The Infectivity and Antigenicity of Epidemic SARS-CoV-2 Variants in the United Kingdom

Youchun Wang (wangyc@nifdc.org.cn)
National Institutes for Food and Drug Control https://orcid.org/0000-0001-9769-5141

Jiajing Wu
National Institutes for Food and Drug Control

Li Zhang
National Institutes for Food and Drug Control

Yue Zhang
National Institutes for Food and Drug Control

Haixin Wang
National Institutes for Food and Drug Control

Ruxia Ding
National Institutes for Food and Drug Control

Jianhui Nie
National Institutes for Food and Drug Control

Qianqian Li
National Institutes for Food and Drug Control

Shuo Liu
National Institutes for Food and Drug Control

Yongxin Yu
National Institutes for Food and Drug Control

Xiao-Ming Yang
China National Biotec Group Company Limited https://orcid.org/0000-0002-2598-4355

Xiaowang Qu
Translational Medicine Institute, The First People's Hospital of Chenzhou, University of South China
https://orcid.org/0000-0002-8135-7292

Kai Duan
Wuhan Institute of Biological Products Co.Ltd.

Weijing Huang
National Institutes for Food and Drug Control (NIFDC) https://orcid.org/0000-0002-4246-8889

Research Article

Keywords: Pseudotyped Virus, Infectivity Enhancement, Neutralization Activity, Convalescent Sera, RBD Protein, Recombinant Spike DNA

DOI: https://doi.org/10.21203/rs.3.rs-153108/v1

Abstract

The SARS-CoV-2 variant VUI/202012/01 has been reported to spread unexpectedly fast in the United Kingdom. It is estimated that its transmissibility may increase by 70%. In this study, the top five variants circulating in the UK including D614G+L18F+A222V, D614G+A222V, D614G+S477N, VUI/202012/01 and D614G+69-70del+439K were analyzed for their infective and neutralizing characteristics. The pseudotyped viruses were constructed for the five variants and 12 single mutants composed those variants. We found that the VUI/202012/01 variant does enhance its infectivity due to the cumulative effect of multiple mutations including 69-70del and 144/145del mutations in NTD, A570D in RBD, and S982A in S2. Meanwhile, mutations N501Y, N439K and S477N in RBD can cause a significant decrease in the neutralization activity for some mAbs. Although VUI/202012/01 did not affect the neutralization effect of convalescent sera, it affected the neutralization activity of animal immunized sera by RBD protein or recombinant spike DNA to some extent.

Main Text

Recently, a new SARS-CoV-2 variant named VOC-202012/01 and classified as lineage B.1.1.7 has been reported to spread unexpectedly fast in the United Kingdom[1]. This variant attracted people's attention also due to its unusually large number of genetic changes on the spike protein, which are speculated to increase infectivity and cause immune escape. Eight of 17 mutations in VOC-202012/01 variant is located at the spike protein including 69-70del, 144/145del, N501Y, A570D, P681H, T716I, S982A and D1118H (COVID-19 Genomics Consortium UK). The 69-70del has been described to become a dominant mutation in an immune suppressed individual treated with convalescent plasma[2-5] and also exists in several natural variants including those transmitted in mink[6]. The N501Y mutation in the receptor binding domain (RBD) has been identified in the mouse-adapted strain, which potentially associated with the increased virulence in mice[7]. It is also suggested that theN501Y mutation increased its binding affinity to human ACE2[8]. Furthermore, as P681H mutation is immediately adjacent to the Furin cleavage site (682-685 RRAR), the proteolytic activity of the enzyme assumed to be changed as well[9].

Up to Jan 13th, 2021, there are 359, 302 SARS-CoV-2 sequences in the GISAID database, of which 44% were from the UK. We first investigated the growth trend of all the mutations with the frequency above 1% globally and in the UK (Fig 1A). The most popular mutations globally or in the UK are D614G, A222V, L18F, and S477N. Since December 2020, the VOC-202012/01 variant including multiple mutations 69-70del, 144/145del, N501Y, A570D, P681H, T716I, S982A and D1118H is found to increase very fast. As the recent SARS-CoV-2 variants appeared as combinations of different mutations, we then chose the five most frequency natural variants happened in the UK for further study, which including D614G+L18F+A222V, D614G+A222V, D614G+S477N, D614G+69-70del+N439K and the VOC-202012/01 strain (Fig1B). To determine whether their infectivity and antigenicity had been altered, we constructed pseudotyped viruses for the five variants and 12 single mutants (L18F, A222V, S477N, 69-70del,

