



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Coronaviridae: 100,000 Years of Emergence and Reemergence

*Yassine Kasmi, Khadija Khataby,
Amal Souiri and Moulay Mustapha Ennaji*

Laboratory of Virology, Microbiology, Quality, Biotechnologies/
Eco-Toxicology and Biodiversity, Faculty of Sciences and Techniques,
Mohammedia, University Hassan II of Casablanca, Casablanca, Morocco

ABBREVIATIONS

CoV	coronavirus
MERS-CoV	Middle East respiratory syndrome coronavirus
SARS-CoV	severe acute respiratory syndrome coronavirus
IBV	infectious bronchitis virus

INTRODUCTION

The Coronaviridae have a global distribution. Most of the humans coronavirus (CoV) infectious have a serious effect. The Coronaviridae includes several animals and human viruses causing a serious epidemics and endemics, as such the severe acute respiratory syndrome (SARS) CoV (SARS-CoV) outbreak in 2003 and the CoV respiratory syndrome outbreak in 2012–14. Severe CoV acute respiratory virus (SARS-CoV) infection and the Middle East respiratory syndrome (MERS) CoV (MERS-CoV) in humans in 2012 caused severe lower respiratory tract disease as well as *IBV* (infectious bronchitis virus) in the avian and poultry field.

EMERGENT AND REEMERGING VIRAL INFECTIONS

An emerging infectious disease is defined as an infectious disease whose incidence has increased over the past 20 years and may increase in the future. Emerging infections account for more than 10% of human diseases (Mackey et al., 2014). An emerging pathogen may be defined as an infectious agent whose frequency or geographic range increases following its first introduction into a new host population, while a re-emerging pathogen is one whose incidence or geographic distribution is increasing in an existing host population as a result of long-term changes in its underlying epidemiology. The emergence of pathogens may be based on subjective criteria, which may reflect increasing awareness, improved diagnosis, discovery of previously unrecognized infectious agents as well as any objective epidemiological data (Woolhouse, 2002; Engering et al., 2013).

Many viruses are classified as emerging pathogens according to the WHO, including *MERS-CoV*, *SARS-CoV*, and *IBV* that are the focus of this work.

TAXONOMY

Phylogenetically, Coronaviridae belongs to Nidovirales in group IV, with a single genomic RNA fragment, oriented in a positive direction.

Nidovirales

Nidovirales is an order that contains four families (Arteriviridae, Coronaviridae, Mesoniviridae, and Roniviridae) according to the genomic classification (Fig. 7.1) (Cavanagh, 1997). The name Nidovirales originates from the fact that the viruses belonging to this order have the capacity to produce during infection a 3'-multiplexed complex of subgenomic messenger RNA (mRNA), hence the word "nidus" in Latin, which means to nest (De Vries et al., 1997).

The main common traits between Nidovirales are as follows:

- Their unfragmented genome of RNA type with positive orientation of the genome (Cavanagh, 1997).
- Nidovirales encodes structural proteins that are separated from nonstructural functional proteins (Balasuriya and Snijder, 2008).
- The attachment to their host cell is done through receptors on the cell surface.
- After which the fusion of the viral and cellular membrane is presumed to be mediated by one of the viral surface glycoproteins.

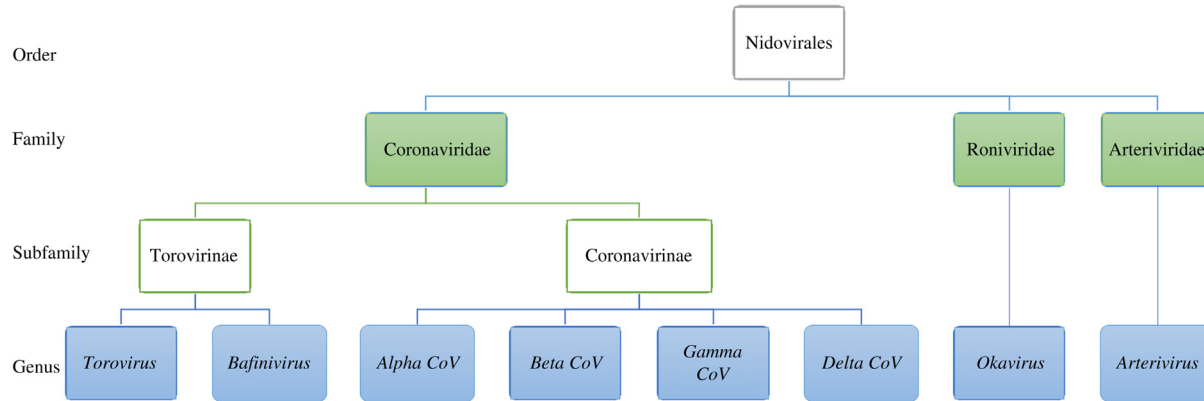


FIGURE 7.1 Nidovirales taxonomy (Chan et al., 2015).

This fusion event (either plasma or endosomal membrane) releases the nucleocapsid into the cytoplasm of the host cell. Following genome takeoff, translation of two open replicase reading frames (ORFs) is initiated by ribosomes of the host, to produce large polyprotein precursors that undergo autoproteolysis to produce a replicase/transcriptase complex (Gorbalenya et al., 2006).

Coronaviridae

The CoV family (Coronaviridae) has been described as a model in virology, because it infects more than 200 different hosts. SARS-CoV is compared to *Cinderella*. They were highlighted in 2003 (Schmidt et al., 2005). CoVs are spherical (120–220 nm in diameter) and appear as special crowns because of the presence of pointed glycoproteins (Fig. 7.2) (Masters, 2006).

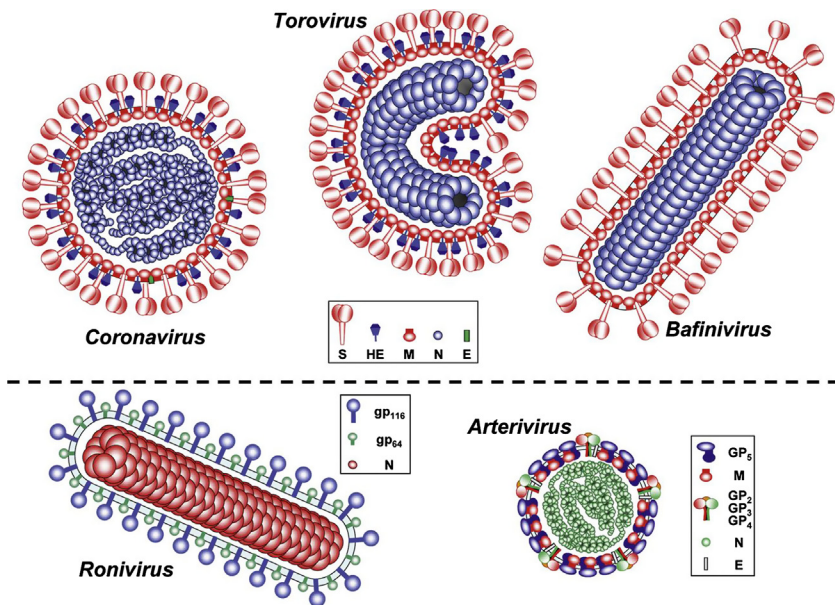


FIGURE 7.2 Structural scheme of the Nidovirales order.

Despite the differences in structure observed at the members of this order, their genomic construction is similar as well as their replication strategies. They use to replicate a similar and distinct “nested set” transcription strategy, in which the expression of genes encoding structural viral proteins is mediated by a nested set of 3′-coterminal subgenomic mRNAs. Source: Reproduced with permission from King, A.M., Lefkowitz, E., Adams, M.J., Carstens, E.B. (Eds.), 2011. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier (King et al., 2011). This figure has license from Elsevier under number 4285901264744.

CoVs are enveloped viruses that contain the largest linear genome of known positive-sense single-stranded viral RNA (Siddell et al., 1983). They are characterized by a structural organization of a crown around the envelope, hence their name Coronaviridae (Fig. 7.4).

Although the first CoV, *IBV*, was discovered in 1932 (Hudson and Beaudette, 1932), Coronaviridae was proposed as a taxonomic family 30 years later after the discovery of human CoV in humans, patients with cold (Tyrrell and Bynoe, 1965; Tyrrell et al., 1975).

