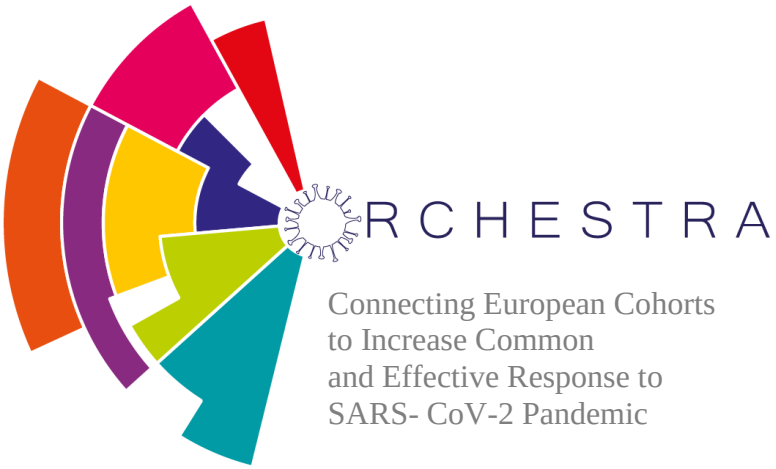

WP3 D3.6

Identification of breakthrough infections in population-based cohorts

Ludwig-Maximilians-Universitaet Muenchen (LMU MUENCHEN)

ORCHESTRA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167

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Executive Summary

Population-based seroprevalence studies aim to measure antibodies generated by silent or symptomatic infections and vaccination in the general population. They have the potential to ascertain the population immunity and to estimate the fraction of asymptomatic infections in the population. This Deliverable aims to report on the assessment of breakthrough infections based on the population-based seroprevalence studies KoCo19 and CON-VINCE, as well as to investigate the seroprevalence of SARS-CoV-2 infection in Munich and Luxembourg.

We present the course of the SARS-CoV-2 pandemic in the general population of Munich and Luxembourg. Based on newly, explicitly for this purpose developed diagnostic methods, SARS-CoV-2 antibodies generated by silent or symptomatic infections and/or vaccination were measured. In a unique, intra-European collaboration, results from the KoCo19 and CON-VINCE cohorts were compared to estimate the development of the pandemic and SARS-CoV-2 immunity in Europe after establishment of vaccination coverage in the general population.

We show that the seroprevalence in Munich and Luxembourg continuously increased over time until December 2021 and drastically increased from about 15% before the spread of the Omicron variant in Munich and Luxembourg, to 57% in Luxembourg thereafter, with a relevant underreporting bias.

Our results illustrate the underreporting of breakthrough infections. The identification of breakthrough infections limited to PCR-confirmed cases might miss the high number of silent infections. With an increasing prevalence of vaccination in the population, as well as the dominance of the Omicron variant, a highly contagious but less virulent variant, silent infections or persons presenting only mild symptoms are common. In this context, population-based seroprevalence studies are important to estimate the true population prevalence.

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Abbreviations

Anti-N	Anti-Nucleocapsid antibodies
Anti-S	Anti-Spike antibodies
BTI	Breakthrough Infection
CIEC	Clinical and Epidemiological Investigation Center
CON-VINCE	COvid-19 National survey for assessing VIral spread by Non-affected CarriErs
cT	Cycle threshold
COVID-19	Coronavirus 2019 disease
DBS	Dried Blood Spot
DII	Department of Infection and Immunity Luxembourg
ELISA	Enzyme-linked immunosorbent assay
IBBL	Integrated Biobank of Luxembourg
INS	Infections in naïve subjects
KoCo19	Prospective Covid-19 cohort Munich
LIH	Luxembourg Institute of Health
LNS	Laboratoire National de la Santé
OD	Optical density
rRT-PCR	Transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
WHO	World Health Organization

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Identification of Breakthrough Infections based on Seroprevalence Studies