144/145del, N501Y, A570D, P681H, T716I, S982A, D1118H and N439K) composed those variants based on D614G virus using VSV vector system

The infectivity of the five variants

The infectivity of the five variants was first tested in four SARS-CoV-2 susceptible cell lines, including two human cell lines Huh-7 and 293T-ACE2, and two non-human primate cell lines LLC-MK2 and Vero. The results showed that the infectivity of VOC-202012/01 variant increased at least 4-fold (Fig2A) compared to the reference strain 614G. Furthermore, the infectivity of D614G +A222V, D614G+69-70del+N439K and D614G+L18F+A222V also slightly increased, whereas the infectivity of D614G+S477N is decreased. To figure out whether any single mutant could determine the infectivity of the variants, we investigated the impact of single mutation that composed the variants on infectivity. As shown in Figure 2B, of the eight mutants composed VOC-202012/01, the infectivity of 69-70del, 144/145del, A570D, P681H, S982A, D1118H were all increased to some extent, except N501Y and T716I (Fig2B). Therefore, the accumulative effects of different mutations may cause the increased infectivity of VOC-202012/01. The enhancement effect of 69-70del might be the reason of the increased infection in D614G+69-70del+N439K. Moreover, the decreased infectivity of L18F may result in the relative low counts of D614G+L18F variant (Fig 1) and the decreased infectivity of D614G+L18F+A222V mutant compared to D614G+A222V (Fig2B). To our surprise, we did not find the increased infectivity in the N501Y single mutated variant, which was not in compliance with other study using yeast-display platform[8]. This may be due to the different glycan between yeast versus human cells. In addition, RBD is just one domain of the viral spike, while dynamic movements of viral entry is complicated in the physiological environment.

As the mutation P681H is immediately adjacent to the Furin or TMPRSS2 cleavage site, the structure changes of the mutation are predicted to influence the function of the enzymes[9]. We subsequently investigated the proteolytic activity of the enzyme by examining the proportion of S1 and S2 protein in the 293T cells expressed with the spike protein of the variants. As shown in the Fig 2D, there is no difference of the S2 proportion in the VOC-202012/01 variant compared to D614G. Furthermore, the infectivity of the variants in the Furin/TMPRSS2/Cathepsin L overexpressed cells were tested, the results showed that the infectivity of VOC-202012/01 was increased in the Furin and TMPRSS2 and Cathepsin L overexpressed cells which was comparable to the reference D614G variant (fig 2C). Therefore, our results suggested the P681H mutation or other mutations VOC-202012/01 did not change the proteolytic activity of Furin as well as TMPRSS2 and Cathepsin L.

The animal tropism of the five variants

To investigate whether the tropism of VOC-202012/01 and the other four epidemic variants to difference species are changed, 14 ACE2s from different species were overexpressed in 293T cells (Fig3A and B).

We found that the infectivity of VOC-202012/01 was significantly changed only in the mouse ACE2 overexpressed cell lines, with enhancement of 79.5-fold compared to 614G. Single mutation tests showed that N501Y and A570D increased their infectivity only in mouse ACE2 overexpressed cell lines. That would be the reason for this dramatic increased infectivity of VOC-202012/01 (Fig3C), which contained both N501Y and A570D mutations. This result was consistent with the previous reported about the mouse-adapted strain containing the N501Y mutation[1]. Meanwhile, the infectivity of other variants was not found to be changed in all overexpressed cell lines with different ACE2.

The neutralization activity of mAbs to the five variants.

