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## **SARS-CoV-2 variants of concern are associated with lower RT-PCR amplification cycles between January and March 2021 in France — Source link**

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1           **SARS-CoV-2 variants of concern are associated with**  
2                           **lower RT-PCR amplification cycles**  
3                           **between January and March 2021 in France**

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## Abstract

SARS-CoV-2 variants raise concern regarding the mortality caused by COVID-19 epidemics. We analyse 88,375 cycle amplification (Ct) values from variant-specific RT-PCR tests performed between January 26 and March 13, 2021. We estimate that on March 12, nearly 85% of the infections were caused by the V1 variant and that its transmission advantage over wild type strains was between 38 and 44%. We also find that tests positive for V1 and V2/V3 variants exhibit significantly lower cycle threshold (Ct) values.

## 1 Context

At least three SARS-CoV-2 lineages are currently a major source of concern: variant V1 from lineage B.1.1.7 [1, 2], variant V2 from lineage B.1.351 [3], and variant V3 from lineage P.1 [4]. V1 and V3 variants have been shown to be more contagious [1, 2, 4], while V2 and V3 variants seem to evade immune responses [3, 4]. Although the mechanistic bases are still being investigated, the increased contagiousness could be driven by the N501Y mutation and the  $\Delta$ 69-70 deletion in the Spike protein [1]. The immune escape could largely be due to the E484K mutation also in Spike [3, 4]. Current evidence regarding potential differences in cycle threshold values (denoted Ct) are still unclear [5, 4] as how well they reflect viral loads [6, 7].

In France, using data from 11,916 tests performed on Jan 6 and 7, 2021, a study estimated that 3.3% of the infections were caused by the V1 variant at that time [8]. With data from 10,261 performed on Jan 27, 2021, their estimate increased to 13.0%. Another study used 40,777 tests performed between Jan 26 and Feb 16, 2021, and estimated that on Feb 16, 55% of the infections were caused by V1, V2, or V3 variants [9].

Here, we estimate the spread of SARS-CoV-2 variants of concern using variant-specific RT-PCRs performed in nasopharyngeal (NP) swabs from the general population and in hospital. Furthermore, we analyse the Ct values of these tests.

## 2 Dataset

We analyse 105,356 variant-specific RT-PCR tests performed in France on the same number of individuals between Jan 26 and Mar 12, 2021. The main assay used was ID<sup>TM</sup> SARS-CoV-2/UK/SA Variant Triplex (ID SOLUTION) but for 3,901 tests (3,7%) performed before Feb 2, 2021 we used the VirSNIp SARS-CoV-2 Spike del+501 (TIB MOLBIOL) assay. The sampling varied between French regions and we excluded from the analysis regions with less than 200 tests. 934 tests were also removed because the sampling region was missing.

These tests have probes with 3 targets: a control one in the virus N gene, the  $\Delta$ 69-70 deletion, and the N501Y mutation. For V1 variants, both the deletion and the mutation are present. For V2 or V3 variants, only the N501Y is detected. For VirSNIp assay, it is based on 501 and 69/70 fragments amplified and analyzed with a melting curve using mutation-specific probes, as described earlier [9]. As indicated in [9], the test specificity was confirmed internally using next-generation sequencing.

The main cofactors in the analysis were the assay used, the patient age, the sampling date, the sampling region, and the sampling facility (hospitals or city screening).

