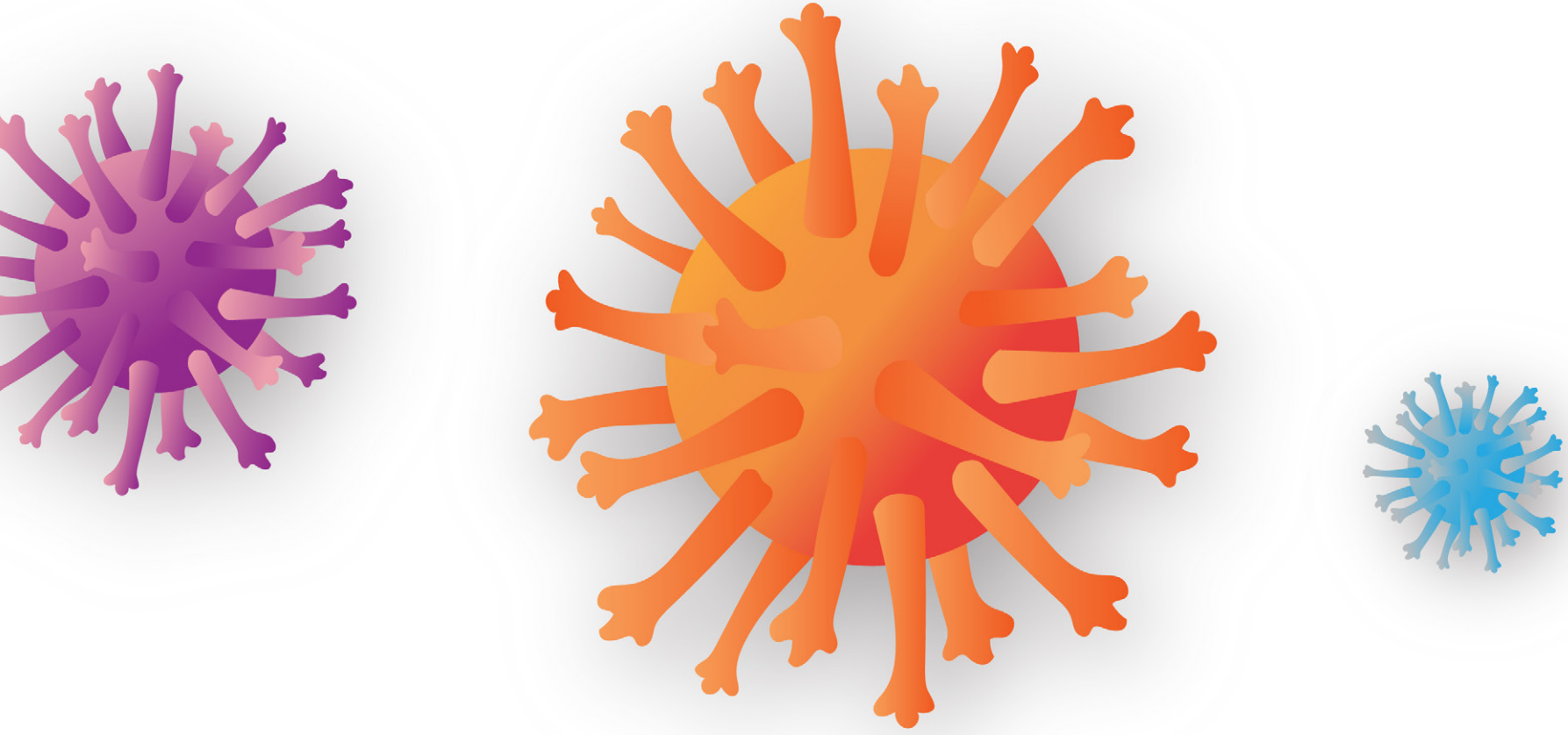
animal and human CORONAVIRUSES



EVOLUTION AND PATHOGENICITY OF THE VIRUS AND DISEASE PREVENTION

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FOREWORD

This is a really great book! It is well written, scientifically precise, well organised and has beautiful graphics. However, what is most impressive is the holistic view of coronavirus pathogenesis and ecology that Veterinarians, Biologists and Doctors capture so well as that is their very nature: to understand an infectious disease in multiple animals and its dynamic relationship with humans.

Viruses are obligate intracellular parasites and as such have co-evolved with other living organisms since the very beginnings of life. When they emerge in a new host it is not due to spontaneous generation, but rather because they have jumped the species barrier from another host. This is precisely what happened with the current pandemic of SARS-CoV-2 where a virus, which likely originated from an unidentified bat species, crossed the species barrier. Now this same virus, SARS-CoV-2, has leaped from humans to minks, where it is more virulent. It has not stopped there, because it has since re-infected humans and we do not know if minks will enable the virus to spread to other animal species. This is nature taking its course, everything is directly or indirectly related. One of the biggest lessons of this pandemic is that humankind must respect nature.

Back to the book; in a world of time travelling I can imagine myself delighting in this book as a high school or university student in the same way as I have as a mature scientist and academic old-timer. This is a book for all ages for those interested in the matters of infectious diseases. Surely professors and academics will recommend this book as enthusiastically as I do right now.

By Pedro Simas

ACKNOWLEDGMENT

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PREFACE

The most recently discovered Human coronavirus, Severe Acute Respiratory Syndrome - Coronavirus - 2 (SARS-CoV-2), is certainly the best-known coronavirus of all, as it is considered a successful invader. First identified in late 2019, it generated the current pandemic of coronavirus disease of 2019 (COVID-19) when it spread across the planet in only a few months. Like some other members of the same family of viruses, SARS-CoV-2 can infect different types of human cells, however, the epithelia of the lungs and larynx are the main targets thus preferentially causing respiratory infections. Mortality associated with COVID-19 alongside the impacts on health, education, the economy and individual and social well-being are currently a concern for the whole world. These are not only due to the effects of the disease, but also the pandemic control measures imposed by governments across the world, namely mandatory isolation, and we are still looking to adapt to this new way of life alongside a virus that was previously unknown. However, humans and other animal species have long lived side by side with other coronaviruses, many of which unknown to most people, some quite harmless whilst other potentially lethal. In that sense, this is no different.

This book aims to assemble and disseminate information about the origin, evolution and pathogenesis of animal and human coronaviruses in a simple and accessible way, and through this clarify readers' doubts and fears as well as the rationale underlying transmission prevention actions.

The main text of the manuscript is accompanied by two types of additional information for optional consultation; more in-depth information about some of the mentioned concepts (know more) and a set of questions & answers, providing the reader with the opportunity to clarify doubts relating to the content of each section. For the preparation of this book, we counted on the collaboration of technicians, researchers and academics in the areas of microbiology, epidemiology, animal health and public health. During the production and editing process, a board game and cards (Beat Corona) were also produced. These further explored some of the concepts mentioned in this book, including behaviours related to life in society and their implications in the transmission of diseases caused by coronaviruses, including COVID-19. This game can be used as a pedagogical resource in the classroom context, or as a mere leisure and learning activity.

TARGET AUDIENCE

This book can be used as supporting material for secondary education in areas of natural sciences and biology. It covers the following topics: cellular biology, genetics, evolution, microbiology, pathogens, principles of immunology, disease transmission and infection control.

It can also be used at higher levels of education in Medicine, Veterinary Medicine, Biology, Dentistry, Nursing, Physiotherapy, Animal Science, Education, Biomedical Sciences, Pharmacy, etc.

CONTENT




1. THE CORONAVIRUSES

1.1. CORONAVIRUS DISCOVERY

Based on recent molecular evolution studies it is estimated that the common ancestor of all current coronaviruses appeared 293 million years ago[1]. However, the first recorded clinical case of a coronavirus infection was reported in the beginning of the 20th century, involving a cat who had developed fever and significant abdominal distension, although only much later was the causative agent identified as being a feline coronavirus. The association between some enzootic diseases (i.e., ones that are sustained within an animal population in a certain geographic region) and coronaviruses was only established in the 1930s, with the identification of the respiratory disease avian infectious bronchitis in domestic chickens [2].

Thus, coronaviruses were only initially associated with enzootic infections restricted to their natural animal hosts (e.g., transmissible gastroenteritis in pigs or infectious peritonitis in cats). Coronaviruses that only infect animal species circulate in their respective domestic or wild populations and are transmissible from infected animals to susceptible animals. Many of these coronaviruses do not constitute any threat to man. Despite the fact that coronaviruses have always coexisted with animals and humans, only in the 1960s were they associated with colds and mild diarrhoea that affect millions of people globally every year. At that time they were recognised and characterised by electron microscopy [3, 4].

1.2. WHY HAVE THEY BEEN CALLED CORONAVIRUSES? THE MORPHOLOGY AND TAXONOMY OF CORONAVIRUS

The development of electronic microscopy techniques enabled the observation of the structure of a coronavirus for the first time in 1965. The images revealed a very distinctive crown-like (crown = corona) morphology and they were thus commonly called coronaviruses. The spikes that give them this unique appearance are formed by viral proteins called S proteins (for spikes) that have an essential role in the virus being able to recognise and bind to the animal or human host cells. Different coronaviruses vary in size, between 80 and 220 nm (manometers), and thus include some of the largest known envelope viruses. They are, nonetheless, only about a thousand times smaller than the thickness of a human hair.

According to the rules of Taxonomy (the scientific area that hierarchizes, organises and classifies living organisms), the Coronaviridae family was created within the order Nidovirales[5].

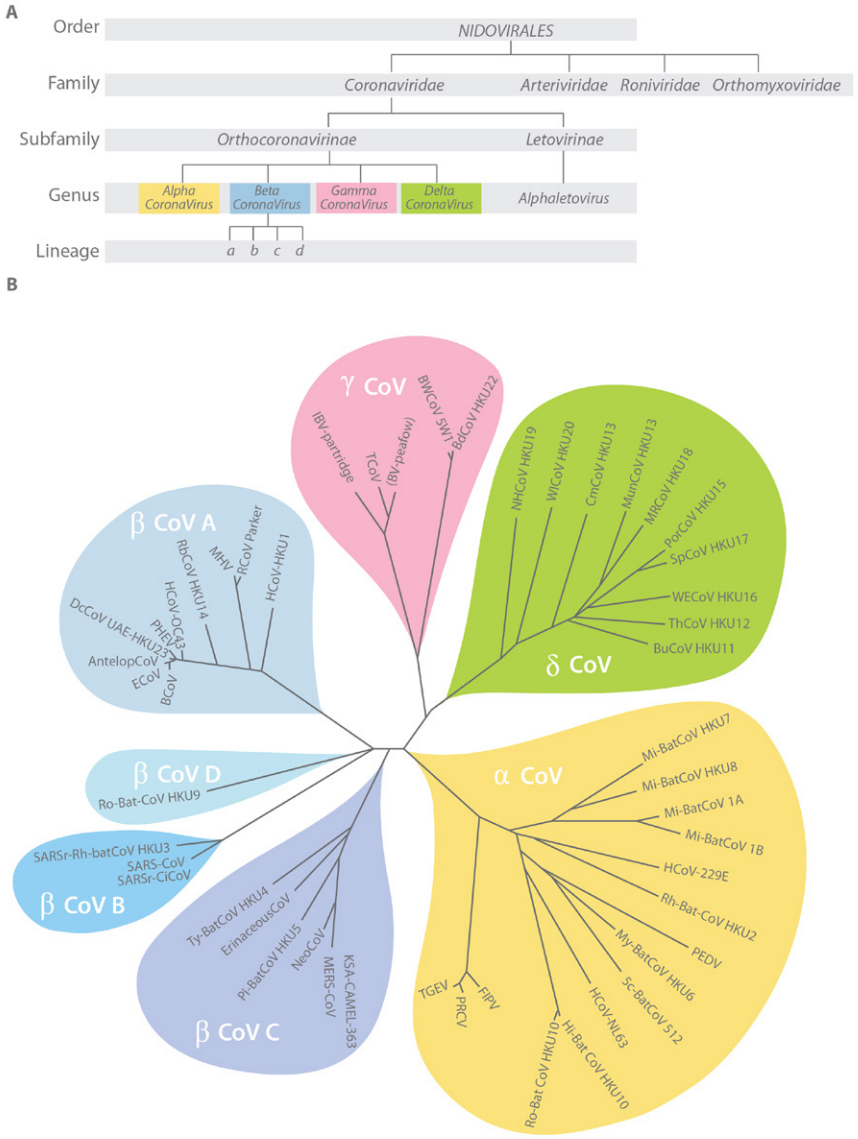


Fig. 1 - Coronavirus phylogenetic classification (phylogenetic tree based on partial sequences of the RNA-dependent RNA polymerase (RdRP) of 50 coronaviruses). Adapted from Fenner and White's 2012[10]

The Coronaviridae family (belonging to the order Nidovirales, suborder Cornidovirineae) is one of about 170 virus families currently recognised by the International Committee on Taxonomy of Viruses (Edition 2019) and includes the subfamilies Orthocoronavirinae and Letovirinae[1].

The genetic material of coronaviruses is made up of non-segmented ribonucleic acid (RNA) (i.e., made up of only one RNA molecule per viral particle). Viral RNA is single-stranded and positive-sense, which means that it is, in itself, potentially infectious. The RNA has, at its 5' end, a cap structure and at the 3' end a poly A tail, which gives it stability and prolongs its life span. Coronaviruses have one of the largest known viral RNA genomes, 27,000 to 32,000 nucleotides in length (revised by Modrow et al, 20132). This genome is three times the size of the genome of the human immunodificiency virus (HIV) and hepatitis C virus, and twice the genome of the influenza virus. The S and HE proteins, which protrude outwards from the surface of the viral particle, are the main targets of the immune system of the host species. In effect, these proteins are immunogenic, that is, they Induce an immune response from the host, with S protein being the dominant antigen and the main inducer of the production of neutralising antibodies (which inactivate the virus).

How are viruses seen and measured?
Most viruses, like coronaviruses, can only be viewed under an electron microscope with a magnifying power of 10,000,000x. Viruses are measured in nanometers (nm), with 1 nm equal to 0.000000001 meter (m). SARS-CoV-2, the COVID-19 agent, is about 100 nm in diameter (0.0000001 m).

What is RNA and DNA?
Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA) are macromolecules that carry genetic instructions. In every living organism, this genetic material stores the information that characterises it. In organisms that undergo sexual reproduction part of this information gets passed down to offspring whereas in organisms that undergo asexual reproduction, like bacteria and viruses, the entirety of the information gets passed down. Unlike animals and plants, whose genetic material consists of a double-stranded DNA (dsDNA), there is a huge variety of viral genomes including double-stranded (dsDNA), single-stranded (ssDNA), linear or circular DNA and also double-stranded (dsRNA) or single-stranded (ssRNA) RNA, as viruses can also have ribonucleic acid (RNA) as their genetic material. Viruses may have a unique or a segmented genome.

The Coronaviridae family, in turn, includes the subfamilies Letovirinae and Orthocoronavirinae. The former includes the genus Alphaletovirus and the latter the genera Alphacoronavirus, Betacoronavirus, Deltacoronavirus and Gammacoronavirus. All coronaviruses were identified and classified according to genetic and antigenic criteria. Only seven are known to infect humans. Figure 1 illustrates the phylogenetic relationships between alphacoronaviruses, betacoronaviruses, gammacoronaviruses and deltacoronaviruses.

1.3. WHAT ARE CORONAVIRUSES MADE OF?

Viruses are essentially made up of genetic material - nucleic acids that can be DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), containing all the information to form new viruses, surrounded by a protective shield of highly resistant protein, called a capsid. The nucleic acid and the capsid form the nucleocapsid. Viruses with only these two components are considered "naked" and can remain intact in the environment for long periods of time given the capsid's resistance to external aggressions. Coronaviruses' genetic material consists of single-stranded RNA (ssRNA). They also have an additional outer structure composed of lipids, called an envelope, making them more fragile to external environmental factors. This outer shell originates from the host cell where the viruses replicate (Figure 2). In the coronavirus, several viral proteins are incorporated into the envelope's surface, including S

proteins (for Spike), M proteins (for Matrix) and E proteins (for Envelope). They are involved in the different stages of the virus' replicative cycle, namely in the assembly of virions (free viral particles), in the formation of the envelope and in the viral pathogenesis [7].

Some coronaviruses, such as HCoV-HKU19 and HCoV-OC43, also have an HE protein (Hemagglutinin Esterase) that is not present in SARS-CoV-2, the COVID-19 (Coronavirus Disease 2019) agent. The S and HE proteins are essential for the binding of the virus to the target host cell (Figure 3), a fundamental step for the occurrence of infection. When the fragile viral envelope is damaged by the action of agents that dissolve fats, the coronaviruses are no longer recognised by the target cell receptors and the fusion with the cell membrane doesn't occur thus preventing intracellular viral replication.

This fragility of enveloped viruses, including the coronavirus, can be exploited through use of detergents and 70% alcohol-based solutions, such as hand sanitiser and surface disinfectants, which effectively neutralise the virus.

