 doi:10.20944/preprints202207.0051.v1

Review

SARS-CoV-2 Intermittent Virulence as A Result of Natural Selection

Alberto Rubio Casillas [1,2,*], Elrashdy M. Redwan [3,4] and Vladimir N. Uversky [5,*]

[1] Autlán Regional Hospital, Health Secretariat, Autlán, Jalisco 48900, México; alberto110966@gmail.com
[2] Biology Laboratory, Autlán Regional Preparatory School, University of Guadalajara, Autlán, Jalisco 48900, Mexico;
[3] Biological Science Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; lradwan@kau.edu.sa
[4] Therapeutic and Protective Proteins Laboratory, Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, New Borg EL-Arab, Alexandria 21934, Egypt
[5] Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA; vuversky@usf.edu
* Correspondence: e-mail@e-mail.com; alberto110966@gmail.com (A.R.C.); vuversky@usf.edu (V.N.U.)

Abstract: For the first time in history, we have witnessed the origin and development of a pandemic. To handle the accelerated accumulation of viral mutations and to comprehend the virus' evolutionary adaptation in humans, an unparalleled program of genetic sequencing and monitoring of SARS-CoV-2 variants has been undertaken. Several scientists have theorized that, with the Omicron surge producing a more contagious but less severe disease, the end of COVID-19 is near. However, by analyzing the behavior shown by this virus for 2 years, we have noted that pandemic viruses do not always show a decreased virulence. Instead, it appears there is an evolutionary equilibrium between transmissibility and virulence. We have termed this concept "intermittent virulence". The present work analyzes the temporal and epidemiological behavior of SARS-CoV-2 and suggests that there is a high possibility that new virulent variants will arise in the near future, although it is improbable that SARS-CoV-2´s virulence will be the same as was seen during the pandemic phase.

Keywords: SARS-CoV-2; Omicron; variant of concern

1. Introduction

The current pandemic has generated a unique opportunity to study SARS-CoV-2 in real time. Several institutions have implemented online monitoring resources to track the progress of the ongoing pandemic. Furthermore, specialized genomic sequencing technologies have contributed to our comprehension of its biology and evolution [1]. The evolution of viral adaptability is the result of selective pressure acting across different mechanisms [2]. Purifying selection removes virus strains with unfavorable characteristics (e.g., disadvantageous conformational changes) within an infected organism, whereas positive selection promotes strains with an evolutionary advantage (e.g., immune evasion) [3,4,5]. Natural selection can have a significant impact on virus binding proteins, which control cell entrance and are the main objective for immune recognition [6]. Genetic changes in the SARS-CoV-2 spike protein may affect the viral efficiency by promoting immune evasion or increasing ACE2 receptor binding. Importantly, variant lineages that appeared at the end of 2020 have numerous spike protein mutations in common, including K417N (discovered in the beta and gamma variants), E484K, N501Y, and D614G (found in the alpha, beta, and gamma variants) [5,7,8,9]. In vitro, both D614G and N501Y cause a structural rearrangement of the spike polypeptide chains, which enhances the probability of attaching to ACE2, improves the infectivity of a cell, and uncovers the cleavage region [8,9]. K417N reduces monoclonal antibody neutralization while moderately increasing

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

ACE2 binding affinity. E484K binds to ACE2 with a higher affinity and has a lower monoclonal antibody neutralizing efficacy [10].

The evolution of a pathogen should be towards a lesser virulence, according to classical evolutionary theory, because killing the host has a detrimental influence on the pathogen's spread [11]. Several works published from 1980 to 1990 explained how a pathogen's evolutionary route to intermediate virulence—rather than zero virulence—might be generated by a positive relationship between disease transmission and virulence [12-16]. Some researchers expect that, with the Omicron surge producing a more contagious but less severe disease, the end of COVID-19 is near. Even though the threat of a "super killer" virus is unfounded, the widespread belief that a virus will change to become more lethal during an outbreak exemplifies this phenomenon [17].

Nevertheless, other experts believe that Omicron's lessened pathogenicity is coincidental, and that continued fast antigenic evolution will certainly yield new variants that will evade immunity and become more virulent. Furthermore, they also believe that one of the most enduring misconceptions about pathogen evolution is that viruses will evolve to become less pathogenic to preserve the life of their hosts [18]. Viruses mutate to maximize their propagation, which can potentially be associated with increased virulence, such as when large viral loads promote transmission while also increasing morbidity. If this is the case, pathogens may change to become more virulent. If higher pathogenicity appears later in the disease, upon the canonical transmission window (as it does in influenza virus, hepatitis C virus, HIV, SARS-CoV-2, and many other viruses) it has a minimal impact on viral fitness, and natural selection will not act against it. Predicting virulence evolution is complicated, and Omicron's decreased virulence isn't a strong forecaster regarding the appearance of new variants [18].

To date, it is very difficult to predict whether Omicron will define the end of the pandemic or new more virulent variants will emerge and the pandemic will continue. Therefore, in this work, we will compare the main genetic mutations in the Delta and Omicron (BA.1, BA.2. BA.4, and BA.5) variants to unravel their relevance in transmission and virulence, since the latest findings [19,20,21] reveal an enhanced Omicron BA.2, BA.4 and BA.5 pathogenicity in animal models that seems to confirm predictions for a higher virulence [18]. However, animal models may not reflect the real situation in humans, where previous exposure to the virus (via either the natural disease or vaccination) confers an effective protection level and could explain why these new Omicron variants are not causing high mortality compared, for example, with the Delta variant.

2. The Delta variant

The B.1.617.2 (Delta) variant was reported for the first time in India in December 2020, and then in several other nations across the globe. Several variants in the Delta variant spike protein have been recently found (L452R, tbl478K, D614G, tbl19R, 157-158, P681R, and D950N) [22]. Due to the greater severity of clinical symptoms elicited by the Delta variant, it quickly outperformed the other variants of concern (VOCs; i.e., variants for which there is an evidence of diagnostic detection failures, or higher disease severity, as evidenced by the increased hospitalizations or deaths, or an increase in the transmissibility, or significant reduction in the neutralization by antibodies generated during previous infection or vaccination, or reduced effectiveness of treatments or vaccines) [23,24]. Delta seems to have enhanced its ability to propagate in human cells as a result. Some hypotheses have been presented to clarify its increased infectiousness, including receptor binding domain (RBD) mutations that enhance its interaction with the receptor [25], a P681R mutation near the S1-S2 zone that leads to more efficacious furin cleavage [25,27], and modifications in its RNA polymerase that increase viral multiplication. In comparison to the Wuhan strain, Delta is also more virulent and highly fusogenic, according to in vitro tests [26]. The Delta variant is distinguished by the P681R mutation in the spike protein. Such

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

change occurred near the SARS-CoV-2 S protein's furin cleavage site (FCS) and as a consequence, a glycosylation site was lost, leaving the furin cleavage site uncovered, thus promoting syncytia formation and greater pathogenicity [28].

