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REVIEW ARTICLE

SARS-CoV-2 does not infect pigs, but this has to be verified regularly

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Abstract

For successful xenotransplantation, freedom of the xenocraft donor from certain viral infections that may harm the organ recipient is important. A novel human coronavirus (CoV) with a respiratory tropism, designated as SARS-CoV-2, was first identified in January 2020 in China, but likely has been circulating unnoticed for some time before. Since then, this virus has reached most inhabited areas, resulting in a major global pandemic which is still ongoing. Due to a high number of subclinical infections, re-infections, geographic differences in diagnostic tests used, and differences in result reporting programs, the percentage of the population infected with SARS-CoV-2 at least once has been challenging to estimate. With continuous ongoing infections in people and an overall high viral load, it makes sense to look into possible viral spillover events in pets and farm animals, who are often in close contact with humans. The pig is currently the main species considered for xenotransplantation and hence there is interest to know if pigs can become infected with SARS-CoV-2 and if so what the infection dynamics may look like. This review article summarizes the latest research findings on this topic. It would appear that pigs can currently be considered a low risk species, and hence do not pose an immediate risk to the human population or xenotransplantation recipients per se. Monitoring the ever-changing SARS-CoV-2 variants appears important to recognize immediately should this change in the future.

KEYWORDS
COVID-19, cross-species transmission, pig models, SARS-CoV-2, therapeutics, xenotransplantation

1 INTRODUCTION

The ongoing COVID-19 pandemic associated with SARS-CoV-2, was initially observed in a cluster of patients with severe respiratory disease in Wuhan, Hubei Province, China during December 2019. The causative agent, a novel coronavirus (CoV), initially called 2019-nCoV but later re-named to SARS-CoV-2, was identified and reported to the World Health Organization (WHO) on January 9, 2020. On March 11, 2020 the WHO upgraded the SARS-CoV-2 outbreak to global pandemic status. Overall the infection pattern of SARS-CoV-2 in the global human population has been remarkable. A pool of approximately 7.753 billion SARS-CoV-2 naive people were present when the virus started infecting humans. Based on data from the United States and eight European countries including France, Italy, Spain, Germany, Belgium, Switzerland, Netherlands, and the United Kingdom, it has been determined that in the early phase of the pandemic, SARS-CoV-2 grew exponentially at rates between 0.18 and 0.29 per day which correlates to epidemic doubling times between 2.4 and 3.9 days. The
virus spread rapidly through most countries and continents resulting in a high number of infected people shedding SARS-CoV-2 for extended periods of time. A recent study estimated that by November 14, 2021 over 40% of the global population had been infected with SARS-CoV-2 at least once.4

2 | ANIMALS IN WHICH NATURAL OR EXPERIMENTAL SARS-CoV-2 INFECTION HAS BEEN CONFIRMED

Pets. As people naturally have close relationships with their pets, it comes as no surprise that human-to-cat transmission was reported on March 28, 2020 in a cat from a Belgian COVID-19 household.5 This first documented spillover of SARS-CoV-2 into animals was quickly followed by detection of the virus in additional cats,6–8 and also in dogs.9–11 Very recently a suspected cat-to-human SARS-CoV-2 transmission was documented for the first time.12 Specifically, a SARS-CoV-2 positive cat from a COVID-19 household sneezed into the face of a veterinarian while it was being examined, and it is hypothesized that the veterinarian was infected by the cat which was previously infected by the owner.12 Moreover, naturally SARS-CoV-2 infected Syrian hamsters in a Hong Kong pet shop have been associated with multiple events of hamster-to-human infection by the Delta variant of SARS-CoV-2, further resulting in a sustained human-to-human transmission.13 Experimentally infected rabbits have also been shown to shed infectious SARS-CoV-2 from the nose and throat.14 At the time of writing, SARS-CoV-2 infection has been confirmed in other companion animals including golden hamsters15 and ferrets.16 Interestingly, guinea pigs appear to be resistant to the virus.17 In contrast, almost 20 years ago when SARS-CoV-1 appeared, guinea pigs were experimentally infected with reovirus alone or concurrent SARS-CoV-1, both isolated from a human case.18 Guinea pigs infected with reovirus alone died between 22 and 30 days after experimental infection while co-infected guinea pigs died between 4 and 7 days and expressed similar gross lung lesions as described in human patients, highlighting the possible importance of coinfections in disease expression.18

Farmed animals/livestock. The first cases of natural SARS-CoV-2 in farmed animals were discovered on two commercial mink farms in the Netherlands in April 2020.19 The mink had developed respiratory symptoms with increased mortality, and it was determined that 68% of the workers on farm had also evidence of SARS-CoV-2 infection. Moreover, besides human-to-mink transmission, mink-to-human transmission could also be confirmed.19 Experimental infection of cattle with SARS-CoV-2 resulted in low levels of virus replication and antibody development; however, transmission to non-infected controls did not occur.20 In a similar study, cattle, sheep, goats, alpacas, and horses were infected intranasally with SARS-CoV-2.21 Virus isolation was successful in one of three cattle; however, neutralizing antibody levels were absent or low in most animals after 1 month, suggesting a minor role of cattle in SARS-CoV-2 epidemiology.21 In a different experimental study, sheep-derived cell cultures supported SARS-CoV-2 replication, but experimental infection of sheep resulted in low viral RNA levels in nasal and oral swabs right after challenge, and in the respiratory tract and lymphoid tissues 4 and 8 days later, indicating a low susceptibility of sheep to SARS-CoV-2 infection.22 Experimentally infected poultry (chickens, ducks, and turkeys) were found to be resistant to SARS-CoV-2 infection.23 Due to their importance in xenotransplantation pigs are discussed in more detail in Section 6.