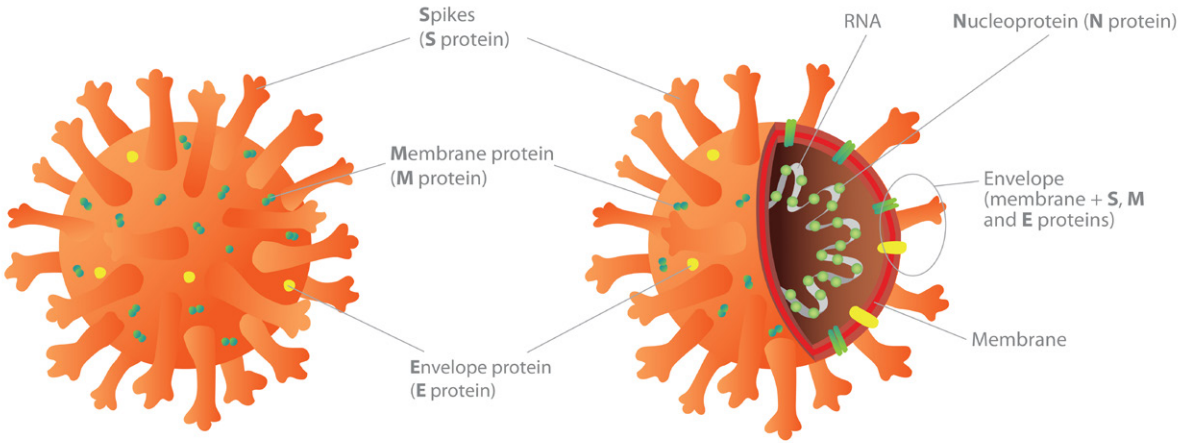


Fig. 2 - Schematic representation of a coronavirus

Once inside the cell, the viral genome is replicated and the viral genes are expressed (transcribed and translated) using the machinery of the living cell. The biological processes necessary for the production of viral progeny can then begin. In coronaviruses, the synthesis of new virions (or viral particles) takes place inside the cell's membrane system - the endoplasmic reticulum and the Golgi apparatus - and is then forwarded to the outside of the cell (Figure 2)[3].

Coronavirus viral RNA is single stranded with a positive polarity (i.e., coding sense). For this reason, if by means of artificial physical or chemical processes (called transfection), the viral nucleic acid is placed inside a susceptible cell, a replicative cycle begins with the production of infectious viruses (revised by Modrow et al, 2013[2]).

S proteins assemble into trimers, forming a tulip-like structure. The base of the stem is inserted in the envelope and the flower projects outwards. Recently, the existence of three articulation zones along the trimer (a foot, knee and hip) was discovered. This gives the S protein greater flexibility which favours the recognition of cellular receptors[4].

1.4. WHERE AND HOW DO CORONAVIRUSES MULTIPLY?

Like any other viruses, coronaviruses are intracellular parasites which depend on the host to replicate. Upon entering a target cell, they recruit the cellular machinery to produce new viral particles that are released and, in turn, able to infect other healthy cells, thus re-starting the replicative cycle. In general, the host does not benefit from this infection but rather suffers injury when the resulting viral disease develops. Throughout the course of the infection, the extent of physiological injury and symptomatic presentation depends on several factors, including the host's responsiveness to invasion. However, the virus can only continue replication and perpetuation in a living host and is, therefore, also dependent on its survival. Thus, the potential for transmission is mainly associated with low virulence. That is why, usually, they are not particularly lethal but instead cause mild or subclinical infections (without symptoms i.e., asymptomatic), as is the case with common colds. Low virulence is, in itself, an adaptive advantage of coronaviruses: the parasite does not sacrifice its host, thus promoting large scale replication and propagation. The new SARS-CoV-2 coronavirus, however, has proved to be more aggressive than other known coronaviruses, probably because it is not yet adapted to its new human host. The same occurred with SARS-CoV (Severe acute respiratory syndrome-associated coronavirus) and MERS-CoV (Middle East respiratory syndrome-associated coronavirus).

The viral replication cycle starts when the S and HE proteins of the viral envelope (if present, as in the case of some betacoronaviruses, but not in SARS-CoV-2) are recognised by specific receptors on the surface of the host cell (Figure 3).

The SARS-CoV-2 virus, for example, recognises a compatible cell receptor found in lungs, blood vessels, heart and kidneys. This receptor, the Angiotensin-converting enzyme 2 (ACE-2), acts as the cell's "door lock", which is recognised by virus' "key", the S protein. In reality, there is no cell "door" to enter so the virus pushes itself into the outer cell membrane, which eventually envelops the virus as it is "swallowed" into the cell. This process is called endocytosis. Inside the vesicle formed by the cell surface membrane the viral envelope and the nucleocapsid are dissociated, releasing the genetic material, viral RNA, directly into the cell. The viral RNA will then serve as a template for the synthesis of the components that will make up new viruses through two different biochemical processes: the synthesis of new viral proteins and of new RNA molecules[8]. These two processes are called transcription and translation of viral proteins, and viral RNA replication, respectively.

The cell's ribosomes, small subcellular structures that viruses lack, are then hijacked for the translation of the information contained in the viral genome into proteins.

The replication of viral genetic material is one of the first tasks to be performed by the newly synthesised viral proteins like RNA dependent RNA polymerases (RdRP), capable of promoting RNA synthesis from a viral RNA template. The synthesis of RNA molecules from an RNA template is a process unique to some viruses, as it does not follow the logic of the Central Dogma of Biology, which states that genetic information flows from DNA to RNA, and from RNA into proteins.

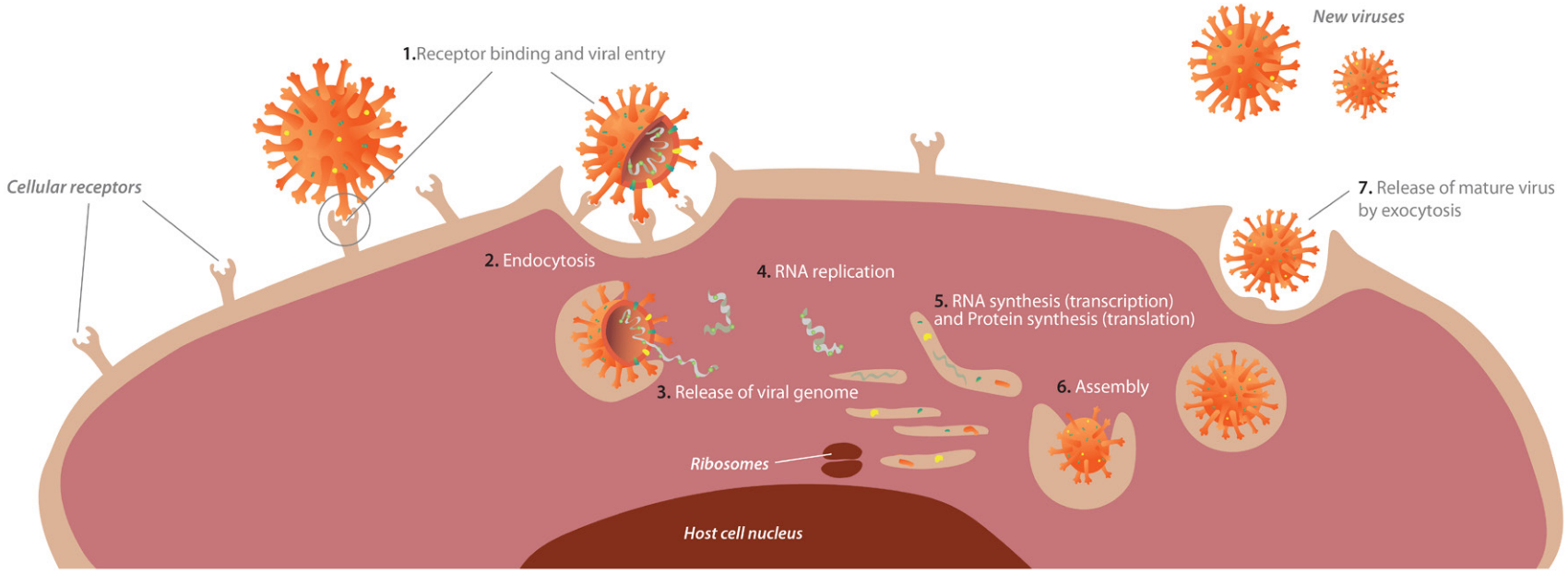


Fig. 3 - Phases of the replication cycle of coronaviruses: 1-Attachment of the virus to the cell receptor. 2-Endocytosis. 3-Digestion of viral proteins and release of viral RNA. 4-RNA replication.5- Synthesis of viral proteins. 6- Assembly of virions. 7-Release of viruses by exocytosis.

The viral proteins that will form the capsid, a structural protein shell enclosing genetic material, surround the new viral RNA molecule forming the nucleocapsid (core) of the new viral particle. The assembly and maturation of the viral particles occurs within the intracellular membrane system and involves the formation of an envelope and subsequent anchoring of viral transmembrane proteins (S and HE). The viral particles are then exported to the cell surface inside vesicles and are expelled to the outside of the cell by the process of exocytosis producing a very high number of new enveloped viruses. Due to this dependence on the cellular system, viruses are referred to as “obligate intracellular parasites” as they hijack the cell that they infect, transforming it into a factory for the mass production of new viruses and in doing so interrupt the processes inherent to the normal cell function. A single virus can take just 10 hours to infect a cell and can produce thousands of new viral particles in only 24 hours. This is where the expression “go viral” originated – it refers to something that quickly spreads everywhere.

How many viral particles are produced in a single cell?

It is estimated that, like other coronaviruses such as the hepatitis virus that infects mice (MHV), the burst size (the average number of newly synthesised virus particles released from a single infected cell), is around 10,000 virus particles[1].

The enzyme ACE2 (angiotensin-converting enzyme[2]), is homologous to ACE which is responsible for the blood pressure regulation through the Renin-Angiotensin System. In addition to the epithelial cells of the lungs, intestines, kidneys, and blood vessels, ACE2 is also present on the surface of the cells in the seminiferous tubules of the testicles. Viral infection can therefore damage the testicular tissue of patients and compromise fertility. SARS-CoV-2 uses this cell receptor to infect cells. However, other coronaviruses use different receptors, such as the MERS-CoV that uses DPP4 (dipeptidylpeptidase[4]) and HCoV-229E, TGEV, PEDV, CCOV that use APN (aminopeptidase N) as cellular receptor, mediating the virus entry into the host cells[5]. The binding of the S protein to the cell receptor is driven by electrostatic interactions.

2. CORONAVIRUS INFECTION

2.1. THE CORONAVIRUSES' CAPACITY FOR EVOLUTION

As we have already seen, infection caused by most coronaviruses depends on the specific recognition of a given host species' target cell receptors (animal or human) (Figure 4). Coronaviruses are, therefore, considered specific to their host.

Which cells are infected by coronaviruses?
Different coronaviruses are capable of infecting different cells, although these are usually cells of the respiratory or digestive tracts. In the case of COVID-19, SARS-CoV-2 infects cells that have receptors for the ACE-2 enzyme, which are compatible with the viral S protein. These cells are found in intestinal and respiratory epithelia, vascular endothelium, heart, kidneys and testicles. The ACE-2 receptor is present on the plasma membrane of all these cells. In the lungs, its concentration increases with age, contributing for the more severe presentation of the disease in the elderly.

What determines cell tropism and host spectrum?
The variation in cell tropism and the range of affected hosts is mainly attributed to the characteristics of the S protein, responsible for the attachment to the host cell.

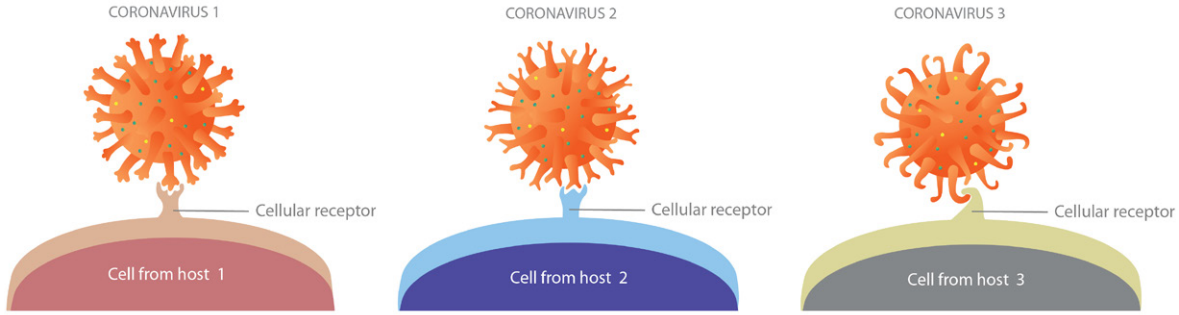


Fig. 4 - Binding specificity between the Coronavirus S protein and the host cell receptor.

What is the mutation rate and evolution of SARS-COV-2?
The estimated evolution rate for SARS-CoV-2 is 0.001 nucleotide per year and the mutation rate is of 0.000001 nucleotide per replicative cycle / viral replication cycle.

Why do new coronaviruses emerge periodically and what favours these appearances?
Coronaviruses, like other viruses, evolve rapidly in an inevitable process that cannot be controlled by humans. The close proximity between humans and animal species from distant natural habitats, the artificial confluence of animal species, the consumption of exotic species and the absence of sanitary control, favour contact between animals and humans, which may result in the emergence of new potentially pathogenic viruses.

The long RNA genome of coronaviruses, combined with a reduced ability to correct errors in the synthesis of new RNA molecules, resulting in genetic mutations, contributes to the diversification and evolution of their genomes over time (Figure 5A). Viruses with unfavourable mutations are at an adaptive disadvantage and tend to disappear in the viral population. Those whose mutations make them more apt will tend to increase in number and settle in their host populations. So, continues the constant perpetuation of the variants that remain at an evolutionary advantage.

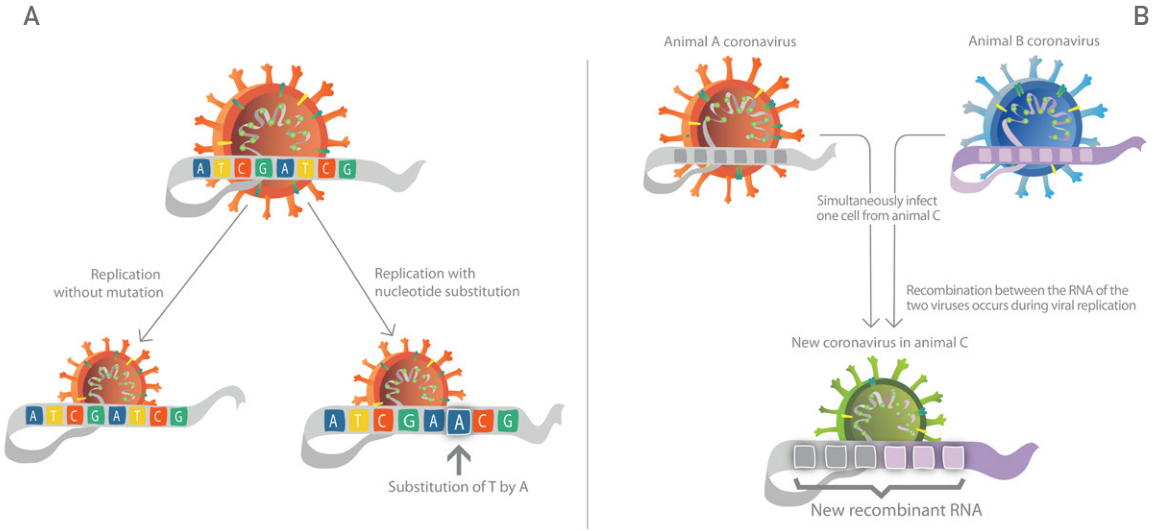


Fig. 5 - Genetic variability in the replication of the viral genome (A- genetic mutation by replacing a base; B - recombination by exchanging RNA fragments from two different viruses found in the same cell).

The viral RNA-dependent RNA polymerase (RdRP) enzyme is responsible for the synthesis of new RNA molecules and, unlike the homologous enzyme of animal and plant cells (DNA polymerase), is more prone to errors during the replication of the viral genome. Viral RdRP lacks the ability to review and correct nucleotides incorporation errors in the complementary strand of the template molecule, enabling the occurrence of genetic mutations at a very high rate. The low rate of correction during the replication process of the coronavirus genome is the result of a long evolutionary process that favours the viral genome diversification, which is essential for its adaptation and evolution. Due to the long length of the coronavirus genome, the accumulation of errors leads to an increasing diversity of the encoding proteins, and consequently, to an accelerated evolutionary rate[6,7].

If two different coronaviruses are simultaneously present in a single cell, during the synthesis of a new RNA molecule from a template molecule, dissociation of the RNA-RNA complex may occur, followed by re-association to the homologous template molecule of the other coronavirus genome, continuing its synthesis and thus generating a recombinant molecule[8]. This results in a recombinant virus.

On the other hand, genetically different coronaviruses can also appear through a process called recombination, which occurs when a cell is simultaneously infected by two different coronaviruses. The combination of genetic segments from different viruses results in new viral RNA sequences (Figure 5B). The molecular recombination process can lead to the production of new viruses with the potential to infect new hosts. The acquisition of this capacity occurs when structural changes in the S protein (the "key") allow coronaviruses to adapt and start recognising new receptors ("locks"), present in the cells of new hosts with which they come into contact with.

When the virus acquires the ability to infect a new animal species, this is described as "jumping the species barrier". In some reservoir host species (animal species that harbour the virus without suffering disease) such as bats, recombination between different coronaviruses occurs frequently[9] and may eventually lead to the genesis of a new viral variant capable of infecting new hosts, namely humans.

