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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Variant

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Abstract: Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in December 2019. The disease has since spread worldwide, leading to an ongoing pandemic. Symptoms of COVID-19 are variable, but often include fever, cough, headache, fatigue, breathing difficulties, and loss of smell and taste. Symptoms begin 1 - 14 days after exposure to the coronavirus. The standard diagnostic method is by detection of the virus' nucleic acid by real-time reverse transcription polymerase chain reaction (rRT-PCR), transcription-mediated amplification (TMA), or by reverse transcription loop-mediated isothermal amplification (RT-LAMP) from a nasopharyngeal swab. (https://en.wikipedia.org/wiki/COVID-19). Upto 12/1/2021 in USA, Total Cases are 48,377,531, Total accines Administered are 460,773,508, Total Deaths 778,489 (https://www.cdc.gov/coronavirus/2019-ncov/index.html). Here gives the gives **SARS-CoV-2** complete genome sequenced by China in 1/13/2020, as a reference for readers (https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3).
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Key words: COVID-19; SARS-CoV-2; genome; sequence; life; research; literature; cell

1. Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in December 2019. The disease has since spread worldwide, leading to an ongoing pandemic. Symptoms of COVID-19 are variable, but often include fever, cough, headache, fatigue, breathing difficulties, and loss of smell and taste. Symptoms begin 1 - 14 days after exposure to the coronavirus. At least 30% of people who are infected do not develop noticeable symptoms. Of those people who develop symptoms noticeable enough to be classed as patients, around 80% develop mild to moderate symptoms, while 14% develop severe, and 5% suffer critical symptoms. Some people continue to experience a range of effects for months after recovery, and damage to organs has been observed. Multi-year studies are underway to further investigate the long-term effects of the disease. COVID-19 transmits when people breathe in air contaminated by droplets and small airborne particles containing the virus. People remain contagious for up to 20 days, and can spread the virus even if they do not develop symptoms. The standard diagnostic method is by detection of the virus' nucleic acid by real-time reverse transcription polymerase chain reaction (rRT-PCR), transcription-mediated amplification (TMA), or by reverse transcription loop-mediated isothermal amplification (RT-LAMP) from a nasopharyngeal swab. Several COVID-19 vaccines have been approved and distributed in various countries, which have initiated mass vaccination campaigns. Other preventive measures include physical or social distancing, quarantining, ventilation of indoor spaces, covering coughs and sneezes, hand washing, and keeping unwashed hands away from the face. The use of face masks or coverings has been recommended in public settings to minimize the risk of transmissions. Management involves the treatment of symptoms, supportive care, isolation, and experimental measures. (https://en.wikipedia.org/wiki/COVID-19). Upto 12/1/2021 in USA, Total Cases are 48,377,531, Total accines Administered are 460,773,508, Total Deaths 778,489 (https://www.cdc.gov/coronavirus/2019-ncov/index.html).

On November 24, 2021, a new variant of SARS-CoV-2, B.1.1.529, was reported to the World Health Organization (WHO). This new variant was first detected in specimens collected on November 11, 2021 in Botswana and on November 14, 2021 in South Africa.

On November 26, 2021, WHO named the B.1.1.529 Omicron and classified it as a Variant of Concern (VOC). On November 30, 2021, the United States designated Omicron as a Variant of Concern,

and on December 1, 2021 the first confirmed U.S. case of Omicron was identified.

CDC has been collaborating with global public health and industry partners to learn about Omicron, as we continue to monitor its course. CDC has been using genomic surveillance throughout the course of the pandemic to track variants of SARS-CoV-2, the virus that causes COVID-19, and inform public health practice. We don't yet know how easily it spreads, the severity of illness it causes, or how well available vaccines and medications work against it (https://www.cdc.gov/coronavirus/2019-ncov/variants/omicron-variant.html).

Here gives the gives SARS-CoV-2 complete genome sequenced by China in 1/13/2020, as a reference for readers (https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3).

Abe, H., et al. (2021). "Unrecognized introduction of SARS-CoV-2 variants of concern to Central Africa: Import and local transmission of B.1.1.7 in Gabon in the very early stage of the variant spread to the African continent." J Med Virol **93**(10): 6054-6058.

The rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern with higher infectivity has already resulted in the enormous increase in infection cases worldwide. We report an unrecognized introduction of the variant B.1.1.7 in Gabon in December 2020, which was the initial phase of the variant introduction to Africa. The B.1.1.7 variant was also detected in a hospitalized patient in January 2021, indicating a rapid spread of the variant in Gabon since its first detection. Phylogenetic analysis revealed that the detected B.1.1.7 variants originated from the distinct regions, strongly suggesting that the B.1.1.7 variant had been repeatedly introduced to Gabon since December 2020. These results provide insights on the unrecognized risks of infections with variants of concern, and show the necessity to conduct continuous genomic monitoring for immediate alert and control of novel SARS-CoV-2 variant infections.

Abou-Hamdan, M., et al. (2021). "Variant analysis of the first Lebanese SARS-CoV-2 isolates." Genomics **113**(1 Pt 2): 892-895.

Recently the first genome sequences for 11 SARS-CoV-2 isolates from Lebanon became available. Here, we report the detection of variants within the genome of these strains. Pairwise alignment analysis using blastx was performed between these sequences and the UniProtKB data for the SARS-CoV-2 coronavirus to identify amino acid variations. Variants analysis was performed using multiple Bioinformatics tools. We noticed for the first time 18 mutations that have never been reported before. Among those, a frame shift (8651A>) in NSP4, a stop codon 6887A > T in NSP3 and two missense mutations in spike S2 were found. In addition, we found 28 variants in ORF1ab alone. A previously reported variant, 23403A > G, in the spike protein S2 was mostly seen. Two other known mutations 25563G > T in ORF3a and 14408C > T in ORF1ab were detected respectively in 6 and 8 out of the 11 isolates. Our results may help to prognose forthcoming infections in this region.

Abraham, R. S., et al. (2021). "Severe SARS-CoV-2 disease in the context of a NF-kappaB2 loss-of-function pathogenic variant." J Allergy Clin Immunol **147**(2): 532-544 e531.

BACKGROUND: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that emerged recently and has created a global pandemic. Symptomatic SARS-CoV-2 infection, termed coronavirus disease 2019 (COVID-19), has been associated with a host of symptoms affecting numerous organ systems, including the lungs, cardiovascular system, kidney, central nervous system, gastrointestinal tract, and skin, among others. OBJECTIVE: Although several risk factors have been identified as related to complications from and severity of COVID-19, much about the virus remains unknown. The host immune response appears to affect the outcome of disease. It is not surprising that patients with intrinsic or secondary immune compromise might be particularly susceptible to complications from SARS-CoV-2 infection. Pathogenic loss-of-function or gain-of-function heterozygous variants in nuclear factor-kappaB2 have been reported to be associated with either a combined immunodeficiency or common variable immunodeficiency phenotype. METHODS: We evaluated the functional consequence and immunologic phenotype of a novel NFKB2 loss of function variant in a 17-year-old male patient and describe the clinical management of SARS-CoV-2 infection in this context. RESULTS: This patient required a 2-week hospitalization for SARS-CoV-2 infection, including 7 days of mechanical ventilation. We used biologic therapies to avert potentially fatal acute respiratory distress syndrome and treat hyperinflammatory responses. The patient had an immunologic phenotype of B-cell dysregulation with decreased switched memory B cells. Despite the underlying immune dysfunction, he recovered from the infection with intense management. CONCLUSIONS: This clinical case exemplifies some of the practical challenges in management of patients with SARS-CoV-2 infection, especially in the context of underlying immune dysregulation.

Agwa, S. H. A., et al. (2021). "Association between Interferon-Lambda-3 rs12979860, TLL1 rs17047200 and DDR1 rs4618569 Variant Polymorphisms with the Course and Outcome of SARS-CoV-2 Patients." Genes (Basel) **12**(6).

BACKGROUND: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection provides a critical host-immunological challenge. AIM: We explore the effect of host-genetic variation in interferon-lambda-3 rs12979860, Tolloid Like-1 (TLL1) rs17047200 and Discoidin domain receptor 1(DDR1) rs4618569 on host response to respiratory viral infections and disease severity that may probe the mechanistic approach of allelic variation in virus-induced inflammatory responses. METHODS: 141 COVID-19 positive patients and 100 healthy controls were tested for interferon-lambda-3 rs12979860, TLL1 rs17047200 and DDR1 rs4618569 polymorphism by TaqMan probe-based genotyping. Different genotypes were assessed regarding the COVID-19 severity and prognosis. RESULTS: There were statistically significant differences between the studied cases and control group with regard to the presence of comorbidities, total leucocytic count, lymphocytic count, CRP, serum LDH, ferritin and D-dimer ($p < 0.01$). The CC genotype of rs12979860 cytokine, the AA genotype of TLL1 rs17047200 and the AA genotype of the rs4618569 variant of DDR1 showed a higher incidence of COVID-19 compared to the others. There were significant differences between the rs4618569 variant of DDR and the outcome of the disease, with the highest mortality in AG genotype 29 (60.4%) in comparison to 16 (33.3%) and 3 (6.2%) in the AA and GG genotypes, respectively ($p = 0.007$*), suggesting that the A allele is associated with a poor outcome in the disease. CONCLUSION: Among people who carry C and A alleles of SNPs IFN-lambda rs12979860 and TLL1 rs17047200, respectively, the AG genotype of the DDR1 rs4618569 variant is correlated with a COVID-19 poor outcome. In those patients, the use of anti-IFN-lambda 3, TLL1 and DDR1 therapy may be promising for personalized translational clinical practice.

Akhmetzhanov, A. R., et al. (2021). "A hospital-related outbreak of SARS-CoV-2 associated with variant Epsilon (B.1.429) in Taiwan: transmission potential and outbreak containment under intensified contact tracing, January-February 2021." Int J Infect Dis **110**: 15-20.

OBJECTIVES: A hospital-related cluster of 22 cases of coronavirus disease 2019 (COVID-19) occurred in Taiwan in January-February 2021. Rigorous control measures were introduced and could only be relaxed once the outbreak was declared over. Each day after the apparent outbreak end, we estimated the risk of future cases occurring in order to inform decision-making. METHODS: Probabilistic transmission networks were reconstructed, and transmission parameters (the reproduction number R and overdispersion parameter k) were estimated. The reporting delay during the outbreak was estimated (Scenario 1). In addition, a counterfactual scenario with less effective interventions characterized by a longer reporting delay was considered (Scenario 2). Each day, the risk of future cases was estimated under both scenarios. RESULTS: The values of R and k were estimated to be 1.30 ((95% credible interval (CI) 0.57-3.80) and 0.38 (95% CI 0.12-1.20), respectively. The mean reporting delays considered were 2.5 days (Scenario 1) and 7.8 days (Scenario 2). Following the final case, ttthe inferred probability of future cases occurring declined more quickly in Scenario 1 than Scenario 2. CONCLUSIONS: Rigorous control measures allowed the outbreak to be declared over quickly following outbreak containment. This highlights the need for effective interventions, not only to reduce cases during outbreaks but also to allow outbreaks to be declared over with confidence.

Alizon, S., et al. (2021). "Rapid spread of the SARS-CoV-2 Delta variant in some French regions, June 2021." Euro Surveill **26**(28).

We analysed 9,030 variant-specific RT-PCR tests performed on SARS-CoV-2-positive samples collected in France between 31 May and 21 June 2021. This analysis revealed rapid growth of the Delta variant in three of the 13 metropolitan French regions and estimated a +79% (95% confidence interval: 52-110%) transmission advantage compared with the Alpha variant. The next weeks will prove decisive and the magnitude of the estimated transmission advantages of the Delta variant could represent a major challenge for public health authorities.

Allen, C. M., et al. (2021). "Guillain-Barre Syndrome Variant Occurring after SARS-CoV-2 Vaccination." Ann Neurol **90**(2): 315-318.

Although SARS-CoV-2 vaccines are very safe, we report 4 cases of the bifacial weakness with paresthesias variant of Guillain-Barre syndrome (GBS) occurring within 3 weeks of vaccination with the Oxford-AstraZeneca SARS-CoV-2 vaccine. This rare neurological syndrome has previously been reported in association with SARS-CoV-2 infection itself. Our cases were given either intravenous immunoglobulin, oral steroids, or no treatment. We suggest vigilance for cases of bifacial weakness with paresthesias variant GBS following vaccination for SARS-CoV-2 and that postvaccination surveillance programs ensure robust data capture of this outcome, to assess for causality. ANN NEUROL 2021;90:315-318.

Allen, H., et al. (2021). "Household transmission of COVID-19 cases associated with SARS-CoV-2 delta variant (B.1.617.2): national case-control study." Lancet Reg Health Eur: 100252.

Background: The SARS-CoV-2 Delta variant (B.1.617.2), first detected in India, has rapidly become the dominant variant in England. Early reports suggest this variant has an increased growth rate suggesting

increased transmissibility. This study indirectly assessed differences in transmissibility between the emergent Delta variant compared to the previously dominant Alpha variant (B.1.1.7). Methods: A matched case-control study was conducted to estimate the odds of household transmission (>/= 2 cases within 14 days) for Delta variant index cases compared with Alpha cases. Cases were derived from national surveillance data (March to June 2021). One-to-two matching was undertaken on geographical location of residence, time period of testing and property type, and a multivariable conditional logistic regression model was used for analysis. Findings: In total 5,976 genomically sequenced index cases in household clusters were matched to 11,952 sporadic index cases (single case within a household). 43.3% (n=2,586) of cases in household clusters were confirmed Delta variant compared to 40.4% (n= 4,824) of sporadic cases. The odds ratio of household transmission was 1.70 among Delta variant cases (95% CI 1.48-1.95, p <0.001) compared to Alpha cases after adjusting for age, sex, ethnicity, index of multiple deprivation (IMD), number of household contacts and vaccination status of index case. Interpretation: We found evidence of increased household transmission of SARS-CoV-2 Delta variant, potentially explaining its success at displacing Alpha variant as the dominant strain in England. With the Delta variant now having been detected in many countries worldwide, the understanding of the transmissibility of this variant is important for informing infection prevention and control policies internationally.

Alpert, T., et al. (2021). "Early introductions and community transmission of SARS-CoV-2 variant B.1.1.7 in the United States." medRxiv.

The emergence and spread of SARS-CoV-2 lineage B.1.1.7, first detected in the United Kingdom, has become a global public health concern because of its increased transmissibility. Over 2500 COVID-19 cases associated with this variant have been detected in the US since December 2020, but the extent of establishment is relatively unknown. Using travel, genomic, and diagnostic data, we highlight the primary ports of entry for B.1.1.7 in the US and locations of possible underreporting of B.1.1.7 cases. Furthermore, we found evidence for many independent B.1.1.7 establishments starting in early December 2020, followed by interstate spread by the end of the month. Finally, we project that B.1.1.7 will be the dominant lineage in many states by mid to late March. Thus, genomic surveillance for B.1.1.7 and other variants urgently needs to be enhanced to better inform the public health response.

Alpert, T., et al. (2021). "Early introductions and transmission of SARS-CoV-2 variant B.1.1.7 in the United States." Cell **184**(10): 2595-2604 e2513.

The emergence and spread of SARS-CoV-2 lineage B.1.1.7, first detected in the United Kingdom, has become a global public health concern because of its increased transmissibility. Over 2,500 COVID-19 cases associated with this variant have been detected in the United States (US) since December 2020, but the extent of establishment is relatively unknown. Using travel, genomic, and diagnostic data, we highlight that the primary ports of entry for B.1.1.7 in the US were in New York, California, and Florida. Furthermore, we found evidence for many independent B.1.1.7 establishments starting in early December 2020, followed by interstate spread by the end of the month. Finally, we project that B.1.1.7 will be the dominant lineage in many states by mid- to late March. Thus, genomic surveillance for B.1.1.7 and other variants urgently needs to be enhanced to better inform the public health response.

Augusto, G., et al. (2021). "In vitro data suggest that Indian delta variant B.1.617 of SARS-CoV-2 escapes neutralization by both receptor affinity and immune evasion." Allergy.

BACKGROUND: Emerged mutations can be attributed to increased transmissibility of the B.1.617 and B.1.36 Indian delta variants of SARS-CoV-2, most notably substitutions L452R/E484Q and N440K, respectively, which occur in the receptor-binding domain (RBD) of the Spike (S) fusion glycoprotein. OBJECTIVE: We aimed to assess the effects of mutations L452R/E484Q and N440K (as well as the previously studied mutation E484K present in variants B.1.351 and P.1) on the affinity of RBD for ACE2, SARS-CoV-2 main receptor. We also aimed to assess the ability of antibodies induced by natural infection or by immunization with BNT162b2 mRNA vaccine to recognize the mutated versions of the RBD, as well as blocking the interaction RBD-ACE2, an important surrogate readout for virus neutralization. METHODS: To this end, we produced recombinant wild-type RBD, as well as RBD containing each of the mutations L452R/E484Q, N440K, or E484K (the latest present in variants of concern B.1.351 and P.1), as well as the ectodomain of ACE2. Using Biolayer Interferometry (BLI), we measured the binding affinity of RBD for ACE2 and the ability of sera from COVID-19 convalescent donors or subjects immunized with BNT162b2 mRNA vaccine to block this interaction. Finally, we correlated these results with total anti-RBD IgG titers measured from the same sera by direct ELISA. RESULTS: The binding assays showed L452R/E484Q double-mutant RBD to interact with ACE2 with higher affinity (KD = 4.6 nM) than wild-type (KD = 21.3 nM) or single mutants N440K (KD = 9.9 nM) and E484K (KD = 19.7 nM) RBDs. Meanwhile, the anti-RBD IgG titration resulted in lower recognition of mutants E484K and

L452R/E484Q by infection-induced antibodies, whereas only mutant E484K was recognized less by antibodies induced by vaccination. More interestingly, sera from convalescent as well as immunized subjects showed reduced ability to block the interaction between ACE2 and RBD mutants E484K and L452R/E484Q, as shown by the inhibition assays. CONCLUSION: Our data suggest that the newly emerged SARS-CoV-2 variant B.1.617, as well as the better-studied variants B.1.351 and P.1 (all containing a mutation at position E484) display increased transmissibility both due to their higher affinity for the cell receptor ACE2 and their ability to partially bypass immunity generated against the wild-type virus. For variant B.1.36 (with a point mutation at position N440), only increased affinity seems to play a role.

Baj, A., et al. (2021). "Spike protein evolution in the SARS-CoV-2 Delta variant of concern: a case series from Northern Lombardy." Emerg Microbes Infect **10**(1): 2010-2015.

The SARS-CoV-2 variant of concern (VOC) "Delta" is currently defined by PANGOLIN as a cluster of 33 different AY sublineages. Delta (in particular B.1.617.2) is largely and rapidly replacing the Alpha VOC as the dominant clade in most countries. To date, variations in the Spike protein of the Delta VOC have largely been limited. We report here the results of a genomic surveillance programme from Northern Italy. We identified several Delta sublineages harbouring mutations previously reported in GISAID at extremely low frequencies and in different combinations. Two patients (one of them vaccinated) tested positive for a Delta sublineage harbouring S71F, T250I, T572I and K854N. More patients tested positive for G769 V plus C1248F, A352S, and R158G and C1248F, respectively. Genomic surveillance of Delta variants should be encouraged to anticipate immune escape and deploy countermeasures.

Bandoy, D. and B. C. Weimer (2021). "Analysis of SARS-CoV-2 genomic epidemiology reveals disease transmission coupled to variant emergence and allelic variation." Sci Rep **11**(1): 7380.

The spread of SARS-CoV-2 created a pandemic crisis with > 150,000 cumulative cases in > 65 countries within a few months. The reproductive number (R) is a metric to estimate the transmission of a pathogen during an outbreak. Preliminary published estimates were based on the initial outbreak in China. Whole genome sequences (WGS) analysis found mutational variations in the viral genome; however, previous comparisons failed to show a direct relationship between viral genome diversity, transmission, and the epidemic severity. COVID-19 incidences from different countries were modeled over the epidemic curve. Estimates of the instantaneous R (Wallinga and Teunis method) with a short and standard serial interval were done. WGS were used to determine the populations genomic variation and that underpinned creation of the pathogen genome identity (GENI) score, which was merged with the outbreak curve in four distinct phases. Inference of transmission time was based on a mutation rate of 2 mutations/month. R estimates revealed differences in the transmission and variable infection dynamics between and within outbreak progression for each country examined. Outside China, our R estimates observed propagating dynamics indicating that other countries were poised to move to the takeoff and exponential stages. Population density and local temperatures had no clear relationship to the outbreak progression. Integration of incidence data with the GENI score directly predicted increases in cases as the genome variation increased that led to new variants. Integrating the outbreak curve, dynamic R, and SNP variation found a direct association between increasing cases and transmission genome evolution. By defining the epidemic curve into four stages and integrating the instantaneous country-specific R with the GENI score, we directly connected changes in individual outbreaks based on changes in the virus genome via SNPs. This resulted in the ability to forecast potential increases in cases as well as mutations that may defeat PCR screening and the infection process. By using instantaneous R estimations and WGS, outbreak dynamics were defined to be linked to viral mutations, indicating that WGS, as a surveillance tool, is required to predict shifts in each outbreak that will provide actionable decision making information. Integrating epidemiology with genome sequencing and modeling allows for evidence-based disease outbreak tracking with predictive therapeutically valuable insights in near real time.

Banu, T. A., et al. (2021). "Genome Sequencing of the SARS-CoV-2 Delta (B.1.617.2) Variant of Concern Detected in Bangladesh." Microbiol Resour Announc **10**(48): e0084921.

We report the near-complete genome sequence and phylogenetic analysis of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant (B.1.617.2) strain. This variant is associated with increased transmission and immune evasion.

Baral, P., et al. (2021). "Mutation-induced changes in the receptor-binding interface of the SARS-CoV-2 Delta variant B.1.617.2 and implications for immune evasion." Biochem Biophys Res Commun **574**: 14-19.

Following the initial surges of the Alpha (B.1.1.7) and the Beta (B.1.351) variants, a more infectious Delta variant (B.1.617.2) is now surging, further deepening the health crises caused by the pandemic. The sharp rise in cases attributed to the Delta variant has made it especially disturbing and is a

variant of concern. Fortunately, current vaccines offer protection against known variants of concern, including the Delta variant. However, the Delta variant has exhibited some ability to dodge the immune system as it is found that neutralizing antibodies from prior infections or vaccines are less receptive to binding with the Delta spike protein. Here, we investigated the structural changes caused by the mutations in the Delta variant's receptor-binding interface and explored the effects on binding with the ACE2 receptor as well as with neutralizing antibodies. We find that the receptor-binding beta-loop-beta motif adopts an altered but stable conformation causing separation in some of the antibody binding epitopes. Our study shows reduced binding of neutralizing antibodies and provides a possible mechanism for the immune evasion exhibited by the Delta variant.

Barua, S., et al. (2021). "Identification of the SARS-CoV-2 Delta variant C22995A using a high-resolution melting curve RT-FRET-PCR." Emerg Microbes Infect: 1-11.

AbstractKnowledge of SARS-CoV-2 variants is essential for formulating effective control policies. Currently, variants are only identified in relatively small percentages of cases as the required genome sequencing is expensive, time-consuming, and not always available. In countries with facilities to sequence the SARS-CoV-2, the Delta variant currently predominates. Elsewhere, the prevalence of the Delta variant is unclear. To avoid the need for sequencing, we investigated a RT-FRET-PCR that could detect all SARS-CoV-2 strains and simultaneously identify the Delta variant. The established Delta RT-FRET-PCR was performed on reference SARS-CoV-2 strains, and human nasal swab samples positive for the Delta and non-Delta strains. The Delta RT-FRET-PCR established in this study detected as few as ten copies of the DNA target and 100 copies of RNA target per reaction. Melting points of products obtained with SARS-CoV-2 Delta variants (around 56.1 degrees C) were consistently higher than products obtained with non-Delta strains (around 52.5 degrees C). The Delta RT-FRET-PCR can be used to diagnose COVID-19 patients and simultaneously identify if they are infected with the Delta variant. The Delta RT-FRET-PCR can be performed with all major thermocycler brands meaning data on Delta variant can now be readily generated in diagnostic laboratories worldwide.

Bashor, L., et al. (2021). "SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection." bioRxiv.

SARS-CoV-2 spillback from humans into domestic and wild animals has been well-documented. We compared variants of cell culture-expanded SARS-CoV-2 inoculum and virus recovered from four species following experimental exposure. Five nonsynonymous changes in nsp12, S, N and M genes were near fixation in the inoculum, but reverted to wild-type sequences in RNA recovered from dogs, cats and hamsters within 1-3 days post-exposure. Fourteen emergent variants were detected in viruses recovered from animals, including substitutions at spike positions H69, N501, and D614, which also vary in human lineages of concern. The rapidity of in vitro and in vivo SARS-CoV-2 selection reveals residues with functional significance during host-switching, illustrating the potential for spillback reservoir hosts to accelerate evolution, and demonstrating plasticity of viral adaptation in animal models. One-Sentence Summary: SARS-CoV-2 variants rapidly arise in non-human hosts, revealing viral evolution and potential risk for human reinfection.

Bashor, L., et al. (2021). "SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection." Proc Natl Acad Sci U S A **118**(44).

SARS-CoV-2 spillback from humans into domestic and wild animals has been well documented, and an accumulating number of studies illustrate that human-to-animal transmission is widespread in cats, mink, deer, and other species. Experimental inoculations of cats, mink, and ferrets have perpetuated transmission cycles. We sequenced full genomes of Vero cell-expanded SARS-CoV-2 inoculum and viruses recovered from cats (n = 6), dogs (n = 3), hamsters (n = 3), and a ferret (n = 1) following experimental exposure. Five nonsynonymous changes relative to the USA-WA1/2020 prototype strain were near fixation in the stock used for inoculation but had reverted to wild-type sequences at these sites in dogs, cats, and hamsters within 1- to 3-d postexposure. A total of 14 emergent variants (six in nonstructural genes, six in spike, and one each in orf8 and nucleocapsid) were detected in viruses recovered from animals. This included substitutions in spike residues H69, N501, and D614, which also vary in human lineages of concern. Even though a live virus was not cultured from dogs, substitutions in replicase genes were detected in amplified sequences. The rapid selection of SARS-CoV-2 variants in vitro and in vivo reveals residues with functional significance during host switching. These observations also illustrate the potential for spillback from animal hosts to accelerate the evolution of new viral lineages, findings of particular concern for dogs and cats living in households with COVID-19 patients. More generally, this glimpse into viral host switching reveals the unrealized rapidity and plasticity of viral evolution in experimental animal model systems.

Bernasconi, A., et al. (2021). "Data-driven analysis of amino acid change dynamics timely reveals SARS-CoV-2 variant emergence." Sci Rep **11**(1): 21068.

Since its emergence in late 2019, the diffusion of SARS-CoV-2 is associated with the evolution of its

viral genome. The co-occurrence of specific amino acid changes, collectively named 'virus variant', requires scrutiny (as variants may hugely impact the agent's transmission, pathogenesis, or antigenicity); variant evolution is studied using phylogenetics. Yet, never has this problem been tackled by digging into data with ad hoc analysis techniques. Here we show that the emergence of variants can in fact be traced through data-driven methods, further capitalizing on the value of large collections of SARS-CoV-2 sequences. For all countries with sufficient data, we compute weekly counts of amino acid changes, unveil time-varying clusters of changes with similar-rapidly growing-dynamics, and then follow their evolution. Our method succeeds in timely associating clusters to variants of interest/concern, provided their change composition is well characterized. This allows us to detect variants' emergence, rise, peak, and eventual decline under competitive pressure of another variant. Our early warning system, exclusively relying on deposited sequences, shows the power of big data in this context, and concurs to calling for the wide spreading of public SARS-CoV-2 genome sequencing for improved surveillance and control of the COVID-19 pandemic.

Bindayna, K. M. and S. Crinion (2021). "Variant analysis of SARS-CoV-2 genomes in the Middle East." Microb Pathog **153**: 104741.

BACKGROUND: Coronavirus (COVID-19) was introduced into society in late 2019 and has now reached over 88 million cases and 1.9 million deaths. The Middle East has a death toll of ~80,000 and over 35000 of these are in Iran, which has over 1.2 million confirmed cases. We expect that Iranian cases caused outbreaks in the neighbouring countries and that variant mapping and phylogenetic analysis can be used to prove this. We also aim to analyse the variants of severe acute respiratory syndrome coronavirus-2 (SARS -CoV-2) to characterise the common genome variants and provide useful data in the global effort to prevent further spread of COVID-19. METHODS: The approach uses bioinformatics approaches including multiple sequence alignment, variant calling and annotation and phylogenetic analysis to identify the genomic variants found in the region. The approach uses 122 samples from the 13 countries of the Middle East sourced from the Global Initiative on Sharing All Influenza Data (GISAID). FINDINGS: We identified 2200 distinct genome variants including 129 downstream gene variants, 298 frame shift variants, 789 missense variants, 1 start lost, 13 start gained, 1 stop lost, 249 synonymous variants and 720 upstream gene variants. The most common, high impact variants were 10818delTinsG, 2772delCinsC, 14159delCinsC and 2789delAinsA. These high impact variant ultimately results in 36 number of mutations on spike glycoprotein. Variant alignment and phylogenetic tree generation indicates that samples from Iran likely introduced COVID-19 to the rest of the Middle East. INTERPRETATION: The phylogenetic and variant analysis provides unique insight into mutation types in genomes. Initial introduction of COVID-19 was most likely due to Iranian transmission. Some countries show evidence of novel mutations and unique strains. Increased time in small populations is likely to contribute to more unique genomes. This study provides more in depth analysis of the variants affecting in the region than any other study.

Blairon, L., et al. (2021). "The challenge of screening SARS-CoV-2 variants of concern with RT-qPCR: One variant can hide another." J Virol Methods **297**: 114248.

INTRODUCTION: Following the emergence of SARS-CoV-2 variants of concern (VOCs) worldwide, it is important to monitor local epidemiology to better understand the occurrence of clusters, reinfections, or infection after vaccination. Detecting mutations by specific RT-qPCR is a rapid and affordable alternative to sequencing. However, care must be taken to ensure that the techniques used are up-to-date and adapted to the variants circulating in the studied population. MATERIAL AND METHODS: All samples tested positive for SARS-CoV-2 were screened for detection of mutations of the spike protein using the Novaplex SARS-CoV-2 Variants I Assay from week 11 of 2021. Target sought were deletion H69/V70 and mutations N501Y and E484K. From week 18 we used in addition the new Novaplex SARS-CoV-2 Variants II Assay for samples with no targets found with the Variants I assay or with the mutation E484K alone, in order to screen the mutations L452R, K417N/T and W152C. RESULTS: Between weeks 11 and 25, 2239 positive samples out of 54,317 were tested with the Variants I Assay. Between weeks 18 and 25, 94 samples met the criteria for being tested with the Variants II Assay. Of these, 47 had the L452R mutation without the W152C mutation, typical in the B.1.617 variant. At week 25, this profile was found in 45.5 % of the samples and was the most frequent. CONCLUSION: According to our observations, variant B.1.617 has become predominant in our institution and most probably in our region. In the absence of the use of the Variants II Assay, they would have been considered wild.

Boogaerts, H. L. F., et al. (2021). "Laboratory analysis of two Delta SARS-CoV-2 variant outbreaks in the Port of Antwerp." Acta Clin Belg: 1-8.

INTRODUCTION: The B.1.617.2 SARS-CoV-2 or Delta variant, first detected in India, has shown a rapid global spread due to its high transmissibility and now represents more than 99% of the currently circulating variants in Europe. METHODS AND RESULT: In May 2021, two ships

that had recently arrived in the Port of Antwerp reported crew members with COVID-like symptoms. SARS-CoV-2 RNA was detected in nasopharyngeal swabs in 30 out of 45 skippers and the B.1.617.2 variant was identified via whole genome sequencing. Crew members were isolated or quarantined and repeatedly tested to assess the evolution of their SARS-CoV-2 viral load based on the cycle threshold (CT) values of the PCR reaction. Viral cultures were also taken at day 7 to detect viable virus and were compared with the subjects CT value at that moment. The shipper's clinical condition was closely observed using a digital home monitoring tool. Eleven crew members (37%) required hospitalization, with CT values of SARS-CoV-2 RNA being a good predictive factor for the hospitalization need. Furthermore, a clear correlation between CT values and positive viral culture was observed, hinting infectiousness even longer than 10 days after the intitial positive PCR test. CONCLUSION: Our study of 2 Delta variant clusters shows that the initial CT value is a good predictor for hospitalization need and suggests that patients infected with this variant may remain infectious for a longer time period.

Boon, S. S., et al. (2021). "Temporal-Geographical Dispersion of SARS-CoV-2 Spike Glycoprotein Variant Lineages and Their Functional Prediction Using in Silico Approach." mBio **12**(5): e0268721.

SARS-CoV-2 is a positive-sense single-stranded RNA virus with emerging mutations, especially on the Spike glycoprotein (S protein). To delineate the genomic diversity in association with geographic dispersion of SARS-CoV-2 variant lineages, we collected 939,591 complete S protein sequences deposited in the Global Initiative on Sharing All Influenza Data (GISAID) from December 2019 to April 2021. An exponential emergence of S protein variants was observed since October 2020 when the four major variants of concern (VOCs), namely, alpha (alpha) (B.1.1.7), beta (beta) (B.1.351), gamma (gamma) (P.1), and delta (delta) (B.1.617), started to circulate in various communities. We found that residues 452, 477, 484, and 501, the 4 key amino acids located in the hACE2 binding domain of S protein, were under positive selection. Through in silico protein structure prediction and immunoinformatics tools, we discovered D614G is the key determinant to S protein conformational change, while variations of N439K, T478I, E484K, and N501Y in S1-RBD also had an impact on S protein binding affinity to hACE2 and antigenicity. Finally, we predicted that the yet-to-be-identified hypothetical N439S, T478S, and N501K mutations could confer an even greater binding affinity to hACE2 and evade host immune surveillance more efficiently than the respective native variants. This study documented the evolution of SARS-CoV-2 S protein over the first 16 months of the pandemic and identified several key amino acid changes that are predicted to confer a substantial impact on transmission and immunological recognition. These findings convey crucial information to sequence-based surveillance programs and the design of next-generation vaccines. IMPORTANCE Our study showed the global distribution of SARS-CoV-2 S protein variants from January 2020 to the end of April 2021. We highlighted the key amino acids of S protein subjected to positive selection. Using computer-aided approaches, we predicted the impact of the amino acid variations in S protein on viral infectivity and antigenicity. We also predicted the potential amino acid mutations that could arise in favor of SARS-CoV-2 virulence. These findings are vital for vaccine designing and anti-SARS-CoV-2 drug discovery in an effort to combat COVID-19.

Borges, V., et al. (2020). "Massive dissemination of a SARS-CoV-2 Spike Y839 variant in Portugal." Emerg Microbes Infect **9**(1): 2488-2496.

Genomic surveillance of SARS-CoV-2 was rapidly implemented in Portugal by the National Institute of Health in collaboration with a nationwide consortium of >50 hospitals/laboratories. Here, we track the geotemporal spread of a SARS-CoV-2 variant with a mutation (D839Y) in a potential host-interacting region involving the Spike fusion peptide, which is a target motif of anti-viral drugs that plays a key role in SARS-CoV-2 infectivity. The Spike Y839 variant was most likely imported from Italy in mid-late February and massively disseminated in Portugal during the early epidemic, becoming prevalent in the Northern and Central regions of Portugal where it represented 22% and 59% of the sampled genomes, respectively, by 30 April. Based on our high sequencing sampling during the early epidemics [15.5% (1275/8251) and 6.0% (1500/24987) of all confirmed cases until the end of March and April, respectively], we estimate that, between 14 March and 9 April (covering the epidemic exponential phase) the relative frequency of the Spike Y839 variant increased at a rate of 12.1% (6.1%-18.2%, CI 95%) every three days, being potentially associated with 24.8% (20.8-29.7%, CI 95%; 3177-4542 cases, CI 95%) of all COVID-19 cases in Portugal during this period. Our data supports population/epidemiological (founder) effects contributing to the Y839 variant superspread. The potential existence of selective advantage is also discussed, although experimental validation is required. Despite huge differences in genome sampling worldwide, SARS-CoV-2 Spike D839Y has been detected in 13 countries in four continents, supporting the need for close surveillance and functional assays of Spike variants.

Boshier, F. A. T., et al. (2021). "The Alpha variant was not associated with excess nosocomial SARS-CoV-2 infection in a multi-centre UK hospital study." J Infect.

OBJECTIVES: Recently emerging SARS-CoV-2 variants have been associated with an increased rate of transmission within the community. We sought to determine whether this also resulted in increased transmission within hospitals. METHODS: We collected viral sequences and epidemiological data of patients with community and healthcare associated SARS-CoV-2 infections, sampled from 16th November 2020 to 10th January 2021, from nine hospitals participating in the COG-UK HOCI study. Outbreaks were identified using ward information, lineage and pairwise genetic differences between viral sequences. RESULTS: Mixed effects logistic regression analysis of 4184 sequences showed healthcare-acquired infections were no more likely to be identified as the Alpha variant than community acquired infections. Nosocomial outbreaks were investigated based on overlapping ward stay and SARS-CoV-2 genome sequence similarity. There was no significant difference in the number of patients involved in outbreaks caused by the Alpha variant compared to outbreaks caused by other lineages. CONCLUSIONS: We find no evidence to support it causing more nosocomial transmission than previous lineages. This suggests that the stringent infection prevention measures already in place in UK hospitals contained the spread of the Alpha variant as effectively as other less transmissible lineages, providing reassurance of their efficacy against emerging variants of concern.

Bourassa, L., et al. (2021). "A SARS-CoV-2 Nucleocapsid Variant that Affects Antigen Test Performance." J Clin Virol **141**: 104900.

More than one year into a global pandemic, SARS-CoV-2 is now defined by a variety of rapidly evolving variant lineages. Several FDA authorized molecular diagnostic tests have been impacted by viral variation, while no reports of viral variation affecting antigen test performance have occurred to date. While determining the analytical sensitivity of the Quidel Sofia SARS Antigen FIA test (Sofia 2), we uncovered a high viral load specimen that repeatedly tested negative by this antigen test. Whole genome sequencing of the specimen uncovered two mutations, T205I and D399N, present in the nucleocapsid protein of the isolate. All six SARS-CoV-2 positive clinical specimens available in our laboratory with a D399N nucleocapsid mutation and CT < 31 were not detected by the Sofia 2 but detected by the Abbott BinaxNOW COVID-19 Ag Card, while clinical specimens with the T205I mutation were detected by both assays. Testing of recombinant SARS-CoV-2 nucleocapsid with these variants demonstrated an approximate 1000-fold loss in sensitivity for the Quidel Sofia SARS Antigen FIA test associated with the D399N mutation, while the BinaxNOW and Quidel Quickvue SARS Antigen tests were unaffected by the mutation. The D399N nucleocapsid mutation has been relatively uncommon to date, appearing in only 0.02% of genomes worldwide at time of writing. Our results demonstrate how routine pathogen genomics can be integrated into the clinical microbiology laboratory to investigate diagnostic edge cases, as well as the importance of profiling antigenic diversity outside of the spike protein for SARS-CoV-2 diagnostics.

Brandt, D., et al. (2021). "Multiple Occurrences of a 168-Nucleotide Deletion in SARS-CoV-2 ORF8, Unnoticed by Standard Amplicon Sequencing and Variant Calling Pipelines." Viruses **13**(9).

Genomic surveillance of the SARS-CoV-2 pandemic is crucial and mainly achieved by amplicon sequencing protocols. Overlapping tiled-amplicons are generated to establish contiguous SARS-CoV-2 genome sequences, which enable the precise resolution of infection chains and outbreaks. We investigated a SARS-CoV-2 outbreak in a local hospital and used nanopore sequencing with a modified ARTIC protocol employing 1200 bp long amplicons. We detected a long deletion of 168 nucleotides in the ORF8 gene in 76 samples from the hospital outbreak. This deletion is difficult to identify with the classical amplicon sequencing procedures since it removes two amplicon primer-binding sites. We analyzed public SARS-CoV-2 sequences and sequencing read data from ENA and identified the same deletion in over 100 genomes belonging to different lineages of SARS-CoV-2, pointing to a mutation hotspot or to positive selection. In almost all cases, the deletion was not represented in the virus genome sequence after consensus building. Additionally, further database searches point to other deletions in the ORF8 coding region that have never been reported by the standard data analysis pipelines. These findings and the fact that ORF8 is especially prone to deletions, make a clear case for the urgent necessity of public availability of the raw data for this and other large deletions that might change the physiology of the virus towards endemism.

Brinkmann, A., et al. (2021). "AmpliCoV: Rapid Whole-Genome Sequencing Using Multiplex PCR Amplification and Real-Time Oxford Nanopore MinION Sequencing Enables Rapid Variant Identification of SARS-CoV-2." Front Microbiol **12**: 651151.

Since the emergence of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) in December 2019, the scientific community has been sharing data on epidemiology, diagnostic methods, and whole-genomic sequences almost in real time. The latter have already facilitated phylogenetic analyses, transmission chain tracking, protein modeling, the

identification of possible therapeutic targets, timely risk assessment, and identification of novel variants. We have established and evaluated an amplification-based approach for whole-genome sequencing of SARS-CoV-2. It can be used on the miniature-sized and field-deployable sequencing device Oxford Nanopore MinION, with sequencing library preparation time of 10 min. We show that the generation of 50,000 total reads per sample is sufficient for a near complete coverage (>90%) of the SARS-CoV-2 genome directly from patient samples even if virus concentration is low (Ct 35, corresponding to approximately 5 genome copies per reaction). For patient samples with high viral load (Ct 18-24), generation of 50,000 reads in 1-2 h was shown to be sufficient for a genome coverage of >90%. Comparison to Illumina data reveals an accuracy that suffices to identify virus mutants. AmpliCoV can be applied whenever sequence information on SARS-CoV-2 is required rapidly, for instance for the identification of circulating virus mutants.

Buenestado-Serrano, S., et al. (2021). "First confirmation of importation and transmission in Spain of the newly identified SARS-CoV-2 B.1.1.7 variant." Enferm Infecc Microbiol Clin (Engl Ed).

INTRODUCTION: A newly identified SARS-CoV-2 variant, VOC202012/01 originating lineage B.1.1.7, recently emerged in the United Kingdom. The rapid spread in the UK of this new variant has caused other countries to be vigilant. MATERIAL AND METHODS: We based our initial screening of B.1.1.7 on the dropout of the S gene signal in the TaqPath assay, caused by the 69/70 deletion. Subsequently, we confirmed the B.1.1.7 candidates by whole genome sequencing. RESULTS: We describe the first three imported cases of this variant from London to Madrid, subsequent post-arrival household transmission to three relatives, and the two first cases without epidemiological links to UK. One case required hospitalization. In all cases, drop-out of gene S was correctly associated to the B.1.1.7 variant, as all the corresponding sequences carried the 17 lineage-marker mutations. CONCLUSION: The first identifications of the SARS-CoV-2 B.1.1.7 variant in Spain indicate the role of independent introductions from the UK coexisting with post-arrival transmission in the community, since the early steps of this new variant in our country.

Bugembe, D. L., et al. (2021). "Emergence and spread of a SARS-CoV-2 lineage A variant (A.23.1) with altered spike protein in Uganda." Nat Microbiol **6**(8): 1094-1101.

