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Analysis of the Dynamics, Outcome, and Prerequisites of the first German SARS-CoV-2 Superspreading Event

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Analysis of the Dynamics, Outcome, and Prerequisites of the first German SARS-CoV-2 Superspreading Event

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Keywords: SARS-CoV-2, COVID-19, superspreading, cohort study, ventilation

Word count: 3903

49 **Abstract**

50

51 **Objectives**

52 Determining the SARS-CoV-2 RT-PCR positivity rate, SARS-CoV-2-specific antibody
53 levels of the participants, and analyzing the conditions and dynamics of
54 superspreading, including ventilation, setting dimensions, distance from infected
55 persons and behavioral patterns.

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57 **Design**

58 51 days after the event all participants were asked to give blood samples, pharyngeal
59 swabs and answer a self-administered questionnaire. Metric room coordinates for all
60 tables, seats, and ventilation-points were assessed.

61

62 **Setting**

63 The superspreading event took place during festivities including 450 people (6-79
64 years of age) in a building of 27 m x 13.20 m x 4.20 m.

65

66 **Participants**

67 All persons who took part in the event which led to superspreading of SARS-CoV-2
68 were invited to participate in this study.

69

70 **Interventions**

71 No interventions were performed.

72

73 **Primary and Secondary Outcome Measures**

74 The primary outcome measure was infection status by combining RT-PCR results with
75 ELISA results. Secondary outcomes were symptoms as stated in the questionnaire.

76

77 **Conclusions**

78 We analyzed infection rates and risk of infection depending on age, alcohol
79 consumption, and ventilation. Overall, 46% of participants had been infected. Spatial
80 distribution of infected participants was associated with proximity to the ventilation
81 system (OR 1.39, 95% KI [0.86; 2.25]). The risk of infection was highly associated with
82 age, thus children (OR: 0.33 [0.267; 0.414]) and young adults had a lower risk than

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3 83 older participants resulting in an average infection risk increase of 28% per 10 years
4 84 age difference. Behavioral differences reduced the risk of infection including time spent
5 85 outside (OR: 0.55 [0.33; 0.91]) or smoking (OR: 0.32 [0.124; 0.81]).
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10 87 **Strengths and Limitations of this Study**

- 11 88 • Strength: The setting and the participant group are extremely well-defined.
- 12 89 • Weakness: Some participants left the venue during the event for short times.
- 13 90 • Strength: Participants were invited by only one criteria, namely their presence
14 91 at the superspreading event; no other preselection/bias took place during
15 92 enrollment.
- 16 93 • Weakness: The event size was below 1000 people (450), therefore it was not
17 94 possible to recruit more than 411 study participants.
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28 97 **Article Summary**

29 98 The scientific literature was searched for the term "superspreading event AND Covid-
30 99 19 OR Sars Cov 2" and identified published papers from China, South Korea, Europe,
31 100 and North America. Most researchers analyzed superspreading events within a health
32 101 care setting e.g. in hospitals or nursing homes, or described the general impact of
33 102 superspreading events on the global pandemic. Only a few metanalyses of
34 103 transmission clusters analyzed party occasions (e.g. a nightclub in Berlin, Germany)
35 104 as superspreading events. These reports describe less than 100 infections and are
36 105 very limited due to missing data or reporting biases. Therefore, the ability to draw
37 106 scientific conclusions is also limited. Additionally, to our knowledge, there are no
38 107 studies, which investigated individual behavior, the location, and role of children during
39 108 a superspreading event. The research for the study started April 2020 and was
40 109 concluded in June 2021.
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51 110 Our report analyzes the first COVID-19 superspreading event in Germany in detail,
52 111 which was not only a unique setting but also included children and adults in the same
53 112 room. We demonstrate that nearly half of the participants were infected with SARS-
54 113 CoV-2 and that the proximity of the seating to the ventilation system was an important
55 114 risk factor for infection. The data showed that low physical distance including singing
56 115 and duration of attendance at this event increased the risk of infection, while regular

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3 116 smoking and spending the break of the event outside lowered the risk of infection. This
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5 117 underlines the benefit of airing to lower the amount of both droplets and aerosols.
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7 118 Furthermore, we found lower infection in children than adults despite being in the same
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9 119 room suggesting differences in infectability in children. Indeed, we observed that an
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11 120 additional 10 years of age is on average associated with 28% increased risk of
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121 infection.

