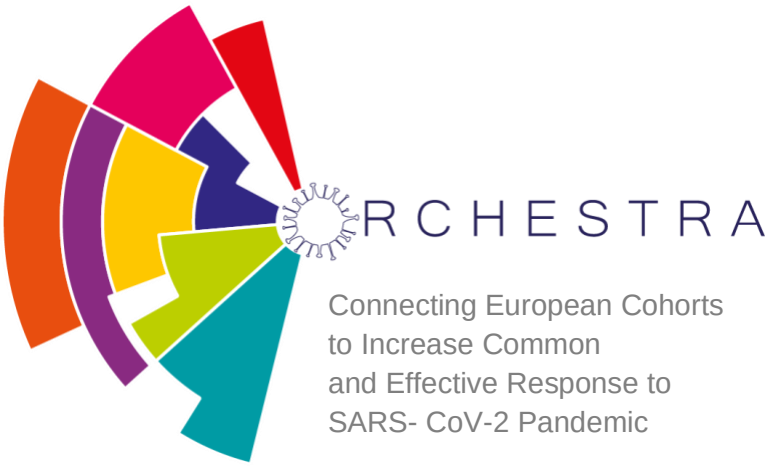

DELIVERABLE

WP5_D5.7,

First interim risk analysis of breakthrough infections

Ludwig-Maximilians-Universität Munich (LMU)

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Executive summary

This report contains preliminary results concerning the risk of SARS-CoV-2 infections in fully vaccinated health-care workers and staff of long-term care facilities (breakthrough infections). That is, unlike D5.8, this report focusses on determinants of breakthrough infections rather than on their frequency.

The analysis is based on two samples of breakthrough infections amongst health-care workers, which were identified from a short-term (i.e., 2 to 6 months) serological follow-up and self-reported, PCR-confirmed SARS-CoV-2-infections, respectively. Since both samples derive from the (COVID-19 vaccinated) KoCoImpf cohort, pre-infection serological data and questionnaire information were available to assess the predictive value of various determinants for subsequent breakthrough infections.

Due to the rather low SARS-CoV-2-incidence in Bavaria in the period between recruitment (June to August 2021) and the first KoCoImpf follow-up (November 2021), both samples are relatively small. An anti-Nucleocapsid-seroconversion (as surrogate for a symptomatic or asymptomatic breakthrough infection) was detected in eight health-care workers out of 280 participating in the first follow-up in November 2021 corresponding to a cumulative incidence of 2.85%. Until February 2022, 32 study participants out of 1743 (health-care workers and staff of long-term care facilities) reported a positive SARS-CoV-2-RNA PCR result constituting a cumulative incidence of 1.84%. The latter incidence is lower since self-reporting was certainly not exhaustive, and a substantial part of asymptomatic cases were likely missed by merely relying on positive PCR results.

In neither sample, post-vaccination anti-Spike-antibody levels were predictive for subsequent SARS-CoV-2 breakthrough infections. This result is in line with our serological findings from the Munich general population investigated in our other cohort studies. So far, non-serological risk-factors were analysed for the 280 participants who underwent and returned samples and questionnaires. At this stage, this rather small sample size limits the external validity of the study substantially. Despite previous publications reporting some risk factors to develop breakthrough infections, no clear trend towards risk- or prediction factors for breakthrough infections were found in this group.

As far as the post-infection anti-Spike-values are concerned, we could see a substantial booster effect of both symptomatic and asymptomatic breakthrough infections. Although expected, this finding underscores the importance of including asymptomatic (i.e., N-seroconverted) cases in the serological models of all Orchestra studies because otherwise the vaccination-related effects on antibody titres might be

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significantly overestimated and the contribution of asymptomatic cases for the outbreak dynamics could be missed.

