

1 **Ineffective neutralization of the SARS-CoV-2 Mu variant by convalescent and**
2 **vaccine sera**

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18 **Abstract**

19 On August 30, 2021, the WHO classified the SARS-CoV-2 Mu variant (B.1.621
20 lineage) as a new variant of interest. The WHO defines “comparative assessment of
21 virus characteristics and public health risks” as primary action in response to the
22 emergence of new SARS-CoV-2 variants. Here, we demonstrate that the Mu variant
23 is highly resistant to sera from COVID-19 convalescents and BNT162b2-vaccinated
24 individuals. Direct comparison of different SARS-CoV-2 spike proteins revealed that
25 Mu spike is more resistant to serum-mediated neutralization than all other currently
26 recognized variants of interest (VOI) and concern (VOC). This includes the Beta
27 variant (B.1.351) that has been suggested to represent the most resistant variant to
28 convalescent and vaccinated sera to date (e.g., Collier et al, Nature, 2021; Wang et
29 al, Nature, 2021). Since breakthrough infection by newly emerging variants is a
30 major concern during the current COVID-19 pandemic (Bergwerk et al., NEJM,
31 2021), we believe that our findings are of significant public health interest. Our results
32 will help to better assess the risk posed by the Mu variant for vaccinated, previously
33 infected and naïve populations.

34 Text

35 During the current pandemic, severe acute respiratory syndrome coronavirus 2
36 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has
37 considerably diversified. As of September 2021, the WHO has defined four variants
38 of concern (VOC), Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta
39 (B.1.617.2 and AY lineages), as well as five variants of interest (VOI), Eta (B.1.525),
40 Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), and Mu (B.1.621).¹

41 The Mu variant represents the most recently recognized VOI.¹ Until August
42 30, 2021, this VOI was detected in 39 countries (**Table S1**). The epicenter of the Mu
43 variant is Colombia, where it was first isolated on January 11, 2021 (GISAID ID:
44 EPI_ISL_1220045; **Figure 1A** and **Table S2**). This country has experienced a huge
45 COVID-19 surge from March to August 2021 that has peaked at 33,594 cases per
46 day (on June 26, 2021; **Figure 1A**). Although the Gamma VOC was dominant during
47 the initial phase, the Mu VOI outcompeted all other variants including the Gamma
48 VOC in May 2021 and has driven the epidemic in Colombia since then (**Figure 1A**).

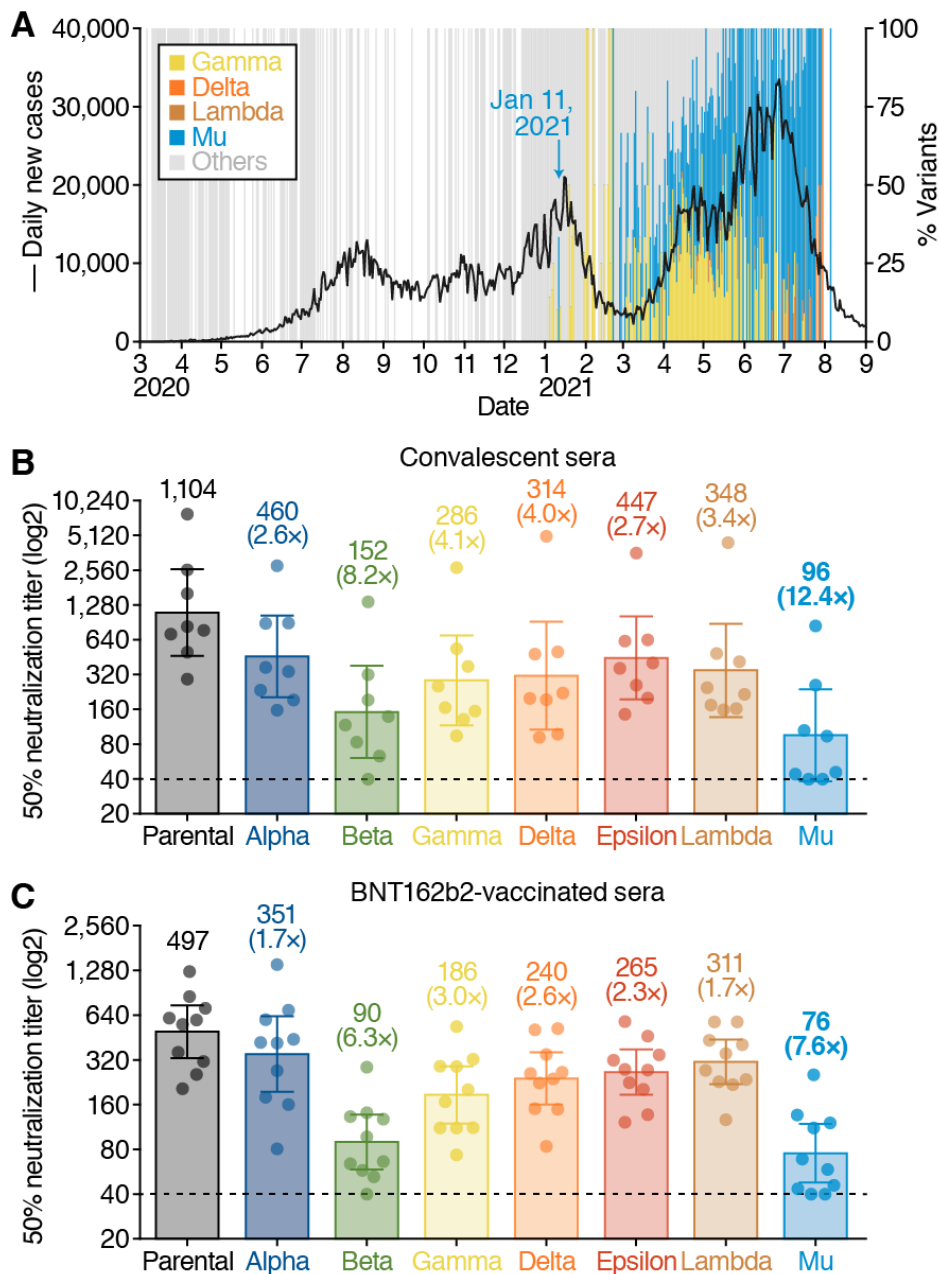
49 Newly emerging SARS-CoV-2 variants need to be carefully monitored for
50 a potential increase in transmission rate, pathogenicity and/or resistance to immune
51 responses. For example, the resistance of VOC/VOIs to humoral immunity elicited
52 by natural SARS-CoV-2 infection or vaccination may allow significant spread of the
53 virus in populations that were initially thought to be protected.² Resistance to COVID-
54 19 convalescent and vaccine recipient sera can be attributed to a variety of
55 mutations in the viral spike protein.² The majority of Mu variants harbors the following
56 eight mutations in spike: T95I, YY144-145TSN, R346K, E484K, N501Y, D614G,
57 P681H, and D950N (**Tables S3 and S4**). These include mutations commonly
58 identified in VOCs: E484K (shared with Beta, Gamma), N501Y (shared with Alpha),
59 P681H (shared with Alpha) and D950N (shared with Delta) (**Table S5**). Of those, the
60 E484K change has been shown to reduce sensitivity towards antibodies induced by
61 natural SARS-CoV-2 infection and vaccination.^{3,4}

