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

CHAPTER

Immunological challenges of the "new" infections: corona viruses

14

The corona viruses have come into high prominence in the 21st century due to serious but contained outbreaks of respiratory disease in 2002 (SARS-CoV) and 2012 (MERS), involving two quite different corona viruses, and an outbreak that began in China in late 2019 caused by a third virus (SARS-CoV-2) which rapidly evolved into a global pandemic. The corona viruses are members of the *coronaviridae* family and are enveloped RNA viruses having the largest genome among all RNA viruses. They are currently classified into four different genera, alpha, beta, gamma, and delta coronaviruses. Phylogenetic evidence suggests that bats and rodents are the main reservoir host for the majority of alpha and beta coronaviruses while birds are the main reservoir host for gamma and delta coronaviruses.[1] There are four different "lineages" in beta viruses, A-D, with lineage D currently having a single virus only found in *Rousettus* bats.

[Note: a lineage is not the same as a strain. Lineages are virus sequences that differ from but are related to a parent virus as a result of mutations. New strains are defined when changes in the properties of lineage members occur, such as, for example, increased pathogenicity, transmission, or rate of reproduction.

Fig. 14.1 shows an example of the phylogenetic relationships for some important members of this family, as cataloged in late 2020.[2] Members of the broader family of these viruses have been identified by RNA sequence analysis in bats, camels, dromedaries, civets, rats, rabbits, horses, pigs, cows, antelopes, birds, dolphins, and whales, although some of this analysis has only thrown up virus fragments. The existence of live, viable virus in all these species has not been verified. Currently only seven coronaviruses, all within the alpha and beta genera, are known to be infective for humans (marked with a Hu prefix in Fig. 14.1).

The pathological effects of some members of this virus family (particularly some of the beta viruses) are serious, affecting both the upper and lower respiratory tracts. Patients experiencing serious disease often present with pneumonia-like symptoms, and in some cases experience more widespread organ damage (e.g., kidneys, heart, CNS, and other organs) as well as disseminated coagulation episodes. The mortality resulting from infection by the more serious family members, while not at the level of for example Ebola disease, is nevertheless of global importance. Since their discovery almost 90 years ago, studies of the origins of coronaviruses, and the development of preventive vaccines as well as postinfection therapeutic treatments, have become an unprecedented global effort.

History of coronaviruses

In 1931, a novel upper respiratory disease of newborn chicks was identified in North Dakota in the US, clinically similar to laryngotracheitis, transmissible by contact and with symptoms of gasping,

A New History of Vaccines for Infectious Diseases. https://doi.org/10.1016/B978-0-12-812754-4.00017-0

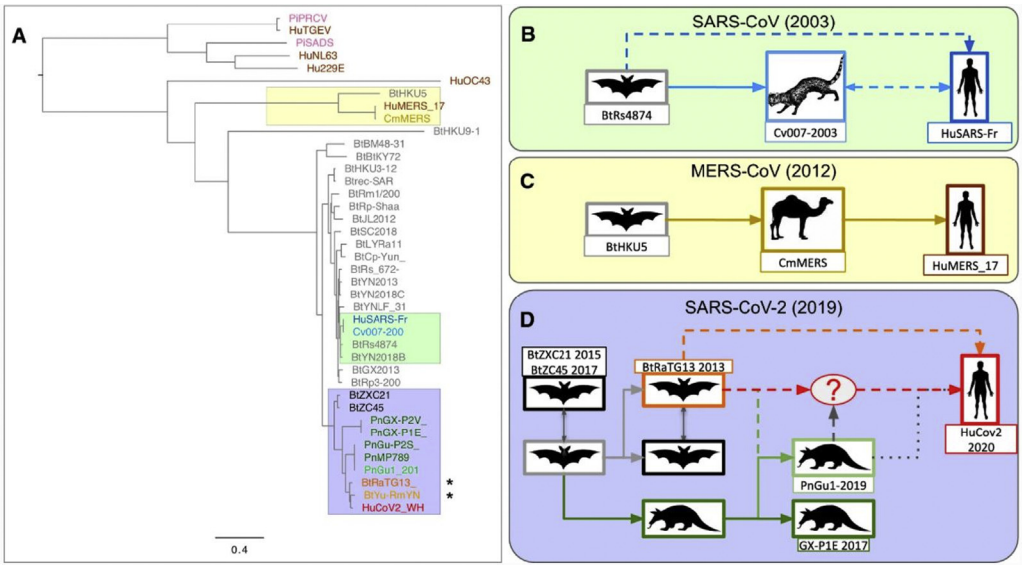

FIGURE 14.1

The left hand panel (A) shows the relationship between different coronaviruses isolated from various animal species (Bt (bat), Hu (human), Pn (pangolin), Cv (civit), Cm (camel), and Pi (pig). The colored boxes represent the coronavirus strain groups a member of which has led to epidemics or pandemics in recent years: green SARS-CoV (2003–04), yellow MERS (2012), and blue SARS-CoV-2 (2019–21). In the right hand panel, the zoonotic origins of the three viruses are indicated with dotted lines in the green and blue panels representing the uncertainty of the SARS-CoV exact origin, and the considerable uncertainly of the SARS-CoV-2 origin.

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listlessness, and depression, and a mortality rate in chicks of 40%–90%.[3] Later studies demonstrated the infection was due to a filterable virus and, based on the overlapping symptoms, it was erroneously thought to be identical with infectious laryngotracheitis. A related infection in chickens, coryza (inflammation of nasal mucous membranes) in a different study, was attributed to a different filterable virus, although the possibility that the respiratory infections previously described were due to one and the same virus was not explored. In 1936, veterinary researchers from the University of California, Berkeley, took samples 6 months apart from two different "broiler plants" that were experiencing the respiratory infection, the two strains taken referred to as M and P. The identical filterable virus was shown to be responsible for the differently observed respiratory infections. Particularly strong support for this conclusion was the observation that chickens that had recovered from infection with one strain of the virus were refractory to infection by the second strain, and further, serum samples from chickens infected with one strain were able to neutralize the second strain, leading to the conclusion that the two strains must be from the same virus. As a final demonstration of Koch's postulates, the authors showed that chickens immune to the new isolated virus were still susceptible to infection with the laryngotracheitis virus, and vice versa, as well as the bacterium *Hemophilus gallinarum* known to cause coryza. In a technical observation that may have been the earliest indication of the "toughness" of

corona viruses the authors also noted that the dried virus, stored for 180 days in a refrigerator still caused an infection.[4]

The corona virus as an entity was not isolated until 1951 when it was grown in embryonated chicken eggs. In the same year, a virus causing hepatitis in laboratory mice was described that would not have been an obvious relative of the respiratory viruses described in chickens. During the early 1960s, June Almeida (St Thomas's Hospital, London) and David Tyrell (MRC Common Cold Research Unit, UK) had been studying upper respiratory tract fluids from individuals suffering from common respiratory infections and had already demonstrated the presence of rhinoviruses, the most frequent cause of common colds. But they, and others, had isolated samples that appeared to contain no infectious agents. When the samples were incubated with human tracheal organ cultures, extracts of the culture given to volunteers retained their ability to cause colds, indicating growth of an infective agent in the culture. Using this approach, three novel viruses were identified, two by Tyrell in 1965 and 1966 (B814 & LAKEY)[5] and one by Dorothy Hamre at the University of Chicago in 1966 (229E). The Chicago virus had been collected from medical students with winter colds and was shown to contain RNA, although serologically distinct from other known respiratory RNA viruses such as the orthomyxoviruses (e.g., influenza) or paramyxoviruses (e.g., measles, mumps, etc.).[6]

The method of using inoculated volunteers for the purpose of characterizing unknown pathogens, however, was not ideal, and ethically debatable. In 1967, Tyrell and Almeida developed an identification method that employed electron microscopy for identifying viruses with similar morphologies. Applying this method to the three unknown viruses established that the 229E and B814 viruses had identical gross structures, morphologically indistinguishable from the avian infectious bronchitis virus (IBV) described more than 30 years earlier.[7] The two viruses showed the same spherical particle crowned with club-ended spikes (Fig. 14.2), a picture now so familiar to the COVID19 world. The third virus (LAKEY) was shown to belong to a completely different family based on its strikingly different morphology.

In parallel studies at the US National Institutes of Health, Kenneth McIntosh, Robert Chanock and colleagues, aware of the recently isolated 229E and B814 virus strains, had noted a sharp drop in the isolation of respiratory viruses during the 1965−66 winter, suggestive of new infectious agents not recoverable by the standard laboratory techniques. Using organ culture methods, six new viruses, with similar morphology to IBV and a mouse hepatitis virus (MHV), were isolated from patients with upper respiratory infections. Further studies of these viruses in organ culture generated two strains (OC38/OC43) that were eventually shown to be identical (now named OC43) and although morphologically similar to both IBV and MHV were serologically distinct.[8]

As a result of these various studies in the UK and USA, the virology community came to a consensus that this must be a new virus family. In November 1968, an informal group of virologists that included June Almeida, David Tyrell, Kenneth McIntosh, and others sent their conclusions to Nature magazine, under the Editorship of John Maddox, recommending that the E229 and B814 and other similar viruses should be classified under a new virus family and suggesting the name "Coronaviruses" to reflect the unusual "crown-like" morphology.[9] The avian bronchitis virus, IBV, was later classified as a gamma coronavirus while 229E was shown to be an alpha virus and OC43 a beta virus (the latter two responsible for up to 30% of all upper respiratory tract infections in humans). The three viruses are formally named Avian-IBV, HCoV-229E, and HCoV-OC43, respectively (see Fig. 14.1 showing the phyletic locations of 229E and OC43).

Until 2002, the coronaviruses were thought to produce relatively mild infections in children and younger adults with little or no mortality. Exceptionally, neonates and older persons, particularly those

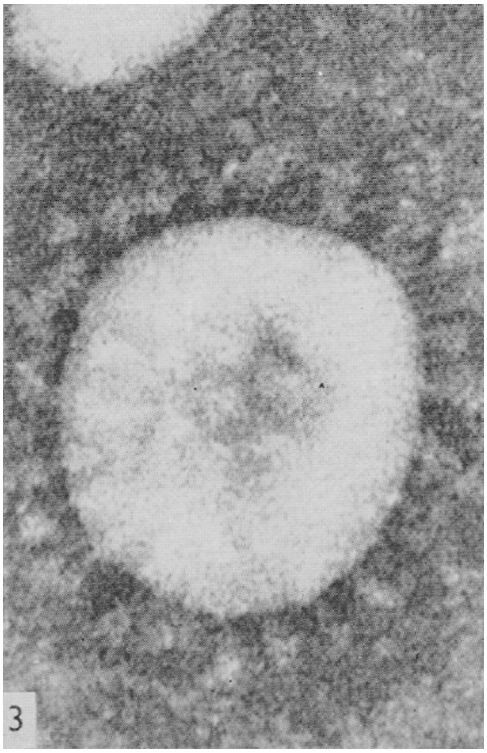

FIGURE 14.2

A particles of virus 229E under the electron microscope with an average diameter of about 800–1200Å. The surface of the particle is covered with a distinct layer of projections roughly 200 Å long with a narrow stalk and a 'head' roughly 100Å across.

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with underlying health conditions, had experienced severe pneumonia. During the winter of 1999–2000, a study in Rochester, New York found that frail, elderly hospitalized patients with various preexisting pulmonary health conditions were susceptible to severe lung disease if infected with rhinoviruses or the OC43 and E229 coronaviruses, but by contrast:

> *"It does not appear that healthy elderly persons are at high risk from these agents. These viruses, although not as common as influenza and RSV among hospitalized adults, also circulate during the winter months, producing similar clinical syndromes."*[10]

That was about to change.

A new viral pathogen arrives

On November 16, 2002 the first case of a patient with an unusual respiratory syndrome was reported in Foshan, Guangdong Province in China. On January 23, 2003, the Guangdong Health Bureau sent

information to other Chinese health authorities describing what appeared to be an atypical pneumonia. Just 7 days later, 69 Guangdong persons fell ill with the reported symptoms, strongly linked to infection by a wet market seafood seller and restaurant supplier. On 10 February, the WHO office in Beijing received an email informing it of a "strange contagious disease" in Guangdong. A day later, Guangdong health officials formally reported that 305 cases and five deaths had occurred between 16 November and 9 February of what was now referred to as "acute respiratory syndrome." The next part of the story will now sound familiar. On 14 February, the Chinese Ministry of Health informed the WHO that the outbreak was under control. In its Weekly Epidemiological Record on February 14, the WHO stated, echoing the Chinese conclusion:

> *"No new cases have been reported in Foshan, Heyuan, and Zhongshan during the first week of February and the number of new cases is decreasing in Guangzhou, Jiangmen and Shenzhen municipalities."*[11]

On February 21st, a Chinese physician staying at the Metropole Hotel in Hong Kong became ill and within 2 weeks similar illnesses were reported in Vietnam, Singapore, and Canada. In Hanoi, the Vietnam French hospital reported a case of an unusual influenza-like illness to the Hanoi WHO office on February 28th. A WHO infectious disease specialist based in Hanoi, Carlo Urbani, attended the hospital and rapidly made the decision to put in place infection control measures, including an isolation ward under guard, while samples were collected and sent for testing. On March 3, Urbani informed the WHO regional office in Manila of the unusual disease. Within a short time, 22 persons working at the Hanoi hospital fell ill with the same symptoms and after a critical meeting on March 9 the hospital was quarantined by the Vietnam government. After assistance from the WHO, the regional CDC and MSF (Médicines sans Frontiers), the rapid measures introduced in Hanoi led to containment of the disease. Dr. Urbani was not so fortunate. On March 11, he became ill *en route* to Bangkok. On arrival, he was met by a colleague from the CDC who he warned to keep his distance while an ambulance in protective gear whisked him to a Bangkok hospital set up with a makeshift isolation ward. After a brave fight with the disease on March 29th Carlo Urbani died. In memory of his brave fight with the disease outbreak, the SARS virus responsible for the Vietnam outbreak was named the SARS-CoV Urbani strain.

In Hong Kong, hospital workers began falling ill around March 7th and by March 11th 26 persons had been admitted to hospital with febrile illness, 11 of whom had signs of pneumonia. In Canada, a multi-generational family of Hong Kong descent, who had visited Hong Kong in the second half of February and stayed at the same Metropole Hotel, fell ill, and were admitted to hospital. A number of deaths were reported and by the end of March contact tracing had identified an additional 100 persons having the suspected new infection. In a report of the outbreak, in the New England Journal of Medicine, May 2003, the large number of Canadian contributors made this comment:

> *"The identification of SARS in Canada only a few weeks after an outbreak on another continent exemplifies the ease with which infectious agents can be transmitted in this era of international travel. It also demonstrates the importance and value of information and alert systems…."*[12]

Cases were also reported in Singapore, Ireland, Germany, and the USA. On March 12, the WHO issued a global "alert." In its Weekly Report of March 14, it suggested that the simultaneous outbreak of bird 'flu in Hong Kong during February appeared not to be connected to the new disease but did not issue a further alert until March 15. In the second alert, the WHO named the disease SARS ("Severe

Acute Respiratory Syndrome") and included a travel advisory that travelers should be mindful of any symptoms that might develop for 10 days after returning home.

Between the initial index case in China on November 16, 2002 and the global alert, 4 months had passed. This train of events elicited several questions from the scientific community. Given its obvious contagiousness why were no travel restrictions, in or out, introduced in the most affected areas? Why were the initial cases in Guangdong downplayed, both within China and by the WHO? The Vietnam example by contrast demonstrated that 19th century methods of hygiene and rapid isolation were still the most effective ways of controlling a pathogen outbreak in the absence of prophylactic or post-infection therapeutic solutions, as experiences with Ebola containment had already shown. To be fair, the WHO office in Beijing expressed concern on 18 April over inadequate case reporting, triggering the firing of Beijing's Minister of Heath and Mayor after 339 further undisclosed cases of the infection had been discovered.

On July 5, 2003, the WHO declared an end to the SARS epidemic that had caused 8096 infections and 774 deaths, an extraordinarily high death rate of 9.6% compared with other respiratory infections. In the event, SARS failed to develop into a global pandemic and although having a relatively high mortality rate the number of cases worldwide was low. Vaccine development projects that had been initiated were mothballed since in the absence of disease, efficacy trials were not possible, other than via volunteer challenge trials. As a result, there is no vaccine for this virus and in the 2018 Edition (seventh) of Plotkin's Vaccines the "bible" of vaccines, there is no section on coronaviruses. Did the absence of a SARS vaccine cause any shuddering in political and health regulatory circles, given the possibility of it returning, either as the identical virus, or more likely as a different variant after mutation? Apparently not.

The SARS disease: its origins and its causative agent

In the April 19th, 2003 issue of The Lancet (available online 8 April), a Hong Kong infectious diseases team reported partial characterization of the agent responsible for the SARS outbreak after a study of samples from 50 hospitalized Chinese patients. In two of the patients, viral isolates were taken from a lung biopsy sample in one patient and an upper respiratory tract aspirate in the second patient. The isolates were grown in monkey kidney cells and analyzed by partial RNA sequencing using PCR (polymerase chain reaction; the RNA is actually copied into DNA for the sequencing). The partial RNA sequences led the authors to conclude the virus was related to a known bovine coronavirus and the murine hepatitis virus, MHV, both members of the Coronaviridae family. While this was a telling result it did not prove causation in the group of infected patients. Once this partial RNA sequence was obtained, however, it allowed design of short sequences of DNA (primers) that would pair specifically with the virus RNA and would then allow PCR to be carried out on the untested patient samples. Of 44 nasal samples taken, 22 showed the presence of the virus by the PCR method while hospital patients with different infections were negative for the virus sequence. Further confirmation of the novel SARS virus as cause of the epidemic was obtained by testing patient sera, 70% of whom had antibodies that were positive for the virus. In concluding their study, the authors attributed the disease to the SARS virus but were also circumspect in allowing for the involvement of other opportunistic pathogens.[13] Two other studies at around the same time were consistent with the Hong Kong conclusions. The Canadian study referred to earlier, published as the earliest of the three studies (online March 21, 2003), identified a novel coronavirus by PCR in five of nine patients, but in addition the presence of a

different virus, metapneumovirus (MPV; a virus causing respiratory disease particularly in children but from the *pneumovirinae* family; similar to respiratory syncytial virus, RSV), in five of nine patients. Since four of the patients tested positive for both viruses, this allowed for the possibility that either one or both of the viruses contributed to the SARS disease.[14]

The third study involved a more extensive analysis. Patient samples from seven different countries were analyzed by a large multinational team of infectious disease scientists from around the world, including members from the early outbreak regions of Hong Kong, Vietnam, Singapore, and Thailand. In this study, the authors used a multitude of analytical methods including virus isolation, animal studies, histology, electron microscopy, serology, and DNA sequencing. The results were in line with the Hong Kong and Canadian studies but further amplified them with significant additional pieces of molecular and epidemiological evidence for the central role of a novel coronavirus in the etiology of SARS.[15] Unlike the Canadian studies, all except one of the 19 patient samples analyzed were negative for other respiratory pathogens, the one exception being also positive for a common cold rhinovirus. A particularly strong correlate was the presence of the SARS virus in the lung tissue of infected patients, the site of lower respiratory tract damage. In concluding, the authors make two points that would have signaled concern to the clinical community. First, that SARS may be the first example of a coronavirus that causes severe disease in healthy humans. More to the point and well understood today, that the ability of different animal coronaviruses to cause serious disease in animals suggests that by adaptation they could become a serious global threat to humanity!