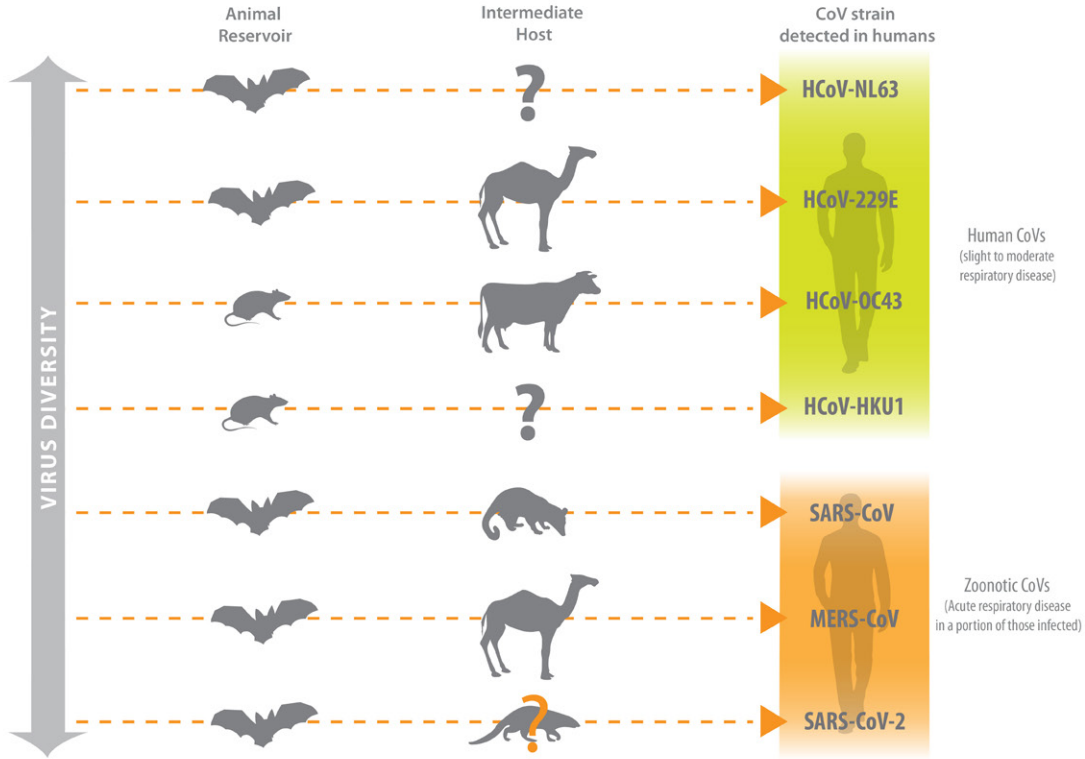


Fig. 6 - Transmission of Coronavirus between animal species and man. Most human coronaviruses originated from bat coronaviruses. Adapted from Cui, 2019.

Once the ability to infect different species has been acquired, the new virus can remain in circulation within susceptible host populations (Figure 6). Depending on the degree of contagiousness and population mobility, the viral spread to different geographic regions may be very fast.

2.2. CORONAVIRUSES' ABILITY TO INFECT A NEW SPECIES – JUMPING THE SPECIES BARRIER

Animals play an essential role in the transmission of infectious diseases to humans. These zoonotic diseases, or zoonoses, constitute about 60% of human infectious diseases reported globally and can be caused by bacteria, parasites, fungi or viruses. The emergence of an increasing number of zoonoses has highlighted the existence of risky practices that favour unrestricted contact between animals and humans. In nature, jumping the species barrier is a rare event, as it requires close and sometimes continuous contact between an infected animal species and individuals of a second susceptible species. With viruses, the jump to a human host often seems to require a third host species, called an "intermediate species," in which the virus possibly acquires the "new abilities" in order to be able to make the jump. In many cases, however, the true link between an animal and humans is never known.

Nonetheless, it is certain that the increase in interactions between humans and wild animals exponentially raises the risk of new zoonoses. These interactions may result from the human invasion of previously unexplored and preserved ecological niches, or from the forced approximation of different wild animal species and humans due to the loss of natural habitats. Scientists estimate that there may be about 800,000 unknown species of viruses in forests, swamps, caves and other unexplored areas of the world and that would have the potential to spread and infect people if there was a disturbance in the ecosystems.

Live meat markets, also known as "wet" markets, are notorious for bringing together a vast range of live animal species and humans, providing and promoting the increased proximity between animal species, that in natural habitats, would never come into contact. This, alongside the consumption of exotic or domestic animals without adequate sanitation and food safety checks, favours viral transmission between species and consequently recombination events between the different viruses that infect them. The animals held, slaughtered and sold in such markets often include bat species[10] where coronaviruses with remarkable genetic diversity are detected, which substantially increases the risk of successful genetic recombination and emergence of new viruses, enhancing the possibility of interspecies transmission.

The recognition of coronaviruses capable of causing serious infections in humans only occurred in 2003, when the outbreak of Severe Acute Respiratory Syndrome (SARS) first appeared in Asia and was attributed to SARS-CoV. The adaptation of the SARS-CoV virus (originating in bats of the genus *Rhinolophus*) to be able to infect humans involved the circulation through an intermediate species, the civet (*Paradoxurus hermaphroditus*).

The same happened later, in 2012, with the identification of another respiratory syndrome, the Middle East Respiratory Syndrome (MERS), this time caused by the coronavirus MERS-CoV, which also originated from coronaviruses found in bats (genus *Pipistrellus*). In this case, the identified intermediate species was the dromedary (*Camelus dromedarius*). The intermediate species of SARS-CoV-2, the causative agent of the current pandemic COVID-19, has not yet been clearly identified, although the Malaysian pangolin (*Manis javanica*), a mammal belonging to the order Pholidota of the family Manidae, has been considered a possibility[11].

Generally, the initial virulence of emerging pathogens tends to decrease over time, because of the host's immune response and the selection of less aggressive (less virulent) strains. Given the high genetic variability of respiratory coronaviruses, the immunity acquired by previous contacts does not protect hosts from new coronaviruses. This provides the virus with an adaptive advantage, allowing it to escape the hosts' immune system thus guaranteeing the permanent availability of susceptible hosts and the maintenance of the virus in the population.

This mechanism is an example of the coevolution of the virus and its host. Natural selection favours viruses (parasites) with different surface antigens (S and HE proteins), while the host's immune system is under selective pressure to produce a specific protective immune response effective for the specific virus present during infection. The accumulation of mutations and genetic variability on both sides "fuels" this evolutionary arms race.

What are intermediate species?
Intermediate species are species that allow the virus to adapt from one host organism to another. It is in intermediate species that different viral genomes undergo recombination, creating new viruses that are then transmitted to humans, through contact or consumption.

What are the sources of infection from coronaviruses?
The infectiousness of coronaviruses found in the environment (outside of a living organism) is very low due to the fragility of the viral particles, namely their outer shell, which consists essentially of lipids. For this reason, the biggest sources of infection are infected animals or humans.

Where did SARS-CoV-2 first appear?
The first clinical cases of SARS-CoV-2 infection were reported in December 2019, in inhabitants of the Wuhan province, in China. However, retrospective serological studies suggest that the virus was already in circulation prior to this[2].

What is the origin of SARS-CoV-2?
The origin of SARS-CoV-2 is not yet fully understood. Although the virus showed a high genomic similarity (96.3%) with a horseshoe bat coronavirus (Rhinolophus affinis), present in southwest China, this virus (Bat-CoV-RaTG13) does not have the same ligand (envelope protein) as SARS-CoV-2. Investigations carried out since the emergence of this pandemic have made it possible to identify a pangolin-malay coronavirus (Manis javanica), with a protein very similar to that of SARS-CoV-2. However, the genome of the pangolin coronavirus is not as similar to the SARS-CoV-2 genome (91.02%). It is hypothesised, therefore, that pangolins may be an intermediate species where a natural genetic recombination event between bat and pangolin coronaviruses may have occurred, resulting in a new coronavirus with the ability to infect human cells. This virus may then have come into contacted with humans through consumption of the infected animal. Alternatively, human infection by a viral variant of the bat coronavirus that did not persist in nature may have occurred. The initially harmless viral variant may have been transmitted between humans with a progressive increase in the virus virulence.

What is the importance of bats in the transmission of SARS-CoV-2 when compared to another species?
Bats have no particular feature, relating to their natural behaviour, that makes them more likely than other species to transmit pathogens to humans. However, the number and vast variety of species alongside higher genetic proximity to humans makes them key potential disease generators and vectors of infection.
Second only to the order Rodentia, which includes mice, rats, beavers, guinea pigs and squirrels, the order Chiroptera, to which bats belong, has the largest number of mammal species. There are around 1,400 different bat species all with the potential to transmit viruses. In fact, one in five of all mammalian species on Earth are bats. They are found throughout the world, except in Antarctica, occupying both urban and natural areas. While some species are found only in certain locations, others are widely distributed over large geographic areas. As mammals, bats have a closer evolutionary relationship with humans, making them more likely to host zoonotic agents than birds or reptiles. Due to the richest in number of bat species, they tend to host a greater variety of viruses and, consequently, the probability of some of these having a zoonotic potential is greater. Due to the diversity and vast geographic distribution of bats it is difficult to identify the group of bats from which SARS-CoV-2 may have originated.

Why do so many diseases emerge from bats?
Bats are hosts/reservoirs, not just of coronavirus, but also of many other viruses, including the Ebola virus, the rabies virus and the Marburg haemorrhagic fever virus. All of these viruses, including coronaviruses, confront bats in an evolutionary race. Viruses continuously evolve to escape the bats' immune system, and bats evolve to resist infections caused by viruses. With coronaviruses, this bilateral war effort translates into the production of a panoply of distinct viruses, increasing the probability that some of them will be able to infect another species, namely humans. Bats are also the only mammals capable of flying, facilitating the spread of disease through their faeces, the main excretion route for most viruses, which they can eject mid-flight. The bats' tolerance to viruses far exceeds that of other mammals, making them the main reservoir hosts for many viruses. Although bats do not develop symptomatic infections, they do still transmit infections. It is thought that the bats' natural resistance to infections is related to the release of a large amount of energy during the flight, increasing their body temperature to 38 to 41ºC, higher than the temperature of most mammals. The pathogens that evolved in bats are therefore able to withstand these high temperatures, thus easily resisting the increase in body temperature that our immune system uses as a mechanism to disable pathogens, the fever.

2.3. ANIMAL AND HUMAN DISEASES ASSOCIATED WITH CORONAVIRUSES

Coronaviruses primarily infect birds and mammals, causing a variety of diseases, having a serious economic impact on cattle, pig, equine, rabbit, chicken, ducks and turkey farms. They also affect pets such as dogs, cats and ferrets and various wild species such as minks. Alphacoronaviruses and betacoronaviruses are mainly found in mammals, such as bats, rodents, civets and humans. Gammacoronaviruses and deltacoronaviruses mainly infect birds, although some can also infect mammals.

Livestock and domestic animals are natural hosts for numerous coronaviruses. Among them are the coronaviruses specific to dogs, cats, ferrets, cattle, pigs and chickens and turkeys.

Dogs are infected with two distinct coronaviruses. The canine enteric coronavirus (CCoV) and the canine respiratory coronavirus (CRCoV). CCoV, which is genetically related to the feline coronavirus (FCoV) and to a porcine coronavirus (TGEV), infects animals, particularly puppies (*Canis lupus familiaris*) less than 1.5 months old, causing mild gastroenteritis. Foxes (Vulpes vulpes), raccoon-dogs (*Nyctereutes procyonoides*) and wild cats (*Felis silvestris silvestris*) are also susceptible to this coronavirus[9]. There are two genotypes of CCoV, type I and II. CCoV-II emerged due to different recombination events between the swine transmissible gastroenteritis virus (TGEV) and one or more unidentified coronavirus[12]. The taxonomy of the current variant of CCoV-II distinguishes CCoV-IIa from CCoV-IIb according to the genetic participation

The rapid evolution of the coronavirus genome can lead to specific structural changes in the S protein enabling the infection of a third host species by the newly emergent virus. The SARS-CoV-2 S protein shared a high similarity with the S protein of a coronavirus detected in the Malaysian pangolin, a small mammal belonging to the order Pholidota that lives in tropical areas of Asia. The remaining genome, however, have a lesser resemblance of only 90%. The SARS-CoV-2 virus RNA is more similar (96%) to the RNA of a coronavirus known to infect a bat (Rhinolophus) that inhabits caves in Yunnan, China, although its S protein (key) is not compatible with human cell receptors (lock) and thus unable to infect humans[9].

The development of feline infectious peritonitis (PIF) appears to be linked to the change of FCoV cell tropism from enterocytes (cells lining the intestinal tract) to monocytes/macrophages (types of white blood cells). The infected monocytes exhibit a particular affinity for the venous endothelium of the different serous membranes (omentum, pleura, meninges). Although the immune response against FCoV infections is preferentially cellular, when an intense humoral response is elicited this causes an exaggerated inflammatory reaction and the formation of oedema (effusive form of FIP). This is due to a type III hypersensitivity reaction that when triggered results in the formation of antigen-antibody complexes that accumulate in the vascular endothelium. The development of ascites (oedema of the abdominal cavity) is characteristic of FIP. However, cats that are able to develop a predominantly cellular immune response are more resistant to the development of the effusive form of PIF. In this "dry" form of PIF, animals mount a partially efficient cellular response in containing the organic spread of the virus, and are therefore able to restrict viral replication to some organs[10,11].

of TGEV in the virus genome. CCoV-IIa is also referred to as pantropic CCoV, due to its ability to induce a systemic disease in dogs that is not restricted to the gastrointestinal tract.

The usefulness of CCoV vaccination is controversial since the disease is generally mild and occurs before the recommended vaccination period.

The other canine coronavirus (CRCoV) has respiratory tropism and, when associated with other viral or bacterial pathogens, induces a highly contagious infectious disease in dogs known as "kennel cough"[13]. This disease has been identified in Europe, North America and Asia. CRCoV is closely related to bovine coronavirus (BCoV) and human HCoV-OC43. There is no vaccine for CRCoV.

Cats are susceptible to infection by several coronaviruses in addition to feline coronaviruses, including dog coronaviruses, porcine coronaviruses (TGEV) and human coronavirus (HCoV-229E and SARS-CoV-2). Feline coronavirus (FCoV) is highly contagious and its prevalence in high-density populations (e.g., catteries and cat shelters) is elevated. There are two serotypes, type I and type II, the latter resulting from a recombination event within the gene encoding the S protein between FCoV serotype I and CCoV. The vast majority of infections caused by feline coronavirus (FCoV) in cats are relatively harmless, ranging from subclinical forms to mild diarrhoea. However, about 5% to 10% of cats infected with FCoVs develop more serious infections, due to the change in the viral tropism to macrophages (cells differentiated from monocytes, a subpopulation of white blood cells) within the intestinal epithelium. In such cases, cats develop a frequently fatal systemic disease, characterised by a peritonitis named feline infectious peritonitis (FIP). The severity of FIP is associated with an exaggerated immune response given that the production of antibodies instead of conferring protection, increases the severity of the disease. The change in FCoV cell tropism is primarily due to viral factors, such as genetic mutations, but also to host-related factors, like the inability to mount an adequate cellular immune response. These two pathological types differ in cell tropism, clinical presentation and prognosis[14]. Since antibody-inducing vaccines are counterproductive to FIP, a vaccine for nasal administration was produced to stimulate mucosal IgAs, limiting the immune response to the upper respiratoty and gastrointestinal tracts. However, this vaccine was only protective when administered before

contact with FCoV, which is extremely difficult in areas where the disease is endemic.

Mink coronavirus (MCoV-1) and ferret coronavirus (FRCoV) are genetically related. MCoV-1 is responsible for catarrhal mink gastroenteritis that affects younger animals, causing high morbidity, but low mortality. FRCoV is the aetiologic agent of catarrhal epizootic enteritis, exhibiting, like FeCoV, two biotypes: i) enteric ferret coronavirus (FRECV), associated with a contagious diarrhoeal disease and ii) systemic ferret coronavirus (FRSCV) responsible for a systemic ferret disease similar to FIP in cats.

Bovine coronavirus (BCoV) was identified in 1973 in the USA and is responsible for three clinical syndromes of different severity in cattle, namely calf diarrhoea, winter dysentery (both gastrointestinal diseases) and bovine respiratory disease. These may or may not be associated with other bacterial or viral infectious agents. Since calves can acquire the infection in the first days of life, immune protection is achieved either by vaccinating the mothers during pregnancy, enabling transfer of passive immunity during colostrum ingestion, or by intranasal vaccination of the calves with attenuated vaccines in the first day of life.

BCoV is thus a paradigmatic example of the ability of coronaviruses to infect different hosts. The high genomic similarity (96%) of BCoV to the human coronavirus HCoV-OC43 suggests a distant previous episode of BCoV zoonotic transmission to humans. By analysing the molecular clock of these two viruses, the most recent common ancestor emerged around 1890[15] coinciding with a pandemic of respiratory disease in humans.

BCoV is not only related to HCoV-OC43, which causes digestive and respiratory disease in humans, but also to PHEV (Porcine haemagglutinating encephalomyelitis virus), horse coronavirus (ECoV) and canine respiratory coronavirus (CRCoV).

Pigs are susceptible hosts to six different coronaviruses that cause intestinal and respiratory disease. Of these, the swine transmissible gastroenteritis virus (TGEV), the porcine epidemic diarrhoea virus (PEDV), the swine acute diarrhoea syndrome virus (SADSV) and the swine deltacoronavirus (PDCoV) induce intestinal disease of differing severities in pigs. The detection of antibodies against TGEV in several species of wild and domestic carnivores, including dogs and cats, suggests that they are also susceptible to infection by this coronavirus. PEDV causes watery diarrhoea, vomiting, anorexia and dehydration in piglets less than 2 weeks old. For this reason, vaccination is widely practiced on pregnant sows to immunise piglets via colostrum. The porcine respiratory coronavirus (PRCoV) appeared after TGEV following genetic deletions in the gene that encodes the S protein in the enteric virus, resulting in the shift from enteric tropism to an almost exclusively respiratory tropism and transmission pattern[16]. PRCoV also has a high genetic similarity to CCoV and FCoV[17]. The swine deltacoronaviruses (PDCoV), like PEDV and SADSV, are agents of emerging diseases. The haemagglutinating encephalomyelitis virus (PHEV) usually causes vomiting and debilitating disease and/or encephalitis in neonate piglets.

Although several wild birds can contribute to the maintenance of the virus in the environment, the natural host of the infectious bronchitis virus (IBV, the first animal coronavirus to be identified), is the chicken. Infectious bronchitis is considered one of the most worrisome avian diseases due to its effect on mortality, animal growth and national and international trade. This coronavirus causes respiratory and renal disease, although it can also affect chickens' reproductive tract. Like other remaining coronaviruses, IBV has a rapid capacity for dissemination and genetic change due to frequent episodes of mutation and recombination. Inactivated, attenuated and recombinant vaccines for IBV are currently available. Inactivated vaccines, as in many other diseases, induce a weak antibody-mediated immune response, requiring multiple vaccine doses. The modified live vaccines containing the most common strains are administered in the drinking water or through sprayers, where ingestion occurs during feather preening (grooming) and are administered on the first days to week of life. Revaccination depends on the animals' average life span. The turkey coronavirus (TCoV) is genetically very similar to IBV although it is considered a new viral species[18].