Background

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), which causes the coronavirus 2019 disease (COVID-19), was first reported in December 2019 in Wuhan, China. Due to its highly contagious nature, it quickly spread within China and worldwide [1]. On March 11, 2020, the World Health Organization (WHO) declared it a pandemic after more than 118 000 cases in 114 countries and 4 291 deaths [2]. Within three months, Europe became the epicenter of the pandemic, surpassing the rest of the world in reported cases and deaths, except for China [3].
In December 2020, COVID-19 vaccines were developed and initially administered to high-risk individuals such as healthcare workers, the elderly, and those with underlying medical conditions [4]. Vaccine distribution among the general population started in 2021, aiming for nationwide coverage [5].
In order to monitor the immune status of the population, studies have been conducted to assess the development of vaccine- and infection-induced antibodies in the population, providing insight into the true rates of immunity, including vaccinations and subsequent infections [6]. Consequently, seroprevalence studies using SARS-CoV-2-induced anti-Nucleocapsid (anti-N) or anti-Spike (anti-S) antibodies have been conducted, but there is heterogeneity in methodologies used, and limited evidence at the national or regional level [7].
To gain a comprehensive understanding of SARS-CoV-2 spread and immunity coverage in the population, prospective-population based cohort studies like KoCo19 and CON-VINCE were initiated in Munich and Luxembourg, respectively [8,9]. This Deliverable aims to report on the assessment of breakthrough infections based on the population-based seroprevalence studies KoCo19 and CON-VINCE, as well as to investigate the seroprevalence of SARS-CoV-2 infection in Munich and Luxembourg.

Development of new diagnostic methods for antibody measurement

The KoCo19 and CON-VINCE cohorts were established with the objective to assess the prevalence and incidence of SARS-CoV-2 antibodies in the population. To achieve this, in a first step, it was necessary to examine the existing diagnostic methods for detecting SARS-CoV-2 antibodies in the blood following infection [10,11]. Subsequently, given the limited medical resources available, new diagnostic methods were developed to measure SARS-CoV-2 antibodies using dried blood spots (DBS) on filter cards. Participants receive DBS filter paper cards and instructions by mail, can prick themselves to collect capillary blood on filter paper cards, and subsequently send the samples back to the laboratory via mail [12,13].

While DBS on filter paper cards have been used before, they have not been commonly used for serology. As a result, we developed a semi-automated protocol for SARS-CoV-2 serology using self-sampled DBS [12,13]. The feasibility of the method was demonstrated in two large serosurveys involving > 10 000 company employees and > 4 400 participants from a population cohort. The sensitivity and specificity of the DBS method were 99.2% and 98.7%, respectively, compared to the standard method of whole blood testing [12]. Subsequently, we further developed the DBS method to measure Ro-RBD-Ig antibodies and validated it in a cohort with matched DBS and venous blood samples (n = 825) [13].

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The sensitivity and specificity of this method were found to be 96.6% and 97.8%, respectively, compared to the same test performed with paired venous blood samples.

The importance of the Ro-RBD-Ig test was crucial during the onset of the vaccination campaign. The Ro-N-Ig and Ro-RBD-Ig tests enable us to distinguish between immune responses induced by vaccination or infection. This is achievable because the Ro-RBD-Ig test identifies antibodies after both infection and vaccination, while the Ro-N-Ig test discerns between antibodies resulting from infection (both anti-S and anti-N present) and those arising from vaccination (only anti-S present). The Ro-N-Ig test can ascertain whether an individual had a previous infection but does not provide information about the exact date of the infection.

Consequently, with this method we can monitor the vaccine-induced immune response based on the presence of anti-S antibodies in the blood sample, and consequently, infection after vaccination based on the presence of anti-N antibodies in the subsequent blood samples.

Epidemic Surveillance based on Seroprevalence

With the effective performance of the DBS methods, it became feasible to establish a study cohort surveillance and acquire significant insights into the SARS-CoV-2 pandemic. Based on these new diagnostic methods, we predict the actual seroprevalence in the population. This analysis often reveals a significant gap between the real infection numbers and those reported officially. [14-16]

Cohorts Description

The KoCo19 Cohort

A more comprehensive description of the KoCo19 cohort until follow-up 4 can be found in [8,14-16]. In short: between April 5th and June 12th, 2020 the Munich cohort of private households was randomly sampled and 5315 participants 14 years and older who gave written informed consent were recruited. For participants younger than 18 years, informed consent was obtained from the parents as well as the participants themselves.
To date, five follow-ups were done at the following times of the pandemic:

- Follow-up 1: December 2020, beginning of the second wave in Munich
- Follow-up 2: March 2021, the peak of the third wave in Munich and the beginning of COVID19-vaccines availability for the general population
- Follow-up 3: August 2021, the end of the third wave in Munich and a vaccination rate of 68% in the general population of 14 years or older
- Follow-up 4: November 2021, the middle of the fourth wave with Delta being the dominant variant in Munich, and
- Follow-up 5: June 2022, the dominance of the Omicron variant in Munich

The CON-VINCE Cohort

The CON-VINCE cohort is described in more detail in [17] until Follow-up 6. In brief, between April 15th and May 4th, 2020, the CON-VINCE cohort was randomly drawn from a representative panel of

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Luxembourgish residents. In total, 1865 participants aged 18 years and older gave a written informed consent and were enrolled.