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Core content

1. Cohorts and Study Profile

KoCoImpf Study: Prospective COVID-19 Post-Immunization Cohort in Munich

The aim of this study is to understand the serological short-, medium- and long-term immune response as well as the patterns of breakthrough infections in vaccinated individuals with focus on health-care workers in the greater Munich area.
Detailed objectives are

- the determination of the baseline immune status and the prevalence of individuals (silently and symptomatically) infected before vaccination
- the follow-up of SARS-CoV-2-antibody dynamics over time and in relation to the vaccine they received, pre-existing immunity, and other covariates
- the determination of relative risks of post-immunization infections with SARS-CoV-2 in relation to antibody titres at the time of infection and other covariates.

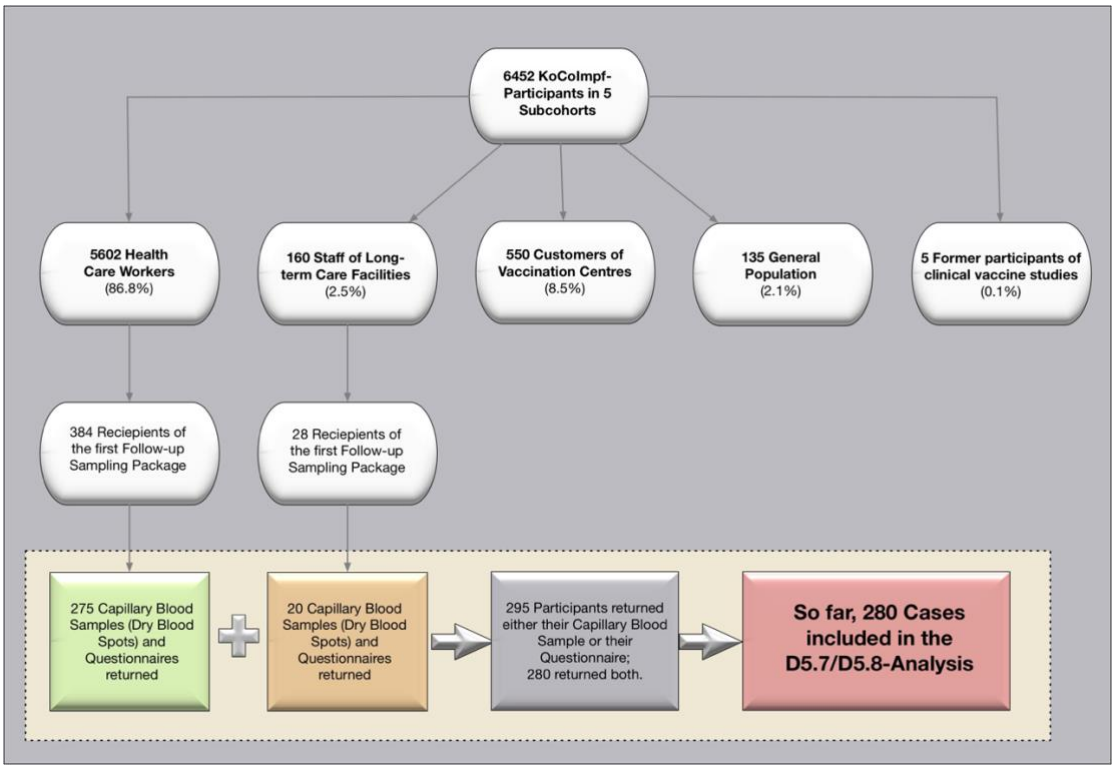

Figure 1: *The KoCoImpf-cohort with its current contribution to D5.7*

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2. Methods Brief

Capillary blood samples were analysed for SARS-CoV-2 antibodies using the Elecsys® Anti-N SARS-CoV-2 (Roche) test. Antibody follow-ups were conducted in the beginning of November 2021 based on participants self-sampled capillary blood (Dry Blood Spot). Anti-Spike-antibodies were additionally measured to quantify the individual serological response to the vaccinations. Newly Anti-N positive participants without anti-Spike in the initial serological analysis were considered naturally infected. Newly anti-N positive cases with anti-Spike-antibodies in the initial round were considered breakthrough infections. Details concerning vaccinations, risk factors and infections were based on questionnaire data.