VIRUS	Abbreviation	Group	Type of disease
DOMESTIC ANIMAL VIRUSES			
Transmissible gastroenteritis virus	TGEV	α-CoVs	Enteric
Porcine respiratory coronavirus	PRCoV	α-CoVs	Respiratory
Porcine epidemic diarrhoea virus	PEDV	α-CoVs	Enteric
Porcine haemagglutinating encephalomyelitis virus	HEV	β-CoVs	Enteric and neurologic
Bovine coronavirus	BCoV	β-CoVs	Enteric and respiratory
Equine coronavirus	ECoV	β-CoVs	Enteric
Infectious Bronchitis virus	IBV	γ-CoVs	Respiratory
Turkey coronavirus	TCoV	γ-CoVs	Enteric
Porcine coronavirus HKU15	PorCoV HKU15	δ-CoVs	Enteric
Canine coronavirus	CCoV	δ-CoVs	Enteric
Canine respiratory coronavirus	CRCoV	β-CoVs	Respiratory
Feline coronavirus	FeCoV	α-CoVs	Enteric and systemic (peritonitis)
WILD ANIMAL VIRUSES			
Hipposideros bat coronavirus HKU10	Hi-BatCoV HKU10	α-CoVs	Asymptomatic

Miniopterus bat coronavirus 1A	Mi-BatCoV 1A	α-CoVs	Asymptomatic
Miniopterus bat coronavirus 1B	Mi-BatCoV 1B	α-CoVs	Asymptomatic
Miniopterus bat coronavirus HKU	Mi-BatCoV HK	α-CoVs	Asymptomatic
Miniopterus bat coronavirus HKU8	Mi-BatCoV HKU8	α-CoVs	Asymptomatic
Myotis bat coronavirus HKU6	My-BatCoV HKU6	α-CoVs	Asymptomatic
Rhinolophus bat coronavirus HKU2	Rh-BatCoV HKU2	α-CoVs	Asymptomatic
Rousettus bat coronavirus HKU10	Ro-BatCoV HKU10	α-CoVs	Asymptomatic
Scotophilus bat coronavirus 512	Sc-BatCoV 512	α-CoVs	Asymptomatic
Rousettus bat coronavirus HKU9	Ro-BatCoV HKU9	β-CoV	Asymptomatic
Murine Hepatitis Virus	MHV	β-CoVs	Hepatic; Enteric; Renal; Encephalic
SARS-related palm civet coronavirus	SARSr-CiCoV	β-CoVs	unknown
SARS related Rhinolophus bat coronavirus	SARSr-Rh-BatCoV HKU3	β-CoVs	Asymptomatic
Sable antelope coronavirus	Antelope CoV	β-CoVs	Unknown
Neoromicia capensis bat coronavirus	NeoCoV	β-CoVs	Unknown
Dromedary camel coronavirus UAE- HKU23	DcCoV UAE-HKU23	β-CoVs	Unknown
European hedgehog coronavirus	ErinaceousCoV	β-CoVs	Unknown
Mink coronavirus 1	MCoV-1	α-CoVs	Enteric
Ferret coronavirus*	FRCoV	α-CoVs	Enteric
Bottlenose dolphin CoV (BdCoV) HKU22	BdCoV HKU22	γ-CoVs	unknown
Beluga Whale coronavirus SW1	BWCoV SW1	γ-CoVs	unknown
Bulbul coronavirus HKU11	BuCoV HKU11	δ-CoVs	unknown
Common moorhen coronavirus HKU21	CMCoV HKU21	δ-CoVs	unknown
Magpie robin coronavirus HKU18	MRCoV HKU18	δ-CoVs	unknown
Munia coronavirus HKU13	MuCoV HKU13	δ-CoVs	unknown
Night heron coronavirus	NH HKU19	δ-CoVs	unknown

Sparrow coronavirus HKU17	SpCoV HKU17	δ-CoVs	unknown
Thrush coronavirus KHU12	ThCoV HKU12	δ-CoVs	unknown
White-eye coronavirus HKU16			
Wigeon coronavirus HKU20	WiCoV HKU20	δ-CoVs	unknown
HUMAN VIRUSES	HCoV-229E	α-CoVs	Mild respiratory disease of the upper respiratory tract. In the elderly and immunocompromised pneumonia may occur
Human coronavirus NL63	HCoV-NL63	α-CoVs	Mild respiratory disease of the upper respiratory tract. In the elderly and immunocompromised pneumonia may occur
Human coronavirus 229E	HCoV-229E	α-CoVs	Mild respiratory disease of the upper respiratory tract. In the elderly and immunocompromised pneumonia may occur
Human enteric coronavirus 4408	HCoV-4408	β-CoVs	Mild to moderate enteric disease in most infected people
Human coronavirus OC43	HCoV-OC43	β-CoVs	Mild respiratory disease of the upper respiratory tract. In the elderly and immunocompromised pneumonia may occur
Human coronavirus HKU1	HCoV-HKU1	β-CoVs	Mild respiratory disease of the upper respiratory tract. In the elderly and immunocompromised pneumonia may occur
Severe acute respiratory syndrome coronavirus	SARS-CoV	β-CoVs	Acute respiratory disease in part of the infected
Middle East Respiratory Syndrome Coronavirus	MERS-CoV	β-CoVs	Acute respiratory disease in part of the infected
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2	β-CoVs	Acute respiratory disease in part of the infected

α-CoVs-alphacoronavirus; β-CoVs-betacoronavirus; δ-CoVs-deltacoronavirus; γ-CoVs-gammacoronavirus

**Considered a domestic species*

The close genetic relationship between coronaviruses of different animal species not only reframes their evolutionary histories (in understanding, for example, that BCoV evolved from a mouse coronavirus), but also reveals some coronaviruses' ability (such as TGEV) to overcome the species barrier and infect several species (Figure 7). This is likely due to the dynamic interactions with its hosts and efficacy of coronaviruses. The recognition of coronavirus reservoir species has actively grown since the identification of SARS-CoV and MERS-CoV[19], with emphasis on different bat species. Very recently, in 2020, six new coronaviruses, unrelated to SARS, MERS or COVID-19, have been identified in bats captured in Myanmar (Southeast Asia).

Although numerous coronaviruses of domestic and wild animal species have been identified, many of the infections they cause are subclinical, taking place without the appearance of clinical symptoms or signs, and therefore go unnoticed.

The most frequent diseases caused by coronaviruses in humans are the common colds[20]. Several coronaviruses circulate in the world population

causing mild upper respiratory tract infections (HCoV-229E[21], HCoV-HKU1[22], HCoV-NL63 and HCoV-OC43[23]). Less often, these same coronaviruses can also infect the lower respiratory tract causing bronchopneumonia and pneumonias.

Contrarily, other coronaviruses such as SARS, MERS and COVID-19 cause acute respiratory syndromes in humans.

Although coronavirus infections most commonly cause respiratory and/or gastrointestinal diseases, some coronaviruses can also cause reproductive diseases, polyserositis (inflammation of serous membranes), sialodacryoadenitis (inflammation of the salivary and lacrimal glands), hepatitis (inflammation of the liver), encephalomyelitis and nephritis (inflammation of the kidneys), in both humans and animals.

2.4. CORONAVIRUSES AND THE ONE HEALTH APPROACH

The concept of One Health recognises the existence of a close link between animal health, human health and the environment. It is based on multisector and transdisciplinary collaboration between professionals from different but interconnected areas relating to human, animal and environmental health, with the aim to control and prevent zoonotic infectious diseases. The need to adopt this approach has been widely emphasised in the context of the COVID-19 pandemic due to the challenges it has posed to public health and the world economy. The situation, which is thought to originate in the market in Wuhan, China, at the end of 2019, was emblematic of how zoonotic diseases can easily emerge and quickly spread at a global level.

The close contact between people and exotic animal species and consequent interspecies disease transmission has had catastrophic consequences. This is a real opportunity to change the relationship between humans and Nature in order to mitigate the risk of future disease outbreaks and further deaths. Assessment of potential interspecies transmission (zoonotic risk) and the development of predictive models for emerging viral diseases alongside specific surveillance and monitoring programmes may allow us to anticipate new epidemics and implement rapid preventative or contingency action.

2.5. TRANSMISSION OF SARS-COV-2

Transmission of SARS-CoV-2 occurs by dissemination of respiratory droplets containing the virus, which are produced by coughing, sneezing and even speaking. These droplets may be inhaled or land on mucous membranes (in the mouth, nose or eyes) of nearby people, spreading the virus and consequently the infection[25]. Additionally, when viruses land on nearby inert surfaces these become contaminated. The transmission potential of

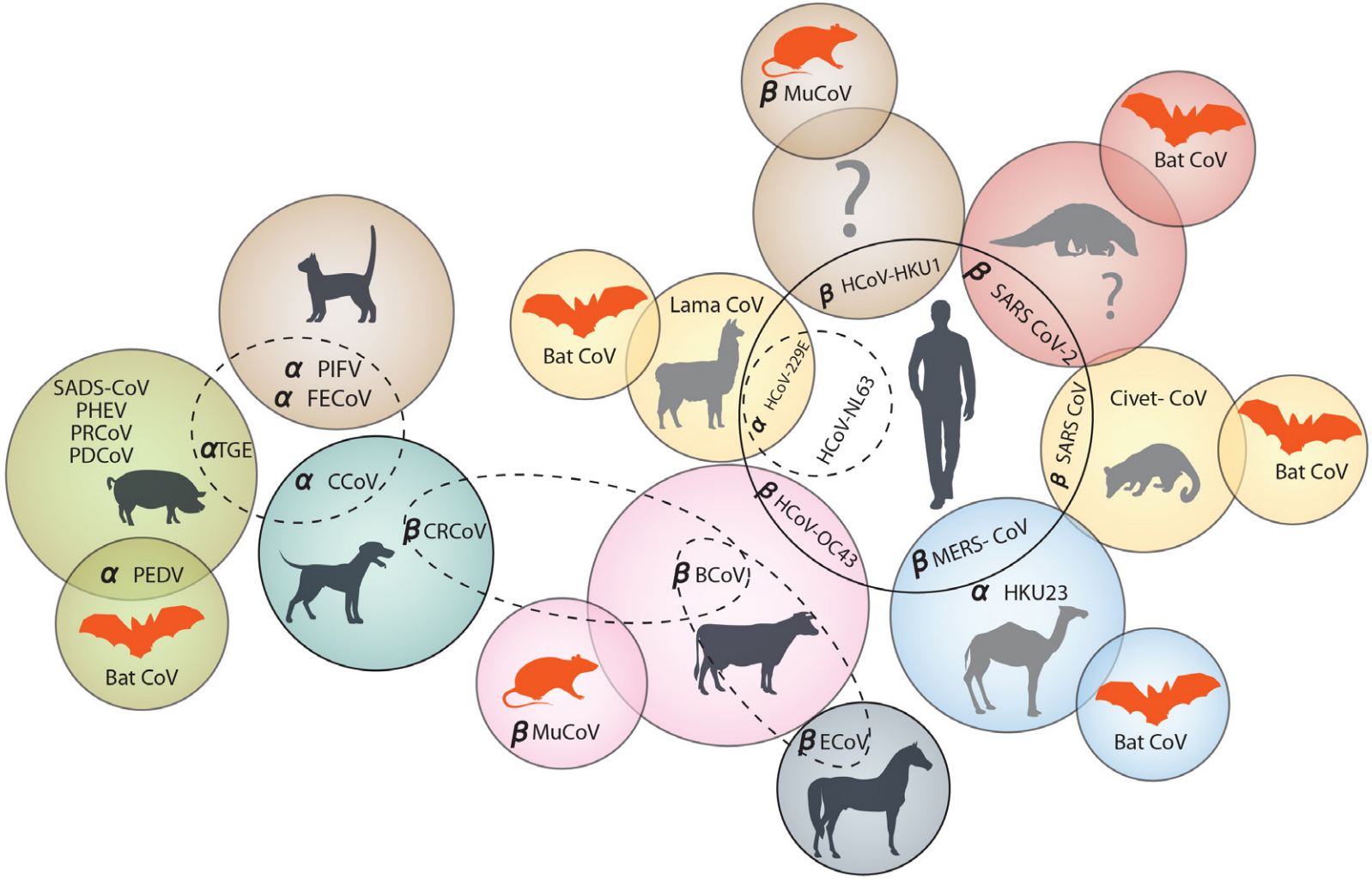


Fig.7 - Illustrative diagram of the chains of transmission of bat and rodent (reservoir hosts, represented in orange) coronaviruses to susceptible hosts (represented in black), through intermediate species (represented in grey). Dotted circles group the coronaviruses of different animal species by close genetic relationships. Adapted from Duarte et al. 2020 [24].

fomites (materials likely to carry infection) is uncertain as it is not clear how long the virus remains active on different surfaces. Given their size, droplets suffer the rapid action of gravity, so their range is relatively short (2 meters). However, under particular conditions such as pressure, high temperature and reduced humidity, droplets can be divided into much smaller particles, called "aerosols". Due to their smaller size, the propulsion force (like a cough or sneeze) is greater than the force of gravity, allowing aerosols to stay suspended in the air for longer and travel longer distances. In environments where there is close and prolonged contact with COVID-19 positive patients undergoing an aerosol-generating procedure (e.g., invasive or non-invasive ventilators, bronchoscopies) the risk of transmission and infection is particularly high.

As with influenza, person-to-person transmission of SARS-CoV-2 frequently occurs before symptoms appear. The asymptomatic period in influenza is shorter, however, only 1 or 2 days. The incubation period of the SARS-CoV-2 infection is currently estimated at about 5 to 6 days, on average[26]. The peak infectiousness (i.e., time of maximum transmission) is still uncertain, as most studies measure viral loads (amount of virus, in this particular case, present in tissues) only after the presentation of symptoms and estimate the peak through extrapolation using mathematical models. However, we know that a large proportion of infections are transmitted during this incubation phase, before symptoms have developed and, therefore, before a person becomes aware that they are infected. Additionally, a significant number of people infected with SARS-CoV-2 never actually develop symptoms. They are commonly called the "asymptomatic carriers", and if they are not identified and quarantined, they can transmit the virus throughout the entirety of the course of the infection.

2.6. CLINICAL SIGNS AND SYMPTOMS OF SARS-COV-2 INFECTION

SARS-CoV-2 characteristically infects upper and lower respiratory tract cells. However, it also infects other cells, resulting in the development of different clinical conditions.

The clinical progression of COVID-19 depends on several factors, including the amount of inhaled viral particles, i.e., the infectious dose to which the person is exposed. If the host is exposed to few viral particles (i.e., at a low infectious dose), the cilia in the cells of the nasal and larynx fossae may

In COVID-19 patients, the lower respiratory tract infection may develop into interstitial pneumonia. In these cases, the tissue and space around the pulmonary alveoli (interstitial space), which includes the alveolar epithelium, the pulmonary capillary endothelium, the basal membrane, and perivascular and perilymphatic tissues) is infected by SARS-CoV-2. The inflammation caused by the host immune response, alongside cell apoptosis (which occurs when viral particles are released from the alveolar cells) result in diffuse alveolar damage. Attempts at healing the damage result in fibrous scarring within the alveolar and interstitial spaces. This compromises the effectiveness of gas exchange and lung elasticity[12].

A delayed unregulated cytokine storm may be responsible for sudden deterioration of COVID-19 patients which appear to be making good recovery. This often occurs between day 7 and 10, when the immune response to the infection is higher[13, 14].

eventually be able to "sweep" them and surround them in mucus. By inactivating the virus and eliminating it the infection remains localised and controlled, resulting only in the development of a cough and/or disturbance of smell and taste, without spreading to the lower respiratory tract. If the infectious dose is high[27], however, or if the infection spreads to neighbouring tissues, the virus may be able to reach the lungs and induce severe interstitial pneumonia, compromising respiratory function. Further spread beyond the lungs can result in a systemic infection which can lead to multiple organ failure.

The diversity of clinical presentations of SARS-CoV-2 infection is due to different factors (Figure 8): the infectious dose an individual is exposed to; the strength of the immune response (diminished with aging and the existence of comorbidities); past exposure to other infectious agents that strengthened non-specific immune defences[28].

The many symptoms and signs associated with COVID-19 include fever, cough, shortness of breath (dyspnoea), headache, muscle and body aches (myalgia), chills, change or loss of smell and taste, sore throat, hoarseness, nasal congestion, nausea or vomiting, diarrhoea, and neurological complications.

An excessive immune response is associated with severe cases of SARS-CoV-2. The cytokine storm triggered by the infection induces severe inflammation which may lead to acute respiratory distress syndrome, coagulation and multi-organ dysfunction. It has also been linked to fibrotic responses resulting in long-term lung damage.

Children are generally more resistant to the disease than adults, often developing asymptomatic infections. However, cases of multisystemic syndrome in children (MIS-C) have been identified in association with SARS-CoV-2[29].

2.7. IMPACT OF COVID-19 ON PUBLIC HEALTH

The impact of COVID-19 in the first 19 months since it was identified has been more than 4 million deaths worldwide[31,32]. Amongst the individuals who have recovered from the infection, estimated at more than 107 million in June 2021, different sequelae have been identified (e.g., neurological, pulmonary, endocrine).

The speed with which SARS-CoV-2 spread across 213 countries reflects the current globalisation and intensity of international transit. However, there have been other epidemics in the recent past which have also had a huge global impact, such as the H1N1 influenza virus. During the first year of circulation since its emergence in 2009 in Mexico the H1N1 virus caused the death of an estimated 151,700 to 575,400 people worldwide and affected more than 60 million people in the USA alone, according to CDC (Centers for Disease Control and Prevention).

