

Coronaviridae - old friends, new enemy!

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Abstract:

Coronaviridae is a family of single-stranded positive enveloped RNA viruses. This article aimed to review the history of these viruses in the last 60 years since their discovery to understand what lessons can be learned from the past. A review of the PubMed database was carried out, describing taxonomy, classification, virology, genetic recombination, host adaptation, and main symptoms related to each type of virus. SARS-CoV-2 is responsible for the ongoing global pandemic, SARS-CoV and MERS-CoV were responsible for causing severe respiratory illness and regional epidemics in the past while the four other strains of CoVs (229-E OC43, NL63, and HKU1) circulate worldwide and normally only cause mild upper respiratory tract infections. Given the enormous diversity of coronavirus viruses in wildlife and their continuous evolution and adaptation to humans, future outbreaks would undoubtedly occur. Restricting or banning all trade in wild animals in wet markets would be a necessary measure to reduce future zoonotic infections.

Introduction

Coronaviruses (CoVs) are zoonotic viruses that belong to the Coronaviridae family with a simple positive-sense RNA genome (Ksiazek et al., 2003). CoVs are similar in the organization and expression of their genome, and are maintained simultaneously in nature, which allows for their constant genetic recombination, resulting in new viruses (Su et al., 2016). The viruses, known since 1960, can infect and cause disease in both animals and humans (Siddell et al., 1983; Ksiazek et al., 2003). The types ~~are~~ known to cause disease in animals include; infectious bronchitis virus (IBV) (Lin & Chen, 2017), canine respiratory coronavirus (CRCoV) (Priestnall, Brownlie, Dubovi & Erles, 2006), mouse hepatitis virus (MHV) (Weiss & Leibowitz, 2011), bovine coronavirus (BCV), turkey coronavirus (TCV) and transmissible gastroenteritis virus (TGEV) (Szczepanski, Owczarek & Bzowska, 2019). Viruses of this family were first identified in humans from the nasal secretions of patients with the common cold (Tyrrel & Byone, 1965). ~~Since~~ Four CoVs are known to produce mild infections in individuals with normal immunity similar to the common 'flu, they are 229-E OC43, NL63, and HKU1 (Esper et al., 2006; Su et al., 2016).

However, in recent years, coronaviruses have given rise to significant diseases such as severe acute respiratory syndrome (SARS-CoV) and Middle Eastern respiratory syndrome coronavirus (MERS-CoV) SARS-CoV infected 8,000 people, from 2002 to 2003 and had a mortality rate of approximately 10% (Marra et al., 2003). Ten years later, in 2012, MERS-CoV, emerged, and infected more than 1,700 people, with a mortality rate of approximately 36% (Zaki et al., 2012). In 2013, it attacked animals again, with a coronavirus epidemic of porcine diarrhoea (PEDV) in the United States, causing a

mortality rate of almost 100% in piglets and wiping out more than 10% of the swine population in less than a year (Mole, 2013). More recently, a new coronavirus (SARS-CoV-2) has emerged that is responsible for the current COVID-19 global pandemic. This virus was first identified in the Chinese province of Hubei in December 2019 (Gudi & Twari, 2020) with possible transmission being suspected to have been from animal to human ~~indicated~~ as the first case was detected from a person working in the local fish and wild animals market – with later transmission from human to human via respiratory droplets or direct contact

It is important to appreciate the ecology of CoVs, its propensity for viral recombination with different CoVs in animal populations, resulting in new CoVs that are transmissible and pathogenic to humans. It is possible that the early cases of the infection went unnoticed as the disease may have been mild, there may have been other comorbidities that would fit with a respiratory infection and/or the initial sporadic distribution of affected individuals may have hidden the insidious nature of this infection. This early period of enigmatic transmission may have allowed SARS-CoV-2 to acquire the facilities to be both easily acquired by humans and to induce disease that aids its own survival.

Taxonomy

The *Coronaviridae* Study Group (CSG) of the International Virus Taxonomy Committee (ICTV), is responsible for the classification and taxonomy of coronaviruses (Gorbalenya et al., 2020). The *Coronaviridae* family is composed of 2 subfamilies, 5 genera, 26 subgenera, and 46 species that are part of the, belonging to the suborder *Cornidovirineae*, order *Nidovirales*, Phylum *Incertae sedis*, and realm *Riboviria*. CoVs

belong to the subfamily *Orthocoronavirinae* covers four genera, namely *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus* (<https://talk.ictvonline.org/taxonomy/> - accessed on April 26, 2020). The *Alphacoronaviruses* has 4 subgenera and 19 species, affecting only mammals, such as *Porcine Transmissible Gastroenteritis Coronavirus* (TGEV), *Porcine Epidemic Diarrhea Coronavirus* (PEDV) and humans are infected with HCoV-229E and HCoV-NL63. The *Betacoronavirus* has 5 subgenera and 14 species among them HCoV-OC43, HKU1, MERS-CoV, SARS-CoV, and SARS-CoV-2 (Gaunt, Hardie, Claas, Simmonds & Templeton, 2010; Gorbalenya et al., 2020). The genera *Deltacoronavirus* and *Gammacoronavirus* have do not infect humans, but cause infections in birds (Woo et al., 2012).