To find out if the existing neutralizing monoclonal antibodies are still effective to the five variants, the neutralizing activity of 17 monoclonal antibodies (mAbs) targeting different area of receptor-binding domain was tested. Nine out of 17 mAbs, including H00S022, A157, A247, 2F7, 11D12, 1F9, 2H10, 10D12 and 10F9 displayed significantly reduced neutralizing activity against the VOC-202012/01 variants as well as variants carrying single N501Y mutant (Fig 4A and4B). Furthermore, mAbs H00S022 and A157 also lost most of their neutralizing activity against D614G+69-70del+N439K and N439K +D614G mutation (Fig 4A and Fig S1). The S477N variant show decreased susceptibility to mAb 7B8, while most of the antibodies were still effective (Fig 4A and Fig S1). No decreased neutralization to all the mAbs was observed for the variants without mutation in RBD. The results indicated the mutations N439K, S477N and N501Y in RBD can affect the neutralizing property of SARS-CoV-2 variants.

The neutralization activity of convalescent sera and immunized sera to the five variants

In order to predict whether the five variants could change their neutralizing property to polyclonal antibodies, we examined the neutralization ability of sera from convalescent patients with COVID-19, as well as sera from animals immunized with purified RBD protein (mouse, horse) and recombinant DNA containing full-length spike gene (guinea pig). As shown in Figure 4B and4C, the neutralization ability of VOC-202012/01 against most of the convalescent sera was not changed. However, the convalescent sera showed increased neutralization ability against D614G+L18F+A222V to some extent, and decreased neutralization ability to D614G+S477N. To our surprise, the pattern of neutralization was different in animal immunized sera compared to convalescent human sera. VOC-202012/01 showed the decreased neutralization in sera from both RBD protein immunized mice and horses and spike DNA immunized guinea pigs. The results indicate that composition of the sera from convalescent patients and immunized animals may be different, which need to be studied further.

In this study, the biological functions of top five variants of SARS-CoV-2 in the UK were analyzed. We found that the VOC-202012/01 variant does enhance virus infectivity. This variant also decreases the neutralization activity of some monoclonal antibodies but does not affect significantly the neutralization

effect of convalescent sera. As the epidemic progresses, the complicated variants of SARS-CoV-2 would occur. It is very important to closely monitor their functional change for prevention and control of SARS-CoV-2 epidemic.

References

1. Kupferschmidt, K. Viral evolution may herald new pandemic phase. *Science***371**, 108-109 (2021).
2. Young, B.E.*, et al.* Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *The Lancet* **396**, 603-611 (2020).
3. Kemp SA*, et al.* Recurrent emergence and transmission of a SARS-CoV-2 Spike deletion 69-70. *bioRxiv* **DOI 10.1101/2020.12.14.422555**(2020).
4. McCarthy, K.R.*, et al.* Natural deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. *bioRxiv* **DOI 10.1101/2020.11.19.389916**(2021).
5. Kemp, S.A.*, et al.* Neutralising antibodies drive Spike mediated SARS-CoV-2 evasion *medRxiv* **DOI 10.1101/2020.12.05.20241927**(2020).
6. Oude Munnink, B.B.*, et al.* Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* **371**, 172-177 (2021).
7. Gu, H.*, et al.* Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* **369**, 1603-1607 (2020).
8. Starr, T.N.*, et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310.e1220 (2020).
9. Hoffmann, M., Kleine-Weber, H. & Pöhlmann, S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Molecular cell* **78**, 779-784.e775 (2020).
10. Li, Q.*, et al.* The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell* **182**, 1284-1294.e1289 (2020).

Declarations

Acknowledgments

We gratefully acknowledge the authors from the originating laboratories and the submitting laboratories where genetic sequence data were generated and shared via GISAID, enabling this research. We thank Dr. Liangzhi Xie from Sino Biological Company for mAbs H014 and H00S022; Prof. Linqi Zhang of Tsinghua University for mAbs P2C-1F11, P2B-2F6 261-262, A151 and A247; Dr. Yuelei Shen of Beijing Biocytogen Co for mAbs 1F9, 7B8, 4E5, 2F7, 2H10, 10D12, 10F9, 9G11, LK+LH and 11D12; This work was supported by General Program of National Natural Science Foundation of China [grant number 82073621], BILL & MELINDA GATES FOUNDATION [Investment ID INV-006379], National Science and Technology Major Projects of Drug Discovery [grant number 2018ZX09101001] and National Science and Technology Major Projects of Infectious Disease [grant number 2017ZX10304402].

Authors' contributions

Y.W, L.Z and W.H conceived, designed and supervised the experiments; L.Z, X.L. and Y.W wrote the manuscript; J.W, Y.Z, R.D, H.W, Q.L, S.L and J.N performed the experiments. X.Y, K, D and X.Q provided the convalescent sera and clinical information. All of the authors approved the final manuscript.