What is the transmission rate of COVID-19?

The rate of transmission of a virus is measured through the "R0" or "basic number of reproduction". It is the average number of people that an infected person will transmit the infection to, in a scenario where everyone is susceptible to the virus and no control measures have been implemented.
In mathematical terms, R0=1 means that each infected individual will infect one other person. When R0 is greater than 1 (R0>1) there is an exponential increase in the number infected individuals, culminating in an epidemic.
Throughout the course of the COVID-19 pandemic understanding the trends and trajectories of the R0 will be more useful that just looking at the R0 as a static measure. This is known as Rt (R0 in time, or contagion speed in time). National health services seek to know how this speed is changed weekly, to assess the effectiveness of local or national infection prevention measures and evaluate the impact of relaxing such measures, although it may take at least two weeks 3, 4 for any change in the Rt value to be observed.

What are the most frequent signs and symptoms of COVID-19?

Fever, cough and fatigue are referred to as the most frequent symptoms. However, there is a significant variation in the presence of these symptoms between individuals and across different countries. For example, a meta-analysis of 148 studies from 9 countries found that, although fever is the most prevalent symptom overall, reported in 78% of all COVID-19-infected patients included in this study, it was only reported in 32% of patients in Korea whereas in Singapore 83% of patients described these symptoms. This is reflected by variations in the 'main' COVID-19 symptoms published across different countries. While in Portugal the warning signs are fever, cough and respiratory difficulty, in the UK they are fever, cough and altered taste and smell. It is also expected that the most frequent signs and symptoms change as the virus infects different age groups and they develop some immunity. Mass immunity will create selective pressure on the virus, resulting in the appearance of strains with different characteristics.

Does COVID-19 also affect different age groups?

Children seem less affected than adults and usually either develop mild conditions or are asymptomatic. The elderly are particularly sensitive often developing more severe clinical conditions. This is attributed to the greater number of ACE2 receptors, which increase with age, the more frequent presence of comorbidities and the reduced strength of the immune system. The mortality rate is also directly proportional to age. However, a multisystemic inflammatory syndrome in children (MIS-C) [5] has been identified in children who have either been infected with SARS-CoV2 or that have been in contact with someone who has. Potentially lethal, this syndrome is characterised by multi-organ generalised inflammation (including heart, lungs, kidneys, brain, skin, eyes or gastrointestinal organs). The presentation and symptoms overlap with those from of other rare paediatric diseases, such as Kawasaki disease shock syndrome (KDSS)[6]

Why is the disease serious in some people and mild in others?

Several factors contribute to the severity of a person's clinical condition. In addition to age, the existence of pulmonary pathology such as chronic obstructive disease, asthma, or emphysema that weaken the lungs and favour the progression of infection, diabetes, cardiovascular pathology, hypertension, and obesity are some of the additional risk factors. Other individual factors have been identified such as variations in the ACE2, TMPRSS2, HLA, CD147, MIF, IFNG and IL6 genes, which may account for the mortality of apparently healthy young and the notorious resistance of some old people[7]

Warning signs of multisystemic inflammatory syndrome in children (MIS-C) include: fever (>38°C) for 24 hours or more, abdominal pain, diarrhoea or vomiting, neck pain, rash or changes in skin colour, redness of the eyes, tiredness, difficulty breathing, chest pain or pressure, confusion, inability to stay awake or wake up, bluish lips or face[15].

Very recently, a study conducted in the UK compared the genome of more than 2,000 people who developed severe forms of COVID-19 with respiratory failure, with the genome of people who developed mild forms. Consequently, genetic variants in the population associated with severe forms of disease were identified. These variants were mapped on chromosomes 12, 19 and 21, affecting the expression the tyrosine kinase 2 gene (TYK2), the dipeptidil peptidase 9 gene (DPP9), the IFNAR2 gene that codes for interferon and of a set of genes encoding antiviral restriction enzyme activators (OAS1, OAS2, OAS3). A causal link was found between the low IFNAR2 expression and high TYK2 expression and life-threatening forms of COVID pneumonitis in COVID-19 patients. These findings highlighted new possibilities for the development of personalised therapies targeting a patient's genetic code to adjust their immune response and prevent disease progression[12].

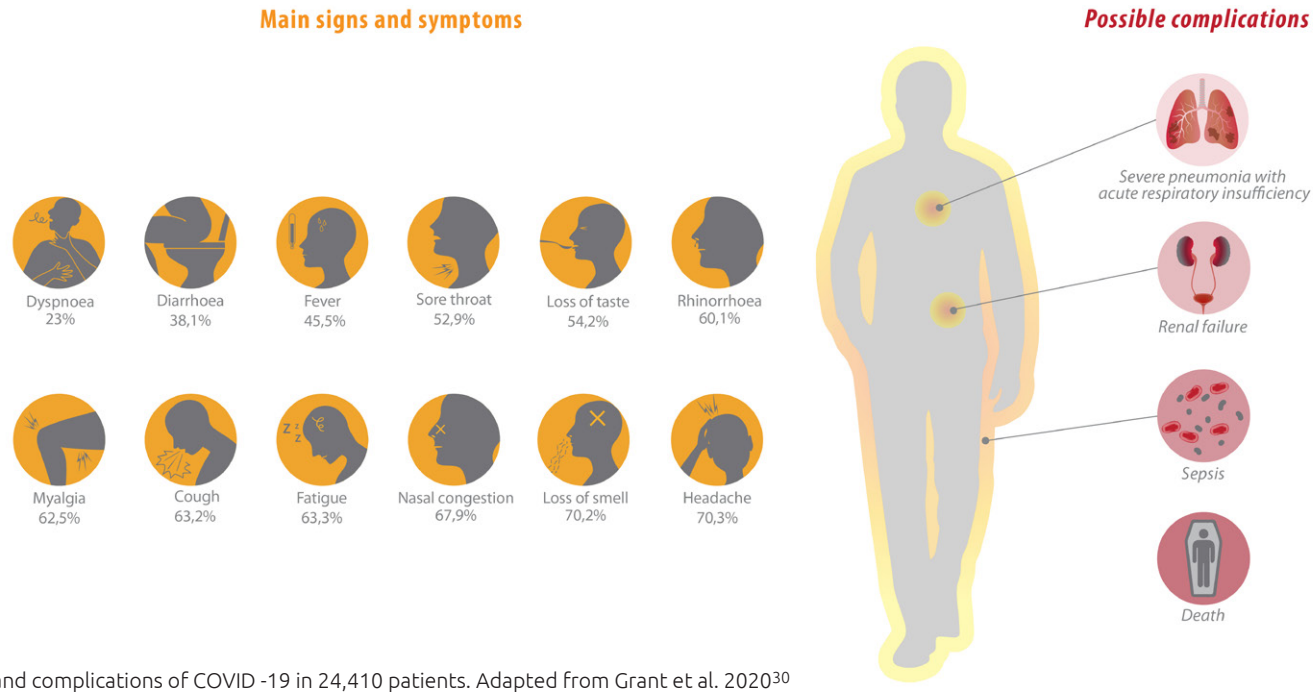


Fig. 8 - Presentation and complications of COVID -19 in 24,410 patients. Adapted from Grant et al. 2020[30]

What is the incubation period (between infection and the onset of symptoms) of COVID-19?

The average time between exposure to the virus and the development of symptoms is 5 days, but can last up to 14 days[8]. For this reason, the current quarantine or isolation period is of 14 days (the time required for the incubation period and symptomatic phase of disease to elapse). However, some studies have described a longer period of incubation in some people.

At what stage of the SARS-CoV-2 infection may transmission to others occur?

The majority of transmissions occurs before the onset of symptoms (60%)[9]. The potential for transmission continues throughout the course of the disease. The presence of viral RNA has been detected more than 20 days after the onset of symptoms, particularly in the intestinal tract. This may suggest potential transmission even after symptom resolution. However, it is uncertain whether the presence of RNA correlates with infection[10], given the limitations mentioned in section 2.2.

How aggressive is COVID-19 to the general population?

Data suggest that 80% of SARS-CoV-2 infections are asymptomatic or mild (and therefore can be treated at home), 15% are severe infections (requiring hospitalisation for oxygen administration), and 5% are critical infections (requiring mechanical ventilation).

How do SARS-CoV-2 variants emerge?

Some mutations can impair the viruses' ability to replicate and/or pass from host to host, but viral variants with such detrimental mutations are quickly eliminated from the viral population. Occasionally, advantageous mutations occur, conferring the new variant with a greater capacity for replication, transmission and evasion from the host's immune system. These variants undergo positive selection and, therefore, become dominant over the other variants.

The impact of the subsequent waves of COVID-19 on the natural immunisation of the world population is still unknown. The small proportion of seropositive people (2-6%) following the first wave of disease[33,34] dissolved the initial expectation that group immunity could be rapidly established, which would have resulted in the progressive reduction of circulation of the virus and consequently its elimination. Given the low proportion of seropositive people following the first wave, the unknown duration of natural acquired immunity and the very high number of casualties that would have resulted from uncontrolled mass infection, efforts are now focused on vaccination programmes to control the pandemic.

Viral S protein, the most immunogenic because of its exposure from within the viral envelope, is one of the proteins that have been used to develop a vaccine against SARS-CoV-2. However, due to its susceptibility to genetic variation, resulting in changes to the structure and behaviour of the protein[35], vaccines will need to be adjusted to emerging variants, as is the case with annual influenza vaccines.

In fact, since its emergence in late 2019, a growing number of SARS-CoV-2 variants have been identified in the world. Some have replaced the initial variants very quickly, demonstrating their clear genetic advantage regarding transmission. These new variants have also contributed to the severity of the COVID-19 pandemic given an increase in transmissibility leads to increased deaths, in the absence of mass immunity. It is not yet clear if those variants are associated with more severe disease.

The **Alpha variant (B.1.1.7)**, also known as the Kent variant, was first detected in the UK at the end of 2020. It carried a large number of mutations, many in the spike protein encoding gene. This variant is substantially more infectious than other variants (with a 50% higher transmissibility rate [30-31]) probably due the N501Y mutation (which means that the amino acid N (Asparagine) in position 501 of the S protein was replaced to a Y (Tyrosine)). This amino acid substitution increases the binding strength of the virus to the ACE2 human receptor. By binding more easily and strongly, the number of viral particles necessary to infect a new person is much lower. Other mutations include the H69/V70 deletion[36], the 144Y deletion, and the N501Y, A570D, D614G and P681H amino acid substitutions. Although initially the variant was not associated with more severe COVID symptoms, recent data raises the possibility that infection with the Alpha variant may be associated with an increased risk of death compared with other variants[37,38]. It spread rapidly throughout the UK, and by mid-February 2021 had already been detected in 70 countries.

The **Beta variant (B.1.351)**, also known as the South African Variant, was detected in Nelson Mandela Bay Municipality, in October 2020, and emerged independently from the Alpha variant, and since then it has been detected in more than 30 countries. Similarly to what happened with the UK variant, the Beta variant quickly replaced other SARS-CoV-2 variants in South Africa. It shares some mutations with the Alpha variant[39], namely the N501Y mutation in the spike protein, but carries also the E484K and K417N mutations, which may reduce the binding of antibodies to the virus. Preliminary evidence suggests that the Moderna mRNA-1273 vaccine may be less effective against this variant[40]. There is no evidence, however, that it is more deadly than the original variants.

The **Gamma variant** (B.1.671.2 or P1), also known as Brazilian variant, was first detected in Japan in a group of Brazilian travellers in January 2021.

This variant has 17 unique mutations, including three in the receptor binding domain of the spike protein (N501Y, E484K and K417N)[41,42]. It's now highly prevalent in the Brazilian state of Amazonas. There is evidence to suggest that some of the mutations in the Gamma variant may affect the ability of antibodies (from natural infection or vaccination) to recognise and neutralise the virus[43], but additional studies are needed to confirm this.

The **Delta variant** (B 1.617.2), also known as the Indian variant, was first identified in the state of Maharashtra in India, in October 2020. This variant has since spread throughout India and more than 96 other countries. Alongside the alpha, beta and gamma variants, it is considered a variant of concern (VOC) by the WHO. The Delta variant has multiple mutations associated with the improvement of the virus' capacity to bind to human cells and escape some immune responses. More recently, a mutation of that variant has emerged, called "delta plus," which is starting to worry global experts.

Several other variants have been detected, such as the Kappa (From India) and Eta (from the UK) variants, among others. It is expected that SARS-CoV-2 will continue to evolve rapidly, through the natural selection of variants that escape the immune response induced by contact with previous variants and vaccines.

What do the UK, South African and Brazilian SARS-CoV-2 variants have in common?
One mutation, which leads to a D (aspartate) to G (glycine) amino acid substitution in the position 614 within the spike protein (D614G), is shared by the UK, South African and Brazilian variants. This mutation gives the variants the ability to spread more quickly than the predominant viruses[11] by enhancing the binding to human ACE2 receptors. This results in increased viral replication in primary human bronchial and nasal airway epithelial cultures and substantially increases replication and transmissibility in hamster and ferret models of SARS-CoV-2 infection[10].

What are the implications of new variant emergence on vaccination success?
Vaccine developers are evaluating the efficacy of their vaccines against the new SARS-CoV-2 variants. The currently licensed vaccines appear to offer relatively good protection against the UK variant, however, the protection the Novavax, Johnson & Johnson and Oxford/AstraZeneca vaccines appear to have reduced protection against the South African variant[12, 13]

Importance of monitoring new variants?
Full genome sequencing of the continuously emerging SARS-CoV-2 variants is paramount to monitor their spread, update the diagnostic techniques, adjust vaccines and to design effective anti-viral drugs.

How is group immunity evolving against SARS-CoV-2 worldwide?
The group (or herd) immunity is evolving very slowly, as indicated by the results of serological studies conducted in several countries. In Portugal, the presence of antibodies was found in about 3% of the 2,302 individuals in the representative sample of the population, a study conducted by the National Institute of Health (INSA I.P.) between May 21 and July 8, 2020; in Spain 9.3% of 578 health professionals tested between 28 of March to 9 April 2020 were seropositive; 4.8% of 2766 participants from Geneva tested between 6 and 9 May 2020 had antibodies; 3.8% of 2,857 blood donors in Brazil tested between April 14 and 27, 2020 were seropositive. Continuous or periodic evaluation of the population immunity is fundamental for a better understanding of herd immunity. However, it is still unclear how long this immunity lasts, and whether it will be effective against new strains of the virus.

3. DIAGNOSIS AND PREVENTIVE MEASURES OF CORONAVIRUS DISEASES

As with most of the other infectious diseases caused by animal coronaviruses the signs and symptoms of COVID-19 infection are nonspecific and, therefore, clinical cases require confirmation by diagnostic tests.

Laboratory diagnosis can be performed by direct and indirect methods. The former is based on the detection of some components of the virus (e.g., viral RNA) through molecular methods such as reverse transcription Polymerase Chain Reaction (RT-PCR) (Figure 9A), or other methodologies such as microarrays, Loop-mediated isothermal amplification (LAMP) and clustered regularly interspaced short palindromic repeats (CRISPR).

For the detection of other viral components such as proteins, ELISA and immunofluorescence techniques can also be used. Rapid Antigen tests (Lateral-flow device) are also available on the market for SARS-CoV-2. Studies carried out in several countries have shown that there is variation in the sensitivity and specificity of these tests. Direct observation of the virus by electron microscopy, or in cell culture through the observation of the morphology of the infected cells (cytopathogenic effect) are techniques that can also be used. However, these two techniques can be extremely laborious, time consuming and require specialised laboratories (e.g., Biosafety level 3 laboratories).

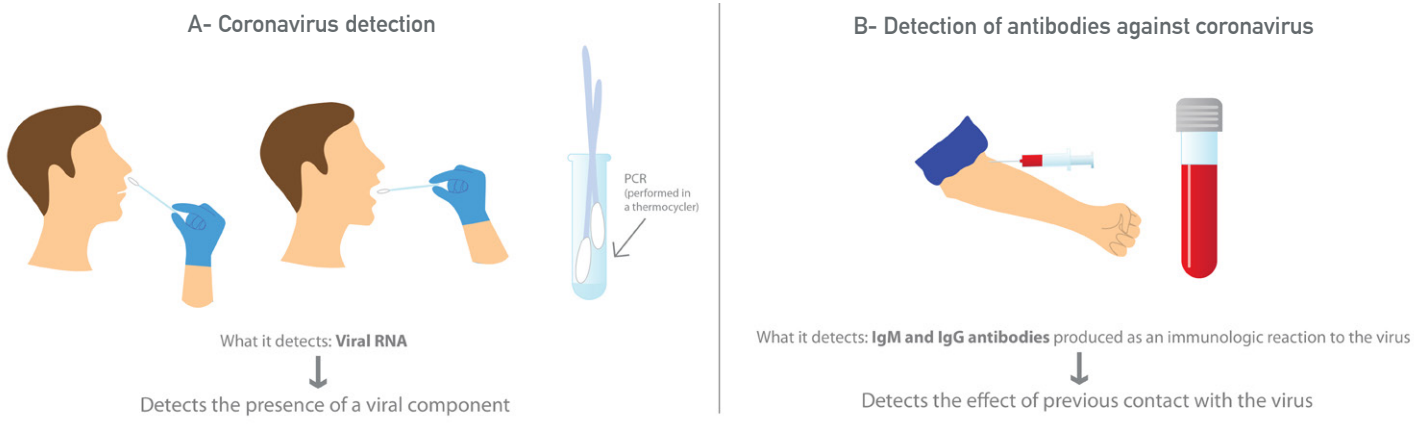


Fig. 9 A- The detection of the viral genome is done by real-time or conventional "RT-PCR" (often refered to as PCR), since it is an RNA virus. Detection of viral RNA, or one of the components of virus, indicates either active or recent SARS-CoV-2 infection. B- The detection of antibodies can be carried out by rapid methods or by immunoassays (ELISA, CLIA). The detection of antibodies indicates previous contact with the virus.