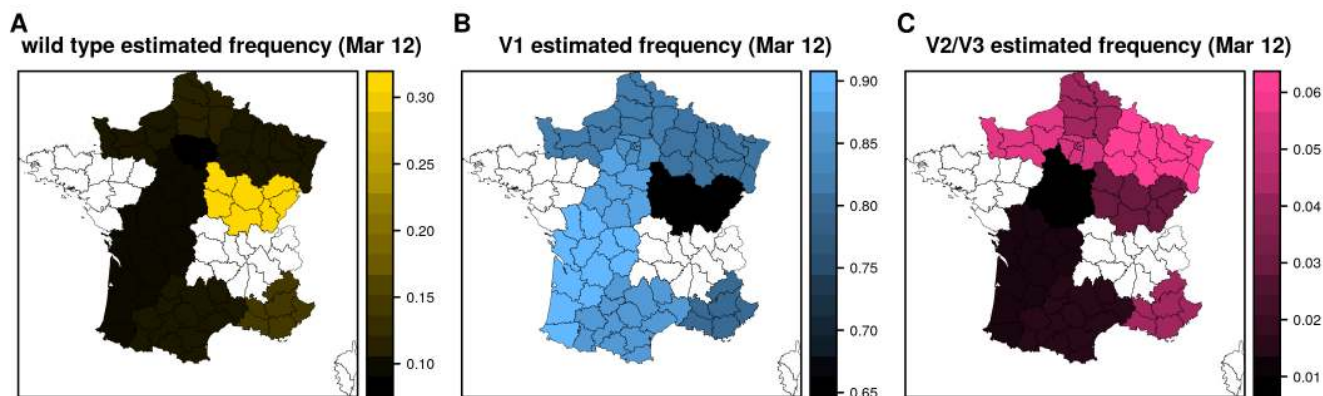


Figure 1: Estimated proportion of new infections caused by A) wild type, B) V1 variant, and C) V2 or V3 variants on Mar 12, 2021, in French regions. Regions with insufficient sampling are in white.

48 For 88,375 ID SOLUTION tests, we also analyse the cycle threshold value (Ct) of the virus control  
49 gene of the assay. Ct values greater than 30 were ignored because they may provide unreliable results  
50 regarding the variant-specific probes LoD (Limit of Detection). Indeed, the latter are located in the S  
51 gene, which tends to exhibit higher Ct values than the N gene [10].

### 52 3 V1 variant is dominant

53 Using the same methodology described in [9] and in Appendix, we calculated the transmission advantage  
54 of each variant compared to the wild type strain after correction for several biases (region, sampling date,  
55 assay, and patient age). This inference was performed without the data from hospitals (in order to avoid  
56 a sampling delay bias) and for individuals from 5 to 80 years old. For V1, the increase in transmission  
57 was 41% (95% confidence interval, CI: [38,44]%). For V2-V3, the estimate was 27% (95%CI: [25,29]%).  
58 These results assume the serial interval reported in [11].

59 Based on these inferences, we estimated the proportion of new infections caused by each type of strain  
60 on Mar 12, 2021. At a national level, the estimate was 84,6% for V1 and 5,4% for V2/V3, but with strong  
61 regional heterogeneity (Figure 1).

### 62 4 Variants have lower Ct values

63 We analysed tests from patients from 1 to 89 years old with Ct values lower than 30. We excluded tests  
64 where the strain could not be determined. Overall, this represents 63,924 tests (96% of all the tests with  
65 Ct values).

66 We used a multivariate linear regression to study variations in Ct values between strains. The linear  
67 model covariates were the age, the sampling facility (hospital or city), the sampling date, and the region.  
68 We also considered an interaction between sampling region and date. We used a type-I analysis of variance  
69 (ANOVA) and added the variant covariate last to avoid favouring it in the analysis.