In correctly drawing a conclusion that the SARS corona virus (named SARS-CoV) was the singular cause of the respiratory disease that began in Guangdong, certain requirements must have been met. One of the requirements of Koch's postulates, in its modified form for viral pathogens, is to demonstrate that the candidate virus produces the disease in the original host species or a related one. Clearly infection of humans was neither desirable nor ethical, leaving nonhuman primates (NHPs) as the obvious second choice. In mid-May 2003, the well-known Dutch "virus hunter" Albert Osterhaus and colleagues from China and Hong Kong established an NHP infection model using SARS-CoV challenge in macaques that mirrored the human infection. The SARS-CoV virus isolated from the upper respiratory tracts of the diseased animals was identical to that administered and the same virus was found in lung tissue. While the authors acknowledged that other viruses may have co-contributed to the disease—the human MPV virus seen in other studies, for example, was not present in the macaques—challenge of the animals with what Osterhaus, Fouchier and colleagues called the SARS-associated coronavirus "*…thus fulfills all of Koch's postulates as the primary etiological agent of SARS*."[16]

A fundamental question that was of equally burning scientific interest was where the SARS virus came from, and if it originated in animals what biological events enabled it to move from animals to humans. Before examining the historical and current scientific opinion, some definitions are useful that will also be helpful when considering the subsequent coronavirus outbreaks in 2012 and 2019.

Evolutionary Host: an animal can serve as an "evolutionary host" of a given virus if it harbors a virus that is an ancestor of a present virus and is closely related (e.g., in RNA sequence). Such an ancestor virus would be normally highly adapted to its animal host and typically nonpathogenic.

Reservoir host: this type of host harbors an infecting virus continuously, long term. It may cause outbreaks of infection in the host but is typically nonfatal. During those outbreaks in the host, the virus will be actively multiplying and may undergo mutation that generate variants that coexist or replace the preexisting virus. In contact with humans a mutant that is fit for human infection may be passed

directly (e.g., the H5N1 strain from birds) but it may also be passed back to the animal host from humans (e.g., the H1N1 (Spanish) influenza virus that underwent the transitions pigs=>humans=>pigs).

Intermediate host: suppose the virus in the reservoir host is introduced into an intermediate host that is not adapted to it and may therefore experience disease. In the transient intermediate host, the virus which is multiplying may also be randomly mutating and by chance create a form able to cross the animal−human species barrier and thereby cause rapid and large-scale infection in humans. If the intermediate host cannot sustain transmission within members of its species, the virus may reach a dead-end. If, however, the virus can adapt to the intermediate host and establish long-term endemicity, it can become the natural reservoir for the new virus with the potential to sustain continuous human infection.

In 2003, several genetic analyses raised the possibility that the SARS-CoV virus was a hybrid virus strain, hybrid in the sense that while in its animal host several coronaviruses that might have been present could exchange parts of their respective genomes creating an assortment of virus "harlequins." This activity, known as "recombination" and a mechanism similar (but not identical) to that by which new influenza virus strains arise, was already known to occur in birds. Several other reports concluded there was no evidence for this diversification mechanism in SARS-CoV, but a mathematical biology (bioinformatics) research team from the pharmaceutical company GSK, and an evolutionary biology group from the University of Michigan, thought otherwise. The renowned evolutionary biologist David Mindell (Michigan) drew attention to the already known host-species shift behavior of coronaviruses such as between mouse and rat, chicken and turkey, mammals and Manx shearwater, and humans and other mammals. In the 2003 analysis of 36 SARS-CoV genomes, Mindell suggested an additional host-species shift, between avian and human coronaviruses. In commenting on this in their published analysis, Mindell and his coauthor made a cautionary observation:

> *"Demonstration of recombination in the SARS-CoV lineage indicates its potential for rapid unpredictable change, a potentially important challenge for public health management and for drug and vaccine development."*[17]

In the GSK team analysis one year later, Michael Stanhope (now at Cornell University) and his colleagues suggested that the coronavirus "groups" normally infective in humans and other animals had recombined with part of a coronavirus "group" only found in birds, in line with the Michigan study, and also based on RNA sequence data. In the conclusion to their publication, they stated:

> *"Mixed animal husbandry practices, in proximity to human populations, could have led to the evolution of the SARS coronavirus and facilitated its progression as an infectious disease in humans. Novel human influenza viruses are thought to have arisen from the reassortment, within porcine hosts, of avian, swine, and human influenza…"*[18]

The conclusions of both groups were a stark reminder that influenza was not the only virus that could undergo change by mixing and matching between and within multiple animal species, a smart mechanism for maintaining its global endemicity.

The exact origin of the SARS-CoV virus that infected the index person in Guangdong, China in November 2002 has been a subject of vigorous investigation, during and since the epidemic. In October 2003, some 3 months after the epidemic was declared, a survey of animals in Shenzhen wet market in Guangdong sold as animal meat for local restaurants was carried out by a combined group

from Hong Kong academic and hospital groups and the Shenzhen Centers for Disease Control and Prevention. The findings by the virologist Yi Guan and his colleagues revealed SARS-CoV-like viruses in Himalayan civets (catlike carnivorous mammals of the *Viverridae* family) but also in racoon dogs, ferret badgers and of course, humans. What was puzzling was the fact that in the virus genomes isolated from the animals, a short piece of RNA was present in one of the genes that was missing in the viral genome isolated from humans. This was significant observation and suggested the SARS-CoV was unlikely to have been transmitted to the animals from a preexisting virus in humans. Notwithstanding the fact that the virus extracted from the civets was 99% identical to the virus present in human samples, it was impossible based on the limited evidence at the time to conclude that any one of the animal species surveyed was the true reservoir for the virus. Later studies showed that Palm civets were susceptible to disease when infected by the virus, a characteristic untenable for a long-time reservoir host. It was still possible, though not proven, that civets had been infected by the true reservoir host to become an intermediate host, where multiplication of the virus during infection would have facilitated mutation and change, leading to virus variants able to infect humans through close contact.[19]

As a result of a separate study by Osterhaus in the Netherlands and the Hong Kong team, published in the same month as the Guan study, the identity of the potential reservoir host species widened even further. Osterhaus showed that in addition to ferrets, domestic cats that had not registered as positive in the Guan study were susceptible to experimental infection by the virus. While the cats were asymptomatic, they had developed antibodies to the virus and, what was more telling, were able to pass the virus to other animals in close proximity. The observation was all the more relevant since domestic cats living in the Amoy Gardens apartment block in Hong Kong where more than 100 residents had been infected with the SARS virus at the end of 2002 were also shown to have been infected with the virus.[20]

The SARS data arriving in the public domain were fast and furious, reflecting a broad scientific commitment to a potentially serious global health challenge. On October 17, 2003, the US CDCP published a report on the results of a survey of traders in three separate Guangdong wet markets carried out by public health authorities in the Guangdong Province.[21] Seven hundred and ninety-two persons, of whom 508 were traders and the remainder healthy controls from three separate locations, were tested for the presence of SARS antibodies. In the animal trader group, 13% were positive while the control groups registered between 1.2% and 2.9% positive antibodies, with the hospital workers at the higher end. As if to suggest a potential reservoir host, the highest antibody prevalence was found in traders of Palm civets (72.7%) although other animal groups traded were also high (wild boars 57%, deer 56%, hares 46%, pheasants 33%). The story was becoming complicated since none of the traders showed evidence at the time of the sampling of having SARS infection themselves, or the atypical pneumonia that had occurred in the province. This further expansion of the list of potential reservoir host species made it impossible to identify a single species with any certainty.

The survey of SARS virus RNA in the various animal species in the Guangdong wet markets, reported in the Guan publication of 2003, was extended in 2005 to a much more extensive survey of animals in the wild that included more than 300 samples from 44 different species present in natural reservoirs or country parks in Hong Kong. The purpose of the study was to attempt to identify the precursor virus of SARS-CoV that may have been circulating in the wild in a region not that far from the outbreak area in Shenzhen (about 30 km from Hong Kong). Curiously, Palm civets that had shown high levels of antibodies in the Guangdong markets were negative when tested in the wild in Hong Kong for SARS-CoV RNA. Using consensus DNA "probes" that would detect coronavirus RNA from

the various coronavirus types in the different animals tested, three species of bats from the same genus were the only species shown to be positive for coronavirus. On the basis of RNA sequence similarity, the BAT-CoV identified was grouped together with the human 229E and NL63 viruses but separate from the SARS-CoV and OC43 viruses. While this was only preliminary data, it was an indication that bats may have been the missing reservoir host for the SARS virus.[22]

In the same year, two reports confirmed the identity of bat species likely to be a reservoir for SARS or SARS-like viruses, with one study carried out in four different locations in southern China and the other in Hong Kong. The Hong Kong study was by the same team that had placed the SARS virus found in the bat species *Miniopterus pusillus* in a different virus group to that of the human SARS-CoV, suggesting a somewhat distant relationship. The second study was carried out by research institutions from Beijing, Guangzhou and Wuhan in China, and Geelong and Queensland in Australia. Their conclusions were based on a much more extensive sequencing of virus RNA samples, necessary to be sure of correct phylogenetic placement. Their findings, which involved sampling of 408 bats from nine different species, six genera, and three families, found a very close relative of the human SARS virus (92% identity at the RNA level) in the horseshoe bat genus Rhinolophidae. This genus of bats has a wide distribution containing 69 species and found from Australasia to Europe. The presence of this bat species *"serendipitously juxtaposed with a susceptible amplifying species, such as P.larvata"* (the Palm civet), as the authors put it, provided a tantalizing scenario where bats and civets in the wet markets engaged in a *pas de deux* resulting in interspecies transmission. While tantalizing, the civet as the intermediate species was not fully established, but for now it was a possibility. In concluding their analysis, the China/Australia team gave the oft repeated warning:

> *"These findings … suggest that genetic diversity exists among zoonotic viruses in bats, increasing the possibility of variants crossing the species barrier and causing outbreaks of disease in human populations …"*[23]

As will shortly become apparent, during 2020−21 little appears to have been learnt from earlier coronavirus outbreaks or epidemics, even if those did not progress to full blown global pandemics. In 2014, Jan Felix Drexler and colleagues drew attention to the global spread of BAT coronaviruses in a chilling map of the world's coronavirus research studies, chilling in the sense that the scientific community was on high pandemic alert even if not yet a reality at the time[24] (Fig. 14.3).

While the bat virus discovery was important the niggling question of why no one had isolated a "live" SARS virus from any of the bat species remained. The uncertainty remained: were bats, civets, or neither, the reservoir species for SARS? The massive culling of wet market civets at the order of the Chinese government was claimed as the reason for containing the SARS epidemic in Guangdong and the spillover in nearby Hong Kong, pointing to civets as the intermediate host culprits. But, as we saw above, the absence of SARS virus in farmed or wild civets raised seriously questions about their role as a genuine reservoir host. Over the years following the bat virus discoveries in 2005, several other reports extended the geographical spread of bat-SARS. SARS-like viruses were discovered throughout China and in horseshoe bats in Slovenia, Bulgaria, and Italy, while related viruses were found in different bat species in Ghana, Kenya, and Nigeria. In all examples, however, the bat viruses found outside China were significantly different in RNA sequence from the SARS-CoV viruses when put under the genome magnifying glass. Distant in one important respect in which the RNA sequence

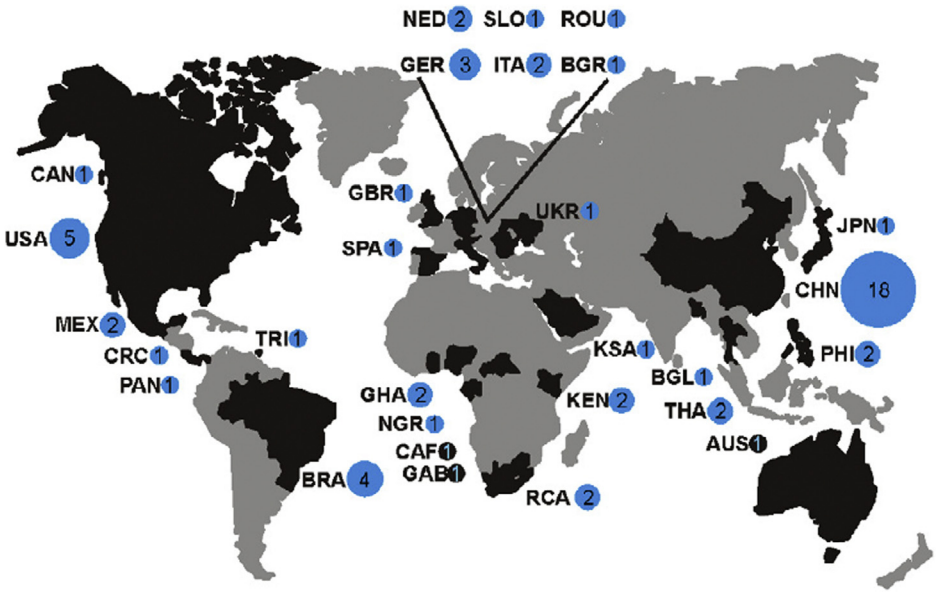

FIGURE 14.3

Distribution of bat coronavirus studies. Studies reporting bat CoV sequences by country are indicated, with the number of studies given in blue circles and adjusted in size accordingly. Country codes: *BGR*, Bulgaria; *BRA*, Brazil; *CAN*, Canada; *CHN*, China; *CRC*, Costa Rica; *GBR*, Great Britain; *GER*, Germany; *GHA*, Ghana; *ITA*, Italy; *JPN*, Japan; *KEN*, Kenia; *MEX*, Mexico; *NED*, Netherlands; *NGR*, Nigeria; *PAN*, Panama; *PHI*, Philippines; *RCA*, South African Republic; *ROU*, Romania; *SLO*, Slovenia; *SPA*, Spain; *THA*, Thailand; *TRI*, Trinidad and Tobago; *UKR*, Ukraine; *USA*, United States of America. Countries where CoV studies have been performed are in black, others in gray.

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encoding the receptor binding domain (RBD) lacked the sequence motifs necessary to recognize and bind to the human receptor for SARS-CoV, known as the ACE2 receptor. The proof of a bat species as a possible precursor to the human SARS-CoV would need to show a much closer similarity to the SARS-CoV RBD to pass the test. Convincing evidence came from a study carried out during 2011–12 in Yunnan Province, China. A team from the Wuhan Institute of Virology (WIV) and Duke-NUS Medical School in Singapore isolated a number of SARS-like viruses from fecal samples of bats but one isolate in particular was to provide a key piece of evidence. The isolate, WIV1, and named SL-CoV-WIV1 (SL for SARS-like) appeared to have the required features. It was 95% identical to the human SARS-CoV and, after purifying the virus, it was able to infect human cells via the ACE2 receptor. Further, it was neutralized by incubation with serum samples collected from humans infected during the 2002–03 epidemic. This was the first evidence of a live virus in bats having all the attributes to promote human infection.[25] But was it proof that WIV1 was the immediate ancestor of SARS-CoV? The Wuhan team in a review of the story in 2015 were rightly circumspect. There were other

differences between WIV1 and SARS-CoV genomes, despite the high RNA similarity, differences that led them to comment:

> *"Despite the fact that … WIV1 is unprecedently close to SARS-CoV in terms of RBD region and genome identity, still there are gaps between them and the immediate ancestor of SARS-CoV."*[26]

Scientific research is like that. Unraveling the biology of complex systems in the human body is difficult. Finding connections that prove a direct link between a pathogen present in a different animal species and its potential for infection in humans is the epidemiologist's nightmare. If WIV1 was not the immediate precursor of SARS-CoV, then was it possible bats were not the true reservoir host? As of 2021, the jury is still out, raising the disturbing prospect that somewhere in the animal kingdom lies a species harboring, mutating, and recombining coronaviruses that one day may again cross the species barrier and cause untold health challenges for humans, even if humans themselves do not play God with such viruses in "gain of function" experiments!

The way out: vaccines to SARS-CoV

When SARS showed the potential to become a pandemic an extensive global effort to develop a vaccine began. The WHO reported 33 different studies employing multiple methods of vaccine design that were ongoing during 2003−04. Of those, only two entered Phase1 human clinical trials, the remainder still sitting at various stages in preclinical (animal) studies.[27] There were four well known and key requirements for development of a human vaccine against SARS. First, the vaccine must induce a robust immune response in selected animal models to whatever element of the virus is inserted into the vaccine vector. Second, the native virus must be shown to induce disease in an appropriate animal that mimics the disease shown in humans, and in which the vaccine induces disease mitigation. Third, the vaccine must reproduce in humans the same level of protective immune responses seen in the animal models, and of course satisfy stringent safety requirements. Finally, when the vaccine is available, the infectious agent should still be circulating in order to assess its protective efficacy during late Phase clinical trials. An alternative type of trial, the "human challenge trial" as used for the early common cold studies, can also be employed when a virus is not naturally circulating in a population, although opinions are frequently divided on the ethics of such a route where a curative method for a potentially dangerous virus infection has not been identified prior to such trials.

The situation with SARS was frustrating. While immune responses to the various vaccine constructions were seen in mice, hamsters, cats, ferrets, and NHPs of various species, only some of the pathologies and symptoms seen in humans—upper ('flu-like) and lower (cytokine elevation, inflammation, atypical pneumonia) respiratory disease—could be repeatedly observed. In NHP studies, effective immune responses were observed but contradictory results were seen when assessing the disease similarities to human SARS infection, as pointed but by Deborah Taylor from the US FDA in 2006:

> *"Development of an animal model that mimics human disease will be the single most important advance in the development of a SARS vaccine."*[28]

Notwithstanding the uncertain behavior of the various animal models, the Chinese company Sinovac developed a SARS vaccine in which the virus, after growth through a large number of

passages (147) in green monkey kidney cells, was inactivated by exposure to the chemical β-propiolactone. It was given permission by the Chinese State FDA to begin Phase 1 trials in a small number (12) of humans, recruited during May to October 2004. The results, not published until mid-2007, were as hoped for with good immune responses to the vaccine that was also well tolerated, with no serious adverse reactions.[29] No further trials (e.g., Phase II or III) to establish efficacy were possible (presumed) since by the time the Phase 1 results had been assembled the SARS epidemic had abated.

The second of the Phase 1 trials was carried out in the US, again involving only a small number (10) of subjects. However, this vaccine developed by a team at the US NIH that included Gary Nabel, differed from the Sinovac approach. The design was a result of various experimental studies that had established the importance of the SARS-CoV spike (S) protein for entry of the virus into cells via the ACE2 receptor. In particular it had been based on preclinical studies in mice, an animal model with varying opinions as to its overlap of symptoms developed during human responses to the virus. The vaccine construct consisted of a circular piece of DNA (plasmid) into which the DNA encoding a slightly modified spike protein (a deletion of part of the S-protein sequence) from the Urbani strain of SARS-CoV had been inserted. The trial involved vaccination of 10 subjects enrolled during December 2004 and May 2005. The subjects were given three intramuscular shots of the vaccine on days 0, 28, and 56. By day 42, all who had received the vaccine had developed anti-SARS antibodies.[30] Cellular (e.g., T-cell) responses to the vaccine were not measured. Again, while the results were promising the authors acknowledged that before approval of any SARS vaccine could be envisaged, an animal model that reflected the pathogenesis in humans would be required. In the post-SARS era, development of SARS vaccines continued. The focus of many of such studies was on identifying animal models in which the virus-induced responses correlated with those seen in human infection, and where vaccination could protect the animal against subsequent virus challenge by the sort of robust antibody and T-cell responses seen in SARS-CoV infected human patients after recovery.