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14 122 Taken together, the results demonstrate the importance of the ventilation system
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16 123 during superspreading events. In particular children and young adults had a lower risk
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18 124 of infection during the event indicating that they have a limited role during this
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20 125 pandemic. Overall, our data demonstrate in detail age-dependent infectability as well
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22 126 as highlights to understand transmission dynamics in order to improve comprehensive
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24 127 public health preparedness measures.

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29 130 **Introduction**

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31 131 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly
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33 132 transmissible and pathogenic RNA virus that emerged in late 2019 and has caused a
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35 133 pandemic threatening human health and public safety worldwide.¹ While factors
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37 134 shaping the dynamics of a pandemic are multifactorial, virulence and reproductive
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39 135 number are important properties of a virus.² For SARS-CoV-2 there is a substantial
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41 136 over-dispersion of the secondary infection distribution (individual R_0) for an individual
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43 137 infected with SARS-CoV-2. An over-dispersed R_0 means that most infected people
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45 138 do not transmit (individual $R_0 = 0$) while a minority of infected people are super-
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47 139 spreaders (individual $R_0 > 5$). Superspreading has been observed for many infectious
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49 140 pathogens, such as measles or SARS.³ During the SARS pandemic in 2003 a
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51 141 superspreading event was defined as one infected person infecting eight others.⁴ For
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53 142 SARS-CoV-2 it has been estimated that 80% of the infections are caused by 10% of
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55 143 infected individuals highlighting the importance of the cluster factor (k).² In Germany
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57 144 an indoor carnival event in the beginning of 2020 is considered as the first major
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59 145 outbreak in a German city and was considered a hotspot during the beginning of the
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146 pandemic in Germany.⁵ Other SARS-CoV-2 superspreading events worldwide have
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148 been linked to indoor gatherings with close proximity of individuals.⁶ Nevertheless,

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3 148 most of the reported superspreading events had less than 100 cases and the reports
4 are limited by missing data or a reporting bias.⁶
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8 151 Here, we closely examined the prerequisite of a unique super-spreading event in
9 152 Germany during the SARS-CoV-2 pandemic, where nearly half of the participants
10 153 became infected including children. We systematically analyzed infection rate,
11 154 potential individual, and environmental risk factors for infection as well as the role of
12 155 the ventilation system.
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18 19 20 157 **Materials and methods**

21 22 158 **Study design and sampling**

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24 159 This cross-sectional epidemiological study was conducted 51 days after a carnival
25 160 celebration in the beginning 2020. Eleven days after the event authorities sent all
26 161 known participants into quarantine after testing 38 out of 99 individuals PCR-positive.
27 162 All adults known to have attended the event were invited to participate in the study.
28 163 About 450 persons attended the event of which 411 participated in the study (**figure**
29 164 **1**, participation rate 91.3%). All study participants provided written informed consent
30 165 before enrolment.
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36 166 Self-administered questionnaires included questions about demographic background,
37 167 symptoms of viral infection as well as detailed information about the behavior during
38 168 the event. Participants' arrival and exit times were assessed in 1-hour categories.
39 169 Study participants were asked to provide blood specimens and pharyngeal swabs for
40 170 further analysis.
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46 172 **Research Ethics Approval**

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48 173 The Ethics Committee of the Medical Faculty of the University of Bonn approved the
49 174 study (approval number 085/20).
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53 176 **Patient and Public Involvement Statement**

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55 177 The concept and organization of the study on-site was in close cooperation and
56 178 agreement with the local administration, the county commissioner and the society-
57 179 leader of the carnival event. Additionally, the community was in need of SARS-CoV-2
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3 180 testing, because at this time the availability of testing was still limited, therefore they
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5 181 invited the collaboration with our team of scientists and physicians.

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9 10 184 **Pharyngeal swab and blood preparation**

11 185 Pharyngeal swabs of participants were performed with FLOQSwabs (Copan) and
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13 186 immediately stored in UTM RT-mini tubes containing UTM Viral Stabilization Media
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15 187 (Copan) at 4 °C. Venous blood was drawn into EDTA tubes (Sarstedt) per volunteer
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17 188 and was transported to the laboratory at the University Hospital Bonn.

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20 21 190 **Anti-SARS-CoV-2 ELISA**

22 191 Anti-SARS-CoV-2 IgA and IgG were determined using enzyme-linked immunosorbent
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24 192 assays (ELISA) on the EUROIMMUN Analyzer I platform.⁵ According to the
25
26 193 manufacturer's instructions a result was considered positive when a ratio (extinction of
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28 194 sample/extinction of calibrator) of 0.8 or higher was reached. The guidelines of the
29
30 195 German Medical Association (RiliBÄK) were abided by, including internal and external
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32 196 quality controls.