The primary COVID-19 vaccination for study participants took place between December 2020 and July 2021. Participants were enrolled from 15th June until 15th August 2021. After enrolment, the first follow-up took place in November and December 2021 with a second DBS sample and a questionnaire. The individual follow-up interval variates between two to six months because the timing of follow-ups was guided rather regarding the local epidemiological situation than a fixed period after recruitment. In case of breakthrough infections, participants' self-reporting of positive SARS-CoV-2 RT-PCR results were collected until February 2022 and led to another blood sampling for further serological and questionnaire-based investigations.

3. Determinants of breakthrough infections

3.1 Risk-related serological determinants of breakthrough infections

3.1.1 Background

The identification of breakthrough infections in KoCoImpf relies mainly on the detection of anti-Nucleocapsid-seroconversions (anti-N-seroconversions) during short-term follow-up periods. On the other hand, post-vaccination anti-Spike-protein antibody titres (anti-Spike) were measured (initially and during follow-ups) to assess a possible serological determinant of the vaccine-derived immune protection. We wanted to investigate if the post-vaccination anti-Spike-protein antibody titre (anti-Spike) prior to

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the breakthrough infection could be utilized as an independent predictor of both probability and severity of subsequent SARS-CoV-2 infections. Previously, higher anti-Spike-levels have been found to be associated with a better protection (1-4).

3.1.2 Findings

Among the 280 study participants included in the first follow-up analysis (see fig. 1), eight new breakthrough infection cases were identified serologically until December 2021. In this relatively small sample of breakthrough infections, the pre-infection anti-Spike-titres of cases (measured as baseline value on the recruitment day) are well spread over the entire spectrum of initial anti-Spike-values of the study population (see fig. 2). Obviously, these post-vaccination anti-Spike-titres were not predictive for the subsequent SARS-CoV-2-infection.