Zoo animals and other wildlife. Several zoo animals have been found to be SARS-CoV-2 positive, thought to be infected by transmission from care staff or zoo visitors. Affected animals include lions and tigers,24 snow leopards, otters, non-human primates, gorillas, a binturong, a fishing cat, a coatimundi, hyenas, hippopotamuses, and manatees.25,26 SARS-CoV-2 infection has also been identified in other wildlife, including white-tailed deer,27 a mule deer,28 a black-tailed marmoset,29 a giant anteater,30 wild otters,31 wild bats,32 fruit bats,33 and raccoon dogs,34 which in the last two cases was experimentally confirmed. Tree shrews have been shown to develop subclinical infection after experimental infection,35 and bank voles could be experimentally infected with SARS-CoV-2 but there was no transmission to contact controls.36 A study of SARS-CoV-2 antibodies in samples from wild jackals, red foxes, and yellow legged gulls in Croatia collected during June–November 2020, assessed by a commercial N-protein-based double antigen enzyme-linked immunosorbent assay (ELISA; ID Screen SARS-CoV-2 Double Antigen Multi-species ELISA; ID.Vet, France), demonstrated that 4.6% (3/65) of jackals, 2.9% (6/204) of red foxes and none of yellow legged gulls (0/111) were positive for SARS-CoV-2 antibody.37 A confirmatory surrogate virus neutralization test based on the S-gene (GenScript, Netherlands) could not confirm the positive ELISA results and all animals were also negative for presence of SARS-CoV-2 RNA in fecal samples.37 When using serology for assessing possible infections it is important to understand that the response could be specific and true, however, there could also be cross-reaction to other circulating CoVs in addition to non-specific binding. Caution is needed when using serology assays validated on few SARS-CoV-2 positive species, and tested in animals where positive controls are not available. The double antigen ELISA described above has been validated on serum, plasma and whole blood and the species range is described by the manufacturer as minks, ferrets, dogs, cattle, sheep, goats, horses, and other susceptible species. In the above study blood was collected from wild boars and yellow-legged gulls, while for all other investigated animal species muscle extracts from carcasses were collected and used, which is not a validated sample.37

Among the cases of SARS-CoV-2 identified in wildlife, those in wild white-tailed deer, raised initial concerns due to the high prevalence rates of SARS-CoV-2 in deer and the presence of white-tailed deer in suburban areas close to humans.27 Based on available data, free-ranging white-tailed deer appear highly susceptible to infection with SARS-CoV-2 and have been exposed to multiple SARS-CoV-2 variants from humans.27,38–40 Furthermore, white-tailed deer are capable of sustaining transmission in the wild population. Importantly, clinical disease has not been observed in free-ranging white-tailed deer but virus and antibodies have been confirmed in wild populations across the United States.27,38–40 Experimental infection of white-tailed deer further confirmed that this species is susceptible to SARS-CoV-2, and the
SARS-CoV-2 VARIANTS CURRENTLY RECOGNIZED

Since its introduction into humans, SARS-CoV-2 has continually evolved. Different labels to describe virus strains have been used, including lineage (i.e., a group of closely related viruses with a common ancestor), and variant (i.e., a viral genome that may contain one or more mutations). The following variant categories are commonly used:

1. **Variants of concern (VOC):** This describes variants that have produced a significant difference in transmissibility, disease severity or interference with the immune system, or a combination of these, which may impact the epidemiological situation. Current VOC include viruses from the Delta (B.1.617.2) and Omicron lineages (BA.1, BA.2, BA.4 and BA.5).

2. **Variants of interest (VOI):** For these variants, genomic, epidemiological or in-vitro evidence is available indicating a potential impact on transmissibility, severity and immunity or any combination, with a high potential to affect the epidemiological situation. No variants currently fall into this category.

3. **Variants under monitoring (VUM) or being monitored (VBM):** This describes SARS-CoV-2 variants that have been detected through epidemic intelligence, rules-based genomic variant screening, or for which preliminary scientific evidence exists. It is possible that these variants have properties similar to those of a VOC, but the evidence is weak or has not yet been assessed. At least one outbreak must have been reported for a variant to fall into this category.

4. **De-escalated variants:** This group includes variants that are no longer circulating and never had any impact or concerning properties.

Other criteria used to differentiate SARS-CoV-2 virus groups include the WHO label (for example: Alpha, Beta, Delta, Omicron), lineage, year and month of discovery, country of discovery, and evidence concerning properties such as transmissibility, immunity or infection severity, or additional mutations such as those described for the Pango lineages and any other characteristic protein changes. Mutations of interest include spike protein mutations such as for example amino acids (aa) 319–541, the receptor binding domain (RBD), and the S1 part of the S1/S2 junction and a small stretch on the S2 side (aa 613–705).

A study investigating lineage diversity of SARS-CoV-2 from human and non-human hosts found 28 lineages in non-human hosts. The majority was represented by Delta (n = 1105, 30.7%) and Alpha variants (n = 466, 12.9%), followed by a Danish B.1.1.298 lineage (n = 458, 12.7%). Non-human hosts with the high numbers of lineages included American mink with 14 lineages, white-tailed deer with 12 lineages, and the domestic cat with 10 lineages. The study authors proposed that interspecies transmission of SARS-CoV-2 between animals and humans could result in novel variants evolving within an animal reservoir, with potential to spill back into humans, and this should be considered in order to establish better pathogen surveillance and containment strategies. The potential for evolution of novel variants in animal species, and spillover back into humans, is an additional risk associated with xenotransplantation.
demonstrated that 55 of 168 of the earliest known COVID-19 cases were clustered in close proximity to the market.49 The second study looked at SARS-CoV-2 sequences obtained early in the pandemic,50 and only two distinct lineages were observed which, based on further analysis, were the result of at least two separate cross-species transmission events into humans close together, estimated to have occurred during October through December 2019. The authors point out that the window between SARS-CoV-2 first jumping into humans and first cases of COVID-19 being reported was very narrow.50