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34 35 198 **Reverse transcription polymerase chain reaction (RT-PCR)**

36 199 Viral RNA was extracted from each 300µl swab sample via the chemagic Viral 300
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38 200 assay (according to manufacturer's instructions) on the Perkin Elmer chemagic™
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40 201 Prime™ instrument platform. The presence of two viral target genes (E and RdRP)
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42 202 was assessed in each sample by real time RT-PCR (SuperScript™III One-Step RT-
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44 203 PCR System with Platinum™ TaqDNA Polymerase, Thermo Fisher). The following
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46 204 primers were used, for E gene: E_Sarbeco_F1 and R, and probe E_Sarbeco_P1, for
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48 205 RdRP gene: RdRP_SARSr_F, and R, and probe RdRP_SARSr-P2.⁷ In addition, an
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50 206 internal control for RNA extraction, reverse transcription, and amplification was applied
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52 207 to each sample (innuDETECT Internal Control RNA Assay, Analytik Jena #845-ID-
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54 208 0007100). If amplification occurred in both virus-specific reactions samples were
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56 209 considered positive.

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58 59 211 **SARS-CoV-2 Neutralization Assay**

60 212 A plaque reduction neutralization test was used to determine SARS-CoV-2
213 213 neutralization capacity as previously described.⁵ Briefly, plasma samples were heat-

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3 214 inactivated and supernatant transferred to a new tube and serially two-fold diluted in
4 215 OptiPROTMSFM (Gibco) performed. 120 mL of each plasma dilution was mixed with
5 216 80 plaque-forming units (PFU) of SARS-CoV-2 in 120 mL OptiPRO SFM (GIBCO) cell
6 217 culture medium and seeded with 1.25×10^5 Vero E6 cells/well. Subsequently, the
7 218 inoculum was removed and cells were overlaid with a mixture of
8 219 carboxymethylcellulose (Sigma) and 2xMEM (Biochrom). Following 3-day incubation,
9 220 the overlay was removed and the 24 well plates were fixed using a 6% formaldehyde
10 221 solution and stained with 1% crystal violet in 20% ethanol revealing the formation of
11 222 plaques. Finally, the neutralizing titers were calculated as the reciprocal of serum
12 223 dilutions resulting in neutralization of 50% input virus (NT50), read out as reduction in
13 224 the number of plaques.
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226 Data management and quality control

227 The Clinical Study Core Unit of the Study Center Bonn (SZB) supported the study by
228 outlining the study protocol and developing the informed consent form as well as
229 participants information sheets with respect to data management and quality control.
230 The data were gathered on paper-based Case Report Forms (pCRF). Data was
231 entered as double-data-entry into the REDCap study database programmed and
232 hosted by SZB. Study personnel was trained by experienced members of the SZB. A
233 quality manager was on site to support the study team. Monitoring of trial data and
234 informed consent forms was performed according to the monitoring plan by qualified
235 SZB staff. The ethics committee of the Medical Faculty of the University of Bonn was
236 involved and approved the study (reference no. 085/20)

237

238 Spatial information

239 Metric room coordinates (length and width [m]) for areas, tables, seats and ventilation
240 shafts were assessed via measurements, seating plan and photos from the event.
241 Persons providing multiple positions were considered as spending an equal amount of
242 time on different positions. When exact seating was unclear and information was
243 available on table or greater area localisation (bar, stage), average coordinate values
244 were used.

245 On the grounds of these coordinates, we calculated pairwise metric distances between
246 all persons and distances to closest inletting and purging airshafts. For all persons their
247 pairwise inverse distances were summarized as mean inverse distance. Inverse metric

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3 248 distances to persons or airshafts were regarded as representing infectious potential
4 249 through local proximity, and inverse distances were capped at 2.5 (the inverse of the
5 250 width of a seat of 0.4 m). Alternatively, we counted all, and all infected persons within
6 251 adjacent rings of 1.5 m width around each participant as a measure of crowdedness
7 252 and infectious potential.
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11 254 **Statistical analysis**