Researchers also have discovered two features that are unique to the Delta variant and could explain its infectiousness. Firstly, when the cell membrane has the highest level of Delta spike protein expression, these cells show a greater capacity to fuse with targeted cells that generate lower amounts of ACE2 compared with cells from other variants [29]. As the degree of ACE2 expression rises, the distinctions between the variations become less pronounced. Second, pseudoviruses with the Delta spike design penetrate cells expressing the ACE2 receptor faster than other variants. These findings show that the Delta spike protein has mutated to improve the merging process for penetrating cells that produce low amounts of ACE2 receptors. This advancement may help to explain why Delta can quickly propagate after contact and infect more host cells, shortening the incubation stage and increasing the viral load during disease [29].

SARS-CoV-2 infectiousness and virulence can be increased by genetic variations in the spike-protein genetic code, which impact its configuration, stability, and functionality [7,30]. SARS-CoV-2 infectiousness was discovered to be increased by the S-protein-D614G mutation [31,32]. Moreover, the structural assessment revealed that changing aspartic acid (D) to glycine (G) at location 614 of the S-protein modified its structure, facilitating the furin site to be cleaved [32,33,34,35]. Patients with the D614G mutation G614 had reduced RT-PCR cycle thresholds, indicating a greater viral load in the upper respiratory system [7]. A study comparing death rates across nations found a link between the G614 variant and greater mortality [36]. Compared to other SARS-CoV-2 strains, the Delta variant has been linked to a 120% enhanced probability of hospitalization, a 287% enhanced probability to be admitted to the intensive care unit, and a 137 % enhanced probability of mortality [37].

3. The Omicron variant

In November 2021, Omicron was first discovered in South Africa [38]. Then, the BA.1 lineage of Omicron expanded quickly over the whole world, outcompeting other variants such as Delta. Another Omicron variant, the BA.2 lineage, was discovered in numerous nations, including Denmark and the United Kingdom, as of February 2022 [39]. BA.2 has recently begun to surpass BA.1, suggesting that BA.2 has a higher transmission rate than BA.1. [19]. Omicron has the same P681H mutation as the Alpha and Delta strains, but it also harbors a new and unique glycosite, threonine (Thr376), that has only been found in Omicron. This was placed close to a proline amino acid that controls O-glycosylation [40]. When compared with wild type or Delta, researchers noticed a notable rise in the use of Core 2 type O-glycans for the Omicron variant, which is compatible with O-glycans′ insertion [41]. This discovery is groundbreaking as it was formerly reported that in vitro, the impairment of GALNT1 glycosyltransferase function (which regulates the insertion of O-glycans in the vicinity of the furin cleavage area) was caused by a mutation of proline 681 [28].

This means that Omicron's new Thr376 mutation recovered the ability to insert O-glycans that cover the furin cleavage site. In conclusion, a mutation in p681 in the Delta variant resulted in glycans loss (**Figure 1**), uncovering the furin cleavage site and making this variant more pathogenic due to enhanced syncytia formation [28]. On the contrary, a mutation in Thr376 in the Omicron variant induced the addition of O-glycans, thus covering the furin cleavage site [40]. As a result, this mutation has increased immune evasion while decreasing pathogenicity. This phenomenon has been also documented by research on the Nipah virus, where numerous N-glycans on the fusion protein were removed, resulting in hyperfusogenic phenotypes but also showing an enhanced susceptibility to antibody neutralization [41]. Although glycans are known to perform an important role in immune evasion, they are also emerging as essential determinants of virulence [42,43].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

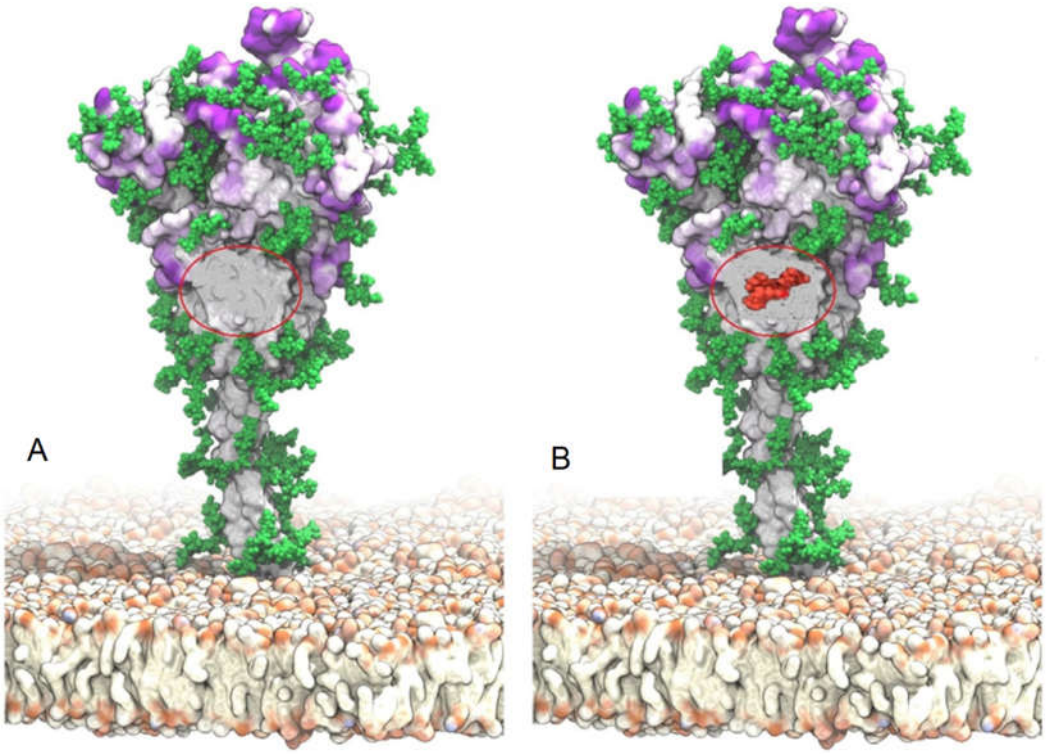

Figure 1. A) Spike protein from the Delta variant showing the glycan hole (an area without glycans depicted with a red oval) where the furin cleavage site is located. In the original Wuhan strain, this site was covered with glycans. However, the P681 mutation in the Delta variant resulted in the loss of glycans, thus promoting a higher cell-to-cell fusion and syncytia formation, which traduced in higher pathogenicity. B) In the Omicron variant, a mutation in Thr376 resulted in the addition of O-glycans (depicted in red) that obstruct the furin cleavage site, and impede cell-to-cell fusion and syncytia formation. This image is reproduced from an open-access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium provided the original work is properly cited. Modified from [44]: Sikora, M.; von Bülow, S.; Blanc, FE.; Gecht, M.; Covino, R.; Hummer, G. Computational epitope map of SARS-CoV-2 spike protein. PLoS Computational Biology 2021, 17, e1008790.