5 | SARS-CoV-2 RECEPTOR BINDING

A requirement for successful replication in both humans and non-human species, including pets and farm animals, is having a suitable angiotensin-converting enzyme 2 (ACE2) which serves as a functional receptor for the spike protein of SARS-CoV-2.51 The ACE2 receptor is widely distributed in animals, particularly in the cardiovascular system and in alveolar epithelial cells.52 It has been shown that adaptive viral genome mutations can potentially change the virulence of the virus.53 For example, a single amino acid exchange can reduce or enhance the ability of the virus to bind a particular ACE2 receptor or evade the immune system, which complicates SARS-CoV-2 spike protein-based vaccine development.53 The spike protein RBD is therefore essential to both successful infection and vaccine escape. The main SARS-CoV-2 strains, categorized initially into Alpha, Beta, Delta, Gamma, and Omicron, all have mutations in the spike protein RBD and the N-terminal domain. The N501Y mutation, located on the RBD, is common to all variants except the Delta variant, and triggers increased affinity of the spike protein to the ACE2 receptors, thereby enhancing the viral attachment and its subsequent entry into the host cells.53 Once the importance of ACE2 in human SARS-CoV-2 infection was realized, numerous studies have focused on identifying animal species that have ACE2 receptors similar enough to humans to become a potential reservoir for the virus.51 In silico structural homology modelling, protein–protein docking, and molecular dynamics simulation studies of the SARS-CoV-2 spike protein’s ability to bind ACE2 from relevant species indicate that the highest binding affinity is to human ACE2, then to pangolin ACE2, whereas the affinity to monkey ACE2 was much lower.54 Interestingly, species with a high predicted ACE2 binding affinity range, for example monkeys, hamsters, dogs, ferrets, and cats, have all been reported to be permissive to SARS-CoV-2 infection based on experimental data or natural infection. These findings would support a correlation between binding affinity and infection susceptibility.54 Other similar studies confirmed these results and also predicted that the ACE2 receptor from other animals such as dogs, tigers, camels, cats, dwarf hamsters, and sheep may have a slightly increased affinity to the SARS-CoV-2 RBD.55

6 | PIGS AND SARS-CoV-2

Whether or not SARS-CoV-2 can infect domestic pigs is of particular concern, not only because these animals are economically important and raised under intensive farming conditions, but also because this species is also being used in xenotransplantation. Pigs are the most commonly used species in xenotransplantation due to their ready availability and the similarity of their organs to human organs. During xenotransplantation the xenograft recipient is usually immunosuppressed, and hence very susceptible to any viral infection that is introduced from the organ donor species. For example, the first human patient to receive a pig heart in January 202256 died 8 weeks after the successful surgery due to a porcine cytomegalovirus infection, a common herpes virus in pigs.57 It is well known that there are certain risk factors for patients developing COVID-19 and requiring re-admittance to hospital, which include solid organ transplantation.58 When liver transplant recipients were assessed following vaccination for SARS-CoV-2, they were found to have a considerably lower immunological response to the Pfizer-BioNTech SARS-CoV-2 mRNA-based vaccine59 compared to normal healthy people. Factors predicting a reduced vaccine response also include older age, reduced renal function and immunosuppressive medications.59 If a virus such as SARS-CoV-2 was introduced during xenotransplantation, this would obviously be detrimental for the patient. Investigations to establish whether or not pigs can support and sustain SARS-CoV-2 infections are therefore of great importance. The work done so far can be divided into studies where pigs were experimentally infected with SARS-CoV-2, studies assessing the ability of pig cells lines to support SARS-CoV-2 replication, and studies determining virus prevalence data from the field.

1. In vivo pig studies. Several studies investigated the ability of SARS-CoV-2 to infect and replicate in pigs (Table 1). While some results are conflicting,50 the body of evidence suggests that pigs are not susceptible to SARS-CoV-2.33,61–65 SARS-CoV-2 infected pigs typically do not develop any clinical signs or lesions, but can mount an antibody response after parenteral virus administration.62–65 Viral RNA can be found for up to 1–3 days after inoculation, likely suggesting inoculum remnants.62–65 SARS-CoV-2 RNA was also found in lung tissue of some pigs on days 3, 6, and 9, without evidence of virus replication.64 A single study reported successful SARS-CoV-2 virus isolation from a lymph node 13 days after experimental infection using the intranasal inoculation route.60 Transmission of SARS-CoV-2 from infected to contact pigs did not occur in any of the studies published so far.33,60–65

2. In vitro studies using porcine cells. SARS-CoV-2 studies using cell lines of porcine origin are summarized in Table 2. It appears that porcine kidney (PK) 15 cells can be readily infected with SARS-CoV-2,33,66 often resulting in cytopathogenic effects (CPE) suggestive of virus-related structural changes in the host cells.62 Cellular restriction factors are important to prevent certain viral infections.67,68 It should be underlined that PK15 cells have lost certain intracellular restriction factors and therefore may be susceptible to not only SARS-CoV-2 but also other viruses, for example porcine endogenous retroviruses (PERV).69 A similar study also reported an increase in SARS-CoV-2 genomic copy number in swine kidney (SK) 6 cells,33 and a study using swine testicle (ST) cells also resulted in virus increase and visible CPE.62 However, as
| Pig breed/age at infection | Infected pigs # | Contact pigs # | Inoculation | Route | Dose | Volume | Day(s) of necropsy | Source farm health status | Concurrent infections | Outcomes | References |
|----------------------------|----------------|----------------|-------------|-------|------|--------|-----------------|--------------------------|---------------------|-----------|------------|
| German Landrace 9 weeks    | 9              | 3 (24 h)                                  | IN           | $10^5$ TCID$_{50}$ | NA     | 4, 8, 12, 21, SPF | SPF $^b$                      | Not described           | RNA shedding  | Virus replication | Serum antibody development | ND | ND | ND | 33 |
| Landrace x Large White 6 weeks | 5              | 3 (0h)                                   | IN           | $10^4.5$ PFU  | NA     | 14               | SPF | Not described | ND | ND | ND | 61 |
| Breed not described 5 weeks | 9              | 6 (0h)                                   | Oral, IN and IT | $10^6$ TCID$_{50}$ | 4 ml   | 4, 8, 21 | SPF | Free of porcine circovirus type 2 (PCV2), swine influenza virus (SIV) and porcine reproductive and respiratory syndrome virus (PRRSV) | ND | ND | ND | 62 |
| Landrace x Large White 5-6 weeks | 5              | None                                      | IN           | $10^5$ TCID$_{50}$ | 3 ml   | 1, 2 | Conventional   | Seropositive for porcine respiratory coronavirus (PRCV). Not described for other pathogens | D1: 1/5 trachea swabs | ND | ND | D22: 5/5 N antibodies and VN titers | 65 |
|                            | 5              | None                                      | IT           | $10^5$ TCID$_{50}$ | 3 ml   | 2, 22 | | ND | ND | D14 and D22: 5/5 spike antibodies | D22: 5/5 VN titers |
|                            | 5              | None                                      | IM           | $10^5$ TCID$_{50}$ | 2 ml   | 2,22 | | ND | ND | D14 and D22: 5/5 spike antibodies | D22: 5/5 VN titers |
| American Yorkshire crossbred 8 weeks | 16             | 2 (10 days)                               | Oral, IN | $10^6$ PFU  | 3 ml   | 3, 5, 7, 9, 11, 13, 15, 22 and 29 | SPF | Not described | D3: 1/2 oral fluids | D3: 2/16 nasal washes | D13: Successful virus isolation from a lymph node | D6: 1/2 oral fluid | D11: 2/8 pigs VN titers | D13: 2/6 pigs VN titers | 60 |