In a small but important step forward, a study in 2010 by the US NIH, the pharma company GlaxoSmithKline Biologicals (Belgium), and the University of Virginia, Charlottesville (US), used the Syrian Golden Hamster for vaccine testing, a species that had been shown to be infectable with SARS-CoV and that developed symptoms mirroring the human response more closely than mice, also part of the study. The vaccine studied was similar to the Sinovac vaccine, an inactivated (by beta-propiolactone) SARS-CoV strain. In hamsters, the vaccine induced robust antibody responses and protected the hamsters from subsequent challenge with live virus. The length and efficacy of the protective response could be enhanced by formulation of the vaccine with "adjuvants" developed and widely used by GSK in other vaccines.[31]

[Note: an adjuvant is typically (but not always) a mixture of lipid-like compounds (as in the GSK case) that enhances an immune response to a particular antigen. It may do so by activating what are known as "pattern recognition" elements of the innate immune system].

As we shall describe shortly, the hamster as a model of disease has become of immense help in the study of coronavirus outbreaks subsequent to SARS, including the current SARS-CoV2 pandemic. But, despite the enormous efforts of the scientific communities worldwide, there are currently no approved SARS-CoV vaccines. Many preliminary vaccine candidates exist, however, a number of which have already been tested in relevant animal models. In the absence of the circulating virus, however, further development will have to focus on appropriate animal models where "correlates of protection" (such as levels of neutralizing antibodies) that are likely to transfer across to humans are identified.[32]

Should the SARS virus emerge as yet a new coronavirus variant, different to MERS or SARS-CoV-2, the global vaccine community should be in a good position to enable rapid development of a protective vaccine. But would it or would laissez-faire political inertia and dilatory planning allow the world to drop needlessly into yet another global health chasm?

Nonsemper erit aestas—MERS attack

On June 13, 2012, a 60-year-old Saudi man was admitted to the Dr. Soliman Fakkeh Hospital in Jeddah, Saudi Arabia with a severe respiratory disease, having had symptoms of fever, cough, and shortness of breath for a week. The attending physicians who had presumed some sort of bacterial infection and administered various antibiotic cocktails without any effect, called on the opinion of the Egyptian virologist Al Mohamed Zaki whose laboratory was attached to the hospital. Screening of samples from the patient's upper respiratory tract for viruses known to produce similar symptoms was negative. After introducing the sample extracts in cultured cells, Zaki observed cellular changes typical of virus infection. Serum samples from the patient reacted strongly with whatever virus was in the cells while 2400 archived serum samples from patients with other diseases collected during the previous two years were all negative. This suggested a novel pathogen that appeared to be a very recent arrival. Additional PCR screening for other viruses was also negative but preliminary PCR by Zaki's laboratory using DNA generic primers for coronaviruses yielded positive results. But, follow-up analysis with primers specific for the recently experienced SARS-CoV virus, his current hypothesis, was negative.

To examine the possibility that this was a novel coronavirus, Zaki coopted the expertise of Fouchier and Osterhaus at the Erasmus Medical Center (EMC) in Rotterdam, sending samples of the virus mixed with cells for more comprehensive genome sequencing. The conclusion of the EMC, based on the resulting RNA sequence divergence from other known coronaviruses, confirmed Zaki's preliminary results that this was a novel species. They further found that it had 88% identity to a coronavirus that had been found in Pipistrellus bats (*Pipistrellus pipistrellus; VM314/2008*) 2 years earlier in the Netherlands.[33] The authors, which included Zaki, named the new virus HCoV-EMC/2012 placing it in the same beta coronavirus genus as the *Pipistrellus* and *Tylonycterus* bat viruses that had been identified in a 2007 Hong Kong study and named BtCoV-HKU4 and BtCoV-HKU5, respectively.[34] To prove that the new virus was a new "species," the RNA and protein sequences of the new virus had to be compared with other members of the genus. The comparison showed that both the RNA and the protein sequences were below the thresholds needed to classify it as simply a strain of the same species. A new corona virus with the potential for serious disease in humans had arrived, subject to ratification by relevant virology classification committees. But where had it come from?

The uncertainty about its origin, despite its relatively close relationship to the bat viruses would, in the event, be resolved in a somewhat unexpected manner and would explain the focused geographical location of the outbreak. In the meantime, the assumption was that it came from bats, possibly via an, as yet unidentified, intermediate animal species.

In a somewhat open interview with the well-known Egyptian virologist, Islam Hussein at the Massachusetts Institute of Technology in Boston, Dr. Zaki recalls the history of the new virus discovery, and the alleged Saudi Arabia attempts to downplay the seriousness of the outbreak.[35] As a

FIGURE 14.4

Dr. Zaki in his office in Cairo after departure from Saudi Arabia.

Photo courtesy of David Degner, with thanks.

result of sending a potentially dangerous sample to the Netherlands and publishing the analyses from patient zero without obtaining permission from the Ministry of Health, Dr. Zaki was removed from his position, his laboratory closed down and patient zero's samples destroyed. Allegedly he was later accused of having spread the virus as a result of an inappropriate laboratory safety environment. While these are only recollections from Dr. Zaki, they emphasize the dilemma academic scientists are faced with when confronted by a new pathogen whose properties are unknown, but which can harbor the potential for a global pandemic. The story of Ali Mohamed Zaki was not the first example of academic physicians or scientists taking it upon themselves to announce the discovery of a potential global health problem while experiencing pushback from government officials, and sadly would not be the last (Fig. 14.4).

The second case of the new infection came to light in London in September 2012. A 49-year-old Qatari man who had developed, and apparently recovered from, a mild respiratory disease in August while in Saudi Arabia, regressed and was admitted to a hospital in Qatar on September 8. His rapid deterioration triggered an air ambulance transfer to a London hospital. His symptoms worsened over the following days and on September 20 he required oxygen delivery directly to the blood using an extracorporeal membrane oxygenation device. Aware of the putative index case in Saudi Arabia the UK team, working with the Institute of Virology in Bonn, Germany and the EMC in Rotterdam, the Netherlands, analyzed the RNA in the patient samples and compared the partial sequences obtained with sequences from the known coronaviruses OC43, 229E, NL63, and SARS-CoV. All the comparisons were negative. When the sequence obtained was compared with sequences in a database of known coronaviruses of all species (Fig. 14.5) a hit was obtained showing that the novel virus, named London1_novel_CoV_2012, was related to the bat viruses HKU4 and HKU5 (2c in Fig. 14.5), but somewhat distant from the SARS virus of 2003 (2b in Fig. 14.5), in agreement with one of the phylogenetic

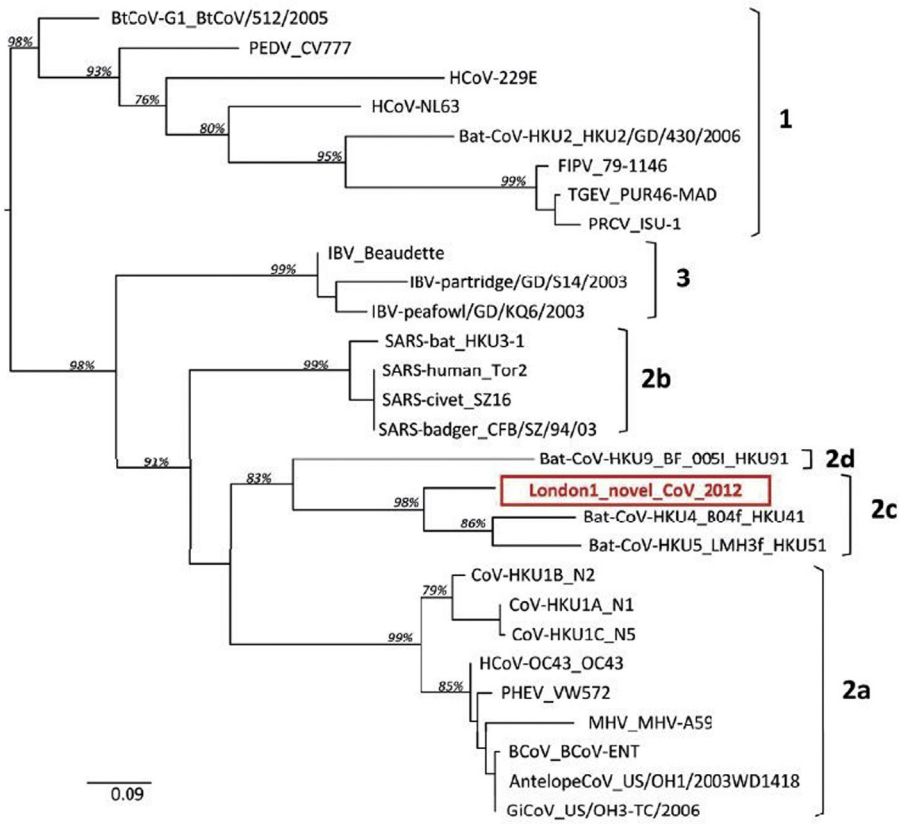

FIGURE 14.5

The relationship of the London virus to other coronaviruses known in 2012. The numbering 1, 2, & 3 corresponds to the alpha, beta, and gamma genera, respectively.

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possibilities suggested for the Netherlands HCoV-EMC/2012 isolate. But uncertainty about the exact classification, and in particular the origin of the virus, was noted by the London authors stating:

> *"The origin for this novel virus is unknown. Epidemiological human and animal investigations in the region of origin are required to distinguish between an animal reservoir that either directly or indirectly transmits the virus occasionally to humans, and a previously unrecognised endemic infection of humans…"*[36]

On December 21, 2012, the WHO issued a notice recognizing the novel coronavirus infection and noted results of its fact finding visit to Jordan. Nine cases in the Middle East had been confirmed by laboratory tests, Qatar (2), Saudi Arabia (5), and Jordan (2). Of the nine cases, five had died. The already published scientific evidence had confirmed this was a novel virus, that its mortality rate was

high (approaching that of Ebola) but, by the end of 2012, the infected numbers were still very low. While there was evidence of intrafamily transmission, the WHO position remained at the "monitoring" stage,[37] despite the fact that serious undiagnosable respiratory infections had been reported in April of 2012 in Jordan, 2 months before the putative zero case in Saudi Arabia and retrospectively diagnosed as due to the new coronavirus. By May 2013, the number of cases was still low, at 34 laboratory-confirmed reports but the mortality rate was at or near 60%. The infections were still largely restricted to the Middle East (Jordan, Saudi Arabia, Qatar, and UAE) with a small number of cases in the UK and France arising from travel to and from the Middle East. In its May 2013 report, the Corona Virus Study Group, a subgroup of the International Committee on Taxonomy of Viruses (ICTV), noted that there was no evidence at the time of sustained community transmission, but that the possibility of adaptation by the virus that facilitated such transmission was not to be ignored. On the question of the, as yet uncertain, virus origin, and questioning the possible direct infection from bats, the Study Group expressed the view:

> *"A more likely scenario is that a single variant from a spectrum of related betacoronaviruses in bats successfully crossed over to and rapidly established itself in (an) intermediate animal host species (at least in the Middle East), with subsequent incidental spillover into the human population."*[38]

In an attempt to rationalize the identity of the novel virus which had been given different labels in the scientific literature, often reflecting where the analyses were carried out, in the same report of May 2013, the Study Group under the auspices of the ICTV took the decision to officially name the novel virus, MERS-CoV (Middle East Respiratory Syndrome CoronaVirus), a name endorsed by the discoverers, other researchers of the virus, the WHO and the Ministry of Health of Saudi Arabia. To reflect the origin of any new or existing strains, a notation was proposed using the example: MERS-CoV Hu/JordanN3/2012 but for the time being noting that the virus was not yet confirmed as a "human virus."

By August 2, 2013, a total of 94 cases of MERS-CoV had been confirmed with 46 deaths. The seriousness of this disease had spawned several studies to identify the zoonotic species passing the virus to humans. The assumption was that it was not a direct bat => human transmission but more likely to be via an intermediate animal host, given the low human-human reproduction number (R_0 number) observed. The first important breakthrough was published in the Lancet on August 9, 2013, by a joint team from The Netherlands, Germany, Spain (Canary Islands), and Oman.[39] In this study, the team had screened camels, llamas, goats, sheep, goats for the presence of antibodies specific for the S-protein of MERS-CoV, and for their reaction with SARS-CoV and OC43 as controls for specificity. Two camel locations were selected, Oman in the Middle East and the Canary Islands, the sampling taking place April–June 2012 (Canary Islands) and March 2013 (Oman). The data were convincing and pointed to the Omani dromedary camels as a potential intermediate host, allowing for the possibility that the virus detected may not be the identical virus infecting humans since no viral RNA sequences were then available for comparison. But dromedaries were certainly a candidate, even if not the true reservoir host, given that 100% of the Omani animals tested were positive for MERS-CoV antibodies, came from different owners and locations, and were often used as racing camels with frequent human contact. The authors speculated that the low percentage of positives (<10%) for the Spanish camels may have been due to vestigial immunity, such as from infection by *Pipistrellus* bats and/or *Rousettus aegyptiacus* (Egyptian fruit bats), both present in the Canary Islands. Confirmatory

studies of anti-MERS antibodies in dromedary camels in Egypt (Sept 5, 2013), Saudi Arabia (Dec 12, 2013) and Jordan (April 2014) rapidly emerged while other animals screened, such as sheep and goats, were largely negative. A study of dromedaries in Kenya (frequently exported to the Arabian Peninsula and Egypt) also showed positive antibodies for the MERS virus. What was particularly new about this latter study was that archived serum samples collected between 1992 and 2013 showed positive antibodies across the range of dates, suggesting that the MERS virus may have been circulating in dromedary camels for decades.[40]

But while all these observations were important, they still failed to cement a direct connection between the presence of dromedary serum antibodies and the transmission of live virus to humans, a requirement for a candidate intermediate host. Several things needed to happen. First, active virus RNA should be detectable in the camels, direct infection by camel-to-human contact should be demonstrated, and it should be observed in multiple locations at different times. MERS-CoV RNA had already been reported in camels in Saudi Arabia and Egypt but no direct relationship with the sequences found in humans had yet been reported.

In November 2013, a Saudi camel herder who had been caring for sick camels was admitted to hospital in Jeddah with respiratory symptoms. Analysis of samples from both the patient and the camels showed close similarity in fragments of viral RNA sequence isolated from two of the camels being cared for. Sequencing of the patient and camel sample by the Sanger Institute, Cambridge, UK and the Institute of Virology in Bonn, Germany confirmed that the human virus was highly likely to have originated in the farmed camels that had also been shown to be seropositive for MERS-CoV. The authors, while concluding that MERS infections in humans "may be" directly acquired from camels, were forced to concede that because this was a retrospective study the camels could have caught the virus from another animal source.[41] In the same scientific journal, the same month and the same issue similar results were reported from a study in Egypt in which camels imported for slaughter from Sudan and Ethiopia were positive for MERS-CoV. On RNA analysis the sequences of the MERS virus in the camels had a similarity of 98%−100% at the amino acid level to the original MERS-CoV/EMC(2012) human sequence. Commenting on the epidemiological consequences of such a widespread incidence of MERS infections, while cautioning that the transmission to humans appears to be uncommon, the authors of this study, from Hong Kong, Egypt, and the US, advised:

> *"The detection of MERS-CoV in dromedaries in Egypt, in animals imported from Sudan and Ethiopia, suggests that cases may occur in humans beyond the Arabian Peninsula. MERS CoV diagnostic tests should be considered for all patients with unexplained severe pneumonia in Egypt, northeastern Africa, and beyond."*[42]

The debate as to whether camels were the intermediate host in the transmission of MERS-CoV continued, despite the mounting evidence of a direct connection. A dromedary-to-man hypothesis published in July 2014 was criticized in the same issue of the scientific journal for the paucity of evidence for the "direct connection" assumption, noting that neither human-to-camel nor human-to-human transmission had been properly studied.[43,44] While it was true that thus far the apparent R number in the human population was low, the potential for global spread of the disease was brought into sharp focus in May of 2015 when the first Korean patient was identified. The 68-year-old man had been traveling in Bahrain, Qatar, and Saudi Arabia and after returning home to Seoul, Korea was admitted to, and transferred between, different medical centers and hospitals. Five days after entering

Korea, he was diagnosed with MERS-CoV. Identification of his immediate family and various medical staff contacts led to a total of 108 infected persons of whom nine died. One of the medical staff involved who had not been confirmed to have been in direct contact with the index case had traveled to China via Hong Kong, triggering a contact tracing activity. Here was a clear case of human-to-human transmission in Korea but the origin of the index person's infection in the Middle East was of course unknown, although it appears unlikely to have been directly from dromedary camels. On June 2015, the WHO published a risk assessment report responding to the Korea situation by which time 1338 confirmed cases had been reported to the WHO since 2012, 166 of whom were from the recent Korea outbreak with 24 deaths (>14%). In all, 26 countries had reported infections, the majority of which (>85%) were in Saudi Arabia.

While all this was going on a multinational team from Saudi Arabia, Hong Kong, Australia, and Egypt published an important study that identified not just a single MERS virus in camels but two betacoronaviruses, and an alpha coronavirus previously well known (229E) cocirculating in the animals, with the highest infection rates present in camel calves. Of particular relevance to the host debate was the fact that the RNA sequences of two MERS-CoV variants circulating in Korea were 99.6% and 96.8% similar to the full genome of a camel MERS virus from Saudi Arabia sampled in March 2015. In the summary of their publication in the prestigious Science journal, the authors took a high line on the origin of MERS, stating:

> *"Camels therefore serve as an important reservoir for the maintenance and diversification of the MERS-CoVs and are the source of human infections with this virus."*[45]

The stuttering path to a MERS-CoV vaccine

Between April 2012 and September 2019, the WHO had recorded 2468 confirmed cases of MERS-CoV in 27 countries and 851 deaths, giving a mortality rate of a little over 34%, mainly occurring among those in the 50–80 years age group. The vaccine development dilemma was that over a period of 7 years there had been too few cases, peaking in 2013–14 and again in 2015, and compared with annual influenza and other infectious diseases MERS was not even close to an epidemic never mind a pandemic. But it was a lethal pathogen killing a third of those infected, so a reasonable question was, at what level of infection and spread would a robust response from the vaccine community be triggered? And what was the WHO position on such an investment? As with SARS, although with even smaller numbers of cases, the biggest clinically important issue with MERS was the absence of regions of widespread infection where efficacy trials with candidate vaccines could be carried out. Also, and understandably, the WHO was not in a position to declare a global emergency on just a few thousand cases with no knowledge of how long the virus would persist and what the risk was of a rapid growth in cases. The prevailing view among research scientists was that the virus could be spread human to human, almost certainly via respiratory aerosols, but its contagiousness was not as high as many other viruses. Notwithstanding this uncertainty, those research teams with experience of vaccine development, or with animal models for other virus infections, took the decision to begin studies with candidate vaccine development. Many of the studies were carried out in mice, a species that while frequently used in animal research would not allow triggering of the FDA "animal rule" since, as disease models, mice had been seen to be refractory to MERS-CoV challenge and typically lacked the

pathological responses seen in humans, despite and perhaps partly because of, producing robust antibody responses. The first attempt to move to a NHP model was made by a large collaborating team in the US that involved the US NIH, the Walter Reed Army Institute, universities in Florida and Tennessee and the pharma company Sanofi-Aventis. Their approach was twofold. DNA plasmid constructions were made that contained three different constructs based on the MERS-CoV spike protein (S) gene and two that used the actual S-protein itself. A modified MERS-CoV virus (pseudovirus) that eliminated the need for a high biosafety facility for testing the route of entry of the virus and its prevention by addition of neutralizing antibodies was also developed. After the mouse immunizations monoclonal antibodies were isolated, and for one antibody an x-ray structure of a complex between the antibody and the region of the MERS-CoV S-protein involved in binding the virus to its target cells, the S-RBD, was determined. Inspection of this structure defined exactly which part of the RBD the MERS-CoV S-protein uses to bind its cell target, known as dipeptidyl-peptidase-4 (DPP4), a receptor different to that used by SARS-CoV. Further, when mutations in the RBD were artificially introduced within this region, binding of the originally neutralizing antibodies was either reduced or eliminated, providing a vivid example of how coronaviruses can easily escape immune surveillance by a simple mutation. While MERS-CoV was not so far known to easily generate "escape mutants," that behavior would soon become a nightmare scenario to control for another coronavirus soon to appear. The three best protocols from the mouse vaccination experiments were tested in Indian macaques (*Macaca mulatta*), and robust antibody responses were seen after a double immunization protocol. While macaque symptom comparison with human disease was not totally congruent, the vaccinated animals showed a significant reduction in lower lung pulmonary disease than unvaccinated animals when challenged with native virus. The completion of this work in early 2015, even before the Korean outbreak, was a testament to the improving effectiveness of vaccine development, even if not as yet tested in human trials.[46] Such rapidity would need to take another major step up within a few short years.