70 The model residuals were normally distributed and a likelihood-ratio test between a model with or

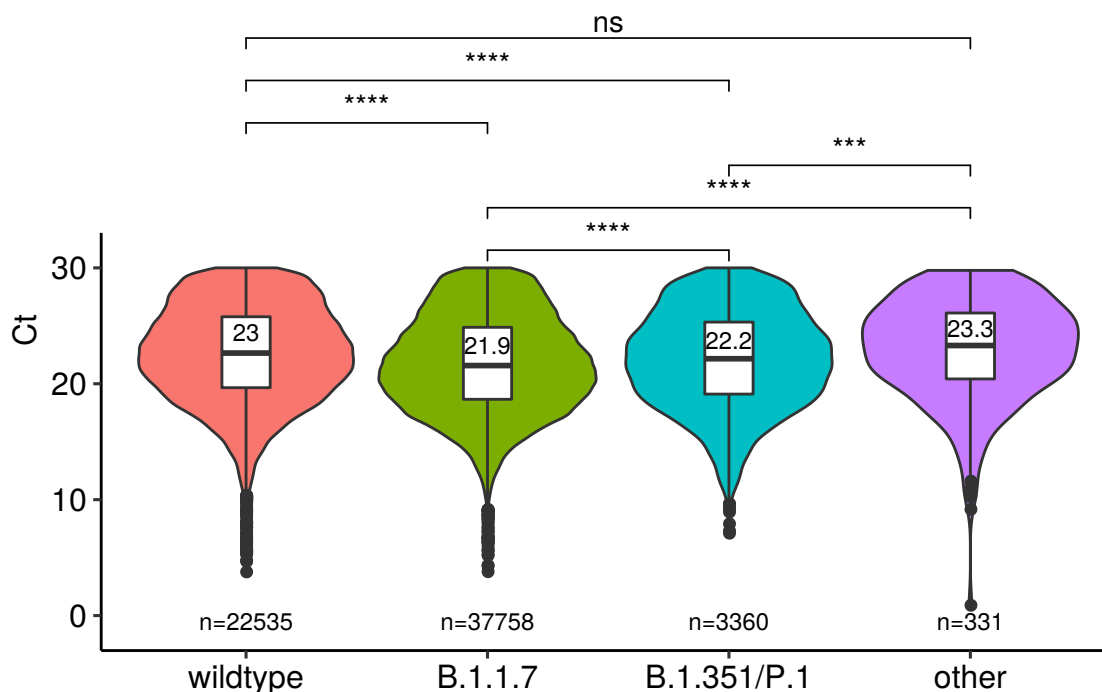


Figure 2: **Cycles threshold (Ct) value for SARS-CoV-2 strains.** Median estimates based on the linear model are shown in the box plot, and number of tests in each class are shown in the bottom of the graph. ‘other’ indicate tests with the  $\Delta 69-70$  deletion and without the N501Y mutation. Start indicate the significance level (\*\*\* for a p-values strictly lower than  $10^{-4}$  and \*\* for a p-value of  $10^{-3}$ , ns : not significant).

71 without strain effect retained the former. Overall, the model explained a small fraction of the variation in  
72 Ct (adj.  $R^2=4.4\%$ ), which is consistent with these values being highly variable [10].

73 The virus strain effect was highly significant in the ANOVA (Figure 2). Samples from V1 variants had  
74 a significantly smaller Ct than that from V2/V3 variants (21.9 vs. 22.2). Both had significantly smaller Ct  
75 values than wild type strains (23) and other variants (without the N501Y mutation but with the  $\Delta 69-70$   
76 deletion).

77 The model also indicated a significant decrease of Ct with age, which is also consistent with existing  
78 data [10]. The sampling date was non-significant but the sampling region mattered and so did the interac-  
79 tion between sampling date and region. Samples from hospitals has a slightly higher Ct, but this is likely  
80 due to the fact that testing in the general population occurs approximately 7 days after infection, whereas  
81 hospital admissions occur after a median time of 14 days [12]. Therefore, data from hospitalised patients  
82 is likely to reflect an older state of the epidemic.

## 83 5 Discussion

84 We show that variant of concern V1 is now vastly dominant in France compared to wild type strains  
85 (84,6% vs. less than 10%). V2 or V3 variants remain limited (approximately 5.4% of the new infections).  
86 These results are consistent with earlier reports of a marked transmission-advantage of the V1 variant

87 [1, 2, 9, 8].

88 By investigating the RT-PCR Ct values, which can inform us on clinical features of the infection [6, 10],  
89 we show that infections caused by variants significantly differ from that caused by wild type strains. That  
90 variants are associated with lower Ct values could be an indication of higher viral load, although care must  
91 be taken because of the biology of SARS-CoV-2 [7] and of the variability inherent to such values [10].

92 This result contrasts with earlier findings. One study did not find a significant result when comparing  
93 Ct values for tests with or without the S-gene target failure [5]. However, our results are based on a variant-  
94 specific PCR. Another study on the V3 variant [4] did not find a significant difference after accounting  
95 for the symptom onset to sampling delay. However, their study was performed on a limited number of  
96 samples ( $n = 147$ ).

## 97 Data availability

98 The data and scripts used for the analysis will be shared upon publication.

## 99 Acknowledgements

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102 This study was approved by the Internal Review Board of the CHU of Montpellier (ClinicalTrials.gov  
103 identifier NCT04738331).