12 255 Associations between positive infection status and exposure variables were analysed
13 256 via logistic regression models. Exposure variables were included crudely, and adjusted
14 257 for potential confounding factors age, sex, and duration of attendance as fixed effects.
15 258 To correct for common household effects a random effects model was used. We
16 259 present odds ratios with 95% confidence intervals. Because we present data on a
17 260 single specific event among a limited number of participants, we completely refrain
18 261 from presenting p-values. All analyses were done with SAS 9.4.
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31 263 **Results**

32 264 411 out of estimated 450 participants of the event responded to our study invitation,
33 265 resulting in a response rate of 91.3%. 404 individuals provided plasma samples and
34 266 316 pharyngeal swabs (**figure 1**). Genders were represented equally among all 404
35 267 participants (48% were male) with a broad range in age ((range 6-79) median age 36
36 268 years) and level of education (**table 1**). 297 individuals were residents of the
37 269 community the event took place in, 103 lived in other parts of the county, and 11 were
38 270 external visitors.

39 271 Overall, 186 out of 404 individuals tested seropositive for IgG- and 161 for IgA-
40 272 antibodies (**suppl. figure 1**). To confirm seropositivity we performed a plaque
41 273 reduction neutralization assay (**suppl. figure 2**) demonstrating neutralizing activity
42 274 against SARS-CoV-2 of their respective antibody responses. Given the low specificity
43 275 of the IgA assay, IgA seropositivity was not further considered **Error! Bookmark not
44 276 defined..** As we tested for seropositivity 51 days after the superspreading event, we
45 277 additionally performed SARS-CoV-2 RT PCR analysis from pharyngeal swabs to
46 278 exclude potential recent infections. Indeed, 19 participants tested positive in RT-PCR,
47 279 and were therefore not considered in the study as there was no likelihood of infection
48 280 during the superspreading event.

| | Not infected N% | Infected N% |
|--|-----------------|-------------|
| Total number | 218 | 186 |
| Female | 114 (52%) | 89 (48%) |
| Age | | |
| <18 years | 31 (14%) | 15 (8%) |
| 18-24 years | 30 (14%) | 20 (11%) |
| 25-39 years | 81 (37%) | 43 (23%) |
| 40-64 years | 71 (33%) | 100 (54%) |
| 65+ years | 5 (2%) | 8 (4%) |
| BMI (kg/m²) (std dev) | 24.3 (5.12) | 26.2 (5.16) |
| Participating house hold member (std dev) | 2.1 (1.12) | 2.4 (1.16) |
| Highest level of formal education | | |
| None | 27 (13%) | 13 (7%) |
| Lower secondary school | 27 (13%) | 23 (13%) |
| Secondary school | 55 (26%) | 71 (39%) |
| Higher education entrance qualification | 54 (25%) | 34 (18%) |
| (Technical) university degree | 52 (24%) | 43 (23%) |
| Duration of attendance [h] (std dev) | 4.7 (2.06) | 5.8 (1.85) |
| Service team | 4 (2%) | 22 (12%) |
| On stage during the event | 80 (37%) | 62 (34%) |
| Member of the 'Council of 11' | 6 (3%) | 18 (10%) |
| On stage during 'finale' | 26 (12%) | 48 (26%) |
| Behavior during break | | |
| Remaining seated | 73 (36%) | 85 (48%) |
| Going outside | 114 (55%) | 72 (39%) |
| Alcohol consumption [drink] (std dev) | 11.3 (7.76%) | 12.2 (7.40) |
| Former smoker | 34 (16%) | 45 (24%) |
| Active smoker (>10 cigarettes per day) | 54 (25%) | 23 (12%) |
| At least one comorbidity | 29 (13%) | 28 (15%) |
| Avg. distance to other participants [m] (std dev) | 9.2 (1.68) | 9.1 (1.70) |
| Distance to air inlet [m] (std dev) | 6.1 (3.22) | 6.0 (3.30) |
| Distance to air outlet [m] (std dev) | 4.8 (2.94) | 5.1 (2.87) |

Table 1: Distribution of demographic factors and exposure information of interest among study participants who tested positive or negative in serology test of SARS-Cov2-infection.

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3 285 Overall, we found that 46.0% (95% CI: [41.2%; 51.0%]) tested seropositive who
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5 286 attended the event, which was significantly higher than the overall estimated infection
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7 287 rate in the same community at large at that time. Indeed, officially 3.1% of the
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9 288 community were reported as positive cases at that time and we estimated the infection
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11 289 rate as 15.5% (95% CI:[12.3%; 19.00%])**Error! Bookmark not defined.** for the
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13 290 community. Taken together, an estimated 46% of participants became infected during
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15 291 a single superspreading event.