The CoVs in humans HCoV-NL63, HCoV-229E, HCoV-OC43, and HKU1 are associated with mild respiratory tract infections (Gaunt et al., 2010; Forni, Cagliani, Clerici & Sironi, 2017). HCoV-229E is associated with infections in immunocompromised individuals (Pene et al., 2003). SARS-CoV and MERS-CoV are highly pathogenic CoVs for humans and have caused two major epidemics (Cui, Li & Shi, 2019). There is still no consensus on a taxonomic position for SARS-CoV-2, but it belongs to a species related to acute respiratory viruses, based on genetic characteristics, but it is a virus independent of SARS-CoV (Gorbalenya et al., 2020) Figures 1a and 1b summarizes the main characteristics of human coronaviruses.

Virology

Coronaviruses are viruses enveloped with positive, non-segmented, single-stranded RNA genomes approximately 26 to 32 kilobases in size. They have phenotypic

and genotypic variety, due to recombinant RNA and large genomes, facilitating, unfortunately, the ability to adapt to new hosts (Woo et al., 2012; Zaki et al., 2012). The activation of S proteins of SARS-CoV-2 occurs through binding with angiotensin-converting enzyme 2 (ACE-2) of the host cells that can be found greatly expressed in nasal epithelial cells, allowing the entry of the virus into the host cells, releasing mRNA for translation of proteins (Guo et al., 2020). The initial portion of the CoVs genome encodes 16 non-structural proteins while the remaining portion encodes 4 essential structural proteins namely small envelope protein (E), spike glycoprotein (S), matrix (M) protein and nucleocapsid (N) protein (Liu et al., 2020).

The bat is suspected to be a natural host and origin for SARS-CoV-2. The present virus may have been transmitted to man via intermediate hosts, such as pangolins. (Zhou et al., 2020), Phylogenetic analysis has shown that SARS-CoV-2 is approximately 89% similar to two bat-derived SARS coronaviruses: bat-SL-CoVZC45 (GenBank MG772933.1) and bat-SL-CoVZXC2 (GenBank MG772934. 1); and is approximately 79% similar to SARS-CoV and 50% similar to the Middle Eastern respiratory syndrome (MERS-CoV) coronavirus (Chen et al., 2020). On the other hand, evolutionary analysis based on the ORF1a / 1b, S and N genes suggests that SARS-CoV-2 is more likely to be a new coronavirus that has been introduced independently from animals to humans due to the inherent mutation property of coronaviruses in nature (Lam et al., 2020). Considering genetic investigations and the presence of some bats and other live wild animals in the Wuhan market, the virus may have originated from bats or bat droppings associated with contaminated materials (Cui, Li & Shi, 2019).

Viral switching overcomes the innate immunity of each host specifically, and coronaviruses can overcome this natural barrier with some ease, as for example MERS-CoV uses Dipeptidyl peptidase 4 as a receptor in several hosts (Muller et al., 2003).

Coronaviruses can acquire new genes that facilitate infection in different hosts through recombination between different viruses, as in the recombination of CoVs with feline FIPV type 1 and CCoV through different parts of the viral ORF1b (Herrewegh et al; 1998). This same type of viral recombination seems to have been involved in the emergence of SARS-CoV (Lau et al., 2010),

Another phenomenon supposedly associated with the adaptation of CoV to different hosts and cells is the variable deletions that cover more than 600 nucleotides in the S1 globular domain of the Spike gene, which is associated with a change in the enteric virus (TGEV) to respiratory tract virus (PRCV). Furthermore, the exclusion of 290 nucleotides from the Spike gene in HCoV-OC43 compared to its BCoV ancestor may have allowed it to adapt to the human host (Vijgen et al., 2005).

The pathogenesis of bat coronavirus is poorly understood when compared to other mammalian hosts and there is little evidence of the consequences of clinical infections in bats. In humans, HCoVs-NL63, -OC43, -HKU1, and -229E circulate constantly in the populations of the world. A comparison and main characteristics of these viruses are shown in Table 1 (Ogimi et al., 2020). SARS-CoV, on the other hand, disappeared a few years after the epidemic, while the new MERS-CoV seems to have remained. All HCoV mainly cause respiratory symptoms in humans (Hamre and Procknow, 1966; Peiris et al., 2003; Woo et al., 2005), although MERS-CoV has also been associated with severe renal complications (Zaki et al., 2012). HCoV is detected in feces (Liu et al., 2004), and there are sporadic reports of CoVs in humans with gastroenteritis, even though they are not consistently related to gastroenteritis in humans (Esper et al., 2010).

SARS-CoV-2 and SARS-CoV have different patterns of tropism and hence transmission profiles. This being due to mutations in NSP2 and NSP3 (Angeletti et al., 2020); this aspect greatly changed the clinical presentation and hence treatment strategies

for each viral infection. Although these mutations were smaller when compared to H7N9 mutations, they significantly worsened the clinical landscape (Zhang, Shen, Chen & Lin, 2020). In the Chinese population, two prevalent types of SARS-CoV-2 evolution have now been observed; type L and type S. Type L strains derive from type S strains and are the more clinically aggressive and contagious (Lai et al., 2020); = hence demonstrating the showing notable adaptive biological capacity of this virus.