## 104 References

- 105 [1] Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated trans-  
106 missibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science*. 2021 Mar; Available from:  
107 <https://science.sciencemag.org/content/early/2021/03/03/science.abg3055>.
- 108 [2] Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, et al. Transmission of SARS-CoV-2  
109 Lineage B.1.1.7 in England: Insights from linking epidemiological and genetic data. *medRxiv*. 2021  
110 Jan;p. 2020.12.30.20249034. Available from: [https://www.medrxiv.org/content/10.1101/2020.](https://www.medrxiv.org/content/10.1101/2020.12.30.20249034v2)  
111 [12.30.20249034v2](https://www.medrxiv.org/content/10.1101/2020.12.30.20249034v2).
- 112 [3] Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Emergence and  
113 rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage  
114 with multiple spike mutations in South Africa. *medRxiv*. 2020 Dec;p. 2020.12.21.20248640. Available  
115 from: <https://www.medrxiv.org/content/10.1101/2020.12.21.20248640v1>.
- 116 [4] Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DdS, Mishra S, et al. Genomics and epidemi-  
117 ology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv*. 2021 Mar;p. 2021.02.26.21252554.  
118 Available from: <https://www.medrxiv.org/content/10.1101/2021.02.26.21252554v1>.
- 119 [5] Walker PGT, Whittaker C, Watson OJ, Baguelin M, Winskill P, Hamlet A, et al. The impact  
120 of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries.

- 121 Science. 2020 Jul;369(6502):413–422. Available from: [https://science.sciencemag.org/content/](https://science.sciencemag.org/content/369/6502/413)  
122 [369/6502/413](https://science.sciencemag.org/content/369/6502/413).
- 123 [6] Néant N, Lingas G, Hingrat QL, Ghosn J, Engelmann I, Lepiller Q, et al. Modeling SARS-CoV-2  
124 viral kinetics and association with mortality in hospitalized patients from the French COVID cohort.  
125 PNAS. 2021 Feb;118(8). Available from: <https://www.pnas.org/content/118/8/e2017962118>.
- 126 [7] Michalakis Y, Sofonea MT, Alizon S. SARS-CoV-2 viral RNA is not viral load. OSF Preprints; 2021.  
127 Type: article. Available from: <https://osf.io/5gra3/>.
- 128 [8] Gaymard A, Bosetti P, Feri A, Destras G, Enouf V, Andronico A, et al. Early assessment of diffusion  
129 and possible expansion of SARS-CoV-2 Lineage 20I/501Y.V1 (B.1.1.7, variant of concern 202012/01)  
130 in France, January to March 2021. Eurosurveillance. 2021 Mar;26(9):2100133. Available from: <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.9.2100133>.
- 132 [9] Haim-Boukobza S, Roquebert B, Trombert-Paolantoni S, Lecorche E, Verdurme L, Foulongne V, et al.  
133 Specific RT-PCR tests reveal that SARS-CoV-2 variants have a significant transmission advantage  
134 over wild type strains and that their spread is faster than anticipated in France. Emerging Infectious  
135 Diseases. 2021;in press.
- 136 [10] Alizon S, Selinger C, Sofonea MT, Haim-Boukobza S, Giannoli JM, Ninove L, et al. Epidemiological  
137 and clinical insights from SARS-CoV-2 RT-PCR cycle amplification values. medRxiv. 2021 Mar;p.  
138 2021.03.15.21253653. Available from: [https://www.medrxiv.org/content/10.1101/2021.03.15.](https://www.medrxiv.org/content/10.1101/2021.03.15.21253653v1)  
139 [21253653v1](https://www.medrxiv.org/content/10.1101/2021.03.15.21253653v1).
- 140 [11] Nishiura H, Linton NM, Akhmetzhanov AR. Serial interval of novel coronavirus (COVID-19)  
141 infections. International Journal of Infectious Diseases. 2020 Apr;93:284–286. Available from:  
142 [https://www.ijidonline.com/article/S1201-9712\(20\)30119-3/abstract](https://www.ijidonline.com/article/S1201-9712(20)30119-3/abstract).
- 143 [12] Sofonea MT, Reyné B, Elie B, Djidjou-Demasse R, Selinger C, Michalakis Y, et al. Epidemio-  
144 logical monitoring and control perspectives: application of a parsimonious modelling framework to  
145 the COVID-19 dynamics in France. medRxiv. 2020 May;p. 2020.05.22.20110593. Available from:  
146 <https://www.medrxiv.org/content/10.1101/2020.05.22.20110593v1>.