Despite the promising results in animal studies, human trials with MERS-CoV vaccines have made slow progress, largely because efficacy testing in the absence of disease is impossible, unless the "ethically debatable" human challenge approach is used. Table 14.1 summarizes the currently ongoing or completed studies as published by the US FDA at the time of writing.[47]

The first human studies initiated at Phase I level were carried out in the US with 75 subjects at the Walter Reed Army Institute between February and July 2016, just 12 months after the outbreak in Korea. The vaccine, GLS-5300 and developed by two cooperating companies, GeneOne Life Science (Korea) and Inovio Pharmaceuticals (US), was a DNA plasmid vaccine that incorporated the entire DNA for the MERS-CoV spike (S-) protein that had already been shown to be broadly immunogenic in mice, camels, and NHPs. The Phase I trial, involving several US institutions and academic centers, showed the vaccine to be well tolerated with no serious adverse effects. It induced antibody and cellular (T-cell) immunity in the majority of subjects, with retention of both close to one year after vaccination. The results were published in 2019, well after the MERS outbreaks had fallen to small sporadic cases mainly in Saudi Arabia.[48] Armed with a promising set of data it was reasonable the group would plan to initiate a Phase II study to further evaluate immunogenicity and tolerability, and importantly efficacy, either in the Middle East or in Korea, or both, where cases of MERS were still occurring. As can be seen from Table 14.1, no efficacy trials have been completed and many were not started, largely down to the impossibility of establishing a trial within a population where the MERS virus was no longer circulating. As a result, the Phase 1/II trial of GLS-5300 involving 60 subjects

Table 14.1 A summary of vaccines for MERS in clinical development as of October 2021.

Status	Description of trial	Intervention	Locations
Phase 1 Terminated (Oct 2021)	Safety and Immunogenicity of a candidate MERS-CoV vaccine (MERS001)	ChAdOx1 MERS	Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, UK
Phase 1 Completed (May 2019)	Safety, Tolerability, and Immunogenicity of Vaccine candidate MVA-MERS-S	Vaccine candidate MVA-MERS-S	CTC North GmbH & Co. KG, University Medical Center, Hamburg-Eppendorf, Hamburg, Germany
Phase 1 Completed (Nov 2020)	A Clinical Trial to Determine the Safety and Immunogenicity of Healthy Candidate MERS-CoV Vaccine (MERS002)	ChAdOx1 MERS	King Abdulaziz Medical City, National Guard Health Affairs Riyadh, Saudi Arabia
Phase 1 Recruiting	Safety and Immunogenicity of the Candidate Vaccine MVA-MERS-S_DF-1 Against MERS	Biological: MVA-MERS-S_DF1 - Low Dose Biological: MVA-MERS-S_DF1 - High Dose Other: Placebo	CTC North, Hamburg, Germany Erasmus Medical Center, Rotterdam, Netherlands
Phase 1 (vaccine) Phase 2 (other placebo). Recruiting	Study of Safety and Immunogenicity of BVRS-GamVac	Biological: BVRS-GamVac Other: Placebo	Research Institute of Influenza, Sankt-Peterburg, Russian Federation
Phase 1 (vaccine) Phase 2 (other placebo). Recruiting	Study of Safety and Immunogenicity of BVRS-GamVac-Combi	Drug: BVRS-GamVac-Combi Other: Placebo	ECO Safety Sankt-Peterburg, Russian Federation
Phase 1 (vaccine); Phase 2 (Electroporation). Completed Apr 2020	Evaluate the Safety, Tolerability, and Immunogenicity Study of GLS-5300 in Healthy Volunteers	Biological: GLS-5300 Device: Cellectra 2000 Electroporation	Seoul National University Bundang Hospital, Seongnam, Republic of Korea, Seoul National University Hospital, Seoul, Republic of Korea
Phase 1 Completed Sept 2017	Open Label Dose Ranging Safety Study of GLS-5300 in Healthy Volunteers	Biological: GLS-5300	Walter Reed Institute of Research, Silver Spring, Maryland, United States

Source: Adapted from data at www.clinicaltrials.gov. Accessed 25 October 2021.

became a trial of immunogenicity, safety, and tolerability, but in addition explored two different methods of vaccination, intradermal (commonly used) and a novel technique of "electroporation." This latter technique, frequently used in the laboratory to deliver DNA into cultured cells, sends electrical signals to the cells (e.g., muscle) during injection of a DNA vaccine causing the cell membranes to become more permeable and allowing more effective entry of the DNA into the cells. The study was

carried out by GeneOne Life Science, Inovio Pharmaceuticals and the International Vaccine Institute based in Seoul, South Korea. According to the FDA records, the trial was completed in April 2020 but as of the time of writing no published results are available.

In December 2017, a different vaccine candidate began human Phase I trials in Hamburg, Germany. It mirrored a vaccine developed during the SARS-CoV outbreak using a modified vaccinia virus (MVA) that incorporated the DNA sequence encoding the spike protein from MERS-CoV. Again, the ability of this vaccine to elicit a good immune response in mice and its ability to inhibit MERS-CoV production (replication) in dromedary camels postvaccination had already been tested. The study, involving several German infectious disease centers and the EMC in Rotterdam, had recruited 26 subjects to receive prime-boost vaccinations, split into a low-dose and a high-dose group. Most from each group received the second boost dose. Adverse events were nonserious, and a high percentage of subjects in the low and high dosing groups gave good immune responses: antibody response (low dose 75%; high dose 100%); T-cell responses (low dose 83%; high dose 91%). However, and disappointingly, immunity declined quite rapidly after vaccination reaching baseline levels after 6 months, as had been seen in earlier SARS-CoV vaccine trials.[49] No further studies were anticipated, largely due to the fact that by its completion date (May 2019) and then data publication date (April 2020) another more serious coronavirus had arrived on the scene.

The third class of vaccine, the name of which is recognizable today in intimate detail to most of the world, was constructed by the Oxford Jenner Institute (UK) team using a non-replicating chimpanzee adenovirus that carried the MERS spike protein DNA, a vaccine formula that had been tried and tested for a number of other viruses, including influenza and ebola. The vaccine, ChAdOx1MERS, was tested in a small Phase I trial on 24 subjects in Oxford between March and August 2018. The subjects were aged 18−50 years and split into three groups for low, intermediate, and high dosing. The primary objective was to assess safety and tolerability, but testing the level, and some degree of longevity, of immune responses were also measured. Both antibody and T-cell responses were seen in most subjects at what could be interpreted as potentially protective levels, but until efficacy trials had been carried out this effect could only be extrapolated from earlier animal studies where the vaccine had mitigated the effects of live virus challenge. The one possible concern noted by the authors of the published data was the frequency of "moderate and severe adverse events" at the high dose of the vaccine, although all resolved without serious effects.[50] At the close of the publication the UK, Germany and South Korean collaborating group expressed their intent to progress the study to Phase 1b/Phase 2 trials, but this time with middle Eastern health workers, camel herders, and susceptible elderly persons. The trial was initiated in late 2019 and completed in late 2020, but results are not yet available, at the time of writing.

In a "Comment" on the Phase I clinical studies using the ChAdOx1 and MVA-MERS-S vaccines Modjarrad and Kim from the Walter Reed Army Institute and the IVI in Seoul, respectively, drew attention to the difficulty of drawing efficacy conclusions based solely on immunogenicity data, particularly when the data are measured or analyzed differently, as in the two studies considered in their commentary.[51] The key question, always relevant, is whether immune responses seen in response to a vaccine correlate with disease protection. This uncertainty can be resolved either by identification of correlates of protection (e.g., neutralizing antibodies as discussed above in the case of 2003 SARS), or through proper Phase II or Phase III efficacy trials during an outbreak or epidemic. With MERS the latter is currently still not feasible, good and bad of course.

Two alternatives to vaccines when an outbreak has already arrived are treatment either with convalescent serum from infected and recovered patients (or from animal sera) containing antibodies

against a particular virus or other pathogen, or with specifically generated human monoclonal antibodies that block the virus activity. The human convalescent serum approach has been shown to be useful only for small numbers of treatments due to the limited quantities of highly immune serum that can be obtained from infected and then recovered human patients. However, a technology developed in the 1990s to enable replacement of animal genes by human genes came to the rescue of this type of antibody therapy. The remarkable immunology development, initially worked out in mice, was the generation of "transgenic" animals whose endogenous antibody genes are replaced by the equivalent human genes, a technology that has allowed the production of human antibodies specific for any antigen in a nonhuman species. Such a technology was used in the first development of human antibodies to MERS-CoV, produced by immunization of transgenic bovine cattle with spike proteins from two different strains of MERS-CoV formulated as nanoparticles by the US vaccine company, Novavax. The "transchromosomic cattle" had been created by deletion of the normal bovine chromosomal region encoding the antibody genes and replacing it with the equivalent human chromosomal region. Using these cattle, specific anti-MERS human polyclonal antibodies from each of the MERS-CoV strains used were isolated and characterized. The characterization of the antibodies was carried out using a separate transgenic animal model, this time in mice. It was already known that mice were refractory to MERS-CoV infection. It was surmised that by replacing the mouse protein on the cell surface (DPP4) that mediates virus entry by the equivalent human protein, viral infection in the mouse might better mimic the human disease. This turned out to be the case and in 2016 an extensive US study led to selection of one of the bovine preparations (SAB301; produced by SAB Biotherapeutics Inc, South Dakota, US), on the basis of its virus neutralization in mice.[52] The other important reason for using transgenic cattle was that the quantity of antibodies that could be isolated per animal was far larger than could be obtained from human patients, reminiscent of the use of horse serum during WWI for preventing tetanus. The perceived advantage of using a "polyclonal" antibody was the fact that many different antibodies recognizing different parts of the MERS spike protein would be present, in theory increasing the chances of neutralizing the virus and any escape mutants that might be developing.

A Phase I trial of SAB301 was approved and carried out at the US NIH, starting in June 2016. The objective of the trial was to establish safety and tolerability of the antibody preparation. SAB301, infused into healthy volunteers, with different groups receiving escalating doses, was shown to be safe, although adverse effects were observed to be more severe in the highest doses.[53] The results were published in January 2018, and the authors were sufficiently encouraged to suggest that further clinical investigation of the antibody treatment would be pursued. In the event, no further results have been published to date (at the time of writing).

The second attempt to identify a passive antibody approach was made by the US biotechnology company, Regeneron Pharmaceuticals. Its approach was different. The company used two different proprietary transgenic mouse strains, one that had been engineered to replace the mouse antibody genes with the human antibody genes enabling the generation of human "monoclonal antibodies" (MAbs; antibodies with a single specificity for a discrete region on the spike protein of MERS-CoV), and a second mouse strain that had the human rather than the mouse DPP4 receptor protein, as with the SAB301 study. In an extension of already completed and published preclinical studies, the protective effect of two MAbs, REGN3048 and REGN3051, was confirmed leading to reductions in viral load, inflammation in the lung and organ and tissue pathology. The Phase I human safety trial was carried out in the US between February and September 2018, involving 48 volunteers at a single location. The subjects were split into six groups of six in the active arm (coadministration of the two MAbs at various doses) and a placebo group of 12. The results, published in early 2021, were promising with a good

safety profile. The antibodies were well tolerated with no dose-limiting adverse events or serious reactions, supporting further development.[54]

By late 2021 and as with SAB301, no further information on these promising passive immunotherapy approaches is available. That is not surprising given the disappearance of MERS-CoV except for small sporadic outbreaks, and the arrival of an even more complicated coronavirus we will come to shortly that would bend the minds of virologists, epidemiologists, immunologists, and clinicians to unforeseen limits of scientific creativity, and turn global health systems and economies upside down.

As of end of May 2021, the WHO Eastern Mediterranean Regional Office MERS Situation Update for the Middle East had recorded 2574 laboratory-confirmed cases of MERS with 886 deaths giving a mortality rate of 34.4%.[55] The majority of cases (2174) were reported from Saudi Arabia with a mortality rate slightly higher than the average at 37.2%. The global update from the United Nations Food & Agriculture Organization (FAO) on March 17th, 2021, began its report with the statement:

> *"Middle East Respiratory Syndrome Coronavirus (MERS-CoV): zoonotic virus with pandemic potential."*[56]

The most worrying data, however, relate to the geographical spread of the virus in animals, detected by serology and/or virology. That spread, covering four out of the five continents, is in addition to countries with confirmed human cases and includes Bangladesh, Burkina Faso, Chile, Ethiopia, Iraq, Israel, Kenya, Mali, Morocco, Nigeria, Pakistan, Senegal, Somalia, Spain (Canary Islands), Sudan, and Uganda, all with potential zoonotic species. While the incidence of MERS-CoV cases between 2012 and July 2021 (Fig. 14.6[57]) shows a welcome decline that may give comfort to many epidemiologists,

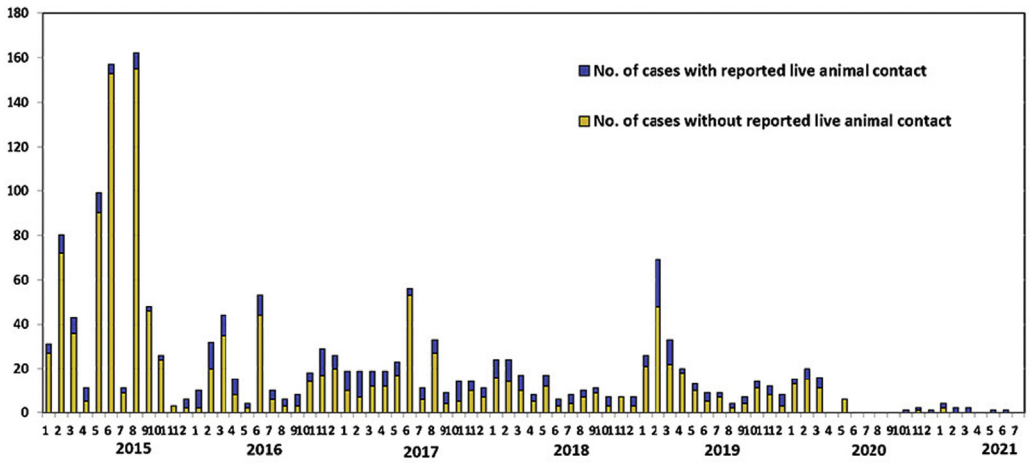

FIGURE 14.6

Human epidemiological timeline (with cases reporting animal exposure in blue), by month of disease onset (from January 2015 to July 2021).

Reproduced with permission from the United Nations FAO—Reference 57.

the potential for a further zoonotic transmission cycle somewhere is the world is ever present, a fact that should be registered indelibly in the minds and vaccine planning documents of all governments worldwide, and especially the WHO.

SARS-CoV2—the darker side of coronaviridae

On December 12, 2019, a 62-year-old male Chinese office worker presented at a hospital in Hubei Province, Wuhan, China with an unidentified pneumonia-type disease, symptoms of which began 4 days earlier. The patient recovered and then returned to hospital 15 days later along with his wife, both having fever. They both recovered, the man for the second time. By the end of December, clusters of new cases were reported by the Wuhan Municipal Health commission, all presenting with fever, dry cough, breathing difficulties (dyspnoea), headaches, and pneumonia. Diagnosis on the basis of chest x-rays showing pulmonary infiltrates, and lack of any effect by antibiotics led to the conclusion the infection had a viral etiology. Samples from the seven ICU patients in the December clusters were provided to the WIV for genome sequencing and diagnosis. Of the seven patients analyzed, six appeared to have direct connections to the Huanan Seafood Wholesale Market, either as sellers or delivery personnel. The obvious early conclusion was that the virus had somehow arrived at the market in some zoonotic animal species, pangolins being an early possible culprit, and had then been passed on to the local workers. On 1 January, WHO set up its Incident Management Support Team, placed on an emergency footing to deal with the outbreak. By January 3, 2020, 44 confirmed cases had been identified in Wuhan, 11 of them serious and 73% of whom were male with a median age of 49 years. Of the 44 patients, more than half had been exposed to the Huanan seafood market. Patients in ICU were already showing elevated levels of various cytokines, something that would later be referred to as the "cytokine storm." Over the next few days, the WHO Twitter account released the following tweet:

> *@WHO Jan 4, 2020*
>
> *#China has reported to WHO a cluster of #pneumonia cases — with no deaths — in Wuhan, Hebei Province…Investigations are underway to identify the cause of this illness.*

On January 5 WHO published its first update via its web site:

> *"Based on the preliminary information from the Chinese investigation team, no evidence of significant human-to-human transmission and no health care worker infections have been reported…-WHO advises against the application of any travel or trade restrictions on China based on the current information available on this event."*[58]

In retrospect some have suggested this was an unfortunate delay in assessing the seriousness of what had already been recognized by local physicians as a dangerous disease of unknown etiology. Historically neither SARS-CoV nor MERS-CoV had developed into global pandemics. Reasons enough for caution on the part of WHO before sending the world into a pandemic panic? Historians of this pandemic will surely have differing views. The key to learning the lessons will be to avoid the frequent creeping determinism that sometimes leads to biased historical accounts.

On January 10, 2020, the genome sequence of the Wuhan virus was posted online at virological.org by Edward Holmes a collaborator with Yong-Zhen Zhang at Fudan University in China, followed by a notification from Holmes on Twitter:[59]

While this rapid release of the virus sequence was allegedly against the instructions of the Chinese authorities, Holmes's interpretation at the time of their response was that the Chinese may have preferred their publication to come out first, or simply were afraid of causing global panic by such a rapid and instantly available release. Others have had and continue to have different opinions. Just 10 days after the genome sequence release, the joint teams from WIV and Beijing submitted their analysis to the international journal Nature, resulting in publication online 2 weeks later (Holmes was not a coauthor). The publication contained a mass of information in addition to the genomic sequence and included critical studies showing that the novel virus used the same ACE-2 cellular receptor to enter human cells as SARS-CoV. In addition, using well-known sequence comparison tests, Zhou and colleagues identified a known bat genome that was more similar to SARS-CoV-2 than the 2003 SARS-CoV genome or some other bat coronavirus genomes. Serum samples from the patients were able to neutralize the virus after it had been grown to sufficient levels for testing in human cells in the laboratory, and the virus morphology showed the established spherical-spike morphology characteristic of other coronaviruses. The results led to the conclusion this was novel coronavirus, initially named 2019-nCoV.[60] The day after the virus genome announcement a first case of infection in Thailand was reported, the person having returned from Wuhan. By 22 January, the WHO indicated it was still uncertain about the extent of human-to-human transmission, despite its extensive experience with many other respiratory diseases. A WHO Emergency Committee (EC) meeting convened during 22−23 January but then decided to wait a further 10 days before meeting again to consider the global implications of the disease. By 30 January in advance of the 10-day waiting plan, the EC met and declared the outbreak was a Public Health Emergency of International Concern, but not yet a pandemic despite the fact that almost 8000 cases had been reported worldwide, albeit the majority in China. On 11 February, the WHO and the UN gave the disease the official name COVID19. Further meetings with Chinese health officials and scientists in Beijing, Wuhan and other cities took place in late February but it was not until 11 March that the WHO declared the disease a pandemic, 2 months after the genome of the new virus, its known tropism for human cells, its serious lower respiratory tract symptoms, and the rapidity with which it was spreading, was known. The responses of different governments were varied. Wuhan went into lockdown imposing a *cordon sanitaire* in late January

while Taiwan and South Korea, unlike many other countries, placed significant emphasis on efficient test and trace, banning international entry as an early precautionary measure. Most of the ROW imposed lockdowns of varying degrees during the second half of March by which time the pandemic was reaching elevated transmission with more than 60,000 cases and more than 3000 deaths.[61] As early as 26 February, daily cases outside China exceeded those within the country, some 2 weeks before the WHO declared COVID19 a pandemic.