It is now recognized that mutations in SARS-CoV-2 have led to clusters of slightly different viruses in different parts of the globe. These clusters, termed A, B and C, vary in their geographic locale. They called the genetic root A; and from it, they identified two sub-clusters of A that are distinguished by the synonymous mutation T29095C. The two other phylogenetic clusters were called B and C. All but 19 of the 93 genomes of type B came from samples from Wuhan and other parts of eastern China and, sporadically, from samples from countries in the adjacent Asian region. Outside East Asia, cluster B was found in 10 viral genomes samples from the United States and Canada, one from Mexico, four from France, two from Germany, one from Italy, and finally one from Australia. Cluster B is derived from A by two mutations: the mutation synonymous with T8782C and the non-synonymous mutation C28144T, changing a leucine to serine. Type C cluster, mainly found in the Americas and Europe, differs from type B by the non-synonymous G26144T mutation that alters a glycine in a valine. This genotype has been found in samples from France, Italy, Sweden, and England, also from California and Brazil, Singapore, Hong Kong, Taiwan, and South Korea but not in mainland China. This phylogenetic classification can be used to design treatment and, eventually, vaccines (Foster et al., 2020).

SARS-CoV

Severe acute respiratory syndrome (SARS) is a human disease associated with severe pneumonia and as noted above is caused by SARS-*Coronavirus* (SARS-CoV) (Drosten et al., 2003). The disease initially occurred in November 2002 in China, and by February 2003, more than 300 cases had reported (Zhong et al., 2003). During the outbreak in 2002-2003, a total of 8,096 cases were reported, including 774 deaths in 27 countries (Ksiazek, et al., 2003). The outbreak was contained, and since 2004 there have been no known cases of SARS-CoV.

SARS-CoV was initially detected in masked palm civets and a raccoon dog. Antibodies against the virus were detected in Chinese ferret badgers in a live-animal market in Shenzhen, China (Guan, 2003), but it is believed that bats were the reservoir (Drexler, Corman & Drosten, 2014). Most of the cases were the result of direct transmission by respiratory droplets during close personal contact, and adequate respiratory precautions taken proved to be effective (Seto et al., 2003).

Of note there was a relatively high incidence of infection in health care professionals managing patients with, or suspected to have SARS-CoV. Peiris et al, (2003) reported that 28% of those infected were health professionals, hospital transmission being a characteristic of the outbreak. The average incubation period depended on the route of transmission but was between 2 to 10 days (Donnelly et al., 2003).

The clinical course of SARS-CoV begins with a flu-like prodrome consisting of fever, chills, fatigue, and malaise. Towards the end of the first week, there is a deterioration of the clinical picture where the lower respiratory tract is involved, and the patient may present with a dry cough, shortness of breath, and possibly hypoxemia (Vijayanand, Wilkins & Woodhead, 2004). Less common symptoms include watery

diarrhea, vomiting, and nausea (Peiris et al., 2003). Laboratory findings include lymphopenia, thrombocytopenia, elevated lactate dehydrogenase (LDH), raised creatine kinase, and alanine aminotransferase (AAT). Infected patients have abnormal chest X-rays in 60-100% of cases, with predominant lower zone and peripheral lung involvement (Goh et al., 2003). Other common findings include lack of cavitation, lymphadenopathy, pleural effusion, mainly ground-glass opacification and sometimes consolidation, interlobular septal, and intralobular interstitial thickening (Wong et al., 2003). Only 20-30% of patients with SARS require intensive care (Peiris et al., 2003). Patients with advanced age and male sex are associated with poor disease outcomes, and the mortality rate is approximately 10% (Leung et al., 2004). Treatment is mainly symptomatic and supportive, no specific therapy (eg.: antivirals) have proven useful.

MERS

A new coronavirus-related outbreak occurred again in 2012 in Saudi Arabia called Middle East Respiratory Syndrome (MERS), it is a severe respiratory illness caused by a variant of the Coronaviruses (CoV), the MERS-CoV, first case detected through sputum analysis in a 60-year-old man (Zaki et al. 2012).

According to WHO (2019) to November 2019, 2494 cases were confirmed, with 858 related deaths. The spread of the outbreak occurred due to people who traveled to the Middle East, with most cases concentrated in Saudi Arabia (Alanazi et al., 2019).

Studies show that MERS-CoV transmission occurs through direct contact with camel dromedaries, such as the consumption of unpasteurized camel milk or undercooked camel meat. The routes of human to human transmission are not yet fully understood, but

possible routes of transmission may occur via human respiratory droplets and contact transmission (Memish et al., 2014). Another characteristic of MERS-CoV was hospital transmission with 43.5% to 100% of outbreaks in different places associated with health care (Chowell et al., 2015). Among the independent risk factors associated with MERS infection are diabetes mellitus, heart disease, smoking, and chronic obstructive pulmonary disease (COPD) (Al-Tawfiq et al., 2014).