- Follow-up 1: May 2020, 2 week follow-up, the very beginning of the pandemic in Luxembourg, 2.4% weighted cohort sero-prevalence
- Follow-up 2: May 2020, 4 week follow-up, precise tracking of the pandemic development, slow linear growth of number of SARS-CoV-2 infections
- Follow-up 3: June 2020, 6 week follow-up, Large Scale Testing programme becomes available in Luxembourg
- Follow-up 4: June 2020, 8 week follow-up, reaching 2.7% weighted cohort sero-prevalence
- Follow-up 5: March 2021, the cases are growing steadily, while the two prevalent variants are the UK variant (B.1.1.7) and the South African variant (B.1.351), simultaneously with the vaccination campaign ongoing actively
- Follow-up 6: April-June 2021, Large Scale Testing is still active, isolation and quarantine measures are still in place, and the weighted prevalence in the cohort is 14.8%, while 36.6% of the country population is fully vaccinated

After joining the ORCHESTRA project, a sub-cohort of the CON-VINCE cohort was invited to take part in additional analyses with the baseline visit taking place in December 2021 - January 2022. This sub-cohort is called ORCHESTRA Luxembourg and comprises 1220 participants aged 18 and older. The CON-VINCE Follow-up 7 is thus considered the initial ORCHESTRA Luxembourg visit (Baseline). To date, 2 follow-ups were done at the following times of the pandemic:

- CON-VINCE Follow-up 8/ORCHESTRA Luxembourg Follow-up 1: May-June 2022, the number of cases increasing rapidly due to the dominance of Omicron variants (BA.2, BA.5), whereas 78.8% of eligible population is vaccinated
- CON-VINCE Follow-up 9/ORCHESTRA Luxembourg Follow-up 2: December 2022 - January 2023, the rate of new infections and re-infections is low, a mix of Omicron variants is prevalent, the vaccination rate of eligible population reached 79.0%

Within the framework of the ORCHESTRA project, the KoCo19 and ORCHESTRA-Luxembourg studies aligned their follow-up assessments to facilitate intra-European analysis.

Specimen Collection and Laboratory Analyses

The KoCo19 cohort

Based on the newly developed diagnostic methods, we used the Elecsys® Anti-SARS-CoV-2 anti-N (Roche) assay (referred to as Ro-N-Ig) for antibody detection post-infection at Baseline and Follow-up 1 [12]. Starting from Follow-up 2, we also employed the Elecsys® Anti-SARS-CoV-2 anti-S (Roche) assay (referred to as Ro-RBD-Ig). This differentiation was crucial for discerning infection-induced antibodies (anti-S and anti-N present) from those solely due to vaccination (only anti-S present). For measurements with full blood samples, a refined cut-off of 0.4218 for Ro-N-Ig was used to indicate seropositivity [16]. For DBS measurements anti-N had a positivity cutoff of 0.105, while for anti-S, the cutoff was set at 0.115.

The CON-VINCE cohort

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Blood samples were collected bi-weekly, starting from April 2020 and continuing through June 2020, which includes Baseline Visit 0 and Follow-up visits 1-4. Additionally, blood samples were taken during the annual Follow-up visit 6. Throughout these collection visits, participants were subjected to SARS-CoV-2 testing via RT-qPCR and were also tested for anti-SARS-CoV-2 antibodies.
The collection of blood samples and swabs was carried out by private partner laboratories, including Laboratoires Réunis, Ketterthill, and BioneXt, as well as by the Laboratoire National de la Santé (LNS) and the Luxembourg Institute of Health (LIH) Clinical and Epidemiological Investigation Center (CIEC) team. Some of these partners offered home collection services under the name Picken Doheem.
To ensure the integrity of biosamples and prevent processing delays, the collected samples were transported on a daily basis from the collection sites to the Integrated Biobank of Luxembourg (IBBL). Swab samples were subjected to analysis either at the LNS or at the Department of Infection and Immunity (DII) of LIH, where RT-qPCR was used to detect the presence of SARS-CoV-2 infection. In cases where sufficient virus material was present, the samples were further sequenced to determine the specific variant. Serology analysis, on the other hand, was conducted at the DII of LIH.
Anti-SARS-CoV-2 IgA and IgG levels were assessed using enzyme-linked immunosorbent assay (ELISA) kits labeled with the CE certification (Euroimmun). To ensure assay accuracy and consistency, internal quality controls were incorporated into all tests, with these controls being specifically prepared to yield an expected optical density (OD) ratio of approximately 2.5.
Results were interpreted as follows: Samples with OD ratios less than 0.8 were classified as negative, those with OD ratios equal to or greater than 1.1 were categorized as positive, and samples exhibiting OD ratios falling within the range of greater than 0.8 but less than 1.1 were considered as intermediate positive.
SARS-CoV-2 detection was conducted utilizing the Allplex 2019 n-CoV Assay developed by Seegene, which targets specific genes, namely RdRP, N (for specific SARS-CoV-2 detection), and E (for pan-Sarbecovirus detection). In instances where results were inconclusive, i.e., only the RdRP or the N gene was amplified, further confirmation was achieved through in-house assays, including the E gene reverse transcription-polymerase chain reaction (rRT-PCR). For the positive control, viral RNA from the BetaCoV/Germany/BavPat1/2020 strain (Reference 026N-03889), generously provided by Charité-Universitätsmedizin Berlin through the European Virus Archive Global platform, was utilized.
In cases where the original rRT-PCR produced inconclusive results, these samples were independently screened using the FTD SARS-CoV-2 assay by Fast Track Diagnostics located in Esch-sur-Alzette, Luxembourg. This assay targeted the N and ORF1ab genes. Samples that initially tested positive for the N gene in the original rRT-PCR were considered positive if a cycle threshold (Ct) value of less than 40 was obtained for at least one replicate in two independent second-round rRT-PCRs performed on the same sample.
For the parallel follow-ups, samples were collected via DBS. After sampling, the CON-VINCE DBS cards were shipped to Munich and analyzed in their lab, performing both anti-N and anti-S measurements.