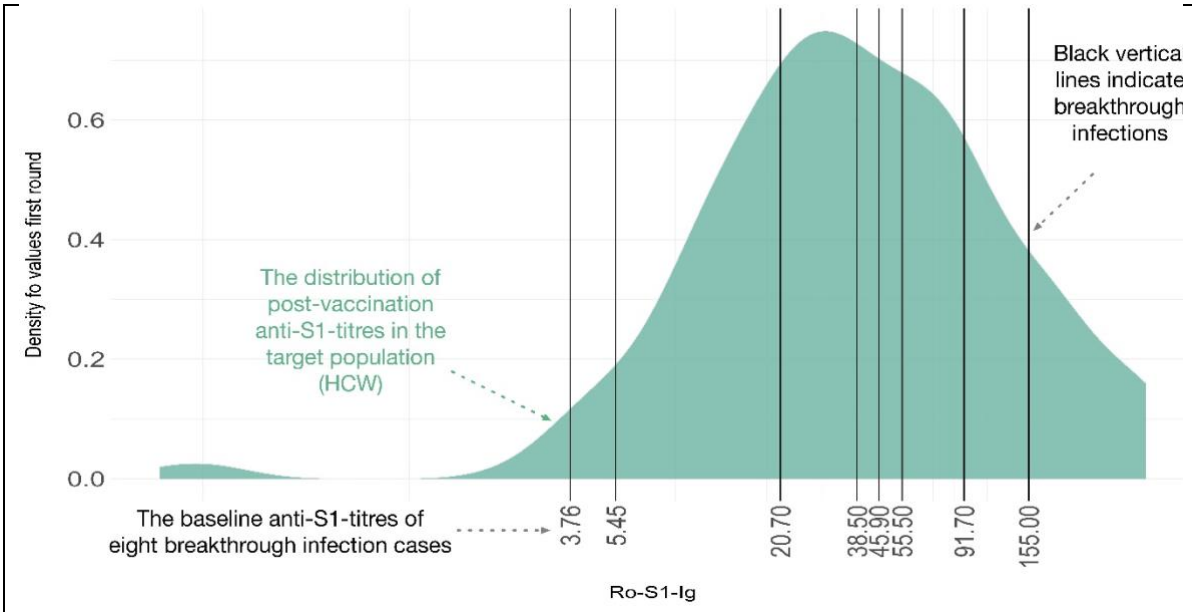

Figure 2: *The initial anti-Spike-antibody titre (vertical lines) of the eight study participants that showed an anti-nucleocapsid seroconversion in their first follow-up (i.e. breakthrough infections) relative to the density distribution (green curve) of anti-Spike-titres of the population (here health care workers)*

Including questionnaire data into this analysis, it was possible to differentiate between symptomatic and asymptomatic infections. In this context, figure 3 seems to suggest that there is a tendency towards symptomatic infections when pre-infection anti-Spike-titres are relatively low. While four out of four cases with a lower pre-infection anti-Spike-value (below the median $_{\text{Ro-Spike-Ig}}$ 42.2 of the eight cases) are symptomatic, there

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is only one such symptomatic case among the four breakthrough infections with a higher pre-infection anti-Spike-value (above the median).

However, looking at the 32 self-reported cases in figure 4 (which are usually symptomatic), the pre-infection serologies are spread again across the entire spectrum of our population-based post-vaccination reference values for anti-Spike.

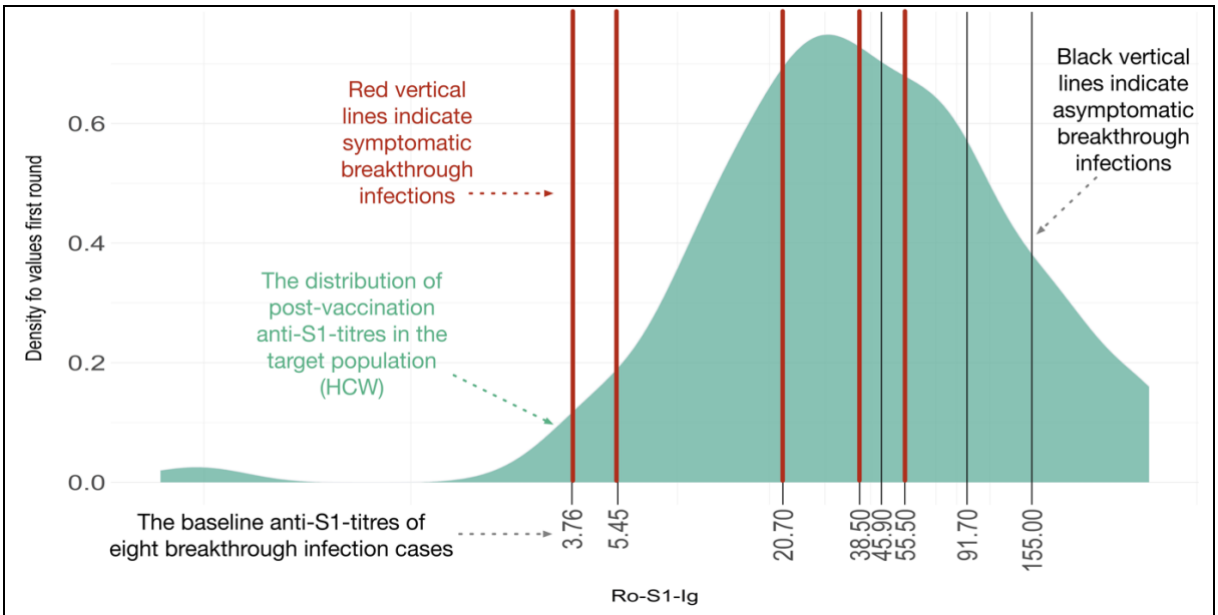

Figure 3: *Differentiation of symptomatic (red lines) and asymptomatic breakthrough infection, identified in the first follow-up in November 2021 (black lines). The vertical lines indicate initial anti-Spike-antibody titre of the eight study participants that showed an anti-nucleocapsid seroconversion in the follow-up relative to the anti-Spike-density distribution of the population*