Declaration of interests:

All authors declare no competing interest.

Materials And Methods

1 Cells

Huh-7 was from Japanese Collection of Research Bioresources (Cat: 0403), LLC-MK2, Vero and 293T were from American Type Culture Collection (Cat: CCL-7, CCL-81and CRL-3216). 293T-hACE2 were human ACE2 stable expressed 293T cells. All the cells were cultured using Dulbecco's modified Eagle medium (DMEM, high glucose; Hyclone) with 100 U/mL of Penicillin-Streptomycin solution (GIBCO), 20mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES, GIBCO) and 10% fetal bovine serum (FBS, Pansera ES, PAN-Biotech) at 37 °C and 5% CO2 environment.

The receptor or proteolytic enzyme transient overexpression cells were prepared by transfecting 293T cells with different species of ACE2, TMPRSS2, Furin, or Cathepsin L plasmids. Lipofectamine 2000 (Invitrogen) was used as transfection reagent. The cells were used 24-48 hours after transfection for the following tests.

2 Monoclonal Antibodies

The monoclonal antibody P2C-1F11, P2B-2F6,261-262, 151 and 247 were from Professor Linqi Zhang of Tsinghua University, H014 and H00S022 were from Sino Biological Company. The antibody 1F9, 7B8, 4E5, 2F7, 2H10, 10D12, 10F9, 9G11, 11D12 and LK+LH were from Beijing Biocytogen Co.

3 Convalescent sera

Convalescent serum of SARS-CoV-2 convalescent patients were collected from Hubei (CS1-CS5) and Hunan province (CS6-CS10) around March to April 2020. The consent forms were signed before blood collection.

4 Immunized sera

Animals were handled in accordance with institutional (NIFDC, Beijing, China) guidelines for laboratory animal care and use, and the Animal Care and Use Committee at the NIFDC approved the study protocol.

Mice were immunized with purified SARS-CoV-2 RBD protein with aluminum adjuvant, 20ug per mouse, once every 7 days for three times. Blood samples were collected 7 days after the third immunization. The serum samples from 9 mice were pooled every 3 mice and labeled as M1-M3 respectively.

Guinea pigs were immunized with pcDNA3.1- SARS-CoV-2-Spike plasmid, 200ug per guinea pig every 14 days for three times. Four serum samples from four guinea pigs 14 days after the third immunization and labeled as G1-G4 respectively.

Horses were immunized with SARS-CoV-2 RBD protein with Freund's incomplete adjuvant at the initial dose of 3mg. Ten days later, 6mg RBD protein with Freund's incomplete adjuvant was injected again. The third immunization was performed 10 days after the second immunization with 12mg of RBD protein with Freund's incomplete adjuvant. Sera were collected 7 days after third immunization (H1-H4 were from four horses respectively).

5 SARS-CoV-2 pseudotyped virus

The SARS-CoV-2 spike protein expression pcDNA3.1 plasmid was constructed based on the GenBank sequence MN908947 as we previously described[10]. The replication-defective VSV G*ΔG-VSV (Kerafast) was used as the backbone virus. Cells were transfected with pcDNA3.1- SARS-CoV-2 and infected with G*ΔG-VSV at the same time, supernatant containing the pseudotyped virus was harvest 24 and 48 hours later, aliquot and stored at −80 ℃ for future use. Site-directed mutagenesis based on circular PCR and DpnI (NEB) cleavage were used to construct the mutants of SARS-CoV-2 pseudotyped virus[5]. Primers used for mutation were listed in supplementary Table 1. Virus are quantified via RT-PCR by detecting the P protein of VSV and diluted to the same copy before use as in our previous paper[5].

6 SARS-CoV-2 pseudotyped virus infection assay

Refer to our previous paper[5], the pseudotyped SARS-CoV-2 variants was series diluted before mixed with the Huh 7 or indicated cells at 37°C for one hour. Duplicated wells were set for each group. The cells were then incubated at 37°C supplied with 5% CO2. Twenty-four hours later, chemiluminescence signal was collected by PerkinElmer Ensight using Britelite plus reporter gene assay system (PerkinElmer). Each experiment was repeated for 3-5 times.