(Continues)
| Pig breed/age at infection | Infected pigs # | Contact pigs # | Route | Dose  | Volume | Day(s) of necropsy | Source farm health status | Concurrent infections | Outcomes | References |
|---------------------------|-----------------|----------------|-------|-------|--------|-------------------|--------------------------|------------------------|-----------|------------|
| Large White or Large White x Norsvin Landrace 12 weeks | 8 | None | IN and IT | 10$^{6.2}$ TCID$_{50}$ | 10 ml | 3, 6, 10, 21 | Conventional | Not described | RNA shedding | Virus replication | Serum antibody development | References |
| | | | | | | | | | D1: 8/8 and D2: 1/8 oropharyngeal swabs | ND | D10: 2/4 pigs VN titers | D14: 1/1 previous positive pig (the other positive pig was euthanized on D10) | 64 |
| Breed not described | 4 | 1 (2 days) | IV | 6.8 × 10$^{5}$ TCID$_{50}$ | 2 ml | 21 | SPF | Not described | RNA shedding | Virus replication | Serum antibody development | References |
| | | | | | | | | D4: 1/4 oronasal swab | ND | D7: 4/4 VN titers | D14: 2/4 VN titers (reduced) | 63 |
| 3 weeks | | | | | | | | D3: 2/4 buffy coat | D21: 0/4 VN titers | |
| | 4 | 1 (2 days) | IT | | 5 ml | 21 | | | | | | |
| | 4 | 1 (2 days) | IN | | 5 ml | 21 | | | | | | |

Abbreviations: D, day post SARS-CoV-2 challenge; IM, intramuscular; IN, intranasal; IT, intratracheal; IV, intravenous; N, nucleocapsid protein; NA, not applicable; ND, not detected; PFU, plaque forming unit; TCID$_{50}$, 50% tissue culture infectious dose; VN, titers determined via a virus neutralization assay.

aTime of introduction of contact pigs after infection of infected pigs.

bSPF, specific pathogen free (SPF). The pig herd is free of certain economically important pig pathogens and is monitored at regular intervals to maintain this status. SPF for the purpose of this table also includes pigs designated as “high health” in contrast to the term “conventional pig farm.” A conventional pig farm is not claiming to be free of one or more specified pathogens, likely has a number of endemic disease pathogens present, but is not required to follow regular disease monitoring programs.
### TABLE 2  Summary of in vitro SARS-CoV-2 infection studies using pig derived cell lines

| Cell line                  | SARS-CoV-2 dose | Passages or hpi | Results                                                                 | Cytopathogenic effects (CPE) | Tests for other viruses | Reference |
|----------------------------|----------------|----------------|-------------------------------------------------------------------------|-----------------------------|-------------------------|-----------|
| Porcine kidney (PK) 15     | $10^{5.5}$ TCID$_{50}$ | 2 and 72 hpi    | Ct 27.7 and 24.2 Titer: negative                                       | No                          | ND                      | 33        |
|                            | 0.05 MOI of passage 3 of the VeroE6-passaged SARS-CoV-2 | 4 passages      |                                                                          | CPE at passage 4            | ND                      | 62        |
|                            | 0.1 MOI for 2 h at 37°C | 2, 24, 72 and 120 hpi | 3 log or greater increase in mean viral load over a period of 120 h    | No                          | ND                      | 66        |
| Swine kidney (SK) 6        | $10^{5.5}$ TCID$_{50}$ | 27.1            | Ct 27.1 → 12.0 Titer: $6.8 \times 10^7$                               | No                          | ND                      | 33        |
| Swine testicle (ST) cells  | $10^{5.5}$ TCID$_{50}$ | 27.8            | Ct 27.8 → 11.2 Titer: $3.1 \times 10^8$                               | No                          | ND                      | 33        |
|                            | 0.05 MOI of passage 3 of the VeroE6-passaged SARS-CoV-2 | 2 passages      |                                                                          | CPE at passage 2            | ND                      | 62        |
| Primary porcine respiratory epithelial cells | MOI 5.0, $5.0 \times 10^{–2}$, $5.0 \times 10^{–4}$ | 120 hpi         | Ct 17.5 with MOI 5.0. CPE dose and time dependent, most prominent with MOI 5.0 infectious dose and 96 hpi | No                          | ND                      | 71        |

Abbreviations: Ct, cycle threshold; hpi, hours post infection; MOI, multiplicity of infection; ND, not described; TCID$_{50}$, 50% tissue culture infectious dose.