## 147 **Supplementary methods**

### 148 **Linear model for the Ct analysis**

149 We used a type I error for the analysis-of-variance. Our response variable was the Ct value. The main  
150 covariate of interest was the strain and it could take 4 values (V1, V2 or V3, wild type, or other). The other  
151 covariates were the age, the sampling facility (hospital or city), the sampling date, and the geographical  
152 region. We also considered an interaction between sampling region and date. We used a type-I analysis of  
153 variance (ANOVA) and added the strain covariate last. The motivation for this is that with the sequential  
154 assumption of the summing of the squares (type I method), the order in which the covariates are tested  
155 matters, and, in the case of an uneven sampling, the last one in the list is less likely to be significant.  
156 Therefore, our assumption decreases the risk of erroneously attributing observed variance to a variant  
157 effect.

158 We used a likelihood-ratio test to determine whether the addition of the strain effect statistically  
159 improved the explanation of the data.

### 160 **Generalised linear model to correct for variant sampling bias**

161 As indicated in [9], for a given variant category (V1 or V2/V3) we first perform a generalised linear model  
162 with a binomial error distribution where the variable of interest is the binary variant variable (with values  
163 ‘variant’ or ‘wild type’) and the explanatory variables are the sampling date, the sampling region, and  
164 the individual age. We also include an interaction between sampling region and date. We then use the  
165 residuals of this model to infer the transmission advantage of the variant.

### 166 **Logistic growth fitting**

167 We used the fitted values of a GLM model applied to the data after removing samples from hospitals  
168 (the sampling location effect was also obviously removed from the model) to perform the inference of a  
169 two-parameter logistic growth kinetic curve:  $f(t) = (1 + e^{-\rho(t-\tau)})^{-1}$ , where  $f(t)$  is the frequency of the  
170 variants in the new infections at time  $t$ ,  $\rho$  is the relative growth rate of the variants and  $\tau$  is the time  
171 at which  $f$  reaches 1/2. This method is indeed more appropriate to deal with temporal auto-correlation  
172 biases in proportion time series [1, 2].

173 The parameter estimation was performed using the `drc` package in R both at the national and the  
174 regional level (for regions with at least 1,000 samples). The confidence intervals of the fitted curves rely  
175 on those of the estimated date of reaching half proportion of new infections ( $\tau$ ).

176 The unitless estimated transmission advantage is expressed in terms of multiplicative gain in repro-  
177 duction number with respect to that of the wild type, such that  $\mathcal{R}_{\text{variant}} = (1 + \text{ETA}) \times \mathcal{R}_{\text{wild type}}$ . Its  
178 calculation was made by solving the Euler-Lotka equation ( $\mathcal{R}_{\text{variant}} \int_0^\infty e^{-\rho t} w(t) dt = 1$ ) assuming a serial  
179 interval  $w$  following a Weibull distribution with a mean and SD of 4.8 and 2.3 days [11] and a constant  
180  $\mathcal{R}_{\text{wild type}}$  equal to 1. The confidence interval rely on those of the estimated relative growth rate.

181 The estimate of the frequency of variant on Mar 12, 2021, was done by first estimating the proportion  
182  $p_x$  of a given variant VX compared to the wild type (while ignoring the other variant VY) and second  
183 performing the same analysis to look at the proportion  $p_x^T$  of wild type and VX compared to the whole  
184 population (VX plus VY plus wild type). The frequency of variant X was then obtained as  $p_x \times p_x^T$ .