Indirect methods are based on the detection of antibodies that are produced in response to the viral infection, through a process called humoral immune response (Figure 9B). The choice of molecular and/or serological diagnostic tests is dependent on the stage of the disease and vaccination status. For example, serological tests are not recommended for the diagnosis of acute infections.

3.1. MOLECULAR DIAGNOSIS OF CORONAVIRUS INFECTIONS

Molecular methods, based on real-time RT-PCR are extremely sensitive and specific (i.e., they only detect the pathogen for which they were developed). They can be easily automated, allowing for the simultaneous analysis of many samples. The high sensitivity of molecular methods enables the detection of small amounts of virus in the sample (1 to 10 copies of viral RNA), which is the case at the beginning of the infection and would, therefore, be undetectable by other methods. The amplification of a single target molecule initially present in the sample to a thousand identical copies in the reaction tube results in specific, sensitive and rapid detection in a matter of hours.

To perform the molecular test, biological samples are taken depending on the tropism of the virus for the different tissues of the host. In coronavirus infections that are characterised by enteritis (FCoV, SuCoV, CCoV, BCoV), faeces are the specimens of choice for laboratory diagnosis. In respiratory coronavirus diseases, such as PRCoV, TCoV and SARS-CoV-2, exudates from the posterior areas of the nasal cavities (nasopharynx) and throat (oropharynx) collected using swabs (like a long cotton bud) are the specimens of choice for analysis. These fluids contain many host cells which will contain viral particles if the individual is infected. The genetic material (host and virus) present in these cells is then extracted to be tested by RT-qPCR. This extraction can be manual or automatic.

What is the difference between PCR and RT-PCR?
PCR (Polymerase Chain Reaction) amplifies a genomic target from a DNA molecule, whereas RT-PCR (Reverse Transcription – Polymerase Chain Reaction) uses RNA molecules as templates for the complementary DNA sequence, generated through reverse transcription, following which the target region can be amplified as with PCR.

What does a positive RT-PCR test for SARS-CoV-2 mean?
A positive test indicates that the person is (or has recently been) infected with SARS-CoV-2. RT-PCR detects viral RNA rather than viable infectious virus (which would require laborious attempts at isolating the virus in a cell culture), and therefore cannot distinguish between the presence of a viable replicating virus, representing an active infection, and inactive or non-viable viruses or viral particles, which would still be present some time after an infection has resolved. However, as a quantitative test the results may suggest which of these situations are more likely (you would expect high quantities of viral RNA to be detected if there is an active infection).

Is a negative RT-PCR test reliable?
It is estimated that up to 20% of patients with negative results have had a 'false-negative[14]. The timing of the test during the course of the infection, the quality of the sample collection and test sensitivity and specificity are crucial factors to ensure a reliable result. Where there is high clinical and epidemiological suspicion of infection, a single negative test, even one of high sensitivity, should not categorically exclude it. In those cases, the test should be repeated.

A positive result in RT-qPCR, indicates the presence of CoV-SARS-2 viral RNA, but does not distinguish between an active infection and an infection that has recently resolved (when the virus has been neutralised by the host's antibodies). However, since this method is quantitative, the longitudinal assessment of viral loads, determined by sequential tests, can reveal the progression of the disease.

3.2. SEROLOGICAL TESTS FOR CORONAVIRUS

Serological tests can detect specific antibodies (immunoglobulins) against coronaviruses present in blood samples. Currently, ELISA tests that detect antibodies against coronaviruses associated with infections in cats, dogs, pigs, cattle and birds are commercially available.

For SARS-CoV-2 multiple tests that are now widely available have already been developed based on different methodologies (Figure 10).

In immunocompetent individuals, exposure to the virus leads to the stimulation of an immune response, which includes the production of specific antibodies against the antigens present on the virus surface, in particular against the S protein. The antigens are viral components (e.g., proteins) that stimulate the production of antibodies.

The first type of antibodies (or immunoglobulins (Ig)) to be produced in the first contact with the virus are IgMs (Figure 10), which can last for more than a month. The presence of IgMs is indicative of recent infection. IgGs against SARS-CoV-2-usually appear 6 to 15 days after the onset of the disease. The production of IgG is generally detected later, and for longer. Serum levels (i.e., in the blood) can reach their peak 1 to 2 weeks after infection and then slowly decrease. It is also known from other coronavirus infections such as MERS that the levels of antibodies are also related

What does a positive serological test mean?
It means that the person has been in contact with the virus and has developed humoral immunity (i.e. it has produced antibodies). Most people infected with SARS-CoV-2, whether symptomatic or asymptomatic, produce antibodies between day 10 and 21 post-infection. However, in some cases, individuals can take more time to develop antibodies, which even may not be produced at all.

Does having antibodies mean that the person is protected from subsequent infections?
The protection conferred by the antibodies depends on their neutralising capacity and concentration (i.e., the antibodies titre in the blood). However, there is evidence that some people who recovered from COVID-19 and developed antibodies then contracted the infection again after a few months. This questions whether the immunity developed provides any protection for subsequent infections. The high rate of virus mutation, may be the reason of this immunity failure.

How long antibodies can last in the blood?
The longevity of the humoral response is still under investigation. A Canadian study indicates that after the onset of symptoms IgA and IgM antibodies are rapidly degraded, whilst IgG antibodies remain relatively stable for up to 150 days in the blood and saliva[15]. This suggests there is a possibility of suffering reinfection by the same virus.

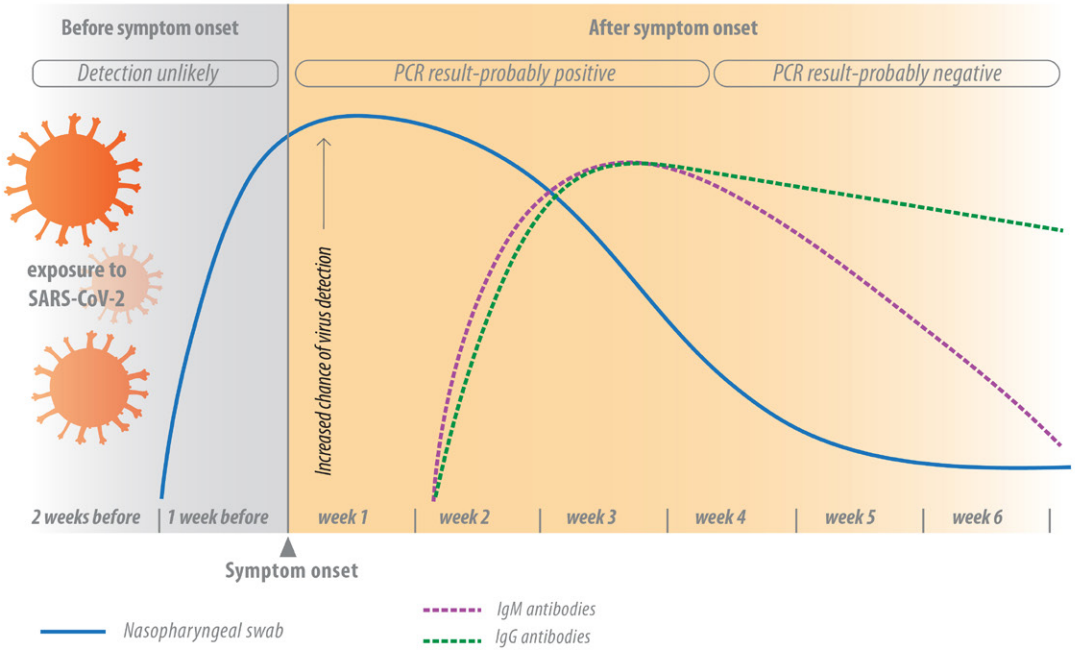


Fig. 10 - Effectiveness of diagnostic methods in detecting SARS-CoV-2 infection from contact to the onset and progression of symptoms. The curves represent the probability of detection of viral RNA (by RT-PCR) and antibodies (IgM and IgG, by ELISA) over time. Adapted from Sethuraman et al 2020[44].

to the severity of the disease. The level of antibodies may be lower and disappear more quickly in asymptomatic individuals. It is still unknown how long this immunity is protective for, but it is estimated that it may be around 6 to 12 months[39]. The World Health Organization (WHO) underlines that there is still no clear evidence that people recovered from COVID-19 are permanently immune and protected against reinfection. After a second contact with SARS-CoV-2, IgG titres can increase substantially.

Antibodies (total immunoglobulins, or their IgG, IgM and IgA classes) developed against SARS-CoV-2 antigens can be detected in whole blood, serum or plasma by conventional tests including immunoenzymatic assays (e.g ELISA), chemiluminescence assays (CLIA) or antibodies rapid immunochromatographic tests (Figure 11).

Rapid tests for which you only need to collect a drop of peripheral blood are quicker and allow you to obtain the result in a few minutes, but to date they are less sensitive and specific.

Immunochromatographic tests that use S protein as an antigen to detect antibodies appear to be more sensitive than those that use nucleoprotein (N).

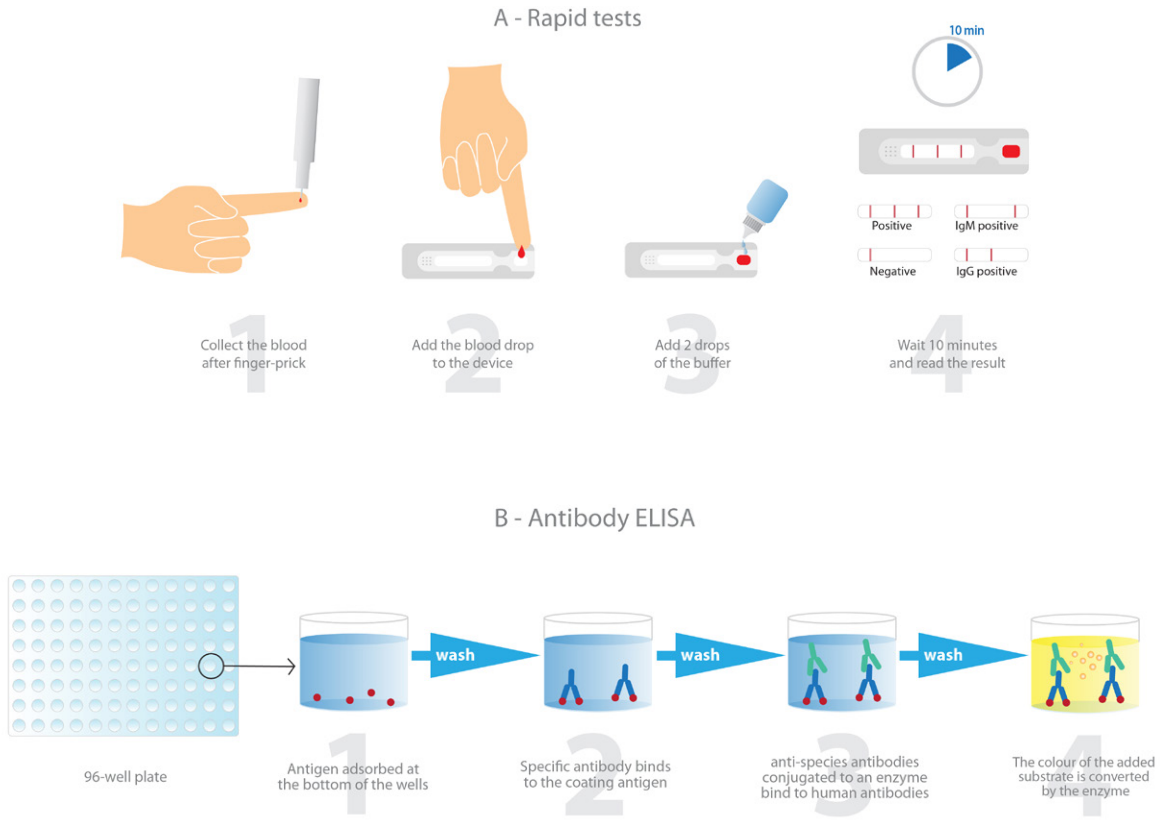


Fig. 11 - Example of tests that detect antibodies against SARS-CoV-2. A- Rapid test that requires a drop of blood used in a specific device. B-ELISA test, an immunoenzymatic assay performed in a laboratory, requiring specific equipment (incubator, plate washer, spectrophotometer).

Is immunity to other human coronaviruses also short?

The immunity conferred by the infection is variable. About 90% of patients infected with SARS-CoV have been shown to maintain IgG antibodies for two years, although this drops to 50% by three years[16]. Immunity to MERS-CoV is shorter. Following infection with the seasonal coronavirus HCoV-229E IgA antibodies are detected in the blood up to one year later[17]. IgA antibodies participate in local mucosal immunity within the nasal cavity by defending mucous membranes which are the gateway for many viruses[17].

The immune response against a pathogen is characterised by humoral and cellular response. The humoral response involves the production of immunoglobulins (antibodies) of different classes (IgM, IgG, IgA, IgD and IgE, which differ in the type of heavy chain found), whose function is to signal and fight infection. Due to differences in heavy chain polypeptides these immunoglobulins are involved in different types of immune responses and in different stages of infection. IgMs, IgGs and IgAs are particularly important in combating viral and bacterial infections. IgMs are produced at the beginning of the infection being very efficient in the initial phase of the infectious processes; IgGs appear after IgMs, usually in higher concentration and stay in the blood for longer. IgAs play an important role in the protection of intestinal, respiratory, and urogenital mucosal epithelia separating the outside environment from the inside of the body. Cellular immunity is associated with activation of specific immune cells (T lymphocytes), capable of identifying and eliminating the pathogen from infected host cells. The two responses (cellular and humoral) work together to eliminate the infection. Sometimes an exacerbation of the immune response process can itself be extremely harmful to the host[16].

3.3. MEASURES TO PREVENT DISEASES CAUSED BY CORONAVIRUSES

As with all other animal diseases, some effective tools for infection prevention include health surveillance, practice of biosecurity measures particularly in animal industries and vaccination.

3.3.1. Social distancing, respiratory etiquette and quarantine

The recommended measures to reduce the transmission rate of SARS-CoV-2 include frequent hand washing/ disinfection, respect for social etiquette (sneezing and coughing into the elbow or into a tissue), wearing a face mask/ cover, physical distancing and self-isolation if symptoms develop or if there has been a recent close contact with someone who has had symptoms, or is suspected to have contracted COVID-19.
A review of the evidence published in June 2020 concluded that quarantine is important both in reducing the number of cases and the number of deaths[45]. The results of several modelling studies suggest that quarantine was more effective and cost less when it was implemented earlier. Quarantine compliance by individuals who have been in contact with a confirmed or suspected case of COVID-19 is thought to have prevented 44-96% of incident cases and 31-76% of deaths[46]. Similarly, there are benefits to quarantining individuals travelling from a country with a COVID-19

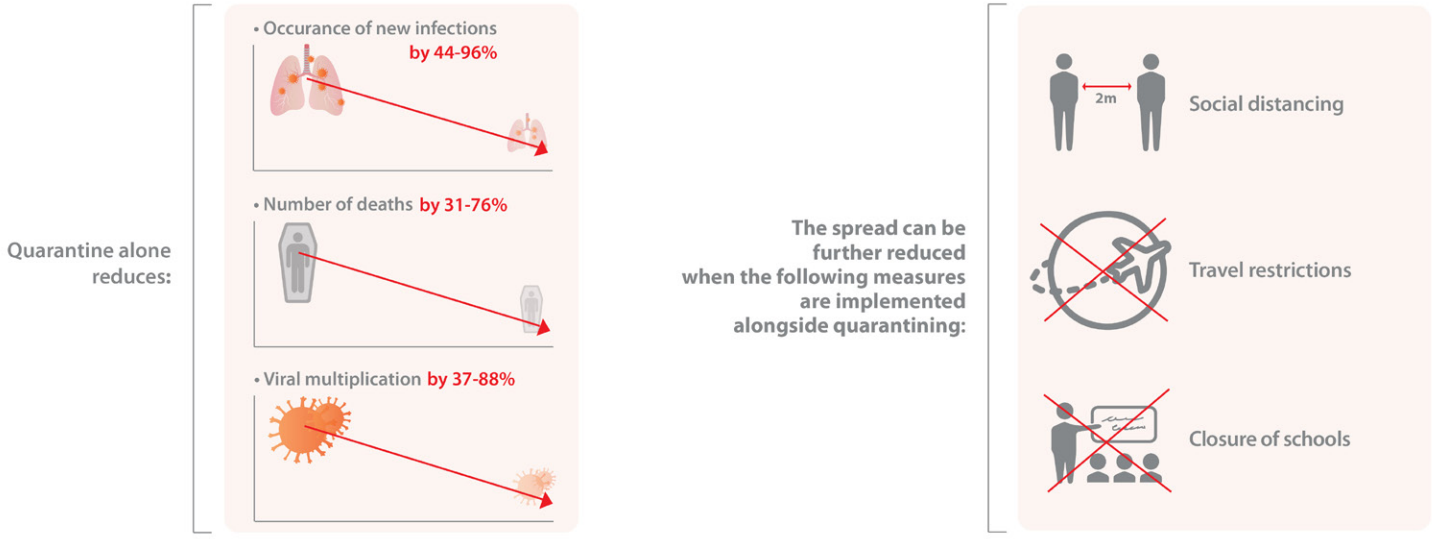


Fig. 12 - Importance of quarantine in reducing transmission of SARS-CoV-2

outbreak or with significantly higher number of COVID-19 cases. Some studies have indicated that there may be a 37-88% reduction in the Rt (the indicator that defines the degree of transmissibility of infection) due to quarantine implementation[47]. The study also concludes that the effect of quarantine is greater when combined with other prevention and control measures (Figure 12).

There is scientific evidence to support handwashing. The mechanical action of washing, in itself, is important for the elimination of a substantial number of viruses and facilitates the subsequent action of detergents and disinfectants, which act on their outer layer. Coronaviruses require an intact viral envelope to be able to infect susceptible host cells and start their replicative cycle. Given the fragile lipid nature of this envelope, it is easily destroyed by detergents that emulsify fats or by alcohol-based disinfectant solutions, which damage it and lead to rapid and irreversible denaturation of proteins (change in its structure, like what happens to egg whites when they are cooked) (Figure 13).