SARS-CoV-2 is a respiratory virus which mainly replicates in the upper respiratory tract and lungs, the importance of successful virus replication in individual cell lines derived from kidneys or testicles is questionable. One recent study infected primary cell cultures from the respiratory tract of various animal species, including pigs, with SARS-CoV-2. Another infected nasal mucosa explants, tracheal epithelial cells cultured at an air-liquid interface and precision cut lung slices from pigs. While productive replication of SARS-CoV-2 was observed in dogs and few other species, none of the pig tissues used supported viral replication. Enhanced apoptosis in primary respiratory epithelial cells has been considered as a possible mechanism to self-limit SARS-CoV-2 replication in the pig respiratory tract. In addition, it may be interesting to know whether the cell lines used for SARS-CoV-2 infection studies were infected with other viruses, for example a porcine circovirus (PCV) 1, which was found to be a contaminant in cells used for production of a human rotavirus vaccine during 2010.

3. **Surveillance studies.** From June to December 2020 blood, muscle extract and fecal samples from 153 free-living wild boars in Croatia were tested and it was found that 6/153 blood samples (3.9%) were positive by a commercial multispecies SARS-CoV-2 antibody ELISA, whereas all fecal samples were negative for SARS-CoV-2 RNA by PCR testing. As outlined in Section 2 under zoo animals and other wildlife, the specificity of the obtained responses should be confirmed to rule out cross-reactions to other CoVs and non-specific binding. In the Netherlands, 417 pig serum samples were obtained at slaughter from 17 farms located in a region with a high human case incidence of SARS-CoV-2 from March to July 2020. These samples were tested with protein micro array, plaque reduction neutralization test (PRNT) and receptor-binding-domain ELISA. None of the serum samples were positive in all three assays, although six samples from one farm returned a low positive result in PRNT (titers 40–80). In addition, the authors also investigated an outbreak of respiratory disease in pigs on one farm, which occurred in February 2021, coinciding with recent exposure to SARS-CoV-2 infected animal caretakers. Tonsil swabs and paired serum samples were tested and there was no evidence for infection with SARS-Co-2. The authors concluded that sporadic infections in the field cannot be excluded, but large-scale SARS-CoV-2 transmission among pigs is unlikely.

7 | DEVELOPMENT OF A HUMANIZED SWINE MODEL FOR COVID-19 RESEARCH

For a virus infection that primarily targets humans, such as SARS-CoV-2, animal models that accurately mimic human disease manifestations and can be used as models for vaccine and therapeutics development,
are incredibly important. A variety of animal SARS-CoV-2 models are currently available, including hamsters,74,75 mice,76,77 ferrets,16 cats,78 and non-human primates.79,80 However, from the perspectives of both ethics and availability, usage of non-human primates should be limited whenever possible, while ferrets, mice, hamsters, and cats simply do not resemble human infections enough to be useful for testing intervention treatments. In contrast, pigs closely resemble human disease processes in general, because their anatomy and physiology are similar. While current data suggest that the ordinary domestic pig is not susceptible to SARS-CoV-2, recent research has been focusing on modifying pigs to change this. To create a COVID-19 pig model, one research group inserted the human ACE2 (hACE2) receptor at the pig ACE2 locus using the CRISPR/Cas9 system to create hACE2 knock-in pigs.81 To determine the replication efficiency of SARS-CoV-2 in primary cells derived from these hACE2 knock-in pigs, lung and kidney epithelial cells were isolated and infected with SARS-CoV-2 at a MOI of 0.01. In contrast to cells from wild-type pigs, these primary hACE2 knock-in pig cells showed significant CPE after 72 h of infection.81 The results of further in vivo studies of SARS-CoV-2 infection in these pigs will hopefully be available soon.

8 PRODUCTION OF SWINE GLYCO-HUMANIZED POLYCLONAL ANTIBODY AS THERAPY FOR COVID PATIENTS

During the early stages of the pandemic few treatment options for severely impacted or immunocompromised patients were available, and passive antibody therapy using convalescent plasma was explored as a treatment for COVID-19. However, the initial reports of therapeutic benefits in immunocompetent patients were not supported in larger studies, possibly because the antibody quality and quantity could not be standardized, and antibodies raised against a certain SARS-CoV-2 variant may not always fully protect against other variants.82,83 As an alternative, the swine glyco-humanized polyclonal antibody XAV-19 was developed using alpha 1,3-galactosyltransferase (GGTA1) and cytidine monophosphate N-acetylneuraminic acid hydroxylase (CMAH) gene double knockout pigs immunized against SARS-CoV-2 spike RBD.84 XAV-19 targets epitopes distributed all over the RBD, and particularly the receptor binding motif. In a subsequent spike/ACE2 interaction assay, XAV-19 showed potent neutralization capacities against the original Wuhan, United Kingdom (Alpha/B.1.1.7) and South African (Beta/B.1.351) variants. These results were confirmed by cytopathogenic assays using Vero E6 cells and live virus variants including the Gamma/P.1 and the Delta/B.1.617.2 variants.85