In recent years, Coronaviridae have been considered among the most popular viral families because a number of its members were responsible for several human and animal epidemiological pathologies. These include the murine epidemic in 2005 (Weiss and Navas-Martin, 2005), the SARS in 2003 (Fleck, 2003), the SARS pandemic in 2015–16 in Russia and Ukraine (Berger, 2017), the MERS-CoV 2012–17 (WHO2), and the CoV *IBV* (Riedel, 2006).

CoVs have diverse host ranges (Fig. 7.3). They affect most terrestrial and marine animals and humans, including dolphins, birds, cattle, woodpeckers, fish, etc. It has been shown that a virus can infect various hosts, for example, SARS and MERS-CoV (Wang et al., 2005; Tang et al., 2006; Belouzard et al., 2012).

The viral infection caused by this family is considered, in most cases, as a severe infection. It is mainly located in the respiratory and gastroenteric tracts by targeting routes according to the host and the infectious virus.

Coronaviridae include two subfamilies: Coronavirinae and Torovirinae. According to the molecular and serological characteristics of Coronavirinae, the classification reveals a subdivision into four groups (alpha coronavirus, beta coronavirus, delta coronavirus, and gamma coronavirus) and two

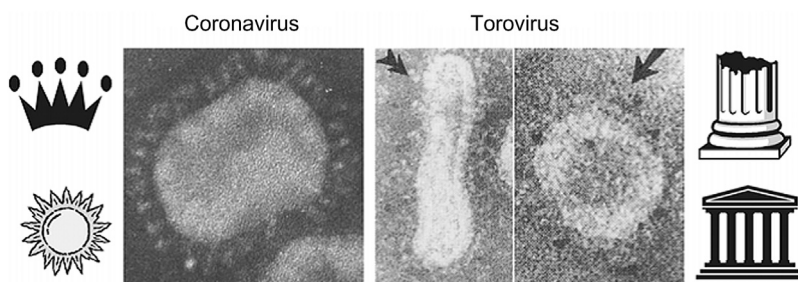


FIGURE 7.3 Coronaviridae structural organization. Comparing the morphology in electron microscopy of coronaviruses whose prototype is the *IBV* virus and the Torovirinae *Torovirus*. *IBV*, Infectious bronchitis virus. Source: Reproduced with permission from Duckmanton L, Luan B, Devenish J, Tellier R, Petric M (1997). Characterization of torovirus from human faecal specimens. *Virology* 239: 158–168. Elsevier (Duckmanton et al., 1997). This figure has license from Elsevier under number 4285901264744.

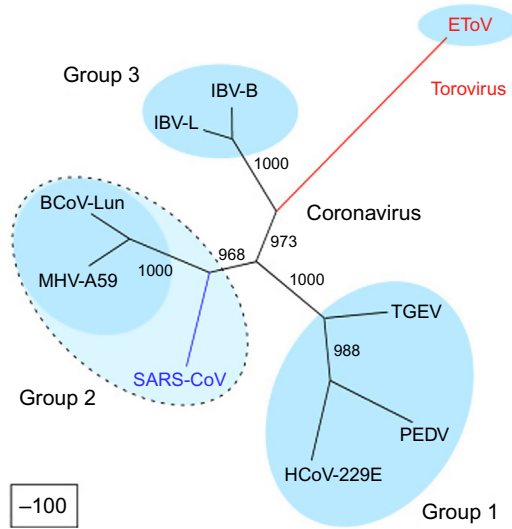


FIGURE 7.4 Phylogram of the Coronaviridae. Source: *Reproduced with permission from Snijder, E.J., Bredenbeek, P.J., Dobbe, J.C., Thiel, V., Ziebuhr, J., Poon, L.L., et al., 2003. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J. Mol. Biol. 331 (5), 991–1004 (Snijder et al., 2003) with permission 4285910843400.*

genera in Torovirinae (*Bafinivirus*, *Torovirus*) (Perlman and Netland, 2009; Birkhead and Paweska, 2015; Appendices 1 and 2) (Fig. 7.4).

GENOMIC ORGANIZATION AND PROTEOMICS OF CORONAVIRIDAE

Coronaviridae is positive RNA viruses with an unsegmented genome of 26–33 kb in length. They are generally of similar genomic and structural construction, containing 5'-end ORF series that encode nonstructural proteins that are primarily involved in pathogenicity. The number of ORFs differs by species, and it has a significant portion of Coronaviridae genomes, followed by the coding regions of structural proteins, more than two-thirds of the CoV genome is composed of an open reading code (ORF) coding for the replicase polyprotein 1a/1b, and the remainder contains ORFs encoding the structural proteins: spicules (S), envelope (E), membrane (M), nucleoprotein (N), and a variable collection of accessory proteins (Woo et al., 2009; Liu et al., 2014) (Fig. 7.4).

CoVs encode membrane-associated proteins that are incorporated into virions: spike (S), envelope (E), membrane (M), and nucleoprotein (N).



FIGURE 7.5 Construction génomique des Coronaviridae.

There is a similarity between human and animal coronaviruses at the organization level of their genome. All coronaviruses encode ORF replica 1a/1b and structural proteins (S), envelope (E), membrane (M), and nucleoprotein (N). In addition, each strain encodes several accessory proteins. Gene sizes are not designed for a specific scale.

These four proteins occur in the S–E–M–N order in all known CoV lineages (Woo et al., 2014).

Among the spike envelope membrane and nucleoprotein (SEMN) genes, CoVs encode species-specific accessory proteins, many of which appear to be incorporated into virions at low levels, ranging from an accessory in alpha-CoVs, including human CoV NL63 (Pyrce et al., 2004), to nine accessories provided in gamma-CoV HKU22 (Woo et al., 2014). The genomic position of these accessory genes varies with S-encoded accessories in some beta-CoV, between S and E in most lineages, between M and N in most lineages, and after N rarely in alpha-CoVs and gamma-CoV and commonly in delta-CoVs. The M gene seems to follow the E gene directly through Coronaviridae, although there is no obvious transcriptional or translational reason for which this should necessarily be the case (Fig. 7.5).

Phylogenetic studies on RNA-dependent RNA polymerase (RdRp) sequences, aimed at studying divergence, suggested that the common ancestor, the most recent of the CoVs infecting mammals, appeared about 7000–8000 years ago, while the most recent common ancestor of avian CoVs dates back 10,000 years (Vijgen et al., 2006). However, current estimates roughly coincide with the dispersion of the human population in the world from about 50,000–100,000 years ago and have increased significantly over the last 10,000 years during the first historical transition (Chan et al., 2013). However, referring to the history of mankind, this epoch corresponds to the beginning of agriculture and animal husbandry.

CoVs are characterized by the property of transcribing code mRNAs for each protein. This property allows the virus to control the rate of protein synthesis according to the state requirements of virus and host cell.

Gene Responsible for Pathogenicity

Protein S plays a key role in the power of pathogenicity. This glycoprotein (S) is an important component in the species specificity, pathogenesis, and escape of immunity. Like human immunodeficiency virus (HIV) gp160, influenza hemagglutinin, and Ebola virus glycoprotein, the CoV spike (S) glycoprotein protein is a class I viral fusion protein that mediates virus binding and fusion, allowing virus to enter the host cell (Xu et al., 2004). Like other class I fusion proteins, the S-glycoprotein contains two functional domains, S1 and S2, linked by a protease cleavage site (Xu et al., 2004). The S1 domain (17–756aa) contains the receptor-binding domain (RBD) (318–510aa) while the S2 region (757–1225aa) contains the two heptad repeat (HR) regions that facilitate viral fusion and a transmembrane domain (1189–1227aa) which anchors the tip on the viral envelope (Xu et al., 2004). CoVs are thought to accumulate in cells by the following sequence of events: cell-receptor binding ACE2, DPP4, and APN to affect tropism in the cell by endocytosis and cleavage of SARS-CoV S by cathepsin-cell protease. L causes a rearrangement of S1 and S2 subunits inducing fusion of the viral membrane and the host to deposit the viral/nucleocapsid genome complex in the cytoplasm where replication occurs.