185 **Supplementary figures**

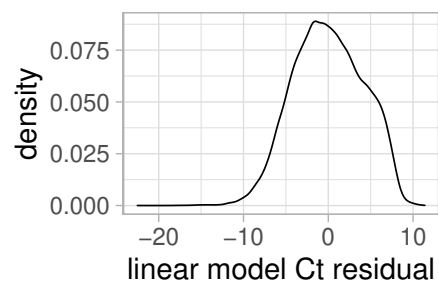


Figure S1: **Distribution of the residual values of the multivariate linear model.**

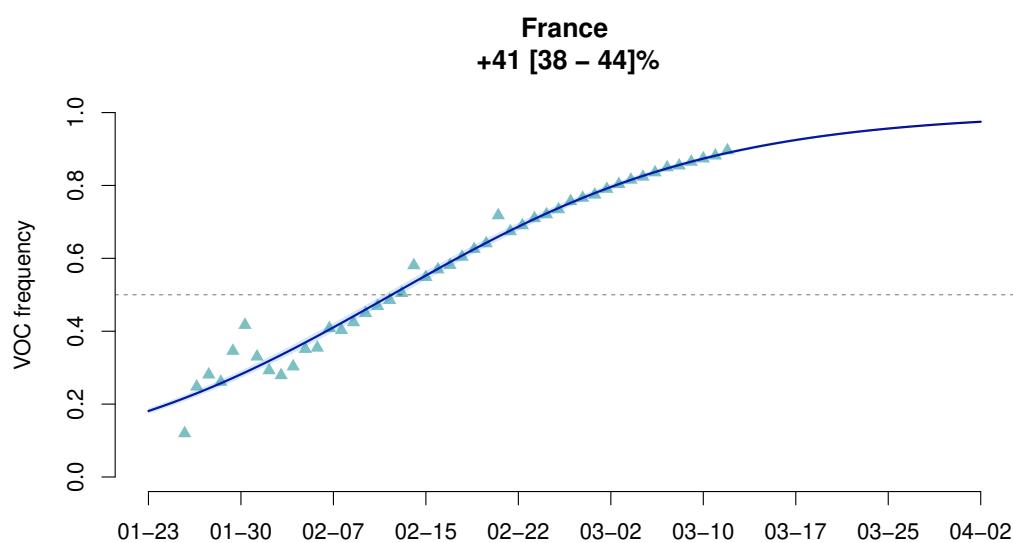


Figure S2: **Estimating the transmission advantage of the V1 variant over the wild type strain.** The dots indicate the GLM-fitted values values and the line is the output of the logistic growth model estimation. The top figures indicate the estimated transmission advantage of the V1 variant (with respect to the wild type reproduction number) and its 95%-confidence interval. The x-axis shows the date (month-day format).

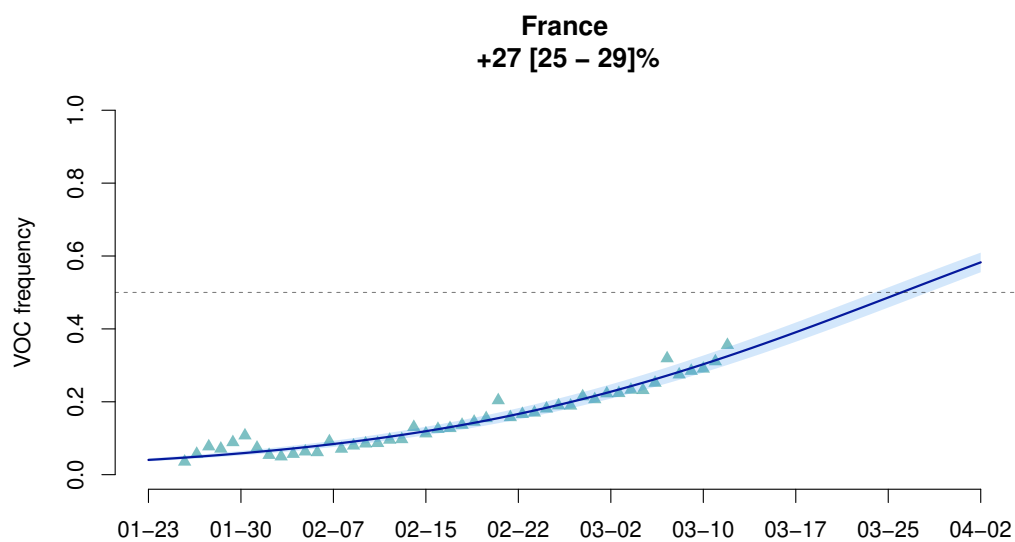


Figure S3: **Estimating the transmission advantage of the V2 or V3 variants over the wild type strain.** See Figure S2 for details.