9 ANIMAL MODELS FOR STUDYING SARS-CoV-2OMICRON AND OTHER VARIANTS

At the beginning of the pandemic there was an immediate need to learn more about SARS-CoV-2 biology, transmission, evolution, and prevention. Due to a lack of knowledge about the virus, and the availability of large research funds almost instantly, a number of studies were conducted during 2020 and 2021. Only at the end of 2020, when many studies were already ongoing or finished, the emergence of different SARS-CoV-2 variants was recognized, which potentially differed in their capacity to cause disease, their transmissibility, or their ability to evade immune responses. To date there is little information on these novel SARS-CoV-2 variants in animal models. Results of studies using transgenic mice carrying human ACE2 indicate that, compared to the Alpha, Beta, and Delta variants, Omicron appears to be milder in virulence, based on insignificant weight loss and a low mortality in Omicron infected mice.86–88 In the hamster model, Alpha and Beta variants were both able to replicate efficiently, and viral infection resulted in bronchopneumonia similar to that observed in COVID-19 patients. However, there was no difference between these two SARS-CoV-2 variants.89 When the Delta and Omicron variants were also compared in this model, infection with the Delta variant resulted in quicker weight loss and more rapid distribution of SARS-CoV-2 protein in the lungs, resulting in increased pathogenicity.90 In ferret models, it was found that both the prototype Wuhan strain and the D614G variant can infect ferrets, but the D614G variant was dominant in terms of progeny virus produced from these animals during dual infection.91 Non-human primates have also been infected with Beta, Gamma, and Delta variants to assess vaccine efficacy against different variants.92 Established nomenclature systems for naming and tracking SARS-CoV-2 genetic lineages by GISAID, Nextstrain and Pango are currently and will remain in use by scientists and in scientific research.93 However, the current system is complicated and, due to different classification schemes of SARS-CoV-2, strategies should be developed to characterize lineages/variants/mutants in a more controlled fashion. A single common classification system of strains should perhaps be implemented so that results in publications can be better compared by people not familiar with virus sequence comparisons. For pigs, to date challenge studies have been done with the following isolates: 2019_nCoV Muc-IMB-1,33 CTan-H,61 hCoV-19/USA/WA1/2020 (GISAID ID EPI ISL 404895) USA-WA1/2020 isolate (GenBank accession # MN985325),62 GISAID ID EPI ISL 510689,65 and hCoV-19/Canada/ON-VIDO-01/2020 (GISAID accession no. EPI ISL 425177).60 All these strains appear to have evolved from the Wuhan SARS-CoV-2 isolate. In addition, SARS-CoV-2/human/NL/Lelystad/2020 (wild-type D614G, Genbank accession number MZ144583),64 and TGR/NY/20 (wild-type D614G, Genbank accession number MT704317), which was obtained from a tiger with contact to a COVID-19 infected zookeeper,65 have also been used. Both contain the D614G S-protein mutation, which has been suggested to alter SARS-CoV-2 fitness,94,95 promote viral transmission,91 and transduce cells more effectively in vitro.96 To the author’s knowledge, no other variants have been assessed.

10 SUMMARY

The SARS-CoV-2 pandemic is now in its third year. While the number of seriously ill people is decreasing, there is still an alarmingly
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REFERENCES

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

2. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Bio Medica Atenei Parmensis. 2020;91(1):157-160.

3. Ke R, Romero-Severson E, Sanche S, Hengartner N. Estimating the reproductive number R0 of SARS-CoV-2 in the United States and eight European countries and implications for vaccination. J Theor Biol. 2021;517:110621.

4. COVID-19 Cumulative Infection Collaborators. Estimating global, regional, and national daily and cumulative infections with SARS-CoV-2 through Nov 14, 2021: a statistical analysis. Lancet. 2022;399(10344):2351-2380.

5. Garigliany M, VanLaere AS, Clerc CX, et al. SARS-CoV-2 natural transmission from human to cat, Belgium, March 2020. Emerg Infect Dis. 2020;26(12):3069-71.

6. Sailleau C, Dumarest M, Vanhomwegen J, et al. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis. 2020;67(6):2324-2328.

7. Ruiz-Arrondo I, Portillo A, Palomar AM, et al. Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe. Transbound Emerg Dis. 2021;68(2):973-6.

8. Teixeira AP. SARS-CoV-2 in animals: what about the cat? Vet Q. 2021;41(1):226-227.

9. Medkour H, Catheland S, Bourcraut-Baralon C, et al. First evidence of human-to-dog transmission of SARS-CoV-2 B.1.160 variant in France. Transbound Emerg Dis. 2022;69(4):e823-e830.

10. Patterson EJ, Ella G, Grassi A, et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun. 2020;11(1):6231.

11. Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARS-CoV-2. Nature. 2020;586(7831):776-778.

12. Sila T, Sunghan J, Laohareonsuk W, et al. Suspected cat-to-human transmission of SARS-CoV-2, Thailand, July-September 2021. Emerg Infect Dis. 2022;28(7):1485-1488.

13. Yen HL, Sit THC, Brackman CJ, et al. Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. Lancet (London, England). 2022;399(10329):1070-1078.

14. Mykytyn AZ, Lamers MM, Okba NMA, et al. Susceptibility of rabbits to SARS-CoV-2. Emerg Infect Dis. 2021;10(1):1-7.

15. Sia SF, Yan LM, Chin AWH, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature. 2020;583(7818):834-838.

16. Kim YJ, Kim SG, Kim SM, et al. Infection and rapid transmission of SARS-CoV-2 in Ferrets. Cell Host Microbe. 2020;27(5):704-709.e2.

17. Yang Y, Zheng M, Liu Y, et al. Analysis of intermediate hosts and susceptible animals of SARS-CoV-2 by computational methods. Zoonoses. 1(1). https://doi.org/10.15212/ZOONOSES-2021-0010

18. Liang L, He C, Lei M, et al. Pathology of guinea pigs experimentally infected with a novel reovirus and coronavirus isolated from SARS patients. DNA Cell Biol. 2005;24(8):485-490.

19. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science (New York, NY). 2021;371(6525):172-177.

20. Ulrich L, Wernike K, Hoffmann D, Mettenleiter TC, Beer M. Experimental infection of cattle with SARS-CoV-2. Emerg Infect Dis. 2020;26(12):2979-2981.

21. Bosco-Lauth AM, Walker A, Guibert L, et al. Susceptibility of livestock to SARS-CoV-2 infection. Emerg Microbes Infect. 2021;10(1):2199-2201.

22. Gaudreault NN, Cool K, Trujillo JD, et al. Susceptibility of sheep to experimental co-infection with the ancestral lineage of SARS-CoV-2 and its alpha variant. Emerg Microbes Infect. 2022;11(1):662-675.

23. Suarez DL, Pantin-Jackwood MJ, Swayne DE, Lee SA, DeBlois SM, Spackman E. Lack of susceptibility to SARS-CoV-2 and MERS-CoV in poultry. Emerg Infect Dis. 2020;26(12):3074-3076.

24. McAloose D, Laverack M, Wang L, et al. From people to panthera: Natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. MBio. 2020;11(5):e02220-20.

25. Animal and Plant Health Inspection Service (USDA). One Health - SARS-CoV-2 in animals. https://www.aphis.usda.gov/aphis/ourfocus/onehealth/one-health-sarscov2-in-animals. Accessed June 09, 2022.