The Contribution of Breakthrough Infections to the Population Spread

In conjunction with the laboratory samples, questionnaires were also collected. The combination of these two datasets culminated in the publication of several papers concerning the analysis of risk factors associated with SARS-CoV-2 and the assessment of underreporting bias over time [14-16]. The combination of serology and questionnaire data also enabled the classification into the following groups based on vaccination and infection status:

- Non-vaccinated, non-infected: Participants who tested negative for both anti-S and anti-N antibodies

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- Vaccinated, non-infected: Participants who tested positive for anti-S antibodies and negative for anti-N antibodies
- Non-vaccinated, infected: Participants who tested positive for both anti-S and anti-N antibodies but provided a negative response to the questionnaire item regarding vaccination
- Vaccinated and infected: Participants who tested positive for both anti-S and anti-N antibodies and provided a positive response to the questionnaire item regarding vaccination.

Based on this combined approach, Figure 1 describes the estimated seroprevalence in the population, taking into account vaccination and infection status. The results reported in this section describe the seroprevalence and incidence in the KoCo19 cohort, i.e. in the Munich population.

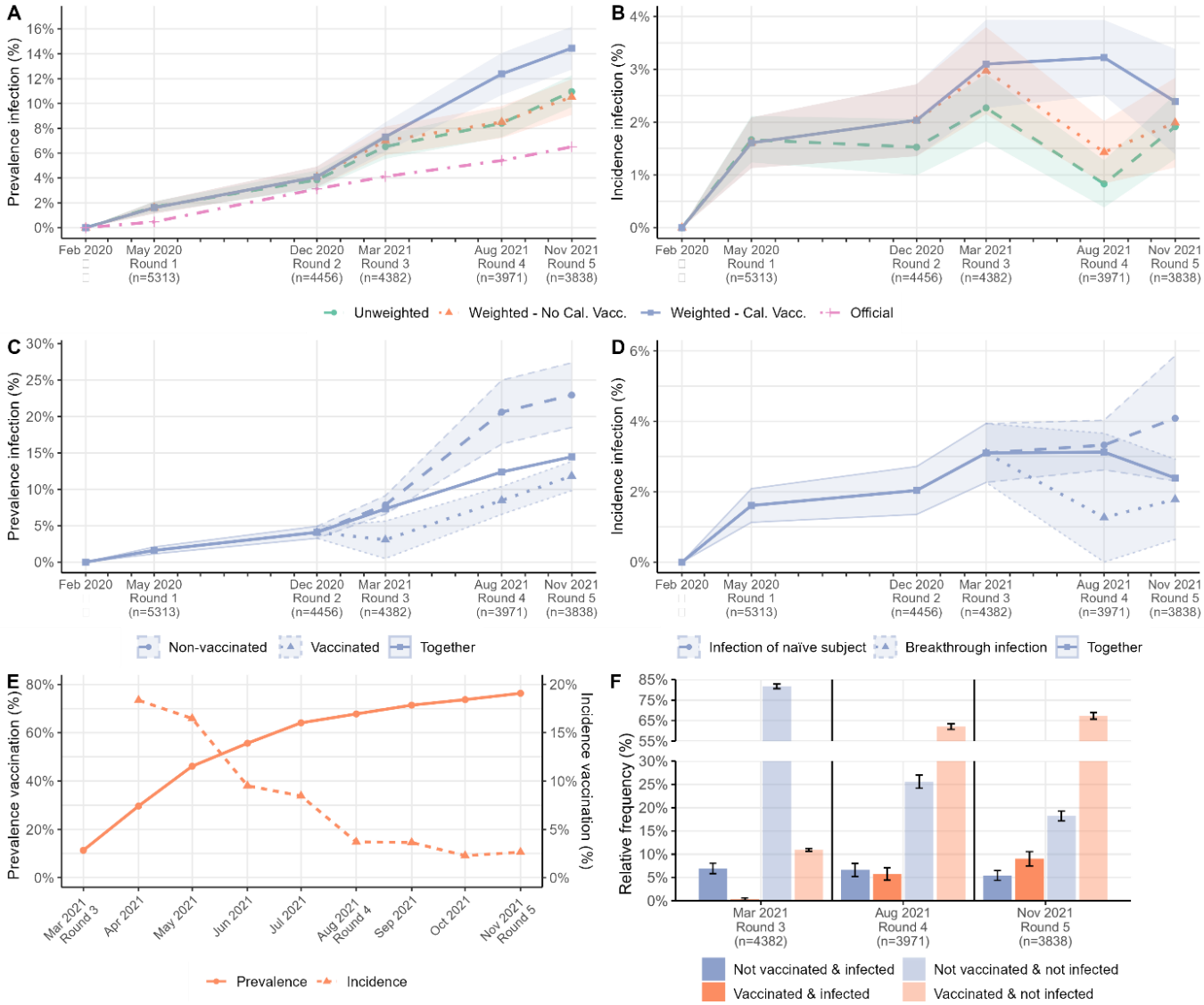

Figure 1: (A) Cumulative anti-N seroprevalence, both weighted and unweighted, in private households compared to official cases reported by the authorities for the Munich population aged 13 and older. **(B)** Anti-N seroincidence, both weighted and unweighted. **(C)** Estimates of seroprevalence for anti-N antibodies, adjusted based on the number of vaccinated individuals, Segmented by their vaccination status in the same round. **(D)** Calibrated estimates for infections of naïve subjects and breakthrough infections. **(E)** Prevalence and incidence of vaccination in Munich (official numbers). **(F)** Relative frequencies based on infection and vaccination status.