Here, we report SARS-CoV-2 genomic surveillance from March 2020 until January 2021 in Uganda, a landlocked East African country with a population of approximately 40 million people. We report 322 full SARS-CoV-2 genomes from 39,424 reported SARS-CoV-2 infections, thus representing 0.8% of the reported cases. Phylogenetic analyses of these sequences revealed the emergence of lineage A.23.1 from lineage A.23. Lineage A.23.1 represented 88% of the genomes observed in December 2020, then 100% of the genomes observed in January 2021. The A.23.1 lineage was also reported in 26 other countries. Although the precise changes in A.23.1 differ from those reported in the first three SARS-CoV-2 variants of concern (VOCs), the A.23.1 spike-protein-coding region has changes similar to VOCs including a change at position 613, a change in the furin cleavage site that extends the basic amino acid motif and multiple changes in the immunogenic N-terminal domain. In addition, the A.23.1 lineage has changes in non-spike proteins including nsp6, ORF8 and ORF9 that are also altered in other VOCs. The clinical impact of the A.23.1 variant is not yet clear and it has not been designated as a VOC. However, our findings of emergence and spread of this variant indicate that careful monitoring of this variant, together with assessment of the consequences of the spike protein changes for COVID-19 vaccine performance, are advisable.

Caccuri, F., et al. (2020). "A persistently replicating SARS-CoV-2 variant derived from an asymptomatic individual." J Transl Med **18**(1): 362.

BACKGROUND: Since the first outbreak of SARS-CoV-2, the clinical characteristics of the Coronavirus Disease 2019 (COVID-19) have been progressively changed. Data reporting a viral intra-host and inter-host evolution favouring the appearance of mild SARS-CoV-2 strains are since being accumulating. To better understand the evolution of SARS-CoV-2 pathogenicity and its adaptation to the host, it is therefore crucial to investigate the genetic and phenotypic characteristics of SARS-CoV-2 strains circulating lately in the epidemic. METHODS: Nasopharyngeal swabs have been analyzed for viral load in the early (March 2020) and late (May 2020) phases of epidemic in Brescia, Italy. Isolation of SARS-CoV-2 from 2 high viral load specimens identified on March 9 (AP66) and on May 8 (GZ69) was performed on Vero E6 cells. Amount of virus released was assessed by quantitative PCR. Genotypic characterization of AP66 and GZ69 was performed by next generation sequencing followed by an in-depth in silico analysis of nucleotide mutations. RESULTS: The SARS-CoV-2 GZ69 strain, isolated in May from an asymptomatic healthcare worker, showed an unprecedented capability of replication in Vero E6 cells in the absence of any evident cytopathic effect. Vero E6 subculturing, up to passage 4, showed that SARS-CoV-2 GZ69 infection was as productive as the one sustained by the cytopathic strain AP66. Whole

genome sequencing of the persistently replicating SARS-CoV-2 GZ69 has shown that this strain differs from the early AP66 variant in 9 nucleotide positions (C2939T; C3828T; G21784T; T21846C; T24631C; G28881A; G28882A; G28883C; G29810T) which lead to 6 non-synonymous substitutions spanning on ORF1ab (P892S; S1188L), S (K74N; I95T) and N (R203K, G204R) proteins. CONCLUSIONS: Identification of the peculiar SARS-CoV-2 GZ69 strain in the late Italian epidemic highlights the need to better characterize viral variants circulating among asymptomatic or paucisymptomatic individuals. The current approach could unravel the ways for future studies aimed at analyzing the selection process which favours viral mutations in the human host.

Camp, J. V., et al. (2021). "RT-PCR based SARS-CoV-2 variant screening assays require careful quality control." J Clin Virol **141**: 104905.

BACKGROUND: Distinctive genotypes of SARS-CoV-2 have emerged that are or may be associated with increased transmission, pathogenicity, and/or antibody escape. In many countries, clinical and diagnostic laboratories are under mandate to identify and report these so-called variants of concern (VOC). OBJECTIVES: We used an external quality assessment scheme to determine the scope, accuracy, and reliability of laboratories using various molecular diagnostic assays to identify current VOC (03 March 2021). STUDY DESIGN: Participant laboratories were sent the same five patient-derived samples and were asked to provide their variant detection methods, variant detection results and interpretation of results. RESULTS: Twenty-five laboratories reported a range of RT-qPCR-based assays to identify specific variations in the SARS-CoV-2 spike protein that are characteristic of three VOC lineages. Laboratories that detected VOC-associated nucleotide mutations at four specific sites had the highest ratio of correct classification. Low template copy number and additional variation in target regions resulted in loss of confidence and accuracy in sample classification. CONCLUSIONS: Melting-curve-based assays to identify genomic variants are less time-consuming and require less bioinformatic analysis compared to partial or whole genome sequencing. However, our results suggest that correct classification of a given genotype/lineage (e.g., a VOC) relies on the ability to detect more than one variant site, adequate template in the sample (i.e., relatively high viral load/copy number) and results may be unclear in certain samples with additional genetic variations. These initial results suggest that some diagnostic laboratories may require additional training to interpret and report complex genetic information about a dynamic emerging virus.

Capoluongo, E., et al. (2021). "Case Report: Discovery a Novel SARS-CoV-2 Variant in a Six-Months Long-Term Swab Positive Female Suffering From Non-Hodgkin Lymphoma." Front Oncol **11**: 705948.

Background: We report the case of a woman with non-Hodgkin lymphoma who remained positive on the molecular assay for SARS-CoV-2 for six months: she has never experienced a severe form of COVID-19 although in absence of seroconversion. Methods: The whole SARS-CoV-2 genome analysis was performed by the CleanPlex SARS-CoV-2 Research and Surveillance NGS Panel (PARAGON GENOMICS, Hayward, USA). Results: We found twenty-two mutations in SARS-CoV-2 genome and a novel deleterious ORF3a frameshift c.766_769del corresponding to a unique and novel lineage. The region affected by this frameshift variant is reported as being important in determining SARS-CoV-2 immunogenicity. Patient's immunophenotype showed the absence of B lymphocytes and significantly reduced T-cell count. Only after the treatment with hyperimmune plasma she finally became negative on the swab. Conclusions: Our findings could be helpful in the management of patients with immunodeficiency, particularly when novel variants, potentially altering the virus immune response, are present.

Capponi, S., et al. (2021). "AI-driven prediction of SARS-CoV-2 variant binding trends from atomistic simulations." Eur Phys J E Soft Matter **44**(10): 123.

We present a novel technique to predict binding affinity trends between two molecules from atomistic molecular dynamics simulations. The technique uses a neural network algorithm applied to a series of images encoding the distance between two molecules in time. We demonstrate that our algorithm is capable of separating with high accuracy non-hydrophobic mutations with low binding affinity from those with high binding affinity. Moreover, we show high accuracy in prediction using a small subset of the simulation, therefore requiring a much shorter simulation time. We apply our algorithm to the binding between several variants of the SARS-CoV-2 spike protein and the human receptor ACE2.

Carcereny, A., et al. (2021). "Monitoring Emergence of the SARS-CoV-2 B.1.1.7 Variant through the Spanish National SARS-CoV-2 Wastewater Surveillance System (VATar COVID-19)." Environ Sci Technol **55**(17): 11756-11766.

Since its first identification in the United Kingdom in late 2020, the highly transmissible B.1.1.7 variant of SARS-CoV-2 has become dominant in several countries raising great concern. We developed a duplex real-time RT-qPCR assay to detect, discriminate, and quantitate SARS-CoV-2 variants containing one of its mutation signatures, the DeltaHV69/70 deletion, and used it to trace the community circulation of the B.1.1.7 variant in Spain through the Spanish National SARS-CoV-2

Wastewater Surveillance System (VATar COVID-19). The B.1.1.7 variant was detected earlier than clinical epidemiological reporting by the local authorities, first in the southern city of Malaga (Andalucia) in week 20_52 (year_week), and multiple introductions during Christmas holidays were inferred in different parts of the country. Wastewater-based B.1.1.7 tracking showed a good correlation with clinical data and provided information at the local level. Data from wastewater treatment plants, which reached B.1.1.7 prevalences higher than 90% for >/=2 consecutive weeks showed that 8.1 +/- 2.0 weeks were required for B.1.1.7 to become dominant. The study highlights the applicability of RT-qPCR-based strategies to track specific mutations of variants of concern as soon as they are identified by clinical sequencing and their integration into existing wastewater surveillance programs, as a cost-effective approach to complement clinical testing during the COVID-19 pandemic.

Cavanaugh, A. M., et al. (2021). "COVID-19 Outbreak Associated with a SARS-CoV-2 R.1 Lineage Variant in a Skilled Nursing Facility After Vaccination Program - Kentucky, March 2021." MMWR Morb Mortal Wkly Rep **70**(17): 639-643.

Although COVID-19 mRNA vaccines demonstrated high efficacy in clinical trials (1), they were not 100% efficacious. Thus, some infections postvaccination are expected. Limited data are available on effectiveness in skilled nursing facilities (SNFs) and against emerging variants. The Kentucky Department for Public Health (KDPH) and a local health department investigated a COVID-19 outbreak in a SNF that occurred after all residents and health care personnel (HCP) had been offered vaccination. Among 83 residents and 116 HCP, 75 (90.4%) and 61 (52.6%), respectively, received 2 vaccine doses. Twenty-six residents and 20 HCP received positive test results for SARS-CoV-2, the virus that causes COVID-19, including 18 residents and four HCP who had received their second vaccine dose >14 days before the outbreak began. An R.1 lineage variant was detected with whole genome sequencing (WGS). Although the R.1 variant has multiple spike protein mutations, vaccinated residents and HCP were 87% less likely to have symptomatic COVID-19 compared with those who were unvaccinated. Vaccination of SNF populations, including HCP, is critical to reduce the risk for SARS-CoV-2 introduction, transmission, and severe outcomes in SNFs. An ongoing focus on infection prevention and control practices is also essential.

Cedro-Tanda, A., et al. (2021). "The Evolutionary Landscape of SARS-CoV-2 Variant B.1.1.519 and Its Clinical Impact in Mexico City." Viruses **13**(11).

The SARS-CoV-2 pandemic is one of the most concerning health problems around the globe. We reported the emergence of SARS-CoV-2 variant B.1.1.519 in Mexico City. We reported the effective reproduction number (Rt) of B.1.1.519 and presented evidence of its geographical origin based on phylogenetic analysis. We also studied its evolution via haplotype analysis and identified the most recurrent haplotypes. Finally, we studied the clinical impact of B.1.1.519. The B.1.1.519 variant was predominant between November 2020 and May 2021, reaching 90% of all cases sequenced in February 2021. It is characterized by three amino acid changes in the spike protein: T478K, P681H, and T732A. Its Rt varies between 0.5 and 2.9. Its geographical origin remain to be investigated. Patients infected with variant B.1.1.519 showed a highly significant adjusted odds ratio (aOR) increase of 1.85 over non-B.1.1.519 patients for developing a severe/critical outcome (p = 0.000296, 1.33-2.6 95% CI) and a 2.35-fold increase for hospitalization (p = 0.005, 1.32-4.34 95% CI). The continuous monitoring of this and other variants will be required to control the ongoing pandemic as it evolves.

Cetin, M., et al. (2021). "Alpha variant (B.1.1.7) of SARS-CoV-2 increases fatality-rate for patients under age of 70 years and hospitalization risk overall." Acta Microbiol Immunol Hung.

The emergence of new SARS-CoV-2 variants is a challenge to the control of this pandemic. It is therefore important to collect and to analyze data related to the infection caused by different variants. We have obtained more than 3,700 COVID-19 patients between April 2020 and March 2021 from Tokat, Turkey (roughly 3,100 outpatients and close to 600 inpatients) where about 30% were infected with Alpha variant (B.1.1.7). Descriptive statistics was used to characterize different subgroups. Both logistic regression and cause-specific Cox survival analysis of competing-risk was run on inpatients, to examine the impact of Alpha variant on hospitalization, on mortality and on other factors. We observed that the Alpha variant is over-represented in inpatients than outpatients so infection by Alpha variant increases the chance for hospitalization. The impact of Alpha variant on mortality seems to depend on the patient's age. For patients under age of 70, the case-fatality-rate was 0.84% (5.3%) for patients without (with) Alpha variant (Fisher's test P-value = 2.4 x 10-10). For patients above age of 70, the trend is opposite: the case-fatality-rate is 31.5% (13.6%) for patients without (with) Alpha variant (Fisher's test P-value = 0.0016). The two opposite trends would cancel each other, making other analyses such as cause-specific Cox regression and logistic regression non-significant. The Alpha variant increases the risk for hospitalization, increases the case-fatality-rate for lower age group, and decreases the case-fatality-rate for the upper age group. If the increase of case-fatality-rate in not the most senior

group holds true, it should provide useful information for a vaccination planning to counter the impact of Alpha variants.

Challen, R., et al. (2021). "Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study." BMJ **372**: n579.

OBJECTIVE: To establish whether there is any change in mortality from infection with a new variant of SARS-CoV-2, designated a variant of concern (VOC-202012/1) in December 2020, compared with circulating SARS-CoV-2 variants. DESIGN: Matched cohort study. SETTING: Community based (pillar 2) covid-19 testing centres in the UK using the TaqPath assay (a proxy measure of VOC-202012/1 infection). PARTICIPANTS: 54 906 matched pairs of participants who tested positive for SARS-CoV-2 in pillar 2 between 1 October 2020 and 29 January 2021, followed-up until 12 February 2021. Participants were matched on age, sex, ethnicity, index of multiple deprivation, lower tier local authority region, and sample date of positive specimens, and differed only by detectability of the spike protein gene using the TaqPath assay. MAIN OUTCOME MEASURE: Death within 28 days of the first positive SARS-CoV-2 test result. RESULTS: The mortality hazard ratio associated with infection with VOC-202012/1 compared with infection with previously circulating variants was 1.64 (95% confidence interval 1.32 to 2.04) in patients who tested positive for covid-19 in the community. In this comparatively low risk group, this represents an increase in deaths from 2.5 to 4.1 per 1000 detected cases. CONCLUSIONS: The probability that the risk of mortality is increased by infection with VOC-202012/01 is high. If this finding is generalisable to other populations, infection with VOC-202012/1 has the potential to cause substantial additional mortality compared with previously circulating variants. Healthcare capacity planning and national and international control policies are all impacted by this finding, with increased mortality lending weight to the argument that further coordinated and stringent measures are justified to reduce deaths from SARS-CoV-2.

Chia, P. Y., et al. (2021). "Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine-breakthrough infections: a multi-center cohort study." Clin Microbiol Infect.

OBJECTIVES: Highly effective vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been developed but variants of concerns are worrisome, especially B.1.617.2 (Delta) which has rapidly spread across the world. We aim to study if vaccination alters virological and serological kinetics in breakthrough infections. METHODS: We conducted a multi-centre retrospective cohort study of patients in Singapore who had received a licensed mRNA vaccine and been admitted to hospital with B.1.617.2 SARS-CoV-2 infection. We compared clinical features, virological and serological kinetics (anti-nucleocapsid, anti-spike and surrogate virus neutralization titres) between fully vaccinated and unvaccinated individuals. RESULTS: Out of 218 individuals with B.1.617.2 infection, 84 received a mRNA vaccine of which 71 were fully vaccinated, 130 were unvaccinated and 4 received a non-mRNA. Despite significantly older age in the vaccine-breakthrough group, only 2.8% (2/71) developed severe COVID-19 requiring oxygen supplementation compared to 53.1% (69/130) in the unvaccinated group ($p<0.001$). Odds of severe COVID-19 following vaccination were significantly lower (adjusted odds ratio 0.07 95%CI: 0.015-0.335, $p=0.001$). PCR cycle threshold values were similar between vaccinated and unvaccinated groups at diagnosis, but viral loads decreased faster in vaccinated individuals. Early, robust boosting of anti-spike protein antibodies was observed in vaccinated patients, however, these titers were significantly lower against B.1.617.2 as compared with wildtype vaccine strain. CONCLUSION: The mRNA vaccines are highly effective at preventing symptomatic and severe COVID-19 associated with B.1.617.2 infection. Vaccination is associated with faster decline in viral RNA load and a robust serological response. Vaccination remains a key strategy for control of COVID-19 pandemic.

Choi, A., et al. (2021). "Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in healthy adults: an interim analysis." Nat Med **27**(11): 2025-2031.

The emergence of SARS-CoV-2 variants of concern (VOCs) and variants of interest (VOIs) with decreased susceptibility to neutralization has generated interest in assessments of booster doses and variant-specific vaccines. Clinical trial participants who received a two-dose primary series of the COVID-19 vaccine mRNA-1273 approximately 6 months earlier entered an open-label phase 2a study (NCT04405076) to evaluate the primary objectives of safety and immunogenicity of a single booster dose of mRNA-1273 or variant-modified mRNAs, including multivalent mRNA-1273.211. As the trial is currently ongoing, this exploratory interim analysis includes preliminary descriptive results only of four booster groups (n = 20 per group). Immediately before the booster dose, neutralizing antibodies against wild-type D614G virus had waned ($P < 0.0001$) relative to peak titers against wild-type D614G measured 1 month after the primary series, and neutralization titers against B.1.351 (Beta), P.1 (Gamma) and B.1.617.2 (Delta) VOCs were either low or undetectable. Both the mRNA-1273 booster and variant-modified boosters were safe and well-tolerated. All boosters, including

mRNA-1273, numerically increased neutralization titers against the wild-type D614G virus compared to peak titers against wild-type D614G measured 1 month after the primary series; significant increases were observed for mRNA-1273 and mRNA-1273.211 ($P < 0.0001$). In addition, all boosters increased neutralization titers against key VOCs and VOIs, including B.1.351, P.1. and B.1.617.2, that were statistically equivalent to peak titers measured after the primary vaccine series against wild-type D614G virus, with superior titers against some VOIs. This trial is ongoing.

Colson, P., et al. (2021). "Spreading of a new SARS-CoV-2 N501Y spike variant in a new lineage." Clin Microbiol Infect **27**(9): 1352 e1351-1352 e1355.

OBJECTIVES: Surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomic epidemiology led us to detect several variants since summer 2020. We report the recent spread of a new SARS-CoV-2 spike 501Y variant. METHODS: SARS-CoV-2 sequences obtained from human nasopharyngeal samples by Illumina next-generation sequencing were analysed using Nextclade and an in-house Python script and were compared using BLASTn to the GISAID database. Phylogeny was investigated using the IQ-TREE software. RESULTS: We identified that SARS-CoV-2 genomes from four patients diagnosed in our institute harboured a new set of amino acid substitutions including L18F, L452R, N501Y, A653V, H655Y, D796Y, G1219V +/- Q677H. These spike N501Y genomes are the first of Nextstrain clade 19B. We obtained partial spike gene sequences of this genotype for an additional 43 patients. All patients infected with this genotype were diagnosed since mid-January 2021. We detected 42 other genomes of this genotype in GISAID, which were obtained from samples collected in December 2020 in four individuals and in 2021 in 38 individuals. The 89 sequences obtained in our institute or other laboratories originated from the Comoros archipelago, western European countries (mostly metropolitan France), Turkey and Nigeria. CONCLUSION: These findings warrant further studies to investigate the spread, epidemiological and clinical features, and sensitivity to immune responses of this variant.

Colson, P., et al. (2021). "Introduction into the Marseille geographical area of a mild SARS-CoV-2 variant originating from sub-Saharan Africa: An investigational study." Travel Med Infect Dis **40**: 101980.

BACKGROUND: In Marseille, France, the COVID-19 incidence evolved unusually with several successive epidemic phases. The second outbreak started in July, was associated with North Africa, and involved travelers and an outbreak on passenger ships. This suggested the involvement of a new viral variant. METHODS: We sequenced the genomes from 916 SARS-CoV-2 strains from COVID-19 patients in our institute. The patients' demographic and clinical features were compared according to the infecting viral variant. RESULTS: From June 26th to August 14th, we identified a new viral variant (Marseille-1). Based on genome sequences (n = 89) or specific qPCR (n = 53), 142 patients infected with this variant were detected. It is characterized by a combination of 10 mutations located in the nsp2, nsp3, nsp12, S, ORF3a, ORF8 and N/ORF14 genes. We identified Senegal and Gambia, where the virus had been transferred from China and Europe in February-April as the sources of the Marseille-1 variant, which then most likely reached Marseille through Maghreb when French borders reopened. In France, this variant apparently remained almost limited to Marseille. In addition, it was significantly associated with a milder disease compared to clade 20A ancestor strains, in univariate analysis. CONCLUSION: Our results demonstrate that SARS-CoV-2 can genetically diversify rapidly, its variants can diffuse internationally and cause successive outbreaks.

Connor, B. A., et al. (2021). "Monoclonal Antibody Therapy in a Vaccine Breakthrough SARS-CoV-2 Hospitalized Delta (B.1.617.2) Variant Case." Int J Infect Dis **110**: 232-234.

We present two Delta (B.1.617.2) vaccine breakthrough individuals, a father and son living in separate households. The older, 63-year-old patient's symptoms were severe enough to require hospitalization. Despite having a high titer of anti-spike IgG in his serum, his symptoms resolved within 24 hours following monoclonal antibody (bamlanivimab/etesevimab) therapy.

Conti, P., et al. (2021). "The British variant of the new coronavirus-19 (Sars-Cov-2) should not create a vaccine problem." J Biol Regul Homeost Agents **35**(1): 1-4.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a highly contagious virus that infects humans and a number of animal species causing coronavirus disease-19 (COVID-19), a respiratory distress syndrome which has provoked a global pandemic and a serious health crisis in most countries across our planet. COVID-19 inflammation is mediated by IL-1, a disease that can cause symptoms such as fever, cough, lung inflammation, thrombosis, stroke, renal failure and headache, to name a few. Strategies that inhibit IL-1 are certainly helpful in COVID-19 and can represent one of the therapeutic options. However, until now, COVID-19 therapy has been scarce and, in many cases, ineffective, since there are no specific drugs other than the vaccine that can solve this serious health problem. Messenger RNA (mRNA) vaccines which are the newest approach, are

already available and will certainly meet the many expectations that the population is waiting for. mRNA vaccines, coated with protected soft fatty lipids, use genetic mRNA (plus various inactive excipients) to make a piece of the coronavirus spike protein, which will instruct the immune system to produce specific antibodies. The soft fatty lipids allow the entry of mRNA into cells where it is absorbed into the cytoplasm and initiates the synthesis of the spike protein. In addition, vaccination also activates T cells that help the immune system respond to further exposure to the coronavirus. mRNA induces the synthesis of antigens of SARS-CoV-2 virus which stimulate the antibody response of the vaccinated person with the production of neutralizing antibodies. The new variant of the coronavirus-19 has been detected in the UK where, at the moment, the London government has imposed a lockdown with restrictions on international movements. The virus variant had already infected 1/4 of the total cases and in December 2020, it reached 2/3 of those infected in the UK. It has been noted that the spreading rate of the British variant could be greater than 70% of cases compared to the normal SARS-CoV-2 virus, with an R index growth of 0.4. Recent studies suggest that coronavirus-19 variation occurs at the level N501Y of the spike protein and involves 23 separate mutations on the spike, 17 of which are linked to the virus proteins, thus giving specific characteristics to the virus. In general, coronaviruses undergo many mutations that are often not decisive for their biological behavior and does not significantly alter the structure and the components of the virus. This phenomenon also occurs in SARS-CoV-2. It is highly probable that the variants recently described in the UK will not hinder vaccine-induced immunity. In fact, the variant will not break the vaccine although it may have some chance of making it a little less effective. Therefore, it is pertinent to think that the vaccine will work against the SARS-CoV-2 variant as well. In today's pandemic, the D614G mutation of the amino acid of corronavirus-19, which emerged in Europe in February 2020 is the most frequent form and causes high viral growth. The previously infrequent D614G mutation is now globally dominant. This variant, which is being tested by many international laboratories, is rapidly spreading across the countries and a series of vaccinated subjects are testing to see if their antibodies can neutralize the new variant of SARS-CoV-2. This variant has a very high viral growth and is less detectable with the RT-PCR technique in the laboratory. It has been reported that the British variant that increases viral load does not cause more severe effects in the respiratory tract and lung disease, therefore, it is certain that the variant is growing rapidly and must be kept under control; for this reason, laboratory data is expected impatiently. The study on the many variants that coronavirus-19 presents is very interesting and complete and clearer data on this topic will be ready in the near future. In addition, it is still unclear whether the different variants discovered in many countries, including Africa, share the same spike protein mutation and therefore, this is another study to elaborate on. In order to be certain and to not have unexpected surprises, we need to reduce the spread and the transmission speed of viral variants that could appear around the world, creating new pandemics. For this reason, the scientific community is on the alert since laboratory tests on serum antibodies from COVID-19 survivors have been reported to be less effective in attacking the variant. In light of the above, the scientific community must be on the alert as larger variants of the spike protein could escape vaccine-induced antibodies, which for now are of great help to the community and can save millions of lives. Deepening the study of spike protein mutations will help to better understand how to combat coronavirus-19 and its variants.

Cool, K., et al. (2021). "Infection and transmission of SARS-CoV-2 and its alpha variant in pregnant white-tailed deer." bioRxiv.

SARS-CoV-2, a novel Betacoronavirus, was first reported circulating in human populations in December 2019 and has since become a global pandemic. Recent history involving SARS-like coronavirus outbreaks (SARS-CoV and MERS-CoV) have demonstrated the significant role of intermediate and reservoir hosts in viral maintenance and transmission cycles. Evidence of SARS-CoV-2 natural infection and experimental infections of a wide variety of animal species has been demonstrated, and in silico and in vitro studies have indicated that deer are susceptible to SARS-CoV-2 infection. White-tailed deer (Odocoileus virginianus) are amongst the most abundant, densely populated, and geographically widespread wild ruminant species in the United States. Human interaction with white-tailed deer has resulted in the occurrence of disease in human populations in the past. Recently, white-tailed deer fawns were shown to be susceptible to SARS-CoV-2. In the present study, we investigated the susceptibility and transmission of SARS-CoV-2 in adult white-tailed deer. In addition, we examined the competition of two SARS-CoV-2 isolates, representatives of the ancestral lineage A (SARS-CoV-2/human/USA/WA1/2020) and the alpha variant of concern (VOC) B.1.1.7 (SARS-CoV-2/human/USA/CA_CDC_5574/2020), through co-infection of white-tailed deer. Next-generation sequencing was used to determine the presence and transmission of each strain in the co-infected and contact sentinel animals. Our results demonstrate that adult white-tailed deer are highly susceptible to SARS-CoV-2 infection and can transmit the virus through

direct contact as well as vertically from doe to fetus. Additionally, we determined that the alpha VOC B.1.1.7 isolate of SARS-CoV-2 outcompetes the ancestral lineage A isolate in white-tailed deer, as demonstrated by the genome of the virus shed from nasal and oral cavities from principal infected and contact animals, and from virus present in tissues of principal infected deer, fetuses and contact animals.

Cool, K., et al. (2021). "Infection and transmission of ancestral SARS-CoV-2 and its alpha variant in pregnant white-tailed deer." Emerg Microbes Infect: 1-39.

AbstractSARS-CoV-2 was first reported circulating in human populations in December 2019 and has since become a global pandemic. Recent history involving SARS-like coronavirus outbreaks have demonstrated the significant role of intermediate hosts in viral maintenance and transmission. Evidence of SARS-CoV-2 natural infection and experimental infections of a wide variety of animal species has been demonstrated, and in silico and in vitro studies have indicated that deer are susceptible to SARS-CoV-2 infection. White-tailed deer (WTD) are amongst the most abundant and geographically widespread wild ruminant species in the US. Recently, WTD fawns were shown to be susceptible to SARS-CoV-2. In the present study, we investigated the susceptibility and transmission of SARS-CoV-2 in adult WTD. In addition, we examined the competition of two SARS-CoV-2 isolates, representatives of the ancestral lineage A and the alpha variant of concern (VOC) B.1.1.7 through co-infection of WTD. Next-generation sequencing was used to determine the presence and transmission of each strain in the co-infected and contact sentinel animals. Our results demonstrate that adult WTD are highly susceptible to SARS-CoV-2 infection and can transmit the virus through direct contact as well as vertically from doe to fetus. Additionally, we determined that the alpha VOC B.1.1.7 isolate of SARS-CoV-2 outcompetes the ancestral lineage A isolate in WTD, as demonstrated by the genome of the virus shed from nasal and oral cavities from principal infected and contact animals, and from virus present in tissues of principal infected deer, fetuses and contact animals.

Coolen, J. P. M., et al. (2021). "SARS-CoV-2 whole-genome sequencing using reverse complement PCR: For easy, fast and accurate outbreak and variant analysis." J Clin Virol **144**: 104993.

During the course of the SARS-CoV-2 pandemic reports of mutations with effects on spreading and vaccine effectiveness emerged. Large scale mutation analysis using rapid SARS-CoV-2 Whole Genome Sequencing (WGS) is often unavailable but could support public health organizations and hospitals in monitoring transmission and rising levels of mutant strains. Here we report a novel WGS technique for SARS-CoV-2, the EasySeq RC-PCR SARS-CoV-2 WGS kit. By applying a reverse complement polymerase chain reaction (RC-PCR), an Illumina library preparation is obtained in a single PCR, thereby saving time, resources and facilitating high-throughput screening. Using this WGS technique, we evaluated SARS-CoV-2 diversity and possible transmission within a group of 173 patients and healthcare workers (HCW) of the Radboud university medical center during 2020. Due to the emergence of variants of concern, we screened SARS-CoV-2 positive samples in 2021 for identification of mutations and lineages. With use of EasySeq RC-PCR SARS-CoV-2 WGS kit we were able to obtain reliable results to confirm outbreak clusters and additionally identify new previously unassociated links in a considerably easier workaround compared to current methods. Furthermore, various SARS-CoV-2 variants of interest were detected among samples and validated against an Oxford Nanopore sequencing amplicon strategy which illustrates this technique is suitable for surveillance and monitoring current circulating variants.

Corbett, K. S., et al. (2021). "Protection against SARS-CoV-2 Beta Variant in mRNA-1273 Boosted Nonhuman Primates." bioRxiv.

Neutralizing antibody responses gradually wane after vaccination with mRNA-1273 against several variants of concern (VOC), and additional boost vaccinations may be required to sustain immunity and protection. Here, we evaluated the immune responses in nonhuman primates that received 100 microg of mRNA-1273 vaccine at 0 and 4 weeks and were boosted at week 29 with mRNA-1273 (homologous) or mRNA-1273.beta (heterologous), which encompasses the spike sequence of the B.1.351 (beta or beta) variant. Reciprocal ID 50 pseudovirus neutralizing antibody geometric mean titers (GMT) against live SARS-CoV-2 D614G and the beta variant, were 4700 and 765, respectively, at week 6, the peak of primary response, and 644 and 553, respectively, at a 5-month post-vaccination memory time point. Two weeks following homologous or heterologous boost beta-specific reciprocal ID 50 GMT were 5000 and 3000, respectively. At week 38, animals were challenged in the upper and lower airway with the beta variant. Two days post-challenge, viral replication was low to undetectable in both BAL and nasal swabs in most of the boosted animals. These data show that boosting with the homologous mRNA-1273 vaccine six months after primary immunization provides up to a 20-fold increase in neutralizing antibody responses across all VOC, which may be required to sustain high-level protection against severe disease, especially for at-risk populations. One-sentence summary: mRNA-

1273 boosted nonhuman primates have increased immune responses and are protected against SARS-CoV-2 beta infection.

Dalwadi, V., et al. (2021). "Axonal-Variant Guillian-Barre Syndrome Temporally Associated With mRNA-Based Moderna SARS-CoV-2 Vaccine." Cureus **13**(9): e18291.

We present a case of an 86-year-old woman who presented with a progressive quadriparesis two days after her second dose of Moderna SARS-CoV-2 vaccine, with cerebrospinal fluid (CSF) evidence of cytoalbuminocytological dissociation and electromyogram/nerve conduction studies (EMG/NCS) findings suggestive of acute axonal motor neuropathy. Her clinical symptoms did not improve with plasmapheresis. There appears to be a potential temporal association between the inoculation of mRNA-based SARS-CoV-2 vaccines and the development of Guillain-Barre Syndrome (GBS). Despite this possible association, infection prevention using highly effective mRNA-based vaccines remains highly recommended. Large epidemiological studies of SARS-CoV-2 vaccine-related adverse events are needed. Physicians should be aware of this possible temporal association since the prompt diagnosis and treatment of GBS can drastically improve outcomes. The aim is to report a case of axonal-variant GBS that was temporally associated with an mRNA-based SARS-CoV-2 vaccine.

Deng, X., et al. (2021). "Transmission, infectivity, and antibody neutralization of an emerging SARS-CoV-2 variant in California carrying a L452R spike protein mutation." medRxiv.

We identified a novel SARS-CoV-2 variant by viral whole-genome sequencing of 2,172 nasal/nasopharyngeal swab samples from 44 counties in California. Named B.1.427/B.1.429 to denote its 2 lineages, the variant emerged around May 2020 and increased from 0% to >50% of sequenced cases from September 1, 2020 to January 29, 2021, exhibiting an 18.6-24% increase in transmissibility relative to wild-type circulating strains. The variant carries 3 mutations in the spike protein, including an L452R substitution. Our analyses revealed 2-fold increased B.1.427/B.1.429 viral shedding in vivo and increased L452R pseudovirus infection of cell cultures and lung organoids, albeit decreased relative to pseudoviruses carrying the N501Y mutation found in the B.1.1.7, B.1.351, and P.1 variants. Antibody neutralization assays showed 4.0 to 6.7-fold and 2.0-fold decreases in neutralizing titers from convalescent patients and vaccine recipients, respectively. The increased prevalence of a more transmissible variant in California associated with decreased antibody neutralization warrants further investigation.

Deng, X., et al. (2021). "Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant." Cell **184**(13): 3426-3437 e3428.

We identified an emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant by viral whole-genome sequencing of 2,172 nasal/nasopharyngeal swab samples from 44 counties in California, a state in the western United States. Named B.1.427/B.1.429 to denote its two lineages, the variant emerged in May 2020 and increased from 0% to >50% of sequenced cases from September 2020 to January 2021, showing 18.6%-24% increased transmissibility relative to wild-type circulating strains. The variant carries three mutations in the spike protein, including an L452R substitution. We found 2-fold increased B.1.427/B.1.429 viral shedding in vivo and increased L452R pseudovirus infection of cell cultures and lung organoids, albeit decreased relative to pseudoviruses carrying the N501Y mutation common to variants B.1.1.7, B.1.351, and P.1. Antibody neutralization assays revealed 4.0- to 6.7-fold and 2.0-fold decreases in neutralizing titers from convalescent patients and vaccine recipients, respectively. The increased prevalence of a more transmissible variant in California exhibiting decreased antibody neutralization warrants further investigation.

Dougherty, K., et al. (2021). "SARS-CoV-2 B.1.617.2 (Delta) Variant COVID-19 Outbreak Associated with a Gymnastics Facility - Oklahoma, April-May 2021." MMWR Morb Mortal Wkly Rep **70**(28): 1004-1007.

The B.1.617.2 (Delta) variant of SARS-CoV-2, the virus that causes COVID-19, was identified in India in late 2020 and has subsequently been detected in approximately 60 countries (1). The B.1.617.2 variant has a potentially higher rate of transmission than other variants (2). During May 12-18, 2021, the Oklahoma State Department of Health (OSDH) Acute Disease Service (ADS) was notified by the OSDH Public Health Laboratory (PHL) of 21 SARS-CoV-2 B.1.617.2 specimens temporally and geographically clustered in central Oklahoma. Public health surveillance data indicated that these cases were associated with a local gymnastics facility (facility A). OSDH ADS and local health department staff members reinterviewed persons with B.1.617.2 variant-positive laboratory results and conducted contact tracing. Forty-seven COVID-19 cases (age range = 5-58 years), including 21 laboratory-confirmed B.1.617.2 variant and 26 epidemiologically linked cases, were associated with this outbreak during April 15-May 3, 2021. Cases occurred among 10 of 16 gymnast cohorts* and three staff members; secondary cases occurred in seven (33%) of 26 interviewed households with outbreak-associated cases. The overall facility and household attack rates were 20% and 53%, respectively. Forty (85%) persons with outbreak-associated COVID-19

had never received any COVID-19 vaccine doses (unvaccinated); three (6%) had received 1 dose of Moderna or Pfizer-BioNTech >/=14 days before a positive test result but had not received the second dose (partially vaccinated); four persons (9%) had received 2 doses of Moderna or Pfizer-BioNTech or a single dose of Janssen (Johnson & Johnson) vaccine >/=14 days before a positive test result (fully vaccinated). These findings suggest that the B.1.617.2 variant is highly transmissible in indoor sports settings and within households. Multicomponent prevention strategies including vaccination remain important to reduce the spread of SARS-CoV-2, including among persons participating in indoor sports(dagger) and their contacts.

Du, M., et al. (2021). "[Progress in research of epidemiologic feature and control of SARS-CoV-2 Delta variant]." Zhonghua Liu Xing Bing Xue Za Zhi **42**(10): 1774-1779.

SARS-CoV-2 Delta variant has the characteristics of stronger infectivity, higher viral load, and shorter incubation period, posing new challenges to the prevention and control of COVID-19 pandemic. SARS-CoV-2 Delta variant was first discovered in India, then quickly spread in many countries and has gradually become one of the main epidemic strains worldwide. Local epidemics caused by SARS-CoV-2 Delta variant also occurred in several provinces in China. This article summarizes the progress in research of etiological characteristics, transmission characteristics or possible mechanism and epidemiological characteristics of SARS-CoV-2 Delta variant, and the protective effects of vaccines and control measures against SARS-CoV-2 Delta variant in order to provide references for the effective prevention and control of COVID-19 epidemic caused by SARS-CoV-2 Delta variant.

Du, Z., et al. (2021). "Risk for International Importations of Variant SARS-CoV-2 Originating in the United Kingdom." Emerg Infect Dis **27**(5): 1527-1529.

A fast-spreading severe acute respiratory syndrome coronavirus 2 variant identified in the United Kingdom in December 2020 has raised international alarm. We analyzed data from 15 countries and estimated that the chance that this variant was imported into these countries by travelers from the United Kingdom by December 7 is >50%.

Dudas, G., et al. (2021). "Emergence and spread of SARS-CoV-2 lineage B.1.620 with variant of concern-like mutations and deletions." Nat Commun **12**(1): 5769.

Distinct SARS-CoV-2 lineages, discovered through various genomic surveillance initiatives, have emerged during the pandemic following unprecedented reductions in worldwide human mobility. We here describe a SARS-CoV-2 lineage - designated B.1.620 - discovered in Lithuania and carrying many mutations and deletions in the spike protein shared with widespread variants of concern (VOCs), including E484K, S477N and deletions HV69Delta, Y144Delta, and LLA241/243Delta. As well as documenting the suite of mutations this lineage carries, we also describe its potential to be resistant to neutralising antibodies, accompanying travel histories for a subset of European cases, evidence of local B.1.620 transmission in Europe with a focus on Lithuania, and significance of its prevalence in Central Africa owing to recent genome sequencing efforts there. We make a case for its likely Central African origin using advanced phylogeographic inference methodologies incorporating recorded travel histories of infected travellers.

Dumont, S., et al. (2021). "Acute SARS-CoV-2 alpha variant infection leading to placental insufficiency and fetal distress." J Med Virol.

The effect of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Alpha variant (also known as B.1.1.7 lineage, 20I/501Y.V1, the UK variant or VOC 202012/01) infection on pregnancy is currently unknown. We present a case of a 37-year-old woman admitted to our tertiary hospital at a gestational age of 29 weeks and 1 day because of oligohydramnios with reduced fetal movements for 10 days. About 20 days before admission, she tested positive for SARS-CoV-2 Alpha variant. The following day, due to abnormal cardiotocography, increased brain sparing, and absent end-diastolic flow in the umbilical artery, an urgent cesarean section was performed. The neonate had an uneventful admission to the neonatal intensive care unit. All neonatal samples proved negative for SARS-CoV-2. Pathological examination of the placenta revealed intervillous fibrin deposition, ischemic necrosis of villi and histiocytic intervillositis, corresponding with the SARS-CoV-2 placentitis triad. The placental tissue demonstrated a high viral load, possibly explaining the acute onset of placental insufficiently and subsequent fetal distress. This case demonstrates the importance of seeking medical care when experiencing reduced fetal movement in SARS-CoV-2 infected patients since acute infection can induce significant placental and subsequent fetal pathology.

Emary, K. R. W., et al. (2021). "Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial." Lancet **397**(10282): 1351-1362.

BACKGROUND: A new variant of SARS-CoV-2, B.1.1.7, emerged as the dominant cause of COVID-19 disease in the UK from November, 2020. We report a post-hoc analysis of the efficacy of the adenoviral vector vaccine, ChAdOx1 nCoV-19

(AZD1222), against this variant. METHODS: Volunteers (aged >/=18 years) who were enrolled in phase 2/3 vaccine efficacy studies in the UK, and who were randomly assigned (1:1) to receive ChAdOx1 nCoV-19 or a meningococcal conjugate control (MenACWY) vaccine, provided upper airway swabs on a weekly basis and also if they developed symptoms of COVID-19 disease (a cough, a fever of 37.8 degrees C or higher, shortness of breath, anosmia, or ageusia). Swabs were tested by nucleic acid amplification test (NAAT) for SARS-CoV-2 and positive samples were sequenced through the COVID-19 Genomics UK consortium. Neutralising antibody responses were measured using a live-virus microneutralisation assay against the B.1.1.7 lineage and a canonical non-B.1.1.7 lineage (Victoria). The efficacy analysis included symptomatic COVID-19 in seronegative participants with a NAAT positive swab more than 14 days after a second dose of vaccine. Participants were analysed according to vaccine received. Vaccine efficacy was calculated as 1 - relative risk (ChAdOx1 nCoV-19 vs MenACWY groups) derived from a robust Poisson regression model. This study is continuing and is registered with ClinicalTrials.gov, NCT04400838, and ISRCTN, 15281137. FINDINGS: Participants in efficacy cohorts were recruited between May 31 and Nov 13, 2020, and received booster doses between Aug 3 and Dec 30, 2020. Of 8534 participants in the primary efficacy cohort, 6636 (78%) were aged 18-55 years and 5065 (59%) were female. Between Oct 1, 2020, and Jan 14, 2021, 520 participants developed SARS-CoV-2 infection. 1466 NAAT positive nose and throat swabs were collected from these participants during the trial. Of these, 401 swabs from 311 participants were successfully sequenced. Laboratory virus neutralisation activity by vaccine-induced antibodies was lower against the B.1.1.7 variant than against the Victoria lineage (geometric mean ratio 8.9, 95% CI 7.2-11.0). Clinical vaccine efficacy against symptomatic NAAT positive infection was 70.4% (95% CI 43.6-84.5) for B.1.1.7 and 81.5% (67.9-89.4) for non-B.1.1.7 lineages. INTERPRETATION: ChAdOx1 nCoV-19 showed reduced neutralisation activity against the B.1.1.7 variant compared with a non-B.1.1.7 variant in vitro, but the vaccine showed efficacy against the B.1.1.7 variant of SARS-CoV-2. FUNDING: UK Research and Innovation, National Institute for Health Research (NIHR), Coalition for Epidemic Preparedness Innovations, NIHR Oxford Biomedical Research Centre, Thames Valley and South Midlands NIHR Clinical Research Network, and AstraZeneca.

Farinholt, T., et al. (2021). "Transmission event of SARS-CoV-2 delta variant reveals multiple vaccine breakthrough infections." BMC Med **19**(1): 255.