26. Centers for Disease Control and Prevention (CDC) Animals and COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html. Accessed June 09, 2022.

27. Hale VL, Dennis PM, McBride DS, et al. SARS-CoV-2 infection in free-ranging white-tailed deer. Nature. 602(7897):481-486.

28. Mallapaty S. COVID is spreading in deer. What does that mean for the pandemic? Nature. 2022;604(7907):612-615.

29. Pereira AH, Vasconcelos AL, Silva VL, et al. Natural SARS-CoV-2 infection in a free-ranging black-tailed marmoset (Mico melanus) from an urban area in Mid-West Brazil. J Comp Pathol. 2022;194:22-27.

30. Sharun K, Dhama K, Pawde AM, et al. SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications. Vet Q. 2021;41(1):181-201.

31. Padilla-Blanco M, Aguilo-Gisbert J, Rubio V, et al. The finding of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) in a wild Eurasian river otter (Lutra lutra) highlights the need for viral surveillance in wild mustelids. Front Vet Sci. 2022;9:826991.

32. Aguilo-Gisbert J, Padilla-Blanco M, Lizana V, et al. First description of SARS-CoV-2 infection in two feral American mink (Neovison vison) caught in the wild. Animals (Basel). 2021;11(5):1422.

33. Schlottau K, Rissmann M, Graaf A, et al. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. Lancet Microbe. 2020;11(5):e218-e225.

high number of people being infected every single day, allowing the virus to continue to move rapidly from person to person and providing the virus with ample opportunities for further mutations. The number of organ recipients has also continued to increase during the pandemic, and solutions to resolve the lack of available organs from human donors are still needed. Pigs are ideal for xenotransplantation and much needed pig-to-human organ transplantation is evolving quickly. However, pigs can harbor both pig- and human-specific viruses which may pose a risk for the human organ recipient. It appears that SARS-CoV-2 infection from pigs is currently not a major threat, but this may change in the future should variants become more adapted to pigs. Because of this, experts should periodically assess the situation to make sure that this has not occurred. In particular, studies of whether pigs are more susceptible to infection with some of the newer SARS-CoV-2 variants, including any variants of concern, is urgently warranted. In transplant organ recipients in general should be cautious of other zoonotic SARS-CoV-2 sources including pets.
34. Freuling CM, Breithaupt A, Müller T, et al. Susceptibility of raccoon dogs for experimental SARS-CoV-2 infection. Emerg Infect Dis. 2020;26(12):2982-2985.
35. Zhao Y, Wang J, Kuang D, et al. Susceptibility of tree shrew to SARS-CoV-2 infection. Sci Rep. 2020;10(1):16007.
36. Ulrich L, Michielsitsch A, Halwe N, Wernike K, Hoffmann D, Beer M. Experimental SARS-CoV-2 infection of bank voles. Emerg Infect Dis. 2021;27(4):1193-1195.
37. Jemeršić L, Lojkić I, Krešić N, et al. Investigating the presence of SARS-CoV-2 in free-living and captive animals. Pathogens. 2021;10(6):635.
38. Palermo PM, Orbeegojo J, Watts DM, Morrill JC. SARS-CoV-2 neutralizing antibodies in white-tailed deer from Texas. Vector Borne Zoonotic Dis. 2022;22(1):62-64.
39. Chandler JC, Bevins SN, Ellis JW, et al. SARS-CoV-2 exposure in wild white-tailed deer (Odocoileus virginianus). Proc Natl Acad Sci U S A. 2021;118(47):e2114828118.
40. Kuchipudi SV, Surendran-Nair M, Ruden RM, et al. Multiple spillovers from humans and onward transmission of SARS-CoV-2 in white-tailed deer. Proc Natl Acad Sci U S A. 2022;119(6):e212644119.
41. Palmer MV, Martins M, Falkenberg S, et al. Susceptibility of white-tailed deer (Odocoileus virginianus) to SARS-CoV-2. J Virol. 2021;95(11):e00083-21.
42. Griffin BD, Chan M, Tailor N, et al. SARS-CoV-2 infection and transmission in the North American deer mouse. Nat Commun. 2021;12(12):3612.
43. European Centre for Disease Prevention and Control. SARS-CoV-2 variants of concern as of 15 July. https://www.ecdc.europa.eu/en/covid-19-variants-concern. Accessed July 21, 2022.
44. Muñoz M, Patiño LH, Ballesteros N, et al. Striking lineage diversity of severe acute respiratory syndrome coronavirus 2 from non-human sources. One Health. 2022;14:100363.
45. Holmes EC, Goldstein SA, Rasmussen AL, et al. The origins of SARS-CoV-2: A critical review. Cell. 2021;184(19):4848-4856.
46. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.
47. Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol. 2020;30(7):1346-1351.e2.
48. Niu S, Wang J, Bai B, et al. Molecular basis of cross-species ACE2 interactions with SARS-CoV-2-like viruses of pangolin origin. EMBO J. 2021;40(16):e107786.
49. Holmes EC, Goldstein SA, Rasmussen AL, et al. The origins of SARS-CoV-2: A critical review. Cell. 2021;184(19):4848-4856.
50. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.
51. Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol. 2020;30(7):1346-1351.e2.
52. Niu S, Wang J, Bai B, et al. Molecular basis of cross-species ACE2 interactions with SARS-CoV-2-like viruses of pangolin origin. EMBO J. 2021;40(16):e107786.
53. Worobey M, Levy JJ, Serrano LM, et al. The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2. Science. 2020;368(6494):1016-1020.
54. Piplani S, Singh PK, Winkler DA, Petrovsky N. In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin. Sci Rep. 2021;11(1):13063.
55. Bouricha EM, Hakmi M, Akachar J, Belyamani L, Ibrahim I. In silico analysis of ACE2 orthologues to predict animal host range with high susceptibility to SARS-CoV-2. 3. Biotech. 2020;10(11):483.
56. Reardon S. First pig-to-human heart transplant: what can scientists learn? Nature. 2022;601(7893):305-306.
57. Rettner R. Man who received 1st pig heart transplant has died. https://www.livescience.com/pig-human-heart-transplant-death. Accessed June 09, 2022.
58. Green H, Yahav D, Elakim-Raz N, et al. Risk-factors for re-admission and outcome of patients hospitalized with confirmed COVID-19. Sci Rep. 2021;11(1):17416.
59. Rabinovich L, Grupper A, Baruch R, et al. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. J Hepatol. 2021;75(2):435-438.
60. Pickering BS, Smith G, Pinette MM, et al. Susceptibility of domestic swine to experimental infection with severe acute respiratory syndrome coronavirus 2. Emerg Infect Dis. 2021;27(1):104-112.
61. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020;368(6494):1016-1020.
62. Meekes DA, Morozov I, Trujillo JD, et al. Susceptibility of swine cells and domestic pigs to SARS-CoV-2. Emerg Infect Dis. 2020;9(1):2278-2288.
63. Buckley A, Falkenberg S, Martins M, et al. Intravenous, intratracheal, and intranasal inoculation of swine with SARS-CoV-2. Viruses. 2021;13(8):1506.
64. Sikkema RS, Tobias T, Oreshkova N, et al. Experimental and field investigations of exposure, replication and transmission of SARS-CoV-2 in pigs in the Netherlands. Emerg Microbes Infect. 2022;11(1):91-94.
65. Vergara-Alert J, Rodon J, Carrillo J, et al. Pigs are not susceptible to SARS-CoV-2 infection but are a model for viral immunogenicity studies. Transbound Emerg Dis. 2021;68(4):1721-1725.
66. Chu H, Chan JF-W, Yuen TT-T, et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. Lancet Microbe. 2021;2(1):e14-e23.
67. Colomer-Lluch M, Ruiz A, Moris A, Prado JG. Restriction factors: From intrinsic viral restriction to shaping cellular immunity against HIV-1. Front Immunol. 2018;9:2876.
68. Duggal NK, Emerman M. Evolutionary conflicts between viruses and restriction factors shape immunity. Nat Rev Immunol. 2012;12(10):687-695.
69. Fieber U, Fischer K, Bähr A, et al. Porcine endogenous retroviruses: quantification of the copy number in cell lines, pig breeds, and organs. Xenotransplantation. 2018;25(4):e12445.
70. Färber I, Krüger J, Rocha C, et al. Investigations on SARS-CoV-2 susceptibility of domestic and wild animals using primary cell culture models derived from the upper and lower respiratory tract. Viruses. 2021;14(4):828.
76. Sun SH, Chen Q, Gu HJ, et al. A mouse model of SARS-CoV-2 infection and pathogenesis. Cell Host Microbe. 2020;28(1):124-133.e4.