The glycoprotein of CoV S is an essential element of species specificity, which is also the main determinant of pathogenesis because a virus that is incapable of infection is unlikely to cause disease. Using reverse genetics, the substitution of mouse hepatitis virus (MHV) protein S for feline infectious peritonitis virus protein S alone was sufficient for the murine tropic virus to infect feline cells. In less extreme examples the host range of CoV can be modulated by a few point mutations in S-glycoprotein that focuses either in RBD or in the fusogenic domain (de Haan et al., 2005).

Although the earlier CoV dogma suggests that expansion of the host range is mediated by mutation in the S1 region, McRoy et al. reported an expansion of the host range of MHV that may also be mediated by changes in the host range, amino acids in fusion equipment of the S2 region. A prime and relevant example of a CoV host range change due to mutations in the S1 region was observed during the evolution of the SARS-CoV epidemic strain, SARS Urbani (de Haan et al., 2005)

The SARS-CoV (S) peak gene sequences isolated from human cases during the early phase of the epidemic in 2002–03 and during the reemergence of 2003–04 are very similar to strain SZ16. SZ16 was isolated from palm tree crops in live animal markets in the Guangdong region of China during the outbreak, and its protein S differs from the epidemic strain, SARS Urbani, in 18 amino acids, 16 of which are in the S1 domain containing the RBD. The crystalline structure of ACE2-

receptor-bound SARS-CoV RBD and biochemical experimentation demonstrated that the critical amino acids (K479, T487) in the SZ16 S civet RBD inhibited its binding to the human ACE2 receptor (hACE2), thereby providing a block in the expansion of host range and human pathogenesis (Sheahan and Baric, 2010). Using a pseudotyped retrovirus with mutant or wild versions of the zoonotic (SZ16) or epidemic (Urbani) glycoprotein, Li et al. demonstrated that K479 and T487 were critical residues inhibiting the binding of the civet tip to the hACE2 receptor.

Unfortunately, the pseudotyped system is able to evaluate the efficiency of binding and entry, but not the growth kinetics of the virus. By using recombinant SARS-CoV carrying a variety of zoonotic, epidermal intermediate S-glycoproteins in a one-step growth pattern, data on binding, entry, and growth can be elucidated. In addition, infection of the cells expressing the civet (cACE2) or hACE2 of the SARS-CoV receptor with recombinant variants of SARS-CoV S-glycoproteins makes it possible to study the ability to grow and use receptors in both the amplifier and the epidemic host. In evaluating the growth of the SARS glycoprotein variant in CACE2 or hACE2 cell cultures, we deduced that the epidemic strain retained growth ability in cell cultures expressed by CACE2 and hACE2 (Sheahan and Baric, 2010).

Viral Cycle of Coronaviridae

Here, we present the viral cycle of Coronaviridae by discussing the replication cycle of MERS-CoV as an example. The viral cycle of MERS-CoV is different from other beta-CoVs that do not code for hemagglutinin esterase (Zaki et al., 2012), (Fig. 7.7).

MERS-CoV binds to its DPP4 cellular receptor via the S protein, then it enters the target cells, followed by fusion of the endosomes and membranes of the virus resulting in the release of the genome of the viral RNA into the cytoplasm. The open reading frame (ORF), 1a and 1b, in the viral genomic RNA is translated into PP1a and PP1ab replicase polyproteins, respectively, and then optionally cleaved by a papain protease (PLpro), (Yang et al., 2014) a type 3C cysteine protease (3CLpro, principle protease) and other viral proteinases, in 16 nonstructural proteins (nsp1–16) (Durai et al., 2015).

A negative-stranded genomic length RNA is synthesized as a template for replication of viral genomic RNA. mRNAs of different lengths of the negative strand subgenome (sg mRNAs) are formed from the viral genome as discontinuous RNAs and used as a template to transcribe sg mRNAs. N viral protein is assembled with genomic RNA in the cytoplasm (Zumla et al., 2015).

The synthesized S, M, and E proteins are collected in the endoplasmic reticulum (ER) and transported to the ER-Golgi intermediate

compartment where they interact with the N-RNA complex and assemble into viral particles. These become mature in the Golgi body and are released into cells (Kuo et al., 2016).

MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

The most infectious members of Coronaviridae belong to the beta group, particularly in lineage C where SARS and MERS-CoV are found to be highly pathogenic to humans and are responsible for severe epidemics worldwide, including the 2015–16 SARS pandemic in Russia, Ukraine (Berger, 2017), and the current global epidemics of MERS according to the epidemiological history of this family (Fig. 7.8).

Lack of vaccines or approved drugs is currently a roadblock to fight the epidemic and the spread of the disease (Wang et al., 2017).

MERS-CoV is a pathogen that infects most mammals and higher animals, as well as humans. Camels represent CoV MERS nature reserves (Alagaili et al., 2014). On the other hand, viruses are isolated from bats, and other mainly domestic animals such as mice, cattle, rats, etc. The infection is mainly due to the interaction with the DPP4 receptor for mammals and the ACE2 in bats. However, recently Widagdo et al. (2017) found that the DPP4 isolated from the intestinal and respiratory tissues from bats resembles those of camels and humans with an absence of DPP4 at the level of respiratory cells.

The CoV responsible for the MERS-CoV was first identified in Saudi Arabia in 2012 from a man with atypical pneumonia (Zaki et al., 2012). Since then, more than 1936 infections and more than 690 deaths have been reported (WHO3, 2017). The ongoing MERS-CoV epidemic is mainly concentrated in the Middle East and Saudi Arabia, but cases have been exported by travelers to over 27 countries causing occasional secondary spread (WHO3, 2017). In addition, the most notable epidemic outside of Saudi Arabia occurred in South Korea in 2015, resulting in 186 new cases (Korea Centers for Disease Control and Prevention, 2015) (Figs. 7.6–7.9).

The Genomic and Proteomic Construction

MERS-CoV is an enveloped virus, with a spherical coronary structure, and a nonsegmented, positively oriented, single-stranded RNA genome with a size of the order of 30 kb. (Fig. 7.4). It is related to strains hCoV-OC43 and hCoV-229E being prototype meadows and IBV being a prototype of all Coronaviridae (Chan et al., 2013).