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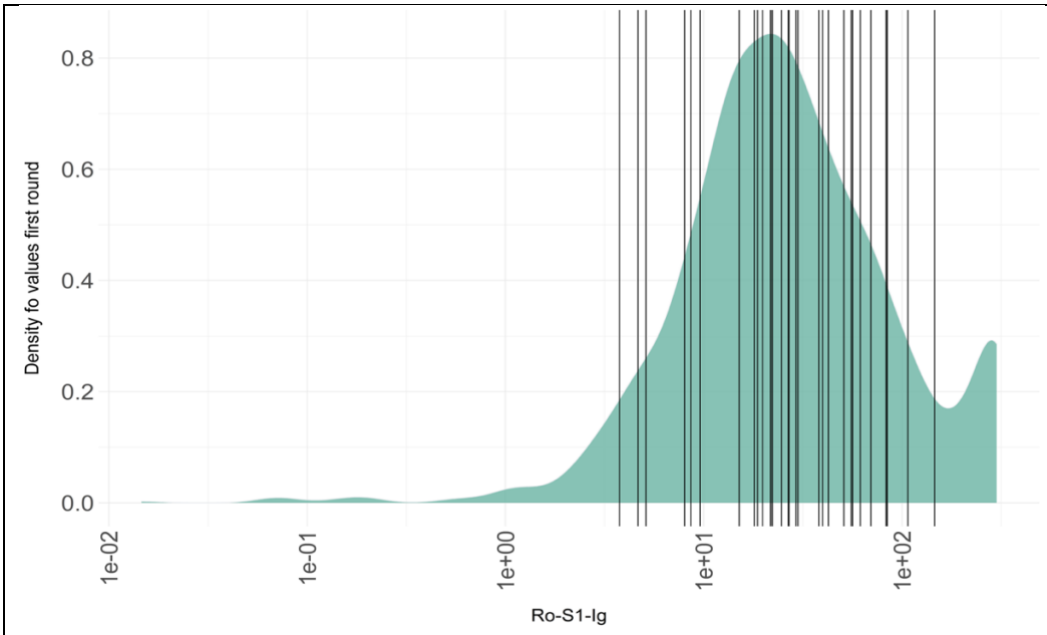

Figure 4: *The initial anti-Spike-antibody titre (vertical lines) of the 32 study participants that reported breakthrough infections until end of January 2022 relative to the density distribution (green curve) of anti-Spike-titres of the population (here health care workers)*

Figure 5 below shows that the post-infection anti-Spike-titres of the eight cases increased substantially to titres which, individually and collectively, none of them had reached in the initial post-vaccination/pre-infection measurements. On the one hand, these results could be seen as a booster effect caused by hybrid immunity (5), which might be an independent determinant of risk especially as far as the clinical severity of infections or breakthrough re-infections are concerned.

Consequently, we might consider these maximum post-infection titres more thoroughly in the upcoming analyses. On the other hand, these result underscore the necessity to include (anti-N-positive) asymptomatic infections in Orchestra's serological studies and risk assessments. Otherwise, these ultra-high anti-Spike-titres might cause a bias towards higher anti-Spike-values misinterpreted as exclusively vaccination-generated titres.