77. Leist SR, Dinmon KH, 3rd, Schäfer A, et al. A mouse-adapted SARS-CoV-2 induces acute lung injury and mortality in standard laboratory mice. Cell. 2020;183(4):1070-1085.e12.

78. Rudd JM, Selvan MT, Cowan S, et al. Clinicopathologic features of a feline SARS-CoV-2 infection model parallel acute COVID-19 in humans. Viruses. 2021;13(8):1550.

79. Rudd JM, Selvan MT, Cowan S, et al. Clinicopathologic features of a feline SARS-CoV-2 infection model parallel acute COVID-19 in humans. Viruses. 2021;13(8):1550.

80. Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature. 2020;586(7830):583-588.

81. Du X, Guo Z, Fan W, et al. Establishment of a humanized swine model for COVID-19. Cell Discov. 2021;7(1):70.

82. Wibmer CK, Ayres F, Hermanus T, et al. SARS-CoV-2 501YV2 escapes neutralization by South African COVID-19 donor plasma. Nat Med. 2021;27(4):622-625.

83. Hammarström L, Marcotte H, Piralla A, Baldanti F, Pan-Hammarström Q. Antibody therapy for COVID-19. Curr Opin Allergy Clin Immunol. 2021;21(6):553-558.

84. Vanhove B, Duvaux O, Rousse J, et al. High neutralizing potency of swine glyco-humanized polyclonal antibodies against SARS-CoV-2. Eur J Immunol. 2021;51(6):1412-1422.

85. Vanhove B, Marot S, So RT, et al. XAV-19, a Swine glyco-humanized polyclonal antibody against SARS-CoV-2 spike receptor-binding domain, targets multiple epitopes and broadly neutralizes variants. Front Immunol. 2021;12:61250.

86. Ding Z, Chen T, Lan J, Wong G. Application of animal models to compare and contrast the virulence of current and future potential SARS-CoV-2 variants. Biosaf Health. 2022;4(3):154-160.

87. Bao L, Deng W, Huang B, et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature. 2020;583(7818):830-833.

88. Shuai H, Chan JF, Hu B, et al. Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. Nature. 2022;603(7902):693-699.

89. Abdelnabi R, Boudewijns R, Foo CS, et al. Comparing infectivity and virulence of emerging SARS-CoV-2 variants in Syrian hamsters. EBioMedicine. 2021;68:103403.

90. Saito A, Irie T, Suzuki R, et al. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature. 2022;602(7896):300-306.

91. Zhou B, Thao TTN, Hoffmann D, et al. SARS-CoV-2 spike D614G change enhances replication and transmission. Nature. 2021;592(7852):122-127.

92. Gagne M, Corbett KS, Flynn BJ, et al. Protection from SARS-CoV-2 Delta one year after mRNA-1273 vaccination in rhesus macaques coincides with anamnestic antibody response in the lung. Cell. 2022;185(1):113-130.e15.

93. World Health Organization. Tracking SARS-CoV-2 variants. https://www.who.int/activities/tracking-SARS-CoV-2-variants. Accessed July 21, 2022.

94. Zhang L, Jackson CB, Mou H, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun. 2020;11(1):6013.

95. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness. Nature. 2021;592(7852):116-121.

96. Daniloski Z, Jordan TX, Ilmain JK, et al. The Spike D614G mutation increases SARS-CoV-2 infection of multiple human cell types. Elife. 2021;10:e65365.

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