BACKGROUND: This study aims to identify the causative strain of SARS-CoV-2 in a cluster of vaccine breakthroughs. Vaccine breakthrough by a highly transmissible SARS-CoV-2 strain is a risk to global public health. METHODS: Nasopharyngeal swabs from suspected vaccine breakthrough cases were tested for SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) by qPCR (quantitative polymerase chain reaction) for Wuhan-Hu1 and alpha variant. Positive samples were then sequenced by Swift Normalase Amplicon Panels to determine the causal variant. GATK (genome analysis toolkit) variants were filtered with allele fraction >/=80 and min read depth 30x. RESULTS: Viral sequencing revealed an infection cluster of 6 vaccinated patients infected with the delta (B.1.617.2) SARS-CoV-2 variant. With no history of vaccine breakthrough, this suggests the delta variant may possess immune evasion in patients that received the Pfizer BNT162b2, Moderna mRNA-1273, and Covaxin BBV152. CONCLUSIONS: Delta variant may pose the highest risk out of any currently circulating SARS-CoV-2 variants, with previously described increased transmissibility over alpha variant and now, possible vaccine breakthrough. FUNDING: Parts of this work was supported by the National Institute of Allergy and Infectious Diseases (1U19AI144297) and Baylor College of Medicine internal funding.

Farinholt, T., et al. (2021). "Transmission event of SARS-CoV-2 Delta variant reveals multiple vaccine breakthrough infections." medRxiv.

Importance: Vaccine breakthrough by an emergent SARS-CoV-2 variant poses a great risk to global public health. Objective: To determine the SARS-CoV-2 variant responsible for 6 cases of vaccine breakthrough. Design: Nasopharyngeal swabs from suspected vaccine breakthrough cases were tested for SARS-CoV-2 by qPCR for Wuhan-Hu1 and Alpha variant. Positive samples were then sequenced by Swift Normalase Amplicon Panels to determine the causal variant. Setting: Transmission event occurred at events surrounding a wedding outside of Houston, TX. Two patients from India, likely transmitted the Delta variant to other guests. Participants: Following a positive SARS-CoV-2 qPCR test at a third-party site, six fully vaccinated patients were investigated. Three males and three females ranged from 53 to 69 years old. One patient suffered from diabetes while three others were classified as overweight. No significant other comorbidities were identified. None of the patients had a history of failed vaccination. Key Points: Question: Which SARS-CoV-2 variant is responsible for 6 cases of vaccine breakthrough, one interventional monoclonal antibody treatment, and one death?Findings: Viral sequencing revealed 6 vaccinated patients were infected with the Delta SARS-

CoV-2 variant. With no histories of vaccine breakthrough, this suggests Delta variant may possess immune evasion in patients that received the Pfizer BNT162b2, Moderna mRNA-1273, and Covaxin BBV152.Meaning: Delta variant may pose the highest risk out of any currently circulating SARS-CoV-2 variants, with increased transmissibility over Alpha variant and possible vaccine breakthrough.

Feder, K. A., et al. (2021). "Linked Clusters of SARS-CoV-2 Variant B.1.351 - Maryland, January-February 2021." MMWR Morb Mortal Wkly Rep **70**(17): 627-631.

In late January 2021, a clinical laboratory notified the Maryland Department of Health (MDH) that the SARS-CoV-2 variant of concern B.1.351 had been identified in a specimen collected from a Maryland resident with COVID-19 (1). The SARS-CoV-2 B.1.351 lineage was first identified in South Africa (2) and might be neutralized less effectively by antibodies produced after vaccination or natural infection with other strains (3-6). To limit SARS-CoV-2 chains of transmission associated with this index patient, MDH used contact tracing to identify the source of infection and any linked infections among other persons. The investigation identified two linked clusters of SARS-CoV-2 infection that included 17 patients. Three additional specimens from these clusters were sequenced; all three had the B.1.351 variant and all sequences were closely related to the sequence from the index patient's specimen. Among the 17 patients identified, none reported recent international travel or contact with international travelers. Two patients, including the index patient, had received the first of a 2-dose COVID-19 vaccination series in the 2 weeks before their likely exposure; one additional patient had a confirmed SARS-CoV-2 infection 5 months before exposure. Two patients were hospitalized with COVID-19, and one died. These first identified linked clusters of B.1.351 infections in the United States with no apparent link to international travel highlight the importance of expanding the scope and volume of genetic surveillance programs to identify variants, completing contact investigations for SARS-CoV-2 infections, and using universal prevention strategies, including vaccination, masking, and physical distancing, to control the spread of variants of concern.

Ferasin, L., et al. (2021). "Infection with SARS-CoV-2 variant B.1.1.7 detected in a group of dogs and cats with suspected myocarditis." Vet Rec **189**(9): e944.

BACKGROUND: Domestic pets can contract severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection; however, it is unknown whether the UK B.1.1.7 variant can more easily infect certain animal species or increase the possibility of human-to-animal transmission. METHODS: This is a descriptive case series reporting SARS-CoV-2 B.1.1.7 variant infections in a group of dogs and cats with suspected myocarditis. RESULTS: The study describes the infection of domestic cats and dogs by the B.1.1.7 variant. Two cats and one dog were positive to SARS-CoV-2 PCR on rectal swab, and two cats and one dog were found to have SARS-CoV-2 antibodies 2-6 weeks after they developed signs of cardiac disease. Many owners of these pets had developed respiratory symptoms 3-6 weeks before their pets became ill and had also tested positive for COVID-19. Interestingly, all these pets were referred for acute onset of cardiac disease, including severe myocardial disorders of suspected inflammatory origin but without primary respiratory signs. CONCLUSIONS: These findings demonstrate, for the first time, the ability for pets to be infected by the B.1.1.7 variant and question its possible pathogenicity in these animals.

Fort, H. (2021). "A very simple model to account for the rapid rise of the alpha variant of SARS-CoV-2 in several countries and the world." Virus Res **304**: 198531.

Since its first detection in the UK in September 2020, a highly contagious version of the coronavirus, the alpha or British variant a.k.a. B.1.1.7 SARS-CoV-2 virus lineage, rapidly spread across several countries and became the dominant strain in the outbreak. Here it is shown that a very simple evolutionary model can fit the observed change in frequency of B.1.1.7 for several countries, regions of countries and the whole world with a single parameter, its relative fitness f, which is almost universal f approximately 1.5. This is consistent with a 50% higher transmissibility than the local wild type and with the fact that the period in which this variant takes over has been in all the studied cases around 22 weeks.

Foster, A. and M. Kinzel (2021). "SARS-CoV-2 transmission in classroom settings: Effects of mitigation, age, and Delta variant." Phys Fluids (1994) **33**(11): 113311.

Traditional, in-person classroom settings have been limited during the COVID-19 pandemic due to their potential to transmit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among students, teachers, and other educational workers. Using computational fluid dynamics simulations, mitigation strategies that span approaches using face coverings, various ventilation schemes, air purifiers/cleaners, and desk shields are systematically evaluated in thermally controlled classrooms. Individually, face coverings and source control were the most effective, which was followed by well-designed ventilation systems. The use of desk shields was also studied and appeared to be ineffective. The best mitigation approach is shown to be through multiple measures-using face coverings and ventilation

systems combined with air purifiers. The studies were extended to elementary schools and consider Delta variants of SARS-CoV-2. In elementary settings, the reduced pulmonary and viral emission rates of small children are observed to drive reduced transmission rates, to values even lower than those observed with several mitigation methods for classrooms with adults. The Delta variant, with adults, was evaluated by considering an increase in quanta and indicated higher transmission probabilities. These increases are levels that are controllable by increasing the mitigation methods. Results indicate several plans of action for schools to return to in-person schooling in the context of age and new variants.

Gaymard, A., et al. (2021). "Early assessment of diffusion and possible expansion of SARS-CoV-2 Lineage 20I/501Y.V1 (B.1.1.7, variant of concern 202012/01) in France, January to March 2021." Euro Surveill **26**(9).

The emergence of SARS-CoV-2 variant 20I/501Y.V1 (VOC-202012/1 or GR/501Y.V1) is concerning given its increased transmissibility. We reanalysed 11,916 PCR-positive tests (41% of all positive tests) performed on 7-8 January 2021 in France. The prevalence of 20I/501Y.V1 was 3.3% among positive tests nationwide and 6.9% in the Paris region. Analysing the recent rise in the prevalence of 20I/501Y.V1, we estimate that, in the French context, 20I/501Y.V1 is 52-69% more transmissible than the previously circulating lineages, depending on modelling assumptions.

Ghosh, A. K., et al. (2021). "Molecular and Serological Characterization of the SARS-CoV-2 Delta Variant in Bangladesh in 2021." Viruses **13**(11).

Novel SARS-CoV-2 variants are emerging at an alarming rate. The delta variant and other variants of concern (VoC) carry spike (S)-protein mutations, which have the potential to evade protective immunity, to trigger break-through infections after COVID-19 vaccination, and to propagate future waves of COVID-19 pandemic. To identify SARS CoV-2 variants in Bangladesh, patients who are RT-PCR-positive for COVID-19 infections in Dhaka were screened by a RT-PCR melting curve analysis for spike protein mutations. To assess the anti-SARS CoV-2 antibody responses, the levels of the anti-S -proteins IgA and IgG and the anti-N-protein IgG were measured by ELISA. Of a total of 36 RT-PCR positive samples (75%), 27 were identified as delta variants, with one carrying an additional Q677H mutation and two with single nucleotide substitutions at position 23029 (compared to Wuhan-Hu-1 reference NC 045512) in the genome sequence. Three (8.3%) were identified as beta variants, two (5.5%) were identified as alpha variants, three (8.3%) were identified as having a B.1.1.318 lineage, and one sample was identified as an eta variant (B.1.525) carrying an additional V687L mutation. The trend of higher viral load (lower Cp values) among delta variants than in the alpha and beta variants was of borderline statistical significance ($p = 0.045$). Prospective studies with larger Bangladeshi cohorts are warranted to confirm the emergence of S-protein mutations and their association with antibody response in natural infection and potential breakthrough in vaccinated subjects.

Head, J. R., et al. (2021). "Model-based assessment of SARS-CoV-2 Delta variant transmission dynamics within partially vaccinated K-12 school populations." Lancet Reg Health Am: 100133.

Background: We examined school reopening policies amidst ongoing transmission of the highly transmissible Delta variant, accounting for vaccination among individuals >/=12 years. Methods: We collected data on social contacts among school-aged children in the California Bay Area and developed an individual-based transmission model to simulate transmission of the Delta variant of SARS-CoV-2 in schools. We evaluated the additional infections in students and teachers/staff resulting over a 128-day semester from in-school instruction compared to remote instruction when various NPIs (mask use, cohorts, and weekly testing of students/teachers) were implemented, across various community-wide vaccination coverages (50%, 60%, 70%), and student (>/=12 years) and teacher/staff vaccination coverages (50% - 95%). Findings: At 70% vaccination coverage, universal masking reduced infections by >57% among students. Masking plus 70% vaccination coverage enabled achievement of <50 excess cases per 1,000 students/teachers, but stricter risk tolerances, such as <25 excess infections per 1,000 students/teachers, required a cohort approach in elementary and middle school populations. In the absence of NPIs, increasing the vaccination coverage of community members from 50% to 70% or elementary teachers from 70% to 95% reduced the excess rate of infection among elementary school students attributable to school transmission by 24% and 37%, respectively. Interpretations: Amidst Delta variant circulation, we found that schools are not inherently low risk, yet can be made so with high community vaccination coverages and masking. Vaccination of adults protects unvaccinated children. Funding: National Science Foundation grant no. 2032210; National Institutes of Health grant nos. R01AI125842 and R01AI148336; MIDAS Coordination Center (MIDASSUP2020-4).

Head, J. R., et al. (2021). "Model-based assessment of SARS-CoV-2 Delta variant transmission dynamics within partially vaccinated K-12 school populations." medRxiv.

Background: We examined school reopening policies amidst rising transmission of the highly

transmissible Delta variant, accounting for vaccination among individuals aged 12 years and older, with the goal of characterizing risk to students and teachers under various within-school non-pharmaceutical interventions (NPIs) combined with specific vaccination coverage levels. Methods: We developed an individual-based transmission model to simulate transmission of the Delta variant of SARS-CoV-2 among a synthetic population, representative of Bay Area cities. We parameterized the model using community contact rates from vaccinated households ascertained from a household survey of Bay Area families with children conducted between February - April, 2021. Interventions and outcomes: We evaluated the additional infections in students and teachers/staff resulting over a 128-day semester from in-school instruction compared to remote instruction when various NPIs (mask use, cohorts, and weekly testing of students/teachers) were implemented in schools, across various community-wide vaccination coverages (50%, 60%, 70%), and student (>/=12 years) and teacher/staff vaccination coverages (50% - 95%). We quantified the added benefit of universal masking over masking among unvaccinated students and teachers, across varying levels of vaccine effectiveness (45%, 65%, 85%), and compared results between Delta and Alpha variant circulation. Results: The Delta variant sharply increases the risk of within-school COVID-transmission when compared to the Alpha variant. In our highest risk scenario (50% community and within-school vaccine coverage, no within-school NPIs, and predominant circulation of the Delta variant), we estimated that an elementary school could see 33-65 additional symptomatic cases of COVID-19 over a four-month semester (depending on the relative susceptibility of children <10 years). In contrast, under the Bay Area reopening plan (universal mask use, community and school vaccination coverage of 70%), we estimated excess symptomatic infection attributable to school reopening among 2.0-9.7% of elementary students (8-36 excess symptomatic cases per school over the semester), 3.0% of middle school students (13 cases per school) and 0.4% of high school students (3 cases per school). Excess rates among teachers attributable to reopening were similar. Achievement of lower risk tolerances, such as <5 excess infections per 1,000 students or teachers, required a cohort approach in elementary and middle school populations. In the absence of NPIs, increasing the vaccination coverage of community members from 50% to 70% or elementary teachers from 70% to 95% reduced the estimated excess rate of infection among elementary school students attributable to school transmission by 24% and 41%, respectively. We estimated that with 70% coverage of the eligible community and school population with a vaccine that is </=65% effective, universal masking can avert more cases than masking of unvaccinated persons alone. Conclusions: Amidst circulation of the Delta variant, our findings demonstrated that schools are not inherently low risk, yet can be made so with high community vaccination coverages and universal masking. Vaccination of adult community members and teachers protects unvaccinated elementary and middle school children. Elementary and middle schools that can support additional interventions, such as cohorts and testing, should consider doing so, particularly if additional studies find that younger children are equally as susceptible as adults to the Delta variant of SARS-CoV-2. Limitations: We did not consider the effect of social distancing in classrooms, or variation in testing frequency, and considerable uncertainty remains in key transmission parameters.

Heinrich, F., et al. (2021). "Dying of VOC-202012/01 - multimodal investigations in a death case of the SARS-CoV-2 variant." Int J Legal Med.

The current pandemic with Severe acute respiratory syndrome-coronavirus-2 has been taking on new dynamics since the emergence of new variants last fall, some of them spreading more rapidly. Many countries currently find themselves in a race to ramp up vaccination strategies that have been initiated and a possible third wave of the pandemic from new variants, such as the Variant of Concern-202012/01 from the B.1.1.7 lineage. Until today, many investigations in death cases of Coronavirus-disease-19 have been conducted, revealing pulmonary damage to be the predominant feature of the disease. Thereby, different degrees of macroscopic and microscopic lung damage have been reported, most of them resembling an Acute Respiratory Distress Syndrome. Far more, systemic complications of the disease such as pulmonary embolisms have been described. However, neither morphologic nor virologic findings of patients dying of the new variants have yet been reported. Here, we report on a comprehensive analysis of radiologic, morphologic, and virologic findings in a fatal case of this variant.

Herlihy, R., et al. (2021). "Rapid Increase in Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant - Mesa County, Colorado, April-June 2021." MMWR Morb Mortal Wkly Rep **70**(32): 1084-1087.

On May 5, 2021, the Colorado Department of Public Health and Environment (CDPHE) identified the first five COVID-19 cases caused by the SARS-CoV-2 B.1.617.2 (Delta) variant in Mesa County in western Colorado (population 154,933, <3% of the state population). All five initial cases were associated with school settings. Through early June, Mesa County experienced a marked increase in the proportion of Delta variant cases identified through sequencing: the 7-day proportion of sequenced specimens identified as

B.1.617.2 in Mesa County more than doubled, from 43% for the week ending May 1 to 88% for the week ending June 5. As of June 6, more than one half (51%) of sequenced B.1.617.2 specimens in Colorado were from Mesa County. CDPHE assessed data from surveillance, vaccination, laboratory, and hospital sources to describe the preliminary epidemiology of the Delta variant and calculate crude vaccine effectiveness (VE). Vaccination coverage in early May in Mesa County was lower (36% of eligible residents fully vaccinated) than that in the rest of the state (44%). Compared with that in all other Colorado counties, incidence, intensive care unit (ICU) admissions, and COVID-19 case fatality ratios were significantly higher in Mesa County during the analysis period, April 27-June 6, 2021. In addition, during the same time period, the proportion of COVID-19 cases in persons who were fully vaccinated (vaccine breakthrough cases) was significantly higher in Mesa County compared with that in all other Colorado counties. Estimated crude VE against reported symptomatic infection for a 2-week period ending June 5 was 78% (95% confidence interval [CI] = 71%-84%) for Mesa County and 89% (95% CI = 88%-91%) for other Colorado counties. Vaccination is a critical strategy for preventing infection, serious illness, and death from COVID-19. Enhanced mitigation strategies, including masking in indoor settings irrespective of vaccination status, should be considered in areas with substantial or high case rates.

Hitchings, M. D. T., et al. (2021). "Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: A test-negative case-control study." Lancet Reg Health Am **1**: 100025.

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant, Gamma, emerged in the city of Manaus in late 2020 during a large resurgence of coronavirus disease (COVID-19), and has spread throughout Brazil. The effectiveness of vaccines in settings with widespread Gamma variant transmission has not been reported. Methods: We performed a matched test-negative case-control study to estimate the effectiveness of an inactivated vaccine, CoronaVac, in healthcare workers (HCWs) in Manaus, where the Gamma variant accounted for 86% of genotyped SARS-CoV-2 samples at the peak of its epidemic. We performed an early analysis of effectiveness following administration of at least one vaccine dose and an analysis of effectiveness of the two-dose schedule. The primary outcome was symptomatic SARS-CoV-2 infection. Findings: For the early at-least-one-dose and two-dose analyses the study population was, respectively, 53,176 and 53,153 HCWs residing in Manaus and aged 18 years or older, with complete information on age, residence, and vaccination status. Among 53,153 HCWs eligible for the two-dose analysis, 47,170 (89%) received at least one dose of CoronaVac and 2,656 individuals (5%) underwent RT-PCR testing from 19 January, 2021 to 13 April, 2021. Of 3,195 RT-PCR tests, 885 (28%) were positive. 393 and 418 case-control pairs were selected for the early and two-dose analyses, respectively, matched on calendar time, age, and neighbourhood. Among those who had received both vaccine doses before the RT-PCR sample collection date, the average time from second dose to sample collection date was 14 days (IQR 7-24). In the early analysis, vaccination with at least one dose was associated with a 0.50-fold reduction (adjusted vaccine effectiveness (VE), 49.6%, 95% CI 11.3 to 71.4) in the odds of symptomatic SARS-CoV-2 infection during the period 14 days or more after receiving the first dose. However, we estimated low effectiveness (adjusted VE 36.8%, 95% CI -54.9 to 74.2) of the two-dose schedule against symptomatic SARS-CoV-2 infection during the period 14 days or more after receiving the second dose. A finding that vaccinated individuals were much more likely to be infected than unvaccinated individuals in the period 0-13 days after first dose (aOR 2.11, 95% CI 1.36-3.27) suggests that unmeasured confounding led to downward bias in the vaccine effectiveness estimate. Interpretation: Evidence from this test-negative study of the effectiveness of CoronaVac was mixed, and likely affected by bias in this setting. Administration of at least one vaccine dose showed effectiveness against symptomatic SARS-CoV-2 infection in the setting of epidemic Gamma variant transmission. However, the low estimated effectiveness of the two-dose schedule underscores the need to maintain non-pharmaceutical interventions while vaccination campaigns with CoronaVac are being implemented. Funding: Fundacao Oswaldo Cruz (Fiocruz); Municipal Health Secretary of Manaus; Fundacao de Vigilancia em Saude do Amazonas.

Hodcroft, E. B., et al. (2021). "Emergence and spread of a SARS-CoV-2 variant through Europe in the summer of 2020." medRxiv.

Following its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic resulting in unprecedented efforts to reduce transmission and develop therapies and vaccines (WHO Emergency Committee, 2020; Zhu et al., 2020). Rapidly generated viral genome sequences have allowed the spread of the virus to be tracked via phylogenetic analysis (Worobey et al., 2020; Hadfield et al., 2018; Pybus et al., 2020). While the virus spread globally in early 2020 before borders closed, intercontinental travel has since been greatly reduced, allowing continent-specific variants to emerge. However, within Europe travel resumed in the summer of 2020, and the impact of this travel on the

epidemic is not well understood. Here we report on a novel SARS-CoV-2 variant, 20E (EU1), that emerged in Spain in early summer, and subsequently spread to multiple locations in Europe. We find no evidence of increased transmissibility of this variant, but instead demonstrate how rising incidence in Spain, resumption of travel across Europe, and lack of effective screening and containment may explain the variant's success. Despite travel restrictions and quarantine requirements, we estimate 20E (EU1) was introduced hundreds of times to countries across Europe by summertime travellers, likely undermining local efforts to keep SARS-CoV-2 cases low. Our results demonstrate how a variant can rapidly become dominant even in absence of a substantial transmission advantage in favorable epidemiological settings. Genomic surveillance is critical to understanding how travel can impact SARS-CoV-2 transmission, and thus for informing future containment strategies as travel resumes.

Hodcroft, E. B., et al. (2021). "Spread of a SARS-CoV-2 variant through Europe in the summer of 2020." Nature **595**(7869): 707-712.

Following its emergence in late 2019, the spread of SARS-CoV-2(1,2) has been tracked by phylogenetic analysis of viral genome sequences in unprecedented detail(3-5). Although the virus spread globally in early 2020 before borders closed, intercontinental travel has since been greatly reduced. However, travel within Europe resumed in the summer of 2020. Here we report on a SARS-CoV-2 variant, 20E (EU1), that was identified in Spain in early summer 2020 and subsequently spread across Europe. We find no evidence that this variant has increased transmissibility, but instead demonstrate how rising incidence in Spain, resumption of travel, and lack of effective screening and containment may explain the variant's success. Despite travel restrictions, we estimate that 20E (EU1) was introduced hundreds of times to European countries by summertime travellers, which is likely to have undermined local efforts to minimize infection with SARS-CoV-2. Our results illustrate how a variant can rapidly become dominant even in the absence of a substantial transmission advantage in favourable epidemiological settings. Genomic surveillance is critical for understanding how travel can affect transmission of SARS-CoV-2, and thus for informing future containment strategies as travel resumes.

Hodgson, D., et al. (2021). "The potential for vaccination-induced herd immunity against the SARS-CoV-2 B.1.1.7 variant." Euro Surveill **26**(20).

We assess the feasibility of reaching the herd immunity threshold against SARS-CoV-2 through vaccination, considering vaccine effectiveness (VE), transmissibility of the virus and the level of pre-existing immunity in populations, as well as their age structure. If highly transmissible variants of concern become dominant in areas with low levels of naturally-acquired immunity and/or in populations with large proportions of < 15 year-olds, control of infection without non-pharmaceutical interventions may only be possible with a VE >/= 80%, and coverage extended to children.

Hoffmann, M., et al. (2021). "SARS-CoV-2 variant B.1.617 is resistant to bamlanivimab and evades antibodies induced by infection and vaccination." Cell Rep **36**(3): 109415.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants threatens efforts to contain the coronavirus disease 2019 (COVID-19) pandemic. The number of COVID-19 cases and deaths in India has risen steeply, and a SARS-CoV-2 variant, B.1.617, is believed to be responsible for many of these cases. The spike protein of B.1.617 harbors two mutations in the receptor binding domain, which interacts with the angiotensin converting enzyme 2 (ACE2) receptor and constitutes the main target of neutralizing antibodies. Therefore, we analyze whether B.1.617 is more adept in entering cells and/or evades antibody responses. B.1.617 enters two of eight cell lines tested with roughly 50% increased efficiency and is equally inhibited by two entry inhibitors. In contrast, B.1.617 is resistant against bamlanivimab, an antibody used for COVID-19 treatment. B.1.617 evades antibodies induced by infection or vaccination, although less so than the B.1.351 variant. Collectively, our study reveals that antibody evasion of B.1.617 may contribute to the rapid spread of this variant.

Hou, Y. J., et al. (2020). "SARS-CoV-2 D614G Variant Exhibits Enhanced Replication ex vivo and Earlier Transmission in vivo." bioRxiv.

The D614G substitution in the S protein is most prevalent SARS-CoV-2 strain circulating globally, but its effects in viral pathogenesis and transmission remain unclear. We engineered SARS-CoV-2 variants harboring the D614G substitution with or without nanoluciferase. The D614G variant replicates more efficiency in primary human proximal airway epithelial cells and is more fit than wildtype (WT) virus in competition studies. With similar morphology to the WT virion, the D614G virus is also more sensitive to SARS-CoV-2 neutralizing antibodies. Infection of human ACE2 transgenic mice and Syrian hamsters with the WT or D614G viruses produced similar titers in respiratory tissue and pulmonary disease. However, the D614G variant exhibited significantly faster droplet transmission between hamsters than the WT virus, early after infection. Our study demonstrated the SARS-CoV2 D614G substitution enhances infectivity, replication fitness, and early transmission.

Hou, Y. J., et al. (2020). "SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo." Science **370**(6523): 1464-1468.

The spike aspartic acid-614 to glycine (D614G) substitution is prevalent in global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strains, but its effects on viral pathogenesis and transmissibility remain unclear. We engineered a SARS-CoV-2 variant containing this substitution. The variant exhibits more efficient infection, replication, and competitive fitness in primary human airway epithelial cells but maintains similar morphology and in vitro neutralization properties, compared with the ancestral wild-type virus. Infection of human angiotensin-converting enzyme 2 (ACE2) transgenic mice and Syrian hamsters with both viruses resulted in similar viral titers in respiratory tissues and pulmonary disease. However, the D614G variant transmits significantly faster and displayed increased competitive fitness than the wild-type virus in hamsters. These data show that the D614G substitution enhances SARS-CoV-2 infectivity, competitive fitness, and transmission in primary human cells and animal models.

Huang, H. C., et al. (2021). "Targeting conserved N-glycosylation blocks SARS-CoV-2 variant infection in vitro." EBioMedicine **74**: 103712.

BACKGROUND: Despite clinical success with anti-spike vaccines, the effectiveness of neutralizing antibodies and vaccines has been compromised by rapidly spreading SARS-CoV-2 variants. Viruses can hijack the glycosylation machinery of host cells to shield themselves from the host's immune response and attenuate antibody efficiency. However, it remains unclear if targeting glycosylation on viral spike protein can impair infectivity of SARS-CoV-2 and its variants. METHODS: We adopted flow cytometry, ELISA, and BioLayer interferometry approaches to assess binding of glycosylated or deglycosylated spike with ACE2. Viral entry was determined by luciferase, immunoblotting, and immunofluorescence assays. Genome-wide association study (GWAS) revealed a significant relationship between STT3A and COVID-19 severity. NF-kappaB/STT3A-regulated N-glycosylation was investigated by gene knockdown, chromatin immunoprecipitation, and promoter assay. We developed an antibody-drug conjugate (ADC) that couples non-neutralization anti-spike antibody with NGI-1 (4G10-ADC) to specifically target SARS-CoV-2-infected cells. FINDINGS: The receptor binding domain and three distinct SARS-CoV-2 surface N-glycosylation sites among 57,311 spike proteins retrieved from the NCBI-Virus-database are highly evolutionarily conserved (99.67%) and are involved in ACE2 interaction. STT3A is a key glycosyltransferase catalyzing spike glycosylation and is positively correlated with COVID-19 severity. We found that inhibiting STT3A using N-linked glycosylation inhibitor-1 (NGI-1) impaired SARS-CoV-2 infectivity and that of its variants [Alpha (B.1.1.7) and Beta (B.1.351)]. Most importantly, 4G10-ADC enters SARS-CoV-2-infected cells and NGI-1 is subsequently released to deglycosylate spike protein, thereby reinforcing the neutralizing abilities of antibodies, vaccines, or convalescent sera and reducing SARS-CoV-2 variant infectivity. INTERPRETATION: Our results indicate that targeting evolutionarily-conserved STT3A-mediated glycosylation via an ADC can exert profound impacts on SARS-CoV-2 variant infectivity. Thus, we have identified a novel deglycosylation method suitable for eradicating SARS-CoV-2 variant infection in vitro. FUNDING: A full list of funding bodies that contributed to this study can be found in the Acknowledgements section.

Huang, H. C., et al. (2021). "Hyperglycosylated spike of SARS-CoV-2 gamma variant induces breast cancer metastasis." Am J Cancer Res **11**(10): 4994-5005.

SARS-CoV-2 exploits the host cellular machinery for virus replication leading to the acute syndrome of coronavirus disease 2019 (COVID-19). Growing evidence suggests SARS-CoV-2 also exacerbates many chronic diseases, including cancers. As mutations on the spike protein (S) emerged as dominant variants that reduce vaccine efficacy, little is known about the relation between SARS-CoV-2 virus variants and cancers. Compared to the SARS-CoV-2 wild-type, the Gamma variant contains two additional NXT/S glycosylation motifs on the S protein. The hyperglycosylated S of Gamma variant is more stable, resulting in more significant epithelial-mesenchymal transition (EMT) potential. SARS-CoV-2 infection promoted NF-kappaB signaling activation and p65 nuclear translocation, inducing Snail expression. Pharmacologic inhibition of NF-kappaB activity by nature food compound, I3C suppressed viral replication and Gamma variant-mediated breast cancer metastasis, indicating that NF-kappaB inhibition can reduce chronic disease in COVID-19 patients. Our study revealed that the Gamma variant of SARS-CoV-2 activates NF-kappaB and, in turn, triggers the pro-survival function for cancer progression.

Huang, S. Y., et al. (2021). "Stability of SARS-CoV-2 Spike G614 Variant Surpasses That of the D614 Variant after Cold Storage." mSphere **6**(2).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) carrying the D614G mutation on the spike protein is the predominant circulating variant and is associated with enhanced infectivity. However, whether this dominant variant can potentially spread through the cold chain and

whether the spike protein affects virus stability after cold storage remain unclear. To compare the infectivity of two SARS-CoV-2 variants, namely, SARS-CoV-2 variants with spike protein with the D614 mutation (S-D614) and G614 mutation (S-G614), after different periods of refrigeration (4 degrees C) and freezing (-20 degrees C). We also determined the integrity of the viral RNA and the ability of the spike protein to bind angiotensin-converting enzyme 2 (ACE2) after storage at these conditions. The results showed that SARS-CoV-2 was more stable and infectious after storage at -20 degrees C than at 4 degrees C. Particularly, the S-G614 variant was found to be more stable than the S-D614 variant. The spike protein of the S-G614 variant had better binding ability with the ACE2 receptor than that of the S-D614 variant after storage at -20 degrees C for up to 30 days. Our findings revealed that SARS-CoV-2 remains stable and infectious after refrigeration or freezing, and their stability and infectivity up to 30 days depends on the spike variant. Stability and infectivity are related to each other, and the higher stability of S-G614 compared to that of S-D614 may contribute to rapid viral spread of the S-G614 variant.IMPORTANCE It has been observed that variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are more stable and infectious after storage at -20 degrees C than at 4 degrees C. A SARS-CoV-2 S-D614G variant is currently the most dominant variant in circulation and is associated with enhanced infectivity. We compared the stability of two SARS-CoV-2 variants: the early S-D614 variant carrying the D614 spike protein and the new S-G614 variant carrying the G614 spike protein, stored at both 4 degrees C and -20 degrees C for different periods. We observed that SARS-CoV-2 remains stable and infectious after refrigeration or freezing, which further depends on the spike variant, that is, the ability of the spike protein to bind with the ACE2 receptor with higher efficiency. The high stability of the S-G614 variant also explains its rapid spread and infectivity. Therefore, precautions should be taken during and after handling food preserved under cold conditions.

Jhun, H., et al. (2021). "SARS-CoV-2 Delta (B.1.617.2) Variant: A Unique T478K Mutation in Receptor Binding Motif (RBM) of Spike Gene." Immune Netw **21**(5): e32.

Over two hundred twenty-eight million cases of coronavirus disease 2019 (COVID-19) in the world have been reported until the 21(st) of September 2021 after the first rise in December 2019. The virus caused the disease called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Over 4 million deaths blame COVID-19 during the last one year and 8 months in the world. Currently, four SARS-CoV-2 variants of concern are mainly focused by pandemic studies with limited experiments to translate the infectivity and pathogenicity of each variant. The SARS-CoV-2 alpha, beta, gamma, and delta variant of concern was originated from United Kingdom, South Africa, Brazil/Japan, and India, respectively. The classification of SARS-CoV-2 variant is based on the mutation in spike (S) gene on the envelop of SARS-CoV-2. This review describes four SARS-CoV-2 alpha, beta, gamma, and delta variants of concern including SARS-CoV-2 epsilon, zeta, eta, iota, kappa, and B.1.617.3 variants of interest and alert. Recently, SARS-CoV-2 delta variant prevails over different countries that have 3 unique mutation sites: E156del/R158G in the N-terminal domain and T478K in a crucial receptor binding domain. A particular mutation in the functional domain of the S gene is probably associated with the infectivity and pathogenesis of the SARS-CoV-2 variant.

Jiang, L., et al. (2021). "Detecting SARS-CoV-2 and its variant strains with a full genome tiling array." Brief Bioinform **22**(6).

Coronavirus disease 2019 pandemic is the most damaging pandemic in recent human history. Rapid detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and variant strains is paramount for recovery from this pandemic. Conventional SARS-CoV-2 tests interrogate only limited regions of the whole SARS-CoV-2 genome, which are subjected to low specificity and miss the opportunity of detecting variant strains. In this work, we developed the first SARS-CoV-2 tiling array that captures the entire SARS-CoV-2 genome at single nucleotide resolution and offers the opportunity to detect point mutations. A thorough bioinformatics protocol of two base calling methods has been developed to accompany this array. To demonstrate the effectiveness of the tiling array, we genotyped all genomic positions of eight SARS-CoV-2 samples. Using high-throughput sequencing as the benchmark, we show that the tiling array had a genome-wide accuracy of at least 99.5%. From the tiling array analysis results, we identified the D614G mutation in the spike protein in four of the eight samples, suggesting the widespread distribution of this variant at the early stage of the outbreak in the United States. Two additional nonsynonymous mutations were identified in one sample in the nucleocapsid protein (P13L and S197L), which may complicate future vaccine development. With around $5 per array, supreme accuracy, and an ultrafast bioinformatics protocol, the SARS-CoV-2 tiling array makes an invaluable toolkit for combating current and future pandemics. Our SARS-CoV-2 tilting array is currently utilized by Molecular Vision, a CLIA-certified lab for SARS-CoV-2 diagnosis.

Johnson, K. E., et al. (2021). "Real-Time Projections of SARS-CoV-2 B.1.1.7 Variant in a University Setting, Texas, USA." Emerg Infect Dis **27**(12): 3188-3190.

We used the incidence of spike gene target failures identified during PCR testing to provide an early projection of the prevalence of severe acute respiratory syndrome coronavirus 2 variant B.1.1.7 in a university setting in Texas, USA, before sequencing results were available. Findings from a more recent evaluation validated those early projections.

Jones, L., et al. (2021). "Isothermal amplification and fluorescent detection of SARS-CoV-2 and SARS-CoV-2 variant virus in nasopharyngeal swabs." PLoS One **16**(9): e0257563.

The COVID-19 pandemic caused by the SARS-CoV-2 is a serious health threat causing worldwide morbidity and mortality. Real-time reverse transcription PCR (RT-qPCR) is currently the standard for SARS-CoV-2 detection. Although various nucleic acid-based assays have been developed to aid the detection of SARS-CoV-2 from COVID-19 patient samples, the objective of this study was to develop a diagnostic test that can be completed in 30 minutes without having to isolate RNA from the samples. Here, we present an RNA amplification detection method performed using reverse transcription loop-mediated isothermal amplification (RT-LAMP) reactions to achieve specific, rapid (30 min), and sensitive (<100 copies) fluorescent detection in real-time of SARS-CoV-2 directly from patient nasopharyngeal swab (NP) samples. When compared to RT-qPCR, positive NP swab samples assayed by fluorescent RT-LAMP had 98% (n = 41/42) concordance and negative NP swab samples assayed by fluorescent RT-LAMP had 87% (n = 59/68) concordance for the same samples. Importantly, the fluorescent RT-LAMP results were obtained without purification of RNA from the NP swab samples in contrast to RT-qPCR. We also show that the fluorescent RT-LAMP assay can specifically detect live virus directly from cultures of both SARS-CoV-2 wild type (WA1/2020), and a SARS-CoV-2 B.1.1.7 (alpha) variant strain with equal sensitivity to RT-qPCR. RT-LAMP has several advantages over RT-qPCR including isothermal amplification, speed (<30 min), reduced costs, and similar sensitivity and specificity.

Keeton, R., et al. (2021). "Prior infection with SARS-CoV-2 boosts and broadens Ad26.COV2.S immunogenicity in a variant-dependent manner." Cell Host Microbe **29**(11): 1611-1619 e1615.

The Johnson and Johnson Ad26.COV2.S single-dose vaccine represents an attractive option for coronavirus disease 2019 (COVID-19) vaccination in countries with limited resources. We examined the effect of prior infection with different SARS-CoV-2 variants on Ad26.COV2.S immunogenicity. We compared participants who were SARS-CoV-2 naive with those either infected with the ancestral D614G virus or infected in the second wave when Beta predominated. Prior infection significantly boosts spike-binding antibodies, antibody-dependent cellular cytotoxicity, and neutralizing antibodies against D614G, Beta, and Delta; however, neutralization cross-reactivity varied by wave. Robust CD4 and CD8 T cell responses are induced after vaccination, regardless of prior infection. T cell recognition of variants is largely preserved, apart from some reduction in CD8 recognition of Delta. Thus, Ad26.COV2.S vaccination after infection could result in enhanced protection against COVID-19. The impact of the infecting variant on neutralization breadth after vaccination has implications for the design of second-generation vaccines based on variants of concern.

Keller, M., et al. (2021). "Detection of SARS-CoV-2 variant B.1.1.7 in a cat in Germany." Res Vet Sci **140**: 229-232.

Several non-variant of concern SARS-CoV-2 infections in pets have been reported as documented in the OIE and GISAID databases and there is only one fully documented case of an alpha variant of concern (VOC)(B.1.1.7) in the United States so far. Here, we describe the first case in a cat infected with the alpha SARS-CoV-2 variant in Germany. A cat suffering from pneumonia was presented to a veterinary practice. The pneumonia was treated symptomatically, but 16 days later the cat was presented again. Since the owner had been tested positive for a SARS-CoV-2 infection in the meantime, swab samples were taken from the cat and analyzed for SARS-CoV-2 specific nucleic acids. The various RT-qPCR analyses and whole-genome sequencing revealed the presence of the SARS-CoV-2 B.1.1.7 variant in this cat. This study shows that pets living in close contact with SARS-CoV-2 B.1.1.7 infected owners can contract this virus and also suffer from a respiratory disease. It is not clear yet whether onward transmissions to other cats and humans can occur. To minimize transmission risks, pet owners and veterinarians should comply to the hygienic rules published by OIE and others. It must be stated, that infections of cats with SARS-CoV-2 is still a rare event. Cats with clinical signs of a respiratory disease should be presented to a veterinarian, who will decide on further steps.

Kerneis, S., et al. (2021). "Transmission of SARS-CoV-2 Alpha Variant (B.1.1.7) From a BNT162b2-Vaccinated Individual." Open Forum Infect Dis **8**(8): ofab369.

Cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) acquisition after vaccination with BNT162b2 have been described, but the risk of secondary transmission from fully vaccinated individuals remains ill defined. Herein we

report a confirmed transmission of SARS-CoV-2 alpha variant (B.1.1.7) from a symptomatic immunocompetent woman 4 weeks after her second dose of BNT162b2, despite antispike seroconversion.

Knabl, L., et al. (2021). "Impact of BNT162b First Vaccination on the Immune Transcriptome of Elderly Patients Infected with the B.1.351 SARS-CoV-2 Variant." Res Sq.

Fast-spreading variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) energize the COVID-19 pandemic. The B.1.351 variant carrying the escape mutation E484K in the receptor binding domain is of particular concern due to reduced immunological protection following vaccination. Protection can manifest as early as 10 days following immunization with full protection two weeks following the second dose, but the course is not well-characterized for variants. Here, we investigated the immune transcriptome of six elderly individuals (average age 82 yr.) from an old peopleaeuros home, who contracted B.1.351, with four having received the first dose of BNT162b eight to 11 days prior to the onset of COVID-19 symptoms. The patients were hospitalized and received dexamethasone treatment. Immune transcriptomes were established from PBMCs approximately 10 and 35 days after the onset of COVID-19 symptomology. RNA-seq revealed a more intensive immune response in vaccinated patients as compared to unvaccinated ones. Specifically, transcription factors linked to the JAK/STAT pathway, interferon stimulated genes, and genes associated with innate antiviral immunity and COVID-19-SARS-CoV-2 infection were highly enriched in vaccinated patients. This rendered the transcriptomes of the older vaccinated group significantly different than older unvaccinated individuals infected at the same institution and more similar to the immune response of younger unvaccinated individuals (ages 48-62) following B.1.351 infection. All individuals in this study whether vaccinated or not were hospitalized due to B.1.351 infection and one vaccinated patient died illustrating that although an enhanced immune response was documented infection it was insufficient to protect from disease. This highlights the need for maintaining physical distancing and prevention measures throughout the time course of vaccination in older adults.

Knabl, L., et al. (2021). "Impact of BNT162b first vaccination on the immune transcriptome of elderly patients infected with the B.1.351 SARS-CoV-2 variant." medRxiv.

Fast-spreading variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) energize the COVID-19 pandemic. The B.1.351 variant carrying the escape mutation E484K in the receptor binding domain is of particular concern due to reduced immunological protection following vaccination. Protection can manifest as early as 10 days following immunization with full protection two weeks following the second dose, but the course is not well-characterized for variants. Here, we investigated the immune transcriptome of six elderly individuals (average age 82 yr.) from an old peopleaeuros home, who contracted B.1.351, with four having received the first dose of BNT162b eight to 11 days prior to the onset of COVID-19 symptoms. The patients were hospitalized and received dexamethasone treatment. Immune transcriptomes were established from PBMCs approximately 10 and 35 days after the onset of COVID-19 symptomology. RNA-seq revealed a more intensive immune response in vaccinated patients as compared to unvaccinated ones. Specifically, transcription factors linked to the JAK/STAT pathway, interferon stimulated genes, and genes associated with innate antiviral immunity and COVID-19-SARS-CoV-2 infection were highly enriched in vaccinated patients. This rendered the transcriptomes of the older vaccinated group significantly different than older unvaccinated individuals infected at the same institution and more similar to the immune response of younger unvaccinated individuals (age range 48-62) following B.1.351 infection. All individuals in this study whether vaccinated or not were hospitalized due to B.1.351 infection and one vaccinated patient died illustrating that although an enhanced immune response was documented infection it was insufficient to protect from disease. This highlights the need for maintaining physical distancing and prevention measures throughout the time course of vaccination in older adults.

Konno, Y., et al. (2020). "SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant." Cell Rep **32**(12): 108185.