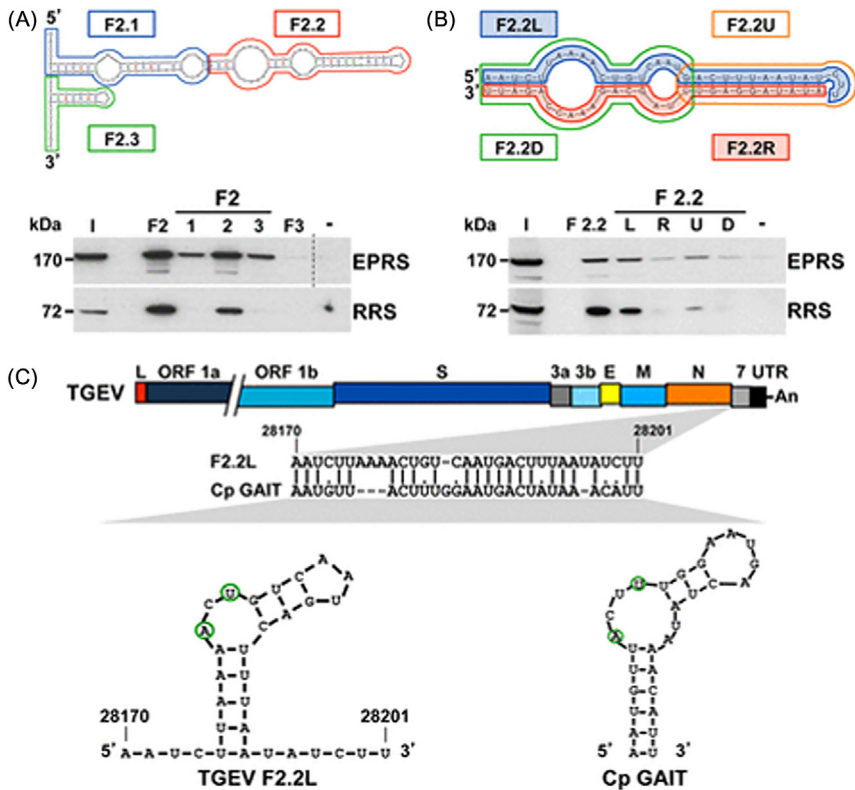


FIGURE 7.6 Mapping of interacting RNA domains with EPRS and RRS. (A) Mapping the region within F2 interacting with EPRS and RRS. F2 was divided into three fragments (F2.1, F2.2, and F2.3) which were used as baits in pulley tests, as well as F2 and F3 fragments or without (-) RNA as controls specificity. The presence of EPRS and RRS in the initial extract (I) and in the fractions drawn was analyzed by western blot. To simplify the information in Fig. 7.6, an empty channel has been removed in the EPRS panel, in which the dotted line indicates the splice site. Molecular weights in kDa of EPRS and RRS are shown on the left. (B) Identification of domain F2.2 in interaction with EPRS and RRS. F2.2 was divided into four fragments (F2.2L, F2.2R, F2.2U, and F2.2D), and their interactions with EPRS and RRS were analyzed by rapid analysis and western blot. The same experiment was performed with the F2.2 fragment or without (-) RNA as specificity controls. The molecular weights in EPRS and RRS kDa are shown on the left. (C) Sequential analysis of the F2.2L fragment. The bar on the top represents the TGEV genome, in which the different genes (ORF 1a, ORF 1b, S, 3a, 3b, E, M, N, and 7 genes), the leader sequence (L) and the 3' UTRs are illustrated by boxes. The sequence alignment of the F2.2L viral fragment with the GAIT element of Cp GAIT and their respective secondary structures, in which residues A and U critical for the function of the element GAIT are described in green, are indicated. The numbers above the sequence alignment and in the secondary structure indicate the position of the F2.2L fragment genome. Cp, Ceruloplasmin; UTR, untranslated region. Source: Adopted after Marquez-Jurado, S., Nogales, A., Zuñiga, S., Enjuanes, L., Almazán, F., 2015. Identification of a gamma interferon-activated inhibitor of translation-like RNA motif at the 3' end of the transmissible gastroenteritis Coronavirus genome modulating innate immune response. *mBio* 6 (2), e00105–e00115 (Marquez-Jurado et al., 2015).

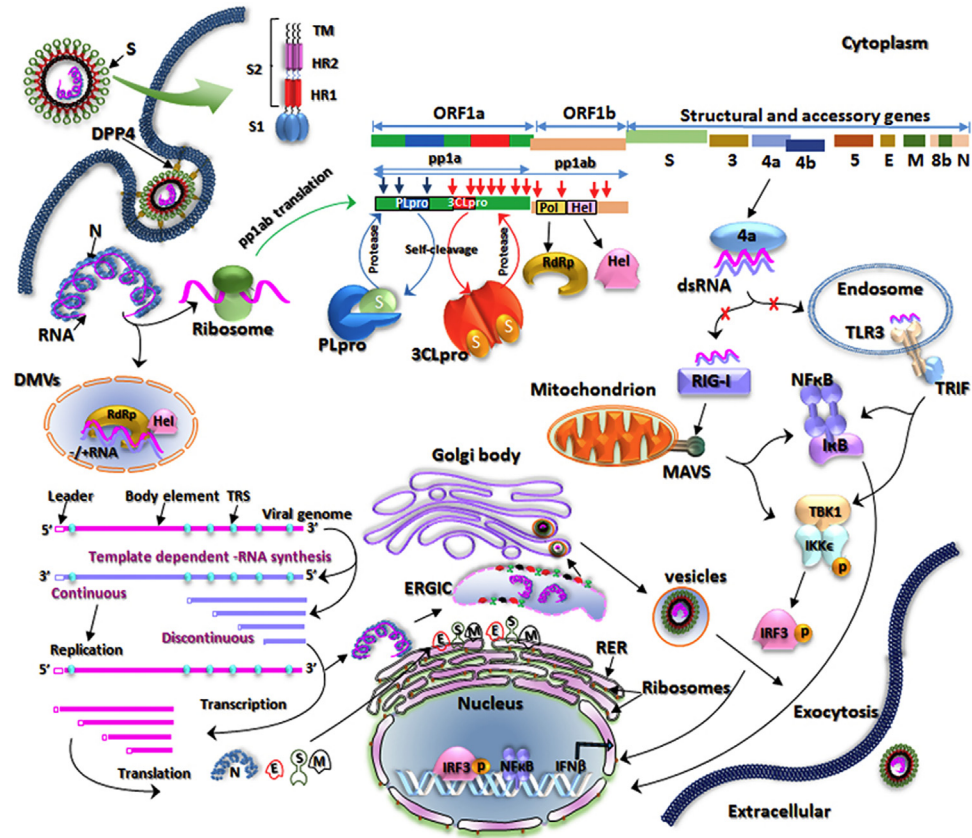


FIGURE 7.7 Viral cycle of MERS coronavirus (Durai et al., 2015). MERS, Middle East respiratory syndrome.

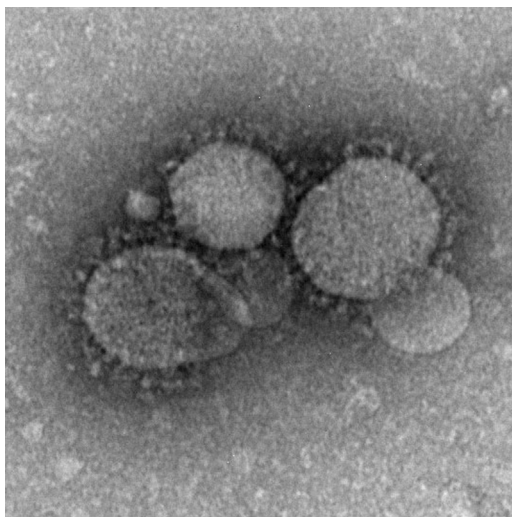


FIGURE 7.8 Morphology of MERS-CoV particles as seen by negative-staining electron microscopy. Virions contain club-specific projections that originate from the viral membrane. Cynthia Goldsmith/Maureen Metcalfe/Azaibi Tamin (microscopic centers for disease control and prevention (CDC photo)). *MERS-CoV*, Middle East respiratory syndrome coronavirus.

GuanineCytosine content (GC) represents 41% of the genome of MERS-CoV ([van Boheemen et al., 2012](#)). The genome is capped at the 5' end and 3' polyadenylated. At the 5' end of MERS-CoV as in all Coronaviridae, there is an untranslated region (5'-UTR) of about 200 nucleotides (nts) before the initiation codon for the ORF.

ORF1 is translated by nonstructural proteins, a second ORF2 exists after the S gene and before the E gene codes for 3 nonstructural proteins which are 3a, 3b, 3c, and 3d. The structural genes are, respectively, spicule (S), envelope (E), membrane (M), and nucleocapsid (N). The MERS virus has the distinction of being more conserved in all hosts and citing humans with the exception of the mutation at position 1020 at the level of the gene coding for S which characterizes the strains infecting humans [Cotten et al. \(2014\)](#).

The ORF1a products have proteolytic roles, interactions with interferon antagonist as well as DeISGylation; however, ORF1b codes for RdRp and Helicase (Hel), which are important enzymes involved in the transcription and replication of CoVs. This is the result of cleavage of the polyprotein, ORF product a/b, by the papain-like protein cysteine protease (PLpro) and the protein 3C-like serine protease (3CLpro) ([Stadler et al., 2003](#)).