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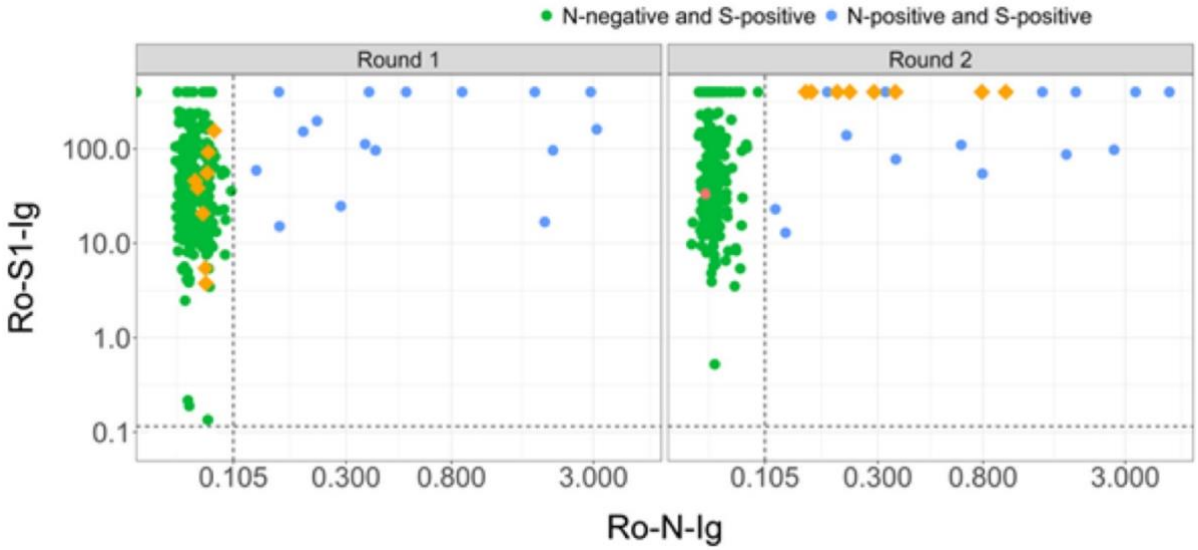

Figure 5: *The four-quadrant-approach: 8 study participants that showed vaccination titres (anti-Spike-antibodies without anti-N in the first round (orange rhombuses) shifted to a positive anti-Spike/anti-N-pattern in the second round thereby indicating a breakthrough infection*

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3.2 Other risk-related determinants of breakthrough infections

The eight breakthrough infection cases identified in the period between recruitment until November 2021 among the 280 study participants included in the first follow-up analysis exhibited a risk factor distribution as shown in the following table:

	Breakthrough Infection cases	Other participants of the 1. follow-up
Total number	8	272
Median age in years (min, max)	35.5 (21.8, 68.3)	40.63 (18.64,78.34)
Gender (female, male)	4, 4	188,84
Smoking status (never smoker, current smoker)	7, 1	192,34,46
Intake of immune drugs (yes, no, unknown)	0, 8, 0	16,255,1
Contact to patients (yes, no, unknown)	3, 0, 5	114,36,122
Type of exposure (Face to face)	1	105
Shift work (yes, no, unknown)	0, 1, 7	31,109,132
First and second vaccination (manufacturers)		
BioNTech - BioNTech	4	157
Moderna - Moderna	1	28
BioNTech – no or unknown second vaccination	0	6
AstraZeneca – BionNTech	0	10
AstraZeneca – AstraZeneca	0	3
AstraZeneca – Moderna	0	6
J&J	0	2
Unknown	3	60

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4 Discussion

Until now, based on the relatively small sample sizes, no clear trend towards risk or prediction factors could be identified for the two groups described above. Non-occupational, arbitrary, and random exposure patterns might have played a greater role for SARS-CoV-2-transmissions in the identified cases than pre-infection antibody titres or the non-serological determinants collected in the KoCoImpf questionnaires. Dimeglio et al. (4) found recently that 90% of Delta and Omicron breakthrough infections occurred with ELISA-Antibodies below 6967 BAU/mL and 2905 BAU/mL respectively. Other cohorts have reported age, sex, comorbidities and close contact to positive SARS-CoV-2 cases (workplace and non-workplace related) as risk factors to develop breakthrough infections in HCWs (6,7).