Common symptoms of people infected with MERS-CoV are fever, cough, shortness of breath, and diarrhea. Pneumonia is not always present and some people are asymptomatic, but test positive on laboratory tests (Chafekar & Fielding, 2018). It has an incubation period of approximately 5 days. Abnormal ~~such as~~ chest X-rays and laboratory tests are common the latter include lymphopenia, thrombocytopenia, ~~high~~ elevated levels of lactate dehydrogenase, and liver enzymes. There are reports of some cases of disseminated intravascular coagulation and hemolysis-(Al-Tawfiq et al., 2014). Up to 50 to 89% of patients require intensive care and mechanical ventilation due to acute respiratory distress syndrome (ARDS). The mortality rate of MERS is approximately 35%, higher when compared to SARS-CoV. There is no specific treatment for MERS, the mainstay of management include supportive, symptomatic measures, and the prevention and management of secondary complications (de Wit et al., 2016).

SARS-Cov-2

Coronavirus disease (COVID-19) originated in December 2019 in Wuhan, Hubei province, China, spread rapidly through China and many other countries, being registered by WHO as the sixth public emergency of international interest. With alarming rates of spread of the disease in many countries outside China, WHO declared COVID-19 a

pandemic in March, 2020. On February 11, 2020, WHO announced a new name for the 2019-nCoV epidemic–COVID-19 (WHO, 2020). The International Virus Taxonomy Committee renamed 2019-nCoV, as severe acute respiratory syndrome coronavirus-2 of the genus betacoronavirus (SARS-CoV-2). SARS-CoV-2 is effectively transmitted between humans, including via asymptomatic carriers (Rabi et al., 2020).

The risk factors associated with the worsening of the disease are patients in the seventh decade of life, male, with associated morbidities and late diagnosis. Laboratory confirmation of COVID-19 is based on detection of SARS COV-2 RNA by reverse-transcription polymerase chain reaction (RT-PCR) on upper respiratory specimens (nasopharyngeal/oropharyngeal swabs) or lower respiratory specimens (sputum, endotracheal aspirates or bronchoalveolar lavage (WHO, 2020: Laboratory testing for coronavirus disease (COVID-19) in suspected human cases interim guidance (WHO, 2020; <https://apps.who.int/iris/handle/10665/331329>).

Clinical characteristics and pathogenesis of COVID-19 are similar to SARS and MERS (Xie & Chen, 2020), with an incubation period of 4 to 7 days, however, this can be up to 14 days. There are reports of high rates of asymptomatic and subclinical infections (Yang et al., 2020). A comparison between these 3 viruses are shown in Table 2. In a study of 1099 patients with Covid-19 confirmed in a laboratory in Mainland China, 5% of patients were admitted to the ICU, 41.3% needed oxygen therapy, 6.1% needed mechanical ventilation and 1.4% died. The average hospital stay was 12 days (Guan et al., 2020). Wang et al (2020) showed that as the number of transmission of COVID-19 cases increases, lethality decreases, this would explain the low mortality rate when comparing SARS and MERS.

The most common presentations in symptomatic patients are fever, dry cough, and shortness of breath. Other features include gastrointestinal symptoms, such as diarrhea, neurological symptoms (stroke, headache, altered mental status, GBS), cardiovascular events (myocarditis, arrhythmias, heart failure), and ocular manifestations (conjunctival hyperemia, chemosis), anosmia and dysgeusia. (Guan et al, 2020). In computed tomography of the chest of COVID-19 pneumonia, subpleural ground-glass opacities were initially observed, which increased, with changes in the paving pattern. In recovering patients, the lesions appeared absorbed with the presence of opacities and subpleural parenchymal bands. (Xie & Chen, 2020). Laboratory findings include leukopenia, lymphocytopenia (sometimes very notable), elevated neutrophilic ratio, C-reactive protein and serum proinflammatory cytokines, the last 3 findings mentioned being associated with more severe disease (Guan et al., 2020)

Immune response in COVID-19

The initial response to CoV infection is carried out through the immune system (Frieman & Baric, 2008). Toll-type receptors, which are receptors for antigen-presenting cells that detect viral RNAs, inducing the signaling cascade of the immune system, consequently increasing cytokines (Alexopolou, Holt, Medzhitov & Flavell, 2001; Wu & Chen, 2014). To prevent recognition of the host's immune system, the virus forms double vesicles on the outside of the cell, it also has non-structural proteins inhibiting functions of the innate immune system, such as Nsp1 to block INF (Guo et al., 2020).