7 Neutralization test

The virus neutralization assay was performed as described in our previous paper[5]. The monoclonal antibodies, animal immunize sera or convalescent sera were pre-diluted to a certain initial concentration, and then 3 times series diluted. They were mixed with pseudotyped virus and incubated at 37°C for 1 hour. Thereafter, the mixture was added to the 96-well cell culture plate with 2×10^4 cells/100 μL Huh 7 cells per well. Cells were then incubation at 37°C in a humidified atmosphere comprising 5% CO2. Chemiluminescence signal was detected after 24 hours. The virus neutralization titer was calculate using Reed-Muench method. The results are based on 3-5 repetitions.

8 Western Blot

Seven milliliter pseudotyped virus of each SARS-CoV-2 variant was added to a 2 mL of 25% sucrose buffer and centrifuged at 10□0000g for 3 hours. The purified pseudotyped virus were then re-suspended in 100 μl PBS. Thirty microliter samples after adding loading buffer and heat at 100℃ for 5 minutes was used for SDS-PAGE and western blot analysis. The homemade mouse anti-S1 and anti-S2 antibodies against SARS-CoV-2 spike protein, and anti-VSV M protein were used as the primary antibody, a 1:10000 dilution of HRP-conjugated goat anti-mouse IgG (CWbiotech) was used as secondary antibody. Immobilon western chemiluminescent HRP substrate (Millipore) was used to develop the immunoreactive bands.

9 Statistical analysis

GraphPad Prism 8 was used for plotting. One-way ANOVA and Holm-Sidak's multiple comparisons test were used for statistical analysis. Values were shown as means ±SEM. * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001.