In our study, however, the absence of evidence so far is not evidence of absence. With the power of the 5,602 Munich health care workers recruited by now (plus the 160 staff of long-term care facilities) that are due for a follow-up in March 2022, we will be able to provide an in-depth risk factor analysis of breakthrough infection in the time of the Omicron variant. Due to our triple detection approach for breakthrough infections (anti-N-seroconversions, self-reported PCR results, and ad-hoc testing of symptomatic participants), we will increasingly be able to distinguish determinants of risk for symptomatic and asymptomatic infections, respectively. Based on the data provided here, it seems already likely that relying exclusively on self-reported diagnoses and PCR results for the identification of breakthrough infections will miss a key element of the outbreak dynamics and introduce a bias into the post-vaccination serological analyses of Orchestra.

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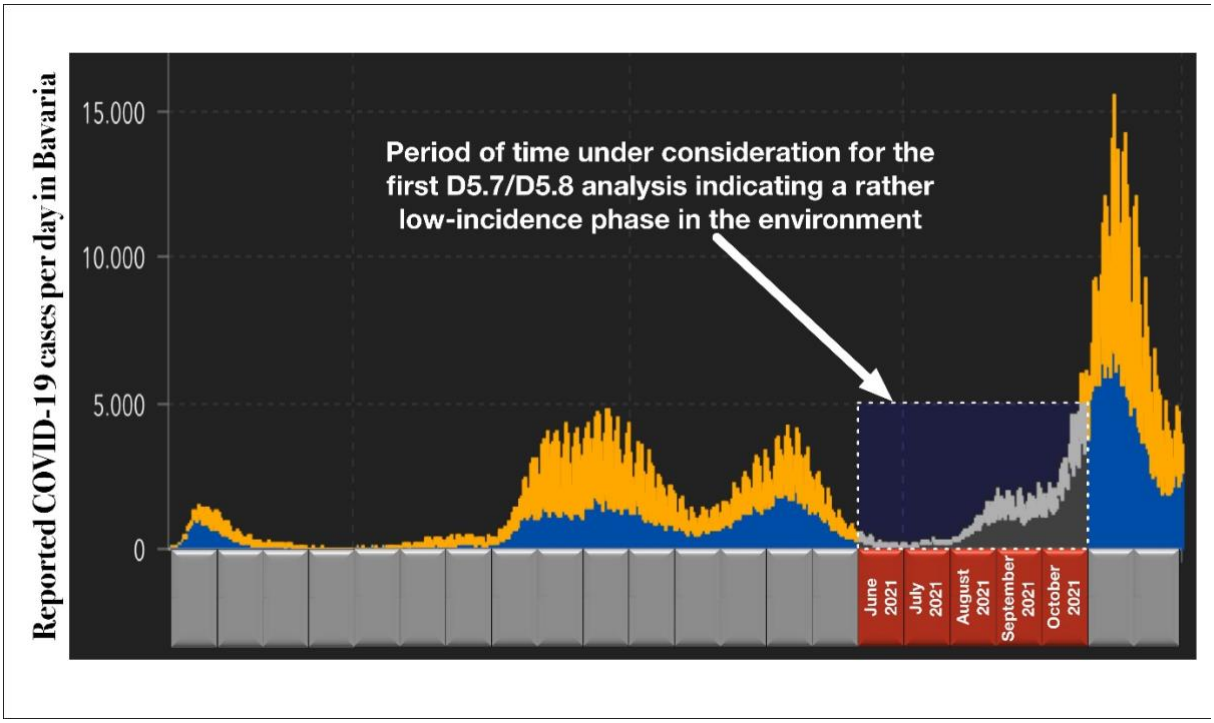

Figure 6: *The Bavarian environmental incidence during the period until the first serological follow-up*

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