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17 293 No association between sex and risk of infection was found ((OR: 1.01 [0.65; 1.58]) for
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19 294 women). On average infected individuals had a higher body mass index (26.2kg/m²
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21 295 compared to 24.3kg/m² for uninfected individuals). Infected participants were more
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23 296 likely to be clustered living in the same household (**table 1**). Having at least one
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25 297 comorbidity, including lung disease (42.3%), cardiovascular disease (53.3%),
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27 298 neurological disease (16.7%), cancer (58.3%) or diabetes (80%), did not increase the
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29 299 risk of infection (OR: 0.64 [0.33; 1.26]). In conclusion, sex and comorbidities did not
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31 300 seem to affect the risk of infection. We next assessed whether age influenced the risk
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33 301 of infection at the event, considering sex, duration of attendance and common
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35 302 household as covariates. Comparison across age-categories showed a lower risk for
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37 303 children (OR: 0.31 [0.14; 0.69]), and also for young adults (18-25 years, OR: 0.53 [0.26;
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39 304 1.09]) as well as adults between 25 and 40 years (OR: 0.48 [0.28; 0.85]) in comparison
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41 305 to older adults (40 to 65 years) (OR: 1, reference), while seniors had a slightly higher
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43 306 risk (older than 65 years, OR: 1.1 [0.31; 3.97]) (**figure 3**). Our data suggest that an
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45 307 additional 10 years of age are on average associated with 28% increased risk of
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47 308 infection (OR: 1.28 [1.10; 1.48]).

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49 309
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51 310 To understand the spreading dynamics of SARS-CoV-2 during the event, we first
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53 311 performed a detailed analysis of potential risk factors and social behavior. The event
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55 312 consisted of speeches, dance, and music performances for a total of five hours, with
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57 313 one large intermission and was hosted at a small community center (320 square
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59 314 meters) with a stage up front and a bar in the back close to the entrance. Alcoholic and
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61 315 nonalcoholic drinks were served in glasses and a food truck was located outside in
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63 316 front of the venue. While most participants were sitting in the hall, a committee of
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65 317 eleven individuals hosting the event were sitting on stage. The eleven people on stage
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67 318 switched after a break. With approximately 450 participants there were about 1.4
68
69 319 individuals per square meter and the tables, each with two benches, were arranged in

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3 320 two blocks with an alley to the stage (**figure 2**). Infected participants had been seated
4 321 mostly at tables close to the bar, at the bar, or on stage. One table with 8 out of 11
5 322 infected people, was located far away from the bar at the other side of the hall and
6 323 close to an air inlet. The group sitting on stage showed high numbers of infection (18
7 324 infected out of 24, **table 1**). We first analyzed whether the ventilation system influenced
8 325 the distribution of SARS-CoV-2 infected individuals. It is important to state that the
9 326 system's air flow consisted of 75% used and 25% fresh air. The air flow can be
10 327 described as clockwise. The air system uses vents along one side of the venue and
11 328 on stage to take in air (**figure 2**, air inlets purple). After 25% of fresh air has been
12 329 added and the air has been filtered, vents along the other side of the venue return the
13 330 air into the room (**figure 2**, air outlets blue). All ventilation points received the same
14 331 amount of air due to throttle valves. For noise protection reasons windows remained
15 332 closed. The air-system used F7-Filters (ISO ePM $\geq 2,5$) and had an air volume flow of
16 333 7500 m³/h.
17 334

18 335 Most tables located close to the air-inlets and showed no or only few infections (**figure**
19 336 **2**, green) also most surrounding tables showed low numbers of infection (**figure 2**,
20 337 yellow). Tables close to the air-outlets (**figure 2**) show high (4 or 5 infected per table)
21 338 and very high (6 or 7 infected per table) numbers of infected individuals. It is important
22 339 to mention that the overall number of participants per table was not equal for all tables.
23 340 Greater proximity to air outlets was associated with increased risk of infection with a
24 341 crude OR=1.39 [0.86; 2.25]. This association remained stable and was hardly
25 342 attenuated from adjustment for proximity to air inlet, age, gender, duration of
26 343 attendance, proximity to other infected persons, stage-activity and going outside during
27 344 the intermission (**figure 4**, multiple adjusted OR=1.26 [0.63; 2.50]). A similar apparent
28 345 effect for proximity to air inlets (crude OR=1.17 [0.72; 1.89]) disappeared when
29 346 duration of attendance was added to the model (**figure 4**, multiple adjusted OR=1.01
30 347 [0.53; 1.94]). Overall, however, we found the increased risk for individuals located
31 348 closer to the air outlet remarkably persistent (**figure 4**).
32 349

