the said error is a biased sampling procedure. Every researcher must seek to establish a sample that is free from bias and is representative of the entire population. In this case, the researcher is able to minimize or eliminate sampling.

There is only one way to eliminate this error. This solution is to eliminate the concept of sample, and to test the entire population. In most cases this is not possible; consequently, what a researcher must to do is to minimize sampling process error. This can be achieved by a proper and unbiased probability sampling and by using a large sample size.

**Q.2 Explain**

A. Statistical Analysis

Ans. Statistical analysis is fundamental to all experiments, observations, analytical approach and research that use statistics as a research methodology. Our exploratory outcomes demonstrate that the factual examples of the above factors fluctuate contingent upon the problem sources. For instance, on account of non-Wi-Fi obstruction, we watched a more prominent number of retried bundles, less FCS blunders, and wider varieties in the bit rates contrasted with channel conflict.

B. Probability Theories

Ans. Probability theory is the mathematical description of research phenomena. Probability plays an increasingly important role in almost all areas of engineering and science. Probability research in ORFE ranges from theoretical to applied, with particular emphasis on stochastic analysis and its applications in various areas including financial mathematics, stochastic networks and queuing, signal and image processing, and stochastic control.

C. Hypothesis Tests

Ans. A research hypothesis is the statement created by researchers when they speculate upon the outcome of a research or experiment. Every true experimental design must have this statement at the core of its structure, as the ultimate aim of any experiment. The hypothesis is generated via a number of means, but is usually the result of a process of inductive reasoning where observations lead to the formation of a theory. Scientists then use a large battery of deductive methods to arrive at a hypothesis that is testable, falsifiable and realistic. Hypothesis test is done as shown in figure 1.

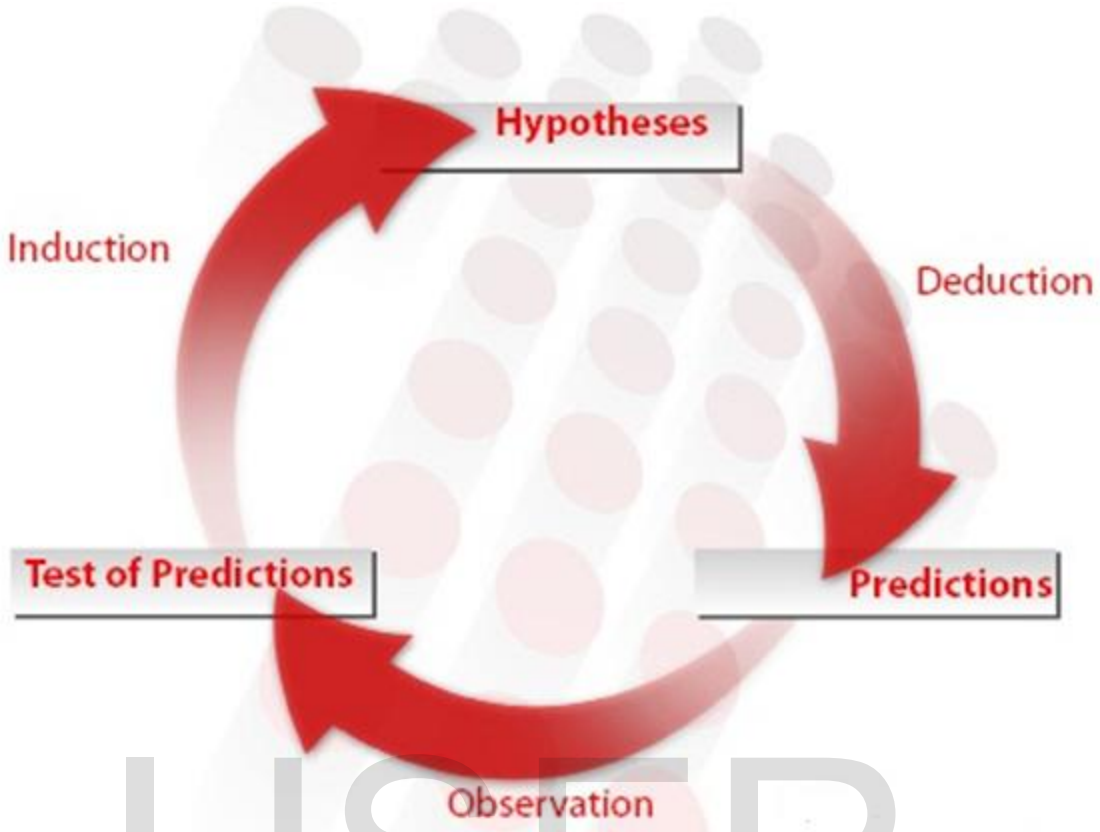





**Figure 1 Hypothesis Test**

D. Sample Test

Ans. A sample is a finite part of a statistical population whose properties are studied to gain information about the whole. When dealing with people, it can be defined as a set of respondents (people) selected from a larger population for the purpose of a survey. The sample size of thesis set in the early stage of the thesis. A sample as per the size set could provide us with needed information quickly. A sample may be more accurate than a census. A sloppily conducted census can provide less reliable information than a carefully obtained sample.