One of the features distinguishing SARS-CoV-2 from its more pathogenic counterpart SARS-CoV is the presence of premature stop codons in its ORF3b gene. Here, we show that SARS-CoV-2 ORF3b is a potent interferon antagonist, suppressing the induction of type I interferon more efficiently than its SARS-CoV ortholog. Phylogenetic analyses and functional assays reveal that SARS-CoV-2-related viruses from bats and pangolins also encode truncated ORF3b gene products with strong anti-interferon activity. Furthermore, analyses of approximately 17,000 SARS-CoV-2 sequences identify a natural variant in which a longer ORF3b reading frame was reconstituted. This variant was isolated from two patients with severe disease and further increased the ability of ORF3b to suppress interferon induction. Thus, our findings not only help to explain the poor interferon response in COVID-19 patients but also

describe the emergence of natural SARS-CoV-2 quasispecies with an extended ORF3b gene that may potentially affect COVID-19 pathogenesis.

Koopsen, J., et al. (2021). "Rapid reinfection with SARS-CoV-2 variant-of-concern Alpha detected in a nurse during an outbreak at a non-covid inpatient ward: lessons learned." Antimicrob Resist Infect Control **10**(1): 137.

We describe the lessons learned during a SARS-CoV-2 variant-of-concern Alpha outbreak investigation at a normal care unit in a university hospital in Amsterdam in December 2020. The outbreak consisted of nine nurses and two roomed-in patient family members. (attack rate 18%). One nurse tested positive with a phylogenetically distinct variant, after a documented infection 83 days prior. Three key points were taken from this investigation. First, it was controlled by adherence to existing guidelines, despite increased transmissibility of the variant. Second, viral sequencing can inform transmission cluster inference, but the epidemiological context is essential to draw appropriate conclusions. Third, reinfections with Alpha variants can occur rapidly after primary infection.

Kostaki, E. G., et al. (2021). "Temporal Dominance of B.1.1.7 over B.1.354 SARS-CoV-2 Variant: A Hypothesis Based on Areas of Variant Co-Circulation." Life (Basel) **11**(5).

Some emergent SARS-CoV-2 variants raise concerns due to their altered biological properties. For both B.1.1.7 and B.1351 variants, named as variants of concern (VOC), increased transmissibility was reported, whereas B.1.351 was more resistant to multiple monoclonal antibodies (mAbs), as well as convalescent and vaccination sera. To test this hypothesis, we examined the proportion of VOC over time across different geographic areas where the two VOC, B.1.1.7 and B.1.351, co-circulate. Our comparative analysis was based on the number of SARS-CoV-2 sequences on GISAID database. We report that B.1.1.7 dominates over B.1.351 in geographic areas where both variants co-circulate and the B.1.1.7 was the first variant introduced in the population. The only areas where B.1.351 was detected at higher proportion were South Africa and Mayotte in Africa, where this strain was associated with increased community transmission before the detection of B.1.1.7. The dominance of B.1.1.7 over B.1.351 could be important since B.1.351 was more resistant to certain mAbs, as well as heterologous convalescent and vaccination sera, thus suggesting that it may be transmitted more effectively in people with pre-existing immunity to other VOC. This scenario would lessen the effectiveness of vaccine and urge the need to update them with new strains.

Koyama, T., et al. (2020). "Variant analysis of SARS-CoV-2 genomes." Bull World Health Organ **98**(7): 495-504.

Objective: To analyse genome variants of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Methods: Between 1 February and 1 May 2020, we downloaded 10 022 SARS CoV-2 genomes from four databases. The genomes were from infected patients in 68 countries. We identified variants by extracting pairwise alignment to the reference genome NC_045512, using the EMBOSS needle. Nucleotide variants in the coding regions were converted to corresponding encoded amino acid residues. For clade analysis, we used the open source software Bayesian evolutionary analysis by sampling trees, version 2.5. Findings: We identified 5775 distinct genome variants, including 2969 missense mutations, 1965 synonymous mutations, 484 mutations in the non-coding regions, 142 non-coding deletions, 100 in-frame deletions, 66 non-coding insertions, 36 stop-gained variants, 11 frameshift deletions and two in-frame insertions. The most common variants were the synonymous 3037C > T (6334 samples), P4715L in the open reading frame 1ab (6319 samples) and D614G in the spike protein (6294 samples). We identified six major clades, (that is, basal, D614G, L84S, L3606F, D448del and G392D) and 14 subclades. Regarding the base changes, the C > T mutation was the most common with 1670 distinct variants. Conclusion: We found that several variants of the SARS-CoV-2 genome exist and that the D614G clade has become the most common variant since December 2019. The evolutionary analysis indicated structured transmission, with the possibility of multiple introductions into the population.

Krafft, E., et al. (2021). "Report of One-Year Prospective Surveillance of SARS-CoV-2 in Dogs and Cats in France with Various Exposure Risks: Confirmation of a Low Prevalence of Shedding, Detection and Complete Sequencing of an Alpha Variant in a Cat." Viruses **13**(9).

Despite the probable zoonotic origin of SARS-CoV-2, only limited research efforts have been made to understand the role of companion animals in SARS-CoV-2 epidemiology. According to recent serological prevalence studies, human-to-companion animal transmission is quite frequent, which led us to consider that the risk of SARS-CoV-2 transmission from animal to human, albeit negligible in the present context, may have been underestimated. In this study, we provide the results of a prospective survey that was conducted to evaluate the SARS-CoV-2 isolation rate by qRT-PCR in dogs and cats with different exposure risks and clinical statuses. From April 2020 to April 2021, we analyzed 367 samples and investigated the presence of SARS-CoV-2 RNA using qRT-PCR. Only

four animals tested positive, all of them being cats. Three cats were asymptomatic and one presented a coryza-like syndrome. We describe in detail the infection in two cats and the associated clinical characteristics. Importantly, we obtained SARS-CoV-2 genomes from one infected animal and characterized them as Alpha variants. This represents the first identification of the SARS-CoV-2 Alpha variant in an infected animal in France.

Laine, P., et al. (2021). "SARS-CoV-2 variant with mutations in N gene affecting detection by widely used PCR primers." J Med Virol.

While most of the spontaneous mutations in the viral genome have no functional, diagnostic, or clinical consequences, some have. In February 2021, we noticed in Southern Finland coronavirus disease 2019 cases where two commercial polymerase chain reaction (PCR) analyses failed to recognize the used N gene target but recognized the other target gene of severe acute respiratory syndrome coronavirus 2. Complete viral genome sequence analysis of the strains revealed several mutations that were not found at that time in public databases. A short 3 bp deletion and three subsequent single nucleotide polymorphisms in the N gene were found exactly at the site where an early published and widely used N gene-based PCR primer is located, explaining the negative results in the N gene PCR. Later the variant strain was identified as a member of the B.1.1.318 Pango lineage that had first been found from Nigerian samples collected in January 2021. This strain shares with the Beta variant the S gene E484K mutation linked to impaired vaccine protection, but differs from this variant in several other ways, for example by deletions in the N gene region. Mutations in the N gene causing diagnostic resistance and on the other hand E484K mutation in the causing altered infectivity warrants careful inspection on virus variants that might get underdiagnosed.

Laiton-Donato, K., et al. (2021). "Characterization of the emerging B.1.621 variant of interest of SARS-CoV-2." Infect Genet Evol **95**: 105038.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genetic diversity has the potential to impact the virus transmissibility and the escape from natural infection- or vaccine-elicited neutralizing antibodies. Here, representative samples from circulating SARS-CoV-2 in Colombia between January and April 2021, were processed for genome sequencing and lineage determination following the nanopore amplicon ARTIC network protocol and PANGOLIN pipeline. This strategy allowed us to identify the emergence of the B.1.621 lineage, considered a variant of interest (VOI) with the accumulation of several substitutions affecting the Spike protein, including the amino acid changes I95I, Y144T, Y145S and the insertion 146 N in the N-terminal domain, R346K, E484K and N501Y in the Receptor binding Domain (RBD) and P681H in the S1/S2 cleavage site of the Spike protein. The rapid increase in frequency and fixation in a relatively short time in Magdalena, Atlantico, Bolivar, Bogota D.C, and Santander that were near the theoretical herd immunity suggests an epidemiologic impact. Further studies will be required to assess the biological and epidemiologic roles of the substitution pattern found in the B.1.621 lineage.

Lam, B., et al. (2021). "In vivo characterization of emerging SARS-CoV-2 variant infectivity and human antibody escape potential." Cell Rep **37**(3): 109838.

As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spreads, variants with enhanced virulence and transmissibility have emerged. Although in vitro systems allow rapid characterization, they do not fully recapitulate the dynamic interaction of virions and neutralizing antibodies in the airway. Here, we demonstrate that the N501Y variant permits respiratory infection in unmodified mice. We utilize N501Y to survey in vivo pseudovirus infection dynamics and susceptibility to reinfection with the L452R (Los Angeles), K417N + E484K (South Africa), and L452R + K417N + E484Q (India) variants. Human coronavirus disease 2019 (COVID-19)+ or vaccinated antibody isotypes, titers, variant receptor binding domain (RBD) binding, and neutralization potential are studied, revealing numerous significant correlations. Immune escape of the K417N + E484K variant is observed because infection can be appreciated in the nasopharynx, but not lungs, of mice transferred with low-antibody-tier plasma. Conversely, near-complete protection is observed in animals receiving high-antibody-tier plasma, a phenomenon that can only be appreciated in vivo.

Lamarca, A. P., et al. (2021). "Genomic Surveillance Tracks the First Community Outbreak of the SARS-CoV-2 Delta (B.1.617.2) Variant in Brazil." J Virol: JVI0122821.

As 2021 comes to a close, the advances in vaccination against COVID-19 allow the world to glimpse an end to the pandemic. In Brazil, the disease has cost more than 600,000 lives and affected more than 21 million people. When the second wave of COVID-19 hit in early 2021, the country saw more than 3,500 daily deaths. As Brazil started to recover from this number, the first reports of infection by the Delta (B.1.617.2) Variant of Concern (VoC) in the country were emerging. The first confirmed case of this variant occurred on 26 April 2021, with five states registering infections by it in the following three months. At the time, these cases were considered isolated or contained imported events. Here we describe the early phase of the first large-scale

community transmission of the Delta variant in Brazil and the associated interstate dispersal.

Lam-Hine, T., et al. (2021). "Outbreak Associated with SARS-CoV-2 B.1.617.2 (Delta) Variant in an Elementary School - Marin County, California, May-June 2021." MMWR Morb Mortal Wkly Rep **70**(35): 1214-1219.

On May 25, 2021, the Marin County Department of Public Health (MCPH) was notified by an elementary school that on May 23, an unvaccinated teacher had reported receiving a positive test result for SARS-CoV-2, the virus that causes COVID-19. The teacher reported becoming symptomatic on May 19, but continued to work for 2 days before receiving a test on May 21. On occasion during this time, the teacher read aloud unmasked to the class despite school requirements to mask while indoors. Beginning May 23, additional cases of COVID-19 were reported among other staff members, students, parents, and siblings connected to the school. To characterize the outbreak, on May 26, MCPH initiated case investigation and contact tracing that included whole genome sequencing (WGS) of available specimens. A total of 27 cases were identified, including that of the teacher. During May 23-26, among the teacher's 24 students, 22 students, all ineligible for vaccination because of age, received testing for SARS-CoV-2; 12 received positive test results. The attack rate in the two rows seated closest to the teacher's desk was 80% (eight of 10) and was 28% (four of 14) in the three back rows (Fisher's exact test; p = 0.036). During May 24-June 1, six of 18 students in a separate grade at the school, all also too young for vaccination, received positive SARS-CoV-2 test results. Eight additional cases were also identified, all in parents and siblings of students in these two grades. Among these additional cases, three were in persons fully vaccinated in accordance with CDC recommendations (1). Among the 27 total cases, 22 (81%) persons reported symptoms; the most frequently reported symptoms were fever (41%), cough (33%), headache (26%), and sore throat (26%). WGS of all 18 available specimens identified the B.1.617.2 (Delta) variant. Vaccines are effective against the Delta variant (2), but risk of transmission remains elevated among unvaccinated persons in schools without strict adherence to prevention strategies. In addition to vaccination for eligible persons, strict adherence to nonpharmaceutical prevention strategies, including masking, routine testing, facility ventilation, and staying home when symptomatic, are important to ensure safe in-person learning in schools (3).

Lascano, A. M., et al. (2020). "SARS-CoV-2 and Guillain-Barre syndrome: AIDP variant with a favourable outcome." Eur J Neurol **27**(9): 1751-1753.

BACKGROUND AND PURPOSE: The spectrum of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 infection (SARS-CoV-2), includes different neurologic manifestations of the central and peripheral nervous system. METHODS: From March through April 2020, in two university hospitals located in western Switzerland, we examined three patients with Guillain-Barre syndrome (GBS) following SARS-CoV-2. RESULTS: These cases were characterized by a primary demyelinating electrophysiological pattern (Acute inflammatory demyelinating polyneuropathy or AIDP) and a less severe disease course compared to recently published case series. Clinical improvement was observed in all patients at week five. One patient was discharged from hospital after full recovery with persistence of minor neurological signs (areflexia). Two of the three patients remained hospitalized: one was able to walk and the other could stand up with assistance. CONCLUSIONS: We report three cases of typical GBS (AIDP) occurring after SARS-CoV-2 infection and presenting with a favourable clinical course. Given the interval between COVID-19-related symptoms and neurological manifestations (mean of 15 days) we postulate a secondary immune-mediated mechanism rather than direct viral damage.

Li, P., et al. (2021). "Rapid differential diagnosis of the B.1.617.2 (delta) variant of SARS-CoV-2 using an automated Cas12a-microfluidic system." Chem Commun (Camb) **57**(92): 12270-12272.

An automated Cas12a-microfluidic system was constructed to distinguish the B.1.617.2 (delta) variant of SARS-CoV-2 from the wild-type virus rapidly and was validated using 30 clinical samples, showing 100% consistency with next-generation sequencing. It will be a potential tool for the rapid differential diagnosis of the delta variant of SARS-CoV-2.

Li, W. Y., et al. (2021). "[Epidemiological characteristics of local outbreak of COVID-19 caused by SARS-CoV-2 Delta variant in Liwan district, Guangzhou]." Zhonghua Liu Xing Bing Xue Za Zhi **42**(10): 1763-1768.

Objective: To analyze the epidemiological characteristics of a local outbreak of COVID-19 caused by SARS-CoV-2 B.1.617.2(Delta) variant in Liwan district, Guangzhou, and provide evidence for the further prevention and control of the Delta variant of COVID-19. Methods: From May 21 to June 18, 2021, the incidence data of COVID-19 caused by Delta variant were obtained from National Notifiable Disease Report System of Chinese Disease Prevention and Control Information System and Liwan District Center for Disease Control and Prevention of Guangzhou.Frequency analysis (proportions), histograms, and percentage stacked area plots were

used to describe the epidemiological characteristics of the outbreaks. The incubation period and time-varying reproduction numbers (Rt) estimations were used for the further analysis. Results: By June 18, 2021, a total of 127 COVID-19 cases caused by Delta variant was reported in Liwan district. The youngest case was aged 2 years and the oldest was aged 85 years. There were 18.9% (24/127) aged <18 years, 43.3% (55/127) aged 18-59 years, and 37.8% (48/127) aged >/=60 years, the male to female ratio of the cases was 1ratio1.35 (54ratio73). The cases were mainly retired people (32.3%, 41/127), the jobless or unemployed (18.1%, 23/127), and students (16.5%, 21/127). The infections mainly occurred in Baihedong (70.1%, 89/127) and Zhongnan street (23.6%, 30/127) communities in the southern area of Liwan district. The median incubation period of the Delta variant infection was 6 days (range: 1-15 days). The clinical classification were mainly common type (64.6%, 82/127). The basic reproduction number (R0) was 5.1, Rt which once increased to 7.3. The transmissions mainly occurred in confined spaces, such as home (26.8%), restaurant (29.1%), neighborhood (3.9%), and market (3.1%), the household clustering was predominant. Close contacts tracing (66.1%) and community screening (33.1%) were the main ways to find the infections. Conclusion: The COVID-19 outbreak caused by Delta variant in Liwan district of Guangzhou was highly contagious, with the obvious characteristics of household clustering and high proportions of cases in adults aged 18-59 years and elderly people aged >/=60 years.

Li, X. N., et al. (2021). "Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study." Emerg Microbes Infect **10**(1): 1751-1759.

The effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant, which has been associated with greater transmissibility and virulence, remains unclear. We conducted a test-negative case-control study to explore the vaccine effectiveness (VE) in real-world settings. We recruited participants aged 18-59 years who consisted of SARS-CoV-2 test-positive cases (n = 74) and test-negative controls (n = 292) during the outbreak of the Delta variant in May 2021 in Guangzhou city, China. Vaccination status was compared to estimate The VE of SARS-CoV-2 inactivated vaccines. A single dose of inactivated SARS-CoV-2 vaccine yielded the VE of only 13.8%. After adjusting for age and sex, the overall VE for two-dose vaccination was 59.0% (95% confidence interval: 16.0% to 81.6%) against coronavirus disease 2019 (COVID-19) and 70.2% (95% confidence interval: 29.6-89.3%) against moderate COVID-19 and 100% against severe COVID-19 which might be overestimated due to the small sample size. The VE of two-dose vaccination against COVID-19 reached 72.5% among participants aged 40-59 years, and was higher in females than in males against COVID-19 and moderate diseases. While single dose vaccination was not sufficiently protective, the two-dose dosing scheme of the inactivated vaccines was effective against the Delta variant infection in real-world settings, with the estimated efficacy exceeding the World Health Organization minimal threshold of 50%.

Lin, J. W., et al. (2021). "Genomic monitoring of SARS-CoV-2 uncovers an Nsp1 deletion variant that modulates type I interferon response." Cell Host Microbe **29**(3): 489-502 e488.

The SARS-CoV-2 virus, the causative agent of COVID-19, is undergoing constant mutation. Here, we utilized an integrative approach combining epidemiology, virus genome sequencing, clinical phenotyping, and experimental validation to locate mutations of clinical importance. We identified 35 recurrent variants, some of which are associated with clinical phenotypes related to severity. One variant, containing a deletion in the Nsp1-coding region (Delta500-532), was found in more than 20% of our sequenced samples and associates with higher RT-PCR cycle thresholds and lower serum IFN-beta levels of infected patients. Deletion variants in this locus were found in 37 countries worldwide, and viruses isolated from clinical samples or engineered by reverse genetics with related deletions in Nsp1 also induce lower IFN-beta responses in infected Calu-3 cells. Taken together, our virologic surveillance characterizes recurrent genetic diversity and identified mutations in Nsp1 of biological and clinical importance, which collectively may aid molecular diagnostics and drug design.

Lind, A., et al. (2021). "Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volume diagnostic laboratory." J Clin Virol **141**: 104906.

OBJECTIVES: The emerging SARS-CoV-2 variants of concern (VoC), B.1.1.7, B.1.351 and P.1, with increased transmission and/or immune evasion, emphasise the need for broad and rapid variant monitoring. Our high-volume laboratory introduced a PCR variant assay (Variant PCR) in January 2021 based on the protocol by Vogels et al. STUDY DESIGN: To assess whether Variant PCR could be used for rapid B.1.1.7, B.1.351 and P.1 screening, all positive SARS-CoV-2 airway samples were prospectively tested in parallel using both the Variant PCR and whole genome sequencing (WGS). RESULTS: In total 1,642 SARS-CoV-2 positive samples from individual patients were tested within a time span of 4 weeks. For all samples with valid results from both Variant PCR and WGS, no VoC was missed by Variant PCR (totalling 399 VoC detected). Conversely, all of the samples identified as "other

lineages" (i.e., "non-VoC lineages") by the Variant PCR, were confirmed by WGS. CONCLUSIONS: The Variant PCR based on the protocol by Vogels et al., is an effective method for rapid screening for VoC, applicable for most diagnostic laboratories within a pandemic setting. WGS is still required to confirm the identity of certain variants and for continuous surveillance of emerging VoC.

Lindstrom, J. C., et al. (2021). "Increased transmissibility of the alpha SARS-CoV-2 variant: evidence from contact tracing data in Oslo, January to February 2021." Infect Dis (Lond): 1-6.

BACKGROUND: Information about the contagiousness of new SARS-CoV-2 variants, including the alpha lineage, and how they spread in various locations is essential. Country-specific estimates are needed because local interventions influence transmissibility. METHODS: We analysed contact tracing data from Oslo municipality, reported from January through February 2021, when the alpha lineage became predominant in Norway and estimated the relative transmissibility of the alpha lineage with the use of Poisson regression. RESULTS: Within households, we found an increase in the secondary attack rate by 60% (95% CI 20-114%) among cases infected with the alpha lineage compared to other variants; including all close contacts, the relative increase in the secondary attack rate was 24% (95% CI -6%-43%). There was a significantly higher risk of infecting household members in index cases aged 40-59 years who were infected with the alpha lineage; we found no association between transmission and household size. Overall, including all close contacts, we found that the reproduction number among cases with the alpha lineage was increased by 24% (95% CI 0%-52%), corresponding to an absolute increase of 0.19, compared to the group of index cases infected with other variants. CONCLUSION: Our study suggests that households are the primary locations for rapid transmission of the new lineage alpha.

Liu, H., et al. (2021). "The Lambda variant of SARS-CoV-2 has a better chance than the Delta variant to escape vaccines." bioRxiv.

The newly emerging variants of SARS-CoV-2 from India (Delta variant) and South America (Lambda variant) have led to a higher infection rate of either vaccinated or unvaccinated people. We found that sera from Pfizer-BioNTech vaccine remain high reactivity toward the receptor binding domain (RBD) of Delta variant while it drops dramatically toward that of Lambda variant. Interestingly, the overall titer of antibodies of Pfizer-BioNTech vaccinated individuals drops 3-fold after 6 months, which could be one of major reasons for breakthrough infections, emphasizing the importance of potential third boost shot. While a therapeutic antibody, Bamlanivimab, decreases binding affinity to Delta variant by ~20 fold, it fully lost binding to Lambda variant. Structural modeling of complexes of RBD with human receptor, Angiotensin Converting Enzyme 2 (ACE2), and Bamlanivimab suggest the potential basis of the change of binding. The data suggest possible danger and a potential surge of Lambda variant in near future.

Lo Menzo, S., et al. (2021). "The first familial cluster of the B.1.1.7 variant of SARS-CoV-2 in the northeast of Italy." Infection **49**(6): 1341-1345.

PURPOSE: We report on the first identified cluster of the B.1.1.7 variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in the northeast of Italy. METHODS: The cluster was recognized in January 2021 with an epidemiological started from the hospitalization of a 68-year-old man suffering from coronavirus disease 2019 (COVID-19) related pneumonia and we surprisingly found three families involved in the same cluster. RESULTS: We retrospectively rebuilt the pathway of infection and performed a virological analysis. CONCLUSION: This allow us to make clear the very high attack rate and the great infective capacity of this B.1.1.7 variant of SARS-CoV-2.

Loconsole, D., et al. (2021). "Rapid Spread of the SARS-CoV-2 Variant of Concern 202012/01 in Southern Italy (December 2020-March 2021)." Int J Environ Res Public Health **18**(9).

Epidemiological and virological studies have revealed that SARS-CoV-2 variants of concern (VOCs) are emerging globally, including in Europe. The aim of this study was to evaluate the spread of B.1.1.7-lineage SARS-CoV-2 in southern Italy from December 2020-March 2021 through the detection of the S gene target failure (SGTF), which could be considered a robust proxy of VOC B.1.1.7. SGTF was assessed on 3075 samples from week 52/2020 to week 10/2021. A subset of positive samples identified in the Apulia region during the study period was subjected to whole-genome sequencing (WGS). A descriptive and statistical analysis of the demographic and clinical characteristics of cases according to SGTF status was performed. Overall, 20.2% of samples showed SGTF; 155 strains were confirmed as VOC 202012/01 by WGS. The proportion of SGTF-positive samples rapidly increased over time, reaching 69.2% in week 10/2021. SGTF-positive cases were more likely to be symptomatic and to result in hospitalization ($p < 0.0001$). Despite the implementation of large-scale non-pharmaceutical interventions (NPIs), such as the closure of schools and local lockdowns, a rapid spread of VOC 202012/01 was observed in southern Italy. Strengthened NPIs and rapid vaccine deployment, first among priority groups and then among the general population, are crucial both to contain the spread of

VOC 202012/01 and to flatten the curve of the third wave.

Loconsole, D., et al. (2021). "An Autochthonous Outbreak of the SARS-CoV-2 P.1 Variant of Concern in Southern Italy, April 2021." Trop Med Infect Dis **6**(3).

The SARS-CoV-2 P.1 variant of concern (VOC) was first identified in Brazil and is now spreading in European countries. It is characterized by the E484K mutation in the receptor-binding domain, which could contribute to the evasion from neutralizing antibodies. In Italy, this variant was first identified in January 2021. Here, we report an autochthonous outbreak of SARS-CoV-2 P.1 variant infections in southern Italy in subjects who had not travelled to endemic areas or outside the Apulia region. The outbreak involved seven subjects, three of whom had received a COVID-19 vaccine (one had received two doses and two had received one dose). Four patients had a mild clinical presentation. Laboratory investigations of nasopharyngeal swabs revealed that all strains were S-gene target failure-negative and molecular tests revealed they were the P.1 variant. Whole-genome sequencing confirmed that five subjects were infected with closely related strains classified as the P.1 lineage. The circulation of VOCs highlights the importance of strictly monitoring the spread of SARS-CoV-2 variants through genomic surveillance and of investigating local outbreaks. Furthermore, public health measures including social distancing, screening, and quarantine for travelers are key tools to slow down the viral transmission and to contain and mitigate the impact of VOC diffusion, and rapid scaling-up of vaccination is crucial to avoid a possible new epidemic wave.

Loenenbach, A., et al. (2021). "SARS-CoV-2 variant B.1.1.7 susceptibility and infectiousness of children and adults deduced from investigations of childcare centre outbreaks, Germany, 2021." Euro Surveill **26**(21).

We investigated three SARS-CoV-2 variant B.1.1.7 childcare centre and related household outbreaks. Despite group cohorting, cases occurred in almost all groups, i.e. also among persons without close contact. Children's secondary attack rates (SAR) were similar to adults (childcare centres: 23% vs 30%; p = 0.15; households: 32% vs 39%; p = 0.27); child- and adult-induced household outbreaks also led to similar SAR. With the advent of B.1.1.7, susceptibility and infectiousness of children and adults seem to converge. Public health measures should be revisited accordingly.

Loney, T., et al. (2021). "Genotype-phenotype correlation identified a novel SARS-CoV-2 variant possibly linked to severe disease." Transbound Emerg Dis.

The geographic location and heterogeneous multi-ethnic population of Dubai (United Arab Emirates; UAE) provide a unique setting to explore the global molecular epidemiology of SARS-CoV-2 and relationship between different viral strains and disease severity. We systematically selected (i.e. every 100th individual in the central Dubai COVID-19 database) 256 patients by age, sex, disease severity and month to provide a representative sample of laboratory-confirmed COVID-19 patients (nasopharyngeal swab PCR positive) during the first wave of the UAE outbreak (January to June 2020). Sociodemographic and clinical data were extracted from medical records and full SARS-CoV-2 genome sequences extracted from nasopharyngeal swabs were analysed. Older age was significantly associated with COVID-19-associated hospital admission and mortality. Overweight/obese or diabetic patients were 3-4 times more likely to be admitted to hospital and intensive care unit (ICU). Sequencing data showed multiple independent viral introductions into the UAE from Europe, Iran and Asia (29 January-18 March), and these early strains seeded significant clustering consistent with almost exclusive community-based transmission between April and June 2020. Majority of sequenced strains (N = 60, 52%) were from the European cluster consistent with the higher infectivity rates associated with the D614G mutation carried by most strains in this cluster. A total of 986 mutations were identified in 115 genomes, 272 were unique (majority were missense, n = 134) and 20/272 mutations were novel. A missense (Q271R) and synonymous (R41R) mutation in the S and N proteins, respectively, were identified in 2/27 patients with severe COVID-19 but not in patients with mild or moderate disease (0/86; p = .05, Fisher's Exact Test). Both patients were women (51-64 years) with no significant underlying health conditions. The same two mutations were identified in a healthy 37-year-old Indian man who was hospitalized in India due to COVID-19. Our findings provide evidence for continued community-based transmission of the European strains in the Dubai population and highlight new mutations that might be associated with severe disease in otherwise healthy adults.

Lopez, A., et al. (2021). "Testing for SARS-CoV-2 in Symptomatic Vaccinated and Unvaccinated Health Care Workers During the Delta Variant Surge." J Occup Environ Med.

BACKGROUND: Infection with SARS-CoV- 2 in health care workers (HCWs) challenges employee health services. METHODS: We analyzed telephone COVID-19 hotline data over eight weeks in 2021 during SARS- CoV- 2 Delta variant surge. We calculated COVID-19 case rates among persons-under-investigation (PUIs) for illness at two health care

centers (HCs). RESULTS: There were 41 COVID-19 cases among the 285 PUIs (14.4%) at the study HC and 549 (16.9%) of 3244 at the comparison HC. At the study HC, 11.7% of vaccinated PUIs vs. 36.6% of unvaccinated PUIs were COVID-19 positive. The COVID-19 positivity rates among vaccinated and unvaccinated PUIs at the comparison HC were 16.1% and 33.3%, respectively. DISCUSSION: In the SARS-CoV-2 Delta variant surge, COVID-19 test positivity rates among unvaccinated symptomatic HCWs are dramatically elevated. Aggressive testing of HCW PUIs is particularly critical during periods of disease upsurge.

Lubinski, B., et al. (2021). "Spike protein cleavage-activation mediated by the SARS-CoV-2 P681R mutation: a case-study from its first appearance in variant of interest (VOI) A.23.1 identified in Uganda." bioRxiv.

The African continent like all other parts of the world with high infection/low vaccination rates can, and will, be a source of novel SARS-CoV-2 variants. The A.23 viral lineage, characterized by three spike mutations F157L, V367F and Q613H, was first identified in COVID-19 cases from a Ugandan prison in July 2020, and then was identified in the general population with the additional spike mutation P681R at the S1/S2 cleavage site to comprise lineage A.23.1 by September 2020 with subsequent spread to 26 other countries. The P681R spike substitution of A.23.1 is of note as it increases the number of basic residues in the sub-optimal SARS-CoV-2 spike protein furin cleavage site; as such, this substitution may affect viral replication, transmissibility, or pathogenic properties. The same P681R substitution has also subsequently appeared in B.1.617 variants, including B.1.617.2 (Delta). Here, we performed assays using fluorogenic peptides mimicking the S1/S2 from A.23.1 and B.1.617 and observed significantly increased cleavability with furin, compared to sequences derived from the original Wuhan-Hu1 S1/S2. We performed cell-cell fusion and functional infectivity assays using pseudotyped particles harboring SARS-CoV-2 spike proteins and observed an increase in transduction for A.23.1-pseudotyped particles compared to Wuhan-Hu-1. However, these changes in activity were not reproduced in the original Wuhan-Hu-1 spike bearing only the P681R substitution. Our findings suggest that while A.23.1 has increased furin-mediated cleavage linked to the P681R substitution-which may affect viral infection and transmissibility-this substitution alone needs to occur on the background of other spike protein changes to enable its full functional consequences. Graphical abstract:

Lubinski, B., et al. (2021). "Functional evaluation of proteolytic activation for the SARS-CoV-2 variant B.1.1.7: role of the P681H mutation." bioRxiv.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the agent behind the current COVID-19 pandemic having emerged in Wuhan China in late 2019 from a yet to be determined animal reservoir. SARS-CoV-2 B.1.1.7, a variant identified in the UK in late 2020, contains a higher than typical level of point mutants across its genome, including P681H in the spike S1/S2 cleavage site. Here, we performed assays using fluorogenic peptides mimicking the S1/S2 sequence from Wuhan-Hu1 and B.1.1.7 and observed no definitive difference in furin cleavage between Wuhan-Hu1 and B.1.1.7 in vitro. We performed functional assays using pseudo-typed particles harboring SARS-CoV-2 spike proteins and observed no significant differences between Wuhan-Hu1, Wuhan-Hu1 P681H or B.1.1.7 spike-carrying pseudo-typed particles in VeroE6 or Vero-TMPRSS2 cells, despite the spikes containing P681H being more efficiently cleaved. Likewise, we or show no differences in cell-cell fusion assays using the spike P681H-expressing cells. Our findings suggest that while the introduction of P681H in the SARS-CoV-2 B.1.1.7 variant may increase spike cleavage by furin-like proteases, this does not significantly impact viral entry or cell-cell spread. We consider that other factors are at play to account for the increased in transmission and disease severity attributed to this variant of concern (VOC).

Lumley, S. F., et al. (2021). "An observational cohort study on the incidence of SARS-CoV-2 infection and B.1.1.7 variant infection in healthcare workers by antibody and vaccination status." Clin Infect Dis.

BACKGROUND: Natural and vaccine-induced immunity will play a key role in controlling the SARS-CoV-2 pandemic. SARS-CoV-2 variants have the potential to evade natural and vaccine-induced immunity. METHODS: In a longitudinal cohort study of healthcare workers (HCWs) in Oxfordshire, UK, we investigated the protection from symptomatic and asymptomatic PCR-confirmed SARS-CoV-2 infection conferred by vaccination (Pfizer-BioNTech BNT162b2, Oxford-AstraZeneca ChAdOx1 nCOV-19) and prior infection (determined using anti-spike antibody status), using Poisson regression adjusted for age, sex, temporal changes in incidence and role. We estimated protection conferred after one versus two vaccinations and from infections with the B.1.1.7 variant identified using whole genome sequencing. RESULTS: 13,109 HCWs participated; 8285 received the Pfizer-BioNTech vaccine (1407 two doses) and 2738 the Oxford-AstraZeneca vaccine (49 two doses). Compared to unvaccinated seronegative HCWs, natural immunity and two vaccination doses provided similar protection against symptomatic infection: no HCW vaccinated twice had symptomatic infection, and incidence was 98% lower in seropositive HCWs

(adjusted incidence rate ratio 0.02 [95%CI <0.01-0.18]). Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result with or without symptoms by 90% (0.10 [0.02-0.38]) and 85% (0.15 [0.08-0.26]) respectively. Single-dose vaccination reduced the incidence of symptomatic infection by 67% (0.33 [0.21-0.52]) and any PCR-positive result by 64% (0.36 [0.26-0.50]). There was no evidence of differences in immunity induced by natural infection and vaccination for infections with S-gene target failure and B.1.1.7. CONCLUSION: Natural infection resulting in detectable anti-spike antibodies and two vaccine doses both provide robust protection against SARS-CoV-2 infection, including against the B.1.1.7 variant.

Luo, C. H., et al. (2021). "Infection with the SARS-CoV-2 Delta Variant is Associated with Higher Infectious Virus Loads Compared to the Alpha Variant in both Unvaccinated and Vaccinated Individuals." medRxiv.

Background: The emerging SARS-CoV-2 variant of concern (VOC) B.1.6.17.2 (Delta) quickly displaced the B.1.1.7 (Alpha) and is associated with increases in COVID-19 cases nationally. The Delta variant has been associated with greater transmissibility and higher viral RNA loads in both unvaccinated and fully vaccinated individuals. Data is lacking regarding the infectious virus load in Delta infected individuals and how that compares to individuals infected with other SARS-CoV-2 lineages. Methods: Whole genome sequencing of 2,785 clinical isolates was used to characterize the prevalence of SARS-CoV-2 lineages circulating in the National Capital Region between January and July 2021. Clinical chart reviews were performed for the Delta, Alpha, and B.1.2 (a control predominant lineage prior to both VOCs) variants to evaluate disease severity and outcome and Cycle threshold values (Cts) were compared. The presence of infectious virus was determined using Vero-TMPRSS2 cells and anti-SARS-CoV-2 IgG levels were determined from upper respiratory specimen. An analysis of infection in unvaccinated and fully vaccinated populations was performed. Results: The Delta variant displaced the Alpha variant to constitute 88.2% of the circulating lineages in the National Capital Region by July, 2021. The Delta variant associated with increased breakthrough infections in fully vaccinated individuals that were mostly symptomatic when compared to the Alpha breakthrough infections, though it is important to note there was a significantly longer period of time between vaccination and infection with Delta infections. The recovery of infectious virus on cell culture was significantly higher with the Delta variant compared to Alpha in both vaccinated and unvaccinated groups. The impact of vaccination on reducing the recovery of infectious virus from clinical samples was only observed with Alpha variant infections but was strongly associated with low localized SARS-CoV-2 IgG for both variants. A comparison of Ct values showed a significant decrease in the Delta compared to Alpha with no significant differences between unvaccinated and vaccinated groups. Conclusions: Our data indicate that the Delta variant is associated with increased infectious virus loads when compared to the Alpha variant and decreased upper respiratory antiviral IgG levels. Measures to reduce transmission in addition to increasing vaccinations rates have to be implemented to reduce Delta variant spread. Funding: NIH/NIAID Center of Excellence in Influenza Research and Surveillance contract HHS N2772201400007C, Johns Hopkins University, Maryland department of health, Centers for Disease Control and Prevention contract 75D30121C11061.

Mandolo, J., et al. (2021). "SARS-CoV-2 exposure in Malawian blood donors: an analysis of seroprevalence and variant dynamics between January 2020 and July 2021." BMC Med **19**(1): 303.

BACKGROUND: By August 2021, the COVID-19 pandemic has been less severe in sub-Saharan Africa than elsewhere. In Malawi, there have been three subsequent epidemic waves. We therefore aimed to describe the dynamics of SARS-CoV-2 exposure in Malawi. METHODS: We measured the seroprevalence of anti-SARS-CoV-2 antibodies amongst randomly selected blood transfusion donor sera in Malawi from January 2020 to July 2021 using a cross-sectional study design. In a subset, we also assessed in vitro neutralisation against the original variant (D614G WT) and the Beta variant. RESULTS: A total of 5085 samples were selected from the blood donor database, of which 4075 (80.1%) were aged 20-49 years. Of the total, 1401 were seropositive. After adjustment for assay characteristics and applying population weights, seropositivity reached peaks in October 2020 (18.5%) and May 2021 (64.9%) reflecting the first two epidemic waves. Unlike the first wave, both urban and rural areas had high seropositivity in the second wave, Balaka (rural, 66.2%, April 2021), Blantyre (urban, 75.6%, May 2021), Lilongwe (urban, 78.0%, May 2021), and Mzuzu (urban, 74.6%, April 2021). Blantyre and Mzuzu also show indications of the start of a third pandemic wave with seroprevalence picking up again in July 2021 (Blantyre, 81.7%; Mzuzu, 71.0%). More first wave sera showed in vitro neutralisation activity against the original variant (78% [7/9]) than the beta variant (22% [2/9]), while more second wave sera showed neutralisation activity against the beta variant (75% [12/16]) than the original variant (63% [10/16]). CONCLUSION: The findings confirm extensive SARS-CoV-2 exposure in Malawi over two epidemic

waves with likely poor cross-protection to reinfection from the first on the second wave. The dynamics of SARS-CoV-2 exposure will therefore need to be taken into account in the formulation of the COVID-19 vaccination policy in Malawi and across the region. Future studies should use an adequate sample size for the assessment of neutralisation activity across a panel of SARS-CoV-2 variants of concern/interest to estimate community immunity.

Manouana, G. P., et al. (2021). "Emergence of B.1.1.318 SARS-CoV-2 viral lineage and high incidence of alpha B.1.1.7 variant of concern in the Republic of Gabon." Int J Infect Dis **114**: 151-154.

OBJECTIVE: Variants of concern (VOCs) associated with relatively high transmissibility appear to be rapidly spreading in Gabon. Therefore, it is imperative to understand the distribution of several VOCs in the population, which could have implications for transmissibility and vaccine efficacy. METHODS: Between February and May 2021, SARS-CoV-2 genomes were sequenced using the Oxford nanopore MinION method and the respective genome diversity was elucidated. Phylogenetic analysis was performed and genomes were classified using pangolin lineages. RESULTS: The results highlighted an increase (46%) in the alpha VOC (B.1.1.7) in the Gabonese population over the study period. In addition, an increase (31%) in the B.1.1.318 lineage, which is associated with high transmission and impaired vaccine efficacy (D614G+E484K+Y144del), was detected. CONCLUSION: With the second wave ongoing, these findings highlight the need for surveillance of the SARS-CoV-2 genome in the Republic of Gabon and should provide useful guidance to policymakers in selecting an appropriate vaccine for this population.

Mansbach, R. A., et al. (2020). "The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State." bioRxiv.

The COVID-19 pandemic underwent a rapid transition with the emergence of a SARS-CoV-2 variant that carried the amino acid substitution D614G in the Spike protein that became globally prevalent. The G-form is both more infectious in vitro and associated with increased viral loads in infected people. To gain insight into the mechanism underlying these distinctive characteristics, we employed multiple replicas of microsecond all-atom simulations to probe the molecular-level impact of this substitution on Spike closed and open states. The open state enables Spike interactions with its human cellular receptor, ACE2. Here we show that changes in the inter-protomer energetics due to the D614G substitution favor a higher population of infection-capable (open) states. The inter-protomer interactions between S1 and S2 subunits in the open state of the D-form are asymmetric. This asymmetry is resolved in the G-form due to the release of tensile hydrogen bonds resulting in an increased population of open conformations. Thus, the increased infectivity of the G-form is likely due to a higher rate of profitable binding encounters with the host receptor. It is also predicted to be more neutralization sensitive due to enhanced exposure of the receptor binding domain, a key target region for neutralizing antibodies.

Mansbach, R. A., et al. (2021). "The SARS-CoV-2 Spike variant D614G favors an open conformational state." Sci Adv **7**(16).

The COVID-19 (coronavirus disease 2019) pandemic underwent a rapid transition with the emergence of a dominant viral variant (from the "D-form" to the "G-form") that carried an amino acid substitution D614G in its "Spike" protein. The G-form is more infectious in vitro and is associated with increased viral loads in the upper airways. To gain insight into the molecular-level underpinnings of these characteristics, we used microsecond all-atom simulations. We show that changes in the protein energetics favor a higher population of infection-capable states in the G-form through release of asymmetry present in the D-form inter-protomer interactions. Thus, the increased infectivity of the G-form is likely due to a higher rate of profitable binding encounters with the host receptor. It is also predicted to be more neutralization sensitive owing to enhanced exposure of the receptor binding domain, a key target region for neutralizing antibodies. These results are critical for vaccine design.

Mazur-Panasiuk, N., et al. (2021). "Expansion of a SARS-CoV-2 Delta variant with an 872 nt deletion encompassing ORF7a, ORF7b and ORF8, Poland, July to August 2021." Euro Surveill **26**(39).

Routine genomic surveillance on samples from COVID-19 patients collected in Poland during summer 2021 revealed the emergence of a SARS-CoV-2 Delta variant with a large 872 nt deletion. This change, confirmed by Sanger and deep sequencing, causes complete loss of ORF7a, ORF7b, and ORF8 genes. The index case carrying the deletion is unknown. The standard pipeline for sequencing may mask this deletion with a long stretch of N's. Effects of this deletion on phenotype or immune evasion needs further study.

McBryde, E. S., et al. (2021). "Modelling direct and herd protection effects of vaccination against the SARS-CoV-2 Delta variant in Australia." Med J Aust **215**(9): 427-432.