Figures

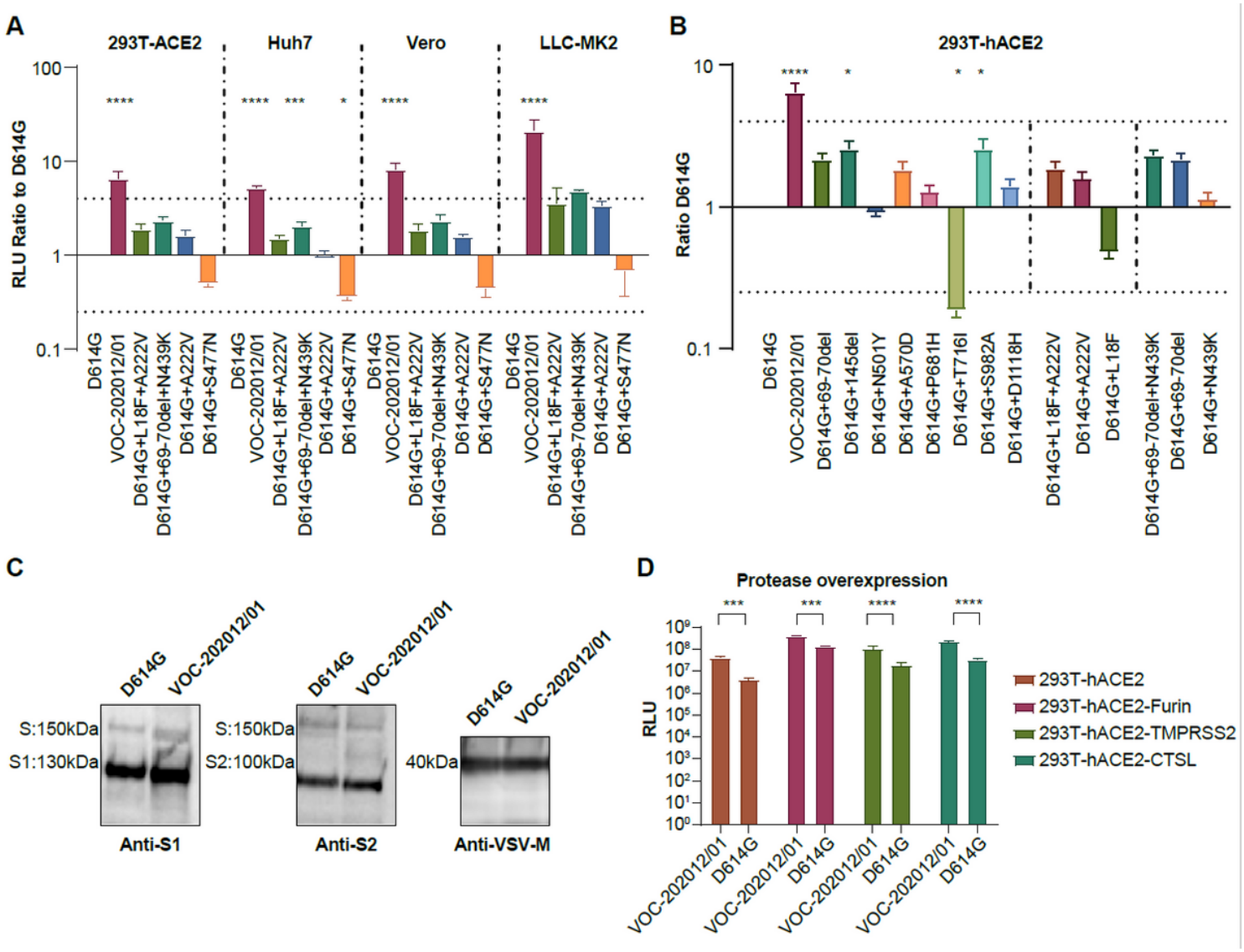

Figure 2

The infectivity analysis of the five variants. A. Four SARS-CoV-2 susceptible cell lines from human and monkey were infected with the pseudotyped viruses of the five SARS-CoV-2 variants after the quantitative of nucleic acid copies. The chemiluminescence signal (RLU) of the target cells were detected 24 hours after the infection. The RLU ratio of the variant compared to D614G were calculated. Three or 12 repeated tests (the 293T-hACE2 data were repeated 12 times) are shown in the figure, with the value of mean±SEM. The dotted line indicates a four-fold change which were considered significant. B. 293T-hACE2 cells were infected with VOC-202012/01, D614G+L18F+A222V, D614G+69-70del+N439K and the single point mutation composing them. The luminescence value of target cells was detected after 24 hours of infection. The ratio of the RLU of each variant to D614G was calculated. The infection dose was adjusted to the same nucleic acid copies. The dotted line represents a 4-fold change. The figure shows the mean value ±SEM of at least three repeated tests. C. VOC-202012/01 and D614G pseudotyped viruses were centrifuged in sucrose buffer, and resuspended in PBS for SDS-PAGE. The western blotting was performed with mouse anti-S1 and anti-S2 polyclonal antibodies. VSV-P was also blotted as internal control. A representative one of three repeated tests were shown. D. Proteolytic enzymes Furin, TMPRSS2

and Cathepsin L (CTSL) were overexpressed in 293T-hACE2 cells respectively. The cells were then infected with VOC-202012/01 or D614G pseudotyped viruses. The chemiluminescence values were detected 24 hours later. The data shown the results of three repeated tests, and the value indicates mean±SEM