But exactly from where and how did this new virus arrive? The evidence based on extraordinary forensic analyses by the most sophisticated global scientific endeavor ever mounted for examining the origin of a pathogen has so far failed to identify an immediate zoonotic source from whom the earliest infection was transmitted to humans while at the same time providing circumstantial evidence for elimination of certain possibilities.

A further dimension of the advice given by WHO for reducing transmission of the virus relates to the interminable debate about whether transmission can occur via aerosols. In 2016, the well-respected virologist Ron Fouchier from the EMC in Rotterdam and a major figure in the analysis of the MERS-CoV outbreak noted in his review on airborne transmission of viruses:

> *"We know that respiratory viruses may spread via small aerosols (generally defined as <5μm) or larger respiratory droplets upon coughing, sneezing or breathing (hereafter collectively referred to as 'airborne transmission') or by direct person-to-person contact or via contaminated surfaces or fomites."*[62]

In their extensive analysis of 41 patients in Wuhan, reported (online) in The Lancet on January 24, 2020, Huang and 27 colleagues from Wuhan and Beijing made the following observation:

> *"We are concerned that 2019-nCoV could have acquired the ability for efficient human transmission … Airborne precautions, such as a fit-tested N95 respirator, and other personal protective equipment, are strongly recommended."*[63]

In July 2020, Nature magazine published a "Feature" article that examined the prevailing scientific view and the WHO position on transmission of COVID19 via aerosols. Eight months after the arrival of the virus in the human population, 7 months after the suggestion the virus was airborne, and 4 months after the WHO had declared the pandemic, arguments about whether or not the virus was "in the air," for and against, were still raging. A WHO statement from Benedetta Allegranzi, technical leader of the WHO task force on infection control, commented that although the WHO acknowledged that airborne transmission is plausible, current evidence falls short of proving the case.[64] Previous experiences of airborne infection, not just by respiratory viruses such as influenza but case reports for poliovirus and even Ebola, could have triggered a more informed message perhaps during the WHO pandemic announcement to the world on that Wednesday in March 2020. An accompanying message could have been that, given it was a respiratory tract pathogen, COVID19 was highly likely to be transmitted by droplets and potentially aerosols, based on past experiences with similar viral pathogens. The clear advice could then have been that avoidance of close proximity to infected persons and perhaps the wearing of masks may be critical for reducing transmission. This view is not really a case of present-day hindsight but rather, when looking back at the state of knowledge at the time a relevant question is whether the delayed advice reflected a lack of foresight by medical agencies and governments alike during the early stages of the outbreak.

The origin of SARS-CoV-2

There has probably never been a more comprehensive or more divisive investigation of a virus history than has surrounded the search for the origins of SARS-CoV-2. As a result, conspiracy theories have gained wide attention and generated by some of the world's most senior government officials in some of the most sophisticated democracies. The phrase "The China virus" rapidly became endemic under the President Trump administration, despite the good offices of the WHO in selecting a name for the disease, COVID19, that carried no implication of origin and certainly none suggesting any direct involvement of the WIV in Wuhan. There do exist, however, vacuums in the scientific information gathered that have led on the one hand to uncertainty about its exact geographical origin, and on the other deep concern that a viable intermediate animal host of COVID19 responsible for transmission to man has not yet been unambiguously identified. These concerns have fueled the emergence of alternative explanations, the supporters of which should note that a validated zoonotic host (other than bats) for the 2003 SARS-CoV with direct infectivity for humans has still not been unequivocally identified.

The question of whether SARS-CoV-2 emerged from a natural (animal) source, or from laboratory virus manipulations at the WIV that somehow then escaped, was attacked head on by Kristian Anderson and colleagues from the US, UK, and Australia in April 2020.[65] Their arguments were somewhat early in the process of data collection but are worth noting, perhaps as a baseline for subsequent theories and arguments. First, the similarity to bat SARS-CoV-like viruses suggested that bats were probably the reservoir host. Sequences of bat viruses by the WIV in Wuhan in early February 2020 had thrown up a close homolog to SARS-CoV-2 from the *Rhinophilus affinis* bat, RaTG13, 96% identical to SARS-CoV-2 at the nucleotide (RNA) level (Fig. 14.7).

While such a close similarity is persuasive of the argument that bats are the primary reservoir for SARS-CoV-2, they are unlikely to have been the species directly transmitting the disease to humans.

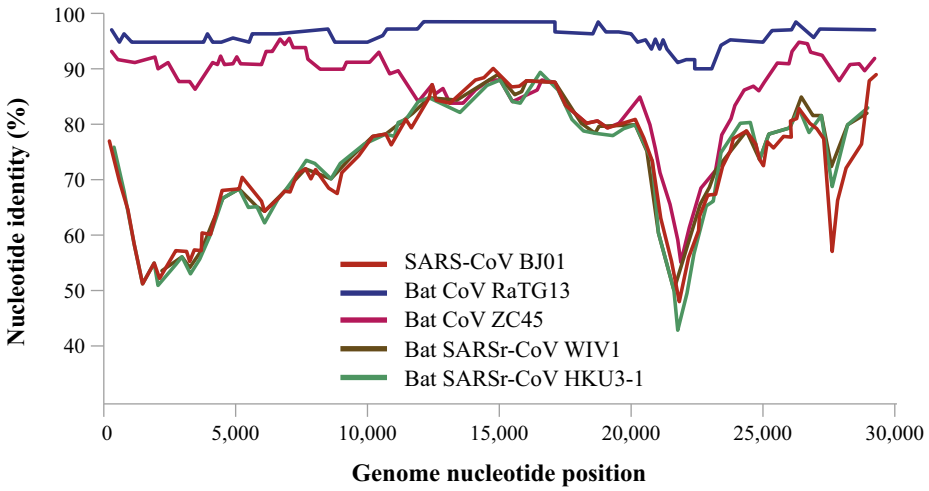

FIGURE 14.7

Full length genome sequence identities (nucleotide %) of SARS-CoV-2 Isolate 2019-nCoVWIV04) versus different bat virus sequences and SARS-CoVBJ01 (=2003 SARS-CoV). The closest match was to RaTG13.

Reproduced under CC BY from Reference 60.

There are two crucial differences between RaTG13 (and other bat viruses) and SARS-CoV-2. First, bat viruses, including RaTG13, lack the specific amino acids in the S-protein required for recognition of the human ACE-2 receptor. Second, a critical feature of SARS-CoV-2 that is thought by some to enhance its infective power is a sequence of amino acids within the S-protein (called the "polybasic cleavage site") that mediates splitting of the S-protein into two parts, with resulting rapid entry of the virus into human cells. Acquisition of those key features would have to have occurred in some intermediate species, or, if direct transmission to humans had occurred the infection would have to have occurred much earlier than the first recorded infections in late November/early December (ex-wet market) or late December (wet market hypothesis) to allow time for mutation and selection of this sequence feature.

A puzzling occurrence reported in late October 2019 concerned an analysis of pangolin virus genomes in Guangdong, more than 800 km from Wuhan although the exact geographical origins of the "trafficked" pangolins is not known. In March 2019, the Guangdong Wildlife Rescue Center examined the 21 sick Malayan pangolins, 16 of which died with swollen lungs and pulmonary fibrosis. Samples from the diseased tissues of 11 of the dead pangolins were screened for various known viruses.[66] Several members of the coronavirus family were identified in two of the 11 pangolins, including the SARS-CoV virus from 2003, which incidentally contains the receptor binding domain able to recognize the human ACE-2 receptor.

In a follow-on study of the 2019 pangolin samples by the Guangdong team and scientists from the University of Missouri (US) further details of the complex genome relationships between the coronavirus detected in the sampled pangolins (named pangolin-CoV-2020), various bat species and SARS-CoV-2 emerged. The story was on the one hand remarkably revealing about the possible bat-pangolin relationship but on the other hand failed to establish a clear path of the SARS-CoV-2 virus from bats through an intermediate species to humans.[67] In Fig. 14.8 the similarities from this study of five different CoV virus RNA sequences encoding the S-protein are shown. The comparison was to SARS-CoV-2 and included SARS-CoV (2003), pangolin-CoV-2020, Bat-CoV-RaTG13, and two other bat viruses. The similarity of the virus sequences at both the RNA and amino acid levels (shown as percent identity on the y-axis) are indicated. The most similar overall was, again, the bat sequence of RaTG13 showing an overall ~96% identity in RNA sequence to SARS-CoV-2, with the next closest the sequence from the sick pangolins (~90%). At first sight this might have reopened the bat sequence as a direct ancestor of SARS-CoV-2 with pangolins as intermediate hosts, but there was an anomaly that did not fit the theory, aside from the rarity of bat-human contact. In the critical region of the S-protein sequence that binds to the ACE-2 receptor on human cells, the pangolin genome was almost identical to SARS-CoV-2 (98.6% identity) but, as can be seen from Fig. 14.8, it diverged significantly in other regions of both the S-protein RNA and amino acid sequences—note the low percentage identity to SARS-CoV-2 of Pangolin-CoV-2020 and RaTG13 within the 200−1000 and the 1500−2000 RNA regions (upper graph), and the variable similarities in different regions of the amino acid sequences (lower graph).

A further conundrum was that, as with RaTG13, the pangolin sequence lacked the critical "polybasic" region within the S-protein that mediates the splitting of the S-protein. This region would have to have arrived by an "insertion recombination" event, as indicated below (inserted cleavage site in red; R is for arginine a basic or positively charged amino acid):

Pangolin CoV-2020 sequence:	… **TNS——RSVSSX** …
SARS-CoV-2 sequence:	… **TNSPRRARSVASQ**
RaTG13 sequence:	… **TNS——RSVASQ**

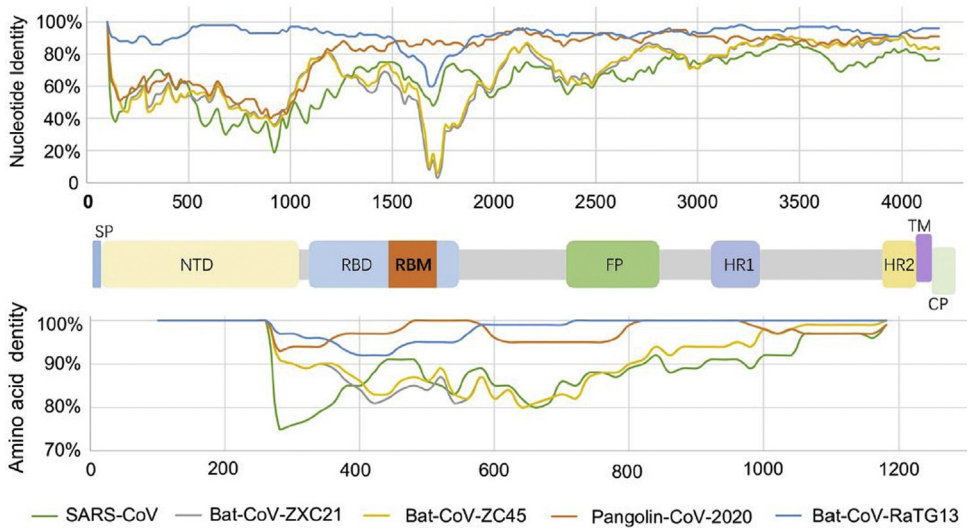

FIGURE 14.8

Similarity plots based on the spike (S) surface glycoprotein nucleotide sequence (upper plot) and amino acid sequence (lower plot) of SARS-CoV-2. Bat-CoV-RaTG13, Bat-CoVZXC21, Bat-CoV-ZC45, SARS-CoV, and pangolin-CoV-2020 were used as comparison sequences. The green lines indicate SARS-CoV, the gray lines indicate Bat-CoV-ZXC21, the yellow lines indicate Bat-CoV-ZC45, the orange lines indicate pangolin-CoV-2020, while the light blue lines indicate Bat-CoV-RaTG13. The RBD is marked in the orange box for the lower plot.

As a result of their analysis, the authors were forced to conclude

> *"However, phylogenetic analyses and a special amino acid sequence in the S-gene of SARS-CoV-2 did not support the hypothesis of SARS-CoV-2 arising directly from the pangolin-CoV-2020."*[67]

There was one more hypothesis the authors of this study were keen to share. By taking pieces of the bat RaTG13 and pangolin genomes, almost the entire genome of SARS-CoV-2 could potentially be reconstructed in a species harboring both viruses (by genetic recombination, manually, or naturally):

> *"Thus, these data suggest that SARS-CoV-2 originated from multiple naturally occurring recombination events among viruses present in bats and other wildlife species."*[67]

If occurring as a natural evolution of the virus, insertion of the missing S-cleavage sequence would then have to arrive by mutation and selection, either in the as yet unknown intermediate species or with infected humans. Several other genomic studies were carried out on the Guangdong diseased pangolins. A critique of these various studies was published in October 2020 drawing attention to the limitation that the set of Guangdong pangolins were the sole source of sequence information used in

those studies that included the RBD able to recognize the human ACE2 target. In commenting on this convergent series of genomic data, the authors noted, however:

> *"Although there is only a single source of pangolin CoVs…there is as yet no direct evidence of pangolins being an intermediate host of SARS-CoV-2, we would like to reinforce that pangolins and other trafficked animals should…be considered as carriers of infectious viruses with the potential to transmit into humans."*[68]

The implication of this analysis was understandably fuzzy: there was as yet no evidence that pangolins were the intermediate host although they may be, and therefore, the hunt for the genuine candidate host should continue.

Meanwhile, conspiracy theories continued to raise the possibility that the complex set of events necessary for this genetic interconversion occurring naturally in an animal over such a short period of time seemed unlikely. The absence of evidence for an intermediate host, whose sampling in the wild has been woefully limited, does not immediately prove of course that SARS-CoV-2 was artificially created. That has not stopped the continuing debate, however. In June 2020, Daoyu Zhang in Australia carried out an exhaustive series of CoV genome forensic analyses and published the results on an open access server managed by CERN in Geneva. His results were startling, and if correct would have turned conspiracy into circumstantial evidence, and with it the opening of a virology Pandora's Box. His claims were that the pangolin CoV sequences contained evidence of synthetic DNA insertions, such as signatures of artificial DNA pieces typically used by molecular biologists when isolating and reconstructing genes.[69] While the data communicated were not peer-reviewed and no vigorous objections appeared to have been published countering the genome inferences, the analysis clearly required expert validation or repudiation before any conclusion on the claims of laboratory manipulations leading to SARS-CoV-2 could be drawn. In ploughing the same furrow Rossana Segreto and Yuri Deigin, from Youthereum Genetics, Toronto, Canada, and the University of Innsbruck, Austria, respectively, speculated in a scientific submission, September 2020, that artificial reconstructions of SARS-CoV-2 should not be dismissed, and that if such manipulation had taken place using the method of "site-directed mutagenesis" it could be done without leaving any trace,[70] a view hotly disputed by Anderson and others. Their central assertion was that gain of function studies may have been ongoing in Wuhan involving manipulation of coronaviruses for infection of pangolins as an intermediate host model. In a vigorous riposte to Segreto and Deigin, Alexander Tyshkovsky, and Alexander Panchin from the Institute of Physico-Chemical Biology at Moscow State University and Harvard University's Department of Medicine, unceremoniously debunked (or attempted to) much of the arguments in the same issue of the scientific journal. Some of their arguments were that, for SARS-CoV-2 to have arrived by mutation of RaTG13 in laboratory cell cultures when used as a starting point, it would have taken 15 years for the 3.8% difference between the pangolin-CoV-2020 (MP789) and bat (RaTG13) viruses to have accumulated. After critiquing many of the other arguments used by Segreto and Deigin, Tyshvovsky and Panchin concluded that the likelihood of an artificially created virus from the bat and pangolin viruses:

> *"…seems incompatible with the high genetic divergence between these viruses and SARS-CoV-2."*[71]

One of the difficulties of reaching a consensus is that the usual methods for establishing the origin of a particular virus strain are to the find the most similar virus sequences in nonhuman species (e.g., bats, pangolins, etc.) and then assume a particular rate of mutation enabling construction of a "phylogenetic tree." This is achieved by ordering the sequences from the present day backwards based on the number of accumulated mutations, allied with a best guesstimate of the mutation rate over time, allowing a range of dates within which the earliest common ancestor appeared. One of the problems with coronaviruses, however, is that they are "highly recombinogenic," that is multiple virus species in the same animal host can mix and match different pieces of their sequences, so that for a given progeny virus, some parts of its sequence may be old while others are very recent, and all dates in between. That makes establishing a date for the emergence of a given sequence such as SARS-CoV-2 extremely difficult unless extensive sampling of viruses in the wild has been carried out to map the "pieces" to particular emergence times. The analysis by Boni and colleagues from the US, Belgium, China, and the UK in July of 2020 suggested that the virus lineage giving rise to SARS-CoV-2 had been "*…circulating unnoticed in bats for decades.*"[72] Their arguments were tinged with a touch of caution given the absence of the "polybasic cleavage sequence" in any bat virus so far sequenced despite the presence of the ACE2 recognition motif in pangolins and the W1V1 SARS-CoV bat virus. Nevertheless, using sophisticated phylogenetic methods, these authors estimate the time at which the most closely related bat virus, RaTG13, and SARS-CoV-2 diverged from a common ancestor was at the end of the 1960s—recall the early origins of the MERS virus. More importantly, Boni et al. estimated the divergence time for the MP789 pangolin virus, the closest to both RaTG13 and SARS-CoV-2, to be in the second half of the 19th century. This led the authors to conclude that pangolins are not implicated as an intermediate host for SARS-CoV-2 and, if their conclusions stand up to a scientific fine-tooth comb, renders the artificial creation theory moot:

> *"While pangolins could be acting as intermediate hosts for bat viruses to get into humans—they develop severe respiratory disease … and commonly come into contact with people through trafficking—there is no evidence that pangolin infection is a requirement for bat viruses to cross into humans."*[72]

This somewhat mirrors the story with SARS-CoV where wet market civets were positive for the virus but when civets in the wild were sampled, they were negative.[22] This is not to say that wet market pangolins were not involved as the initial conduit to the SARS-CoV-2 human index infection, but simply they may not be a stable intermediate host. The implication of this comprehensive study implicating horseshoe bat species as the direct source of SARS-CoV-2, sitting in an as yet unidentified eco-niche, has an air of doom about it. There are more than 1400 species of bats and more than 100 species of *Rhinolophus* (horseshoe) bats, the genus most closely associated with SARS viruses. The somewhat pessimistic conclusion of Boni and colleagues echoed that of one of the world's most experienced virologists, Jeffery Taubenberger, who commented in 2019:

> *"…viral phenotypic properties associated with human adaptation and transmissibility cannot yet be predicted from genetic sequences. The implications are sobering: identifying pre-pandemic viruses by increased viral surveillance in mammals and birds may be difficult or impossible."*[73]

Despite all the expert analysis, it must be said that the arguments about naturally evolved versus experimentally constructed SARS-CoV-2 are likely to continue. On March 11, 2021 Nature magazine

in its News in Focus and commenting on the WHO investigation in Wuhan added fuel to that debate with the article title "Where did COVID come from? Five Mysteries that Remain."[74] The questions, put by Smriti Mallapaty to some of the investigators preparing the 2021 WHO report, were wide ranging but at the same time epidemiologically challenging. For example, was the virus circulating in Wuhan before the first known cases? The supplementary question is "which virus?" Are we talking about the strain experienced by the index case in early December, or a less virulent strain circulating much earlier with only mild symptoms, making its detection as a novel infection difficult but giving the virus time to mutate toward SARS-CoV-2? As Boni et al. indicated the latter scenario is feasible but not proven until the exact phylogeny of SARS-CoV-2 is known. That may never be possible. Analysis of a US group of evolutionary and systems biologists and computer scientists from California and Arizona in late March 2021 suggested SARS-CoV-2 to have emerged in mid-October to mid-November 2019, and that the virus strains causing the majority of infections during that period could have died out. Their salutary concluding sentence in the abstract of their publication was:

> *"Our findings highlight the shortcomings of zoonosis surveillance approaches for detecting highly contagious pathogens with moderate mortality rates."*[75]

An observation consistent with this view is the sickness reported by a group of Swedish military personnel who had been in Wuhan during October 2019 for the World Military Games. Similar reports of unusual illnesses came from France, Germany, Italy, the US, and other countries, all of whom had attended the games. As a result, suggestions, or better theories, were propagated in the media supported by some of the athletes themselves that SARS-CoV-2 was already in Wuhan at the time of the games. If the reports that military establishments in the home countries on return of the athletes became epicenters of COVID19 outbreaks, then the stories might have some merit. However, without firm virus sequence analysis (do samples still exist?- in Wuhan some athletes were reported to have contracted malaria), now not possible, the epidemiological assertions are just that, unproven and probably now, unprovable assertions. Notwithstanding the diagnostic uncertainties a study by Harvard University and Boston University in 2020 based on satellite images of increased hospital visits in Wuhan during October/November 2019 is consistent with an unusual disease outbreak occurrence of some sort.[76]

Mallapaty's question on the role of the Wuhan Seafood Market is equally difficult to answer. The 2019 events in Guangdong where infected pangolins were identified, indicates just how easily coronaviruses are spread by bats to animals in the wild. This relates to the last of the five questions raised by Mallapaty, and perhaps most important from an epidemiological point of view: Was the virus circulating in animals in China before the pandemic? Again, the question 'Which version of the virus?' is relevant. The Nature article and perhaps the WHO investigators failed to mention the Guangdong diseased pangolins story of 2019 and the closeness of one of the pangolin sequences to SARS-CoV-2 when analyzed in detail during the following year. But the conspiracy theories will not go away, as evidenced by the multitude of Twitter messages still occurring.

In February 2021 Segreto and Deigin, now joined by colleagues from Spain, Japan, and the US, called for an open debate on the origin of SARS-CoV-2, and a month later asked the question "Should we discount the laboratory origin of COVID19?"[77] Their continuing message was that certain characteristics of SARS-CoV-2 are not consistent with a "natural zoonotic origin hypothesis." In defense of their *cause celèbre,* also taken up by many scientists and politicians on social media, as of November 2021, no intermediate species has been unequivocally identified that could have received a bat CoV virus and which, through an extensive series of in vivo cutting and pasting recombination of multiple virus segments followed by mutations, could have arrived at the December 2019 SARS-CoV-2 isolated in Wuhan from an infected patient. While the scientific world holds its metaphorical breath, the reality

is that without complete data transparency from China and the Wuhan Institute of Virology on the one hand, and the absence of firm identification of a relevant intermediate host and its reservoir host interactions over time arising from an extensive wildlife screening program on the other, the origin of SARS-CoV-2 will remain cloaked in mystery. The former alternative has been the subject of immense conspiratorial rhetoric and, until now, has been little more than conjecture. However, the recent report by Bloom[78] has brought the question of scientific veracity under the spotlight again. Bloom asserts that SARS-CoV-2 sequences deleted by Wuhan scientists may hold the key to viral sequences present in the Wuhan laboratory that are closer to the published sequence that infected the index patient in December and reported to the world in January 2021. Bloom notes that the timing and provenance of such sequences cannot be determined since only partial sequences were recovered and makes the important point that just because sequences are deleted does not immediately translate into "scientific malfeasance." In a further series of exchanges the sickness and eventual deaths of three out of six Chinese workers carrying out cleaning operations on bat faeces in the Mojiang mine (1500km from Wuhan) in 2012 has been raised as a possible origin of SARS-CoV-2, or its precursor. The workers suffered from persistent coughs, fevers, head and chest pains and breathing difficulties, conditions that have been suggested by some as evidence of a coronavirus present in China that would have had considerable time to mutate to the observed SARS-CoV-2 virus of December 2019. The basis for this assertion is flimsy although the three surviving workers allegedly had a persistent infection for as long as 6 months. A recent analysis by French researchers has an alternative view:

> *"We analyzed the clinical reports. The diagnosis is not that of COVID-19 or SARS. SARS-CoV-2 was not present in the Mojiang mine. We also bring arguments against the laboratory leak narrative*."[79]

But this is not just about the science. As the Washington Post reported on July 22, 2021, the funding of virus research at the Wuhan Institute of Biology by the US NIH, revealed in a report by the Trump administration, was for "gain of function" research, an assertion repeated by Senator Rand Paul in a recent exchange with Anthony Fauci, head of the US NIAID and vociferously denied.[80] It was further asserted, although no evidence has been made public, that the WIV had connections to the Chinese Military. Recently a new book, "*Viral: The Search for the origin of COVID-19*" [81], written by the well-known Oxford zoologist and science writer Matt Ridley, and the Harvard molecular biologist Alina Chan, has raised the portcullis on the artificially created SARS-CoV-2 story. While the arguments restore the need for further investigation, the current evidence remains circumstantial, bolstered by recent and more extensive revelations of alleged "Gain of Function" experimental plans between the US and WIV on coronavirus manipulations. Until transparency on whether such experiments were actually carried out by the WIV on ancestor coronaviruses that led to COVID-19, and its possible accidental escape, the debate will continue to bubble. Alternatively, if and when a viable zoonotic species is identified that carries a virus genome close enough to COVID-19 that unequivocally identifies it as the progenitor virus, will the conspiracy debate subside? Currently, the jury has its hands tied. The world awaits transparency from all those involved.

Pandemic models and the vaccine solutions for COVID19

During the early days, weeks, and months of the COVID19 outbreak notions of how to deal with a rapidly spreading virus varied from country to country. The two extremes were complete lock down with a rapidly employed test and trace system, or partial measures that kept invasion of people's normal life activities to a minimum, while others implemented a range of measures in between. Interspersed with these types of policy discussions were suggestions that the low mortality rate,

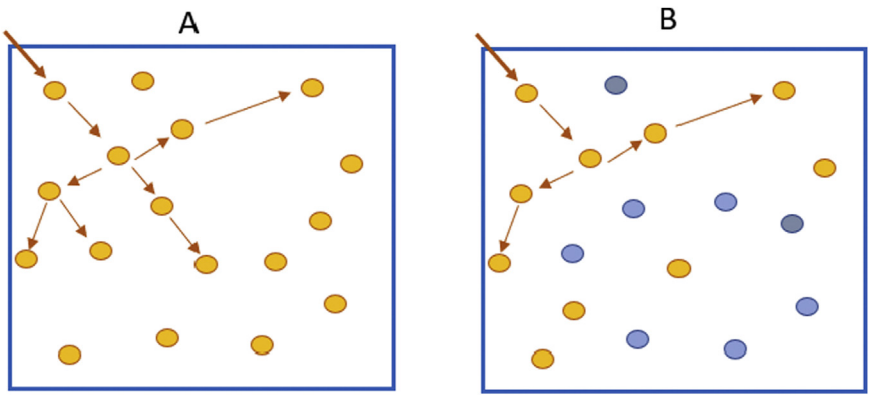

FIGURE 14.9

Example showing three successive transmission events of a pathogen in a susceptible population. In A, the pathogen infects all susceptibles but as some persons become immune from natural exposure to the pathogen others are less likely to be infected. In B, the effect of a controlled vaccination program produces more and more immune persons (blue) until eventually the pathogen has few or no susceptibles to infect. If the most susceptible are vaccinated first the incidence of serious disease will diminish more rapidly.

particularly for younger persons, favored a "herd immunity" approach, a notion that, as it became public, caused embarrassment among some scientific advisors to government and even government itself, and in some countries (Sweden and the UK) denial that this was a chosen policy. Before looking at the development of the vaccination programs initiated early in the COVID19 spread, and well before the WHO defined it as a pandemic, it will be useful to consider the origins and theoretical framework behind the herd immunity concept and whether it was a viable option. The term first arose in the early 1920s from experiments by UK bacteriologists Topley and Wilson in the context of reduced infection in laboratory mice among whom were both susceptible (to infection) and immune mice, both groups living in close proximity to one another.[82] As immunization against human diseases such as smallpox and polio began to eliminate those pathogens from a given population, it was understandable that theoreticians would begin to develop models that predicted the protective effect of not just those immunized but also susceptible nonimmunized individuals in the same population. While some commentators and even scientists outside the field of immunology have linked the concept solely to vaccination, it is also the case that even without vaccination immunity developed in some members of the population through infection and recovery can also protect uninfected individuals, although such a process would be uncontrollable if left to chance and would not ensure that the most susceptible are protected. An example of the two scenarios, one of which (A) would have been operating in Topley and Wilson's mice experiments, is shown in Fig. 14.9.

As we have seen earlier (Chapter 2) and about which the reader will have heard probably more than enough during 2020 and 2021, the measure of the transmissibility of an infection is the R_0 number (R-naught). As a reminder, R_0 is the basic reproduction number defined as the average number of transmissions expected from a single infectious person introduced into a fully susceptible population. If immune individuals are present in the population, either as a result of infection and recovery, or from vaccination, the R number becomes a "net R" known as Rn. In that case, the actual number of transmissions is equal to R_0 times the proportion of susceptibles (S), or $R_n = R_0 \times S$.

As the proportion of susceptibles reduces due to growth of immunity in the population, a point will be reached where transmission drops below an R_n value of 1. It follows then that the

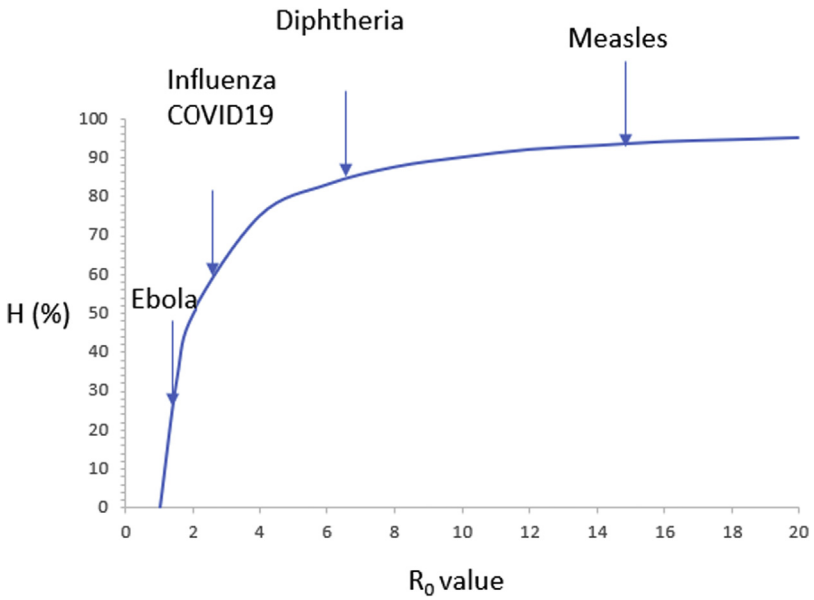

FIGURE 14.10

The approximate relationship between the herd immunity threshold (H %) in a fully susceptible population and the pathogen transmission number R_0. Examples of the value of H for several viral infections are shown. Note: The estimates of R_0 for particular infections can vary widely, depending on when (historically) they were estimated, and the assumptions made (e.g., socio-geographic conditions) in their calculation. See Delamater et al. 2019. Emerging Infectious Diseases volume 25 pages one to four for a perspective on its hidden complexity.

proportion of susceptibles is the complement of the proportion immune, and the point where the population see no further growth in transmission is called the herd immunity threshold (H), given by $H = 1 - 1/R_0 = (R_0 - 1)/R_0$.

To illustrate this graphically using the equation for H, different values for R_0 will show different values for the herd immunity threshold. Fig. 14.10 shows that COVID19 has a similar R_0 value to influenza leading to a much lower herd immunity threshold than either diphtheria, or measles.

While these numbers are only crude estimates, they indicate the likely percentage of individuals in a population that will need to be vaccinated to ensure disappearance, or at least maintenance at a low level, of a viral or other type of pathogen. The reason they are only estimates is due to a number of different variables in the calculations not visible in the simple relationships shown above. These variations introduce uncertainty into the exact values of H which depends on a correctly estimated value of R_0. For example, the R_0 value is influenced by the serial interval—the time between successive cases of transmission. For influenza, it is approximately 3 days, for COVID19 at about 5 days, and for measles about 15 days. To illustrate the impact of this single factor, the number of infection events in a 30-day period would be 10 for influenza (30/3), ~6 for COVID19 (30/5) and only ~2 for measles (30/15). Using the estimated R_0 numbers of two for influenza, 2.5 for COVID19 and 14 for measles, and allowing for the different serial intervals, leads to 1024 'flu, 244 COVID19, and 146 measles new cases of infection per 30-day period, assuming the population is 100% susceptible. While the transmission spread is higher in influenza the lower R_0 leads to a smaller percentage of vaccinated

individuals required to achieve herd immunity (~50%) compared to measles, whose high R_0 requires ~93% to achieve the same level of herd immunity. Estimates suggest vaccination against COVID19 requires around 60%–70% to reach herd immunity, although that can also be affected by the arrival of COVID19 variants that have more effective transmission leading to higher R_0 numbers.[83] The challenge with COVID19 is the fact that its serial interval is about the same period as the arrival of first symptoms (5–7 days), allowing for presymptomatic transmission. A further critical and more complex factor that influences the transmission dynamics of a circulating pathogen, and the associated calculation of susceptibility to infection, is the population heterogeneity. P*arri passu* this confounds accurate modeling of the course of any pandemic. For example, differences in age, health status, race, immune competence, gender, social behavior affecting contact patterns, and others, are all variables that introduce enormous complexity into the calculation of susceptibility to the virus. If valid models of the pandemic that can be used to guide government decision making are to be realistic, all of these factors must be considered. Some of these variables can only be crude estimates, such as immune competence status, social behavior, and even those health conditions or ethnicities that predispose some individuals to more serious disease. As an example of the continuing shift of the susceptibility variables, a recent study has shown mutational differences in the structure (sequence) of the ACE2 receptors in different ethnicities. Some of these mutations increase the affinity of SARS-CoV-2 for the ACE2 receptor, potentially leading to more severe disease consequences.[84]

The foregoing is of course grossly oversimplified, but if one can imagine a process 10 times more complex than stated here, the enormous burden on epidemiologists attempting to find a pandemic model that best fits the population they are concerned with might be understandably heavy. Those modeling the development of COVID19 in various countries have often used different epidemiological algorithms in the hope of reflecting the situation in their target population. This has sometimes been seen by scientists outside the immediate circle of those with such expertise as crude and sometimes plainly wrong. One of the leading young Oxford mathematical biologists, Robin Thompson (recently moved to Warwick University) while extolling the importance of epidemiological models in March 2020 also drew attention to the limitations as well as the value of such models, noting:

> *"Perfect data are not available, so modelling requires assumptions…"* and *"…despite unavoidable uncertainties, models can demonstrate important principles about outbreaks and determine which interventions are most likely to reduce case numbers…."*[85]

The fact is that while some models developed early in the COVID19 outbreak were somewhat pessimistic (some would say realistic) in their mortality projections than has turned out to be the case, others were overly optimistic. An example of an optimistic model is that proposed by Gupta and colleagues in March 2020 that stated:

> *"Our overall approach rests on the assumption that only a very small proportion of the population is at risk of hospitalisable illness. This proportion is itself only a fraction of the risk groups already well described in the literature … including the elderly and those carrying critical comorbidities (e.g. asthma)."*[86]

On the pessimistic side, and some say "rightly so" in retrospect, Neil Ferguson, the well-publicized British epidemiologist, reported an analysis of COVID19 with colleagues from London and Oxford that highlighted the potential for significant infection fatality in older persons (~80y+) and a lower

but significant fatality rate observed for those hospitalized, even exceeding the fatality rates of recent influenza pandemics. In placing their analysis in context, the authors stated:

> *"Our estimates of the case fatality ratio for COVID-19, although lower than some of the crude estimates made to date, are substantially higher than for recent influenza pandemics … With the rapid geographical spread observed to date, COVID-19 therefore represents a major global health threat in the coming weeks and months."*[87]

In hindsight, the pessimistic model, rather than being criticized, could be seen in a positive light for two reasons. First, the anticipated seriousness of this viral infection triggered a massive effort to develop vaccines, and second, it catalyzed the launch of creative "physical" solutions to lower transmission, not all of which it must be said as I reflect on the past year in my Stockholm home office, have been deployed everywhere. At the time of writing, the enormously rapid development, clinical testing, and production of COVID19 vaccines around the world has brought into sharp focus the tremendous global scientific cooperation that can be summoned in times of critical health crises, made possible by the rapid genome sequencing of the Wuhan SARS-CoV-2 virus, its publication and deposition in genome databases many months before pandemic status was declared by the WHO. That genomic information enabled the vaccine war against COVID19 to begin, a war that would experience an unfair change of tactics by a virus with a remarkable ability to generate variants, a further confounding factor in the modeling of the course of any pandemic.

The recent history of infectious diseases is a rich source of vaccinology, a patchwork of vaccine constructions and methods that have worked well, those that have disappointed, and others than have been abandoned either for lack of efficacy or induction of undesirable adverse effects. By July 15, 2021, a total of 19 different vaccines were either approved or in various stages of obtaining approval, as reported by the WHO (Fig. 14.11).[88] A number of vaccines in the Table have the same composition but are produced in different countries having gone through separate regulatory approval processes. For example, numbers 2 and 3 (the Oxford/Astra Zeneca vaccines) use the same adenovirus vaccine construction.

In addition to the candidate vaccines under regulatory evaluation, there are 108 vaccines in various phases of clinical development and 184 vaccines in pre-clinical (animal studies) development, as of July 27th, 2021.[89] This represents the largest global vaccine development initiative in the history of infectious diseases, or indeed any other diseases amenable to vaccine treatment.