OBJECTIVES: To analyse the outcomes of COVID-19 vaccination by vaccine type, age group eligibility, vaccination strategy, and population coverage. DESIGN: Epidemiologic modelling to assess the final size of a COVID-19 epidemic in Australia, with vaccination program (Pfizer, AstraZeneca, mixed), vaccination strategy (vulnerable first,

transmitters first, untargeted), age group eligibility threshold (5 or 15 years), population coverage, and pre-vaccination effective reproduction number (R eff v) for the SARS-CoV-2 Delta variant as factors. MAIN OUTCOME MEASURES: Numbers of SARS-CoV-2 infections; cumulative hospitalisations, deaths, and years of life lost. RESULTS: Assuming R eff v = 5, the current mixed vaccination program (vaccinating people aged 60 or more with the AstraZeneca vaccine and people under 60 with the Pfizer vaccine) will not achieve herd protection unless population vaccination coverage reaches 85% by lowering the vaccination eligibility age to 5 years. At R eff v = 3, the mixed program could achieve herd protection at 60-70% population coverage and without vaccinating 5-15-year-old children. At R eff v = 7, herd protection is unlikely to be achieved with currently available vaccines, but they would still reduce the number of COVID-19-related deaths by 85%. CONCLUSION: Vaccinating vulnerable people first is the optimal policy when population vaccination coverage is low, but vaccinating more socially active people becomes more important as the R eff v declines and vaccination coverage increases. Assuming the most plausible R eff v of 5, vaccinating more than 85% of the population, including children, would be needed to achieve herd protection. Even without herd protection, vaccines are highly effective in reducing the number of deaths.

McCallum, M., et al. (2021). "SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern." Science **373**(6555): 648-654.

A novel variant of concern (VOC) named CAL.20C (B.1.427/B.1.429), which was originally detected in California, carries spike glycoprotein mutations S13I in the signal peptide, W152C in the N-terminal domain (NTD), and L452R in the receptor-binding domain (RBD). Plasma from individuals vaccinated with a Wuhan-1 isolate-based messenger RNA vaccine or from convalescent individuals exhibited neutralizing titers that were reduced 2- to 3.5-fold against the B.1.427/B.1.429 variant relative to wild-type pseudoviruses. The L452R mutation reduced neutralizing activity in 14 of 34 RBD-specific monoclonal antibodies (mAbs). The S13I and W152C mutations resulted in total loss of neutralization for 10 of 10 NTD-specific mAbs because the NTD antigenic supersite was remodeled by a shift of the signal peptide cleavage site and the formation of a new disulfide bond, as revealed by mass spectrometry and structural studies.

McCallum, M., et al. (2021). "SARS-CoV-2 immune evasion by variant B.1.427/B.1.429." bioRxiv.

SARS-CoV-2 entry is mediated by the spike (S) glycoprotein which contains the receptor-binding domain (RBD) and the N-terminal domain (NTD) as the two main targets of neutralizing antibodies (Abs). A novel variant of concern (VOC) named CAL.20C (B.1.427/B.1.429) was originally detected in California and is currently spreading throughout the US and 29 additional countries. It is unclear whether antibody responses to SARS-CoV-2 infection or to the prototypic Wuhan-1 isolate-based vaccines will be impacted by the three B.1.427/B.1.429 S mutations: S13I, W152C and L452R. Here, we assessed neutralizing Ab responses following natural infection or mRNA vaccination using pseudoviruses expressing the wildtype or the B.1.427/B.1.429 S protein. Plasma from vaccinated or convalescent individuals exhibited neutralizing titers, which were reduced 3-6 fold against the B.1.427/B.1.429 variant relative to wildtype pseudoviruses. The RBD L452R mutation reduced or abolished neutralizing activity of 14 out of 35 RBD-specific monoclonal antibodies (mAbs), including three clinical-stage mAbs. Furthermore, we observed a complete loss of B.1.427/B.1.429 neutralization for a panel of mAbs targeting the N-terminal domain due to a large structural rearrangement of the NTD antigenic supersite involving an S13I-mediated shift of the signal peptide cleavage site. These data warrant closer monitoring of signal peptide variants and their involvement in immune evasion and show that Abs directed to the NTD impose a selection pressure driving SARS-CoV-2 viral evolution through conventional and unconventional escape mechanisms.

Medkour, H., et al. (2021). "First evidence of human-to-dog transmission of SARS-CoV-2 B.1.160 variant in France." Transbound Emerg Dis.

Since the start of the coronavirus disease of 2019 (COVID-19) pandemic, several episodes of human-to-animal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission have been described in different countries. The role of pets, especially domestic dogs, in the COVID-19 epidemiology is highly questionable and needs further investigation. In this study, we report a case of COVID-19 in a French dog living in close contact with its owners who were COVID-19 patients. The dog presented rhinitis and was sampled 1 week after its owners (a man and a woman) were tested positive for COVID-19. The nasal swabs for the dog tested remained positive for SARS-CoV-2 by reverse transcription quantitative real-time PCR (RT-qPCR) 1 month following the first diagnosis. Specific anti-SARS-CoV-2 antibodies were detectable 12 days after the first diagnosis and persisted for at least 5 months as tested using enzyme-linked immunoassay (ELISA) and automated western blotting. The whole-genome sequences from the dog and its owners were 99%-100% identical (with the man and the woman's sequences, respectively) and matched the B.1.160 variant of concern (Marseille-4 variant), the most widespread in France at the time the dog was infected.

This study documents the first detection of B.1.160 in pets (a dog) in France, and the first canine genome recovery of the B.1.160 variant of global concern. Moreover, given the enhanced infectivity and transmissibility of the Marseille-4 variant for humans, this case also highlights the risk that pets may potentially play a significant role in SARS-CoV-2 outbreaks and may transmit the infection to humans. We have evidence of human-to-dog transmission of the Marseille-4 variant since the owners were first to be infected. Finally, owners and veterinarians must be vigilent for canine COVID-19 when dogs are presented with respiratory clinical signs.

Meng, B., et al. (2021). "Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7." Cell Rep **35**(13): 109292.

We report severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike DeltaH69/V70 in multiple independent lineages, often occurring after acquisition of receptor binding motif replacements such as N439K and Y453F, known to increase binding affinity to the ACE2 receptor and confer antibody escape. In vitro, we show that, although DeltaH69/V70 itself is not an antibody evasion mechanism, it increases infectivity associated with enhanced incorporation of cleaved spike into virions. DeltaH69/V70 is able to partially rescue infectivity of spike proteins that have acquired N439K and Y453F escape mutations by increased spike incorporation. In addition, replacement of the H69 and V70 residues in the Alpha variant B.1.1.7 spike (where DeltaH69/V70 occurs naturally) impairs spike incorporation and entry efficiency of the B.1.1.7 spike pseudotyped virus. Alpha variant B.1.1.7 spike mediates faster kinetics of cell-cell fusion than wild-type Wuhan-1 D614G, dependent on DeltaH69/V70. Therefore, as DeltaH69/V70 compensates for immune escape mutations that impair infectivity, continued surveillance for deletions with functional effects is warranted.

Meyer, M., et al. (2021). "The Alpha Variant (B.1.1.7) of SARS-CoV-2 in Children: First Experience from 3544 Nucleic Acid Amplification Tests in a Cohort of Children in Germany." Viruses **13**(8).

In May 2021, the Alpha variant (B.1.1.7) of SARS-CoV-2 was found in 91% of the SARS-CoV-2 cases in Germany. Not much is known about the symptoms, courses of disease, and infectiousness in pediatric patients with the Alpha variant. OBJECTIVE: The aim of this retrospective analysis was to gain information on the infection with the Alpha variant in children and adolescents. METHODS: Between 12 January 2021 and 3 June 2021, all nucleic acid amplification tests (NAATs) of children who received a swab for SARS-CoV-2 were included. Data were collected on standardized questionnaires. The analysis of data was anonymized and retrospective. RESULTS: We investigated 3544 NAATs; 95 children were tested positive (2.7%) for SARS-CoV-2. For the sub-analysis, 65 children were analyzed. In 59 children, the Alpha variant was found (90.8%), and 54.2% (n = 32/59) were symptomatic. The most common symptoms were fever, cough, and rhinitis. The median Ct value was 24.0 (min 17.0; max 32.7). CONCLUSIONS: We can underline early findings that children are still less effected by SARS-CoV-2 infection with the spread of the Alpha variant. We found no evidence that children infected with the Alpha variant showed more severe symptoms or suffered from a more severe clinical course than those infected with the wild type.

Mhatre, S., et al. (2021). "Entry-inhibitory role of catechins against SARS-CoV-2 and its UK variant." Comput Biol Med **135**: 104560.

BACKGROUND: The global pandemic caused by a RNA virus capable of infecting humans and animals, has resulted in millions of deaths worldwide. Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infects the lungs, and the gastrointestinal tract to some extent. Rapid structural mutations have increased the virulence and infectivity of the virus drastically. One such mutated strain known as the UK variant has caused many deaths in the United Kingdom. HYPOTHESIS: Among several indigenous natural ingredients used for prevention and cure of many diseases, the catechins have been reported for their antiviral activity, even against SARS-CoV-2. Characteristic mutations present on the spike protein have presented the newer strain its enhanced infectivity. The spike protein helps the virus bind to ACE2 receptor of the host cell and hence is a drug target. Catechins have been reported for their entry-inhibitory activity against several viruses. METHOD: In this study, we performed molecular docking of different catechins with the wild and mutant variants of the spike protein of SARS-CoV-2. The stability of the best docked complexes was validated using molecular dynamics simulation. RESULTS: The in-silico studies show that the catechins form favourable interactions with the spike protein and can potentially impair its function. Epigallocatechin gallate (EGCG) showed the best binding among the catechins against both the strains. Both the protein-ligand complexes were stable throughout the simulation time frame. CONCLUSION: The outcomes should encourage further exploration of the antiviral activity of EGCG against SARS-CoV-2 and its variants.

Mishra, S., et al. (2021). "Changing composition of SARS-CoV-2 lineages and rise of Delta variant in England." EClinicalMedicine **39**: 101064.

Background: Since its emergence in Autumn 2020, the SARS-CoV-2 Variant of Concern (VOC) B.1.1.7 (WHO label Alpha) rapidly became the

dominant lineage across much of Europe. Simultaneously, several other VOCs were identified globally. Unlike B.1.1.7, some of these VOCs possess mutations thought to confer partial immune escape. Understanding when and how these additional VOCs pose a threat in settings where B.1.1.7 is currently dominant is vital. Methods: We examine trends in the prevalence of non-B.1.1.7 lineages in London and other English regions using passive-case detection PCR data, cross-sectional community infection surveys, genomic surveillance, and wastewater monitoring. The study period spans from 31st January 2021 to 15th May 2021. Findings: Across data sources, the percentage of non-B.1.1.7 variants has been increasing since late March 2021. This increase was initially driven by a variety of lineages with immune escape. From mid-April, B.1.617.2 (WHO label Delta) spread rapidly, becoming the dominant variant in England by late May. Interpretation: The outcome of competition between variants depends on a wide range of factors such as intrinsic transmissibility, evasion of prior immunity, demographic specificities and interactions with non-pharmaceutical interventions. The presence and rise of non-B.1.1.7 variants in March likely was driven by importations and some community transmission. There was competition between non-B.1.17 variants which resulted in B.1.617.2 becoming dominant in April and May with considerable community transmission. Our results underscore that early detection of new variants requires a diverse array of data sources in community surveillance. Continued real-time information on the highly dynamic composition and trajectory of different SARS-CoV-2 lineages is essential to future control efforts. Funding: National Institute for Health Research, Medicines and Healthcare products Regulatory Agency, DeepMind, EPSRC, EA Funds programme, Open Philanthropy, Academy of Medical Sciences Bill,Melinda Gates Foundation, Imperial College Healthcare NHS Trust, The Novo Nordisk Foundation, MRC Centre for Global Infectious Disease Analysis, Community Jameel, Cancer Research UK, Imperial College COVID-19 Research Fund, Medical Research Council, Wellcome Sanger Institute.

Mitchell, P. K., et al. (2021). "SARS-CoV-2 B.1.1.7 Variant Infection in Malayan Tigers, Virginia, USA." Emerg Infect Dis **27**(12): 3171-3173.

We report infection of 3 Malayan tigers with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) B.1.1.7 (Alpha) variant at a zoologic park in Virginia, USA. All tigers exhibited respiratory signs consistent with SARS-CoV-2 infection. These findings show that tigers are susceptible to infection with the SARS-CoV-2 B.1.1.7 variant.

Mlcochova, P., et al. (2020). "Combined Point-of-Care Nucleic Acid and Antibody Testing for SARS-CoV-2 following Emergence of D614G Spike Variant." Cell Rep Med **1**(6): 100099.

Rapid COVID-19 diagnosis in the hospital is essential, although this is complicated by 30%-50% of nose/throat swabs being negative by SARS-CoV-2 nucleic acid amplification testing (NAAT). Furthermore, the D614G spike mutant dominates the pandemic and it is unclear how serological tests designed to detect anti-spike antibodies perform against this variant. We assess the diagnostic accuracy of combined rapid antibody point of care (POC) and nucleic acid assays for suspected COVID-19 disease due to either wild-type or the D614G spike mutant SARS-CoV-2. The overall detection rate for COVID-19 is 79.2% (95% CI 57.8-92.9) by rapid NAAT alone. The combined point of care antibody test and rapid NAAT is not affected by D614G and results in very high sensitivity for COVID-19 diagnosis with very high specificity.

Mlcochova, P., et al. (2021). "SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion." Nature **599**(7883): 114-119.

The B.1.617.2 (Delta) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in the state of Maharashtra in late 2020 and spread throughout India, outcompeting pre-existing lineages including B.1.617.1 (Kappa) and B.1.1.7 (Alpha)(1). In vitro, B.1.617.2 is sixfold less sensitive to serum neutralizing antibodies from recovered individuals, and eightfold less sensitive to vaccine-elicited antibodies, compared with wild-type Wuhan-1 bearing D614G. Serum neutralizing titres against B.1.617.2 were lower in ChAdOx1 vaccinees than in BNT162b2 vaccinees. B.1.617.2 spike pseudotyped viruses exhibited compromised sensitivity to monoclonal antibodies to the receptor-binding domain and the amino-terminal domain. B.1.617.2 demonstrated higher replication efficiency than B.1.1.7 in both airway organoid and human airway epithelial systems, associated with B.1.617.2 spike being in a predominantly cleaved state compared with B.1.1.7 spike. The B.1.617.2 spike protein was able to mediate highly efficient syncytium formation that was less sensitive to inhibition by neutralizing antibody, compared with that of wild-type spike. We also observed that B.1.617.2 had higher replication and spike-mediated entry than B.1.617.1, potentially explaining the B.1.617.2 dominance. In an analysis of more than 130 SARS-CoV-2-infected health care workers across three centres in India during a period of mixed lineage circulation, we observed reduced ChAdOx1 vaccine effectiveness against B.1.617.2 relative to non-B.1.617.2, with the caveat of possible residual confounding. Compromised vaccine efficacy against the highly fit and immune-evasive B.1.617.2

Delta variant warrants continued infection control measures in the post-vaccination era.

Mohammad, A., et al. (2021). "Structural modelling of SARS-CoV-2 alpha variant (B.1.1.7) suggests enhanced furin binding and infectivity." Virus Res **303**: 198522.

The B.1.1.7 SARS-CoV-2 strain that has emerged in the UK in early December presents seven mutations and three deletions on S-protein structure that could lead to a more infective strain. The P681H mutation in the "PRRAR" furin cleavage site might affect the binding affinity to furin enzyme and hence its infectivity. Therefore, in this study, various structural bioinformatics approaches were used to model the S-protein structure with the B.1.1.7 variant amino acid substitutions and deletions. In addition to modelling the binding of furin to the cleavage site of the wild-type and the B.1.1.7 variant. Conclusively the B.1.1.7 variant resulted in dynamic stability, conformational changes and variations in binding energies in the S-protein structure, resulting in a more favourable binding of furin enzyme to the SARS-CoV-2 S-protein.

Mohandas, S., et al. (2021). "Comparison of SARS-CoV-2 Variants of Concern 202012/01 (U.K. Variant) and D614G Variant Transmission by Different Routes in Syrian Hamsters." Vector Borne Zoonotic Dis **21**(8): 638-641.

Introduction: Many SARS-CoV-2 variants of concern (VOC) have been reported recently that were linked to increased transmission. In our earlier study using VOC 202012/01 (U.K. variant) and D614G variant in the hamster model, we observed higher viral RNA shedding through nasal wash in the case of U.K. variant with lower pathogenicity in lung. In this study, we have studied transmission of these two variants by direct contact, aerosol, and fomite routes in Syrian hamsters and compared the viral load and body weight changes in hamsters exposed by both variants to understand the transmission efficiency. Methods: Nasal, throat, and rectal swabs were collected sequentially to assess viral load till 14 days. Results: Transmission could be established by direct, aerosol, and fomite contact in Syrian hamsters. Body weight loss or viral load in the contact animals exposed did not show any statistical significance. Conclusion: The study demonstrated comparable transmission of both U.K. and D614G variants of SARS-CoV-2 in Syrian hamsters in the given conditions. Provided these data, it seems that all the routes of exposure are effective leading to higher transmission.

Mohandas, S., et al. (2021). "SARS-CoV-2 Delta Variant Pathogenesis and Host Response in Syrian Hamsters." Viruses **13**(9).

B.1.617 is becoming a dominant Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) lineage worldwide with many sublineages, of which B.1.617.2 is designated as a variant of concern. The pathogenicity of B.1.617.2 (Delta) and B.1.617.3 lineage of SARS-CoV-2 was evaluated and compared with that of B.1, an early virus isolate with D614G mutation in a Syrian hamster model. Viral load, antibody response, and lung disease were studied. There was no significant difference in the virus shedding pattern among these variants. High levels of SARS-CoV-2 sub genomic RNA were detected in the respiratory tract of hamsters infected with the Delta variant for 14 days, which warrants further transmission studies. The Delta variant induced lung disease of moderate severity in about 40% of infected animals, which supports the attributed disease severity of the variant. Cross neutralizing antibodies were detected in animals infected with B.1, Delta, and B.1.617.3 variant, but neutralizing capacity was significantly lower with B.1.351 (Beta variant).

Mok, B. W., et al. (2021). "Low dose inocula of SARS-CoV-2 Alpha variant transmits more efficiently than earlier variants in hamsters." Commun Biol **4**(1): 1102.

Emerging variants of SARS-CoV-2 have been shown to rapidly replace original circulating strains in humans soon after they emerged. There is a lack of experimental evidence to explain how these natural occurring variants spread more efficiently than existing strains of SARS-CoV-2 in transmission. We found that the Alpha variant (B.1.1.7) increased competitive fitness over earlier parental D614G lineages in in-vitro and in-vivo systems. Using hamster transmission model, we further demonstrated that the Alpha variant is able to replicate and shed more efficiently in the nasal cavity of hamsters than other variants with low dose and short duration of exposure. The capability to initiate effective infection with low inocula may be one of the key factors leading to the rapid transmission of emerging variants of SARS-CoV-2.

Molenkamp, R., et al. (2021). "Supplementing SARS-CoV-2 genomic surveillance with PCR-based variant detection for real-time actionable information, the Netherlands, June to July 2021." Euro Surveill **26**(40).

We evaluated routine testing with SARS-CoV-2 Delta variant-specific RT-PCR in regional hospital laboratories in addition to centralised national genomic surveillance in the Netherlands during June and July 2021. The increase of the Delta variant detected by RT-PCR correlated well with data from genomic surveillance and was available ca 2 weeks earlier. This rapid identification of the relative abundance and increase of SARS-CoV-2 variants of concern may have important benefits for implementation of local public health measures.

Mor, O., et al. (2021). "The Rise and Fall of a Local SARS-CoV-2 Variant with the Spike Protein Mutation L452R." Vaccines (Basel) **9**(8).

Emerging SARS-CoV-2 variants may threaten global vaccination efforts and the awaited reduction in outbreak burden. In this study, we report a novel variant carrying the L452R mutation that emerged from a local B.1.362 lineage, B.1.362+L452R. The L452R mutation is associated with the Delta and Epsilon variants and was shown to cause increased infection and reduction in neutralization in pseudoviruses. Indeed, the B.1.362+L452R variant demonstrated a X4-fold reduction in neutralization capacity of sera from BNT162b2-vaccinated individuals compared to a wild-type strain. The variant infected 270 individuals in Israel between December 2020 and March 2021, until diminishing due to the gain in dominance of the Alpha variant in February 2021. This study demonstrates an independent, local emergence of a variant carrying a critical mutation, L452R, which may have the potential of becoming a variant of concern and emphasizes the importance of routine surveillance and detection of novel variants among efforts undertaken to prevent further disease spread.

Mor, O., et al. (2021). "BNT162b2 vaccine effectiveness was marginally affected by the SARS-CoV-2 beta variant in fully vaccinated individuals." J Clin Epidemiol **142**: 38-44.

OBJECTIVE: To evaluate the effectiveness of the Pfizer BNT162b2 vaccine against the SARS-Cov-2 Beta variant. STUDY DESIGN AND SETTING: Israel's mass vaccination program, using two doses of the Pfizer BNT162b2 vaccine, successfully curtailed the Alpha variant outbreak during winter 2020-2021, However, the virus may mutate and partially evade the immune system. To monitor this, sequencing of selected positive swab samples of interest was initiated. Comparing vaccinated with unvaccinated PCR positive persons, we estimated the odds ratio for a vaccinated case to have the Beta vs. the Alpha variant, using logistic regression, controlling for important confounders. RESULTS: There were 19 cases of Beta variant (3.2%) among those vaccinated more than 14 days before the positive sample and 79 (3.4%) among the unvaccinated. The estimated odds ratio was 1.26 (95% CI: 0.65-2.46). Assuming the effectiveness against the Alpha variant to be 95%, the estimated effectiveness against the Beta variant was 94% (95% CI: 88%-98%). CONCLUSION: Despite concerns over the Beta variant, the BNT162b2 vaccine seemed to provide substantial immunity against both the Beta and the Alpha variants. From 14 days following the second vaccine dose, the effectiveness of BNT162b2 vaccine was at most marginally affected by the Beta variant.

Moss, D. L. and J. Rappaport (2021). "SARS-CoV-2 beta variant substitutions alter spike glycoprotein receptor binding domain structure and stability." J Biol Chem **297**(6): 101371.

The emergence of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) and the subsequent COVID-19 pandemic have visited a terrible cost on the world in the forms of disease, death, and economic turmoil. The rapid development and deployment of extremely effective vaccines against SARS-CoV-2 have seemingly brought within reach the end of the pandemic. However, the virus has acquired mutations. and emerging variants of concern are more infectious and reduce the efficacy of existing vaccines. Although promising efforts to combat these variants are underway, the evolutionary pressures leading to these variants are poorly understood. To that end, here we have studied the effects on the structure and function of the SARS-CoV-2 spike glycoprotein receptor-binding domain of three amino-acid substitutions found in several variants of concern, including alpha (B.1.1.7), beta (B.1.351), and gamma (P.1). We found that these substitutions alter the receptor-binding domain structure, stability, and ability to bind to angiotensin converting enzyme 2, in such a way as to possibly have opposing and compensatory effects. These findings provide new insights into how these variants of concern may have been selected for infectivity while maintaining the structure and stability of the receptor binding domain.

Motozono, C., et al. (2021). "SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity." Cell Host Microbe **29**(7): 1124-1136 e1111.

Many SARS-CoV-2 variants with naturally acquired mutations have emerged. These mutations can affect viral properties such as infectivity and immune resistance. Although the sensitivity of naturally occurring SARS-CoV-2 variants to humoral immunity has been investigated, sensitivity to human leukocyte antigen (HLA)-restricted cellular immunity remains largely unexplored. Here, we demonstrate that two recently emerging mutations in the receptor-binding domain of the SARS-CoV-2 spike protein, L452R (in B.1.427/429 and B.1.617) and Y453F (in B.1.1.298), confer escape from HLA-A24-restricted cellular immunity. These mutations reinforce affinity toward the host entry receptor ACE2. Notably, the L452R mutation increases spike stability, viral infectivity, viral fusogenicity, and thereby promotes viral replication. These data suggest that HLA-restricted cellular immunity potentially affects the evolution of viral phenotypes and that a further threat of the SARS-CoV-2 pandemic is escape from cellular immunity.

Mujwar, S. (2021). "Computational repurposing of tamibarotene against triple mutant variant of SARS-CoV-2." Comput Biol Med **136**: 104748.

The outbreak of the triple mutant strain of severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) was more virulent and pathogenic than

its original strain. The viral triple mutant strain of SARS-COV-2 is extremely adaptive and increases penetrability into the host. The triple mutant viral strain was first reported in Brazil and South Africa and then communicated to different countries responsible for the second wave of the coronavirus disease (COVID-19) global pandemic with a high mortality rate. The reported genomic mutations are responsible for the alterations in the viral functional and structural proteins, causing the ineffectiveness of the existing antiviral therapy targeting these proteins. Thus, in current research, molecular docking simulation-based virtual screening of a ligand library consisting of FDA-approved existing drugs followed by molecular dynamics simulation-based validation of leads was performed to develop a potent inhibitor molecule for the triple mutant viral strain SARS-CoV-2. Based on the safety profile, tamibarotene was selected as a safe and effective drug candidate for developing therapy against the triple mutant viral spike protein of SARS-CoV-2.

Muller, K., et al. (2021). "Emerging SARS-CoV-2 variant B.1.1.7 reduces neutralisation activity of antibodies against wild-type SARS-CoV-2." J Clin Virol **142**: 104912.

Spike-specific antibodies contribute significantly to the neutralising activity against SARS-CoV-2 and are important for the therapeutic effect of convalescent plasma. B.1.1.7 is a recently emerged variant of SARS-CoV-2 that has several mutations in the gene encoding for the spike-protein. To assess the potential effect these mutations could have on the neutralising efficacy of antibodies, we evaluated 96 serum samples from convalescent plasma donors collected before the first occurrence of B.1.1.7 and tested their neutralising effect on wild-type SARS-CoV-2 and B.1.1.7. We found that B.1.1.7 is more resistant to neutralisation by convalescent plasma from patients infected with wild-type SARS-CoV-2 with an overall decrease in neutralising activity of 47.7%. Thus, the neutralising effect of convalescent plasma should be determined against the major circulating virus clades whenever possible to ensure the best possible therapeutic effect.

Munitz, A., et al. (2021). "BNT162b2 vaccination effectively prevents the rapid rise of SARS-CoV-2 variant B.1.1.7 in high-risk populations in Israel." Cell Rep Med **2**(5): 100264.

Since the emergence of the SARS-CoV-2 pandemic, various genetic variants have been described. The B.1.1.7 variant, which emerged in England during December 2020, is associated with increased infectivity. Therefore, its pattern of spread is of great importance. The Israeli government established three national programs: massive RT-PCR testing, focused surveillance in nursing homes, and robust prioritized vaccination with BNT162b2. To define the impact of the aforementioned programs, we analyze data from approximately 300,000 RT-PCR samples collected from December 6, 2020, to February 10, 2021. We reveal that the B.1.1.7 is 45% (95% confidence interval [CI]: 20%-60%) more transmissible than the wild-type strain and has become the dominant strain in Israel within 3.5 weeks. Despite the rapid increase in viral spread, focused RT-PCR testing and prioritized vaccination programs are capable of preventing the spread of the B.1.1.7 variant in the elderly. Therefore, proactive surveillance programs, combined with prioritized vaccination, are achievable and can reduce severe illness and subsequent death.

Mutnal, M. B., et al. (2021). "Surveillance genome sequencing reveals multiple SARS-CoV-2 variants circulating in central Texas, USA, with a predominance of delta variant and review of vaccine breakthrough cases." J Med Virol.

As surges in the COVID-19 pandemic have continued worldwide, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has mutated, spawning several new variants, and impacting, to various degrees, transmission, disease severity, diagnostics, therapeutics, and natural and vaccine-induced immunity. Baylor Scott & White Health has implemented, along with laboratory diagnosis, SARS-CoV-2 sequencing to identify variants in its geographical service area. We analyzed virus sequencing results of specimens collected across Central Texas and found dramatic changes in variant distribution in the first half of 2021. The alpha variant (B 1.1.7) became predominant at week 13 and continued dominance until week 25. A growth rate of 1.20 (R(2) = 0.92) for the first 15 weeks was noted and this growth gradually declined to -0.55 (R(2) = 0.99) for the final 13 weeks. Currently, B.1.1.7 is being displaced with B.1.617.2 at a 0.58 growth rate (R(2) = 0.97). We also investigated vaccine breakthrough cases (VBCs) within our healthcare system and present clinical data on 28 symptomatic patients.

Mwenda, M., et al. (2021). "Detection of B.1.351 SARS-CoV-2 Variant Strain - Zambia, December 2020." MMWR Morb Mortal Wkly Rep **70**(8): 280-282.

The first laboratory-confirmed cases of coronavirus disease 2019 (COVID-19), the illness caused by SARS-CoV-2, in Zambia were detected in March 2020 (1). Beginning in July, the number of confirmed cases began to increase rapidly, first peaking during July-August, and then declining in September and October (Figure). After 3 months of relatively low case counts, COVID-19 cases began rapidly rising throughout the country in mid-December. On December 18, 2020, South Africa published the

genome of a SARS-CoV-2 variant strain with several mutations that affect the spike protein (2). The variant included a mutation (N501Y) associated with increased transmissibility.(dagger)(,)(section sign) SARS-CoV-2 lineages with this mutation have rapidly expanded geographically.(paragraph sign)(,)** The variant strain (PANGO [Phylogenetic Assignment of Named Global Outbreak] lineage B.1.351(daggerdagger)) was first detected in the Eastern Cape Province of South Africa from specimens collected in early August, spread within South Africa, and appears to have displaced the majority of other SARS-CoV-2 lineages circulating in that country (2). As of January 10, 2021, eight countries had reported cases with the B.1.351 variant. In Zambia, the average number of daily confirmed COVID-19 cases increased 16-fold, from 44 cases during December 1-10 to 700 during January 1-10, after detection of the B.1.351 variant in specimens collected during December 16-23. Zambia is a southern African country that shares substantial commerce and tourism linkages with South Africa, which might have contributed to the transmission of the B.1.351 variant between the two countries.

Nagy-Szakal, D., et al. (2021). "Targeted Hybridization Capture of SARS-CoV-2 and Metagenomics Enables Genetic Variant Discovery and Nasal Microbiome Insights." Microbiol Spectr **9**(2): e0019721.

The emergence of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genetic variants that may alter viral fitness highlights the urgency of widespread next-generation sequencing (NGS) surveillance. To profile genetic variants of the entire SARS-CoV-2 genome, we developed and clinically validated a hybridization capture SARS-CoV-2 NGS assay, integrating novel methods for panel design using double-stranded DNA (dsDNA) biotin-labeled probes, and built accompanying software. This test is the first hybrid capture-based NGS assay given Food and Drug Administration (FDA) emergency use authorization for detection of the SARS-CoV-2 virus. The positive and negative percent agreement (PPA and NPA, respectively) were defined in comparison to the results for an orthogonal real-time reverse transcription polymerase chain reaction (RT-PCR) assay (PPA and NPA, 96.7 and 100%, respectively). The limit of detection was established to be 800 copies/ml with an average fold enrichment of 46,791. Furthermore, utilizing the research-use-only analysis to profile the variants, we identified 55 novel mutations, including 11 in the functionally important spike protein. Finally, we profiled the full nasopharyngeal microbiome using metagenomics and found overrepresentation of 7 taxa and evidence of macrolide resistance in SARS-CoV-2-positive patients. This hybrid capture NGS assay, coupled with optimized software, is a powerful approach to detect and comprehensively map SARS-CoV-2 genetic variants for tracking viral evolution and guiding vaccine updates. IMPORTANCE This is the first FDA emergency-use-authorized hybridization capture-based next-generation sequencing (NGS) assay to detect the SARS-CoV-2 genome. Viral metagenomics and the novel hybrid capture NGS-based assay, along with its research-use-only analysis, can provide important genetic insights into SARS-CoV-2 and other emerging pathogens and improve surveillance and early detection, potentially preventing or mitigating new outbreaks. Better understanding of the continuously evolving SARS-CoV-2 viral genome and the impact of genetic variants may provide individual risk stratification, precision therapeutic options, improved molecular diagnostics, and population-based therapeutic solutions.

Nakaya, A., et al. (2021). "Red face may be a specific sign of SARS-CoV-2 alpha variant." IDCases **25**: e01214.

Japan is currently suffering the fourth wave of the COVID-19 pandemic, with the dominant type being SARS-CoV-2 alpha variant. Patients with COVID-19 variant types show more aggressive symptoms. In the present study, three patients developed a red face during treatment. Two of them suddenly worsened shortly after. We assumed that the red face reflected a cytokine storm and conjectured that it may be a specific sign of variant type COVID-19, because we have never seen it in patients with non-variant type. Moreover, we believe that red face may be predictive of a sudden deterioration.

Nanduri, S., et al. (2021). "Effectiveness of Pfizer-BioNTech and Moderna Vaccines in Preventing SARS-CoV-2 Infection Among Nursing Home Residents Before and During Widespread Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant - National Healthcare Safety Network, March 1-August 1, 2021." MMWR Morb Mortal Wkly Rep **70**(34): 1163-1166.

Nursing home and long-term care facility residents live in congregate settings and are often elderly and frail, putting them at high risk for infection with SARS-CoV-2, the virus that causes COVID-19, and severe COVID-19-associated outcomes; therefore, this population was prioritized for early vaccination in the United States (1). Following rapid distribution and administration of the mRNA COVID-19 vaccines (Pfizer-BioNTech and Moderna) under an Emergency Use Authorization by the Food and Drug Administration (2), observational studies among nursing home residents demonstrated vaccine effectiveness (VE) ranging from 53% to 92% against SARS-CoV-2 infection (3-6). However, concerns about the potential for waning vaccine-induced immunity and the recent emergence of the highly transmissible SARS-CoV-2 B.1.617.2 (Delta) variant(dagger) highlight the need to continue to monitor VE (7).

Weekly data reported by the Centers for Medicaid & Medicare (CMS)-certified skilled nursing facilities or nursing homes to CDC's National Healthcare Safety Network (NHSN)(section sign) were analyzed to evaluate effectiveness of full vaccination (2 doses received >/=14 days earlier) with any of the two currently authorized mRNA COVID-19 vaccines during the period soon after vaccine introduction and before the Delta variant was circulating (pre-Delta [March 1-May 9, 2021]), and when the Delta variant predominated(paragraph sign) (Delta [June 21-August 1, 2021]). Using 17,407 weekly reports from 3,862 facilities from the pre-Delta period, adjusted effectiveness against infection for any mRNA vaccine was 74.7% (95% confidence interval [CI] = 70.0%-78.8%). Analysis using 33,160 weekly reports from 11,581 facilities during an intermediate period (May 10-June 20) found that the adjusted effectiveness was 67.5% (95% CI = 60.1%-73.5%). Analysis using 85,593 weekly reports from 14,917 facilities during the Delta period found that the adjusted effectiveness was 53.1% (95% CI = 49.1%-56.7%). Effectiveness estimates were similar for Pfizer-BioNTech and Moderna vaccines. These findings indicate that mRNA vaccines provide protection against SARS-CoV-2 infection among nursing home residents; however, VE was lower after the Delta variant became the predominant circulating strain in the United States. This analysis assessed VE against any infection, without being able to distinguish between asymptomatic and symptomatic presentations. Additional evaluations are needed to understand protection against severe disease in nursing home residents over time. Because nursing home residents might remain at some risk for SARS-CoV-2 infection despite vaccination, multiple COVID-19 prevention strategies, including infection control, testing, and vaccination of nursing home staff members, residents, and visitors, are critical. An additional dose of COVID-19 vaccine might be considered for nursing home and long-term care facility residents to optimize a protective immune response.

Neerukonda, S. N., et al. (2021). "SARS-COV-2 Delta variant displays moderate resistance to neutralizing antibodies and spike protein properties of higher soluble ACE2 sensitivity, enhanced cleavage and fusogenic activity." bioRxiv.

The SARS-CoV-2 B.1.617 lineage variants, Kappa (B.1.617.1) and Delta (B.1.617.2, AY) emerged during the second wave of infections in India, but the Delta variants have become dominant worldwide and continue to evolve. The spike proteins of B.1.617.1, B.1.617.2, and AY.1 variants have several substitutions in the receptor binding domain (RBD), including L452R+E484Q, L452R+T478K, and K417N+L452R+T478K, respectively, that could potentially reduce effectiveness of therapeutic antibodies and current vaccines. Here we compared B.1.617 variants, and their single and double RBD substitutions for resistance to neutralization by convalescent sera, mRNA vaccine-elicited sera, and therapeutic neutralizing antibodies using a pseudovirus neutralization assay. Pseudoviruses with the B.1.617.1, B.1.617.2, and AY.1 spike showed a modest 1.5 to 4.4-fold reduction in neutralization titer by convalescent sera and vaccine-elicited sera. In comparison, similar modest reductions were also observed for pseudoviruses with C.37, P.1, R.1, and B.1.526 spikes, but seven- and sixteen-fold reduction for vaccine-elicited and convalescent sera, respectively, was seen for pseudoviruses with the B.1.351 spike. Four of twenty-three therapeutic neutralizing antibodies showed either complete or partial loss of neutralization against B.1.617.2 pseudoviruses due to the L452R substitution, whereas six of twenty-three therapeutic neutralizing antibodies showed either complete or partial loss of neutralization against B.1.617.1 pseudoviruses due to either the E484Q or L452R substitution. Against AY.1 pseudoviruses, the L452R and K417N substitutions accounted for the loss of neutralization by four antibodies and one antibody, respectively, whereas one antibody lost potency that could not be fully accounted for by a single RBD substitution. The modest resistance of B.1.617 variants to vaccine-elicited sera suggest that current mRNA-based vaccines will likely remain effective in protecting against B.1.617 variants, but the therapeutic antibodies need to be carefully selected based on their resistance profiles. Finally, the spike proteins of B.1.617 variants are more efficiently cleaved due to the P681R substitution, and the spike of Delta variants exhibited greater sensitivity to soluble ACE2 neutralization, as well as fusogenic activity, which may contribute to enhanced spread of Delta variants.

Ng, O. T., et al. (2021). "Impact of Delta Variant and Vaccination on SARS-CoV-2 Secondary Attack Rate Among Household Close Contacts." Lancet Reg Health West Pac **17**: 100299.

Background: Impact of the Delta variant and vaccination on SARS-CoV-2 transmission remains unclear. In Singapore, quarantine of all close contacts, including entry and exit PCR testing, provided the opportunity to determine risk of infection by the Delta variant compared to other variants, vaccine efficacy against SARS-CoV-2 acquisition, symptomatic or severe COVID-19, and risk factors associated with SARS-CoV-2 acquisition and symptomatic disease. Methods: This retrospective cohort study included all close contacts between September 1, 2020 and May 31, 2021. Regardless of symptoms, all were quarantined for 14 days with entry and exit PCR testing. Household contacts were defined as individuals who shared a

residence with a Covid-19 index case. Secondary attack rates among household close contacts of Delta variant-infected indexes and other variant-infected indexes were derived from prevalence of diagnosed cases among contacts. Relative risk ratios and bootstrapping at the cluster level was used to determine risk of infection by the Delta variant compared to other variants and vaccine efficacy against SARS-CoV-2 acquisition, symptomatic or severe COVID-19. Logistic regression using generalized estimating equations was used to determine risk factors associated with SARS-CoV-2 acquisition and symptomatic disease. Findings: Of 1024 household contacts linked to 301 PCR-confirmed index cases, 753 (73.5%) were linked to Delta-infected indexes and 248 (24.2%) were exposed to indexes with other variants. Household secondary attack rate among unvaccinated Delta-exposed contacts was 25.8% (95% boostrap confidence interval [BCI] 20.6-31.5%) compared with 12.9% (95%BCI 7.0-20.0%) among other variant-exposed contacts. Unvaccinated Delta-exposed contacts were more likely to be infected than those exposed to other variants (Relative risk 2.01, 95%CI 1.24-3.84). Among Delta-exposed contacts, complete vaccination had a vaccine effectiveness of 56.4% (95%BCI 32.6-75.8%) against acquisition, 64.1% (95%BCI 37.8-85.4%) against symptomatic disease and 100% against severe disease. Among Delta-exposed contacts, vaccination status (adjusted odds ratio [aOR] 0.33, 95% robust confidence interval [RCI] 0.17-0.63) and older age of the index (aOR 1.20 per decade, 95%RCI 1.03-1.39) was associated with increased risk of SARS-CoV-2 acquisition by the contact. Vaccination status of the index was not associated with a statistically-significant difference for contact SARS-CoV-2 acquisition (aOR 0.73, 95%RCI 0.38-1.40). Interpretation: Increased risk of SARS-CoV-2 Delta acquisition compared with other variants was reduced with vaccination. Close-contacts of vaccinated Delta-infected indexes did not have statistically significant reduced risk of acquisition compared with unvaccinated Delta-infected indexes.

Nonaka, C. K. V., et al. (2021). "SARS-CoV-2 variant of concern P.1 (Gamma) infection in young and middle-aged patients admitted to the intensive care units of a single hospital in Salvador, Northeast Brazil, February 2021." Int J Infect Dis **111**: 47-54.

OBJECTIVES: To evaluate changes in the characteristics of patients with coronavirus disease 2019 (COVID-19) after the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern (VOC) P.1 (Gamma), by comparing the clinical, demographic, and laboratory profiles of patients hospitalized during the first (May to July 2020) and second (December 2020 to February 2021) pandemic waves. METHODS: Data were collected from the records of COVID-19 patients (n = 4164) admitted to a single hospital in Salvador, Northeast Brazil. SARS-CoV-2 genome sequencing was performed on nasopharyngeal swab samples from 12 patients aged <60 years admitted to the intensive care unit (ICU) in February 2021. RESULTS: Between June 2020 and February 2021, the median age of patients admitted to the ICU decreased from 66 to 58 years ($P < 0.05$). This was accompanied by an increased proportion of patients without comorbidities (15.32% vs 32.20%, $P < 0.0001$). A significant reduction in the cycle threshold values of SARS-CoV-2 RT-PCR tests was observed in the second wave ($P < 0.0001$). Sequencing analysis detected lineage Gamma in all 12 ICU patients sampled in February 2021. CONCLUSIONS: The results of this study demonstrated an increased proportion of younger adults without comorbidities with severe disease during the second COVID-19 wave, shortly after the confirmation of local Gamma circulation.

Noureddine, F. Y., et al. (2021). "The Emergence of SARS-CoV-2 Variant(s) and Its Impact on the Prevalence of COVID-19 Cases in the Nabatieh Region, Lebanon." Med Sci (Basel) **9**(2).

Background: An outbreak of an unknown respiratory illness caused by a novel coronavirus, SARS-CoV-2, emerged in the city of Wuhan in Hubei Province, China, in December 2019 and was referred to as coronavirus disease-2019 (COVID-19). Soon after, it was declared as a global pandemic by the World Health Organization (WHO) in March 2020. SARS-CoV-2 mainly infects the respiratory tract with different outcomes ranging from asymptomatic infection to severe critical illness leading to death. Different SARS-CoV-2 variants are emerging of which three have raised concerns worldwide due to their high transmissibility among populations. Objective: To study the prevalence of COVID-19 in the region of Nabatieh-South Lebanon during the past year and assess the presence of SARS-CoV-2 variants and their effect on the spread of infection during times of lockdown. Methods: In our study, 37,474 nasopharyngeal swab samples were collected and analyzed for the detection of SARS-CoV-2 virus in suspected patients attending a tertiary health care center in South Lebanon during the period between 16 March 2020 and 21 February 2021. Results: The results demonstrated a variation in the prevalence rates ranging from less than 1% during full lockdown of the country to 8.4% upon easing lockdown restrictions and reaching 27.5% after the holidays and 2021 New Year celebrations. Interestingly, a new variant(s) appeared starting January 2021 with a significant positive association between the prevalence of positive tests and the percentage of the variant(s). Conclusions: Our results indicate that the lockdown implemented by the Lebanese officials was an effective intervention to

contain COVID-19 spread. Our study also showed that lifting lockdown measures during the holidays, which allowed indoor crowded gatherings to occur, caused a surge in COVID-19 cases and rise in the mortality rates nationwide. More importantly, we confirmed the presence of a highly transmissible SARS-CoV-2 variant(s) circulating in the Lebanese community from at least January 2021 onwards.