33 350 We further studied the sum of the inverse distance to all infected participants as a
34 351 measure of proximity to either one common virus source or mutual infection. However,
35 352 there was no evidence for increased risk of infection from greater proximity to other
36 353 infected persons (**suppl. table 1**). Furthermore, we found no evidence for a single

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3 354 person being the source of the infection using 401 quantile-plot analysis conducted for
4 each participant as potential source of infection separately (**suppl. figure 3**).

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8 357 To understand the association of risk with behavior patterns we next investigated the
9 influence of several factors on SARS-CoV-2 infection including time spent outside,
10 358 smoking, performing on stage and participation during the final act (“Finale”) for 30
11 359 minutes. Results were all adjusted for age, sex, common household, and duration of
12 360 attendance. Participation in multiple performances did not increase the risk of infection
13 361 (OR per performance: 1.08 [0.91; 1.27]) while participation in the last “Finale” indicated
14 362 a trend towards increased risk of infection (OR: 1.41 [0.65; 3.02]) (**figure 4**). Duration
15 363 of attendance was persistently and strongly associated with an increased infection risk
16 364 of 32% with each additional hour spent at the party (OR per hour: 1.32 [1.16; 1.49]).
17 365 All further analyses were adjusted for this variable as potential confounding factor.
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368 We next determined the level of alcohol consumption as number of drinks (high-proof
369 liquor or beer) and did not observe any influence for the amount of alcohol consumption
370 on the risk of becoming infected (OR per drink: 1.00 [0.96; 1.05]). Furthermore,
371 participants who spent the break outside were less likely to be infected (OR: 0.55 [0.33;
372 0.91]) compared to individuals who spent the break inside the venue hall (**figure 4**).
373 Interestingly, however, when we determined the impact of being regular smoker
374 (defined as smoking of at least 10 cigarettes a day) on the risk of SARS-CoV-2 infection
375 we observed a reduced risk of infection (OR: 0.32 [0.12; 0.81]) even after adjustment
376 for “time spent outside”. In conclusion, duration of attendance at the carnival party
377 increased the risk of infection, the number of alcoholic drinks was not associated with
378 infection risk, while regular smoking and spending the break of the event outside
379 lowers the risk of infection.

380

381 We next stratified seropositive individuals by their reported symptoms. Odds-ratios for
382 each symptom were calculated for the timespan of 14 days following the event (**figure**
383 **5**). Similar to previous reports⁸ loss of smell (OR: 8.78 [4.81; 16.02]) and taste (OR:
384 10.09 [5.13; 19.88]) were strongest associated with SARS-CoV-2 infection. Other
385 symptoms which were strongly associated with COVID-19 were: sweats and chills
386 (OR: 5.28 [3.08; 9.07]), muscle and joint ache (OR: 5.19 [3.19; 8.44]), fatigue (OR:
387 4.22 [2.76; 6.45]) and fever (OR: 3.73 [2.10; 6.63]) (**figure 5**). Importantly, 15.1% of

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3 388 the infected individuals reported no symptoms at all in a period of 14 days after the
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5 389 event. The rate of asymptomatic infections of participants of the event was lower than
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7 390 generally observed in the community the event took place in (36%).⁵ Overall, there
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9 391 was a lower proportion of asymptomatic cases among individuals infected after the
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11 392 event compared to members of the community, while loss of smell and taste showed
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13 393 the strongest association with an infection.
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16 395 **Discussion**