In a recent study, it was found that co-culturing spike-expressing cells with HEK293-ACE2/TMPRSS2 cells dramatically increased the amount of syncytia formation caused by BA.2 spike compared to BA.1 spike, but less than Delta (**Figure 2**) [19]. Since the efficiency of S1/S2 dissociation has been related to cell fusion induced by SARS-CoV-2´s spike protein [26,45], it was proposed that BA.2 spike is cleaved in a more effective way than the BA.1 spike. Nevertheless, a western blot test revealed that BA.2 S is cleaved less efficiently than the BA.1 Spike, implying that BA.2 Spike produces more syncytia than the BA.1 spike by using an S1/S2 cleavage-independent mechanism. To find out whether BA.2 S uses TMPRSS2, 293-ACE2 cells with or without TMPRSS2 expression were used in a cell-based fusion assay [19].

The findings demonstrated that BA.2's relatively greater fusogenic potential depends on TMPRSS2 expression in target cells [19]. The Omicron BA.1 variant harbors O-glycans in the FCS region (**Figure 1B**), thus blocking access to furin and limiting cell fusion and syncytia formation [40]. It is highly likely that BA.2 also harbors O-glycans in the FCS region, which could block cell fusion through this mechanism and produce a shift to TMPRSS2-mediated syncytia formation.

It's worth noting that BA.1 makes inefficient use of TMPRSS2 during infection [46]. Thus, the new mutations found in BA.2 apparently restored its capacity to use TMPRSS2, which has resulted in a higher fusogenicity and pathogenic potential compared with BA.1. [19].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

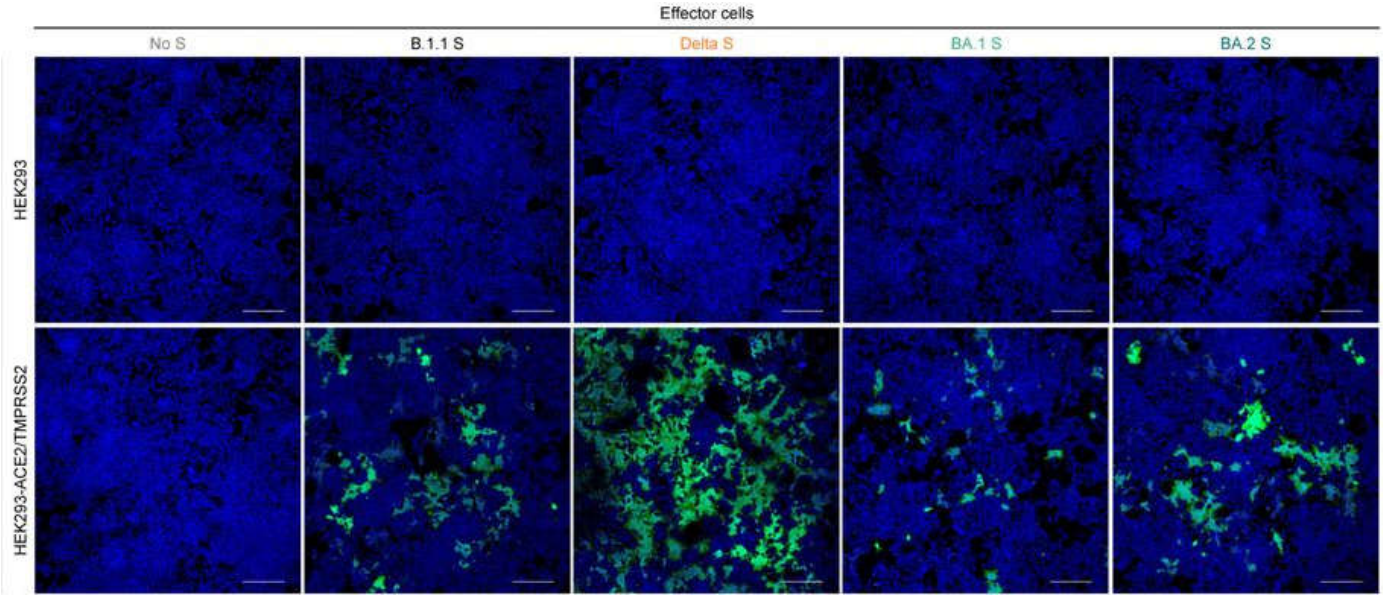

Figure 2. BA.2 spike produced a greater (2.9-fold) syncytia formation than BA.1, but lesser than Delta. Because syncytia generation has long been related to pathogenesis, these findings propose that BA.2 could be more pathogenic than BA.1 but not as pathogenic as Delta. This image is reproduced from an open-access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium provided the original work is properly cited. Source [19]: Yamasoba, D.; Kimura, I.; Nasser, H.; Morioka, Y.; Nao, N.; Ito, J.; Uriu, K.; Tsuda, M.; Zahradnik, J.; Shirakawa, K. Viro-logical characteristics of SARS-CoV-2 BA. 2 variant. Cell 2022, 185, 2103-2115.

Analyses of these genomic differences between Delta and Omicron variants allow us to understand the relevance of glycans for virulence. Notably, Delta showed a greater fusogenicity due to the p681 mutation that eliminated an O-glycan molecule from the furin cleavage site [28], and also the D614G mutation increased syncytia creation and viral load through enhanced furin-induced spike cleavage [47]. Interestingly, a similar mutation occurred with the Influenza virus. When virulence of H3N2 strains in mice was compared, it was discovered that viruses isolated after 1980 had a high glycans number and caused mild disease in mice. An N-linked glycan from the hemagglutinin receptor of influenza virus was lost due to a mutation in the gene codifying for such receptor in Beijing/89 strain, which was linked to enhanced virulence in mice [48]. The virulence of the 2009 H1N1 virus also was driven by a sudden glycan loss near the receptor binding site [49]. A subsequent study in mice confirmed this discovery, providing evidence that a comparable mutation increased the virulence of the 1918 H1N1 pandemic virus [50]. The 1918 H1N1 virus contained fewer glycosylation sites on the hemagglutinin motif than seasonal influenza viruses with lower virulence. Utilizing site-directed mutagenesis, it was discovered that by incorporating 2 extra glycosylation domains (asparagine Asn71 and Asn286) on 1 flank of the hemagglutinin receptor, a highly virulent 1918 HA chimeric virus was considerably attenuated in mice [50].