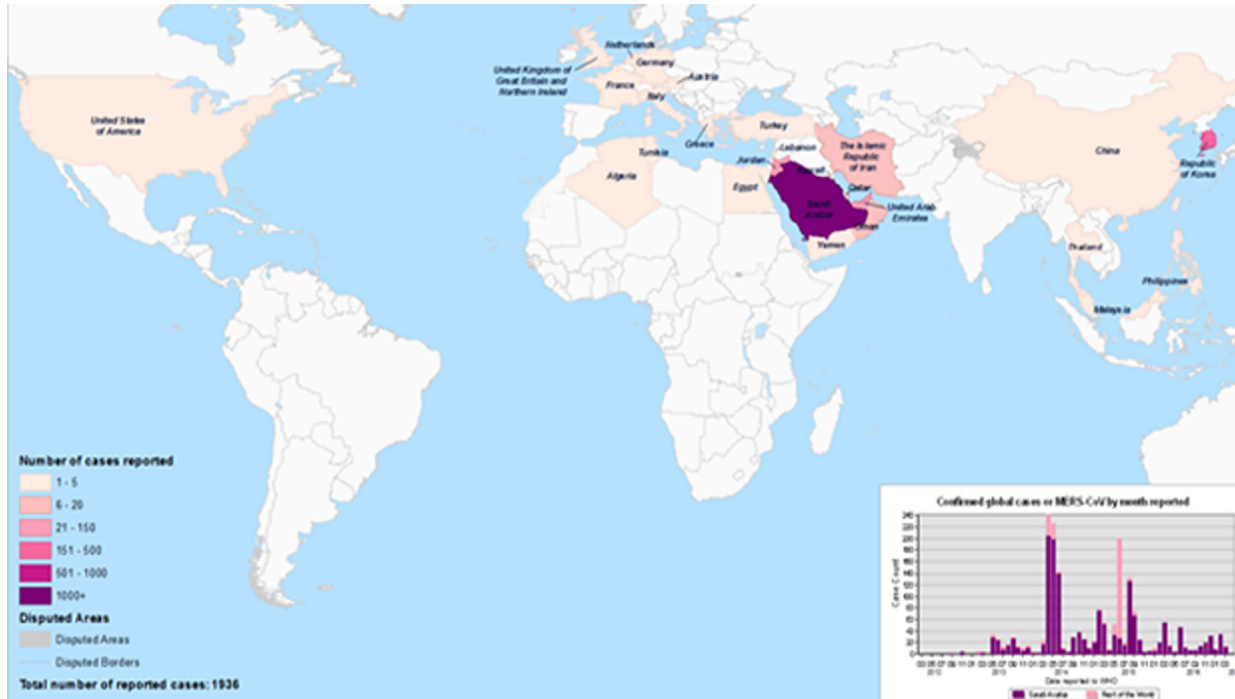


FIGURE 7.9 Spread of MERS-CoV worldwide (WHO1). MERS-CoV, Middle East respiratory syndrome coronavirus. Plus de 27 pays des cinq continents, et 2000 cas d'infection avec un ratio de mortalité de 39%. A noter ici, que ces statistiques concernent seulement l'Homme, alors que des études au niveau vétérinaire ont démontré la présence et la circulation du virus dans plusieurs pays chez des animaux domestiques.

Protein S is a glycoprotein expressed on the surface of viruses, and it plays key roles in the internalization of the virus and attachment to the host cell. It has a molecular weight of about 200 kDa (Qian et al., 2013). The attachment of the virus to the host cell is essentially ensured by the dominance of RDB links and the HR regions (Xia et al., 2014). It consists of two subunits: the subunit S1 which ensures the fusion with the host cell at the amino-terminal and the subunit S2 which ensures the fusion at the carboxyl-terminal (Xia et al., 2014).

The S1 subunit (including the N-terminal domain and the RBD) and S2 (encoding fusion peptides and conserved HR). RBD is characterized by two core and external subdomains, the latter of which is less conserved and involved in the binding of CoVs with their receptors (Wang et al., 2016).

Recently, Lu et al. (2016) showed a deletion mutation of 530 nucleotides in the S2 subunit, but the RDB region is highly conserved. Other studies have also revealed that the S gene has developed several mutations that can negatively influence the therapeutic strategies of protein targeting S.

Spicules S bind to MERS-CoV with DPP4, which is a proteolytic enzyme dipeptidyl peptidase 4, they are intrinsic glycoproteins and assigned exopeptidases (Silva Júnior et al., 2015), which cleave dipeptides at the N-terminus end of a peptide as they have immune and vital roles for cells to cite: the interaction with CD26 (Raj et al., 2013).

INFECTIOUS BRONCHITIS VIRUS

The IBV, a member of Coronaviridae (Cavanagh, 2007), is a highly pathogenic respiratory agent responsible for infectious bronchitis that is a major disease in the poultry field and may be associated fertility, nephritis, and respiratory problems as well as effects on the production of hatched eggs. (Cavanagh, 2007) Like all Coronaviridae, it has a single-strand linear positive-sense RNA genome. Although the structural similarity of Coronaviridae is round, IBV has a diameter of 100–160 nm and long petal-shaped spikes (spicules S) on the surface of the virus (Gonzalez et al., 2003). Characterized by S, E, M, and N proteins, it differs in accessory proteins and genome size (Figs. 7.2 and 7.4). IBVs are counted as prototypes of Coronaviridae (Chan et al., 2013).

The first replications of the virus are at the level of tracheal epithelial cells. Viral infection with IBV causes several pathological mucosal changes, including ciliary loss, degeneration and necrosis of epithelial cells, glandular degeneration, inflammatory cell infiltration, and epithelial hyperplasia (Okino et al., 2014). According to Cavanagh (2007), IBV

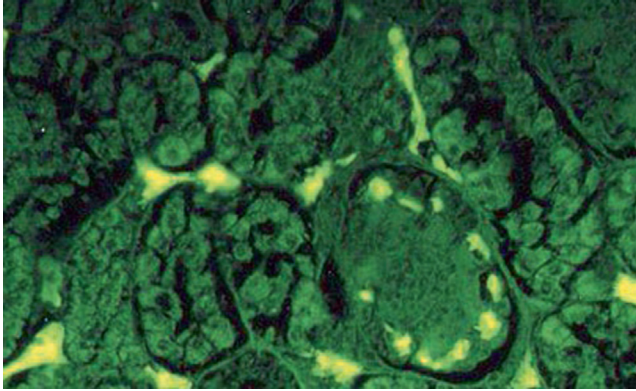


FIGURE 7.10 Coloration immunofluorescente de la section de paraffine rénale du rein 5 jours après la fièvre avec Egypte/Beni-Suef/01 (Abdel-Moneim et al., 2005). La fluorescence intracytoplasmique dans la touffe glomérulaire et la doublure endothéliale des vaisseaux sanguins rénaux dans les zones inter tubulaires (40 ×).

serotypes that share more than 95% amino acid identity in S1 should have cross protection, while IBV strains share less than 85% amino acid identity but do not protect each other (Cavanagh, 2007) (Fig. 7.10).

Although a large number of IBV strains and variants have been described in the recent years (De Wit et al., 2011), most studies have been limited to the classification and differentiation of IBV strains in genotyping, pathotyping, protections, and serotypes, with a remarkable lack of studies dealing with the specific immunity components and mechanisms involved in the pathogenesis of this virus: this would allow us to understand the depth and the system semantic epidemic virus. At the level of virus replication, they are similar to all CoVs.

Specific receptors are still poorly known, but α -2, 3-linked sialic acid has been shown to be essential for attachment of spicules (Abd El Rahman et al., 2009; Promkuntod et al., 2014). In addition to the replicase gene, the final 5' and 3' UTR sequences, with certain specific secondary structures, are required for replication of the genomic RNA. Nucleocapsid (N) is also needed for efficient synthesis of viral RNA (Verheije et al., 2010; Zuniga et al., 2010).

Isolation and Diagnostic of Infectious Bronchitis Virus

Isolation of Infectious Bronchitis Virus From Eggs

Specific pathogen-free embryonated chicken eggs are recommended for primary isolation of IBV. Treated samples (10%–20% w/v) in phosphate buffered saline are used for egg inoculation, after being clarified by low-speed centrifugation and filtration through bacteriological filters.

A volume of 100–200 μL of the treated sample are inoculated into the allantoic cavity of 9–11 day embryos (Delaplane, 1947). Embryo mortality in the first 24 hours is considered nonspecific death. The allantoic fluids of the inoculated eggs (36–48 hours postinoculation) are harvested and pooled (Cunningham, 1973; Cunningham and El Dardiry, 1948). Blind passage to another set of eggs for up to three to four passes is made.