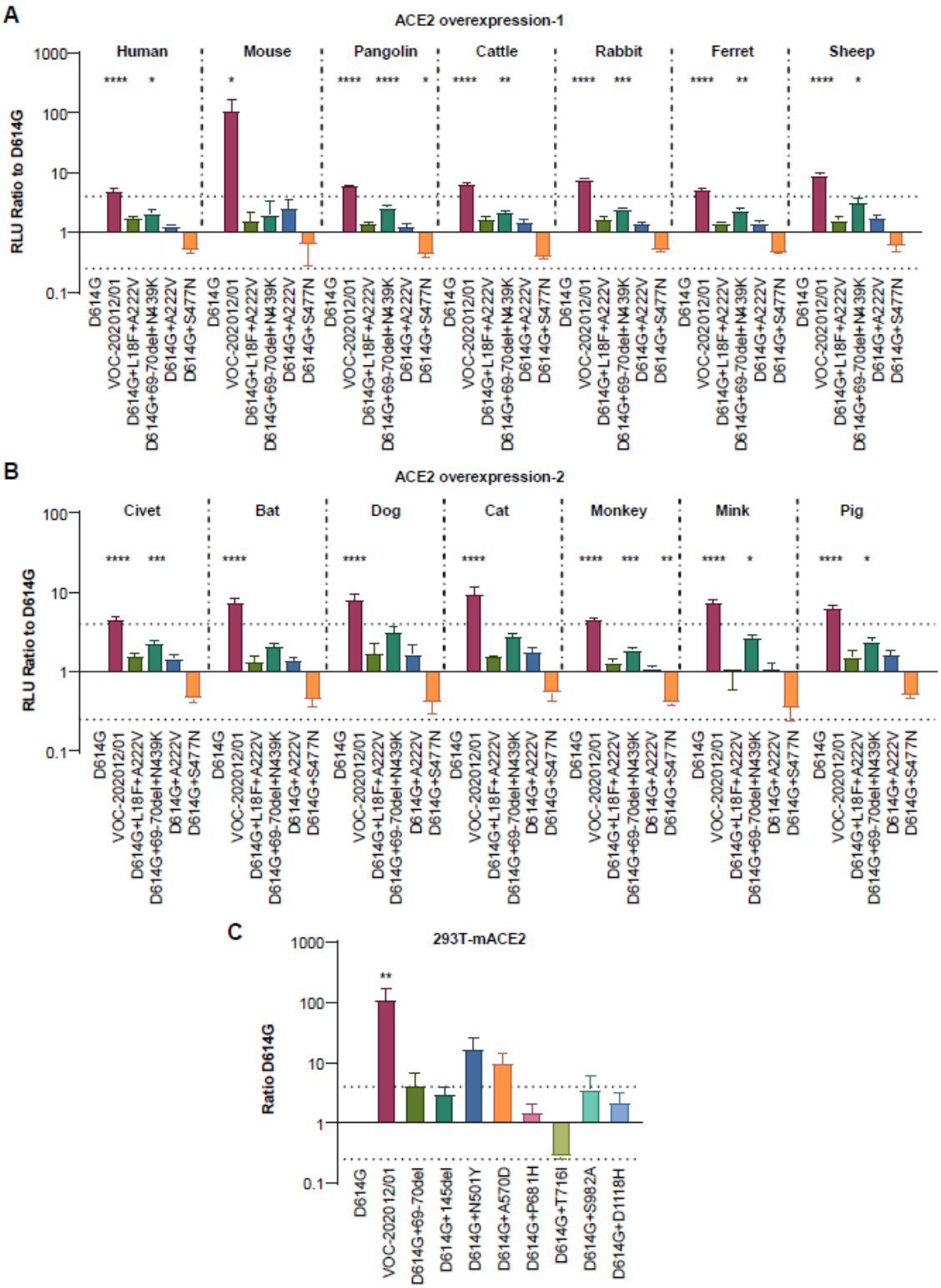

Figure 3

The infectivity of the five variants to fourteen different ACE2 expressed 293T cells. A&B. Equal amount of ACE2 expressing plasmid from different species was transfected in 293T cells. The cells were then infected with five SARS-CoV-2 variants after quantification. The chemiluminescence signal was collected 24hours later. The ratio between the variants and the D614G was calculated. The data shown the results of three repeated tests, and the value indicated mean±SEM. C. Mouse ACE2 was overexpressed in 293T cells, and then infected with VOC-202012/01 and the single point mutated pseudotyped virus composing the variant. The chemiluminescence ratio between the mutants and D614G infected target cells was analyzed. The data show the results of three repeated tests, and the value indicates the mean±SEM.