Similarly, the Omicron variant BA.1 developed a mutation in Thr376, which is located near the FCS and consisted of the addition of O-glycan molecules, thus covering the FCS (**Figure 1**). That interference was traduced in a lower fusogenicity and pathogenicity [40,45]. This is also consistent with the clinical symptomatology being milder, as a significantly reduced Omicron reproduction velocity was recently detected in lung epithelial cells [51,52]. In vitro experiments imply that the Omicron variant BA.1 is less likely to disseminate by cell merging than other variants, adding to proof that the virus on its own may cause less severe illness [46,51].

All these data may clarify why Omicron-BA.1-infected individuals suffer fewer serious complications [53,54]. Regarding the pathogenicity of BA.2 in humans, the risk of hospitalization in Germany after a BA.1 or BA.2 Omicron variant infection was approximately

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

80% lower than after a Delta variant infection, especially in people under 35 years old [55]. Both BA.1 and BA.2 showed a similar impact on hospitalization or intensive care unit (ICU) admission, implying that, despite evidence of greater transmissibility for BA.2 [19], this variant is pathogenically equivalent to BA.1 [55]. Confirming these results, no clinical differences among individuals infected with BA.1 and BA.2 were detected in Denmark [56] and South Africa [57]. In a study carried out in France, the first 207 Omicron BA.2 cases were recorded, and it was discovered that severe Omicron BA.2 infections were exclusively seen in individuals over the age of 80. Three patients (1.5%) died at the ages of 80, 97, and 99, and two of them had diabetes. Two people (ages 80 and 97) received the COVID-19 vaccine (three doses). Individuals who passed away from Omicron BA.2 infections were much older than those who passed away from Omicron BA.1 infection [58].

The latest research findings showed that a genetic mutation in the E protein from Omicron also contributes to its reduced pathogenic potential. The SARS-CoV-2 envelope protein (2- E) creates a homopentameric cation channel that is essential for virulence. SARS-CoV-2 does not travel through the traditional biosynthetic secretory channel; instead, it enters lysosomes and exits via lysosomal deacidification (alkalization). The lysosomes are where coronaviruses' envelope (E) protein channels are found. The E protein is therefore in charge of calcium outflow to achieve an alkaline pH since otherwise the virus would be destroyed by the lysosome's acidic pH. While mutated T9I channels had less of an impact on the luminal pH, the expression of wild-type channels significantly alkalized the lysosomal pH. Viral load was decreased as a result of decreased lysosomal deacidification. T9I's cytotoxic potential and cytokine production were both roughly 150-fold lower than those of wild type, which could further diminish the virulence of Omicron variants [59].

4. Discussion

Our proposal of an “intermittent virulence” reconciles two contrary hypotheses. The first claims that the Omicron variant will define the end of the current pandemic, whereas the other suggests that new virulent variants will arise in the future. We propose that in order to survive, viruses may develop what we have termed “intermittent virulence”. This concept implies that when the virus has become more contagious but less virulent, natural selection could create new variants with a higher pathogenic capacity; otherwise, the dominant variant could disappear or become endemic due to the community having reached herd immunity through natural immunity or to global vaccination programs. In fact, during the ongoing pandemic, SARS-CoV-2-associated mortality has shown interesting fluctuations. For example, on 20 January 2021, the highest mortality was reached with 18,144 deaths (**Figure 3**). Afterward, a significant decrease in mortality was observed worldwide. Such behavior has been repeated several times, giving rise to the so-called “waves”. By April 28, the third wave reached its peak level with 15,978 deaths. After a peak there is a sharp decrease in mortality; that could be because the population has reached herd immunity.

However, a new rise in mortality numbers occurred by August 23, 2021, and again by February 09, 2022 (**Figure 3**) which contradicts the idea that the population reached herd immunity. If it were true, the number of infections or deaths would decrease, which is not the case. The Omicron variant was first discovered in South Africa in November 2021 [38]. This variant rapidly outcompeted Delta, and by February 2022 it was the dominant variant. Although it has been demonstrated that Omicron is less pathogenic than Delta, the mortality peak shown in **Figure 3** by February 2022 could be due to the fact that at that time both variants were co-circulating or because the global population had no immunity against Omicron. After the February peak, there has been a consistent decrease in mortality worldwide, leading some experts to ask that “public health officials need to declare the end of the pandemic” [60].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0051.v1

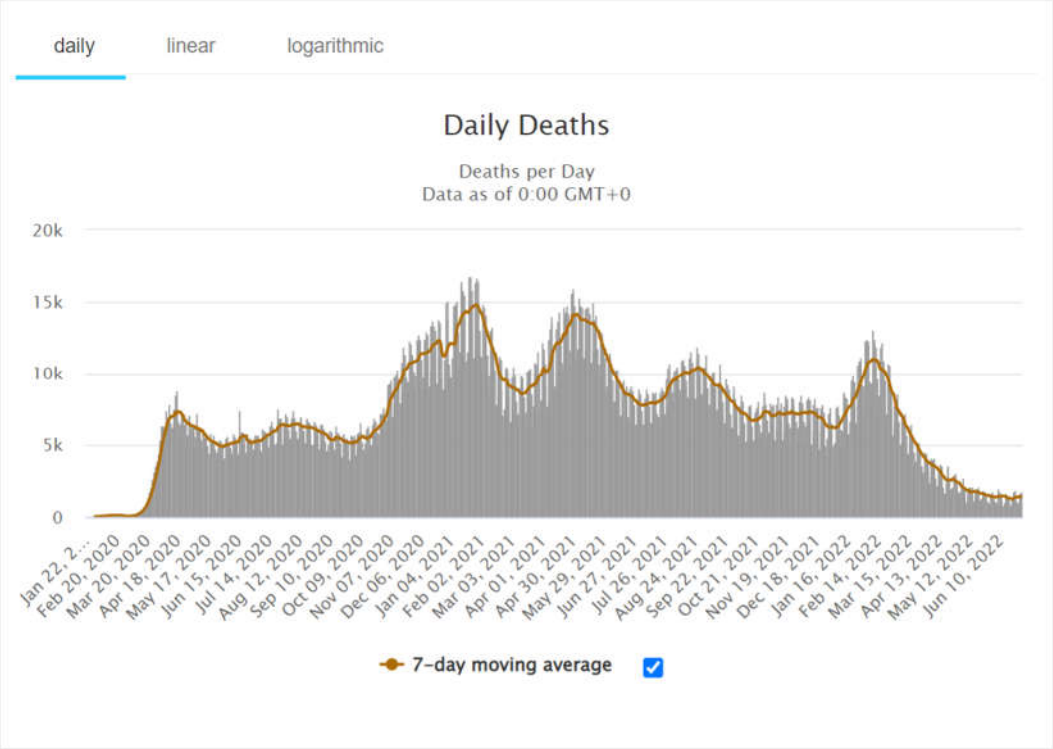

Figure 3. Graph showing global mortality numbers and the so-called "pandemic waves". Source: https://www.worldometers.info/coronavirus/.