The last passage is left for 7 days to detect the presence of pathognomonic embryonic changes: stunted embryos and wound with feather dystrophy and urate deposits in the mesonephros. These lesions could also appear at the second pass (Delaplane, 1947). The embryo-adapted strains induce greater embryo mortality. Isolation of IBV should be confirmed by serum neutralization or reverse transcription polymerase chain reaction (PCR).

Tracheal Culture

Tracheal cycle culture (0.5–1.0 mm thick) of 19- to 20-day embryos can be used for primary isolation of IBV directly from field samples (Cook et al., 1996). The rings are maintained in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid Eagle's medium in roller drums (15 revolutions/h) (OIE, 2013). Ciliostasis within 24–48 hours is an indication for virus multiplication; however, other viruses could produce similar lesions so further identification of the virus is needed.

The diagnosis of infectious diseases is made by the direct and/or indirect detection of infectious agents. By direct methods, the particles of the agents and/or their components, such as nucleic acids, structural or nonstructural proteins, enzymes, etc., are detected. Indirect methods demonstrate the presence of antibodies induced by infections.

The most common methods for direct detection are isolation or in vitro culture, electron microscopy, immunofluorescence, immunohistochemistry, enzyme immunoassays (ELISA), nucleic acid hybridization, and various nucleic acid amplification techniques such as the PCR.

The most common methods of indirect detection of infectious agents are serological tests, such as viral neutralization, ELISA, hemagglutination inhibition tests, etc.

INTERSPIECES VIRAL TRANSMISSION

Although viruses and microorganisms have the property of host specificity due to various factors, we quote

The sensitivity of the cell to a virus via the presence of receptors allows the tropism of viruses and the presence of the factors necessary for the replication of the virus in the host cell (Segondy, 2010). Coronaviridae are the exception, as several

viruses that are detected in humans have phylogenetic and genetic similarity to those isolated from other animal hosts (Woo et al., 2009; Chan et al., 2013). These nonspecific properties that CoVs possess may be due to accessory CoV genes, which are already thought to play a role in host tropism and adaptation to a new host. S-Glycoprotein appears to be the main determinant for the success of initial events of infection between species.

Bats are home to a wide range of CoVs, including the acute respiratory syndrome (SARS-CoV) and acute respiratory syndrome virus (MERS-CoV) CoV viruses. SARS-CoV has crossed the species barrier in masked palm civets and other animals in live animal markets in China; genetic analysis suggests that this occurred at the end of 2002. Several people in the immediate vicinity of palm civets were infected with SARS-CoV. Ancestral MERS-CoV virus crossed the species barrier in camel camels; serological evidence suggests that this occurred more than 30 years ago (de Wit et al., 2016). Abundant MERS-CoV circulation in camel camels results in frequent zoonotic transmission of this virus. SARS-CoV and MERS-CoV spread among humans primarily through nosocomial transmission, resulting in the infection of healthcare workers and patients at a higher frequency than the infection of their loved ones. The transmission is done by several horizontal levels animal–animal and animal–man and man–man.

References

- Abdel-Moneim, A., Madbouly, H., El-Kady, M., 2005. *In vitro* characterization and pathogenesis of Egypt/Beni-Suef/01; a novel genotype of infectious bronchitis virus. *Beni Suef Vet. Med. J.* 15 (2), 127–133.
- Abd El Rahman, S., El-Kenawy, A.A., Neumann, U., Herrler, G., Winter, C., 2009. Comparative analysis of the sialic acid binding activity and the tropism for the respiratory epithelium of four different strains of avian infectious bronchitis virus. *Avian Pathol.* 38 (1), 41–45.
- Alagaili, A.N., Briese, T., Mishra, N., Kapoor, V., Sameroff, S.C., de Wit, E., et al., 2014. Middle East respiratory syndrome Coronavirus infection in dromedary camels in Saudi Arabia. *MBio* 5 (2), e00884-14.
- Balasuriya and Snijder, 2008. Arteriviruses. *Animal Viruses: Molecular Biology*. Caister Academic Press.
- Belouzard, S., Millet, J.K., Licitra, B.N., Whittaker, G.R., 2012. Mechanisms of Coronavirus cell entry mediated by the viral spike protein. *Viruses* 4 (6), 1011–1033.
- Berger, S., 2017. SARS and MERS: Global Status. GIDEON Informatics, Inc.
- Birkhead, M., Paweska, J., 2015. A microscopic introduction to virus taxonomy. *Commun. Dis. Surveill. Bull.* 13, 52–61.
- Cavanagh, D., 1997. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. *Arch. Virol.* 142 (3), 629.
- Cavanagh, D., 2007. Coronavirus avian infectious bronchitis virus. *Vet. Res.* 38 (2), 281–297.
- Chan, J.F., To, K.K., Tse, H., Jin, D.Y., Yuen, K.Y., 2013. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. *Trends Microbiol.* 21 (10), 544–555.

- Chan, J.F., Lau, S.K., To, K.K., Cheng, V.C., Woo, P.C., Yuen, K.Y., 2015. Middle East respiratory syndrome Coronavirus: another zoonotic betaCoronavirus causing SARS-like disease. *Clin. Microbiol. Rev.* 28 (2), 465–522.
- Cook, J.K., Orbell, S.J., Woods, M.A., Huggins, M.B., 1996. A survey of the presence of a new infectious bronchitis virus designated 4/91 (793B). *Vet. Rec.* 138 (8), 178–180.
- Cotten, M., Watson, S.J., Zumla, A.I., Makhdoom, H.Q., Palser, A.L., Ong, S.H., et al., 2014. Spread, circulation, and evolution of the Middle East respiratory syndrome Coronavirus. *MBio* 5 (1), e01062-13.
- Cunningham, C., 1973. *A Laboratory Guide in Virology*, seventh ed Burgess, Minneapolis, MN, pp. 67–80.
- Cunningham, C.H., El Dardiry, A., 1948. Distribution of the virus of infectious bronchitis of chickens in embryonated chicken eggs. *Cornell. Vet.* 38, 381–388.
- Delaplaine, J., 1947. Technique for the isolation of infectious bronchitis or Newcastle virus including observations on the use of streptomycin in overcoming bacterial contaminants. In: *NE. Conf. Lab. Workers Pullorum Dis. Control Proc.*, vol. 19, pp. 11–13.
- de Haan, C.A., Li, Z., te Lintelo, E., Bosch, B.J., Haijema, B.J., Rottier, P.J., 2005. Murine Coronavirus with an extended host range uses heparan sulfate as an entry receptor. *J. Virol.* 79, 14451–14456.
- De Vries, A.A., Horzinek, M.C., Rottier, P.J., De Groot, R.J., 1997. The genome organization of the *Nidovirales*: similarities and differences between arteri-, toro-, and Coronaviruses, *Seminars in VIROLOGY*, vol. 8. Academic Press, pp. 33–47, No. 1.
- De Wit, J.J., Cook, J.K.A., Heijden, H.M.J.F., 2011. Infectious bronchitis virus variants: a review of the history current situation and control measures. *Avian Pathol.* 40, 223–235.
- de Wit, E., van Doremalen, N., Falzarano, D., Munster, V.J., 2016. SARS and MERS: recent insights into emerging Coronaviruses. *Nat. Rev. Microbiol.* 14 (8), 523–534.
- Duckmanton, L., Luan, B., Devenish, J., Tellier, R., Petric, M., 1997. Characterization of Torovirus from human faecal specimens. *Virology* 239, 158–168.
- Durai, P., Batool, M., Shah, M., Choi, S., 2015. Middle East respiratory syndrome Coronavirus: transmission, virology and therapeutic targeting to aid in outbreak control. *Exp. Mol. Med.* 47 (8), e181.
- Engering, A., Hogerwerf, L., Slingenbergh, J., 2013. Pathogen–host–environment interplay and disease emergence. *Emerg. Microbes Infect.* 2 (2), e5.
- Fleck, F., 2003. How SARS changed the world in less than six months. *Bull. World Health Organ.* 81 (8), 625–626.
- Gonzalez, J.M., Gomez-Puertas, P., Cavanagh, D., Gorbalenya, A.E., Enjuanes, L., 2003. A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. *Arch. Virol.* 148 (11), 2207–2235.
- Gorbalenya, A.E., Enjuanes, L., Ziebuhr, J., Snijder, E.J., 2006. *Nidovirales*: evolving the largest RNA virus genome. *Virus Res.* 117 (1), 17–37.
- Hudson, C.B., Beaudette, F.R., 1932. Infection of the cloaca with the virus of infectious bronchitis. *Science* 76 (1958), 34.
- King, A.M., Lefkowitz, E., Adams, M.J., Carstens, E.B. (Eds.), 2011. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier.
- Korea Centers for Disease Control and Prevention, 2015. Middle East respiratory syndrome coronavirus outbreak in the Republic of Korea, 2015. *Osong Public Health Res. Persp.* 6 (4), 269–278.
- Kuo, L., Hurst-Hess, K.R., Koetzner, C.A., Masters, P.S., 2016. Analyses of Coronavirus assembly interactions with interspecies membrane and nucleocapsid protein chimeras. *J. Virol.* 90 (9), 4357–4368.
- Liu, D.X., Fung, T.S., Chong, K.K., Shukla, A., Hilgenfeld, R., 2014. Accessory proteins of SARS-CoV and other Coronaviruses. *Antiviral Res.* 109, 97–109.