The increase in pro-inflammatory cytokines, including as IFN- α , IFN- γ , IL-1 β , IL-6, IL-12, IL-18, IL-33, TNF- α , TGF β and CCL2, CCL3, CCL5 chemokines, CXCL8, CXCL9, CXCL10, is the first line of defense against infection Wu & Chen, 2014). However, the massive proliferation of immune cells can result in hyper inflammation of the lungs and

other viscera causing acute respiratory distress syndrome (ARDS) and notable myocardial damage. (Huang et al., 2020)

Asymptomatic cases

As noted above, there are reports of asymptomatic COVID-19 cases from several groups. For example, nearly 46% of positive cases in a quarantined cruise ship were asymptomatic. A study conducted in an Italian village revealed that 50- 75% of cases were asymptomatic. In children, 15-30% who tested positive may be asymptomatic. Notably, SARS-CoV-2 has been found in upper respiratory tract secretions of asymptomatic individuals (Zou et al., 2020), raising concerns that even asymptomatic patients can transmit COVID-19.

Conclusions

SARS-CoV-2 has become the seventh Coronavirus disease in humans responsible for this current global pandemic. Once again, it demonstrated its ability to cross-species and adapt to the human host. Critically, Coronavirus surveillance in animals must include animals other than bats, as the role of intermediate hosts is likely to be of great importance, providing a more direct route for the virus to emerge in humans. Given the enormous diversity of coronavirus viruses in wildlife and their continuous evolution and adaptation to humans, mainly causing respiratory or gastrointestinal disorders it is not difficult to predict other outbreaks. Restricting or banning all trade in wild animals in wet markets would be a necessary measure to reduce future zoonotic infections.

REFERENCES

- Alanazi, K.H., Killerby, M.E., Biggs, H.M., Abedi, G.R., Jokhdar, H., Alsharef, A.A., ...Watson, J. T. (2019). Scope and extent of healthcare-associated Middle East respiratory syndrome coronavirus transmission during two contemporaneous outbreaks in Riyadh, Saudi Arabia, 2017. *Infection control and hospital epidemiology*, *40*(1), 79–88. doi:10.1017/ice.2018.290
- Alexopoulou, L., Holt, A. C., Medzhitov, R., & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature*, *413*(6857), 732–738. doi:10.1038/35099560
- Al-Tawfiq, J. A., Hinedi, K., Ghandour, J., Khairalla, H., Musleh, S., Ujayli, A., & Memish, Z. A. (2014). Middle East respiratory syndrome coronavirus: a case-control study of hospitalized patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *59*(2), 160–165. doi:10.1093/cid/ciu226
- Annan A, Baldwin HJ, Corman VM, Klose S. M., Owusu, M., Nkrumah E.E.... Jan Felix Drexler, J.F.(2013). Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerg Infect Dis.*;19(3):456-459. doi:10.3201/eid1903.121503
- Angeletti, S., Benvenuto, D., Bianchi, M., Giovanetti, M., Pascarella, S., & Ciccozzi, M. (2020). COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. *Journal of Medical Virology*. doi:10.1002/jmv.25719
- Centre for Infectious Disease Epidemiology and Research-NUS. COVID-19 Situation Report 80- WHO 2020
- Chafekar, A., & Fielding, B. C. (2018). MERS-CoV: Understanding the Latest Human Coronavirus Threat. *Viruses*, *10*(2), 93. doi:10.3390/v10020093
- Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., ... Zhang, X. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* *395* (10223) 507–513. doi: 10.1016/S0140-6736(20)30211-7
- Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., ... Xu, G. (2020). Kidney impairment is associated with in-hospital death of COVID-19 patients. *MedRxiv*. doi 10.1101/2020.02.18.20023242
- Chinese Clinical Trial Registry. <http://www.chictr.org.cn/> (accessed February 18, 2019)
- Chowell, G., Abdirizak, F., Lee, S., Lee, J., Jung, E., Nishiura, H., & Viboud, C. (2015). Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC medicine*, *13*, 210. doi:10.1186/s12916-015-0450-0
- Coman, V. M., Eckerle, I., Memish, Z. A., Liljander, A. M., Dijkman, R., Jonsdottir, H., ... Drosten, C. (2016). Link of a ubiquitous human coronavirus to dromedary camels. *Proceedings of the National Academy of Sciences*, *113*(35), 9864–9869. doi:10.1073/pnas.1604472113

Crossley, B., Mock, R., Callison, S., & Hietala, S. (2012). Identification and Characterization of a Novel Alpaca Respiratory Coronavirus Most Closely Related to the Human Coronavirus 229E. *Viruses*, 4(12), 3689–3700. doi:10.3390/v4123689

Cui, J., Li, F., & Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*. 17(3):181–92, doi:10.1038/s41579-018-0118-9

Dangers, L., Montlahuc, C., Kouatchet, A., Jaber, S., Meziani, F., Perbet, S., ... Demoule, A. (2018). Dyspnoea in patients receiving noninvasive ventilation for acute respiratory failure: prevalence, risk factors and prognostic impact. *European Respiratory Journal*, 52(2), 1702637. doi:10.1183/13993003.02637-2017

de Wit, E., van Doremalen, N., Falzarano, D., & Munster, V. J. (2016). SARS and MERS: recent insights into emerging coronaviruses. *Nature reviews. Microbiology*, 14(8), 523–534. doi: 10.1038/nrmicro.2016.81