Nyberg, T., et al. (2021). "Risk of hospital admission for patients with SARS-CoV-2 variant B.1.1.7: cohort analysis." BMJ **373**: n1412.

OBJECTIVE: To evaluate the relation between diagnosis of covid-19 with SARS-CoV-2 variant B.1.1.7 (also known as variant of concern 202012/01) and the risk of hospital admission compared with diagnosis with wild-type SARS-CoV-2 variants. DESIGN: Retrospective cohort analysis. SETTING: Community based SARS-CoV-2 testing in England, individually linked with hospital admission data. PARTICIPANTS: 839 278 patients with laboratory confirmed covid-19, of whom 36 233 had been admitted to hospital within 14 days, tested between 23 November 2020 and 31 January 2021 and analysed at a laboratory with an available TaqPath assay that enables assessment of S-gene target failure (SGTF), a proxy test for the B.1.1.7 variant. Patient data were stratified by age, sex, ethnicity, deprivation, region of residence, and date of positive test. MAIN OUTCOME MEASURES: Hospital admission between one and 14 days after the first positive SARS-CoV-2 test. RESULTS: 27 710 (4.7%) of 592 409 patients with SGTF variants and 8523 (3.5%) of 246 869 patients without SGTF variants had been admitted to hospital within one to 14 days. The stratum adjusted hazard ratio of hospital admission was 1.52 (95% confidence interval 1.47 to 1.57) for patients with covid-19 infected with SGTF variants, compared with those infected with non-SGTF variants. The effect was modified by age (P<0.001), with hazard ratios of 0.93-1.21 in patients younger than 20 years with versus without SGTF variants, 1.29 in those aged 20-29, and 1.45-1.65 in those aged >/=30 years. The adjusted absolute risk of hospital admission within 14 days was 4.7% (95% confidence interval 4.6% to 4.7%) for patients with SGTF variants and 3.5% (3.4% to 3.5%) for those with non-SGTF variants. CONCLUSIONS: The results suggest that the risk of hospital admission is higher for people infected with the B.1.1.7 variant compared with wild-type SARS-CoV-2, likely reflecting a more severe disease. The higher severity may be specific to adults older than 30 years.

Ohki, S., et al. (2021). "Similarities and differences in the conformational stability and reversibility of ORF8, an accessory protein of SARS-CoV-2, and its L84S variant." Biochem Biophys Res Commun **563**: 92-97.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has the characteristic accessory protein ORF8. Although clinical reports indicate that ORF8 variant strains (Delta382 and L84S variants) are less likely to cause severe illness, functional differences between wild-type and variant ORF8 are unknown. Furthermore, the physicochemical properties of the ORF8 protein have not been analyzed. In this study, the physicochemical properties of the wild-type ORF8 and its L84S variant were analyzed and compared. Using the tobacco BY-2 cell production system, which has been successfully used to produce the wild-type ORF8 protein with a single conformation, was used to successfully produce the ORF8 L84S variant protein at the same level as wild-type ORF8. The produced proteins were purified, and their temperature and pH dependencies were examined using nuclear magnetic resonance spectra. Our data suggested that the wild-type and L84S variant ORF8 structures are highly stable over a wide temperature range. Both proteins displayed an aggregated conformation at higher temperature that reverted when the temperature was decreased to room temperature. Moreover, ORF8 precipitated at acidic pH and this precipitation was reversed when the solution pH was shifted to neutral. Interestingly, the L84S variant exhibited greater solubility than wild-type ORF8 under acidic conditions. Thus, the finding indicated that conformational stability and reversibility of ORF8 are key properties related to function in oppressive environments.

Ojelade, M., et al. (2021). "Travel from the United Kingdom to the United States by a Symptomatic Patient Infected with the SARS-CoV-2 B.1.1.7 Variant - Texas, January 2021." MMWR Morb Mortal Wkly Rep **70**(10): 348-349.

In December 2020, the B.1.1.7 genetic variant of SARS-CoV-2, the virus that causes COVID-19, was first reported after emergence and rapid circulation in the United Kingdom (1). Evidence suggests that the B.1.1.7 variant is more efficiently transmitted than are other SARS-CoV-2 variants, and widespread circulation could thereby increase SARS-CoV-2 infection and hospitalization rates (1,2). The first reported SARS-CoV-2 B.1.1.7 variant case in the United States was confirmed by sequencing in Colorado on December 29, 2020.* This report describes a person who traveled from the United Kingdom to the United States after experiencing COVID-19-compatible symptoms(dagger) and was eventually confirmed to be infected with the B.1.1.7 variant.

Pachetti, M., et al. (2020). "Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant." J Transl Med **18**(1): 179.

BACKGROUND: SARS-CoV-2 is a RNA coronavirus responsible for the pandemic of the Severe Acute Respiratory Syndrome (COVID-19). RNA viruses are characterized by a high mutation rate, up to a million times higher than that of their hosts. Virus mutagenic capability depends upon several factors, including the fidelity of viral enzymes that replicate nucleic acids, as SARS-CoV-2 RNA dependent RNA polymerase (RdRp). Mutation rate drives viral evolution and genome variability, thereby enabling viruses to escape host immunity and to develop drug resistance. METHODS: We analyzed 220 genomic sequences from the GISAID database derived from patients infected by SARS-CoV-2 worldwide from December 2019 to mid-March 2020. SARS-CoV-2 reference genome was obtained from the GenBank database. Genomes alignment was performed using Clustal Omega. Mann-Whitney and Fisher-Exact tests were used to assess statistical significance. RESULTS: We characterized 8 novel recurrent mutations of SARS-CoV-2, located at positions 1397, 2891, 14408, 17746, 17857, 18060, 23403 and 28881. Mutations in 2891, 3036, 14408, 23403 and 28881 positions are predominantly observed in Europe, whereas those located at positions 17746, 17857 and 18060 are exclusively present in North America. We noticed for the first time a silent mutation in RdRp gene in England (UK) on February 9th, 2020 while a different mutation in RdRp changing its amino acid composition emerged on February 20th, 2020 in Italy (Lombardy). Viruses with RdRp mutation have a median of 3 point mutations [range: 2-5], otherwise they have a median of 1 mutation [range: 0-3] (p value < 0.001). CONCLUSIONS: These findings suggest that the virus is evolving and European, North American and Asian strains might coexist, each of them characterized by a different mutation pattern. The contribution of the mutated RdRp to this phenomenon needs to be investigated. To date, several drugs targeting RdRp enzymes are being employed for SARS-CoV-2 infection treatment. Some of them have a predicted binding moiety in a SARS-CoV-2 RdRp hydrophobic cleft, which is adjacent to the 14408 mutation we identified. Consequently, it is important to study and characterize SARS-CoV-2 RdRp mutation in order to assess possible drug-resistance viral phenotypes. It is also important to recognize whether the presence of some mutations might correlate with different SARS-CoV-2 mortality rates.

Peiffer-Smadja, N., et al. (2021). "Emergence of E484K Mutation Following Bamlanivimab Monotherapy among High-Risk Patients Infected with the Alpha Variant of SARS-CoV-2." Viruses **13**(8).

An Emergency Use Authorization was issued in the United States and in Europe for a monoclonal antibody monotherapy to prevent severe COVID-19 in high-risk patients. This study aimed to assess the risk of emergence of mutations following treatment with a single monoclonal antibody. Bamlanivimab was administered at a single dose of 700 mg in a one-hour IV injection in a referral center for the management of COVID-19 in France. Patients were closely monitored clinically and virologically with nasopharyngeal RT-PCR and viral whole genome sequencing. Six patients were treated for a nosocomial SARS-CoV-2 infection, all males, with a median age of 65 years and multiple comorbidities. All patients were infected with a B.1.1.7 variant, which was the most frequent variant in France at the time, and no patients had E484 mutations at baseline. Bamlanivimab was infused in the six patients within 4 days of the COVID-19 diagnosis. Four patients had a favorable outcome, one died of complications unrelated to COVID-19 or bamlanivimab, and one kidney transplant patient treated with belatacept died from severe COVID-19 more than 40 days after bamlanivimab administration. Virologically, four patients cleared nasopharyngeal viral shedding within one month after infusion, while two presented prolonged viral excretion for more than 40 days. The emergence of E484K mutants was observed in five out of six patients, and the last patient presented a Q496R mutation potentially associated with resistance. CONCLUSIONS: These results show a high risk of emergence of resistance mutants in COVID-19 patients treated with monoclonal antibody monotherapy.

Penetra, S. L. S., et al. (2021). "Post-acute COVID-19 syndrome after reinfection and vaccine breakthrough by the SARS-CoV-2 Gamma variant in Brazil." Int J Infect Dis **114**: 58-61.

We describe a case of prolonged COVID-19 caused by the SARS-CoV-2 Gamma variant in a fully vaccinated healthcare worker, 387 days after an infection caused by lineage B.1.1.33. Infections were confirmed by whole-genome sequencing and corroborated by the detection of neutralizing antibodies in convalescent serum samples. Considering the permanent exposure of this healthcare worker to SARS-CoV-2, the waning immunity after the first infection, the low efficacy of the inactivated vaccine at preventing COVID-19, the immune escape of the Gamma variant (VOC), and the burden of post-COVID syndrome, this individual would have benefited from an additional dose of a heterologous vaccine.

Pereira, F., et al. (2021). "Genomic surveillance activities unveil the introduction of the SARS-CoV-2 B.1.525 variant of interest in Brazil: Case report." J Med Virol **93**(9): 5523-5526.

The appearance of new variants of SARS-CoV-2 has recently challenged public health authorities with respect to tracking transmission and mitigating the impact in the evolving pandemic across countries.

B.1.525 is considered a variant under investigation since it carries specific genetic signatures present in P.1, B.1.1.7, and B.1.351. Here we report genomic evidence of the first likely imported case of the SARS-CoV-2 B.1.525 variant, isolated in a traveler returning from Nigeria.

Peters, M. H., et al. (2021). "Transformations, Lineage Comparisons, and Analysis of Down to Up Protomer States of Variants of the SARS-CoV-2 Prefusion Spike Protein Including the UK Variant B.1.1.7." bioRxiv.

Monitoring and strategic response to variants in SARS-CoV-2 represents a considerable challenge in the current pandemic, as well as potentially future viral outbreaks of similar magnitude. In particular mutations and deletions involving the virion's prefusion Spike protein have significant potential impact on vaccines and therapeutics that utilize this key structural viral protein in their mitigation strategies. In this study, we have demonstrated how dominant energetic landscape mappings ("glue points") coupled with sequence alignment information can potentially identify or flag key residue mutations and deletions associated with variants. Surprisingly, we also found excellent homology of stabilizing residue glue points across the lineage of beta coronavirus Spike proteins, and we have termed this as "sequence homologous glue points". In general, these flagged residue mutations and/or deletions are then computationally studied in detail using all-atom biocomputational molecular dynamics over approximately one microsecond in order to ascertain structural and energetic changes in the Spike protein associated variants. Specifically, we examined both a theoretically-based triple mutant and the so-called UK or B.1.1.7 variant. For the theoretical triple mutant, we demonstrated through Alanine mutations, which help "unglue" key residue-residue interactions, that these three key stabilizing residues could cause the transition of Down to Up protomer states, where the Up protomer state allows binding of the prefusion Spike protein to hACE2 host cell receptors, whereas the Down state is believed inaccessible. Thus, we are able to demonstrate the importance of glue point residue identification in the overall stability of the prefusion Spike protein. For the B.1.1.7 variant, we demonstrated the critical importance of D614G and N5017 on the structure and binding, respectively, of the Spike protein. Notably, we had previously identified D614 as a key glue point in the inter-protomer stabilization of the Spike protein prior to the emergence of its mutation. The mutant D614G is a structure breaking Glycine mutation demonstrating a relatively more distal Down state RBD and a more stable conformation in general. In addition, we demonstrate that the mutation N501Y may significantly increase the Spike protein binding to hACE2 cell receptors through its interaction with Y41 of hACE2 forming a potentially strong hydrophobic residue binding pair. We note that these two key mutations, D614G and N501Y, are also found in the so-called South African (SA; B.1.351) variant of SARS-CoV-2. Future studies along these lines are, therefore, aimed at mapping glue points to residue mutations and deletions of associated prefusion Spike protein variants in order to help identify and analyze possible "variants of interest" and optimize efforts aimed at the mitigation of this current and future virions.

Peters, M. H., et al. (2021). "Transformations, Lineage Comparisons, and Analysis of Down-to-Up Protomer States of Variants of the SARS-CoV-2 Prefusion Spike Protein, Including the UK Variant B.1.1.7." Microbiol Spectr **9**(1): e0003021.

Monitoring and strategic response to variants in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represent a considerable challenge in the current pandemic and for future viral outbreaks. Mutations/deletions of the virion's prefusion Spike protein may have significant impact on vaccines and therapeutics that utilize this key structural protein in their mitigation strategies. In this study, we have demonstrated how dominant energetic landscape mappings ("glue points") based on ab inito all-atom force fields coupled with phylogenetic sequence alignment information can identify key residue mutations and deletions associated with variants. We also found several examples of excellent homology of stabilizing residue glue points across the lineages of betacoronavirus Spike proteins that we have called "sequence homologous glue points." SARS-CoV-2 demonstrates the least number of stabilizing glue points associated with interchain interactions among Down-state protomers across lineages. Additionally, we computationally studied variants among the trimeric Spike protein of SARS-CoV-2 using all-atom molecular dynamics to ascertain structural and energetic changes among variants. We examined both a theoretically based triple mutant and the UK or B.1.1.7 variant. For the theoretical triple mutant, we demonstrated through alanine substitutions that three key residues could cause the transition of Down-to-Up protomer states, where the transition is characterized by the "arm" length of the receptor-binding domain (RBD) rather than the hinge angle. For the B.1.1.7 variant, we demonstrated the critical importance of mutations D614G and N501Y on the structure and binding, respectively, of the Spike protein. We note that these same two key mutations are also found in the South African B.1.351 variant. IMPORTANCE Viral variants represent a major challenge to monitoring viral outbreaks and formulating strategic health care responses. Variants represent transmitting viruses that have specific mutations and deletions associated with

their genome. In the case of SARS-CoV-2 and other related viruses (betacoronaviruses), many of these mutations and deletions are associated with the Spike protein that the virus uses to infect cells. Here, we have analyzed both SARS-CoV-2 variants and related viruses, such as Middle Eastern respiratory syndrome coronavirus (MERS-CoV), in order to understand not only differences, but also key similarities between them. Understanding similarities can be as important as differences in determining key functional features of a class of viruses, such as the betacoronaviruses. We have used both phylogenetic analysis, which traces genetic similarities and differences, along with independent biophysics analysis, which adds function or behavior, in order to determine possible functional differences and hence possible transmission and infection differences among variants and lineages.

Petersen, E., et al. (2021). "Emergence of new SARS-CoV-2 Variant of Concern Omicron (B.1.1.529) - highlights Africa's research capabilities, but exposes major knowledge gaps, inequities of vaccine distribution, inadequacies in global COVID-19 response and control efforts." Int J Infect Dis.

Pirnay, J. P., et al. (2021). "Variant Analysis of SARS-CoV-2 Genomes from Belgian Military Personnel Engaged in Overseas Missions and Operations." Viruses **13**(7).

More than a year after the first identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the causative agent of the 2019 coronavirus disease (COVID-19) in China, the emergence and spread of genomic variants of this virus through travel raise concerns regarding the introduction of lineages in previously unaffected regions, requiring adequate containment strategies. Concomitantly, such introductions fuel worries about a possible increase in transmissibility and disease severity, as well as a possible decrease in vaccine efficacy. Military personnel are frequently deployed on missions around the world. As part of a COVID-19 risk mitigation strategy, Belgian Armed Forces that engaged in missions and operations abroad were screened (7683 RT-qPCR tests), pre- and post-mission, for the presence of SARS-CoV-2, including the identification of viral lineages. Nine distinct viral genotypes were identified in soldiers returning from operations in Niger, the Democratic Republic of the Congo, Afghanistan, and Mali. The SARS-CoV-2 variants belonged to major clades 19B, 20A, and 20B (Nextstrain nomenclature), and included "variant of interest" B.1.525, "variant under monitoring" A.27, as well as lineages B.1.214, B.1, B.1.1.254, and A (pangolin nomenclature), some of which are internationally monitored due to the specific mutations they harbor. Through contact tracing and phylogenetic analysis, we show that isolation and testing policies implemented by the Belgian military command appear to have been successful in containing the influx and transmission of these distinct SARS-CoV-2 variants into military and civilian populations.

Planas, D., et al. (2021). "Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization." Nature **596**(7871): 276-280.

The SARS-CoV-2 B.1.617 lineage was identified in October 2020 in India(1-5). Since then, it has become dominant in some regions of India and in the UK, and has spread to many other countries(6). The lineage includes three main subtypes (B1.617.1, B.1.617.2 and B.1.617.3), which contain diverse mutations in the N-terminal domain (NTD) and the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein that may increase the immune evasion potential of these variants. B.1.617.2-also termed the Delta variant-is believed to spread faster than other variants. Here we isolated an infectious strain of the Delta variant from an individual with COVID-19 who had returned to France from India. We examined the sensitivity of this strain to monoclonal antibodies and to antibodies present in sera from individuals who had recovered from COVID-19 (hereafter referred to as convalescent individuals) or who had received a COVID-19 vaccine, and then compared this strain with other strains of SARS-CoV-2. The Delta variant was resistant to neutralization by some anti-NTD and anti-RBD monoclonal antibodies, including bamlanivimab, and these antibodies showed impaired binding to the spike protein. Sera collected from convalescent individuals up to 12 months after the onset of symptoms were fourfold less potent against the Delta variant relative to the Alpha variant (B.1.1.7). Sera from individuals who had received one dose of the Pfizer or the AstraZeneca vaccine had a barely discernible inhibitory effect on the Delta variant. Administration of two doses of the vaccine generated a neutralizing response in 95% of individuals, with titres three- to fivefold lower against the Delta variant than against the Alpha variant. Thus, the spread of the Delta variant is associated with an escape from antibodies that target non-RBD and RBD epitopes of the spike protein.

Port, J., et al. (2021). "Increased aerosol transmission for B.1.1.7 (alpha variant) over lineage A variant of SARS-CoV-2." Res Sq.

Airborne transmission, a term combining both large droplet and aerosol transmission, is thought to be the main transmission route of SARS-CoV-2. Here we investigated the relative efficiency of aerosol transmission of two variants of SARS-CoV-2, B.1.1.7 (alpha) and lineage A, in the Syrian hamster. A novel transmission caging setup was designed and validated, which allowed the assessment of transmission efficiency at various distances. At 2 meters distance,

only particles <5 microm traversed between cages. In this setup, aerosol transmission was confirmed in 8 out of 8 (N = 4 for each variant) sentinels after 24 hours of exposure as demonstrated by respiratory shedding and seroconversion. Successful transmission occurred even when exposure time was limited to one hour, highlighting the efficiency of this transmission route. Interestingly, the B.1.1.7 variant outcompeted the lineage A variant in an airborne transmission chain after mixed infection of donors. Combined, this data indicates that the infectious dose of B.1.1.7 required for successful transmission may be lower than that of lineage A virus. The experimental proof for true aerosol transmission and the increase in the aerosol transmission potential of B.1.1.7 underscore the continuous need for assessment of novel variants and the development or preemptive transmission mitigation strategies.

Port, J. R., et al. (2021). "Increased aerosol transmission for B.1.1.7 (alpha variant) over lineage A variant of SARS-CoV-2." bioRxiv.

Airborne transmission, a term combining both large droplet and aerosol transmission, is thought to be the main transmission route of SARS-CoV-2. Here we investigated the relative efficiency of aerosol transmission of two variants of SARS-CoV-2, B.1.1.7 (alpha) and lineage A, in the Syrian hamster. A novel transmission caging setup was designed and validated, which allowed the assessment of transmission efficiency at various distances. At 2 meters distance, only particles <5 microm traversed between cages. In this setup, aerosol transmission was confirmed in 8 out of 8 (N = 4 for each variant) sentinels after 24 hours of exposure as demonstrated by respiratory shedding and seroconversion. Successful transmission occurred even when exposure time was limited to one hour, highlighting the efficiency of this transmission route. Interestingly, the B.1.1.7 variant outcompeted the lineage A variant in an airborne transmission chain after mixed infection of donors. Combined, this data indicates that the infectious dose of B.1.1.7 required for successful transmission may be lower than that of lineage A virus. The experimental proof for true aerosol transmission and the increase in the aerosol transmission potential of B.1.1.7 underscore the continuous need for assessment of novel variants and the development or preemptive transmission mitigation strategies.

Pouwels, K. B., et al. (2021). "Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK." Nat Med.

The effectiveness of the BNT162b2 and ChAdOx1 vaccines against new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections requires continuous re-evaluation, given the increasingly dominant B.1.617.2 (Delta) variant. In this study, we investigated the effectiveness of these vaccines in a large, community-based survey of randomly selected households across the United Kingdom. We found that the effectiveness of BNT162b2 and ChAdOx1 against infections (new polymerase chain reaction (PCR)-positive cases) with symptoms or high viral burden is reduced with the B.1.617.2 variant (absolute difference of 10-13% for BNT162b2 and 16% for ChAdOx1) compared to the B.1.1.7 (Alpha) variant. The effectiveness of two doses remains at least as great as protection afforded by prior natural infection. The dynamics of immunity after second doses differed significantly between BNT162b2 and ChAdOx1, with greater initial effectiveness against new PCR-positive cases but faster declines in protection against high viral burden and symptomatic infection with BNT162b2. There was no evidence that effectiveness varied by dosing interval, but protection was higher in vaccinated individuals after a prior infection and in younger adults. With B.1.617.2, infections occurring after two vaccinations had similar peak viral burden as those in unvaccinated individuals. SARS-CoV-2 vaccination still reduces new infections, but effectiveness and attenuation of peak viral burden are reduced with B.1.617.2.

Pymm, P., et al. (2021). "Nanobody cocktails potently neutralize SARS-CoV-2 D614G N501Y variant and protect mice." Proc Natl Acad Sci U S A **118**(19).

Neutralizing antibodies are important for immunity against SARS-CoV-2 and as therapeutics for the prevention and treatment of COVID-19. Here, we identified high-affinity nanobodies from alpacas immunized with coronavirus spike and receptor-binding domains (RBD) that disrupted RBD engagement with the human receptor angiotensin-converting enzyme 2 (ACE2) and potently neutralized SARS-CoV-2. Epitope mapping, X-ray crystallography, and cryo-electron microscopy revealed two distinct antigenic sites and showed two neutralizing nanobodies from different epitope classes bound simultaneously to the spike trimer. Nanobody-Fc fusions of the four most potent nanobodies blocked ACE2 engagement with RBD variants present in human populations and potently neutralized both wild-type SARS-CoV-2 and the N501Y D614G variant at concentrations as low as 0.1 nM. Prophylactic administration of either single nanobody-Fc or as mixtures reduced viral loads by up to 10(4)-fold in mice infected with the N501Y D614G SARS-CoV-2 virus. These results suggest a role for nanobody-Fc fusions as prophylactic agents against SARS-CoV-2.

Qiu, H., et al. (2021). "Development and characterization of SARS-CoV-2 variant-neutralizing monoclonal antibodies." Antiviral Res **196**: 105206.

Vaccination and administration of monoclonal antibody cocktails are effective tools to control the

progression of infectious diseases and to terminate pandemics such as COVID-19. However, the emergence of SARS-CoV-2 mutants with enhanced transmissibility and altered antigenicity requires broad-spectrum therapies. Here we developed a panel of SARS-CoV-2 specific mouse monoclonal antibodies (mAbs), and characterized them based on ELISA, Western immunoblot, isotyping, and virus neutralization. Six neutralizing mAbs that exhibited high-affinity binding to SARS-CoV-2 spike protein were identified, and their amino acid sequences were determined by mass spectrometry. Functional assays confirmed that three mAbs, F461G11, F461G15, and F461G16 neutralized four variants of concern (VOC): B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) These mAbs are promising candidates for COVID-19 therapy, and understanding their interactions with virus spike protein should support further vaccine and antibody development.

Queiros-Reis, L., et al. (2021). "SARS-CoV-2 Virus-Host Interaction: Currently Available Structures and Implications of Variant Emergence on Infectivity and Immune Response." Int J Mol Sci **22**(19).

Coronavirus disease 19, or COVID-19, is an infection associated with an unprecedented worldwide pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which has led to more than 215 million infected people and more than 4.5 million deaths worldwide. SARS-CoV-2 cell infection is initiated by a densely glycosylated spike (S) protein, a fusion protein, binding human angiotensin converting enzyme 2 (hACE2), that acts as the functional receptor through the receptor binding domain (RBD). In this article, the interaction of hACE2 with the RBD and how fusion is initiated after recognition are explored, as well as how mutations influence infectivity and immune response. Thus, we focused on all structures available in the Protein Data Bank for the interaction between SARS-CoV-2 S protein and hACE2. Specifically, the Delta variant carries particular mutations associated with increased viral fitness through decreased antibody binding, increased RBD affinity and altered protein dynamics. Combining both existing mutations and mutagenesis studies, new potential SARS-CoV-2 variants, harboring advantageous S protein mutations, may be predicted. These include mutations S13I and W152C, decreasing antibody binding, N460K, increasing RDB affinity, or Q498R, positively affecting both properties.

Resende, P. C., et al. (2021). "A Potential SARS-CoV-2 Variant of Interest (VOI) Harboring Mutation E484K in the Spike Protein Was Identified within Lineage B.1.1.33 Circulating in Brazil." Viruses **13**(5).

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic in Brazil was dominated by two lineages designated as B.1.1.28 and B.1.1.33. The two SARS-CoV-2 variants harboring mutations at the receptor-binding domain of the Spike (S) protein, designated as lineages P.1 and P.2, evolved from lineage B.1.1.28 and are rapidly spreading in Brazil. Lineage P.1 is considered a Variant of Concern (VOC) because of the presence of multiple mutations in the S protein (including K417T, E484K, N501Y), while lineage P.2 only harbors mutation S:E484K and is considered a Variant of Interest (VOI). On the other hand, epidemiologically relevant B.1.1.33 deriving lineages have not been described so far. Here we report the identification of a new SARS-CoV-2 VOI within lineage B.1.1.33 that also harbors mutation S:E484K and was detected in Brazil between November 2020 and February 2021. This VOI displayed four non-synonymous lineage-defining mutations (NSP3:A1711V, NSP6:F36L, S:E484K, and NS7b:E33A) and was designated as lineage N.9. The VOI N.9 probably emerged in August 2020 and has spread across different Brazilian states from the Southeast, South, North, and Northeast regions.

Reuschl, A. K., et al. (2021). "Host-directed therapies against early-lineage SARS-CoV-2 retain efficacy against B.1.1.7 variant." bioRxiv.

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in millions of deaths worldwide and massive societal and economic burden. Recently, a new variant of SARS-CoV-2, known as B.1.1.7, was first detected in the United Kingdom and is spreading in several other countries, heightening public health concern and raising questions as to the resulting effectiveness of vaccines and therapeutic interventions. We and others previously identified host-directed therapies with antiviral efficacy against SARS-CoV-2 infection. Less prone to the development of therapy resistance, host-directed drugs represent promising therapeutic options to combat emerging viral variants as host genes possess a lower propensity to mutate compared to viral genes. Here, in the first study of the full-length B.1.1.7 variant virus , we find two host-directed drugs, plitidepsin (aplidin; inhibits translation elongation factor eEF1A) and ralimetinib (inhibits p38 MAP kinase cascade), as well as remdesivir, to possess similar antiviral activity against both the early-lineage SARS-CoV-2 and the B.1.1.7 variant, evaluated in both human gastrointestinal and lung epithelial cell lines. We find that plitidepsin is over an order of magnitude more potent than remdesivir against both viruses. These results highlight the importance of continued development of host-directed therapeutics to combat current and future coronavirus variant outbreaks.

Rocheleau, L., et al. (2021). "Identification of a High-Frequency Intrahost SARS-CoV-2 Spike Variant with

Enhanced Cytopathic and Fusogenic Effects." mBio **12**(3): e0078821.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus that is continuously evolving. Although its RNA-dependent RNA polymerase exhibits some exonuclease proofreading activity, viral sequence diversity can be produced by replication errors and host factors. A diversity of genetic variants can be observed in the intrahost viral population structure of infected individuals. Most mutations will follow a neutral molecular evolution and will not make significant contributions to variations within and between infected hosts. Herein, we profiled the intrasample genetic diversity of SARS-CoV-2 variants, also known as quasispecies, using high-throughput sequencing data sets from 15,289 infected individuals and infected cell lines. Despite high mutational background, we identified recurrent intragenetic variable positions in the samples analyzed, including several positions at the end of the gene encoding the viral spike (S) protein. Strikingly, we observed a high frequency of C-->A missense mutations resulting in the S protein lacking the last 20 amino acids (SDelta20). We found that this truncated S protein undergoes increased processing and increased syncytium formation, presumably due to escaping M protein retention in intracellular compartments. Our findings suggest the emergence of a high-frequency viral sublineage that is not horizontally transmitted but potentially involved in intrahost disease cytopathic effects. IMPORTANCE The mutation rate and evolution of RNA viruses correlate with viral adaptation. While most mutations do not make significant contributions to viral molecular evolution, some are naturally selected and produce variants through positive selection. Many SARS-CoV-2 variants have been recently described and show phenotypic selection toward more infectious viruses. Our study describes another type of variant that does not contribute to interhost heterogeneity but rather phenotypic selection toward variants that might have increased cytopathic effects. We identified that a C-terminal truncation of the spike protein removes an important endoplasmic reticulum (ER) retention signal, which consequently results in a spike variant that easily travels through the Golgi complex toward the plasma membrane in a preactivated conformation, leading to increased syncytium formation.

Rodriguez-Grande, C., et al. (2021). "SARS-CoV-2 B.1.1.7 Decline Is Not Driven by the Introduction of a More Successful Variant." Microbiol Spectr: e0112821.

The SARS-CoV-2 variant of concern (VOC) Delta (B.617.2 lineage) displaced the predominant VOC Alpha (B.1.1.7 lineage) in the United Kingdom. In Madrid, recent start of the decline of predominant VOC Alpha suggested an equivalent phenomenon. However, 11 different variants, none overrepresented in frequency, occupied progressively over a period of 7 weeks the niche previously dominated by VOC Alpha. Only after these 7 weeks, VOC Delta started to emerge. Viral competition due to the entry of VOC Delta is not the major force driving the start of VOC Alpha decline in Madrid. IMPORTANCE Our data indicate that the dynamics of SARS-CoV-2 VOCs turnover in our setting differ from those proposed for other countries. A systematic genomic analysis, updated on a weekly basis, of representative randomly selected samples of SARS-CoV-2 circulating variants allowed us to define a lapse of 7 weeks between the start of VOC Alpha decline and the final emergence of VOC Delta. During this period, VOC Alpha showed a sustained decline, while 11 VOCs, variants of interest (VOIs), and other identified variants, none overrepresented, occupied the niche left by VOC Alpha. Only after these 7 weeks, emergence of VOC Delta occurred, indicating that viral competition involving VOC Delta was not the exclusive direct driving force behind the starting of VOC Alpha decline.

Rodriguez-Maldonado, A. P., et al. (2021). "Emergence and spread of the potential variant of interest (VOI) B.1.1.519 of SARS-CoV-2 predominantly present in Mexico." Arch Virol **166**(11): 3173-3177.

SARS-CoV-2 variants emerged in late 2020, and at least three variants of concern (B.1.1.7, B.1.351, and P1) have been reported by WHO. These variants have several substitutions in the spike protein that affect receptor binding; they exhibit increased transmissibility and may be associated with reduced vaccine effectiveness. In the present work, we report the identification of a potential variant of interest, harboring the mutations T478K, P681H, and T732A in the spike protein, within the newly named lineage B.1.1.519, that rapidly outcompeted the preexisting variants in Mexico and has been the dominant virus in the country during the first trimester of 2021.

Roltgen, K., et al. (2021). "mRNA vaccination compared to infection elicits an IgG-predominant response with greater SARS-CoV-2 specificity and similar decrease in variant spike recognition." medRxiv.

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, new vaccine strategies including lipid nanoparticle delivery of antigen encoding RNA have been deployed globally. The BioNTech/Pfizer mRNA vaccine BNT162b2 encoding SARS-CoV-2 spike protein shows 95% efficacy in preventing disease, but it is unclear how the antibody responses to vaccination differ from those generated by infection. Here we compare the magnitude and breadth of antibodies targeting SARS-

CoV-2, SARS-CoV-2 variants of concern, and endemic coronaviruses, in vaccinees and infected patients. We find that vaccination differs from infection in the dominance of IgG over IgM and IgA responses, with IgG reaching levels similar to those of severely ill COVID-19 patients and shows decreased breadth of the antibody response targeting endemic coronaviruses. Viral variants of concern from B.1.1.7 to P.1 to B.1.351 form a remarkably consistent hierarchy of progressively decreasing antibody recognition by both vaccinees and infected patients exposed to Wuhan-Hu-1 antigens.

Ryu, D. K., et al. (2021). "Therapeutic effect of CT-P59 against SARS-CoV-2 South African variant." Biochem Biophys Res Commun **566**: 135-140.

The global circulation of newly emerging variants of SARS-CoV-2 is a new threat to public health due to their increased transmissibility and immune evasion. Moreover, currently available vaccines and therapeutic antibodies were shown to be less effective against new variants, in particular, the South African (SA) variant, termed 501Y.V2 or B.1.351. To assess the efficacy of the CT-P59 monoclonal antibody against the SA variant, we sought to perform as in vitro binding and neutralization assays, and in vivo animal studies. CT-P59 neutralized B.1.1.7 variant to a similar extent as to wild type virus. CT-P59 showed reduced binding affinity against a RBD (receptor binding domain) triple mutant containing mutations defining B.1.351 (K417N/E484K/N501Y) also showed reduced potency against the SA variant in live virus and pseudovirus neutralization assay systems. However, in vivo ferret challenge studies demonstrated that a therapeutic dosage of CT-P59 was able to decrease B.1.351 viral load in the upper and lower respiratory tracts, comparable to that observed for the wild type virus. Overall, although CT-P59 showed reduced in vitro neutralizing activity against the SA variant, sufficient antiviral effect in B.1.351-infected animals was confirmed with a clinical dosage of CT-P59, suggesting that CT-P59 has therapeutic potential for COVID-19 patients infected with SA variant.

Sah, P., et al. (2021). "Quantifying the potential for dominant spread of SARS-CoV-2 variant B.1.351 in the United States." medRxiv.

Recent evidence suggests that the SARS-CoV-2 variant B.1.351 exhibits partial immune evasion to antibodies generated by natural infection or vaccination. We used a dynamic transmission model to evaluate whether this variant could become dominant in the United States given mounting vaccination coverage and other circulating variants. We show that B.1.351 is unlikely to become dominant even when all fully vaccinated individuals return to their pre-pandemic behavior. However, an improved selection advantage of B.1.351 arising from a combination of increased transmission and immune escape could drive this variant to dominance as early as July 2021 and fuel a resurgence of cases and hospitalizations. Our study underscores the urgency for continued rollout of the current generation of vaccines despite the emergence of immune escape variants.

Sakai-Tagawa, Y., et al. (2021). "Comparative Sensitivity of Rapid Antigen Tests for the Delta Variant (B.1.617.2) of SARS-CoV-2." Viruses **13**(11).

Rapid antigen tests (RATs) for COVID-19 based on lateral flow immunoassays are useful for rapid diagnosis in a variety of settings. Although many kinds of RATs are available, their respective sensitivity has not been compared. Here, we examined the sensitivity of 27 RATs available in Japan for the detection of the SARS-CoV-2 delta variant. All of the RATs tested detected the delta variant albeit with different sensitivities. Nine RATs (ESPLINE SARS-CoV-2, ALSONIC COVID-19 Ag, COVID-19 and Influenza A+B Antigen Combo Rapid Test, ImmunoArrow SARS-CoV-2, Fuji Dri-chem immuno AG cartridge COVID-19 Ag, 2019-nCoV Ag rapid detection kit, Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit, and Rabliss SARS-CoV-2 antigen detection kit COVID19 AG) showed superior sensitivity to the isolated delta variant. Although actual clinical specimens were not examined, the detection level of most of the RATs was 7500 pfu, indicating that individuals whose test samples contained less virus than that would be considered negative. Therefore, it is important to bear in mind that RATs may miss individuals shedding low levels of infectious virus.

Sallam, M. and A. Mahafzah (2021). "Molecular Analysis of SARS-CoV-2 Genetic Lineages in Jordan: Tracking the Introduction and Spread of COVID-19 UK Variant of Concern at a Country Level." Pathogens **10**(3).

The rapid evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is manifested by the emergence of an ever-growing pool of genetic lineages. The aim of this study was to analyze the genetic variability of SARS-CoV-2 in Jordan, with a special focus on the UK variant of concern. A total of 579 SARS-CoV-2 sequences collected in Jordan were subjected to maximum likelihood and Bayesian phylogenetic analysis. Genetic lineage assignment was undertaken using the Pango system. Amino acid substitutions were investigated using the Protein Variation Effect Analyzer (PROVEAN) tool. A total of 19 different SARS-CoV-2 genetic lineages were detected, with the most frequent being the first Jordan lineage (B.1.1.312), first detected in August 2020 (n = 424, 73.2%). This was followed by the second Jordan lineage (B.1.36.10), first detected in September 2020 (n = 62, 10.7%), and the UK variant of concern (B.1.1.7;

n = 36, 6.2%). In the spike gene region, the molecular signature for B.1.1.312 was the non-synonymous mutation A24432T resulting in a deleterious amino acid substitution (Q957L), while the molecular signature for B.1.36.10 was the synonymous mutation C22444T. Bayesian analysis revealed that the UK variant of concern (B.1.1.7) was introduced into Jordan in late November 2020 (mean estimate); four weeks earlier than its official reporting in the country. In Jordan, an exponential increase in COVID-19 cases due to B.1.1.7 lineage coincided with the new year 2021. The highest proportion of phylogenetic clustering was detected for the B.1.1.7 lineage. The amino acid substitution D614G in the spike glycoprotein was exclusively present in the country from July 2020 onwards. Two Jordanian lineages dominated infections in the country, with continuous introduction/emergence of new lineages. In Jordan, the rapid spread of the UK variant of concern should be monitored closely. The spread of SARS-CoV-2 mutants appeared to be related to the founder effect; nevertheless, the biological impact of certain mutations should be further investigated.

Shimada, R., et al. (2021). "SARS-CoV-2 Variant Identification Using a Genome Tiling Array and Genotyping Probes." bioRxiv.

With over three million deaths worldwide attributed to the respiratory disease COVID-19 caused by the novel coronavirus SARS-CoV-2, it is essential that continued efforts be made to track the evolution and spread of the virus globally. We previously presented a rapid and cost-effective method to sequence the entire SARS-CoV-2 genome with 95% coverage and 99.9% accuracy. This method is advantageous for identifying and tracking variants in the SARS-CoV-2 genome when compared to traditional short read sequencing methods which can be time consuming and costly. Herein we present the addition of genotyping probes to our DNA chip which target known SARS-CoV-2 variants. The incorporation of the genotyping probe sets along with the advent of a moving average filter have improved our sequencing coverage and accuracy of the SARS-CoV-2 genome.

Shitrit, P., et al. (2021). "Nosocomial outbreak caused by the SARS-CoV-2 Delta variant in a highly vaccinated population, Israel, July 2021." Euro Surveill **26**(39).

A nosocomial outbreak of SARS-CoV-2 Delta variant infected 42 patients, staff and family members; 39 were fully vaccinated. The attack rate was 10.6% (16/151) among exposed staff and reached 23.7% (23/97) among exposed patients in a highly vaccinated population, 16-26 weeks after vaccination (median: 25 weeks). All cases were linked and traced to one patient. Several transmissions occurred between individuals wearing face masks. Fourteen of 23 patients became severely sick or died, raising a question about possible waning immunity.

Shrestha, R., et al. (2021). "Whole Genome Sequence Analysis to Identify SARS-CoV-2 Variant in Nepal." Kathmandu Univ Med J (KUMJ) **19**(74): 137-142.

Background The spread of SARS-CoV-2 has become a global public health crisis. Nepal is facing the second wave of COVID-19 pandemic but, there is still a limited data on the genomic sequence of SARS-CoV-2 variants circulating in Nepal. Objective The objective of this study is to sequence the whole genome of SARS-CoV-2 in Nepal to detect possible mutation profiles and phylogenetic lineages of circulating SARSCoV-2 variants. Method In this study, swab samples tested positive for SARS-CoV-2 were investigated. After RNA extraction, the investigation was performed through real-time PCR followed by whole genome sequencing. The consensus genome sequences were, then, analyzed with appropriate bioinformatics tools. Result Sequence analysis of two SARS-CoV-2 genomes from patient without travel history (Patient A1 and A2) were found to be of lineage B.1.1. Similarly, among other four samples from subjects returning from the United Kingdom, genomes of two samples were of lineage B.1.36, and the other two were of lineage B.1.1.7 (Alpha Variant). The mutations in the consensus genomes contained the defining mutations of the respective lineages of SARS-CoV-2. Conclusion We confirmed two genomic sequences of variant of concern VOC-202012/01 in Nepal. Our study provides the concise genomic evidence for spread of different lineages of SARS-CoV-2 - B.1.1, B.1.36 and B.1.1.7 of SARS-CoV-2 in Nepal.

Siddle, K. J., et al. (2021). "Evidence of transmission from fully vaccinated individuals in a large outbreak of the SARS-CoV-2 Delta variant in Provincetown, Massachusetts." medRxiv.

Multiple summer events, including large indoor gatherings, in Provincetown, Massachusetts (MA), in July 2021 contributed to an outbreak of over one thousand COVID-19 cases among residents and visitors. Most cases were fully vaccinated, many of whom were also symptomatic, prompting a comprehensive public health response, motivating changes to national masking recommendations, and raising questions about infection and transmission among vaccinated individuals. To characterize the outbreak and the viral population underlying it, we combined genomic and epidemiological data from 467 individuals, including 40% of known outbreak-associated cases. The Delta variant accounted for 99% of sequenced outbreak-associated cases. Phylogenetic analysis suggests over 40 sources of Delta in the dataset, with one responsible for a single cluster containing 83% of outbreak-associated genomes. This

cluster was likely not the result of extensive spread at a single site, but rather transmission from a common source across multiple settings over a short time. Genomic and epidemiological data combined provide strong support for 25 transmission events from, including many between, fully vaccinated individuals; genomic data alone provides evidence for an additional 64. Together, genomic epidemiology provides a high-resolution picture of the Provincetown outbreak, revealing multiple cases of transmission of Delta from fully vaccinated individuals. However, despite its magnitude, the outbreak was restricted in its onward impact in MA and the US, likely due to high vaccination rates and a robust public health response.