Of the vaccines in extensive use, four employ one of the adenoviruses as a backbone for the vaccine construction and two use messenger RNA (mRNA) that encodes only the spike protein of COVID19, the latter a breakthrough vaccine technology for infectious diseases and one which has achieved enormous success. Vaccines based on adenoviruses (a DNA virus) have been used in the past for a number of infectious diseases, including adenovirus respiratory disease (ARD) caused by the adenoviruses (AdV) themselves. The early vaccines for protection against ARD were developed in the late 1950s and used formalin inactivated AdV for vaccination of US military recruits who experienced a high rate of ARD in high density training establishments. The less than fully effective inactivated vaccine was later replaced by "live vaccines" targeting the most frequently met virus serotypes (Ad4 and Ad7) formulated as oral tablets for ease of distribution in a military context. The safety and efficacy of this vaccine platform led to its use for many other infectious diseases, such as influenza A, Zika virus, MERS-CoV, Ebola, Japanese encephalitis, and others, most frequently now in an injectable

	Manufacturer/WHO EUL Holder	Vaccine Name	Vaccine type	Status
1	Pfizer/BioNTech, USA, GE	Comirnaty	modified mRNA	Finalized
2	Astra Zeneca/Univ Oxford, UK	AZD1222	Recombinant adenovirus encoding SARS-CoV-2 spike protein	Finalized. EU, Korea, Japan & Australia
3	Serum Institute of India	Covishield	As 2	Finalized
4	Janssen/J&J USA	AD26.COV2.S	Recombinant adenovirus encoding SARS-CoV-2 spike protein	Finalized
5	Moderna, USA	mRNA-1273	mRNA-based nanoparticle	Finalized
6	Sinopharm/BIBP, China	SARS-CoV-2 Vaccine lnCoV	Inactivated SARS-CoV-2 produced in Vero cells	Finalized
7	Sinovac, China	Coronavac™	Inactivated SARS-CoV-2 produced in Vero cells	Finalized
8	The Gamelaya National Center, Russia	Sputnik V	Human Adenovirus Vector based COVID-19	Ongoing
9	Bharat Biotech India	Covaxin	SARS-CoV-2 inactivated, Vero cell	Ongoing
10	CanSinoBio, China	Ad5-nCoV	Recombinant adenovirus type 5	rolling data collection starting August 2021
11	Novavax, USA	NVX-CoV2373 /Covovax	Recombinant spike protein - subunit vaccine with Matrix-M adjuvant	Pre-submission
12	Sinopharm/WIBP	SARS-CoV-2 Vaccine lnCoV	Inactivated SARS-CoV-2 produced in Vero cells	Pre-submission
13	Curevac, USA	Zorecimeran (INN)	mRNA-based lipid nanoparticle	Pre-submission
14	Sanofi Pasteur, France	CoV2 preS dTM-AS03 vaccine	Recombinant spike protein - subunit vaccine with AS03 adjuvant	Pre-submission
15	Vector State Research Center, Russia	EpiVacCorona	Peptide antigen	EOI pending
16	Zhifei, China	Recombinant Novel Coronavirus Vaccine (CHO Cell)	Recombinant protein subunit	EOI pending
17	IMBCAMS, China	SARS-CoV-2 Vaccine, inactivated (Vero cell)	Inactivated SARS-CoV-2	EOI not yet accepted
18	Clover Biopharmaceuticals, China	SCB-2019	Recombinant spike protein (trimer)	EOI under discussion
19	BioCubaFarma, Cuba	Soberana 01, Soberana 02, Soberana Plus, Abdala	SARS-CoV-2 spike protein conjugated to meningococcal B or tetanus toxoid or Aluminum	EOI awaiting information

FIGURE 14.11

Status of COVID-19 vaccines within the WHO EUL/PQ evaluation process, as of July 15, 2021, showing the type of biological constructions used.

Reproduced with permission from Reference 88. EOI=expression of interest for WHO to evaluate.

form. The vaccines produced were engineered to carry the gene encoding a key protein antigen from the target pathogen. In the so-called nonreplicating version, the virus is first disabled so that it cannot reproduce whole virus copies after entering cells, but its DNA can be transcribed into RNA and then turned into proteins. By replacing part of the virus normal genome with a gene that encodes the spike protein of COVID19, the cellular apparatus produces copies of this spike protein which, after exiting the cell (or being displayed on its surface), can induce an immune response that generates antibodies and T-cells against COVID19.

There are many different AdVs that cause a range of respiratory infections in humans with 88 viruses within seven different species that give rise to ~50 different serotypes—virus types that can be distinguished by antibody tests. A challenge with using any of the common AdVs that infect humans is that because they cause ~30% of common cold infections, humans already have some immunity to some of those serotypes. If such common serotypes were used to construct the vaccine, while new antibodies would be generated against the added COVID19 spike protein antigen, there could also be preexisting antibodies that recognize the non-COVID viral proteins due to previous exposure to that AdV serotype. If present, such antibodies when encountering the vaccine could activate a "vaccine clearance" process that obviously would reduce its efficacy.

[Note: In some instances, such as in the suspended STEP trial of an AdV-based HIV vaccine, those with a high preexisting level of antibodies to the adenovirus serotype used in the vaccine (Ad5) were considered to be at a higher risk of HIV infection if vaccinated.[90]]

To avoid immune clearance, vaccine design can either select a serotype that is rarely seen in human infections, or better use AdVs from NHPs, such as chimpanzees (ChAdVs). The theory here is that antigens in the ChAdVs should not have previously been met by humans (unless inhabiting areas close to NHPs) so that no preexisting antibodies or T-cells, or any memory B- or T-cells, will be present. This is not foolproof, however, and some studies have demonstrated antibodies in human subjects that react with both human AdV and certain chimpanzee serotypes, particularly in African countries harboring NHP species.[91,92] Acutely aware of this, the very experienced University of Oxford team that included scientists and clinicians from the Jenner Institute and Oxford hospitals, under the guidance of Sarah Gilbert, drew on their experience with adenoviruses, employing a chimpanzee serotype (ChAdV.Y25) that had been shown in previous trials to have zero seroprevalence in adults living in the UK, the region in which the initial COVID19 clinical trials would be carried out. Modifications to strain Y25 were then made to remove the genes used to replicate the virus, plus other regions of the genome known to encode immune evasion factors, and then adding in the gene for the complete COVID19 spike protein. This generated the candidate vaccine "ChAdOx1 n-CoV-19." In addition to these design features, the Oxford team had also examined the frequency with which the chimpanzee AdV genes still present in the vaccine were transcribed (DNA converted to mRNA, a requirement for making proteins) in human cells in culture, a surrogate test for their production during an actual vaccination. The important result was that COVID19 spike protein production was far in excess of the remaining adenovirus "backbone" virus proteins and confirming that the major protein antigen presented to the human immune system by the vaccine would be the COVID19 spike protein.[93]

After a rapid development phase, enabled by the past use of this vaccine backbone in other diseases, ChAdOx1 n-CoV-19 became the first COVID19 vaccine to be used in a vaccination safety trial. During April/May 2020, a Phase I trial established safety and immunogenicity (antibodies and T-cells) with a small number of UK subjects, aged 18−55.[94] In the ensuing Phase III trials involving subjects in the UK, Brazil, and South Africa, safety and efficacy were tested in four clinical trials. In the first interim analysis (December 2020), vaccine efficacy was at >70% for those who received two doses and ~60% efficacy after one dose. Due to a dosing error (only revealed after publication), a subset of the vaccine participants had received a first dose that was only 50% of the planned dosing. In assessing efficacy, the results of both dosing regimens were conflated, the two standard dose regimen at 62.1% efficacy, and the low dose regimen at 90% efficacy giving a mean efficacy of 70.4%[95] On December 30th, the UK Medicines and Healthcare products Regulatory Agency (MHRA) authorized the vaccine under emergency rules to be given to all those over 18 years.

In the slightly delayed US trials, delayed while the regulatory authorities debated the dose regimen errors of the UK, and allegedly also because of communication issues between the commercial partner Astra Zeneca and the FDA, the results of vaccination of 32,000 individuals in the US, Chile, and Peru were reported via an Astra Zeneca press release on March 22, 2021. Efficacy in preventing symptomatic COVID19 was at 79% (later downgraded to 76%) while prevention of severe disease and hospitalization was 100%. In a follow-on press release 3 days later, addressing public accessibility to the clinical trial primary data results, the head of Biopharmaceuticals at Astra Zeneca declared:

> *"The primary analysis…is consistent with our previously released interim analysis and confirms that our COVID-19 vaccine is highly effective in adults, including those aged 65 years and over. We look forward to filing our regulatory submission for Emergency Use Authorization in the US …"*[96]

The new name VaxZevria was confirmed by the European Medicines Agency (EMA) the following day. By early April 2021, more than 20 million doses of Vaxzevria had been administered to UK persons in the elderly age groups and those at the front line of clinical practice.

Despite the excellent safety profile of the vaccine in the clinical trials, by the end of February 2021 reports of an unusual blood clotting condition began to emerge, first in Germany and then more widely. Vaxzevria had been mainly used in the UK and the EU, and while the clotting cases reported were not conclusively proved to be causally linked to the adenovirus-based vaccine, the EMA was understandably cautious if a little ahead of the science:

> *As for the mechanism, it is thought that the vaccine may trigger an immune response leading to an atypical heparin-induced thrombocytopenia-like disorder. At this time, it is not possible to identify specific risk factors.*[97]

As of April 4, 2021, the EMA (EU) and MHRA (UK) had received reports of 169 cases of cerebral venous sinus thrombosis and 53 cases of splanchnic vein (abdomen) thrombosis with most of the cases occurring in women under 60 years of age. By the same date, some 34 million EU and UK persons had received the vaccine, giving an incidence of ~6.5 cases per million persons vaccinated. On April 14, 2021, the Danish Health Authority took the decision to permanently stop use of Vaxzevria, claiming blood clot events at a frequency of one in 40,000, puzzlingly well above that indicated by the EMA. At first sight, the Danish decision may have seemed perplexing, but it appears to have been judicious caution because of the rapidly declining COVID19 cases in Denmark, and the availability of other vaccines allowing reappraisal of the Vaxzevria benefit−risk equation.[98] Given the rarity of the disorder, the EMA continued to express the view that the overall benefit−risk ratio was positive despite the observed serious reactions. The story, however, became more complicated when a second vaccine based on a human adenovirus and developed by Janssen in Belgium, a Johnson & Johnson company, began to show similar cases. On February 27th the vaccine, known as the Janssen COVID-19 Vaccine and by its more technical name, Ad26.COV2.S, was given emergency use authorization by the US FDA. It was to be used as a single dose vaccine. By April 12th more than 6.8 million doses had been administered, mainly in the US. However, six cases of the severe blood clotting disorder similar to that seen with Vaxzevria had been reported, with one death and a second person in a critical condition, occurring 6−13 days after vaccination in women between the ages of 18 and 48. On April 13, the US, South Africa, and the UK paused rollout of the vaccine while J&J itself paused its EU roll out pending investigation. The Janssen vaccine is based on a human adenovirus backbone (Ad26) and as with the AZ vaccine is replication incompetent and shares with other COVID vaccines the addition of the

COVID19 spike protein gene. It had been used successfully in the Ebola vaccine Ad26.ZEBOV in combination with a MVA (see Chapter 13), and in other vaccines where its extensive use has shown no evidence of the types of disorder seen with the COVID19 vaccines.[99] We will return to the subject of vaccine adverse effects in the next short chapter, and in particular the vexed question of causal inference, and the important roles of "epidemiology" and "biological mechanism" in establishing causality.

A number of other vaccines based on adenoviruses have also been developed. The Sputnik V vaccine (also called Gam-COVID-Vac), developed by the Gameleya National Center in Moscow, uses two different human adenovirus serotypes, AdV26 and AdV5, one for the prime dose and the other as a boost dose, respectively. This heterologous protocol was designed to offset any immune reaction after the prime injection that may cause a vaccine clearance reaction on arrival of the boost. In February 2021, Denis Luganov and colleagues reported the interim results from the SputnikV Phase III vaccine trials which showed a strong protective effect across all age groups within the trial cohorts, comprising more than 20,000 participants.[100] This vaccine, which is in extensive use in Russia and other countries, differs from both the AZ vaccine which uses the same vaccine serotype for both prime and boost injections and the single adenovirus serotype used in the Janssen vaccine. The protective effect of single versus prime-boost vaccine protocols and the longevity of those protections are still unknown.

In China early vaccine developments involved two different approaches. The company CanSino Biologics in a recurring vaccine design theme used the single adenovirus serotype AdV5 in a non-replicating form with the addition of the COVID19 spike protein gene. However, Ad5 occurs as one of the more common cold viruses in humans and significant preexisting immunity may therefore exist, varying in intensity with the geographical distribution of the serotype. In February 2021, the single dose vaccine was approved in China having shown around 65% efficacy in preventing mild disease and 91% for preventing serious disease.[101] The phase III trials involved more than 40,000 participants in several countries. The vaccine, trade named Convidecia, has received emergency authorization for use in Pakistan, Indonesia, Hungary, Mexico, Chile, and Argentina.

One of the most frequent methods for vaccine development, employed in the early years of vaccines, was inactivation of the whole virus, usually by formalin treatment. Three Chinese companies working with academic institutions developed three different COVID19 vaccines all of which are in emergency use in China and a few other countries. The IMBCAMS vaccine began its Phase 3 study in January 2021 enrolling 34,000 participants in Malaysia, after showing good safety and immunogenicity in Phase 1/2 trials. No news on efficacy was available at the time of writing. Similar inactivation platforms were employed by Sinovac and Sinopharm who reported 75% and 70% efficacy, respectively, against infection, the UAE reporting 86% efficacy for the Sinopharm vaccine. The inactivated vaccine, Covaxin, was developed by the Indian biotech company Bharat Biotech, located in Hyderabad, and is a two-dose vaccine which showed 81% interim efficacy during its Phase 3 trial, and importantly was particularly effective against the UK variant, B.1.1.7. The two remaining vaccines in use are both protein subunit vaccines. The Chinese biotech company Anhui Zhifel Longcom Pharmaceutical produces the COVID19 spike protein as a tandem repeat of the region of the spike protein that recognizes the ACE2 receptor, known as the S-protein receptor binding domain, or RBD-dimer. In Phase1/2 trials reported in late March 2021, the RBD-dimer vaccine was shown to be safe and generated effective immune responses after a 3-dose protocol but only moderate T-cell responses. Since none of the participants in that study developed COVID19 infections, no efficacy indications were possible.

A different protein subunit approach was taken by the US company Novavax. The vaccine consisted of a recombinant version of the COVID19 S(spike)-protein stabilized in its prefusion state—the structure the S-protein normally adopts before its attachment and fusion with the host cell. After production (in insect cells) and purification of the protein, spontaneous trimers form. When these trimeric molecules are mixed with the surfactant polysorbate-80, which contains both a hydrophilic (water loving) and hydrophobic (water hating) region, the trimeric spike protein assembles into nanoparticles, held together on the inside of the particle away from the water environment by tight interactions between the hydrophobic parts of the protein and the polysorbate, while the hydrophilic portions of the protein sit on the outside bathed by the hydrophilic water environment. The vaccine also contains an adjuvant Matrix-M1 that contains agents that stabilize the nanoparticles, some components of which are there to improve the activation of the cellular response (T-cells) to foreign antigens. In early September 2020, Novavax reported interim results of the Phase 1 part of its Phase 1-2 trial, carried out in Australia and involving vaccination of 131 healthy adults, split into groups receiving placebo, or vaccine (NVX-CoV2373) with and without adjuvant. The trial was conducted by Novavax, University of Maryland School of Medicine, Baltimore, USA, Baylor College of Medicine, Houston, USA, Nucleus Network (Australia's largest Phase 1 clinical trials organization) and Q-Pharm also an Australian clinical trials organization. The purpose of the Phase 1 trial was to assess safety and immunogenicity of the vaccine regimens. In summarizing the results, the authors commented:

> *"At 35 days, NVX-CoV2373 appeared to be safe, and it elicited immune responses that exceeded levels in Covid-19 convalescent serum."*[102]

On the safety aspects although adverse effects were seen in a small number of cases categorized as serious according to a trial scale of reactogenicity, the study conclusion was

> *"No serious adverse events or adverse events of special interest were reported, and vaccination pause rules were not implemented….No adverse event extended beyond 7 days after the second vaccination."*[102]

In commenting on the results, the independent US organization, The National Vaccine Information Center (NVIC), in its Vaccine Reaction newsletter published 2 weeks after the New England Journal of Medicine publication of the Phase 1 results, had the headline:

> ***"Novavax's Adjuvanted COVID-19 Vaccine Caused Severe Adverse Reactions in Clinical Trials."***[103]

No comments were made about the excellent immune response of the vaccine, and it would not be unreasonable to presume that readers of the short article might take away an unwarranted negative view of this vaccine and vaccines in general. In February 2021, Nature magazine commented on more recent data from the Novavax trials in South Africa (4400 participants) and the UK (15,000 participants). The title of the short news article, with a somewhat contrasting message from that given by NVIC, was

> **"Novavax vaccine protects people against variants."**[104]

In the preliminary announcement of the UK Phase 3 trial, efficacy against the original virus was 95.6% and 85.6% against the UK variant (B1.1.7). In the South Africa Phase 2b trial, NVX-CoV2373

was seen to be 60% efficacy in prevention of mild, moderate, and serious disease in 94% of the study population but only 50% effective against the SA variant B.1.351, mirroring results with the other vaccines.[105] In May 2021, further results from trials in South Africa confirmed the impact the B.1.351 variant, circulating at the time of the trial, had on infection, generating an overall efficacy against this South Africa variant of only 50%.[106] In June 2021, Novavax published an updated analysis of the UK Phase 3 trial conducted at 33 sites around the UK in adults with an age range of 19−84 years, concluding

> *"…the NVX-CoV2373 vaccine administered to adult participants conferred 89.7% protection against SARS-CoV-2 infection and showed high efficacy against the B.1.1.7 variant… no hospitalizations or deaths were reported among the 10 cases in the vaccine group. Five cases of severe infection were reported, all of which were in the placebo group."*[107]

In commenting on the results of the Phase 3 clinical trial in the UK and the Phase 2b trial in South Africa, Clive Dix Chair of the UK Vaccine Task force used the phrase "spectacular results."[108] The data from the Novavax studies and those of other vaccine responses clearly indicate that immunization with a spike protein vaccine, whether as the gene or the protein the gene encodes, can generate neutralizing antibodies with a significant protection against severe diseases.

While most of the vaccines already described have employed tried and tested constructions, used in vaccination against a number of infectious diseases during the past 60 years, two biotechnology companies, one in Germany and one in the US, made a brave move to apply a technology that had already been tested for treatment of other types of disease but had never been used for a vaccine. The technology was messenger RNA, or mRNA therapy. While gene technology using DNA to replace or correct a genetic deficiency was well understood the introduction of DNA that modifies the normal genome typically introduces a permanent change. The alternative notion was that if you needed to make a transient change, such as augmenting a protein deficiency, DNA would not be the first choice. If mRNA could be introduced into the region where the protein operated, for example, a liver enzyme with a critical metabolic function, the mRNA once homed in on the target tissue or organ would be directly translated by the ribosomes in the cell cytoplasm to produce the required protein locally. This would then be a "replacement therapy" that in theory could correct many such protein deficiency-based diseases. It would be transient because mRNA once degraded would no longer be active. As a therapy it would then have to be regularly introduced into the body as required. Antibodies that typically attack viruses typically do so by reacting to accessible proteins located on the surface of the virus it uses to gain entry to cells and tissues. If enough of the key virus protein could be produced in the body for the immune system to react, no other components of the foreign virus, or a surrogate for it such as the adenoviruses, would be required. Since exposure to a protein antigen is only required for a short period, long enough for the immune system to recognize it and begin the antibody generation, the short lifetime of the mRNA would not be an issue. This was the approach taken by BioNTech, located in Mainz, Germany, and Moderna, a company based in Cambridge, Massachusetts in the US. Both companies had already explored mRNA for other types of therapy, and it would have been a logical if unproven extension of the technology to apply it to antiviral vaccines. But RNA is much less stable than DNA. Even a small amount of sweat from the human hand contains enough enzyme (a ribonuclease) that can degrade RNA to a useless mixture of the monomers (the nucleotides) that link together to make the RNA chain. Using sophisticated techniques, BioNTech constructed an mRNA that contained only the sequence coding for the COVID19 spike protein. Aside from some additives to give

the RNA molecule stability during injection no other virus genetic material was present. The naturally occurring nucleotide building locks used to make the RNA were also modified, to increase the RNA stability but also to minimize any immune response to the RNA itself. It was then enclosed in a nanoparticle with a fatty (lipid) coat to protect it from degradation during storage and the vaccination process itself. BioNTech teamed up with Pfizer to carry out the clinical trials who employed a creative Phase I/2/3 continuous trial method that cut the normal trial times down to just an 11-month period and involved more than 40,000 participants in the US and Germany. The results were nothing less than astounding. The Phase 1 safety and immunogenicity trial demonstrated robust antibody and T-cell responses with only mild vaccine reactions. In the combined Phase 2/3 trial, the results were summarized by the trial teams as follows:

> *"BNT162b2 was 95% effective in preventing Covid-19 (95% credible interval, 90.3 to 97.6). Similar vaccine efficacy (generally 90 to 100%) was observed across subgroups defined by age, sex, race, ethnicity, baseline body-mass index, and the presence of coexisting conditions."*[109]

On December 11, 2020, the US FDA gave the vaccine, trade named Comirnaty, "emergency use authorization" triggering its roll out in the US. Ten days later, the European EMA followed suit with its equivalent "conditional marketing authorization."