Donnelly, C. A., Ghani, A. C., Leung, G. M., Hedley, A. J., Fraser, C., Riley, S., ... Anderson, R. M. (2003). Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *The Lancet*, 361(9371), 1761–1766. doi:10.1016/s0140-6736(03)13410-1

Drexler, J. F., Corman, V. M., & Drosten, C. (2014). Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research*, 101, 45–56. doi:10.1016/j.antiviral.2013.10.013

Drosten, C., Günther, S., Preiser W., van der Werf, S., Brodt, HR., Becker, S., ... Doerr, HW. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 348, 1967–1976. doi:10.1056/NEJMoa0307

Esper, F., Weibel, C., Ferguson, D., Landry, M. L., & Kahn, J. S. (2006). Coronavirus HKU1 infection in the United States. *Emerging infectious diseases*, 12(5), 775–779. doi: 10.3201/eid1205.051316

Fan, E., Del Sorbo, L., Goligher, E. C., Hodgson, C. L., Munshi, L., Walkey, A. J., ... Brochard, L. J. (2017). An Official American Thoracic Society/European Society of Intensive Care Medicine/Society of Critical Care Medicine Clinical Practice Guideline: Mechanical Ventilation in Adult Patients with Acute Respiratory Distress Syndrome. *American Journal of Respiratory and Critical Care Medicine*, 195(9), 1253–1263. doi:10.1164/rccm.201703-0548st

Frieman, M., & Baric, R. (2008). Mechanisms of severe acute respiratory syndrome pathogenesis and innate immunomodulation. *Microbiology and molecular biology reviews: MMBR*, 72(4), 672–685. doi:10.1128/MMBR.00015-08

Forni, D., Cagliani, R., Clerici, M., Sironi, M. (2017). Molecular evolution of human coronavirus genomes. *Trends Microbiol.* 25:35–48. doi: 10.1016/j.tim.2016.09.001

Forster, P., Forster, L., Renfrew, C., Forster, M. (2020). Phylogenetic network analysis of SARS-CoV-2 genomes. *Lancet*; doi:10.1073/pnas.2004999117

Gandhi, R.T., Lynch, J.B. & Del Rio, C. (2020). Mild or Moderate COVID-19. *N Engl J Med.* Apr 24. doi: 10.1056/NEJMcp2009249

Gaunt, E. R., Hardie, A., Claas, E. C. J., Simmonds, P., & Templeton, K. E. (2010). Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method. *Journal of Clinical Microbiology*, 48(8), 2940–2947. doi:10.1128/jcm.00636-10

Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont H T, Honoré S, Colson P, Chabrière E, La Scola B, Rolain J-M, Brouqui P, Raoult D. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents*; doi.org/10.1016/j.ijantimicag.2020.105949

Goh, J.S.-K., Tsou, I.Y.-Y., & Kaw, G.J.-L. (2003). Severe Acute Respiratory Syndrome (SARS): Imaging Findings During the Acute and Recovery Phases of Disease. *Journal of Thoracic Imaging*, 18(3), 195–199. doi:10.1097/00005382-200307000-00010

Gorbalenya, A.E., Baker, S.C., Baric, R.S., de Groot, R.J., Drosten, C., Gulyaeva, A.A., ... Ziebuhr, (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*, 5, 536-544. doi: 10.1038/s41564-020-0695-z

Guan, Y. (2003). Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China. *Science*, 302(5643), 276–278. doi:10.1126/science.1087139

Guan, WJ., Ni, ZY., Hu, Y., Liang, WH., Ou, CQ., He, JX., ... Zhong, NS. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* doi: 10.1056/NEJMoa2002032

Gudi, S.K. & Tiwari, K.K. (2020). Preparedness and Lessons Learned from the Novel Coronavirus Disease. *The IJOEM*; 11 (Num 2); April, 108:112. doi: 10.34172/ijoem.2020.1977

Guidance for health workers – who.int 2019. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/health-workers>.

Guo, Y-R., Cao, Q-D., Hong, Z-S., Tan, Y-Y., Chen, S-D., Jin, H-J., ... Yan, Y. (2020) The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. *Military Medical Research*. 2020;7:1–10. doi: 10.1186/s40779-020-00240-0

Herrewegh AA, Smeenk I, Horzinek MC, Rottier PJ, de Groot RJ. (1998) Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. *J Virol.*;72(5):4508-4514

Ho AS, Sung JJ, Chan-Yeung M. (2003). An outbreak of severe acute respiratory syndrome among hospital workers in a community hospital in Hong Kong. *Ann Intern Med.*;139(7):564-567. doi:10.7326/0003-4819-139-7-200310070-00008

Hu, B., Ge, X., Wang, L. F., & Shi, Z. (2015). Bat origin of human coronaviruses. *Virology journal*, 12, 221. doi: 10.1186/s12985-015-0422-1
Huynh, J., Li, S., Yount, B., Smith, A., Sturges, L., Olsen, J. C., Nagel, J., Johnson, J. B., Agnihothram, S., ..., Donaldson, E. F. (2012). Evidence supporting a zoonotic origin of human coronavirus strain NL63. *Journal of virology*, 86(23), 12816–12825. doi:10.1128/JVI.00906-12