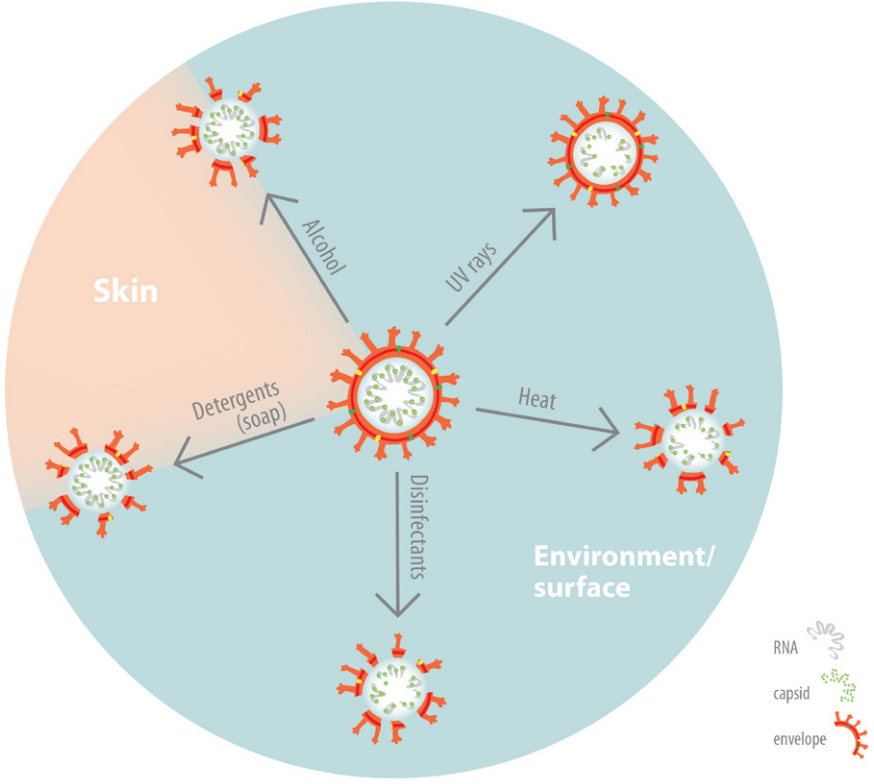


Fig. 13 - Methods of virus inactivation and its efficacy. Adapted from Pfaender et al. 2015[63].

What are the recommendations of the World Health Organization (WHO) to prevent the spread of COVID-19 from person to person?

1 - Limit close contact between infected people and others;
2 - Ensure a physical distance of at least 1 meter between people who do not belong to the same household, in the workplace and public spaces;
3 - Wear a mask when social distancing cannot be guaranteed;
4 - Quickly identify infected people, promote their isolation and, where necessary, facilitate treatment. Isolate all their recent close contacts;
5 - Sanitise and disinfect hands frequently (actions that reduce microbial load on hands and directly inactivate the virus);
6 - Avoid crowded places, and closed or confined spaces with poor ventilation;
7 - Ensure good ventilation indoors, including homes and offices;
8 - Stay at home when you feel sick and contact your doctor as soon as possible to determine if medical attention is needed;
9 - In countries or areas where COVID-19 is circulating, healthcare professionals should wear medical masks continuously during all routine activities in clinical areas in health facilities;
10 - Health professionals should also use personal protective equipment and additional precautions when caring for patients with COVID-19;
11 - Workplaces must comply with existing protection measures.

What is the importance of hand disinfection in the prevention of COVID-19?

SARS-CoV-2 infection occurs by oronasal route through inhalation and ingestion of viral particles. However, the ocular conjunctiva is also a gateway to infection with this virus. Even when wearing a mask, if our hands touch a contaminated object, and then our mouth or eyes[18] it promotes contact between the virus and mucous membranes, which may subsequently lead to infection.

Why use soap and 70% alcohol to disinfect your hands?

Hand washing with soap is the most effective mechanical method of elimination of most microorganisms present in the skin. The use of 70% alcohol (7 parts of alcohol, 3 parts of water) ensures the inactivation of a wider spectrum of microorganisms, including enveloped viruses, such as SARS-CoV-2. It is important to mention that the hands must be thoroughly washed if visibly dirty before using the alcoholic solution because excess dirt (organic matter) impairs antimicrobial action of the alcoholic compounds. Soap and alcohol dissolve and, therefore, alter the lipids of the viral envelope, thus preventing the virus from binding to host cell receptors and causing infection.

Why is 70% alcohol more effective than pure alcohol?

The disinfectant capacity of alcohol diluted in water to 50-80% is higher than undiluted alcohol. Alcohol denatures proteins and dissolves the fats in bacteria, fungi and some viruses. However, when applied pure, alcohol quickly denatures only its outermost layers, encasing and protecting their interior, allowing its survival. For alcohol to be more effective at penetrating deeper into microorganisms it must be mixed with water, which serves as a vehicle so that direct contact between molecules can occur.

Why should diluted bleach be used in surface disinfection?

Bleach diluted in water (0.5% to 1%) is more effective at killing microorganisms. In addition, bleach at high concentrations is irritating to mucous membranes (eyes, nose, mouth), skin and airways. Undiluted, bleach decomposes with heat and light, and reacts quickly with other chemicals, so it should be used in the disinfection of surfaces with great care and always diluted.

Is the use of masks effective in preventing transmission?

The mask is an essential barrier to limit contamination and must be used alongside other measures to prevent the transmission of the virus. Wearing a mask essentially allows you to protect others and is, therefore, a gesture of respect and social awareness. Masks are physical and electrostatic barriers blocking droplets emitted by the mouth and nose, which may contain infectious coronaviruses. The mask helps to reduce the spread of SARS-CoV-2 coronavirus particles into the atmosphere, released by infected people (even asymptomatic ones)[19], and thus is particularly important in reducing the environmental load. The mask also protects those who use it, since it filters the air we breathe and in doing so reduces the infectious dose (i.e. the concentration of viruses that can infect us), which in turn also reduces the severity of the infection. It should be noted that the mask does not offer any added protection against the risk of indirect transmission through the hands.
The composition of the mask and its seal are very important factors that influence its effectiveness. The use of cloth masks (handmade, washable) and surgical masks (disposable) significantly reduce the emission of droplets and aerosols by the people who wear them, thus conferring some degree of protection to surrounding people and contributing to an effective reduction of viral load. However, neither face covering nor surgical masks effectively filter aerosols from the air surrounding the mask user. Only masks or respirators of category N95 /FFP2 have the ability to filter about 95% of environmental aerosols (protecting both the users and those around them) and are advised for healthcare professionals working in environments with high viral loads or for people working in environments with high occupancy and poor aeration.

Is the use of gloves an important tool in the prevention of transmission?
Although SARS-CoV-2 is not able to infect us transdermally, the hands frequently transport viruses to recognised ports of entry including mucous membranes in the mouth, nose and eyes. By frequently washing and disinfecting the hands, we significantly reduce the likelihood of infection when we unconsciously touch our mouth, nose or eyes. While gloves can effectively keep the hands protected, their outer surface can still become contaminated and transport the virus, just like bare hands. The use of gloves can, therefore, provide a false sense of security.

What other measures are recommended to stop the transmission of the virus?
The use of masks, alone, is not enough to prevent the transmission of the virus. Other measures that effectively reduce transmission include respect for physical distancing, hand washing and disinfection of surfaces. Furthermore, when we sneeze, even when using a mask, we should do so into our elbow and then disinfect our hands.

How can vaccination control the infection?
Vaccination against SARS-CoV-2 has been shown to significantly reduce disease severity. Immunised people can still become infected, but, unlike non-immunised people, they will quickly develop specific antibodies that neutralise the virus thus controlling and eliminating the infection. If the vaccines are found to significantly reduce the transmissibility of the virus the infection chain will be broken, resulting in a reduction in the number of viruses in circulation. The frequency of vaccinations will depend on the length of time the vaccine is able to confer immunity.

What is the treatment for COVID-19?
There are no therapies licensed for COVID-19 yet. Supportive treatment is aimed at symptomatic relief and includes antipyretics, analgesics, anti-inflammatory drugs and if necessary, oxygen supplementation. Remdesivir is currently the most used antiviral, and appears to reduce the recovery time of patients with lower respiratory tract infection from 15 to 10 days[20] and immunomodulation therapy are used in more severe cases.

As with other coronaviruses, heat treatment and ultraviolet (UV) radiation applied to inanimate objects and surfaces induce irreparable damage to viral RNA by causing structural changes in viral proteins and thus compromising replication. SARS-CoV-2 can withstand a range of temperatures from below 0°C to just below 60ºC, which is thought to be the inactivation temperature[46]. Without their envelope, coronaviruses are not infectious because they cannot naturally enter the host cells to start their replication cycle. For all these reasons, hand hygiene with lipid solvents, like detergents or 70% alcohol solutions, and disinfection of all surfaces that have been touched with products containing chlorine, peracetic acid and chloroform are effective measures to contain the contagion.

Efforts to contain the pandemics through the application of biosecurity measures have proved to be insufficient due to difficulty in their application as well as public compliance. The return to social and economic normalities relies on successful vaccination to prevent disease (particularly severe disease) and limit the spread of the virus. The development of immunity through vaccination reduces the risk of becoming ill and of suffering long-term sequelae. Immunity also protects others, as a vaccinated person who contracts the virus is less likely to excrete and transmit it. Vaccination is, therefore, particularly important to protect people at increased risk of severe illness from COVID-19, such as the elderly and people with underlying medical conditions.

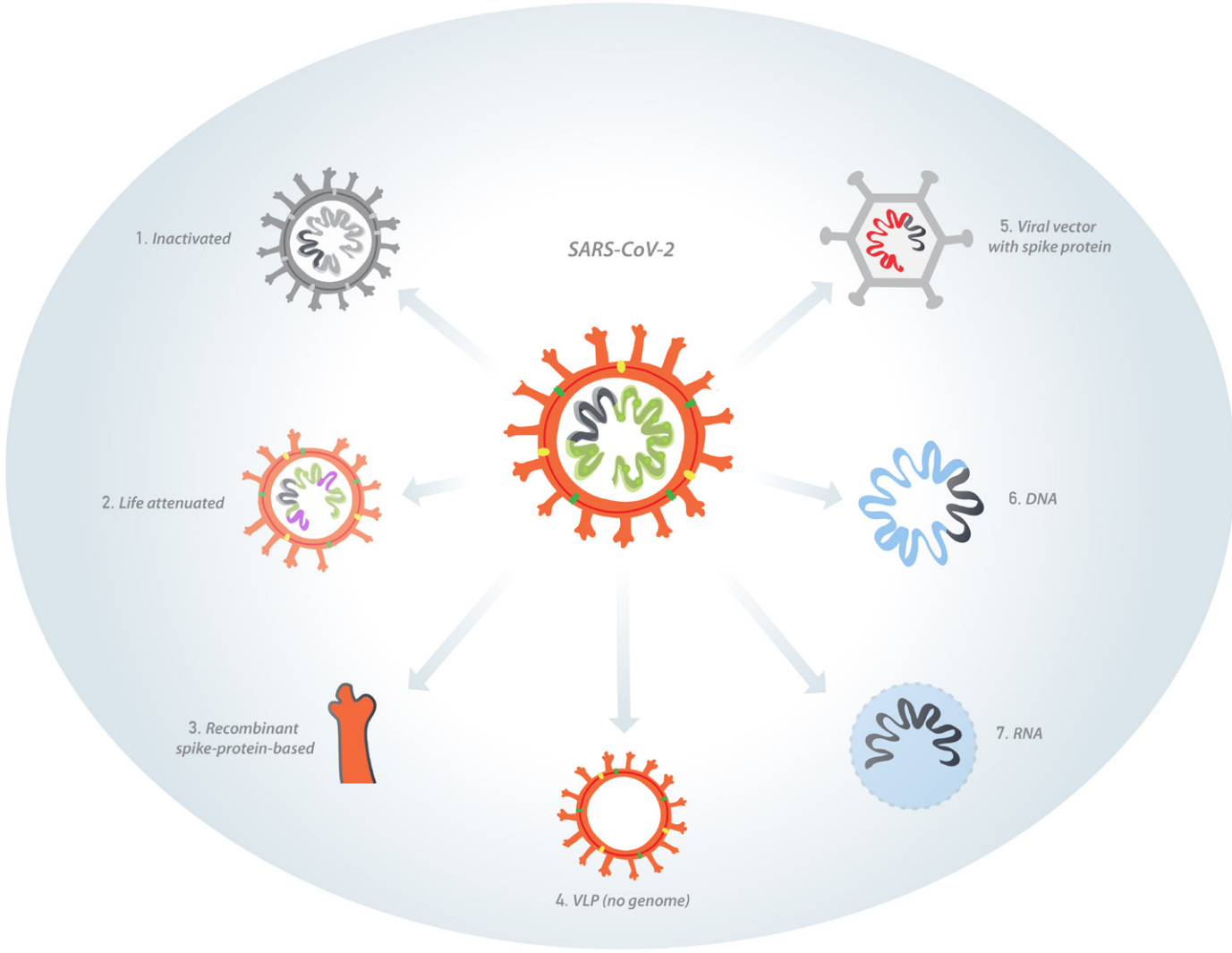


Fig. 14 - Schematic representation of the type of vaccines presently available for Covid-19. In the centre of the figure is represented a particle of SARS-CoV-2, surrounded by the types of vaccines prepared from SARS-CoV-2 or its components: 1. Inactivated vaccines (dead virus); 2. Live attenuated vaccines (low pathogenic virus); 3. Recombinant vaccines (consisting of S protein or the receptor binding domain of the S-protein); 4. Virus Like Protein (VLP) based vaccines, consisting of empty capsids; 5. Viral vector vaccines expressing the S protein; 6. DNA based vaccines expressing the S protein; 7. messenger RNA based vaccines (RNA encoding the S protein involved in a lipid nanoparticle. Adapted from Krammer, 2020.[49]

3.3.2. Vaccines

More than 180 vaccine candidates against SARS-CoV-2 have been developed around the world. The World Health Organization (WHO) maintains a working document that includes most of the vaccines in development[48]. The developmental approaches used for SARS-CoV-2 vaccines can be divided into “traditional”, which include inactivated or live-virus vaccines, “recombinant protein vaccines”, “vectored vaccines”, “DNA” and “RNA vaccines”.

Inactivated vaccines are produced by multiplying SARS-CoV-2 in VERO cells or in other cell lines in biosafety level 3 laboratories (BSL-3), followed by chemical inactivation of the virus, with betapropriolactone (an organic denaturating compound) for example[50,51]. As the whole virus is present in the RNA vaccines (figure 14), immune responses are then developed against all the structural proteins (not only the S protein but also the M, N and the E proteins). These vaccines are usually administered intramuscularly and can contain adjuvants such as aluminium hydroxide. Examples of inactivated vaccines for COVID-19 are the BBIBP-CorV (Sinopharm), the CoronaVac (SinoVac) and the Covaxin (Bharat Biotech) vaccines.

Live attenuated vaccines use a weaker live version of the virus, which can only replicate to a limited extent, causing infection but not overt disease. This induces an immune response similar to what would occur in natural infection. Attenuation can be achieved by adapting the virus in unfavourable conditions or through genetic modifications[48,52]. These vaccines can be administered via the intranasal route, inducing mucosal immune responses that can protect the upper respiratory tract, the primary natural entry route of SARS-CoV-2. As the virus replicates in the vaccinated person, strong humoral and cellular responses are induced against the structural and non-structural viral proteins (only present when viral replication occurs). Concerns regarding the safety of the vaccine are one of the drawbacks to this type of vaccine[53].

Recombinant protein vaccines are created from viral proteins that are produced by biotechnological processes. These mostly include the S-protein, located on the virus’ receptor binding domain, or a set of structural proteins that spontaneously assemble into a virus-like particle (VLP) whose structure mimics the natural virus but that has no genetic material inside and therefore cannot replicate. All these recombinant proteins can be expressed in different systems including mammalian cells, yeast, plants or insect cells[53–55]. Similar to inactivated vaccines, these are injected intramuscularly and are not expected to result in robust mucosal immunity. The NVX-CoV2373 (VLP, Novavax) vaccine is a recombinant protein vaccine against COVID-19.

Viral vector vaccines consist of a virus (the vector) that is altered to express a specific protein/antigen belonging to the virus the vaccine is protecting against. Since viral vectors can infect cells and deliver antigens (protein S, in SARS-CoV-2 vaccines[48]) inside them very effectively they trigger a strong immune response. Most of these vaccines are administered intramuscularly.

There are two types of viral vectors; non-replicative and replicative viral vectors. In non-replicative viral vectors, the vector virus, usually an adenovirus (AdV), is unable to replicate and cause disease[56]. The advantage of this approach is that the inactivation process renders the vectors safe, even in an

immunocompromised host, by impeding replication. By using inactivated vectored vaccines, the amount of antigen to be administered can be standardised as is the case in inactivated or recombinant protein vaccines. Examples of COVID-19 non-replicative viral vectors vaccines include the Johnson & Johnson, the Oxford-AstraZeneca and the Sputnik V.

The replicative viral vector vaccines use attenuated viral vectors capable of replication. Since the vector is able to multiply to some extent in the vaccinated person, a more robust immunity is expected compared to inactivated, recombinant or non-replicating vector vaccines. Some of these vaccines can also be administered through mucosal surfaces, which may trigger robust mucosal immune responses. Influenza virus[48], rabies virus[48], vesicular stomatitis virus (VSV)[60], vaccinia virus and Newcastle disease virus (NDV)[61,62] are currently being used[45]. These vaccines can be manufactured using similar technologies to those used to produce the flu vaccines[59].