Nevertheless, the recently reported virological characteristics of the new Omicron variant BA.2 suggested a return to a higher virulence than its previous antecessor, BA.1. [19]. This variant rapidly displaced Delta around the world, showing remarkable transmissibility but with lower pathogenicity. Compared to BA.1 and Delta, BA.2 is approximately 1.5 and 4.2 times more infectious, respectively [61,62]. In vitro studies have shown BA.2 has a higher fusogenicity, and histological studies demonstrated that BA.2 replicates faster and more efficiently in hamsters´ lungs, and causes greater damage to this organ than BA.1 [19]. Interestingly, BA.2 showed a lower fusogenic capacity compared with Delta (**Figure 2**). This result suggests that BA.2 could produce a less severe clinical symptomatology in humans than the Delta variant, but higher than BA.1. As of May 2022, Omicron sub-variants harboring a mutation at the L452 residue of the spike (S) protein, including BA.4 and BA.5, have been frequently discovered [63,64].

Neutralization studies demonstrated that the immunity conferred by BA.1 and BA.2 outbreaks is less effective against BA.4/5, according to a preprint paper [20]. Cell culture investigations demonstrated that BA.2.12.1 and BA.4/5 propagate more effectively in human alveolar epithelial cells than BA.2, and specifically, BA.4/5 produces more syncytia than BA.2. In addition, hamster infection tests revealed that BA.4/5 is more virulent than BA.2, "and could represent a greater threat to world health than the original BA.2 because SARS-CoV-2 does not necessarily evolve to attenuate its pathogenicity" [20]. However, as we mentioned before, there is an important difference between these in vitro studies, in vivo investigations using animal models, and our species. In contrast to animals, humans have received different COVID-19 vaccines which have induced artificial immunity, and that could explain why mortality continues to decline. Epidemiological surveillance organizations like Worldometer (worldometers.info), or Our World in Data provide information in near real-time regarding the number of cases and deaths.

It is possible that the pandemic phase of COVID-19 is about to end, but the virus is not going to disappear and an endemic phase will begin, where intermittent virulence could guarantee the survival of this pathogen. Recent findings show that it is not a rule

 doi:10.20944/preprints202207.0051.v1

that viruses become less virulent over time. A particularly aggressive variant of the human immunodeficiency virus (HIV) has been discovered in the Netherlands, where it has been circulating for some years. More than 100 individuals with HIV-1 subtype B infection experienced a twofold decline in CD4+ cell numbers than predicted. These people were already at risk of having AIDS within two to three years of being diagnosed. This virus lineage, which appears to have emerged de novo around the 1990s, has undergone considerable changes in its genes, changing almost 300 amino acids, making it difficult to determine the reason for the increased virulence [65].

Because it is known that genetic recombination can result in the appearance of new extremely pathogenic variants, the most concerning evolutionary aspect of SARS-CoV-2 is that it could experiment with widespread recombination [66,67]. Simultaneous infection with various subtypes of the same virus might cause this outcome. The activity of the protein Nsp14 regulates this mechanism in SARS-CoV-2 [68]. In February 2022, for example, evidence of Deltacron XD recombinant SARS-CoV-2 transmission and circulation in northwest France was reported. Following virological and epidemiological studies, 17 cases of this recombinant SARS-CoV-2 were validated by genotyping or inferred due to epidemiological relationships, indicating an extensive propagation incident and transmission of this virus, but not showing evidence of severe clinical symptoms [69]. Another contemporary research discovered a Delta variant sub-lineage spreading throughout the United States, particularly in Colorado (CO), Texas (TX), and Wyoming (WY). This sublineage is characterized by a spike protein mutation at position 112 that has been found in low-prevalence lineages around the world, as well as in circulating U.S. isolates since the end of April 2021. Two unique mutations are found in this Delta S: S112L sublineage group: ORF1b: V2354F, which corresponds to nonstructural protein NSP15 at position 303 (NSP15:V303F), and a premature stop codon (Q94*) that truncates ORF7a [70]. Unfortunately, the clinical characteristics of people infected with this sublineage were not examined in this investigation.

To predict the epidemic's future, researchers must accurately investigate how the virus' tropism evolves. A respiratory tropism is prevalent at this time, but it is known from other coronaviruses that this can evolve very quickly [71]. The avian coronavirus spike protein, for example, had three amino acid modifications that enabled the virus to attach to kidney cells [72]. Coronaviruses also are neurotropic in mice [73] and SARS-CoV-2 also shows neurotropism [74,75,76]. The changing structure of the spike protein is significantly related to natural selection on cell ingress and fusion. SARS-CoV-2 was able to swap host and adapt to the human receptor ACE2 [77] after the acquisition of a furin cleavage site. At least in vitro, the virus has encountered another receptor for entry, specifically the CD147 receptor, a protein present in several tissues, which include epithelial and neural cells [78]. This is significant because using a variety of receptors allows for switching between different cell types and, thus, different entry gateways [71]. Notably, SARS-CoV-2 can infect non-permissive cells (which do not harbor ACE2 receptors) by using an innovative intra-cytoplasmic connection mechanism that could serve as an alternative viral transmission pathway, independent of the canonical extra-cytoplasmic ACE2 binding mechanism [79].

We conclude that the end of the current pandemic is near and the disease will enter the endemic phase, and the virus will continue to cause a low mortality rate between the non-vaccinated, old people and those with co-morbidities. An increased virulence produced by new variants could guarantee the survival of this pathogen, thus confirming the validity of the term “intermittent virulence”. However, it is improbable that SARS-CoV-2´s virulence will be the same as was seen during the pandemic phase.