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020; 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

Kirk-Bayley, J., Combes, J., Sunkaraneni, S., Challacombe, S. (2020). The Use of Povidone Iodine Nasal Spray and Mouthwash During the Current COVID-19 Pandemic May Reduce Cross Infection and Protect Healthcare Workers. (March 28, 2020). doi: 10.2139/ssrn.3563092

Ksiazek, T.G., Erdman D., Goldsmith C.S., Zaki S.R., Peret T., Emery S., ..., Anderson L.J. (2003). A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 348, 1953–1966. doi: 10.1056/NEJMoa030781

Lai, C.C., Shih, T.P., Ko, W.C., Tang, H.J., & Hsueh, P.R. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *International journal of antimicrobial agents*, 55(3), 105924. doi: 10.1016/j.ijantimicag.2020.105924

Lau SK, Li KS, Huang Y, Shek, C-T, Tse, H., Wang, M., ... Kwok-Yung Yuen, K-Y. (2010). Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related Rhinolophus bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. *J Virol.*;84(6):2808-2819. doi:10.1128/JVI.02219-09

Lam, T.T., Shum, M.H., Zhu, H-C., Tong, Y-G., Ni, X-B., Liao, Y-S., ... Guan, Y. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature*. doi:10.1038/s41586-020-2169-0

Leung, G. M., Hedley, A. J., Ho, L.-M., Chau, P., Wong, I. O. L., Thach, T. Q., ... Lam, T.-H. (2004). The Epidemiology of Severe Acute Respiratory Syndrome in the 2003 Hong Kong Epidemic: An Analysis of All 1755 Patients. *Annals of Internal Medicine*, 141 (9), 662. doi: 10.7326/0003-4819-141-9-200411020-00006

Li W., Moore, M.J., Vasilieva, N., N.J., Wong, S.K., Berne, M.A., ... Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. ;426(6965):450-454. doi:10.1038/nature02145

Li F. (2016). Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annual review of virology*, 3(1), 237–261. doi:10.1146/annurev-virology-110615-042301

Lin SY & Chen HW. (2017) Infectious Bronchitis Virus Variants: Molecular Analysis and Pathogenicity Investigation. *Int J Mol Sci*. 2017;18(10):2030. Published 2017 Sep 22. doi:10.3390/ijms18102030

Liu, Q., Wang, R., Qu, G., Wang, Y., Liu, P., Zhu, Y., ... Liu, L. (2020). Gross examination report of a COVID-19 death autopsy. *Journal of Forensic Medicine* Feb;36(1):21-23. doi: 10.12116/j.issn.1004-5619.2020.01.005

Lu, H. (2020). Drug treatment options for the 2019-new coronavirus (2019-nCoV). *BioScience Trends*. Vol. 14, No. 1 pp. 69-71. doi:10.5582/bst.2020.01020.

Marra, M.A., Jones, S.J., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S., ..., Roper, R.L. (2003). The Genoma sequence of the SARS-associated coronavirus. *Science*. May 30;300(5624):1399-404. doi: 10.1126/science.1085953

Memish, Z.A., Cotten, M., Meyer, B., Watson, S. J., Alshafi, A.J., Al Rabeeah, A.A., ... Drosten, C. (2014). Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. *Emerging infectious diseases*, 20(6), 1012–1015. doi:10.3201/eid2006.140402

Mole, B. (2013). Deadly pig virus slips through US borders. *Nature*, 499(7459), 388–388. doi:10.1038/499388a

Müller, N.L., Ooi, G.C., Khong, P.L., & Nicolaou, S. (2003). Severe Acute Respiratory Syndrome: Radiographic and CT Findings. *American Journal of Roentgenology*, 181(1), 3–8. doi:10.2214/ajr.181.1.1810003

Ogimi C, Kim YJ, Martin ET, Huh HJ, Chiu CH, Englund JA. (2020) What's New With the Old Coronaviruses? *Journal of the Pediatric Infectious Diseases Society*. 9(2): 210–217

Pene, F., Merlat, A., Vabret, A., Rozenberg, F., Buzyn, A., Dreyfus, F., Cariou, A., Freymuth, F., & Lebon, P. (2003). Coronavirus 229E-related pneumonia in immunocompromised patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 37(7), 929–932. doi: 10.1086/377612

Peiris, J.S., Lai, S.T., Poon, L.L., Guan, Y., Yam, L.Y., Lim, W., Yuen, K.Y., group Ss (2003). Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*, 361: 1319-1325. doi: 10.1016/s0140-6736(03)13077-2

Priestnall, S., Brownlie, J., Dubovi, E., & Erles, K. (2006). Serological prevalence of canine respiratory coronavirus. *Veterinary Microbiology*, 115(1-3), 43–53. doi:10.1016/j.vetmic.2006.02.008

Rabi, F. A., Al Zoubi, M. S., Kasasbeh, G. A., Salameh, D. M., & Al-Nasser, A. D. (2020). SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. *Pathogens*, 9(3), 231. doi:10.3390/pathogens9030231