The blue estimate in **Figure 1A** shows the calibrated seroprevalence (adjusted for sensitivity and specificity) in private households for the Munich population aged 14 years and older:

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- Baseline: 1.6% (1.1 – 2.1%)
- Follow-up 1: 4.1% (3.3 – 4.9%), and after adjustment for vaccination status
- Follow-up 2: 7.3% (6.1 – 8.5%),
- Follow-up 3: 12.4% (10.7 – 14.1%),
- Follow-up 4: 14.5% (12.7 – 16.2%).

As expected, the seroprevalence is increasing over time. The official number of positive cases is reported in pink for the general population of Munich, which includes institutions like nursing homes and potential reinfections. Given that the KoCo19 cohort is limited to private households and the estimated seroprevalence does not account for multiple infections, comparing this estimate with the official number over time allows us to estimate a lower bound for the underreporting factor. The estimated underreporting factor varies across the rounds:

- Baseline: 3.4 (2.4 – 4.4),
- Follow-up 1: 1.3 (1.0 – 1.6),
- Follow-up 2: 1.8 (1.5 – 2.1),
- Follow-up 3: 2.3 (2.0 – 2.6),
- Follow-up 4: 2.2 (2.0 – 2.5).

To gain a better understanding of the impact of the vaccination campaign, the calibrated cumulative seroprevalence was analyzed separately for vaccinated and non-vaccinated individuals (**1C**):

- Follow-up 2: 3.1% (0.5 – 5.6%) versus 7.8% (6.6 – 9.1%),
- Follow-up 3: 8.5% (6.6 – 10.4%) versus 20.6% (16.2 – 25.0%),
- Follow-up 4: 11.8% (9.8 – 13.8%) versus 22.9% (18.5 – 27.4%).

The seroprevalence of the vaccinated group is lower compared to the non-vaccinated group. In **Figure 1D**, we compare the adjusted (for sensitivity and specificity) incidence rates for breakthrough infections (BTI) versus infections in naïve subjects (INS) over the rounds:

- Follow-up 3: 1.3% (0 – 3.7%) versus 3.3% (2.6 – 4%),
- Follow-up 4: 1.8% (0.6 – 2.9%) versus 4.1% (2.3 – 5.9%).