- Mackey, T.K., Liang, B.A., Cuomo, R., Hafen, R., Brouwer, K.C., Lee, D.E., 2014. Emerging and reemerging neglected tropical diseases: a review of key characteristics, risk factors, and the policy and innovation environment. *Clin. Microbiol. Rev.* 27 (4), 949–979.
- Marquez-Jurado, S., Nogales, A., Zuñiga, S., Enjuanes, L., Almazán, F., 2015. Identification of a gamma interferon-activated inhibitor of translation-like RNA motif at the 3' end of the transmissible gastroenteritis Coronavirus genome modulating innate immune response. *mBio* 6 (2), e001105–e001115.
- Masters, P.S., 2006. The molecular biology of Coronaviruses. *Adv. Virus Res.* 66, 193–292.
- OIE, 2013. Avian infectious bronchitis virus. *Terrestrial Manual.*, pp. 1–15. Chapter 2.3.2.
- Okino, C.H., Santos, I.L.D., Fernando, F.S., Alessi, A.C., Wang, X., Montassier, H.J., 2014. Inflammatory and cell-mediated immune responses in the respiratory tract of chickens to infection with avian infectious bronchitis virus. *Viral Immunol.* 27 (8), 383–391.
- Perlman, S., Netland, J., 2009. Coronaviruses postSARS: update on replication and pathogenesis. *Nat. Rev. Microbiol.* 7 (6), 439–450.
- Promkuntod, N., van Eijndhoven, R.E., de Vrieze, G., Grone, A., Verheije, M.H., 2014. Mapping of the receptor-binding domain and amino acids critical for attachment in the spike protein of avian Coronavirus infectious bronchitis virus. *Virology* 448, 26–32.
- Pyrç, K., Jebbink, M.F., Berkhout, B., van der Hoek, L., 2004. Genome structure and transcriptional regulation of human Coronavirus NL63. *Viol. J.* 1, 7.
- Qian, Z., Dominguez, S.R., Holmes, K.V., 2013. Role of the spike glycoprotein of human Middle East respiratory syndrome Coronavirus (MERS-CoV) in virus entry and syncytia formation. *PLoS one* 8 (10), e76469.
- Raj, V.S., Mou, H., Smits, S.L., 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human Coronavirus-EMC. *Nature* 495 (7440), 251–254.
- Riedel, S., 2006, January. Crossing the species barrier: the threat of an avian influenza pandemic. In: *Baylor University Medical Center Proceedings*, vol. 19, no. 1. Baylor University Medical Center, p. 16.
- Schmidt, A., Wolff, M.H., Weber, O. (Eds.), 2005. *Coronaviruses With Special Emphasis on First Insights Concerning SARS*. Springer Science & Business Media.
- Segondy, M., 2010. Spécificité d'hôte des virus et passages inter-espèces. *Revue Francophone des Laboratoires* 2010 (423), 37–42.
- Sheahan, T.P., Baric, R.S., 2010. *SARS Coronavirus Pathogenesis and Therapeutic Treatment Design*. Springer Berlin Heidelberg, pp. 195–230.
- Siddell, S., Wege, H., Ter Meulen, V., 1983. The biology of Coronaviruses. *J. Gen. Virol.* 64 (Pt 4), 761–776.
- Silva Júnior, W.S.D., Godoy-Matos, A.F.D., Kraemer-Aguiar, L.G., 2015. Dipeptidyl peptidase 4: a new link between diabetes mellitus and atherosclerosis? *BioMed Res. Int.* 2015.
- Snijder, E.J., Bredenbeek, P.J., Dobbe, J.C., Thiel, V., Ziebuhr, J., Poon, L.L., et al., 2003. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J. Mol. Biol.* 331 (5), 991–1004.
- Stadler, K., Masignani, V., Eickmann, M., Becker, S., Abrignani, S., Klenk, H.D., et al., 2003. SARS—beginning to understand a new virus. *Nat. Rev. Microbiol.* 1 (3), 209–218.
- Tang, X.C., Zhang, J.X., Zhang, S.Y., Wang, P., Fan, X.H., Li, L.F., et al., 2006. Prevalence and genetic diversity of Coronaviruses in bats from China. *J. Virol.* 80 (15), 7481–7490.
- Tyrrell, D.A., Bynoe, M.L., 1965. Cultivation of a novel type of common-cold virus in organ cultures. *Br. Med. J.* 1 (5448), 1467–1470.
- Tyrrell, D.A., Almeida, J.D., Cunningham, C.H., Dowdle, W.R., Hofstad, M.S., McIntosh, K., et al., 1975. Coronaviridae. *Intervirology* 5 (1-2), 76–82.
- van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Raj, V.S., Zaki, A.M., et al., 2012. Genomic characterization of a newly discovered Coronavirus associated with acute respiratory distress syndrome in humans. *MBio* 3 (6).

- Verheije, M.H., Hagemeijer, M.C., Ulasli, M., Reggiori, F., Rottier, P.J., Masters, P.S., et al., 2010. The Coronavirus nucleocapsid protein is dynamically associated with the replication transcription complexes. *J. Virol.* 84 (21), 11575–11579.
- Vijgen, L., Keyaerts, E., Lemey, P., Maes, P., Van Reeth, K., Nauwynck, H., et al., 2006. Evolutionary history of the closely related group 2 Coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine Coronavirus, and human Coronavirus OC43. *J. Virol.* 80 (14), 7270–7274.
- WHO1. 2017. Available from: <<http://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/>>.
- WHO2. Available from: <<http://www.who.int/emergencies/mers-cov/en/>>.
- WHO3, 2017. Middle East respiratory syndrome Coronavirus (MERS-CoV). Available from: <www.who.int/emergencies/mers-cov/en/>.
- Wang, M., Jing, H.Q., Xu, H.F., Jiang, X.G., Kan, B., Liu, Q.Y., et al., 2005. Surveillance on severe acute respiratory syndrome associated Coronavirus in animals at a live animal market of Guangzhou in 2004.. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxing-bingxue zazhi* 26 (2), 84–87.
- Wang, Q., Wong, G., Lu, G., Yan, J., Gao, G.F., 2016. MERS-CoV spike protein: Targets for vaccines and therapeutics. *Antiviral Res.* 133, 165–177.
- Wang, C., Zheng, X., Gai, W., Zhao, Y., Wang, H., Wang, H., et al., 2017. MERS-Cov virus-like particles produced in insect cells induce specific humoral and cellular immunity in rhesus macaques. *Oncotarget* 8 (8), 12686.
- Weiss, S.R., Navas-Martin, S., 2005. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome Coronavirus. *Microbiol. Mol. Biol. Rev.: MMBR* 69 (4), 635–664.
- Widagdo, W., Begeman, L., Schipper, D., van Run, P.R., Cunningham, A.A., Kley, N., et al., 2017. Tissue distribution of the MERS-coronavirus receptor in bats. *Nat. Sci. Rep.* 7.
- Woo, P.C., Lau, S.K., Huang, Y., Yuen, K.Y., 2009. Coronavirus diversity, phylogeny and interspecies jumping. *Exp. Biol. Med. (Maywood, N.J.)* 234 (10), 1117–1127.
- Woo, P.C., Lau, S.K., Lam, C.S., Tsang, A.K., Hui, S.W., Fan, R.Y., et al., 2014. Discovery of a novel bottlenose dolphin coronavirus reveals a distinct species of marine mammal Coronavirus in Gammacoronavirus. *J. Virol.* 88 (2), 1318–1331.
- Woolhouse, M.E., 2002. Population biology of emerging and re-emerging pathogens. *Trends Microbiol.* 10 (10), s3–s7.
- Xia, S., Liu, Q., Wang, Q., Sun, Z., Su, S., Du, L., et al., 2014. Middle East respiratory syndrome Coronavirus (MERS-CoV) entry inhibitors targeting spike protein. *Virus Res.* 194, 200–210.
- Xu, Y., Lou, Z., Liu, Y., Pang, H., Tien, P., Gao, G.F., et al., 2004. Crystal structure of severe acute respiratory syndrome Coronavirus spike protein fusion core. *J. Biol. Chem.* 279, 49414–49419.
- Yang, X., Chen, X., Bian, G., Tu, J., Xing, Y., Wang, Y., et al., 2014. Proteolytic processing, deubiquitinase and interferon antagonist activities of Middle East respiratory syndrome Coronavirus papain-like protease. *J. Gen. Virol.* 95 (3), 614–626.
- Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D., Fouchier, R.A., 2012. Isolation of a novel Coronavirus from a man with pneumonia in Saudi Arabia. *New Engl. J. Med.* 367 (19), 1814–1820.
- Zumla, A., Hui, D.S., Perlman, S., 2015. Middle East respiratory syndrome. *The Lancet* 386 (9997), 995–1007.
- Zuniga, S., Cruz, J.L., Sola, I., Mateos-Gomez, P.A., Palacio, L., Enjuanes, L., 2010. Coronavirus nucleocapsid protein facilitates template switching and is required for efficient transcription. *J. Virol.* 84 (4), 2169–2175.