Author Contributions: Conceptualization, A.R.C., E.M.R., V.N.U.; Study design, A.R.C., E.M.R., V.N.U.; Literature Collection and Analysis, A.R.C., E.M.R., V.N.U.; Investigation, A.R.C., E.M.R., V.N.U.; Writing - Original Draft, A.R.C., E.M.R., V.N.U.; Writing - Review & Editing, A.R.C., E.M.R., V.N.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

 doi:10.20944/preprints202207.0051.v1

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health. Geneva: World Health Organization. (2021). https://www.who.int/publications/i/item/9789240018440.
2. Park, M.; Loverdo, C.; Schreiber, S. J.; Lloyd-Smith, J. O. Multiple scales of selection influence the evolutionary emergence of novel pathogens. Philos Trans R Soc Lond B Biol Sci 2013, 368 (1614), 20120333.
3. Wasik, B. On the biological success of viruses. Annu Rev Microbiol: 2013, 67, 519-541.
4. van Dorp, L.; Richard, D.; Tan, C. C. S.; Shaw, L. P.; Acman, M.; Balloux, F. No evidence for increased transmissibility from recurrent mutations in SARS-CoV-2. Nat Commun 2020, 11, 5986.
5. Thomson, E. C.; Rosen, L. E.; Shepherd, J. G.; Spreafico, R.; da Silva Filipe, A.; Wojcechowskyj, J. A.; Davis, C.; Piccoli, L.; Pascall, D. J.; Dillen, J.; et al. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. Cell 2021, 184, 1171-1187.e1120.
6. Piccoli, L.; Park, Y. J.; Tortorici, M. A.; Czudnochowski, N.; Walls, A. C.; Beltramello, M.; Silacci-Fregni, C.; Pinto, D.; Rosen, L. E.; Bowen, J. E.; et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell 2020, 183, 1024-1042.e1021.
7. Korber, B.; Fischer, W. M.; Gnanakaran, S.; Yoon, H.; Theiler, J.; Abfalterer, W.; Hengartner, N.; Giorgi, E. E.; Bhattacharya, T.; Foley, B.; et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell 2020, 182, 812-827.e819.
8. Yurkovetskiy, L.; Wang, X.; Pascal, K. E.; Tomkins-Tinch, C.; Nyalile, T. P.; Wang, Y.; Baum, A.; Diehl, W. E.; Dauphin, A.; Carbone, C.; et al. Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant. Cell 2020, 183, 739-751.e738.
9. Greaney, A. J.; Loes, A. N.; Crawford, K. H. D.; Starr, T. N.; Malone, K. D.; Chu, H. Y.; Bloom, J. D. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe 2021, 29, 463-476.e466.
10. Wang, P.; Nair, M. S.; Liu, L.; Iketani, S.; Luo, Y.; Guo, Y.; Wang, M.; Yu, J.; Zhang, B.; Kwong, P. D. Antibody resistance of SARS-CoV-2 variants B. 1.351 and B. 1.1. 7. Nature 2021, 593, 130-135.
11. Burnet, FM.; White, D. Natural history of infectious disease; Cambridge, Eng, 1972.
12. Levin, S.; Pimentel, D. Selection of intermediate rates of increase in parasite-host systems. The American Naturalist 1981, 117, 308-315.
13. Anderson, RM.; May, RM. Coevolution of hosts and parasites. Parasitology 1982, 85, 411-426.
14. May, RM.; Anderson, RM. Epidemiology and genetics in the coevolution of parasites and hosts. Proceedings of the Royal Society of London. Series B. Biological sciences 1983, 219, 281-313.
15. Frank, SA. Models of parasite virulence. The Quarterly review of biology 1996, 71, 37-78.
16. Galvani, AP. Epidemiology meets evolutionary ecology. Trends in Ecology & Evolution 2003, 18, 132-139.
17. Grubaugh, ND.; Petrone, ME.; Holmes, EC. We shouldn't worry when a virus mutates during disease outbreaks. Nature microbiology 2020, 5, 529-530.
18. Markov, PV.; Katzourakis, A.; Stilianakis, NI. Antigenic evolution will lead to new SARS-CoV-2 variants with unpredictable severity. Nature Reviews Microbiology 2022, 20, 251-252.
19. Yamasoba, D.; Kimura, I.; Nasser, H.; Morioka, Y.; Nao, N.; Ito, J.; Uriu, K.; Tsuda, M.; Zahradnik, J.; Shirakawa, K. Virological characteristics of SARS-CoV-2 BA. 2 variant. Cell 2022, 185, 2103-2115.
20. Kimura, I.; Yamasoba, D.; Tamura, T.; Nao, N.; Oda, Y.; Mitoma, S.; Ito, J.; Nasser, H.; Zahradnik, J.; Uriu, K. Virological characteristics of the novel SARS-CoV-2 Omicron variants including BA. 2.12. 1, BA. 4 and BA. 5. bioRxiv 2022.
21. Qu, P.; Evans, JP.; Faraone, JN.; Zou, X.; Zheng, YM.; Carlin, C.; Bednash, JS.; Lozanski, G.; Mallampalli, RK.; Saif, L. J. Differential Evasion of Delta and Omicron Immunity and Enhanced Fusogenicity of SARS-CoV-2 Omicron BA. 4/5 and BA. 2.12. 1 Subvariants. bioRxiv 2022.
22. European Centre for Disease Prevention and Control. Threat assessment brief: emergence of SARS-CoV-2 B.1.617 variants in India and situation in the EU/EEA. Available from https://www.ecdc.europa.eu/en/publications-data/threat-assessment-emergence-sars-cov-2-b1617-variants
23. Sheikh, A.; McMenamin, J.; Taylor, B.; Robertson, C. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. The Lancet 2021, 397, 2461-2462.
24. Twohig, KA.; Nyberg, T.; Zaidi, A.; Thelwall, S.; Sinnathamby, MA.; Aliabadi, S.; Seaman, SR.; Harris, RJ.; Hope, R.; Lopez-Bernal, J. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B. 1.617. 2) compared with alpha (B. 1.1. 7) variants of concern: a cohort study. The Lancet Infectious Diseases 2022, 22, 35-42.
25. Kim, S.; Liu, Y.; Lei, Z.; Dicker, J.; Cao, Y.; Zhang, XF.; Im, W. Differential interactions between human ACE2 and Spike RBD of SARS-CoV-2 variants of concern. Journal of Chemical Theory and Computation 2021, 17, 7972-7979.