In August and November 2021, the incidence rates of INS were greater than the ones of BTI. Despite the cumulative seroprevalence appearing higher among the unvaccinated population compared to the vaccinated population (**Figure 1C** and **D**), BTIs relevantly contributed to community spread, considering that the population of vaccinated individuals was much larger than the non-vaccinated one during the last rounds of investigation (**1E**). **Figure 1F** provides a more detailed illustration of this effect, showing that the proportion of vaccinated and infected individuals increased over time, becoming significantly greater than the proportion of infected and non-vaccinated individuals by follow-up four.

Comparing the representative COVID-19 cohorts in Munich (KoCo19) and in Luxembourg (CON-VINCE): Insights from the KoCo19 Munich and CON-VINCE Luxembourg Cohorts

Within the framework of the ORCHESTRA project, the KoCo19 and ORCHESTRA Luxembourg studies aligned their follow-up assessments to facilitate intra-European analysis. Specifically, KoCo19 Follow-up 4 and 5 coincided with CON-VINCE Follow-up 7 and 8. Following this, CON-VINCE conducted an additional follow-up (Follow-up 9). Furthermore, after data collection, the samples from the CON-VINCE cohort were transported to Munich and analyzed in the KoCo19 laboratory. The analyses presented in this report concentrate on these parallel follow-up assessments (Figure 2).

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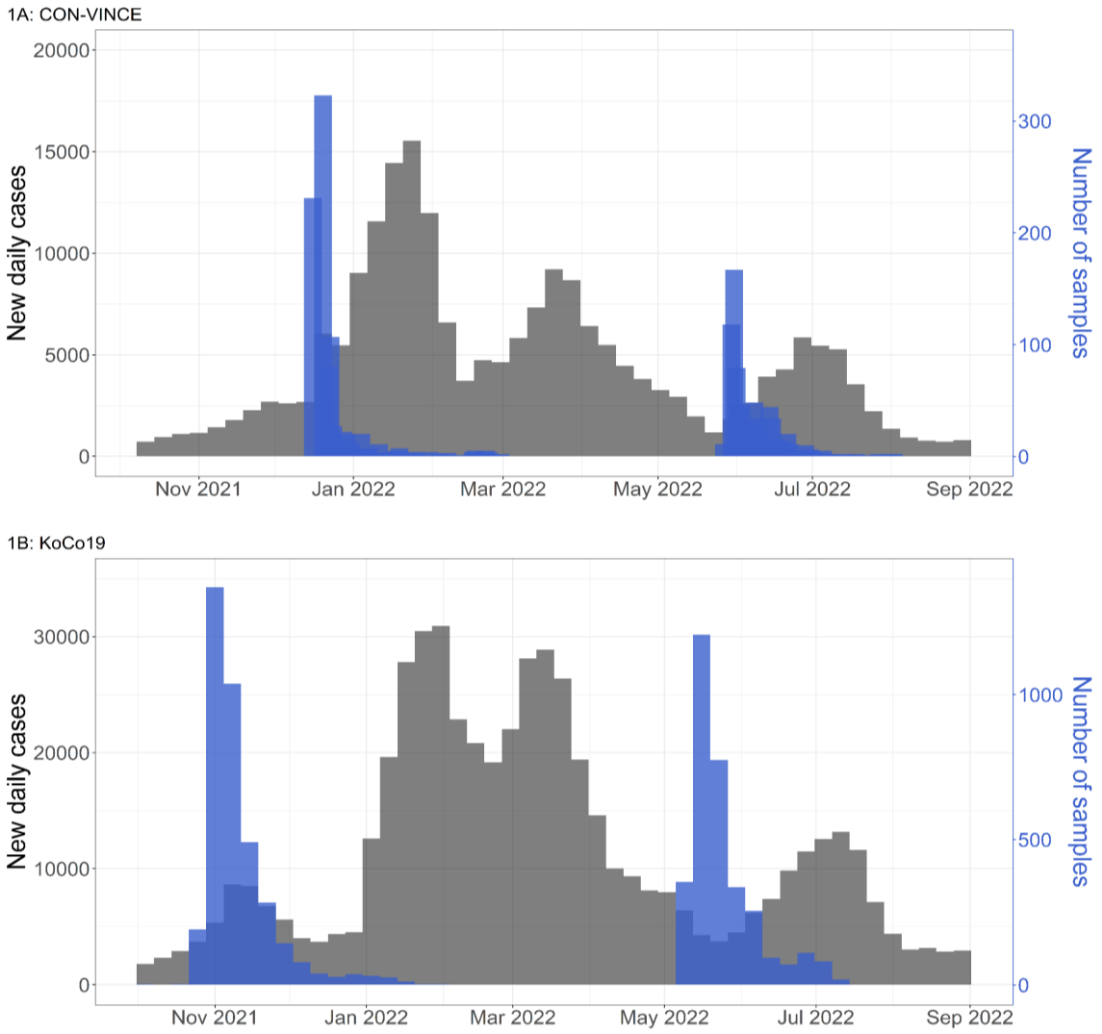

Figure 2: Epidemic evolution with description of the sample collection. The number of officially reported daily SARS-CoV-2 cases are represented in gray, while the number of daily DBS samples collected in blue. (**A**): The CON-VINCE in Luxembourg: the officially reported cases are taken from the World Health Organization (WHO) [18]. (**B**) The KoCo19 in Munich: the officially reported cases are taken from the Robert Koch Institute (RKI) [19].