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18 396 The high overdispersion characteristics of SARS-CoV2 and its ability to be transmitted
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20 397 via aerosols under certain conditions are one of the main reasons that the beginning
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22 398 of the SARS-CoV2 pandemic was shaped by superspreading events.^{9,10} Germany's
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24 399 first superspreading event was an indoor carnival event in the beginning of 2020 in a
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26 400 rural the community. In this naturally occurring experiment, we demonstrate that nearly
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28 401 half of the participants became infected and demonstrate multiple prerequisites of such
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30 402 an event and risk factors for becoming infected. While our study population is not a
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32 403 representative sample of the general population the event may be regarded as
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34 404 exemplary for similar party occasions and may help reduce the number of infected in
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36 405 the future.
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41 407 An important factor associated with infection risk was the ventilation system and the
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43 408 individual proximity to the ventilation outlets. Individuals close to the air-outlets that
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45 409 contained air with low amount of fresh air had the highest infection risk compared to
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47 410 those close to the air-inlets. This is in line with previous studies that demonstrated
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49 411 SARS-CoV-2 to be able to become air-borne under certain conditions and that the
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51 412 ventilation system can have an influence on virus spread.^{11,12,13} The air filters in the
52
53 413 venue were not capable of intercepting virus particles supporting the notion on the
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55 414 importance of proper indoor ventilation systems.^{14,15} Indeed, spending the break of the
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57 415 event outside decreased the possibility of infection underscoring the benefit of proper
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59 416 ventilation to lower the amount of aerosols. Due to the nature of the event, the spatial
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417 distribution of the participants was not fixed throughout the evening, and not perfectly
418 recapitulated, so this information carries some error. However, allowing for multiple
419 positions per person we used all available information. Assuming further error in the
420 spatial data to be random, this might lead to a dilution of effects, i.e. true associations

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3 421 may remain undetected. Complementary analyses including e.g. the persons'
4 422 functions during the event show consistent results, so we see no evidence suggesting
5 423 bias in our findings.
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11 425 The consumption of alcoholic drinks did not increase the risk of infection. While it has
12 426 been assumed that the alcoholic effect of decreased social inhibition may increase
13 427 likelihood of infection, we did not find any evidence for this association questioning
14 428 measures of a ban on alcohol to reduce numbers of infected. It is known that current
15 429 and former smokers disproportionately suffer from severe COVID-19 and their
16 430 numbers are relatively increased among those patients that need intensive care
17 431 treatment compared to non-smokers.^{16,17} However, it has been previously speculated
18 432 that the risk of infection is lower for smokers.¹⁸ Furthermore, a meta-analysis of seven
19 433 studies suggests that smokers have a reduced risk of testing positive for SARS-CoV-
20 434 2.¹⁹ Interestingly, we also observed a protective effect for an infection with SARS-CoV-
21 435 2, thus our findings support those statements and show an even greater protective
22 436 effect. The association might for example be explained by a role of the nicotinic
23 437 acetylcholine receptor.²⁰ While we strongly advise that smoking should not be
24 438 considered as a protective habit to prevent risk of infection, this knowledge may lead
25 439 to the investigation of a therapeutic or prophylactic treatment on the basis of this
26 440 molecular target.²¹
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40 442 Our results indicate a trend that younger people are less likely to be infected compared
41 443 to older age groups. This trend is strongest for people under 18 but levels out over 40
42 444 years of age. The risk of infection for children in superspreading events has not been
43 445 investigated but the overall risk for infection in children seems to be lower than for
44 446 adults as a systematic review and its recent update reported, which is further supported
45 447 by our findings.^{22,23} As all individuals were exposed at the same event and time our
46 448 study is a perfect model for the previously described notion, that children are less likely
47 449 to become infected. Indeed, a recently published meta-analysis by Viner et al. showed
48 450 a low susceptibility for children and adolescents (OR of 0.56 (95% CI, 0.37-0.85))
49 451 which strongly supports our findings of a lower risk of infection in that age group, which
50 452 is even lower in our study.²⁴ Our finding supports the previously shown subordinate
51 453 influence on the spreading of the virus by children. The finding that each 10 years of
52 454 age increase the risk of infection during an event indicates that younger people and
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3 455 their limited role should be considered when measures to contain the pandemic are
4 456 implemented. Taken together, we could demonstrate important risk factors for infection
5 457 during a superspreading event, which helps to understand transmission dynamics in
6 458 order to improve comprehensive public health preparedness measures.
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12 461 **Acknowledgments**

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14
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11 496 parties, including the government of North Rhine-Westphalia, Germany.
12 497

498

499 **Author Contributions**

500 L.W. wrote the manuscript. L.W., RMS, and ER organized and ran the testing center.
501 ER and BS organized and performed sample processing, analysis, and corrected the
502 manuscript. NL and AH performed statistical analysis. MC, CF, and AK monitored the
503 study. ME, KHJ and H.S oversaw the study and corrected the manuscript.