One of the disadvantages is the possibility of pre-existing immunity to the vector[57], which may neutralise the vaccine before it can induce the production of a viral particle. This can be overcome, however, by using animal viruses[58] as vectors, thus ensuring humans would have had no previous contact or immunity to it.

DNA vaccines are based on plasmid DNA (smaller than chromosomes' self-replicating molecules) that can be produced on a large scale in bacteria. These plasmids contain mammalian expression promoters and the S-gene, which is expressed in the vaccinated person. The great advantages of this technology include the high degree of stability of plasmid DNA. However, DNA vaccines often show low immunogenicity, and have to be administered via delivery devices to make them more efficient.

RNA vaccines are the most recent vaccines, consisting of a portion of the viral RNA, which is usually delivered via lipid nanoparticles (LNPs) given the lability of this molecule. In this case, the genetic information encoding the antigen (the S-protein) is delivered in the form of mRNA (with certain modifications) or self-replicating RNA, instead of the S-protein itself. The S-protein is then expressed within the cells after vaccination. Through this technology the vaccine can be totally produced in vitro. However, frozen storage is required. In addition, these vaccines are administered by injection and are therefore unlikely to induce strong mucosal immunity[63]. Examples of these vaccines are the BNT162b2 from Pfizer BioNTech and the mRNA-1273 from Moderna.

4. CORONAVIRUSES IN THE WORLD

Most animal (e.g., FCoV, CCoV and TGE) and human (e.g., HCoV2-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43) coronaviruses are found world-wide.

The first reported outbreak of SARS-CoV, the causative agent of SARS in humans, emerged between 2002 and 2003. Around 8096 people from 33 countries were affected and there were 774 deaths[65]. Some studies suggest that the first cases emerged in the province of Guangdong where the virus circulated for about 2 months before causing a major outbreak in the city of Guangzhou[66]. International transmission occurred later, from the 9th floor of the Metropole Hotel in Hong Kong, following contact with a doctor infected with SARS-CoV. Other guests on the same floor were infected and later returned to their countries of origin, on different continents, quickly promoting the spread of the virus worldwide [67,68].

MERS-CoV was first detected in a patient in Saudi Arabia (Middle East) in 2012 and as of March 1st, 2020 the World Health Organization confirmed about 2553 cases and 856 deaths which have occurred since[69]. Although this coronavirus (MERS-CoV) has spread to other parts of the globe like France, Germany, Italy, Tunisia, United Kingdom, through people who had travelled to or temporarily worked in the Middle East, most of the historic and new cases occurred and continue to appear in the Arabian Peninsula.

The current COVID-19 pandemic has reached 213 countries and regions, infecting millions of people. Its spread is considerably higher than that of both SARS and MERS. The number of infected people as of December 10th, 2020 is 68 million.

What is the mortality rate associated with COVID-19 infection?
Although the real COVID-19 mortality is not accurately known, current data suggests that the crude mortality rate (the total number of deaths to residents in a specified geographic area (country, state, county, etc.) divided by the total population for the same geographic area (for a specified period, usually a calendar year) and multiplied by 100,000) is between 3-4 %, although varies widely between countries, from 0.1% and 25%[21]. However, the real mortality rate will be lower due to the cases that aren't identified or recorded. Mortality rate is impacted by the access to and quality of health care, the virus' infectious dose and the virulence of the strains.

How will the pandemic evolve?
The future of the pandemic is still unknown and will depend on the equilibrium between the virus' ability to evolve and on the population's immune response. The latter will either be acquired naturally through infection or artificially through a vaccine. The outcome of this co-evolution is still unknown as our immunity will push the virus to evolve.

5. WHAT NOW?

Viruses are very peculiar. Not least because of the ongoing controversial issue of whether or not they are living beings. Although they depend on the living cells which they access through surface receptors, they are considerably more efficient than bacteria, parasites and fungi at replicating. Just as scientists think they have developed a solution to the challenges posed by a virus, they are often surprised either by a new mutation or a rapid emergence of a new virus, which nullify those same solutions.

No human, animal or zoonotic virus has ever been talked about and reported in international media as SARS-CoV-2 has, despite the fact that viruses have evolved in parallel to us shaping people and societies, like smallpox, HIV and seasonal influenza viruses which have had a significantl impact to this day. The emergence of the COVID-19 coronavirus at the end of 2019 represented an unprecedented challenge for governments and health services worldwide in being able to respond to a lethal unknown virus with high capacity of dissemination.

International and multi-institutional collaboration allowed the rapid sequencing of the SARS-CoV-2 genome. The exchange of this information was essential for the rapid development of diagnostic tests and vaccines based on RNA or DNA. The international collection and publication of health and epidemiological data enabled the monitoring of the number of new cases in real time. The information shared between the vast majority of affected countries includes death figures and data from studies, including the impact of regulating travel across national borders, imposing quarantines and self-isolation on the reduction of transmission rates and disease spread.

The frequency with which widely available information is updated is unprecedented. This is facilitated by the variety and comprehensiveness of current technology. The information raises awareness about how to identify the disease as well as about practices and behaviours that individuals should follow if they develop symptoms or have had close contact with an infected individual.

More than a year after the first COVID-19 deaths there is still no specific recommended antiviral treatment. Patients receive symptomatic treatment with antipyretics and analgesics. In more severe cases, medical care focuses on the treatment of complications from COVID-19, including severe pneumonia, Acute Respiratory Distress Syndrome, and sepsis. When respiratory function is compromised, oxygen therapy is the first line of action. In cases of respiratory failure, non-invasive and invasive mechanical ventilation may be necessary. Corticosteroid anti-inflammatory drugs seem to have beneficial effects in these cases, reducing the mortality rate by 35%[70]. The benefits of using some antiviral agents such as remdesivir[71], lopinavir/ ritonavir and arbidol, and drugs with immunomodulatory effects, such as chloroquine and hydroxychloroquine, are still being analysed.

Promising research is being developed to find new targets for drug design. A new study in Portugal revealed viral ribonuclease nsp14 as a promising target for the discovery of new drug inhibitors that interfere with the replication of coronaviruses and improve the prognosis of severe cases of the disease[72].

Although this pandemic has surprised the world, the emergence and intercontinental spread of a new pathogenic virus has long been anticipated and the need for profound changes in the way humans interacts with the Planet. Indeed, the relentless and insidious invasion of wild ecosystems alongside the destruction of wild habitats will inevitably result in the emergence of more new pathogens. This is a result of the increasing proximity to previously isolated exotic species, which act as reservoirs for those agents, facilitating cross-species transmission. Compounded with international travel and commerce, this enhances the rapid spread of emerging pathogens, leading to epidemics and pandemics.

Long after the report of the first alleged case of COVID-19 in November 2019, there are still many uncertainties about the origin and its future evolution[73]. However, it is encouraging to note that those newly infected with SARS-CoV-2 are now more likely to survive the infection than those who were infected in early 2020. Since then, many uncertainties about the best hospital treatment and infection prevention measures have been clarified. Therefore, it is essential to abide by the precautions and advice highlighted by the WHO which are founded on evidence-based scientific knowledge, in particular the use of masks, in frequent hand-washing and physical distance between people, especially from those with suspected or confirmed infection. These infection prevention measures reduce the transmission of all respiratory infectious agents, such as the other respiratory coronavirus mentioned above and the influenza virus.

Access to updated information communicated in a clear and simple way is essential for adequate understanding and for consciously adaptation of behaviours, ultimately aiming to break the chains of infection and protect the most vulnerable.

Remdesivir is a precursor to the adenosine nucleotide that, once inside cells, is metabolised to pharmacologically active nucleoside triphosphate. Remdesivir triphosphate acts as an analogue of adenosine triphosphate which, in competing for incorporation into the RNA chain synthesised by viral RpRd, induces premature chain termination during viral RNA replication. Although Coronaviruses have an error correction process that detects and removes other nucleoside analogues during RNA synthesis, resulting in drug resistance, Remdesivir seems to be unaffected by this mechanism[17].

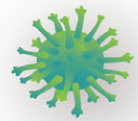
Could SARS-CoV-2 have been created in the laboratory?

The idea that the coronavirus was created in a laboratory in China, from where it escaped due to the lack of biosafety conditions or negligence, emerged as a conspiracy theory that destabilised public opinion.

The vast majority of scientists agree that this virus originated from an animal species, probably a bat, and then evolved naturally in an intermediate species, perhaps even in humans. This is supported by the evolutionary history of the virus and the understanding of the ecology of the reservoir species[22]. The SARS-CoV-2 genome is very similar to that of other bat coronaviruses, and pangolin coronaviruses. They all share the same genomic organisation/structure but their differences reveal their divergences during natural pattern of evolution[23].

Analysis of its genetic material provided evidence that SARS-CoV-2 has a natural zoonotic origin. It demonstrated that the “backbone” of the SARS-CoV-2 genome is novel and does not correspond to that of a pre-existing coronavirus, which would have been used as the genetic template for artificial construction. In addition, there are no obviously inserted or removed genetic elements which would be highly likely if SARS-CoV-2 were genetically manipulated.






6. GLOSSARY

ACE2 – Angiotensin-converting enzyme 2. An enzymatic protein on the surface of many human cells that is involved in blood pressure control by catalysing the hydrolysis of angiotensin II (a vasoconstrictor peptide) into angiotensin (a vasodilator). Some coronaviruses recognise it and use it as receptors to infect human cells.

Antibody - A protein produced by B lymphocytes that recognises, adheres to and neutralises foreign (non-self) molecules that represent a potential threat to the body.

Antigen – A molecule (usually proteins or polysaccharides) that is foreign to the organism which, once recognised by B cells of the host's immune system, triggers a series of defence mechanisms, including the development of antibodies.

Antiviral - Medication used to treat viral infections.

COVID-19 – Coronavirus disease 2019. The infection caused by SARS-CoV-2.

CRISPR – Clustered regularly interspaced short palindromic repeats. CRISPR/CAS9 is a RNA-guided targeted genome editing tool which allows researchers to do gene knockout, insertions and deletions in cell lines and in animals.

DNA –Deoxyribonucleic acid. A macromolecule that contains the genes of all cells and life forms, with the exception of some viruses, consisting of nucleotides A, T, G and C.

ELISA - Immunoenzymatic assay.

Endemic disease - Disease that affects humans and is present in a certain region with a low and stable prevalence (number of cases); the same as enzootic disease in animals.

Enzootic disease or enzooty - Animal disease that is present in a certain region with a low and stable prevalence (number of cases); the same as endemic disease in human beings.

Enzyme - Protein whose function is to catalyse a chemical reaction.

Epidemic disease - A disease that quickly spreads, until it reaches a large number of people in a given territory and that goes extinct after a period or becomes endemic; in the animals it is referred as epizootic disease or epizooty.

Evolution - Gradual changes occurring in populations over the generations.

Exponential growth - It is said when a value increases with a rate proportional to its current size, i.e. as the value increases, the growth rate also increases. When related to a disease, it means that the more infected individuals exist in the early stages of a disease outbreak, more individuals will become infected and more the number of cases will increase.

Genes - Regions of DNA that codify for proteins.
Genome - Set of genes that make up the genetic material of a living organism.

Infectious dose - Number of microorganisms capable of triggering an infection.

LAMP - Loop-Mediated Isothermal Amplification is a nucleic acid detection technique based on DNA amplification at low temperature.

Microarray – A DNA chip consisting of a collection of microscopic DNA spots (specific DNA sequences known as probes) attached to a solid surface that are used to measure the expression levels of a large number of genes or to genotype multiple regions of a genome. Probe-target hybridization is detected and quantified to determine relative abundance of nucleic acid sequences in the sample.

Mutation - Alteration in the nucleotide sequence of DNA or RNA that may involve the replacement, the insertion or the removal (deletion) of nucleotides.

Nucleic acid - Molecule that stores genetic information and is involved in different stages of gene expression. It consists of nucleotides and can be two types: DNA or RNA.

Pandemic disease - According to the World Health Organization it is "an epidemic that occurs worldwide, or in a very wide area, crossing international borders and generally affecting a large number of people"; in the animals it is referred as panzootic disease or panzooty.

Pathogen - Microorganism capable of causing disease.

Pathogenic - Which causes injury or illness.

PCR - Polymerization chain reaction. Allows synthesizing multiple copies of a region of the genome using a thermoresistant polymerase.

Polymerase - Enzyme that synthesizes nucleic acid molecules (polymers).

Protein - Essential molecules for all living systems. They have numerous functions such as forming structures (structural proteins), controlling chemical reactions (catalysts or enzymes), sending and receiving messages. They are basically formed by long chains, like a rosary, of simpler molecules - amino acids -, which fold into secondary and tertiary, three-dimensional, more complex structures. There are 20 main amino acids and their sequence in a protein is encoded by genes.

RdRp - RNA-dependent RNA polymerase. A viral enzyme that promotes the synthesis of an RNA chain using a RNA chain as a template.

Recombination - Alteration of the DNA or RNA sequence by exchanging segments between molecules.

Reservoir - Animal species, susceptible to infection, where the virus replicates without causing clinical disease. The reservoir host acts as a virus source for infection of other hosts.

Ribosome - A cellular organelle found in all living cells that translates the instructions contained in genes into proteins.

RNA - Ribonucleic Acid.

Subclinical infection - Infection without symptoms.

Taxonomy - Discipline that deals with the organization and classification of living organisms.

Transmembrane protein - Integral membrane protein crossing it from side to side.

Viral envelope - Outer layer present in some viruses, consisting of a phospholipid membrane, similar to the plasma membrane, modified by the addition of transmembrane viral proteins.

Virulence - Ability to invade and colonize new niches, namely infection of a host. Degree of pathogenicity of a microorganism.

Zoonotic diseases - Diseases transmitted from animals to humans.

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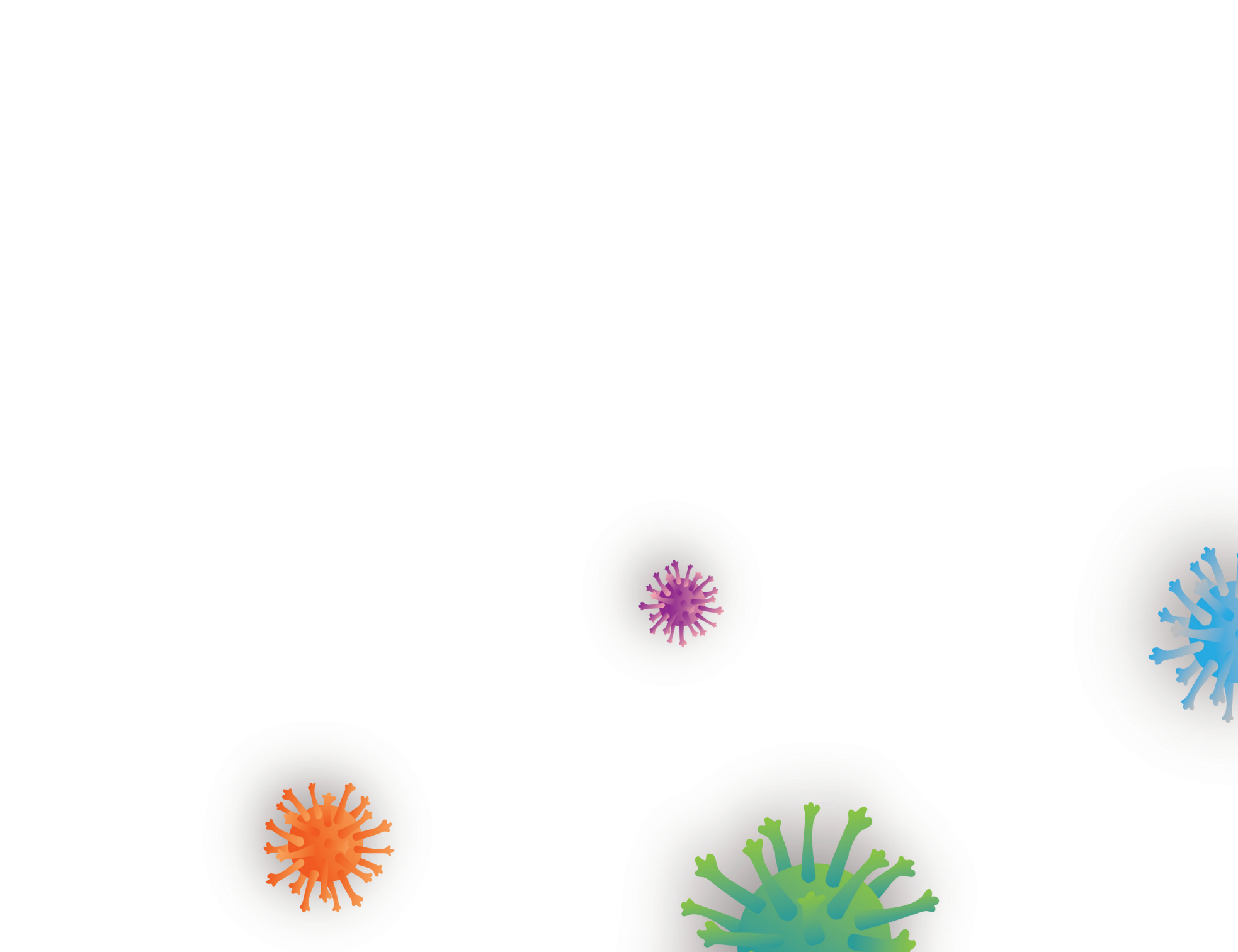

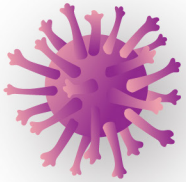

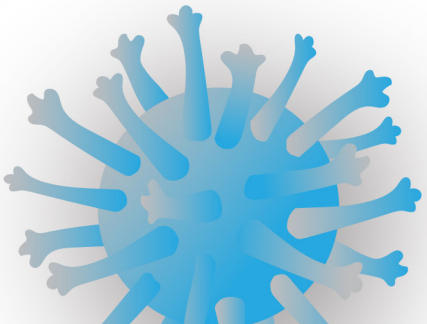

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