These aligned follow-up sampling periods and the intra-European collaboration allowed us to measure the samples collected in the CON-VINCE cohort in the same lab as the samples collected in the KoCo19 cohort, therefore avoiding the problems of heterogeneity in methodologies used. This unique, collaborative approach enables us to investigate the seroprevalence of SARS-CoV-2 infection in Munich and Luxembourg from November 2021 on, after population-wide vaccination coverage was established.

The red and gray estimates in Figure 3 show the calibrated cumulative seroprevalence (adjusted for sensitivity, specificity, and re-infections) in the population of Munich and Luxembourg, respectively.

The overall weighted and adjusted SARS-CoV-2 seroprevalence for the KoCo19 cohort in Munich was:

- KoCo19 Follow-up 2 (March 2021): 7.3% (6.1-8.5%)
- KoCo19 Follow-up 3 (August 2021): 12.4% (10.7-14.1%)
- KoCo19 Follow-up 4 (November 2021): 14.5% (12.7-16.2%)

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Simultaneously, the overall weighted and adjusted SARS-CoV-2 seroprevalence for the CON-VINCE cohort in Luxembourg was:

- CON-VINCE Follow-up 6 (May 2021): 11.7% (10.2-13.3%)
- CON-VINCE Follow-up 7/ORCHESTRA Luxembourg Baseline (December 2021): 18.3% (16.1-20.4%)
- CON-VINCE Follow-up 8/ORCHESTRA Luxembourg Follow-up 1 (June 2022) 56.7% (53.9-59.5%)

It can be observed that the seroprevalence in the KoCo19 cohort is continuously slightly lower than the seroprevalence in the CON-VINCE cohort. However, given that the KoCo19 samples were collected some weeks before the respective samples of the CON-VINCE cohort, a continuous upward trend can be observed from March 2021 to December 2021. From December 2021 on, the seroprevalence increases dramatically up to over 57% in Luxembourg, with the respective seroprevalence for the KoCo19 cohort being yet to be calculated.

It can be observed that the weighted CON-VINCE seroprevalence is continuously higher than the official numbers reported in Luxembourg, indicating underreporting of infections. A massive increase of this underreporting factor can be observed from December 2021 on. A similar trend can be observed for the weighted KoCo19 sero-prevalence compared to the official numbers reported in Munich. While the underreporting factor was already reported in detail above, this intra-European comparison shows that since the beginning of the vaccination campaigns in Munich and Luxembourg, infections in the population have continuously been underreported.