Further Reading

- Butler, D., 2012. SARS veterans tackle Coronavirus: genome sequence of new virus speeds up testing. *Nature* 490 (7418), 20–21.
- Chinese SARS Molecular Epidemiology Consortium, 2004. Molecular evolution of the SARS Coronavirus during the course of the SARS epidemic in China. *Science* 303 (5664), 1666–1669.
- Chu, D.K., Leung, C.Y., Gilbert, M., Joyner, P.H., Ng, E.M., Tsemay, M.T., et al., 2011. Avian Coronavirus in wild aquatic birds. *J. Virol.* JVI-05838.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.
- Ehrlich, M., Wang, R., 1981. 5-Methylcytosine in eukaryotic DNA. *Science* 212 (4501), 1350–1357.
- Ehrlich, M., Gama-Sosa, M.A., Huang, L.-H., Midgett, R.M., Kuo, K.C., McCune, R.A., et al., 1982. Amount and distribution of 5-methylcytosine in human DNA from different types of tissues or cells. *Nucl. Acids Res.* 10 (8), 2709–2721.
- Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using Phred. I. *Accuracy assessment.* *Genome Res.* 8 (3), 175–185.
- Fellahi, S., Ducatez, M., Harrak, M.E., Guérin, J.L., Touil, N., Sebbar, G., et al., 2015. Prevalence and molecular characterization of avian infectious bronchitis virus in poultry flocks in Morocco from 2010 to 2014 and first detection of Italy 02 in Africa. *Avian Pathol.* 44 (4), 287–295.
- George, K.S., Zhao, X., Gallahan, D., Shirkey, A., Zareh, A., Esmaeli-Azad, B., 1997. Capillary electrophoresis methodology for identification of cancer related gene expression patterns of fluorescent differential display polymerase chain reaction. *J. Chromatogr. B: Biomed. Sci. Appl.* 695 (1), 93–102.
- Green, P.J., Mardia, K.V., 2006. Bayesian alignment using hierarchical models, with applications in protein bioinformatics. *Biometrika* 235–254.
- Holder, M., Lewis, P.O., 2003. Phylogeny estimation: traditional and Bayesian approaches. *Nat. Rev. Genet.* 4 (4), 275–284.
- Khataby, K., Souiri, A., Kasmi, Y., Loutfi, C., Ennaji, M.M., 2016a. Current situation, genetic relationship and control measures of infectious bronchitis virus variants circulating in African regions. *J. Basic Appl. Zool.* 76, 20–30.
- Khataby, K., Fellahi, S., Loutfi, C., Mustapha, E.M., 2016b. Avian infectious bronchitis virus in Africa: a review. *Vet. Q.* 36 (2), 71–75.
- Li, F., Berardi, M., Li, W., Farzan, M., Dormitzer, P.R., Harrison, S.C., 2006. Conformational states of the severe acute respiratory syndrome Coronavirus spike protein ectodomain. *J. Virol.* 80, 6794–6800.
- Liais, E., Croville, G., Mariette, J., Delverdier, M., Lucas, M.N., Klopp, C., et al., 2014. Novel avian Coronavirus and fulminating disease in guinea fowl, France. *Emerg. Infect. Dis.* 20 (1), 105.
- Mardia, K.V., Taylor, C.C., Westhead, D.R., 2003. Structural bioinformatics revisited. In: *Proceedings in Stochastic Geometry, Biological Structure and Images*, pp. 11–18.
- Mariella, R., 2008. Sample preparation: the weak link in microfluidics-based biodetection. *Biomed. Microdev.* 10 (6), 777.
- McRoy, W.C., Baric, R.S., 2007. Amino acid substitutions in the S2 subunit of mouse hepatitis virus variant V51 encode determinants of host range expansion. *J. Virol.*
- Miguel, B., Pharr, G.T., Wang, C., 2002. The role of feline aminopeptidase N as a receptor for infectious bronchitis virus. *Arch. Virol.* 147 (11), 2047–2056.
- NIAD1. 2018. Available from: <<https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens>>.

- Parrish, C.R., Holmes, E.C., Morens, D.M., Park, E.C., Burke, D.S., Calisher, C.H., et al., 2008. Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol. Mol. Biol. Rev.* 72 (3), 457–470.
- Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26 (1), 139–140.
- Shendure, J.A., Porreca, G.J., Church, G.M., Gardner, A.F., Hendrickson, C.L., Kieleczawa, J., et al., 2008. Overview of DNA sequencing strategies. *Curr. Protoc. Mol. Biol.* 7-1.
- Smith, L.M., Sanders, J.Z., Kaiser, R.J., Hughes, P., Dodd, C., Connell, C.R., et al., 1986. Fluorescence Detection in Automated DNA Sequence Analysis.
- Song, C.X., Clark, T.A., Lu, X.Y., Kislyuk, A., Dai, Q., Turner, S.W., et al., 2012. Sensitive and specific single-molecule sequencing of 5-hydroxymethylcytosine. *Nat. Methods* 9 (1), 75–77.
- Stehle, T., Casasnovas, J.M., 2009. Specificity switching in virus-receptor complexes. *Curr. Opin. Struct. Biol.* 19, 181–188.
- Swerdlow, H., Gesteland, R., 1990. Capillary gel electrophoresis for rapid, high resolution DNA sequencing. *Nucl. Acids Res.* 18 (6), 1415–1419.
- Wang, N., Shi, X., Jiang, L., Zhang, S., Wang, D., Tong, P., et al., 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res.* 23 (8), 986–993.
- Wolfe, N.D., Dunavan, C.P., Diamond, J., 2007. Origins of major human infectious diseases. *Nature* 447 (7142), 279–283.
- Xie, W., Lewis, P.O., Fan, Y., Kuo, L., Chen, M.H., 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Syst. Biol.* 60 (2), 150–160.
- Ying, T., Du, L., Ju, T.W., Prabakaran, P., Lau, C.C., Lu, L., et al., 2014. Exceptionally potent neutralization of Middle East respiratory syndrome coronavirus by human monoclonal antibodies. *J. Virol.* 88 (14), 7796–7805.