Siedner, M. J., et al. (2021). "Duration of viral shedding and culture positivity with post-vaccination SARS-CoV-2 delta variant infections." JCI Insight.

Isolation guidelines for severe acute respiratory syndrome-cornavirus-2 (SARS-CoV-2) are largely derived from data collected prior to emergence of the delta variant. We followed a cohort of ambulatory patients with post-vaccination breakthrough SARS-CoV-2 infections with longitudinal collection of nasal swabs for SARS-CoV-2 viral load quantification, whole genome sequencing, and viral culture. All delta variant infections (10/10, 100%) in our cohort were symptomatic, compared with 64% (9/14) of non-delta variant infections. Symptomatic delta variant breakthrough infections were characterized by higher initial viral load, longer duration of virologic shedding by PCR, greater likelihood of replication-competent virus at early stages of infection, and longer duration of culturable virus compared to non-delta variants. The duration of time since vaccination was also correlated with both duration of PCR positivity and duration of detection of replication-competent virus. Nonetheless, no individuals with symptomatic delta variant infections had replication-competent virus by day 10 after symptom onset or 24 hours after resolution of symptoms. These data support current US Center for Disease Control isolation guidelines and reinforce the importance of prompt testing and isolation among symptomatic individuals with delta variant breakthrough infections. Additional data are needed to evaluate these relationships among asymptomatic and more severe delta variant breakthrough infections.

Silva, M. S. D., et al. (2021). "Early detection of SARS-CoV-2 P.1 variant in Southern Brazil and reinfection of the same patient by P.2." Rev Inst Med Trop Sao Paulo **63**: e58.

Multiple variants of the Severe Acute Respiratory Syndrome coronavirus 2 virus (SARS-CoV-2) have been constantly reported across the world. The B.1.1.28 lineage has been evolving in Brazil since February 2020 and originated the P.1 variant of concern (VOC), recently named as the Gamma variant by the newly WHO nomenclature proposal, and P.2 as a variant of interest (VOI). Here we describe an early case of P.1 primary infection in Southern Brazil in late November 2020, soon after the emergence of the variant in Manaus, Northern Brazil. The same male patient was reinfected by another B.1.1.28 variant, namely P.2, in March, 2021. The genomic analysis confirmed genetically significant differences between the two viruses recovered in both infections, the P.1 lineage in the first episode and P.2 in the reinfection. Due the very early detection of P.1, we have also investigated the circulation of P.1 in the same region by differential RT-qPCR, showing that this was an isolated case of P.1 at the time of detection, and this variant has disseminated and became prominent from late January to the end of March, 2021. SARS-CoV-2 recent reports of reinfection have raised critical questions on whether and how well a first infection protects against reinfection.

Singanayagam, A., et al. (2021). "Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study." Lancet Infect Dis.

BACKGROUND: The SARS-CoV-2 delta (B.1.617.2) variant is highly transmissible and spreading globally, including in populations with high vaccination rates. We aimed to investigate transmission and viral load kinetics in vaccinated and unvaccinated individuals with mild delta variant infection in the community. METHODS: Between Sept 13, 2020, and Sept 15, 2021, 602 community contacts (identified via the UK contract-tracing system) of 471 UK COVID-19 index cases were recruited to the Assessment of Transmission and Contagiousness of COVID-19 in Contacts cohort study and contributed 8145 upper respiratory tract samples from daily sampling for up to 20 days. Household and non-household exposed contacts aged 5 years or older were eligible for recruitment if they could provide informed consent and agree to self-swabbing of the upper respiratory tract. We analysed transmission risk by vaccination status for 231 contacts exposed to 162 epidemiologically linked delta variant-infected index cases. We compared viral load trajectories from fully vaccinated individuals with delta infection (n=29) with unvaccinated individuals with delta (n=16), alpha (B.1.1.7; n=39), and pre-alpha (n=49) infections. Primary outcomes for the epidemiological analysis were to assess the secondary attack rate (SAR) in household contacts stratified by contact vaccination status and the index cases' vaccination status. Primary outcomes for the viral load kinetics analysis were to detect differences in the peak viral load, viral growth rate, and viral decline rate between participants according to SARS-CoV-2 variant

and vaccination status. FINDINGS: The SAR in household contacts exposed to the delta variant was 25% (95% CI 18-33) for fully vaccinated individuals compared with 38% (24-53) in unvaccinated individuals. The median time between second vaccine dose and study recruitment in fully vaccinated contacts was longer for infected individuals (median 101 days [IQR 74-120]) than for uninfected individuals (64 days [32-97], p=0.001). SAR among household contacts exposed to fully vaccinated index cases was similar to household contacts exposed to unvaccinated index cases (25% [95% CI 15-35] for vaccinated vs 23% [15-31] for unvaccinated). 12 (39%) of 31 infections in fully vaccinated household contacts arose from fully vaccinated epidemiologically linked index cases, further confirmed by genomic and virological analysis in three index case-contact pairs. Although peak viral load did not differ by vaccination status or variant type, it increased modestly with age (difference of 0.39 [95% credible interval -0.03 to 0.79] in peak log10 viral load per mL between those aged 10 years and 50 years). Fully vaccinated individuals with delta variant infection had a faster (posterior probability >0.84) mean rate of viral load decline (0.95 log10 copies per mL per day) than did unvaccinated individuals with pre-alpha (0.69), alpha (0.82), or delta (0.79) variant infections. Within individuals, faster viral load growth was correlated with higher peak viral load (correlation 0.42 [95% credible interval 0.13 to 0.65]) and slower decline (-0.44 [-0.67 to -0.18]). INTERPRETATION: Vaccination reduces the risk of delta variant infection and accelerates viral clearance. Nonetheless, fully vaccinated individuals with breakthrough infections have peak viral load similar to unvaccinated cases and can efficiently transmit infection in household settings, including to fully vaccinated contacts. Host-virus interactions early in infection may shape the entire viral trajectory. FUNDING: National Institute for Health Research.

Singer, S. R., et al. (2021). "Effectiveness of BNT162b2 mRNA COVID-19 vaccine against SARS-CoV-2 variant Beta (B.1.351) among persons identified through contact tracing in Israel: A prospective cohort study." EClinicalMedicine **42**: 101190.

Background: SARS-CoV-2 variant Beta (B.1.351) was designated as a Variant of Concern (VoC) after becoming the dominant strain in South Africa and spreading internationally. BNT162b2 showed lower levels of neutralizing antibodies against Beta than against other strains raising concerns about effectiveness of vaccines against infections caused by Beta. We estimated BNT162b2 vaccine effectiveness (VE) against Beta infections in Israel, a country with high vaccine uptake. Methods: The Ministry of Health (MoH) identified Beta cases through mandatory reporting of SARS-CoV-2 cases and whole genome sequencing (WGS) of specimens from vaccination-breakthrough infections, reinfections, arriving international travelers, and a selection of other infected persons. A cohort analysis was conducted of exposure events of contacts of primary Beta cases. WGS was conducted on available PCR-positive specimens collected from contacts. VE estimates with 95% confidence intervals (CIs) against confirmed and probable Beta infections were determined by comparing infection risk between unvaccinated and fully-vaccinated (>/=7 days after the second dose) contacts, and between unvaccinated and partially-vaccinated (<7 days after the second dose) contacts. Findings: MoH identified 310 Beta cases through Jun 27, 2021. During the study period (Dec 11, 2020 - Mar 25, 2021), 164 non-institutionalized primary Beta cases, with 552 contacts aged >/=16 years, were identified. 343/552 (62%) contacts were interviewed and tested. 71/343 (21%) contacts were PCR-positive. WGS was performed on 7/71 (10%) PCR-positive specimens; all were Beta. Among SARS-CoV-2-infected contacts, 48/71 (68%) were symptomatic, 10/71 (14%) hospitalized, and 2/71 (3%) died. Fully-vaccinated VE against confirmed or probable Beta infections was 72% (95% CI -5 - 97%; p=0.04) and against symptomatic confirmed or probable Beta infections was 100% (95% CI 19 - 100%; p=0.01). There was no evidence of protection in partially-vaccinated contacts. Interpretation: In a prospective observational study, two doses of BNT162b2 were effective against confirmed and probable Beta infections. Through the end of June 2021, introductions of Beta did not interrupt control of the pandemic in Israel. Funding: Israel Ministry of Health and Pfizer.

Singh, S., et al. (2021). "SARS-CoV-2 and its beta variant of concern infect human conjunctival epithelial cells and induce differential antiviral innate immune response." Ocul Surf.

PURPOSE: SARS-CoV-2 RNA has been detected in ocular tissues, but their susceptibility to SARS-CoV-2 infection is unclear. Here, we tested whether SARS-CoV-2 can infect human conjunctival epithelial cells (hCECs) and induce innate immune response. METHODS: Conjunctival tissue from COVID-19 donors was used to detect SARS-CoV-2 spike and envelope proteins. Primary hCECs isolated from cadaver eyes were infected with the parental SARS-CoV-2 and its beta variant of concern (VOC). Viral genome copy number, and expression of viral entry receptors, TLRs, interferons, and innate immune response genes were determined by qPCR. Viral entry receptors were examined in hCECs and tissue sections by immunostaining. Spike protein was detected in the cell culture supernatant by dot blot. RESULTS: Spike and envelope proteins were found in conjunctiva from COVID-19 patients. SARS-CoV-2 infected hCECs

showed high viral copy numbers at 24-72h post-infection; spike protein levels were the highest at 24hpi. Viral entry receptors ACE2, TMPRSS2, CD147, Axl, and NRP1 were detected in conjunctival tissue and hCECs. SARS-CoV-2 infection-induced receptor gene expression peaked at early time points post-infection, but gene expression of most TLRs peaked at 48 or 72hpi. SARS-CoV-2 infected hCECs showed higher expression of genes regulating antiviral response, RIG-I, interferons (alpha, beta, & lambda), ISG15 & OAS2, cytokines (IL6, IL1beta, TNFalpha), and chemokines (CXCL10, CCL5). Compared to the parental strain, beta VOC induced increased viral copy number and innate response in hCECs. CONCLUSIONS: Conjunctival epithelial cells are susceptible to SARS-CoV-2 infection. Beta VOC is more infectious than the parental strain and evokes a higher antiviral and inflammatory response.

Siqueira, J. D., et al. (2021). "Distinguishing SARS-CoV-2 bonafide re-infection from pre-existing minor variant reactivation." Infect Genet Evol **90**: 104772.

Different groups have recently reported events of SARS-CoV-2 reinfection, where patients had a sequence of positive-negative-positive RT-PCR tests. However, such events could be explained by different scenarios such as intermittent viral shedding, bonafide re-infection or multiple infection with alternating predominance of different viruses. Analysis of minor variants is an important tool to distinguish between these scenarios. Using ARTIC network PCR amplification and next-generation sequencing, we obtained SARS-CoV-2 sequences from two timepoints (with a time span of 102 days) of a patient followed at the Brazilian National Cancer Institute. Within-host variant analysis evidenced three single nucleotide variants (SNVs) at the consensus viral sequence in the second timepoint that were already present in the first timepoint as minor variants. Another five SNVs found in the second timepoint were not detected in the first sample sequenced, suggesting an additional infection by a yet another new virus. Our observation shed light into the existence of different viral populations that are present in dynamic frequencies and fluctuate during the course of SARS-CoV-2 infection. The detection of these variants in distinct disease events of an individual highlights a complex interplay between viral reactivation from a pre-existing minority variant and reinfection by a different virus.

Skidmore, P. T., et al. (2021). "Genomic Sequencing of SARS-CoV-2 E484K Variant B.1.243.1, Arizona, USA." Emerg Infect Dis **27**(10): 2718-2720.

Genomic surveillance can provide early insights into new circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants. While conducting genomic surveillance (1,663 cases) from December 2020-April 2021 in Arizona, USA, we detected an emergent E484K-harboring variant, B.1.243.1. This finding demonstrates the importance of real-time SARS-CoV-2 surveillance to better inform public health responses.

Slavov, S. N., et al. (2021). "Genomic monitoring unveil the early detection of the SARS-CoV-2 B.1.351 (beta) variant (20H/501Y.V2) in Brazil." J Med Virol **93**(12): 6782-6787.

Sao Paulo State, currently experiences a second COVID-19 wave overwhelming the healthcare system. Due to the paucity of SARS-CoV-2 complete genome sequencing, we established a Network for Pandemic Alert of Emerging SARS-CoV-2 Variants to rapidly understand and monitor the spread of SARS-CoV-2 variants into the state. Through analysis of 210 SARS-CoV-2 complete genomes obtained from the largest regional health departments we identified cocirculation of multiple SARS-CoV-2 lineages such as B.1.1 (0.5%), B.1.1.28 (23.2%), B.1.1.7 (alpha variant, 6.2%), B.1.566 (1.4%), B.1.544 (0.5%), C.37 (0.5%) P.1 (gamma variant, 66.2%), and P.2 (zeta variant, 1.0%). Our analysis allowed also the detection, for the first time in Brazil, the South African B.1.351 (beta) variant of concern, B.1.351 (501Y.V2) (0.5%), characterized by the following mutations: ORF1ab: T265I, R724K, S1612L, K1655N, K3353R, SGF 3675_F3677del, P4715L, E5585D; spike: D80A, D215G, L242_L244del, A262D, K417N, E484K, N501Y, D614G, A701V, C1247F; ORF3a: Q57H, S171L, E: P71L; ORF7b: Y10F, N: T205I; ORF14: L52F. The most recent common ancestor of the identified strain was inferred to be mid-October to late December 2020. Our analysis demonstrated the P.1 lineage predominance and allowed the early detection of the South African strain for the first time in Brazil. We highlight the importance of SARS-CoV-2 active monitoring to ensure the rapid detection of potential variants for pandemic control and vaccination strategies. Highlights Identification of B.1.351 (beta) variant of concern in the Sao Paulo State. Dissemination of SARS-CoV-2 variants of concern and interest in the Sao Paulo State. Mutational Profile of the circulating variants of concern and interest.

Solo, P. and M. A. Doss (2021). "Potential inhibitors of SARS-CoV-2 (COVID 19) spike protein of the delta and delta plus variant: In silico studies of medicinal plants of North-East India." Curr Res Pharmacol Drug Discov **2**: 100065.

Phytochemicals of 38 Medicinal plants of North-East India, with anti-viral, anti-oxidant or anti-bacterial properties were screened for properties of drug likeness. 231 phytochemicals were screened with LIPINSKI rule of five to obtain 131 candidates, which were further screened with SWISS-ADME, to obtain 50 phytochemicals. These phytochemicals were docked with the spike protein of the Delta variant (B.1.617.2)

and Delta-Plus (AY.1) variant of SARS-CoV-2 using Autodock Vina and MOE 09. The target proteins were constructed by homology modeling using Swiss-Model. Hydroxychloroquine, taken as a standard in docking analysis, exhibited a binding energy of -6.5 kcal/mol and -6.1 kcal/mol with respect to the Delta variant and Delta-Plus variant respectively. Among the 50 docked results most flavones showed very good docking scores. 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone, a Poly-Methoxyflavone, produced a highest docking score of -8.7 kcal/mol with respect to both the spike protein targets. Poly-Methoxyflavones and Poly-Ethoxyflavones exhibited good binding affinity for the target spike protein of SARS-CoV-2, and can be potential anti-viral drug candidates against the existing Delta variant of the SARS-CoV-2.

Somekh, I., et al. (2021). "Intrafamilial Spread and Altered Symptomatology of SARS-CoV-2, During Predominant Circulation of Lineage B.1.1.7 Variant in Israel." Pediatr Infect Dis J **40**(8): e310-e311.

The dynamics of intrafamilial spread of SARS-CoV-2 during January-February 2021 when variant B.1.1.7 predominated were compared with data from April to May 2020, when other circulating variants prevailed. Much higher intrafamilial transmission rates among all age groups, in particular in young children, and lower rates of sensory impairment were demonstrated during January-February 2021.

Sonabend, R., et al. (2021). "Non-pharmaceutical interventions, vaccination, and the SARS-CoV-2 delta variant in England: a mathematical modelling study." Lancet **398**(10313): 1825-1835.

BACKGROUND: England's COVID-19 roadmap out of lockdown policy set out the timeline and conditions for the stepwise lifting of non-pharmaceutical interventions (NPIs) as vaccination roll-out continued, with step one starting on March 8, 2021. In this study, we assess the roadmap, the impact of the delta (B.1.617.2) variant of SARS-CoV-2, and potential future epidemic trajectories. METHODS: This mathematical modelling study was done to assess the UK Government's four-step process to easing lockdown restrictions in England, UK. We extended a previously described model of SARS-CoV-2 transmission to incorporate vaccination and multi-strain dynamics to explicitly capture the emergence of the delta variant. We calibrated the model to English surveillance data, including hospital admissions, hospital occupancy, seroprevalence data, and population-level PCR testing data using a Bayesian evidence synthesis framework, then modelled the potential trajectory of the epidemic for a range of different schedules for relaxing NPIs. We estimated the resulting number of daily infections and hospital admissions, and daily and cumulative deaths. Three scenarios spanning a range of optimistic to pessimistic vaccine effectiveness, waning natural immunity, and cross-protection from previous infections were investigated. We also considered three levels of mixing after the lifting of restrictions. FINDINGS: The roadmap policy was successful in offsetting the increased transmission resulting from lifting NPIs starting on March 8, 2021, with increasing population immunity through vaccination. However, because of the emergence of the delta variant, with an estimated transmission advantage of 76% (95% credible interval [95% CrI] 69-83) over alpha, fully lifting NPIs on June 21, 2021, as originally planned might have led to 3900 (95% CrI 1500-5700) peak daily hospital admissions under our central parameter scenario. Delaying until July 19, 2021, reduced peak hospital admissions by three fold to 1400 (95% CrI 700-1700) per day. There was substantial uncertainty in the epidemic trajectory, with particular sensitivity to the transmissibility of delta, level of mixing, and estimates of vaccine effectiveness. INTERPRETATION: Our findings show that the risk of a large wave of COVID-19 hospital admissions resulting from lifting NPIs can be substantially mitigated if the timing of NPI relaxation is carefully balanced against vaccination coverage. However, with the delta variant, it might not be possible to fully lift NPIs without a third wave of hospital admissions and deaths, even if vaccination coverage is high. Variants of concern, their transmissibility, vaccine uptake, and vaccine effectiveness must be carefully monitored as countries relax pandemic control measures. FUNDING: National Institute for Health Research, UK Medical Research Council, Wellcome Trust, and UK Foreign, Commonwealth and Development Office.

Song, Y., et al. (2021). "COVID-19 Cases from the First Local Outbreak of the SARS-CoV-2 B.1.1.7 Variant in China May Present More Serious Clinical Features: A Prospective, Comparative Cohort Study." Microbiol Spectr **9**(1): e0027321.

The SARS-CoV-2 B.1.1.7 variant has increased sharply in numbers worldwide and is reported to be more contagious than the nonvariant. Little is known regarding the detailed clinical features of B.1.1.7 variant infection. Data on 74 COVID-19 cases from two outbreaks in two districts of Beijing, China were extracted from a cloud database, including 41 cases from Shunyi District (Shunyi B.1.470 group) and 33 from Daxing (Daxing B.1.1.7 group) from December 25, 2020 to January 17, 2021. We conducted a comparison of the clinical characteristics. Seven clinical indicators of the Daxing B.1.1.7 group were significantly higher than those of the Shunyi group, including the proportion with fever over 38 degrees C, the levels of C-reactive protein (CRP), serum amyloid

A (SAA), creatine kinase (CK), d-dimer (DD), and CD4(+) T lymphocytes (CD4(+) T), and the proportion with ground-glass opacity (GGO) in the lung (P values of </=0.05). After adjusting for age, B.1.1.7 variant infection was a risk factor for elevated CRP (P = 0.045), SAA (P = 0.011), CK (P = 0.034), and CD4(+) T (P = 0.029) and for the presence of GGO (P = 0.005). The median threshold cycle (CT) value of reverse transcriptase quantitative PCR (RT-qPCR) tests of the N gene target in the Daxing B.1.1.7 group was significantly lower (P = 0.036) than that in the Shunyi B.1.470 group. Clinical features, including a more serious inflammatory response, pneumonia, and a possibly higher viral load, were detected in the cases infected with B.1.1.7 SARS-CoV-2. The B.1.1.7 variant may have increased pathogenicity. IMPORTANCE The SARS-CoV-2 B.1.1.7 variant, which was first identified in the United Kingdom, has increased sharply in numbers worldwide and was reported to be more contagious than the nonvariant. To our knowledge, no studies investigating the detailed clinical features of COVID-19 cases infected with the B.1.1.7 variant have been published. Local epidemics have rarely occurred in China, but occasionally, a small clustered outbreak triggered by an imported SARS-CoV-2 strain with only one chain of transmission could happen. From late 2020 to early 2021, two clustered COVID-19 outbreaks occurred in Beijing, one of which was caused by the B.1.1.7 variant. The COVID-19 patients from the two outbreaks received similar clinical tests, diagnoses, and treatments. We found that the B.1.1.7 variant infection could lead to a more serious inflammatory response, acute response process, more severe pneumonia, and probably higher viral loads. This therefore implies that the B.1.1.7 variant may have increased pathogenicity.

Spinello, A., et al. (2021). "Allosteric Cross-Talk among Spike's Receptor-Binding Domain Mutations of the SARS-CoV-2 South African Variant Triggers an Effective Hijacking of Human Cell Receptor." J Phys Chem Lett **12**(25): 5987-5993.

The rapid and relentless emergence of novel highly transmissible SARS-CoV-2 variants, possibly decreasing vaccine efficacy, currently represents a formidable medical and societal challenge. These variants frequently hold mutations on the Spike protein's receptor-binding domain (RBD), which, binding to the angiotensin-converting enzyme 2 (ACE2) receptor, mediates viral entry into host cells. Here, all-atom molecular dynamics simulations and dynamical network theory of the wild-type and mutant RBD/ACE2 adducts disclose that while the N501Y mutation (UK variant) enhances the Spike's binding affinity toward ACE2, the concomitant N501Y, E484K, and K417N mutations (South African variant) aptly adapt to increase SARS-CoV-2 propagation via a two-pronged strategy: (i) effectively grasping ACE2 through an allosteric signaling between pivotal RBD structural elements and (ii) impairing the binding of antibodies elicited by infected or vaccinated patients. This information unlocks the molecular terms and evolutionary strategies underlying the increased virulence of emerging SARS-CoV-2 variants, setting the basis for developing the next-generation anti-COVID-19 therapeutics.

Srivastava, V. K., et al. (2021). "A Bioinformatics Approach for the Prediction of Immunogenic Properties and Structure of the SARS-COV-2 B.1.617.1 Variant Spike Protein." Biomed Res Int **2021**: 7251119.

Background: B.1.617.1, a variant of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causing respiratory illness is responsible for the second wave of COVID-19 and associated with a high incidence of infectivity and mortality. To mitigate the B.1.617.1 variant of SARS-CoV-2, deciphering the protein structure and immunological responses by employing bioinformatics tools for data mining and analysis is pivotal. Objectives: Here, an in silico approach was employed for deciphering the structure and immune function of the subunit of spike (S) protein of SARS-CoV-2 B.1.617.1 variant. Methods: The partial amino acid sequence of SARS-CoV-2 B.1.617.1 variant S protein was analyzed, and its putative secondary and tertiary structure was predicted. Immunogenic analyses including B- and T-cell epitopes, interferon-gamma (IFN-gamma) response, chemokine, and protective antigens for SARS-CoV 2 S proteins were predicted using appropriate tools. Results: B.1.617.1 variant S protein sequence was found to be highly stable and amphipathic. ABCpred and CTLpred analyses led to the identification of two potential antigenic B cell and T cell epitopes with starting amino acid positions at 60 and 82 (for B cell epitopes) and 54 and 98 (for T cell epitopes) having prediction scores > 0.8. Further, RAMPAGE tool was used for determining the allowed and disallowed regions of the three-dimensional predicted structure of SARS-CoV-2 B.1.617.1 variant S protein. Conclusion: Together, the in silico analysis revealed the predicted structure of partial S protein, immunogenic properties, and possible regions for S protein of SARS-CoV-2 and provides a valuable prelude for engineering the targeted vaccine or drug against B.1.617.1 variant of SARS-CoV-2.

Stamatatos, L., et al. (2021). "mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection." Science.

Emerging SARS-CoV-2 variants have raised concerns about resistance to neutralizing antibodies elicited by previous infection or vaccination. We examined whether sera from recovered and naive

donors collected prior to, and following immunizations with existing mRNA vaccines, could neutralize the Wuhan-Hu-1 and B.1.351 variants. Pre-vaccination sera from recovered donors neutralized Wuhan-Hu-1 and sporadically neutralized B.1.351, but a single immunization boosted neutralizing titers against all variants and SARS-CoV-1 by up to 1000-fold. Neutralization was due to antibodies targeting the receptor binding domain and was not boosted by a second immunization. Immunization of naive donors also elicited cross-neutralizing responses, but at lower titers. Our study highlights the importance of vaccinating both uninfected and previously infected persons to elicit cross-variant neutralizing antibodies.

Stamatatos, L., et al. (2021). "A single mRNA immunization boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection." medRxiv.

Emerging SARS-CoV-2 variants have raised concerns about resistance to neutralizing antibodies elicited by previous infection or vaccination. We examined whether sera from recovered and naive donors collected prior to, and following immunizations with existing mRNA vaccines, could neutralize the Wuhan-Hu-1 and B.1.351 variants. Pre-vaccination sera from recovered donors neutralized Wuhan-Hu-1 and sporadically neutralized B.1.351, but a single immunization boosted neutralizing titers against all variants and SARS-CoV-1 by up to 1000-fold. Neutralization was due to antibodies targeting the receptor binding domain and was not boosted by a second immunization. Immunization of naive donors also elicited cross-neutralizing responses, but at lower titers. Our study highlights the importance of vaccinating both uninfected and previously infected persons to elicit cross-variant neutralizing antibodies.

Stange, M., et al. (2021). "SARS-CoV-2 outbreak in a tri-national urban area is dominated by a B.1 lineage variant linked to a mass gathering event." PLoS Pathog **17**(3): e1009374.

The first case of SARS-CoV-2 in Basel, Switzerland was detected on February 26th 2020. We present a phylogenetic study to explore viral introduction and evolution during the exponential early phase of the local COVID-19 outbreak from February 26th until March 23rd. We sequenced SARS-CoV-2 naso-oropharyngeal swabs from 746 positive tests that were performed at the University Hospital Basel during the study period. We successfully generated 468 high quality genomes from unique patients and called variants with our COVID-19 Pipeline (COVGAP), and analysed viral genetic diversity using PANGOLIN taxonomic lineages. To identify introduction and dissemination events we incorporated global SARS-CoV-2 genomes and inferred a time-calibrated phylogeny. Epidemiological data from patient questionnaires was used to facilitate the interpretation of phylogenetic observations. The early outbreak in Basel was dominated by lineage B.1 (83.6%), detected first on March 2nd, although the first sample identified belonged to B.1.1. Within B.1, 68.2% of our samples fall within a clade defined by the SNP C15324T ('Basel cluster'), including 157 identical sequences at the root of the 'Basel cluster', some of which we can specifically trace to regional spreading events. We infer the origin of B.1-C15324T to mid-February in our tri-national region. The other genomes map broadly over the global phylogenetic tree, showing several introduction events from and/or dissemination to other regions of the world via travellers. Family transmissions can also be traced in our data. A single lineage variant dominated the outbreak in the Basel area while other lineages, such as the first (B.1.1), did not propagate. A mass gathering event was the predominant initial source of cases, with travel returners and family transmissions to a lesser extent. We highlight the importance of adding specific questions to epidemiological questionnaires, to obtain data on attendance of large gatherings and their locations, as well as travel history, to effectively identify routes of transmissions in up-coming outbreaks. This phylogenetic analysis in concert with epidemiological and contact tracing data, allows connection and interpretation of events, and can inform public health interventions. Trial Registration: ClinicalTrials.gov NCT04351503.

Staub, T., et al. (2021). "Case series of four re-infections with a SARS-CoV-2 B.1.351 variant, Luxembourg, February 2021." Euro Surveill **26**(18).

We describe four SARS-CoV-2 re-infections with a B.1.351 variant in 2021, in healthcare workers (HCWs) previously infected in 2020, before detection of this variant in Europe. Cases live in France, near the border with Luxembourg, where variants B.1.351 and B.1.1.7 circulated. All work in the same hospital unit where a cluster of COVID 19 with B1.351 variant occurred, affecting patients and HCWs. Before the cluster onset, HCWs used surgical masks, as per recommendations. After cluster onset, HCWs used FFP2 masks.

Stauft, C. B., et al. (2021). "The G614 pandemic SARS-CoV-2 variant is not more pathogenic than the original D614 form in adult Syrian hamsters." Virology **556**: 96-100.

Dynamic tracking of variant frequencies among viruses circulating in the global pandemic has revealed the emergence and dominance of a D614G mutation in the SARS-CoV-2 spike protein. To address whether pandemic SARS-CoV-2 G614 variant has evolved to become more pathogenic, we infected adult hamsters (>10 months old) with two natural SARS-CoV-2 variants carrying either D614 or G614 spike

protein to mimic infection of the adult/elderly human population. Hamsters infected by the two variants exhibited comparable viral loads and pathology in lung tissues as well as similar amounts of virus shed in nasal washes. Altogether, our study does not find that naturally circulating D614 and G614 SARS-CoV-2 variants differ significantly in pathogenicity in hamsters.

Sun, Y. S., et al. (2020). "A SARS-CoV-2 variant with the 12-bp deletion at E gene." Emerg Microbes Infect **9**(1): 2361-2367.

The coronavirus disease 2019 (COVID-19) pandemic is still ongoing and has become an important public health threat. This disease is caused by a new coronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, and so far, little is known about this virus. In this study, by using plaque purification, we purified two SARS-CoV-2 virus strains from the same specimen, one named F8 containing a 12-bp deletion in the E gene and the other named 8X containing the wild-type E gene. There was no significant difference in the viral titer and infectivity of these two strains. The S protein content of the F8 viral culture was 0.39 mug/ml, much higher than that of 8X. An inactivated vaccine made from the F8 strain could trigger high levels of the IgG titer and neutralizing antibody titer, which could last for at least 6 weeks and were significantly higher than those from the 8X strain at 1 and 3 weeks post vaccination, respectively. In conclusion, we reported that both the E gene mutant and wild-type SARS-CoV-2 strains were isolated from the same clinical sample by plaque purification. A 12-bp deletion in the E gene was important for SARS-CoV-2 replication and immunogenicity.

Supasa, P., et al. (2021). "Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera." Cell **184**(8): 2201-2211 e2207.

SARS-CoV-2 has caused over 2 million deaths in little over a year. Vaccines are being deployed at scale, aiming to generate responses against the virus spike. The scale of the pandemic and error-prone virus replication is leading to the appearance of mutant viruses and potentially escape from antibody responses. Variant B.1.1.7, now dominant in the UK, with increased transmission, harbors 9 amino acid changes in the spike, including N501Y in the ACE2 interacting surface. We examine the ability of B.1.1.7 to evade antibody responses elicited by natural SARS-CoV-2 infection or vaccination. We map the impact of N501Y by structure/function analysis of a large panel of well-characterized monoclonal antibodies. B.1.1.7 is harder to neutralize than parental virus, compromising neutralization by some members of a major class of public antibodies through light-chain contacts with residue 501. However, widespread escape from monoclonal antibodies or antibody responses generated by natural infection or vaccination was not observed.

Susky, E. K., et al. (2021). "Hospital outbreak of the severe acute respiratory coronavirus virus 2 (SARS-CoV-2) delta variant in partially and fully vaccinated patients and healthcare workers in Toronto, Canada." Infect Control Hosp Epidemiol: 1-4.

The severe acute respiratory coronavirus virus 2 (SARS-CoV-2) delta variant is highly transmissible, and current vaccines may have reduced effectiveness in preventing symptomatic infection. Using epidemiological and genomic analyses, we investigated an outbreak of the variant in an acute-care setting among partially and fully vaccinated individuals. Effective outbreak control was achieved using standard measures.

Tada, T., et al. (2021). "Convalescent-Phase Sera and Vaccine-Elicited Antibodies Largely Maintain Neutralizing Titer against Global SARS-CoV-2 Variant Spikes." mBio **12**(3): e0069621.

The increasing prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with spike protein mutations raises concerns that antibodies elicited by natural infection or vaccination and therapeutic monoclonal antibodies will become less effective. We show that convalescent-phase sera neutralize pseudotyped viruses with the B.1.1.7, B.1.351, B.1.1.248, COH.20G/677H, 20A.EU2, and mink cluster 5 spike proteins with only a minor loss in titer. Similarly, antibodies elicited by Pfizer BNT162b2 vaccination neutralized B.1.351 and B.1.1.248 with only a 3-fold decrease in titer, an effect attributable to E484K. Analysis of the Regeneron monoclonal antibodies REGN10933 and REGN10987 showed that REGN10933 has lost neutralizing activity against the B.1.351 and B.1.1.248 pseudotyped viruses, and the cocktail is 9- to 15-fold decreased in titer. These findings suggest that antibodies elicited by natural infection and by the Pfizer vaccine will maintain protection against the B.1.1.7, B.1.351, and B.1.1.248 variants but that monoclonal antibody therapy may be less effective for patients infected with B.1.351 or B.1.1.248 SARS-CoV-2. IMPORTANCE The rapid evolution of SARS-CoV-2 variants has raised concerns with regard to their potential to escape from vaccine-elicited antibodies and anti-spike protein monoclonal antibodies. We report here on an analysis of sera from recovered patients and vaccinated individuals and on neutralization by Regeneron therapeutic monoclonal antibodies. Overall, the variants were neutralized nearly as well as the wild-type pseudotyped virus. The B.1.351 variant was somewhat resistant to vaccine-elicited antibodies but was still readily neutralized. One of the two Regeneron therapeutic monoclonal antibodies seems to have lost most of its activity against the B.1.351 variant, raising

concerns that the combination therapy might be less effective for some patients. The findings should alleviate concerns that vaccines will become ineffective but suggest the importance of continued surveillance for potential new variants.

Tada, T., et al. (2021). "Neutralization of viruses with European, South African, and United States SARS-CoV-2 variant spike proteins by convalescent sera and BNT162b2 mRNA vaccine-elicited antibodies." bioRxiv.

The increasing prevalence of SARS-CoV-2 variants with mutations in the spike protein has raised concerns that recovered individuals may not be protected from reinfection and that current vaccines will become less effective. The B.1.1.7 isolate identified in the United Kingdom and B.1.351 isolate identified in the Republic of South Africa encode spike proteins with multiple mutations in the S1 and S2 subunits. In addition, variants have been identified in Columbus, Ohio (COH.20G/677H), Europe (20A.EU2) and in domesticated minks. Analysis by antibody neutralization of pseudotyped viruses showed that convalescent sera from patients infected prior to the emergence of the variant viruses neutralized viruses with the B.1.1.7, B.1.351, COH.20G/677H Columbus Ohio, 20A.EU2 Europe and mink cluster 5 spike proteins with only a minor decrease in titer compared to that of the earlier D614G spike protein. Serum specimens from individuals vaccinated with the BNT162b2 mRNA vaccine neutralized D614G virus with titers that were on average 7-fold greater than convalescent sera. Vaccine elicited antibodies neutralized virus with the B.1.1.7 spike protein with titers similar to D614G virus and neutralized virus with the B.1.351 spike with, on average, a 3-fold reduction in titer (1:500), a titer that was still higher than the average titer with which convalescent sera neutralized D614G (1:139). The reduction in titer was attributable to the E484K mutation in the RBD. The B.1.1.7 and B.1.351 viruses were not more infectious than D614G on ACE2.293T cells in vitro but N501Y, an ACE2 contacting residue present in the B.1.1.7, B.1.351 and COH.20G/677H spike proteins caused higher affinity binding to ACE2, likely contributing to their increased transmissibility. These findings suggest that antibodies elicited by primary infection and by the BNT162b2 mRNA vaccine are likely to maintain protective efficacy against B.1.1.7 and most other variants but that the partial resistance of virus with the B.1.351 spike protein could render some individuals less well protected, supporting a rationale for the development of modified vaccines containing E484K.

Tagliamonte, M. S., et al. (2021). "Rapid emergence and spread of SARS-CoV-2 gamma (P.1) variant in Haiti." Clin Infect Dis.

After an initial wave of COVID-19 in Haiti in summer 2020 (primarily lineage B.1), seropositivity for anti-SARS-CoV-2 IgG was ~40%. Variant P.1 (gamma) was introduced in February 2021, with an initially limited introduction followed by exponential local dissemination within this unvaccinated population with prior exposure to earlier SARS-CoV-2 lineages.

Tahan, S., et al. (2021). "SARS-CoV-2 E Gene Variant Alters Analytical Sensitivity Characteristics of Viral Detection Using a Commercial Reverse Transcription-PCR Assay." J Clin Microbiol **59**(7): e0007521.

Diagnostic assays for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are essential for patient management, infection prevention, and the public health response for coronavirus disease 2019 (COVID-19). The efficacy and reliability of these assays are of paramount importance in both tracking and controlling the spread of the virus. Real-time reverse transcription-PCR (RT-PCR) assays rely on a fixed genetic sequence for primer and probe binding. Mutations can potentially alter the accuracy of these assays and lead to unpredictable analytical performance characteristics and false-negative results. Here, we identify a G-to-U transversion (nucleotide 26372) in the SARS-CoV-2 E gene in three specimens with reduced viral detection efficiency using a widely available commercial assay. Further analysis of the public GISAID repository led to the identification of 18 additional genomes with this mutation, which reflect five independent mutational events. This work supports the use of dual-target assays to reduce the number of false-negative PCR results.

Tan, E., et al. (2021). "Use of Lateral Flow Immunoassay to Characterize SARS-CoV-2 RBD-Specific Antibodies and Their Ability to React with the UK, SA and BR P.1 Variant RBDs." Diagnostics (Basel) **11**(7).

Identifying anti-spike antibodies that exhibit strong neutralizing activity against current dominant circulating variants, and antibodies that are escaped by these variants, has important implications in the development of therapeutic and diagnostic solutions and in improving understanding of the humoral response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We characterized seven anti-SARS-CoV-2 receptor binding domain (RBD) antibodies for binding activity, pairing capability, and neutralization activity to SARS-CoV-2 and three variant RBDs via lateral flow immunoassays. The results allowed us to group these antibodies into three distinct epitope bins. Our studies showed that two antibodies had broadly potent neutralizing activity against SARS-CoV-2 and these variant RBDs and that one antibody did not neutralize the South African (SA) and Brazilian P.1 (BR P.1) RBDs. The antibody escaped by the SA and BR P.1 RBDs retained binding

activity to SA and BR P.1 RBDs but was unable to induce neutralization. We demonstrated that lateral flow immunoassay could be a rapid and effective tool for antibody characterization, including epitope classification and antibody neutralization kinetics. The potential contributions of the mutations (N501Y, E484K, and K417N/T) contained in these variants' RBDs to the antibody pairing capability, neutralization activity, and therapeutic antibody targeting strategy are discussed.

Tanaka, H., et al. (2021). "Increased Transmissibility of the SARS-CoV-2 Alpha Variant in a Japanese Population." Int J Environ Res Public Health **18**(15).

To assess the relative transmissibility of the SARS-CoV-2 Alpha variant compared to the pre-existing SARS-CoV-2 in Japan, we performed a cross-sectional study to determine the secondary attack rate of COVID-19 in household contacts before and after the Alpha variant became dominant in Osaka. We accessed 290 household contacts whose index cases were diagnosed between 1 and 20 December 2020 (the third epidemic group), at a time when Osaka was free of the Alpha variant. We also accessed 398 household contacts whose index cases were diagnosed between 20 April and 3 May 2021 (the fourth epidemic group), by which time the Alpha variant had become dominant. We identified 124 household contacts whose index case was determined positive for the Alpha variant (Alpha group) in this fourth group. The secondary attack rates in the fourth group (34.7%) and the Alpha group (38.7%) were significantly higher than that in the third group (19.3%, $p < 0.001$). Multivariable Poisson regression analysis with a robust error variance showed a significant excess risk in the fourth group (1.90, 95% CI = 1.47-2.48) and the Alpha group (2.34, 95% CI = 1.71-3.21). This finding indicates that the SARS-CoV-2 Alpha variant has an approximately 1.9-2.3-fold higher transmissibility than the pre-existing virus in the Japanese population.

Tasakis, R. N., et al. (2021). "SARS-CoV-2 variant evolution in the United States: High accumulation of viral mutations over time likely through serial Founder Events and mutational bursts." PLoS One **16**(7): e0255169.

Since the first case of COVID-19 in December 2019 in Wuhan, China, SARS-CoV-2 has spread worldwide and within a year and a half has caused 3.56 million deaths globally. With dramatically increasing infection numbers, and the arrival of new variants with increased infectivity, tracking the evolution of its genome is crucial for effectively controlling the pandemic and informing vaccine platform development. Our study explores evolution of SARS-CoV-2 in a representative cohort of sequences covering the entire genome in the United States, through all of 2020 and early 2021. Strikingly, we detected many accumulating Single Nucleotide Variations (SNVs) encoding amino acid changes in the SARS-CoV-2 genome, with a pattern indicative of RNA editing enzymes as major mutators of SARS-CoV-2 genomes. We report three major variants through October of 2020. These revealed 14 key mutations that were found in various combinations among 14 distinct predominant signatures. These signatures likely represent evolutionary lineages of SARS-CoV-2 in the U.S. and reveal clues to its evolution such as a mutational burst in the summer of 2020 likely leading to a homegrown new variant, and a trend towards higher mutational load among viral isolates, but with occasional mutation loss. The last quartile of 2020 revealed a concerning accumulation of mostly novel low frequency replacement mutations in the Spike protein, and a hypermutable glutamine residue near the putative furin cleavage site. Finally, end of the year data and 2021 revealed the gradual increase to prevalence of known variants of concern, particularly B.1.1.7, that have acquired additional Spike mutations. Overall, our results suggest that predominant viral genomes are dynamically evolving over time, with periods of mutational bursts and unabated mutation accumulation. This high level of existing variation, even at low frequencies and especially in the Spike-encoding region may become problematic when super-spreader events, akin to serial Founder Events in evolution, drive these rare mutations to prominence.

team, S. A.-C.-v. w. l. B. c. i. and S.-C.-v. w. l. B. c. i. t. Members of the (2021). "Linked transmission chains of imported SARS-CoV-2 variant B.1.351 across mainland France, January 2021." Euro Surveill **26**(13).

Two cases of confirmed SARS-CoV-2 infection with the B.1.351 variant were reported in France in mid-January, 2020. These cases attended a gathering in Mozambique in mid-December 2020. Investigations led to the identification of five imported cases responsible for 14 transmission chains and a total 36 cases. Epidemiological characteristics seemed comparable to those described before the emergence of the South African variant B.1.351. The lack of tertiary transmission outside of the personal sphere suggests that distancing and barrier measures were effective.

Tegally, H., et al. (2021). "Detection of a SARS-CoV-2 variant of concern in South Africa." Nature **592**(7854): 438-443.

Continued uncontrolled transmission of SARS-CoV-2 in many parts of the world is creating conditions for substantial evolutionary changes to the virus(1,2). Here we describe a newly arisen lineage of SARS-CoV-2 (designated 501Y.V2; also known as B.1.351 or 20H) that is defined by eight mutations in the spike protein, including three substitutions (K417N, E484K and N501Y) at residues in its receptor-binding

domain that may have functional importance(3-5). This lineage was identified in South Africa after the first wave of the epidemic in a severely affected metropolitan area (Nelson Mandela Bay) that is located on the coast of the Eastern Cape province. This lineage spread rapidly, and became dominant in Eastern Cape, Western Cape and KwaZulu-Natal provinces within weeks. Although the full import of the mutations is yet to be determined, the genomic data-which show rapid expansion and displacement of other lineages in several regions-suggest that this lineage is associated with a selection advantage that most plausibly results from increased transmissibility or immune escape(6-8).