26. Saito, A.; Irie, T.; Suzuki, R.; Maemura, T.; Nasser, H.; Uriu, K.; Kosugi, Y.; Shirakawa, K.; Sadamasu, K.; Kimura, I. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature 2022, 602, 300-306.
27. Liu, Y.; Liu, J.; Johnson, BA.; Xia, H.; Ku, Z.; Schindewolf, C.; Widen, SG.; An, Z.; Weaver, SC.; Menachery, V. D. Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. Cell Reports 2022, 39, 110829.
28. Zhang, L.; Mann, M.; Syed, ZA.; Reynolds, HM.; Tian, E.; Samara, NL.; Zeldin, DC.; Tabak, LA.; Ten Hagen, KG. Furin cleavage of the SARS-CoV-2 spike is modulated by O-glycosylation. Proceedings of the National Academy of Sciences 2021, 118 (47).
29. Cai, Y.; Zhang, J.; Xiao, T.; Lavine, CL.; Rawson, S.; Peng, H.; Zhu, H.; Anand, K.; Tong, P.; Gautam, A. Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants. Science 2021, 373, 642-648.
30. Berger, I.; Schaffitzel, C. The SARS-CoV-2 spike protein: balancing stability and infectivity. Cell research 2020, 30, 1059-1060.
31. Phan, T. Genetic diversity and evolution of SARS-CoV-2. Infection, genetics and evolution 2020, 81, 104260.
32. Zhang, L.; Jackson, CB.; Mou, H.; Ojha, A.; Peng, H.; Quinlan, BD.; Rangarajan, ES.; Pan, A.; Vanderheiden, A.; Suthar, MS. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nature communications 2020, 11, 1-9.
33. Eaaswarkhanth, M.; Al Madhoun, A.; Al-Mulla, F. Could the D614G substitution in the SARS-CoV-2 spike (S) protein be associated with higher COVID-19 mortality? International Journal of Infectious Diseases 2020, 96, 459-460.
34. Jackson, CB.; Zhang, L.; Farzan, M.; Choe, H. Functional importance of the D614G mutation in the SARS-CoV-2 spike protein. Biochemical and Biophysical Research Communications 2021, 538, 108-115.
35. Mohammad, A.; Alshawaf, E.; Marafie, SK.; Abu-Farha, M.; Abubaker, J.; Al-Mulla, F. Higher binding affinity of furin for SARS-CoV-2 spike (S) protein D614G mutant could be associated with higher SARS-CoV-2 infectivity. International journal of infectious diseases 2021, 103, 611-616.
36. Becerra-Flores, M.; Cardozo, T. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. International journal of clinical practice 2020, 74, e13525.
37. Fisman, DN.; Tuite, AR. Evaluation of the relative virulence of novel SARS-CoV-2 variants: a retrospective cohort study in Ontario, Canada. Cmaj 2021, 193, E1619-E1625.
38. WHO. "Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of concern (November 26, 2021)" https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern. (2021).
39. UKHSA. "SARS-CoV-2 variants of concern and variants under investigation in England. Technical briefing 35 (January 28, 2022)". https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1050999/Technical-Briefing-35-28January2022.pdf.
40. Roberts, DS.; Mann, M.; Li, BH.; Kim, D.; Brasier, AR.; Jin, S.; Ge, Y. Distinct Core Glycan and O-Glycoform Utilization of SARS-CoV-2 Omicron Variant Spike Protein RBD Revealed by Top-Down Mass Spectrometry. bioRxiv 2022.
41. Aguilar, HC.; Matreyek, KA.; Filone, CM.; Hashimi, ST.; Levroney, EL.; Negrete, OA.; Bertolotti-Ciarlet, A.; Choi, DY.; McHardy, I.; Fulcher, JA. N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. Journal of virology 2006, 80, 4878-4889.
42. Watanabe, Y.; Bowden, TA.; Wilson, IA.; Crispin, M. Exploitation of glycosylation in enveloped virus pathobiology. Biochimica et Biophysica Acta (BBA)-General Subjects 2019, 1863, 1480-1497.
43. Yang, Q.; Hughes, TA.; Kelkar, A.; Yu, X.; Cheng, K.; Park, S.; Huang, WC.; Lovell, JF.; Neelamegham, S. Inhibition of SARS-CoV-2 viral entry upon blocking N-and O-glycan elaboration. Elife 2020, 9, e61552.
44. Sikora, M.; von Bülow, S.; Blanc, FE.; Gecht, M.; Covino, R.; Hummer, G. Computational epitope map of SARS-CoV-2 spike protein. PLoS Computational Biology 2021, 17, e1008790.
45. Suzuki, R.; Yamasoba, D.; Kimura, I.; Wang, L.; Kishimoto, M.; Ito, J.; Morioka, Y.; Nao, N.; Nasser, H.; Uriu, K. Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant. Nature 2022, 603, 700-705.
46. Meng, B.; Abdullahi, A.; Ferreira, IA.; Goonawardane, N.; Saito, A.; Kimura, I.; Yamasoba, D.; Gerber, PP.; Fatihi, S.; Rathore, S. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. Nature 2022, 603, 706-714.
47. Cheng, YW.; Chao, TL.; Li, CL.; Wang, SH.; Kao, HC.; Tsai, YM.; Wang, HY.; Hsieh, CL.; Lin, YY.; Chen, PJ. D614G substitution of SARS-CoV-2 spike protein increases syncytium formation and virus titer via enhanced furin-mediated spike cleavage. Mbio 2021, 12, e00587-00521.
48. Reading, PC.; Pickett, DL.; Tate, MD.; Whitney, PG.; Job, ER.; Brooks, AG. Loss of a single N-linked glycan from the hemagglutinin of influenza virus is associated with resistance to collectins and increased virulence in mice. Respiratory research 2009, 10, 1-11.
49. Wei, CJ.; Boyington, JC.; Dai, K.; Houser, KV.; Pearce, MB.; Kong, WP.; Yang, Zy.; Tumpey, TM.; Nabel, GJ. Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. Science translational medicine 2010, 2, 24ra21-24ra21.
50. Sun, X.; Jayaraman, A.; Maniprasad, P.; Raman, R.; Houser, KV.; Pappas, C.; Zeng, H.; Sasisekharan, R.; Katz, JM.; Tumpey, TM. N-linked glycosylation of the hemagglutinin protein influences virulence and antigenicity of the 1918 pandemic and seasonal H1N1 influenza A viruses. Journal of virology 2013, 87, 8756-8766.