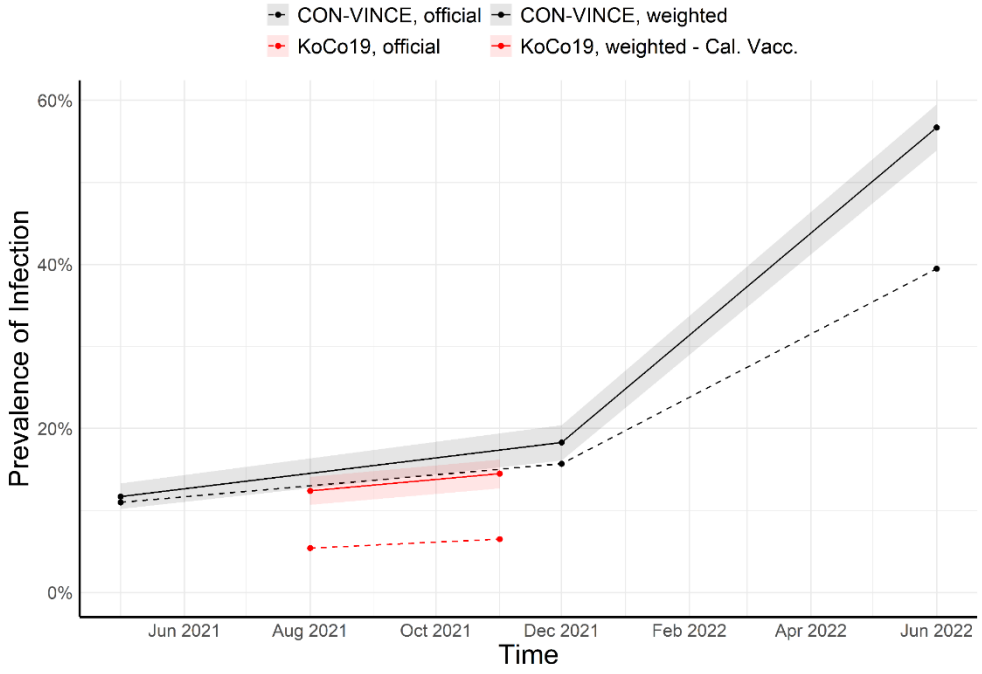

Figure 3: Comparison of weighted cumulative anti-N sero-prevalence in private households with officially reported cases reported by authorities. Black: CON-VINCE in Luxembourg, as reported by the World Health Organization (WHO) [18]. Red: KoCo19 in Munich as reported by the Robert Koch Institute (RKI) [19]. The KoCo19 analysis pertains specifically to the population in Munich aged 14 years and older.

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Discussion

In this report, we present the course of the SARS-CoV-2 pandemic in the general population of Munich and Luxembourg. To assess the real number of SARS-CoV-2 infections, the members of the representative, population-based KoCo19 and CON-VINCE cohorts were asked multiple times to donate blood for epidemic surveillance purposes. Based on newly, explicitly for this purpose developed diagnostic methods, SARS-CoV-2 antibodies generated by silent or symptomatic infections and/or vaccination were measured. In a unique, intra-European collaboration, results from the KoCo19 and CON-VINCE cohorts were compared to estimate the development of the pandemic and SARS-CoV-2 immunity in Europe after establishment of vaccination coverage in the general population.

As a first step, we compared the sero-prevalence of vaccinated individuals to that of non-vaccinated individuals to quantify the effect of the vaccination campaign in Munich. The share of infected persons (sero-prevalence) was shown to be greater in the non-vaccinated population in comparison to the vaccinated one, in non-Omicron variants. The findings show that vaccination lowered the risk of infection for all non-Omicron variants. The sero-incidence of (most likely asymptomatic) infections among vaccinated people in the population was lower than the one in non-vaccinated people; however, the difference was statistically non-significant. BTIs might thus relevantly contribute to the community spread, considering also the fact that the vaccinated population is much larger compared to the non-vaccinated one.

In a unique, intra-European collaborative approach we could also show that the sero-prevalence in Munich and Luxembourg continuously increased over time until December 2021. In Luxembourg, the Large Scale Testing scheme was in place from March 2021 to September 2021, and monitored retirement homes and the education sector in particular. This close monitoring of a larger part of these populations might contribute to a relatively low underreporting of infections in the CONVINCE/ORCHESTRA until December 2021.

From then on, with the emergence of the omicron variant, the sero-prevalence drastically increased from 14.5% / 18.3% before the spread of the omicron variant in Munich / Luxembourg, respectively, to 56.7% in Luxembourg thereafter, with a relevant underreporting bias. This is consistent with previous research from the COVID-19 Cumulative Infection Collaborators [20] that revealed a cumulative sero-prevalence of 43.9% globally to Nov 14, 2021, with high heterogeneity in the European population (10 to ≥ 80%, 10 to <20% in Germany and 20 to <30% in Luxembourg). The highest cumulative infection rates were assessed in sub-Saharan Africa, Europe and Asia. Another meta-analysis by Bergeri et al. [21] showed rising sero-prevalence up to 47.9% in Europe High Income Countries after the emergence of the Omicron variant in 2022, indicating also a high level of underreporting.

Outlook

Our results illustrate the under-reporting of breakthrough infections. The identification of breakthrough infections based on PCR tests might miss the high number of silent infections. With an increasing prevalence of vaccination in the population, as well as the dominance of the Omicron

Major strengths of our analysis are its population-based approach, the appropriate weighting of results for the general population, the high number of participants, as well as the thorough variant, a highly contagious but less virulent variant, silent infections or persons presenting only mild symptoms are common. In this context, population-based sero-prevalence studies are important to estimate the true population prevalence. validation of the assays used. Moreover, the intra-European collaboration

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enables us to use homogeneous testing and therefore to compare the results from two cohorts representative for the population in Munich and Luxembourg. Based on this collaborative research, the serological immune responses of breakthrough infected individuals, as well as of non-vaccinated infected and vaccinated individuals was further investigated *in Deliverable 3.5 (submitted in November 2023).* The results presented in both Deliverables will be published.

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