Tejedor Vaquero, S., et al. (2021). "The mRNA-1273 Vaccine Induces Cross-Variant Antibody Responses to SARS-CoV-2 With Distinct Profiles in Individuals With or Without Pre-Existing Immunity." Front Immunol **12**: 737083.

mRNA-based vaccines effectively induce protective neutralizing antibodies against SARS-CoV-2, the etiological agent of COVID-19. Yet, the kinetics and compositional patterns of vaccine-induced antibody responses to the original strain and emerging variants of concern remain largely unknown. Here we characterized serum antibody classes and subclasses targeting the spike receptor-binding domain of SARS-CoV-2 wild type and alpha, beta, gamma and delta variants in a longitudinal cohort of SARS-CoV-2 naive and COVID-19 recovered individuals receiving the mRNA-1273 vaccine. We found that mRNA-1273 vaccine recipients developed a SARS-CoV-2-specific antibody response with a subclass profile comparable to that induced by natural infection. Importantly, these antibody responses targeted both wild type SARS-CoV-2 as well as its alpha, beta, gamma and delta variants. Following primary vaccination, individuals with pre-existing immunity showed higher induction of all antibodies but IgG3 compared to SARS-CoV-2-naive subjects. Unlike naive individuals, COVID-19 recovered subjects did not mount a recall antibody response upon the second vaccine dose. In these individuals, secondary immunization resulted in a slight reduction of IgG1 against the receptor-binding domain of beta and gamma variants. Despite the lack of recall humoral response, vaccinees with pre-existing immunity still showed higher titers of IgG1 and IgA to all variants analyzed compared to fully vaccinated naive individuals. Our findings indicate that mRNA-1273 vaccine triggered cross-variant antibody responses with distinct profiles in vaccinees with or without pre-existing immunity and suggest that individuals with prior history of SARS-CoV-2 infection may not benefit from the second mRNA vaccine dose with the current standard regimen.

Temsah, M. H., et al. (2021). "SARS-CoV-2 B.1.1.7 UK Variant of Concern Lineage-Related Perceptions, COVID-19 Vaccine Acceptance and Travel Worry Among Healthcare Workers." Front Public Health **9**: 686958.

Background: Healthcare workers' (HCWs') travel-related anxiety needs to be assessed in light of the emergence of SARS-CoV-2 mutations. Methods: An online, cross-sectional questionnaire among HCWs between December 21, 2020 to January 7, 2021. The outcome variables were HCWs' knowledge and awareness of the SARS-CoV-2 B.1.1.7 lineage that was recently reported as the UK variant of concern, and its associated travel worry and Generalized Anxiety Disorder (GAD-7) score. Results: A total of 1,058 HCWs completed the survey; 66.5% were female, 59.0% were nurses. 9.0% indicated they had been previously diagnosed with COVID-19. Regarding the B.1.1.7 lineage, almost all (97.3%) were aware of its emergence, 73.8% were aware that it is more infectious, 78.0% thought it causes more severe disease, and only 50.0% knew that current COVID-19 vaccines are effective in preventing it. Despite this, 66.7% of HCWs were not registered to receive the vaccine. HCWs' most common source of information about the new variant was social media platforms (67.0%), and this subgroup was significantly more worried about traveling. Nurses were more worried than physicians (P = 0.001). Conclusions: Most HCWs were aware of the emergence of the SARS-CoV-2 B.1.1.7 variant and expressed substantial travel worries. Increased worry levels were found among HCWs who used social media as their main source of information, those with lower levels of COVID-19 vaccine uptake, and those with higher GAD-7 scores. The utilization of official social media platforms could improve accurate information dissemination among HCWs regarding the Pandemic's evolving mutations. Targeted vaccine campaigns are warranted to assure HCWs about the efficacy of COVID-19 vaccines toward SARS-CoV-2 variants.

Umair, M., et al. (2021). "Whole-genome sequencing of SARS-CoV-2 reveals the detection of G614 variant in Pakistan." PLoS One **16**(3): e0248371.

Since its emergence in China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide including Pakistan. During the pandemic, whole genome sequencing has played an important role in understanding the evolution and genomic diversity of SARS-CoV-2. Although an unprecedented number of SARS-CoV-2 full genomes have been submitted in GISAID and NCBI, data from Pakistan is scarce. We report the sequencing, genomic characterization, and phylogenetic analysis of five SARS-CoV-2 strains isolated from patients in Pakistan. The oropharyngeal swabs of patients that were confirmed positive for SARS-CoV-2 through real-time RT-PCR at National Institute of Health, Pakistan, were

selected for whole-genome sequencing. Sequencing was performed using NEBNext Ultra II Directional RNA Library Prep kit for Illumina (NEW ENGLAND BioLabs Inc., MA, US) and Illumina iSeq 100 instrument (Illumina, San Diego, US). Based on whole-genome analysis, three Pakistani SARS-CoV-2 strains clustered into the 20A (GH) clade along with the strains from Oman, Slovakia, United States, and Pakistani strain EPI_ISL_513925. The two 19B (S)-clade strains were closely related to viruses from India and Oman. Overall, twenty-nine amino acid mutations were detected in the current study genome sequences, including fifteen missense and four novel mutations. Notably, we have found a D614G (aspartic acid to glycine) mutation in spike protein of the sequences from the GH clade. The G614 variant carrying the characteristic D614G mutation has been shown to be more infectious that lead to its rapid spread worldwide. This report highlights the detection of GH and S clade strains and G614 variant from Pakistan warranting large-scale whole-genome sequencing of strains prevalent in different regions to understand virus evolution and to explore their genetic diversity.

Umair, M., et al. (2021). "Proliferation of SARS-CoV-2 B.1.1.7 Variant in Pakistan-A Short Surveillance Account." Front Public Health **9**: 683378.

The emergence of a more transmissible variant of SARS-CoV-2 (B1. 1.7) in the United Kingdom (UK) during late 2020 has raised major public health concerns. Several mutations have been reported in the genome of the B.1.1.7 variant including the N501Y and 69-70deletion in the Spike region that has implications on virus transmissibility and diagnostics. Although the B.1.1.7 variant has been reported by several countries, only three cases have been reported in Pakistan through whole-genome sequencing. Therefore, the objective of the study was to investigate the circulation of B.1.1.7 variant of concern (VOC) in Pakistani population. We used a two-step strategy for the detection of B.1.1.7 with initial screening through TaqPath(TM) COVID-19 CE-IVD RT-PCR kit (ThermoFisher Scientific, Waltham, US) followed by partial spike (S) gene sequencing of a subset of samples having the spike gene target failure (SGTF). From January 01, 2021, to February 21, 2021, a total of 2,650 samples were tested for SARS-CoV-2 and 70.4% (n = 1,867) showed amplification of all the 3 genes (ORF, N, and S). Notably, 29.6% (n=783) samples have been SGTF that represented numbers from all the four provinces and suggest a rather low frequency during the first 3 weeks of January (n = 10, n = 13, and n = 1, respectively). However, the numbers have started to increase in the last week of January, 2021. During February, 726 (93%) cases of SGTF were reported with a peak (n = 345) found during the 3rd week. Based on the partial sequencing of SGTF samples 93.5% (n = 29/31) showed the characteristic N501Y, A570D, P681H, and T716I mutations found in the B.1.1.7 variant. In conclusion, our findings showed an upsurge of B.1.1.7 cases in Pakistan during February, 2021 affecting 15 districts and warranting large scale genomic surveillance, strengthening of laboratory network and implementation of appropriate control measures in the country.

Wang, H., et al. (2021). "Mutation-Specific SARS-CoV-2 PCR Screen: Rapid and Accurate Detection of Variants of Concern and the Identification of a Newly Emerging Variant with Spike L452R Mutation." J Clin Microbiol **59**(8): e0092621.

The emergence of more transmissible and/or more virulent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOC) has triggered intensive genomic surveillance, which is costly and difficult to sustain operationally over the long term. To address this problem, we developed a set of four multiplex mutation-specific PCR-based assays with same-day reporting that can detect five VOC and three variants of interest (VOI), as defined in the March 2021 guidelines from the U.S. Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/). The screening results were compared to the whole-genome sequencing (WGS) and showed 100% concordance for strain typing for B.1.1.7 (n = 25) and P.1 (n = 5) variants using spike (S) mutation S-N501Y, S-E484K, and S-H69-V70del assays. The S-L450R assay, designed to detect the B.1.427/429 VOC, also identified multiple isolates of a newly emerging multiply mutated B.1.526.1 variant that is now rapidly increasing in the eastern United States. PCR approaches can be easily adopted in clinical laboratories, providing rapid screening methods to allow early detection of newly emergent variants and to efficiently triage cases for full genomic sequencing.

Wang, H., et al. (2021). "Multiplex SARS-CoV-2 Genotyping Reverse Transcriptase PCR for Population-Level Variant Screening and Epidemiologic Surveillance." J Clin Microbiol **59**(8): e0085921.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with concerning phenotypic mutations is of public health interest. Genomic surveillance is an important tool for a pandemic response, but many laboratories do not have the resources to support population-level sequencing. We hypothesized that a nucleic acid amplification test (NAAT) to genotype mutations in the viral spike protein could facilitate high-throughput variant surveillance. We designed and analytically validated a one-step multiplex allele-specific reverse transcriptase PCR (RT-qPCR) to detect three nonsynonymous spike protein mutations (L452R, E484K, N501Y). Assay specificity was validated with

next-generation whole-genome sequencing. We then screened a large cohort of SARS-CoV-2-positive specimens from our San Francisco Bay Area population. Between 1 December 2020 and 1 March 2021, we screened 4,049 unique infections by genotyping RT-qPCR, with an assay failure rate of 2.8%. We detected 1,567 L452R mutations (38.7%), 34 N501Y mutations (0.84%), 22 E484K mutations (0.54%), and 3 (0.07%) E484K plus N501Y mutations. The assay had perfect (100%) concordance with whole-genome sequencing of a validation subset of 229 specimens and detected B.1.1.7, B.1.351, B.1.427, B.1.429, B.1.526, and P.2 variants, among others. The assay revealed the rapid emergence of the L452R variant in our population, with a prevalence of 24.8% in December 2020 that increased to 62.5% in March 2021. We developed and clinically implemented a genotyping RT-qPCR to conduct high-throughput SARS-CoV-2 variant screening. This approach can be adapted for emerging mutations and immediately implemented in laboratories already performing NAAT worldwide using existing equipment, personnel, and extracted nucleic acid.

Wang, M., et al. (2021). "Reduced sensitivity of the SARS-CoV-2 Lambda variant to monoclonal antibodies and neutralizing antibodies induced by infection and vaccination." Emerg Microbes Infect: 1-30.

AbstractSevere acute respiratory syndrome coronavirus 2 variants have continued to emerge in diverse geographic locations with a temporal distribution. The Lambda variant containing multiple mutations in the spike protein, has thus far appeared mainly in South America. The variant harbours two mutations in the receptor binding domain, L452Q and F490S, which may change its infectivity and antigenicity to neutralizing antibodies. In this study, we constructed 10 pseudoviruses to study the Lambda variant and each individual amino acid mutation's effect on viral function, and used eight cell lines to study variant infectivity. In total, 12 monoclonal antibodies, 14 convalescent sera, and 23 immunized sera induced by mRNA vaccines, inactivated vaccine, and adenovirus type 5 vector vaccine were used to study the antigenicity of the Lambda variant. We found that compared with the D614G reference strain, Lambda demonstrated enhanced infectivity of Calu-3 and LLC-MK2 cells by 3.3-fold and 1.6-fold, respectively. Notably, the sensitivity of the Lambda variant to 5 of 12 neutralizing monoclonal antibodies, 9G11, AM180, R126, X593, and AbG3, was substantially diminished. Furthermore, convalescent- and vaccine-immunized sera showed on average 1.3-2.5-fold lower neutralizing titres against the Lambda variant. Single mutation analysis revealed that this reduction in neutralization was caused by L452Q and F490S mutations. Collectively, the reduced neutralization ability of the Lambda variant suggests that the efficacy of monoclonal antibodies and vaccines may be compromised during the current pandemic.

Wang, P., et al. (2021). "Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization." Cell Host Microbe **29**(5): 747-751 e744.

The emergence of SARS-CoV-2 variants has raised concerns about altered sensitivity to antibody-mediated immunity. The relative resistance of SARS-CoV-2 variants B.1.1.7 and B.1.351 to antibody neutralization has been recently investigated. We report that another emergent variant from Brazil, P.1, is not only refractory to multiple neutralizing monoclonal antibodies but also more resistant to neutralization by convalescent plasma and vaccinee sera. The magnitude of resistance is greater for monoclonal antibodies than vaccinee sera and evident with both pseudovirus and authentic P.1 virus. The cryoelectron microscopy structure of a soluble prefusion-stabilized spike reveals that the P.1 trimer adopts exclusively a conformation in which one of the receptor-binding domains is in the "up" position, which is known to facilitate binding to entry receptor ACE2. The functional impact of P.1 mutations thus appears to arise from local changes instead of global conformational alterations. The P.1 variant threatens current antibody therapies but less so protective vaccine efficacy.

Wang, P., et al. (2021). "Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization." bioRxiv.

The relative resistance of SARS-CoV-2 variants B.1.1.7 and B.1.351 to antibody neutralization has been described recently. We now report that another emergent variant from Brazil, P.1, is not only refractory to multiple neutralizing monoclonal antibodies, but also more resistant to neutralization by convalescent plasma (3.4 fold) and vaccinee sera (3.8-4.8 fold). The cryo-electron microscopy structure of a soluble prefusion-stabilized spike reveals the P.1 trimer to adopt exclusively a conformation in which one of the receptor-binding domains is in the "up" position, with the functional impact of mutations appearing to arise from local changes instead of global conformational alterations. The P.1 variant threatens current antibody therapies but less so the protective efficacy of our vaccines.

Wang, P., et al. (2021). "Characterization of an attenuated SARS-CoV-2 variant with a deletion at the S1/S2 junction of the spike protein." Nat Commun **12**(1): 2790.

SARS-CoV-2 is of zoonotic origin and contains a PRRA polybasic cleavage motif which is considered critical for efficient infection and transmission in humans. We previously reported on a panel of attenuated SARS-CoV-2 variants with

deletions at the S1/S2 junction of the spike protein. Here, we characterize pathogenicity, immunogenicity, and protective ability of a further cell-adapted SARS-CoV-2 variant, Ca-DelMut, in in vitro and in vivo systems. Ca-DelMut replicates more efficiently than wild type or parental virus in Vero E6 cells, but causes no apparent disease in hamsters, despite replicating in respiratory tissues. Unlike wild type virus, Ca-DelMut causes no obvious pathological changes and does not induce elevation of proinflammatory cytokines, but still triggers a strong neutralizing antibody and T cell response in hamsters and mice. Ca-DelMut immunized hamsters challenged with wild type SARS-CoV-2 are fully protected, with little sign of virus replication in the upper or lower respiratory tract, demonstrating sterilizing immunity.

Wang, R., et al. (2021). "Analysis of SARS-CoV-2 variant mutations reveals neutralization escape mechanisms and the ability to use ACE2 receptors from additional species." Immunity **54**(7): 1611-1621 e1615.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants continue to emerge during the global pandemic and may facilitate escape from current antibody therapies and vaccine protection. Here we showed that the South African variant B.1.351 was the most resistant to current monoclonal antibodies and convalescent plasma from coronavirus disease 2019 (COVID-19)-infected individuals, followed by the Brazilian variant P.1 and the United Kingdom variant B.1.1.7. This resistance hierarchy corresponded with Y144del and 242-244del mutations in the N-terminal domain and K417N/T, E484K, and N501Y mutations in the receptor-binding domain (RBD) of SARS-CoV-2. Crystal structure analysis of the B.1.351 triple mutant (417N-484K-501Y) RBD complexed with the monoclonal antibody P2C-1F11 revealed the molecular basis for antibody neutralization and escape. B.1.351 and P.1 also acquired the ability to use mouse and mink ACE2 receptors for entry. Our results demonstrate major antigenic shifts and potential broadening of the host range for B.1.351 and P.1 variants, which poses serious challenges to current antibody therapies and vaccine protection.

Wilton, T., et al. (2021). "Rapid Increase of SARS-CoV-2 Variant B.1.1.7 Detected in Sewage Samples from England between October 2020 and January 2021." mSystems **6**(3): e0035321.

SARS-CoV-2 variants with multiple amino acid mutations in the spike protein are emerging in different parts of the world, raising concerns regarding their possible impact on human immune response and vaccine efficacy against the virus. Recently, a variant named lineage B.1.1.7 was detected and shown to be rapidly spreading across the UK since November 2020. As surveillance for these SARS-CoV-2 variants of concern (VOCs) becomes critical, we have investigated the use of environmental surveillance (ES) for the rapid detection and quantification of B.1.1.7 viruses in sewage as a way of monitoring its expansion that is independent on the investigation of identified clinical cases. Next-generation sequencing analysis of amplicons synthesized from sewage concentrates revealed the presence of B.1.1.7 mutations in viral sequences, first identified in a sample collected in London on 10 November 2020 and shown to rapidly increase in frequency to >95% in January 2021, in agreement with clinical data over the same period. We show that ES can provide an early warning of VOCs becoming prevalent in the population and that, as well as B.1.1.7, our method can detect VOCs B.1.351 and P.1, first identified in South Africa and Brazil, respectively, and other viruses carrying critical spike mutation E484K, known to have an effect on virus antigenicity. Although we did not detect such mutation in viral RNAs from sewage, we did detect mutations at amino acids 478, 490, and 494, located close to amino acid 484 in the spike protein structure and known to also have an effect on antigenicity. IMPORTANCE The recent appearance and growth of new SARS-CoV-2 variants represent a major challenge for the control of the COVID-19 pandemic. These variants of concern contain mutations affecting antigenicity, which raises concerns on their possible impact on human immune response to the virus and vaccine efficacy against them. Here, we show how environmental surveillance for SARS-CoV-2 can be used to help us understand virus transmission patterns and provide an early warning of variants becoming prevalent in the population. We describe the detection and quantification of variant B.1.1.7, first identified in southeast England in sewage samples from London (UK) before widespread transmission of this variant was obvious from clinical cases. Variant B.1.1.7 was first detected in a sample from early November 2020, with the frequency of B.1.1.7 mutations detected in sewage rapidly increasing to >95% in January 2021, in agreement with increasing SARS-CoV-2 infections associated with B.1.1.7 viruses.

Wu, K., et al. (2021). "Variant SARS-CoV-2 mRNA vaccines confer broad neutralization as primary or booster series in mice." bioRxiv.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of a global pandemic that has led to more than 2.8 million deaths worldwide. Safe and effective vaccines are now available, including Moderna's COVID-19 vaccine (mRNA-1273) that showed 94% efficacy in prevention of symptomatic COVID-19 disease in a phase 3 clinical study. mRNA-1273 encodes for a prefusion stabilized full length spike (S) protein of the Wuhan-Hu-1 isolate.

However, the emergence of SARS-CoV-2 variants has led to concerns of viral escape from vaccine-induced immunity. Several emerging variants have shown decreased susceptibility to neutralization by vaccine induced immunity, most notably the B.1.351 variant, although the overall impact on vaccine efficacy remains to be determined. Here, we present the initial evaluation in mice of two updated COVID-19 mRNA vaccines designed to target emerging SARS-CoV-2 variants: (1) monovalent mRNA-1273.351 encodes for the S protein found in the B.1.351 lineage and (2) mRNA-1273.211 comprising a 1:1 mix of mRNA-1273 and mRNA-1273.351. Both vaccines were evaluated as a 2-dose primary series in mice; mRNA-1273.351 was also evaluated as a booster dose in animals previously vaccinated with 2-doses of mRNA-1273. The results demonstrated that a primary vaccination series of mRNA-1273.351 was effective at increasing neutralizing antibody titers against the B.1.351 lineage, while mRNA-1273.211 was most effective at providing broad cross-variant neutralization in mice. In addition, these results demonstrated a third dose of mRNA-1273.351 significantly increased both wild-type and B.1.351-specific neutralization titers. Both mRNA-1273.351 and mRNA-1273.211 are currently being evaluated in additional pre-clinical challenge models and in phase 1/2 clinical studies.

Wu, K., et al. (2021). "Variant SARS-CoV-2 mRNA vaccines confer broad neutralization as primary or booster series in mice." Vaccine.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of a global pandemic. Safe and effective COVID-19 vaccines are now available, including mRNA-1273, which has shown 94% efficacy in prevention of symptomatic COVID-19 disease. However, the emergence of SARS-CoV-2 variants has led to concerns of viral escape from vaccine-induced immunity. Several variants have shown decreased susceptibility to neutralization by vaccine-induced immunity, most notably B.1.351 (Beta), although the overall impact on vaccine efficacy remains to be determined. Here, we present the initial evaluation in mice of 2 updated mRNA vaccines designed to target SARS-CoV-2 variants: (1) monovalent mRNA-1273.351 encodes for the spike protein found in B.1.351 and (2) mRNA-1273.211 comprising a 1:1 mix of mRNA-1273 and mRNA-1273.351. Both vaccines were evaluated as a 2-dose primary series in mice; mRNA-1273.351 was also evaluated as a booster dose in animals previously vaccinated with mRNA-1273. The results demonstrated that a primary vaccination series of mRNA-1273.351 was effective at increasing neutralizing antibody titers against B.1.351, while mRNA-1273.211 was effective at providing broad cross-variant neutralization. A third (booster) dose of mRNA-1273.351 significantly increased both wild-type and B.1.351-specific neutralization titers. Both mRNA-1273.351 and mRNA-1273.211 are being evaluated in pre-clinical challenge and clinical studies.

Wu, L., et al. (2021). "Exploring the immune evasion of SARS-CoV-2 variant harboring E484K by molecular dynamics simulations." Brief Bioinform.

Although the current coronavirus disease 2019 (COVID-19) vaccines have been used worldwide to halt spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the emergence of new SARS-CoV-2 variants with E484K mutation shows significant resistance to the neutralization of vaccine sera. To better understand the resistant mechanism, we calculated the binding affinities of 26 antibodies to wild-type (WT) spike protein and to the protein harboring E484K mutation, respectively. The results showed that most antibodies (~85%) have weaker binding affinities to the E484K mutated spike protein than to the WT, indicating the high risk of immune evasion of the mutated virus from most of current antibodies. Binding free energy decomposition revealed that the residue E484 forms attraction with most antibodies, while the K484 has repulsion from most antibodies, which should be the main reason of the weaker binding affinities of E484K mutant to most antibodies. Impressively, a monoclonal antibody (mAb) combination was found to have much stronger binding affinity with E484K mutant than WT, which may work well against the mutated virus. Based on binding free energy decomposition, we predicted that the mutation of four more residues on receptor-binding domain (RBD) of spike protein, viz., F490, V483, G485 and S494, may have high risk of immune evasion, which we should pay close attention on during the development of new mAb therapeutics.

Wu, S. S., et al. (2021). "[Survey on source of infection of the first local outbreak caused by SARS-CoV-2 Alpha variant in China]." Zhonghua Yu Fang Yi Xue Za Zhi **55**(11): 1311-1315.

Objective: To investigate the epidemiological characteristics and the chain of infection of a local outbreak, which was the first outbreak caused by severe acute respiratory syndrome corona virus 2 Alpha variant in China and occurred in Daxing district, Beijing. Methods: Epidemiological investigation and big data technology were used to verify the exposure points of the cases. Close contacts were traced from the exposure points, and their human and environmental samples were collected for nucleic acid tests. Serum samples were collected from key persons for antibody detection. Results: A total of 33 corona virus disease 2019(COVID-19) cases were reported in the local outbreak, from January 17, 2021 to January 29, 2021 in Daxing district, Beijing, and there was epidemiological association in 32 cases. Except for one case who was

infected in the workplace, other cases were all infected in the community and family. All cases involved 14 families, of which 6 families were all infected. The attack rate of all family members was 69%(33/48), and the secondary attack rate was 56%(19/34). There was no obvious source of infection found after the investigation of entry-exit personnel and goods. Conclusion: The first outbreak caused by severe acute respiratory syndrome corona virus 2 Alpha variant in China is found and handled in time, and thus the scope of influence is limited, but the family clustering characteristics are more obvious than previous outbreaks.

Wuertz, K. M., et al. (2021). "A SARS-CoV-2 spike ferritin nanoparticle vaccine protects hamsters against Alpha and Beta virus variant challenge." NPJ Vaccines **6**(1): 129.

The emergence of SARS-CoV-2 variants of concern (VOC) requires adequate coverage of vaccine protection. We evaluated whether a SARS-CoV-2 spike ferritin nanoparticle vaccine (SpFN), adjuvanted with the Army Liposomal Formulation QS21 (ALFQ), conferred protection against the Alpha (B.1.1.7), and Beta (B.1.351) VOCs in Syrian golden hamsters. SpFN-ALFQ was administered as either single or double-vaccination (0 and 4 week) regimens, using a high (10 mug) or low (0.2 mug) dose. Animals were intranasally challenged at week 11. Binding antibody responses were comparable between high- and low-dose groups. Neutralizing antibody titers were equivalent against WA1, B.1.1.7, and B.1.351 variants following two high dose vaccinations. Dose-dependent SpFN-ALFQ vaccination protected against SARS-CoV-2-induced disease and viral replication following intranasal B.1.1.7 or B.1.351 challenge, as evidenced by reduced weight loss, lung pathology, and lung and nasal turbinate viral burden. These data support the development of SpFN-ALFQ as a broadly protective, next-generation SARS-CoV-2 vaccine.

Wysocki, J., et al. (2021). "A Novel Soluble ACE2 Variant with Prolonged Duration of Action Neutralizes SARS-CoV-2 Infection in Human Kidney Organoids." J Am Soc Nephrol.

BACKGROUND: There is an urgent need for approaches to prevent and treat SARS-CoV-2 infection. Administration of soluble ACE2 protein acting as a decoy to bind to SARS-CoV-2 should limit viral uptake mediated by binding to membrane-bound full-length ACE2, and further therapeutic benefit should result from ensuring enzymatic ACE2 activity to affected organs in patients with COVID-19. METHODS: A short variant of human soluble ACE2 protein consisting of 618 amino acids (hACE2 1-618) was generated and fused with an albumin binding domain (ABD) using an artificial gene encoding ABDCon, with improved albumin binding affinity. Human kidney organoids were used for infectivity studies of SARS-CoV-2 in a BSL-3 facility to examine the neutralizing effect of these novel ACE2 variants. RESULTS: Whereas plasma ACE2 activity of the naked ACE2 1-618 and ACE2 1-740 lasted about 8 hours, the ACE2 1-618-ABD resulted in substantial activity at 96 hours, and it was still biologically active 3 days after injection. Human kidney organoids express ACE2 and TMPRSS2, and when infected with SARS-CoV-2, our modified long-acting ACE2 variant neutralized infection. CONCLUSIONS: This novel ACE2 1-618-ABD can neutralize SARS-CoV-2 infectivity in human kidney organoids, and its prolonged duration of action should ensure improved efficacy to prevent viral escape and dosing convenience.

Yang, W. and J. Shaman (2021). "COVID-19 pandemic dynamics in India, the SARS-CoV-2 Delta variant, and implications for vaccination." medRxiv.

Background: The COVID-19 Delta pandemic wave in India surged and declined within 3 months; cases then remained low despite the continued spread of Delta elsewhere. Here we aim to estimate key epidemiological characteristics of the Delta variant based on data from India and examine the underpinnings of its dynamics. Methods: We utilize multiple datasets and model-inference methods to reconstruct COVID-19 pandemic dynamics in India during March 2020 - June 2021. We further use model estimates to retrospectively predict cases and deaths during July - mid-Oct 2021, under various vaccination and vaccine effectiveness (VE) settings to estimate the impact of vaccination and VE for non-Delta-infection recoverees. Findings: We estimate that Delta escaped immunity in 34.6% (95% CI: 0 - 64.2%) of individuals with prior wildtype infection and was 57.0% (95% CI: 37.9 - 75.6%) more infectious than wildtype SARS-CoV-2. Models assuming higher VE among those with prior non-Delta infection, particularly after the 1 (st) dose, generated more accurate predictions than those assuming no such increases (best-performing VE setting: 90/95% vs. 30/67% baseline for the 1 (st) /2 (nd) dose). Counterfactual modeling indicates that high vaccination coverage for 1 (st) vaccine-dose in India (approximately 50% by mid-Oct 2021) combined with the boosting of VE among recoverees averted around 60% of infections during July - mid-Oct 2021. Interpretation: Non-pharmaceutical interventions, infection seasonality, and high coverage of 1-dose vaccination likely all contributed to pandemic dynamics in India during 2021. Given the shortage of COVID-19 vaccines globally and boosting of VE, for populations with high prior infection rates, prioritizing the first vaccine-dose may protect more people. Research in context: Evidence before this study: We searched PubMed for studies published through Nov 3,

2021 on the Delta (B.1.617.2) SARS-CoV-2 variant that focused on three areas: 1) transmissibility [search terms: ("Delta variant" OR "B.1.617") AND ("transmission rate" OR "growth rate" OR "secondary attack rate" OR "transmissibility")]; 2) immune response ([search terms: ("Delta variant" OR "B.1.617") AND ("immune evas" OR "immune escape")]; and 3) vaccine effectiveness ([search terms: ("Delta variant" OR "B.1.617") AND ("vaccine effectiveness" OR "vaccine efficacy" OR "vaccination")]. Our search returned 256 papers, from which we read the abstracts and identified 54 relevant studies.Forty-two studies addressed immune evasion and/or vaccine effectiveness. Around half (n=19) of these studies measured the neutralizing ability of convalescent sera and/or vaccine sera against Delta and most reported some reduction (around 2-to 8-fold) compared to ancestral variants. The remainder (n=23) used field observations (often with a test-negative or cohort-design) and reported lower VE against infection but similar VE against hospitalization or death. Together, these laboratory and field observations consistently indicate that Delta can evade preexisting immunity. In addition, five studies reported higher B-cell and/or T-cell vaccine-induced immune response among recovered vaccinees than naive vaccinees, suggesting potential boosting of pre-existing immunity; however, all studies were based on small samples (n = 10 to 198 individuals).Sixteen studies examined transmissibility, including 1) laboratory experiments (n=6) showing that Delta has higher affinity to the cell receptor, fuses membranes more efficiently, and/or replicates faster than other SARS-CoV-2 variants, providing biological mechanisms for its higher transmissibility; 2) field studies (n=5) showing higher rates of breakthrough infections by Delta and/or higher viral load among Delta infections than other variants; and 3) modeling/mixed studies (n=5) using genomic or case data to estimate the growth rate or reproduction number, reporting a 60-120% increase. Only one study jointly estimated the increase in transmissibility (1.3-1.7-fold, 50% CI) and immune evasion (10-50%, 50% CI); this study also reported a 27.5% (25/91) reinfection rate by Delta.Added value of this study: We utilize observed pandemic dynamics and the differential vaccination coverage for two vaccine doses in India, where the Delta variant was first identified, to estimate the epidemiological properties of Delta and examine the impact of prior non-Delta infection on immune boosting at the population level. We estimate that Delta variant can escape immunity from prior wildtype infection roughly one-third of the time and is around 60% more infectious than wildtype SARS-CoV-2. In addition, our analysis suggests the large increase in population receiving their first vaccine dose (approximately 50% by end of Oct 2021) combined with the boosting effect of vaccination for non-Delta infection recoverees likely mitigated epidemic intensity in India during July - Oct 2021.Implications of all the available evidence: Our analysis reconstructs the interplay and effects of non-pharmaceutical interventions, infection seasonality, Delta variant emergence, and vaccination on COVID-19 pandemic dynamics in India. Modeling findings support prioritizing the first vaccine dose in populations with high prior infection rates, given vaccine shortages.

Yurkovetskiy, L., et al. (2020). "Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant." bioRxiv.

The SARS-CoV-2 spike (S) protein variant D614G supplanted the ancestral virus worldwide in a matter of months. Here we show that D614G was more infectious than the ancestral form on human lung cells, colon cells, and cells rendered permissive by ectopic expression of various mammalian ACE2 orthologs. Nonetheless, D614G affinity for ACE2 was reduced due to a faster dissociation rate. Assessment of the S protein trimer by cryo-electron microscopy showed that D614G disrupts a critical interprotomer contact and that this dramatically shifts the S protein trimer conformation toward an ACE2-binding and fusion-competent state. Consistent with the more open conformation, neutralization potency of antibodies targeting the S protein receptor-binding domain was not attenuated. These results indicate that D614G adopts conformations that make virion membrane fusion with the target cell membrane more probable but that D614G retains susceptibility to therapies that disrupt interaction of the SARS-CoV-2 S protein with the ACE2 receptor.

Yurkovetskiy, L., et al. (2020). "Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant." Cell **183**(3): 739-751 e738.

The SARS-CoV-2 spike (S) protein variant D614G supplanted the ancestral virus worldwide, reaching near fixation in a matter of months. Here we show that D614G was more infectious than the ancestral form on human lung cells, colon cells, and on cells rendered permissive by ectopic expression of human ACE2 or of ACE2 orthologs from various mammals, including Chinese rufous horseshoe bat and Malayan pangolin. D614G did not alter S protein synthesis, processing, or incorporation into SARS-CoV-2 particles, but D614G affinity for ACE2 was reduced due to a faster dissociation rate. Assessment of the S protein trimer by cryo-electron microscopy showed that D614G disrupts an interprotomer contact and that the conformation is shifted toward an ACE2 binding-competent state, which is modeled to be on pathway for virion membrane fusion with target cells. Consistent with this more open conformation,

neutralization potency of antibodies targeting the S protein receptor-binding domain was not attenuated.

Zahradnik, J., et al. (2021). "SARS-CoV-2 variant prediction and antiviral drug design are enabled by RBD in vitro evolution." Nat Microbiol **6**(9): 1188-1198.

SARS-CoV-2 variants of interest and concern will continue to emerge for the duration of the COVID-19 pandemic. To map mutations in the receptor-binding domain (RBD) of the spike protein that affect binding to angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2, we applied in vitro evolution to affinity-mature the RBD. Multiple rounds of random mutagenic libraries of the RBD were sorted against decreasing concentrations of ACE2, resulting in the selection of higher affinity RBD binders. We found that mutations present in more transmissible viruses (S477N, E484K and N501Y) were preferentially selected in our high-throughput screen. Evolved RBD mutants include prominently the amino acid substitutions found in the RBDs of B.1.620, B.1.1.7 (Alpha), B1.351 (Beta) and P.1 (Gamma) variants. Moreover, the incidence of RBD mutations in the population as presented in the GISAID database (April 2021) is positively correlated with increased binding affinity to ACE2. Further in vitro evolution increased binding by 1,000-fold and identified mutations that may be more infectious if they evolve in the circulating viral population, for example, Q498R is epistatic to N501Y. We show that our high-affinity variant RBD-62 can be used as a drug to inhibit infection with SARS-CoV-2 and variants Alpha, Beta and Gamma in vitro. In a model of SARS-CoV-2 challenge in hamster, RBD-62 significantly reduced clinical disease when administered before or after infection. A 2.9 A cryo-electron microscopy structure of the high-affinity complex of RBD-62 and ACE2, including all rapidly spreading mutations, provides a structural basis for future drug and vaccine development and for in silico evaluation of known antibodies.

Zhang, J., et al. (2021). "Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant." bioRxiv.

The Delta variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has outcompeted previously prevalent variants and become a dominant strain worldwide. We report here structure, function and antigenicity of its full-length spike (S) trimer in comparison with those of other variants, including Gamma, Kappa, and previously characterized Alpha and Beta. Delta S can fuse membranes more efficiently at low levels of cellular receptor ACE2 and its pseudotyped viruses infect target cells substantially faster than all other variants tested, possibly accounting for its heightened transmissibility. Mutations of each variant rearrange the antigenic surface of the N-terminal domain of the S protein in a unique way, but only cause local changes in the receptor-binding domain, consistent with greater resistance particular to neutralizing antibodies. These results advance our molecular understanding of distinct properties of these viruses and may guide intervention strategies.

Zhang, J., et al. (2021). "Potential transmission chains of variant B.1.1.7 and co-mutations of SARS-CoV-2." Cell Discov **7**(1): 44.

The presence of SARS-CoV-2 mutants, including the emerging variant B.1.1.7, has raised great concerns in terms of pathogenesis, transmission, and immune escape. Characterizing SARS-CoV-2 mutations, evolution, and effects on infectivity and pathogenicity is crucial to the design of antibody therapies and surveillance strategies. Here, we analyzed 454,443 SARS-CoV-2 spike genes/proteins and 14,427 whole-genome sequences. We demonstrated that the early variant B.1.1.7 may not have evolved spontaneously in the United Kingdom or within human populations. Our extensive analyses suggested that Canidae, Mustelidae or Felidae, especially the Canidae family (for example, dog) could be a possible host of the direct progenitor of variant B.1.1.7. An alternative hypothesis is that the variant was simply yet to be sampled. Notably, the SARS-CoV-2 whole-genome represents a large number of potential co-mutations. In addition, we used an experimental SARS-CoV-2 reporter replicon system to introduce the dominant co-mutations NSP12_c14408t, 5'UTR_c241t, and NSP3_c3037t into the viral genome, and to monitor the effect of the mutations on viral replication. Our experimental results demonstrated that the co-mutations significantly attenuated the viral replication. The study provides valuable clues for discovering the transmission chains of variant B.1.1.7 and understanding the evolutionary process of SARS-CoV-2.

Zhou, B., et al. (2020). "SARS-CoV-2 spike D614G variant confers enhanced replication and transmissibility." bioRxiv.

During the evolution of SARS-CoV-2 in humans a D614G substitution in the spike (S) protein emerged and became the predominant circulating variant (S-614G) of the COVID-19 pandemic (1) . However, whether the increasing prevalence of the S-614G variant represents a fitness advantage that improves replication and/or transmission in humans or is merely due to founder effects remains elusive. Here, we generated isogenic SARS-CoV-2 variants and demonstrate that the S-614G variant has (i) enhanced binding to human ACE2, (ii) increased replication in primary human bronchial and nasal airway epithelial cultures as well as in a novel human ACE2 knock-in mouse model, and (iii) markedly increased replication and transmissibility in hamster and ferret models of

SARS-CoV-2 infection. Collectively, our data show that while the S-614G substitution results in subtle increases in binding and replication in vitro , it provides a real competitive advantage in vivo , particularly during the transmission bottle neck, providing an explanation for the global predominance of S-614G variant among the SARS-CoV-2 viruses currently circulating.

Zhou, D., et al. (2021). "Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera." Cell **184**(9): 2348-2361 e2346.

The race to produce vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began when the first sequence was published, and this forms the basis for vaccines currently deployed globally. Independent lineages of SARS-CoV-2 have recently been reported: UK, B.1.1.7; South Africa, B.1.351; and Brazil, P.1. These variants have multiple changes in the immunodominant spike protein that facilitates viral cell entry via the angiotensin-converting enzyme-2 (ACE2) receptor. Mutations in the receptor recognition site on the spike are of great concern for their potential for immune escape. Here, we describe a structure-function analysis of B.1.351 using a large cohort of convalescent and vaccinee serum samples. The receptor-binding domain mutations provide tighter ACE2 binding and widespread escape from monoclonal antibody neutralization largely driven by E484K, although K417N and N501Y act together against some important antibody classes. In a number of cases, it would appear that convalescent and some vaccine serum offers limited protection against this variant.

Zhou, W., et al. (2021). "N439K Variant in Spike Protein Alter the Infection Efficiency and Antigenicity of SARS-CoV-2 Based on Molecular Dynamics Simulation." Front Cell Dev Biol **9**: 697035.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing an outbreak of coronavirus disease 2019 (COVID-19), has been undergoing various mutations. The analysis of the structural and energetic effects of mutations on protein-protein interactions between the receptor binding domain (RBD) of SARS-CoV-2 and angiotensin converting enzyme 2 (ACE2) or neutralizing monoclonal antibodies will be beneficial for epidemic surveillance, diagnosis, and optimization of neutralizing agents. According to the molecular dynamics simulation, a key mutation N439K in the SARS-CoV-2 RBD region created a new salt bridge with Glu329 of hACE2, which resulted in greater electrostatic complementarity, and created a weak salt bridge with Asp442 of RBD. Furthermore, the N439K-mutated RBD bound hACE2 with a higher affinity than wild-type, which may lead to more infectious. In addition, the N439K-mutated RBD was markedly resistant to the SARS-CoV-2 neutralizing antibody REGN10987, which may lead to the failure of neutralization. The results show consistent with the previous experimental conclusion and clarify the structural mechanism under affinity changes. Our methods will offer guidance on the assessment of the infection efficiency and antigenicity effect of continuing mutations in SARS-CoV-2.

Zoccola, R., et al. (2021). "First detection of an Italian human-to-cat outbreak of SARS-CoV-2 Alpha variant - lineage B.1.1.7." One Health **13**: 100295.

The emergence of new SARS-CoV-2 variants and their rapid spread pose a threat to both human and animal health and may conceal unknown risks. This report describes an Italian human-to-cat outbreak of SARS-CoV-2 lineage B.1.1.7 (the Alpha variant) . On March 7th, 2021, approximately ten days after COVID-19 appeared in the family, the onset of respiratory signs in a cat by COVID-19-affected owners led to an in-depth diagnostic investigation, combining clinical and serological data with rt-qPCR-based virus detection and whole genome sequencing. The Alpha variant was confirmed first in the owners and a few days later in the cat that was then monitored weekly: the course was similar with one-week lag time in the cat. In addition, based on comparative analysis of genome sequences from our study and from 200 random Italian cases of Alpha variant, the familial cluster was confirmed. The temporal sequence along with the genomic data support a human-to-animal transmission. Such an event emphasizes the importance of studying the circulation and dynamics of SARS-CoV-2 variants in humans and animals to better understand and prevent potential spillover risks or unwarranted alerts involving our pet populations.

Zuckerman, N., et al. (2021). "The SARS-CoV-2 Lambda variant and its neutralisation efficiency following vaccination with Comirnaty, Israel, April to June 2021." Euro Surveill **26**(45).

The SARS-CoV-2 Lambda (Pango lineage designation C.37) variant of interest, initially identified in Peru, has spread to additional countries. First detected in Israel in April 2021 following importations from Argentina and several European countries, the Lambda variant infected 18 individuals belonging to two main transmission chains without further spread. Micro-neutralisation assays following Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer) vaccination demonstrated a significant 1.6-fold reduction in neutralising titres compared with the wild type virus, suggesting increased susceptibility of vaccinated individuals to infection.

Zuckerman, N. S., et al. (2021). "A Unique SARS-CoV-2 Spike Protein P681H Variant Detected in Israel." Vaccines (Basel) **9**(6).

The routine detection, surveillance, and reporting of novel SARS-CoV-2 variants is crucial, as these threaten to hinder global vaccination efforts. Herein we report a novel local variant with a non-synonymous mutation in the spike (S) protein P681H. This local Israeli variant was not associated with a higher infection rate or higher prevalence. Furthermore, the local variant was successfully neutralized by sera from fully vaccinated individuals at a comparable level to the B.1.1.7 variant and an Israel wild-type strain. While it is not a variant of concern, routine monitoring by sequencing is still required.

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