51. Bentley, EG.; Kirby, A.; Sharma, P.; Kipar, A.; Mega, DF.; Bramwell, C.; Penrice-Randal, R.; Prince, T.; Brown, JC.; Zhou, J. SARS-CoV-2 Omicron-B. 1.1. 529 Variant leads to less severe disease than Pango B and Delta variants strains in a mouse model of severe COVID-19. bioRxiv 2021.
52. McMahan K.; Giffin, V.; Tostanosky, L.; et al. Reduced pathogenicity of the SARS-CoV-2 Omicron variant in hamsters. Med (New York, N.Y.), 2022 3, 262–268.e4.
53. Shuai, H.; Chan, JFW.; Hu, B.; Chai, Y.; Yuen, TTT.; Yin, F.; Huang, X.; Yoon, C.; Hu, JC.; Liu, H. Attenuated replication and pathogenicity of SARS-CoV-2 B. 1.1. 529 Omicron. Nature 2022, 1-1.
54. Wolter, N.; Jassat, W.; Walaza, S.; Welch, R.; Moultrie, H.; Groome, M.; Amoako, DG.; Everatt, J.; Bhiman, JN.; Scheepers, C. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. The Lancet 2022, 399,437-446.
55. Sievers, C.; Zacher, B.; Ullrich, A.; Huska, M.; Fuchs, S.; Buda, S.; Haas, W.; Diercke, M.; an der Heiden, M.; Kröger, S. SARS-CoV-2 Omicron variants BA. 1 and BA. 2 both show similarly reduced disease severity of COVID-19 compared to Delta, Germany, 2021 to 2022. Eurosurveillance 2022, 27, 2200396.
56. Fonager, J.; Bennedbæk, M.; Bager, P.; Wohlfahrt, J.; Ellegaard, KM.; Ingham, AC.; Edslev, SM.; Stegger, M.; Sieber, RN.; Lassauniere, R. Molecular epidemiology of the SARS-CoV-2 variant Omicron BA. 2 sub-lineage in Denmark, 29 November 2021 to 2 January 2022. Eurosurveillance 2022, 27, 2200181.
57. Wolter, N.; Jassat, W.; von Gottberg, A.; Cohen, C. Clinical severity of Omicron sub-lineage BA. 2 compared to BA. 1 in South Africa. MedRxiv 2022.
58. Gautret, P.; Hoang, VT.; Jimeno, MT.; Lagier, JC.; Rossi, P.; Fournier, PE.; Colson, P.; Raoult, D. The severity of the first 207 infections with the SARS-CoV-2 Omicron BA. 2 variant, in Marseille, France, December 2021–February 2022. Journal of medical virology 2022, 1:10.1002/jmv.27760.
59. Xia, B.; Wang Y.; Pan X.; et al. Why is the SARS-CoV-2 Omicron variant milder? The Innovation 2022, 3, 100251.
60. Ioannidis JPA. The end of the COVID-19 pandemic. Eur J Clin Invest 2022, 52, :e13782.
61. Lyngse, FP.; Kirkeby, CT.; Denwood, M.; Christiansen, LE.; Mølbak, K.; Møller, CH.; Skov, RL.; Krause, TG.; Rasmussen, M.; Sieber, RN. Transmission of SARS-CoV-2 Omicron VOC subvariants BA. 1 and BA. 2: evidence from Danish Households. MedRxiv 2022.
62. Chen, J.; Wei, GW. Omicron ba. 2 (b. 1.1. 529.2): High potential for becoming the next dominant variant. The journal of physical chemistry letters 2022, 13, 3840-3849.
63. Tegally, H.; Moir, M.; Everatt, J.; Giovanetti, M.; Scheepers, C.; Wilkinson, E.; Subramoney, K.; Moyo, S.; Amoako, DG.; Althaus, CL. Continued emergence and evolution of Omicron in South Africa: New BA. 4 and BA. 5 lineages. medRxiv 2022.
64. WHO. Tracking SARS-CoV-2 variants (who.int), June 18, 2022.
65. Wymant, C.; Bezemer, D.; Blanquart, F.; Ferretti, L.; Gall, A.; Hall, M.; Golubchik, T.; Bakker, M.; Ong, SH.; Zhao, L. A highly virulent variant of HIV-1 circulating in the Netherlands. Science 2022, 375, 540-545.
66. Zhang, X.; Yap, Y.; Danchin, A. Testing the hypothesis of a recombinant origin of the SARS-associated coronavirus. Archives of virology 2005, 150, 1-20.
67. Goldstein, SA.; Brown, J.; Pedersen, BS.; Quinlan, AR.; Elde, NC. Extensive recombination-driven coronavirus diversification expands the pool of potential pandemic pathogens. BioRxiv 2021.
68. Gribble, J.; Stevens, LJ.; Agostini, ML.; Anderson-Daniels, J.; Chappell, JD.; Lu, X.; Pruijssers, AJ.; Routh, AL.; Denison, MR. The coronavirus proofreading exoribonuclease mediates extensive viral recombination. PLoS pathogens 2021, 17, e1009226.
69. Moisan, A.; Mastrovito, B.; De Oliveira, F.; Martel, M.; Hedin, H.; Leoz, M.; Nesi, N.; Schaeffer, J.; Ar Gouilh, M.; Plantier, JC. Evidence of transmission and circulation of Deltacron XD recombinant SARS-CoV-2 in Northwest France. Clinical Infectious Diseases 2022, 10: ciac360.
70. Brinkac, L.; Diepold, S.; Mitchell, S.; Sarnese, S.; Kolakowski, LF.; Nelson, WM.; Jennings, K. SARS-CoV-2 Delta variant isolates from vaccinated individuals. BMC genomics 2022, 23, 1-11.
71. Luo, R.; Delaunay-Moisan, A.; Timmis, K.; Danchin, A. SARS-CoV-2 biology and variants: anticipation of viral evolution and what needs to be done. Environ Microbiol 2021, 23:2339-2363.
72. Bouwman, KM.; Parsons, LM.; Berends, AJ.; De Vries, RP.; Cipollo, JF.; Verheije, MH. Three amino acid changes in avian coronavirus spike protein allow binding to kidney tissue. Journal of Virology 2020, 94, e01363-01319.
73. Pasick, J.; Kalicharran, K.; Dales, S. Distribution and trafficking of JHM coronavirus structural proteins and virions in primary neurons and the OBL-21 neuronal cell line. Journal of virology 1994, 68, 2915-2928.
74. Wang, C.; Zhang, M.; Garcia Jr, G.; Tian, E.; Cui, Q.; Chen, X.; Sun, G.; Wang, J.; Arumugaswami, V.; Shi, Y. ApoE-isoform-dependent SARS-CoV-2 neurotropism and cellular response. Cell Stem Cell 2021, 28, 331-342. e335.
75. Cantuti-Castelvetri, L.; Ojha, R.; Pedro, LD.; Djannatian, M.; Franz, J.; Kuivanen, S.; van der Meer, F.; Kallio, K.; Kaya, T.; Anastasina, M. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science 2020, 370, 856-860.
76. Bullen, CK.; Hogberg, HT.; Bahadirli-Talbott, A.; Bishai, WR.; Hartung, T.; Keuthan, C.; Looney, MM.; Pekosz, A.; Romero, JC.; Sillé, FC. Infectability of human Brain Sphere neurons suggests neurotropism of SARS-CoV-2. ALTEX-Alternatives to animal experimentation 2020, 37, 665-671.

 doi:10.20944/preprints202207.0051.v1

77. Coutard, B.; Valle, C.; de Lamballerie, X.; Canard, B.; Seidah, NG.; Decroly, E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral research 2020, 176, 104742.
78. Wang, K.; Chen, W.; Zhang, Z.; Deng, Y.; Lian, JQ.; Du, P.; Wei, D.; Zhang, Y.; Sun, XX.; Gong, L. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal transduction and targeted therapy 2020, 5, 1-10.
79. Merolli A, Kasaei L, Ramasamy S, et al. An intra-cytoplasmic route for SARS-CoV-2 transmission unveiled by Helium-ion microscopy. Sci Rep 2022, 12 :3794.