62 To assess the sensitivity of the Mu variant to antibodies induced by SARS-
63 CoV-2 infection and vaccination, we generated pseudoviruses harboring the spike
64 proteins of Mu or the other VOC/VOIs. Virus neutralization assays revealed that the
65 Mu variant is 12.4-fold more resistant to sera of eight COVID-19 convalescents, who
66 were infected during the early pandemic (April–September, 2020), than the parental
67 virus ($P=0.0078$; **Figure 1B**). Also, the Mu variant was 7.6-fold more resistant to sera
68 obtained from ten BNT162b2-vaccinated individuals compared to the parental virus
69 ($P=0.0020$; **Figure 1C**). Notably, although the Beta VOC was thought to be the most
70 resistant variant to date,^{3,4} Mu pseudoviruses were significantly more resistant to
71 convalescent serum-mediated neutralization than Beta pseudoviruses ($P=0.031$;

72 **Figure 1B**). Thus, the Mu variant shows a pronounced resistance to antibodies
73 elicited by natural SARS-CoV-2 infection and the BNT162b2 mRNA vaccine. Since
74 breakthrough infections are a major threat of newly emerging SARS-CoV-2
75 variants,⁵ we strongly suggest to further characterize and monitor the Mu variant.

76 **References**

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89 **Figure 1. Characterization of the Mu variant.**

90 (A) SARS-CoV-2 epidemic in Colombia. New COVID-19 cases per day (black line,
 91 left y-axis) and percentage of different SARS-CoV-2 variants spreading in Colombia
 92 (right y-axis) are shown. The daily frequency of Gamma (P.1), Delta (B.1.617.2, AY.4,
 93 AY.5, AY.12), Lambda (C.37), Mu (B.1.621), and other variants are shown in the
 94 indicated colors. Note that there are a few Delta VOC (the currently most dominant
 95 variant in the world) and Lambda VOI (a variant mainly spreading in South American
 96 countries) have been isolated in this country so far. The date when the Mu variant

97 was first isolated (January 11, 2021) is indicated in the figure. The raw data are
98 summarized in **Table S2** in the Supplementary Appendix. (**B and C**) Virus
99 neutralization assays. A neutralization assay was performed using pseudoviruses
100 harboring the SARS-CoV-2 spike proteins of the Alpha, Beta, Gamma, Delta, Epsilon,
101 Lambda, Mu variants or the D614G-harboring parental virus. Eight COVID-19
102 convalescent sera (**B**) and ten sera from BNT162b2-vaccinated individuals (**C**) were
103 tested. The assay of each serum was performed in triplicate to determine the 50%
104 neutralization titer, and each data point represents the 50% neutralization titer
105 obtained with a serum sample against the indicated pseudovirus. The bar graphs
106 indicate geometric mean titers with 95% confidence. The numbers over the bars
107 indicate geometric mean titers. The numbers over the bars in parentheses (with "X")
108 indicate the average of fold change in neutralization resistance of the indicated spike
109 variants compared to that with the parental spike in each serum. Statistical analysis
110 was performed with the use of the Wilcoxon signed-rank test. Horizontal dashed
111 lines indicate limit of detection. The raw data are summarized in **Tables S6 and S7**
112 in the Supplementary Appendix.