A parallel development was taking place in the laboratories of the biotech company Moderna Therapeutics, located in Cambridge, Massachusetts, and collaborators at the NIAID within the US NIH. Again, an RNA molecule, mRNA-1273, encoding the COVID19 spike protein was the target vaccine construct which, after production and purification was encapsulated in a lipid particle to ensure its stability. In addition, the Moderna scientists had introduced two amino acid changes in the spike protein RNA sequence that prevented it from changing its shape (called the prefusion state) after entry into cells, a piece of smart "engineering" that would ensure the best possible presentation of the protein to the human immune system. The vaccine had demonstrated safety and immunogenicity in earlier Phase 1/2 trials after which more than 30,000 US participants from 99 different US sites were enrolled in a Phase 3 trial receiving two injections of the RNA vaccine 28 days apart. The results were also outstanding, with the vaccine returning 94.1% efficacy at preventing all forms of the disease and no serious safety concerns. On December 18, 2020, Moderna received emergency use authorization for use in the US with individuals of 18 years and older. A more recent update of the Moderna vaccine efficacy, reflecting analysis of a larger number of participants, showed a slight decrease to 90% for all forms of the disease and 95% against serious disease. Curiously, although using very similar technology the Moderna vaccine showed a different temperature sensitivity to the Pfizer vaccine, the latter requiring storage at very low temperatures (−80°C) while the Moderna vaccine while normally stored at −25°C to −15°C was stable at normal refrigerator temperatures (2 degrees-8°C) for up to 30 days prior to use. This difference would turn out to be an important factor for any rollout in geographical regions where low temperature storage is challenging. While the Pfizer and Moderna mRNA vaccines have had outstanding success, as a new solution the technology is not yet problem free. A third player, Curevac in Germany who developed an "unmodified mRNA" as a vaccine (different to both Pfizer and Moderna), presented preliminary results of its clinical trial on June 16, 2021. The reported efficacy was only 48% from a 40,000 person trial. Several reasons have been put forward, from too low dosing (higher doses had side effects) to inflammatory responses when a "foreign RNA" molecule enters the body, a potential issue mitigated in the other mRNA vaccines by RNA modifications designed to reduce such responses. The field of mRNA vaccine development is watching with interest as Curevac

continues to explore its unique application of natural, unmodified mRNA that has the added advantage of longer stability at refrigerator temperatures than its competitors.[110]

With several vaccines rolling out in many countries, the big questions are whether the vaccine is able to reduce the infection rate (get R_n below or well below 1), and the rate at which herd immunity is reached allowing opening up of society. An increasingly important additional question was whether a vaccine based on the original SARS-CoV-2 virus spike protein sequence would protect against ever increasing virus variants. Recent (at the time of writing) data from the Johns Hopkins resource web site suggests a mixed bag of answers. Israel whose vaccination rate has outshone most other countries, with the US and UK rapidly catching up, showed a big decline in cases despite opening up society and was the first to undertake a third booster vaccination as variants begin to compromise the earlier vaccination protection, particularly in the more elderly, and immunity begins to decline. By contrast, Chile and Brazil both with less stringent lockdown policies than many other countries, and whose vaccination levels are at a low level, have experienced high case levels and mortality rates that have been extremely concerning.

A number of recent studies have begun to tease out the relationship between the immune responses elicited by direct infection from the virus, or from vaccination, or both. In June 2021, Michel Nussenzweig and colleagues at Rockefeller University and the Howard Hughes Institute in New York described the broad-based immune responses seen in convalescent individuals post-infection and the long-lived neutralizing antibodies retained for up to 12 months as a result of the induction of stable memory B cells, particularly with specificity for the SARS-CoV-2 receptor binding domain. What was more revealing was that post-infection vaccination (with mRNA) of convalescent individuals who had recovered, boosted their neutralizing antibody response by up to 30 times. The improved response appeared to involve recruitment of preexisting memory B cells into the plasma cell compartment where high levels of antibody are produced. The consequence of infection-plus-vaccination led to the following conclusion by these authors:

> *"…the robust enhancement of serologic responses and B cell memory achieved with mRNA vaccination suggests that convalescent individuals who are vaccinated should enjoy high levels of protection against emerging variants without a need to modify existing vaccines."*[111]

In a follow-on study that compared the response of SARS-CoV-2 naïve individuals who had been vaccinated by an mRNA vaccine, a much narrower range of antibodies was elicited the breadth of which was not increased by booster shots of the vaccine. The authors concluded that the infection plus vaccination responses:

> *"…have greater potency and breadth than antibodies elicited by vaccination."*[112]

The suggestion that even those who have been infected by the virus could experience a much broader and longer lived protection against variants by a follow-on vaccination, if proven to be widely seen, is not unexpected given that the natural virus will present many different antigens to the immune system, some of which will be conserved and much less likely to accept mutations. However, the breadth of response to the single spike protein contained in all COVID19 vaccines currently available and the effectiveness of antibodies to that single protein to fight off variants with altered sequences in individuals who have not been previously infected (naïve) with the virus is a potential limitation of the "one-antigen" vaccine approach, a limitation already being seen with reduced immunity to the

SARS-CoV-2 delta variant. In their recent review, Gunilla Karlsson Hedestam and her colleagues from the Karolinska Institute in Stockholm drew attention to the reduced efficacy of the current vaccines against SARS-CoV-2 variants, in particular the alpha, beta, P1 and delta variants. Given this decrease in effectiveness of current vaccines, although still preventing serious illness, and the waning of antibody levels over time, these authors cautioned:

> *"Given the high global transmission levels and the risk of new variants … continued virus surveillance will be needed for a foreseeable future. This is especially important as vaccine-induced Ab levels wane over time, in some cases below protective levels … boosting the immune response with a variant vaccine may be needed…"*[113]

This slightly depressing view is supported by the Nussenzweig studies, which nevertheless provided a consistent story about the limitations of a single antigen vaccine. The concluding sentence of the opening abstract to their July 2021 publication contains the bottom line message:

> *"These results suggest that boosting vaccinated individuals with currently available mRNA vaccines would produce a quantitative increase in plasma neutralizing activity but not the qualitative advantage against variants obtained by vaccinating convalescent individuals."*[112]

The development of variants that can elude the antibody repertoire generated by the wildtype virus would seem to suggest a rethinking of vaccine compositions, a not too unfamiliar story in the influenza vaccine arena and suggested by the observations of Nussenzweig and others.[114] But a multicenter study in the UK, coordinated by Public Health England and published on July 21, 2021, offered a somewhat more optimistic message when comparing vaccine effectiveness against either the alpha (B.1.1.7) or the delta (B.1.617.2) variants. Efficacies after two doses of either the AZ (ChAdOx1) or Pfizer (BNT162b2) vaccines were 74.5% (alpha) and 68.4% (delta) for the AZ vaccine and 93.7% (alpha) and 88% (delta) for the Pfizer vaccine.[115] If these differences are seen in more widespread populations, there are several questions to consider. Is the greater prevalence of delta cases in both unvaccinated and vaccinated individuals seen in some countries linked to the type of vaccine they have received? Do the two types of vaccine present the spike protein antigen differently to the immune system, generating different antibody specificities? Whatever the explanation the current data might suggest adoption of a "chimeric vaccine strategy," such as an adenovirus vaccine first dose followed by an mRNA second dose. While such a vaccine "mix" approach might elicit better immune responses the vexed question of immunity longevity still remains. If that cannot be resolved SARS-CoV-2 may join influenza as an endemic virus whose mutational drift over time will require constant redesign of vaccines to address those variants with a sufficient immune escape velocity. By the time this book is published, the story will have moved on, happily toward a solution that protects the world against any and all SARS-CoV-2 variants.

COVID19 and the role of passive antibody therapy

When infection has already occurred in a susceptible individual from a pathogen that has a relatively short incubation time before serious disease develops (e.g., days), vaccination is normally not a useful option. During 2020, various ad hoc trials were made using antibodies (monoclonal and polyclonal)

that recognized the COVID19 spike protein and which could be used to treat already infected persons. The most publicized example of this "passive immune therapy" was that of President Trump in early October 2020 who, after showing symptoms of infection, received an experimental mixture of two monoclonal antibodies (REGN-COV2) produced by Regeneron Inc in the US, that recognized the virus spike protein. Although the antibody mixture was not approved by the FDA permission was obtained to use the drug on "compassionate" grounds, granted infrequently by the FDA on a "case-by-case" basis. Similar experimental treatments were being explored elsewhere in the US. For example, Eli Lilly had isolated a monoclonal antibody from a convalescent patient after infection with COVID19 that bound to the SARS-CoV2 spike protein. The antibody, bamlanivimab/LY-COV555 entered clinical trial as a passive immunotherapy treatment of infected and hospitalized patients with mild to moderate disease.

[Note: the formal naming scheme for antibodies is a little complicated. As an example there are three elements to the bamlanivimab name: "bamlani" (an Eli Lilly internally decided prefix)—"vi" meaning against a virus—"mab" meaning a monoclonal antibody. The name LY-COV555 is an Eli Lilly company name for the antibody].

While positive effects on reducing viral load were seen, the Phase III clinical trial (Activ-3) was put on hold in mid-October for safety reasons after 5 days of treatment. This was widely reported in the media, including a short report in the British Medical Journal which at the same time reported a pause in the Astra Zeneca UK vaccine trial for safety reasons, triggering a delay in the US regulatory review for that vaccine, and a similar safety pause for the Johnson & Johnson vaccine.[116] The Eli Lilly parallel trial (Activ-2) in non-hospitalized patients with mild to moderate disease was unaffected by the pause. On the ninth of November, the FDA issued an EUA for the investigational LY-COV555 antibody for the post-infection treatment of mild to moderate cases of COVID19 in adult and pediatric patients. It was not authorized for hospitalized patients since as the FDA noted:

> *"A benefit of bamlanivimab treatment has not been shown in patients hospitalized due to COVID-19. Monoclonal antibodies, such as bamlanivimab, may be associated with worse clinical outcomes when administered to hospitalized patients with COVID-19 requiring high flow oxygen or mechanical ventilation."*[117]

Passive antibody therapy is not new and has an important role to play when vaccines are not available. There is currently no vaccine for the dangerous RSV, although candidate vaccines are in clinical trial. In the US, this virus causes annual hospitalizations of up to 58,000 children under 5 years of age with somewhere between 0.7% and 1.5% mortality, while global estimates from 2017 put annual infant mortality from this virus at between 90,000 and 150,000.[118] There is, however, an approved passive antibody therapy (Synagis) that provides a measure of protection in infants with underlying health conditions and who are at risk of developing serious lung (pneumonia) disease.[119] In Chapter 13, we also saw how passive antibody therapy has had moderate success in reducing the mortality associated with Ebola infection. For those who contract rabies and have not been vaccinated passive antibody therapy ((HYPERAB) is essential, but since this virus travels slowly to the CNS antibodies introduced in a timely manner are able to neutralize the virus effectively.

On October 21, 2020, Nature magazine published an extensive article in which a number of experts were asked for their views on the use of passive antibody therapy for COVID19.[120] The experts were all from the US. The timing of such a discussion was important, however, since at the time there were 11 other antibody-based experimental therapies in clinical trials, by US, Chinese, and UK companies and/or academic institutions, listed in the Nature article. It would be presumptuous to attempt to condense the

views of the "magnificent 7" to a few words but it would not be unfair to state that opinions were varied. In some instances, examples of antibody therapy were cited that did not involve direct recognition of the infectious pathogen by the antibody therapeutic. One example was the antibody bavituximab used in hepatitis C infection which recognizes lipid-like molecules made and then exposed on liver cell surfaces that have been infected by the hepatitis C virus. These molecules then act as a beacon so that when the antibody has bound to them other elements of the immune system can home in on the infected cells and destroy them. Such an approach is not a direct parallel to an immune response elicited by a vaccine containing antigens from the virus of course. For hepatitis C, no vaccine is currently available.

On the question of whether there should be concerted efforts to develop antibodies that have broad neutralizing capability by targeting conserved regions of a given virus, there was universal agreement. The other important conclusion, highlighted by one of the experts having the last words (literally), is best quoted verbatim:

> *"T.G.: The identification and stockpiling of broadly protective coronavirus mAbs and vaccines should be a priority going forward. We have now seen the emergence of four endemic coronaviruses (HKU1, NL63, OC43 and 229E), two highly pathogenic coronaviruses that caused deadly outbreaks (SARS and MERS), and one coronavirus that caused the current COVID-19 pandemic (SARS-CoV-2). Given that the latter three viruses all emerged over the past two decades, it appears not to be a matter of 'if' but a matter of 'when' the next pathogenic coronavirus will spill over from zoonotic reservoirs into the human population."*[121]

In May 2021, the results of a clinical trial became available (RECOVERY) in which more than 16,000 hospitalized patients at 177 UK sites were enrolled in a randomized trial, the convalescent plasma arm receiving high-titre convalescent plasma (containing anti-COVID19 antibodies donated by infected and recovered persons). The conclusion of this part of the trial was disappointingly low key:

> *"In patients hospitalised with COVID-19, high-titre convalescent plasma did not improve survival or other prespecified clinical outcomes."*[122]

A cohort of patients in the same RECOVERY trial received the Regeneron double antibody mixture, REGEN-COV, the results for which were published in June 2021. While the treatment showed some efficacy in hospitalized patients who were sero-negative (no COVID19 antibodies measurable) at the time of infusion, in sero-positive patients no advantage was seen. The overall efficacy combining the data for both sets of patients was disappointing:

> *"Consequently, when all patients were considered together (including those with unknown 341 antibody status), allocation to REGEN-COV was associated with non-significant differences in clinical outcomes."*[123]

In their July 2021 review of various "immune-enhancing" therapies, Wittebole et al., commenting on the role of convalescent plasma or other forms of antibody therapy in critical care settings, were somewhat downbeat:

> *"The other strategy involve* (sic) *a passive improvement of the immune function through the administration of IvIg or convalescent plasma. Unfortunately, results from large randomized controlled trial (RCT) in this setting were contrasting, and could currently not serve as a recommendation for treating critically ill."*[124]
> [Note: IvIg = intravenous immunoglobulin, or antibodies.]

In April 2021, "The Antibody Society" released a summary of the ongoing clinical trials using antibodies for treatment of COVID19. Of the 28 studies, there were 8 Ph1, two Ph1/2, 5 Ph2, 4 Ph 2/3, five Ph3, and four that had received emergency use authorization.[125] This is an impressive set of developments but will require careful selection to achieve the necessary efficacy and safety profiles, and most importantly will need a drastic reduction in cost of treatment ($10,000 per gram not unusual; needs to get to $100−500/gram to become widely available through funding by health organizations in different countries) in order for this approach to therapy to become a globally accessible treatment.

In June 2021, the US biotechnology company Regeneron announced its own results of a multipart Phase III study of its passive antibody treatment. The participants had no COVID19 symptoms at the time of enrollment but were resident in the same household as persons who had tested positive in the previous 4 days. After enrollment, the participants were tested for the virus and those who tested positive were assigned to the "prevention trial" (Part A) while those who tested positive were assigned to the "treatment" trial (Part B). In the randomized prevention arm (1505 participants: 752 placebo, 753 REGEN-COV), administration of the antibody cocktail reduced symptomatic infection by more than 80% and either symptomatic or asymptomatic infections by ∼66% compared with the placebo.[126] In the treatment arm (314 participants: 156 placebo, 155 REGEN-COV), antibody administration had a significant effect on progression of the infection from asymptomatic to symptomatic, likely as a result of its ability to reduce the viral load leading to an almost 6 day reduction in the symptoms period. What was also impressive was the activity of the antibody cocktail in neutralizing certain COVID19 variants of concern (B.1.1.7—Alpha; P.1—gamma; B.1.351 - beta).[127]

The continued development of passive antibody therapies such as those discussed will open up an important parallel route to pathogen control and will also provide those, who for various reasons are not able to receive vaccines, a route to disease prevention or treatment. We await the results of the many clinical trials in progress with interest.

Interim epilogue: vaccine nationalism and effective use of global resources

The number of COVID19 vaccines in current use and in development is an extraordinary testament to the response of the scientific community, both academic and commercial, and the governments who support them. The unfortunate term "vaccine nationalism" was coined early on to reflect the "me first" attitude of some countries who had developed vaccines and then placed orders with the producers that pushed the production capacity to such a limit that supply to other countries or regions was delayed, often by many months. In the middle of a pandemic affecting the entire world, this was seen by some as lacking the humanitarian spirit that ought to exist at a time when everyone in the world is potentially susceptible to serious disease. To repeat what the head of the UK Wellcome Trust, Jeremy Farrar, said, "*Nobody is safe until everyone is safe.*" There is, however, another aspect of this type of nationalism that ought to be debated by world governments in cooperation with the WHO, and that is the sheer number of vaccines being developed, often by groups with limited experience of vaccine clinical development. The more than 300 candidate vaccines either in clinical or preclinical stages is clearly a drain on precious scientific financial resource, given the cost of clinical trials in particular, and while development teams should be praised for "stepping up to the plate," these exaggerated efforts are likely to have little impact on a pandemic that has already struck. The solution for a future that will surely

bring forth yet more COVID19-like viruses is to assemble a global vaccine task force led by a multinational committee of experts in epidemiology, virology, immunology, and clinical development. It should sit outside the WHO whose role as always is to monitor global health problems and to ensure that effective vaccines and other therapeutic treatments are available and supplied to the areas of the world where they are needed most. The task force remit would be to initiate and fund via multinational government grants the development of new vaccine technologies that address the special challenges of rapidly mutating pathogens, and in addition improve our understanding of how to engineer vaccines that ensure long lived immunity. A second focus should be the creation of novel, fast track preclinical and clinical trial structures that enable future pandemics to be addressed safely, rapidly, and effectively. Vaccines identified as the most effective would then be manufactured by a global consortium of companies adhering to strict GMP protocols and supplied simultaneously to all countries in need. If something similar to this global approach is not implemented with urgency—Dr. Anthony Fauci at the US National Institutes of Health has recently announced the beginnings of such an initiative—the human race will face an increasing danger from promiscuous pathogens that may challenge even the most sophisticated health systems.

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