Seto, W., Tsang, D., Yung, R., Ching, T., Ng, T., Ho, M., ... Peiris, J. (2003). Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). *The Lancet*, 361(9368), 1519–1520. doi:10.1016/s0140-6736(03)13168-6

Siddell, S.G., Anderson R., Cavanagh, D., Fujiwara, K., Klenk, H.D., Macnaughton, M. R., ..., van der Zeijst, B.A.M. (1983). Coronaviridae. *Intervirology*. 20:181-189. doi: 10.1159/000149390

Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C.K., Zhou, J., ... Gao, G.F. (2016). Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends in Microbiology*, 24(6), 490–502. doi:10.1016/j.tim.2016.03.003

Szczepanski, A., Owczarek, K., Bzowska, M. (2019). Canine Respiratory Coronavirus, Bovine Coronavirus, and Human Coronavirus OC43: Receptors and Attachment Factors. *Viruses*. 11(4):328. doi:10.3390/v11040328

Tyrrell, D. A., & Bynoe, M. L. (1965). Cultivation of a novel type of common-cold virus in organ cultures. *British medical journal*, 1(5448), 1467–1470. doi:10.1136/bmj.1.5448.1467

Vijaykrishna D, Smith GJ, Zhang JX, Peiris JS, Chen H, Guan Y. (2007) Evolutionary insights into the ecology of coronaviruses [published correction appears in J Virol. 2007 Aug;81(15):8371]. *J Virol.*;81(8):4012-4020. doi:10.1128/JVI.02605-06

Vijayanand, P., Wilkins, E., & Woodhead, M. (2004). Severe acute respiratory syndrome (SARS): a review. *Clinical Medicine*, 4(2), 152–160. doi:10.7861/clinmedicine.4-2-152

Vijgen, L., Keyaerts, E., Lemey, P., Moës, E., Li, S., Vandamme, A. M., & Van Ranst, M. (2005). Circulation of genetically distinct contemporary human coronavirus OC43 strains. *Virology*, 337(1), 85–92. doi: 10.1016/j.virol.2005.04.010

Xie, M., & Chen, Q. (2020). Insight into 2019 novel coronavirus - an updated interim review and lessons from SARS-CoV and MERS-CoV. *International Journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*, S1201-9712(20)30204-6. Advance online publication. doi: 10.1016/j.ijid.2020.03.071

Wang, L., Li, J., Guo, S., Xie, N., Yao, L., Cao, Y., Day, S. W., Howard, S. C., Graff, J. C., Gu, T., Ji, J., Gu, W., & Sun, D. (2020). Real-time estimation and prediction of mortality caused by COVID-19 with patient information based algorithm. *The Science of the total environment*, 727, 138394. doi:10.1016/j.scitotenv.2020.138394

Weiss SR, Leibowitz JL. (2011). Coronavirus pathogenesis. *Adv Virus Res.*;81:85-164. doi:10.1016/B978-0-12-385885-6.00009-2

WHO. Middle East Respiratory Syndrome Coronavirus (MERS-CoV). 2019. Available at: <https://www.who.int/emergencies/mers-cov/en/>. Accessed April 17, 2020.

WHO. Situation report - Coronavirus disease – 100 (Accessed April 29, 2020)

Wong, R. S., Wu, A., To, K. F., Lee, N., Lam, C. W., Wong, C. K., ... Sung, J. J. (2003). Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. *BMJ (Clinical research ed.)*, 326(7403), 1358–1362. <https://doi.org/10.1136/bmj.326.7403.1358>

Woo PC, Lau SK, Lam CS, Lai KK, Huang Y, Lee P, ... Yuen KY (2009). Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. *Journal of Virology*, 83: 908-917. doi: 10.1128/JVI.01977-08

Woo, P.C., Lau, S.K., Lam, C.S., Lau, C.C., Tsang, A.K., Lau, J. H., ... Yuen, K.Y. (2012). Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *Journal of virology*, 86(7), 3995–4008. <https://doi.org/10.1128/JVI.06540-11>

Wu, J., & Chen, Z. J. (2014). Innate Immune Sensing and Signaling of Cytosolic Nucleic Acids. *Annual Review of Immunology*, 32(1), 461–488. doi:10.1146/annurev-Immunol-032713-120156

Yang, Y., Lu, Q., Liu, M., Wang, Y., Zhang, A., Jalali, N., ... & Zhang, X. (2020). Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. *medRxiv*. doi: 10.1101/2020.02.10.20021675

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. *N Engl J Med*. 367:1814–20. doi: 10.1056/NEJMoa1211721

Zhang, L., Shen, F., Chen, F., & Lin, Z. (2020). Origin and evolution of the 2019 novel coronavirus. *Clinical Infectious Diseases*. doi:10.1093/cid/ciaa112

Zhong, N.S., Zheng, B.J., Li, Y.M., Poon, Xie, Z.H., Chan, K.H., ... Guan, Y. (2003). Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February. *Lancet*. Oct 25;362(9393):1353-8. doi: 10.1016/s0140-6736(03)14630-2

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., ... Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. doi:10.1038/s41586-020-2012-7