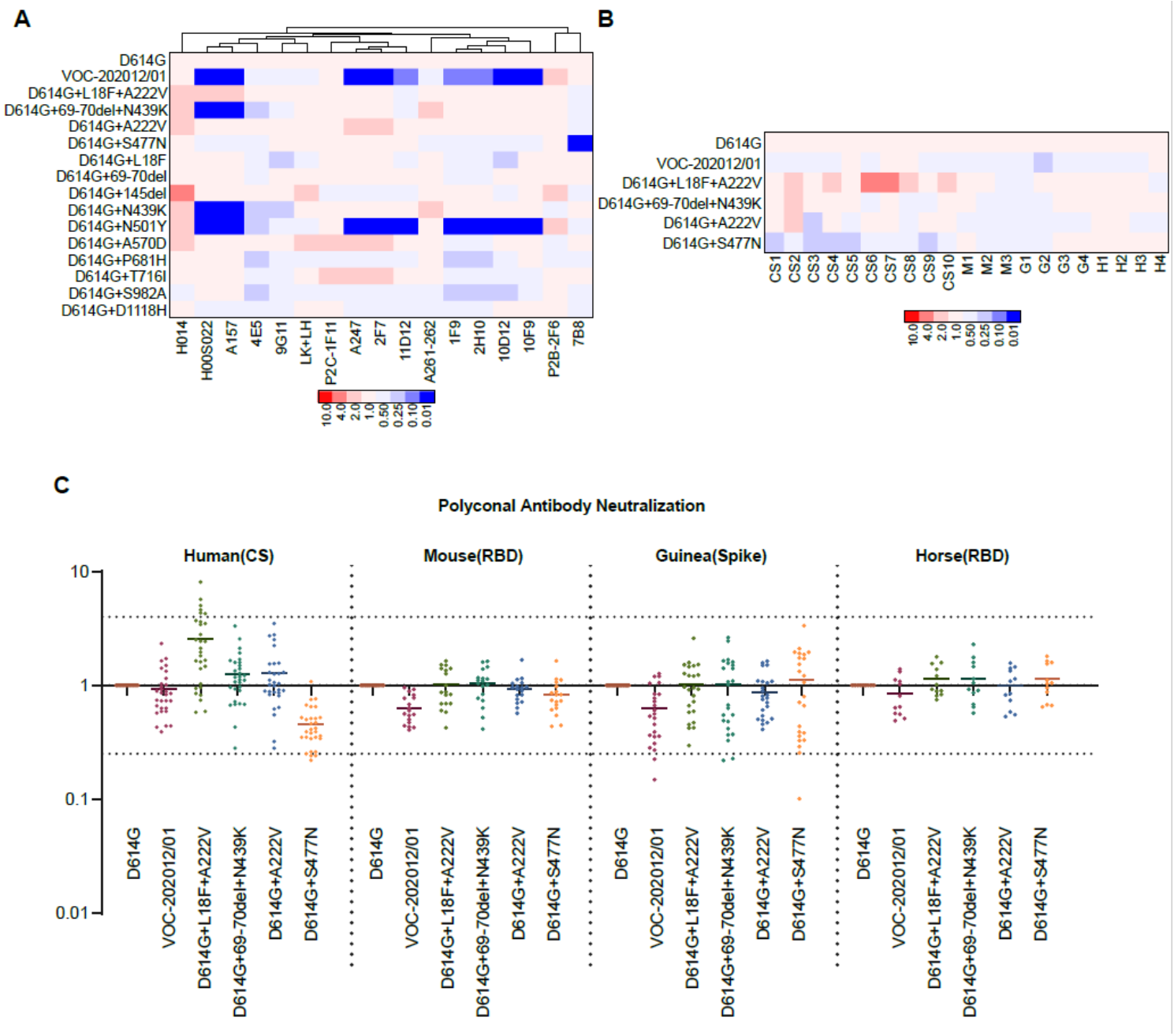

Figure 4

The antigenicity of the five variants Monoclonal antibodies or polyclonal antibodies were serials diluted and mixed with equal amount of the five different SARS-CoV-2 variants as well as those containing single

mutation. After pre-incubation at 37℃ for one hour, Huh 7 cells digested by trypsin were added. After co-culture for 24 hours, the luminescence value of target cells was detected. The neutralization inhibition rate of antibody and EC50 were calculated using the Reed-Muench method. The data shows the EC50 ratio of each variant to D614G. Red represents an increase in neutralization capacity, while blue represents a decrease in neutralization capacity. Four times changes are considered to be statistically significant. A. Heatmap of neutralization ability of 17 different monoclonal antibodies against 16 SARS-CoV-2 variants and mutations, B. Heatmap of neutralization ability 21 different polyclonal antibodies from different sources. CS: convalescent sera; M1-M3: Spike RBD protein immunized mouse sera; G1-G4: Spike DNA immunized guinea pig sera; H1-H4: Spike RBD protein immunized horse sera. C. Scatter graph of the relative neutralization activities of polyclonal antibodies, each point represents a single result, and the dash line represents the mean value. The result is a summary of at least 3 repeated experiments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.pdf