504

505 **Data statement**

506 All data from the study will be made available upon reasonable request.

507

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511

512 **Conflicts of interest**

513 The authors declare no conflict of interest.

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516 **References**

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602 Figure Legends

603

604 **Fig. 1: Study participants.** Of the 400 people contacted originally (left) 362 adults and
605 49 children agreed to enroll in the study. An overview of the number of samples
606 collected is given on the right. Downstream sample processing included centrifugation
607 of blood samples for plasma collection (SARS-CoV-2 ELISAs), and viral RNA
608 extraction from swab samples (SARS-CoV-2 RT-PCR).

609

610 **Fig. 2: Reconstructed 3D-Model of the venue hall.** Self-administered questionnaires
611 included questions about main seating-position of the participants during the evening
612 event as specifying table and seat with the help of a schematic seating plan. Metric
613 room coordinates for all tables, seats, and ventilation-points were assessed and the
614 seating was reconstructed from pictures taken during the event. Therefore, the location
615 of the stage, the bar, the exit as well as the tables and the air-inlets/outlets were
616 reconstructed in a 3D-Model. The original external dimensions of the building were
617 27m x 13.20m x 4.20m. Tables, where more than 7 infected individuals have stayed
618 are colored in dark red, this includes the stage and bar as well. Air-inlets are colored
619 in violet and the air-outlets in blue. Infected participants had been seated mostly at
620 tables close to the bar, the bar itself and on stage. One table with 8 out of 11 infected
621 people, was located far away from the bar at the other side of the hall and close to an
622 air inlet. The group sitting on stage showed as well high numbers of infection (18
623 infected out of 24). Greater proximity to air outlets seems to be associated with
624 increased risk of infection with a crude OR=1.39 [0.86; 2.25].

625

626 **Fig. 3 Odds-Ratio for the likelihood of SARS-CoV-2 infection by age groups.**
627 Participants were divided into age groups of 8, 15, or 25 years, participants younger
628 than 18 or older than 65 years. Participants were considered to have been infected
629 during the event if they were SARS-CoV-2 antibody positive (ELISA).

630

631 **Fig. 4: Odds ratios for the association of SARS-CoV-2 infection with specific
632 activities of the participants and their location in the venue relative to ventilation
633 shafts.** The model was additionally adjusted for age, sex, duration of attendance,
634 participation in multiple activities, and cumulative proximity to other infected persons,
635 and common household.

636

637 **Fig. 5: Odds ratios for symptoms of SARS-CoV-2 antibody-positive participants
638 in the 14 days following the super spreading event.** The information on symptoms
639 was derived from the self-administered questionnaire, which was filled out on the day
640 of sample collection. Odds ratio estimates (OR) are shown with confidence intervals

641

642 **Suppl. Fig. 1: Correlation of SARS-CoV-2 Euroimmun ELISA results for IgA and
643 IgG.** The correlation of IgA levels to IgG levels in the same person was significant (r:
644 Pearson coefficient, $p < 0.0001$, 95 % CI, 0.7043 to 0.7902). The dotted lines mark the
645 ratios above which each ELISA result is considered positive.

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647 **Suppl. Fig. 2: Correlation of plasma neutralization capacity and IgG ELISA
648 results (Euroimmun) from each donor.** The dotted line marks the ratio above which
649 the ELISA result is considered positive. The correlation coefficient (Pearson) was

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3 650 0.3667 (95 % CI, 0.2275 to 0.4192, $p < 0.0001$). Samples with a negative result in the
4 651 neutralization assay were set as 0.1 here so as to appear on the logarithmic axis.
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6 653 **Supplemental Figure 3:** Quantile plot of observed p-values from analyses of inverse
7 654 distance [1/m] to single specific study participants as risk factor for corona-virus
8 655 infection. In case of no association, the ordered log-transformed p-values are expected
9 656 to lie on, or below the diagonal. Panel A: results from crude analyses, Panel B:
10 657 analyses were adjusted for age, sex, common household and duration of attendance.
11 658

12 659 **Supplementary table 1:** Estimated relative risk of SARS-CoV-2 infection (IGG-
13 660 positive) from logistic regression on summary measures of spatial proximity between
14 661 participants in terms of odds ratio estimates (OR) with confidence interval and p-
15 662 values. a) adjusted for sex, age, common household and duration. b) multivariate
16 663 analysis, mutually adjusted for distance to ventilation system, participation in (multiple)
17 664 performances, going out of doors during the intermission, and participating in the grand
18 665 finale. c) multivariate analysis, mutually adjusted for distance to ventilation system,
19 666 participation in (multiple) performances, going out of doors during the intermission, and
20 667 participating in the grand finale and adjusted for sex, age, common household and
21 668 duration.
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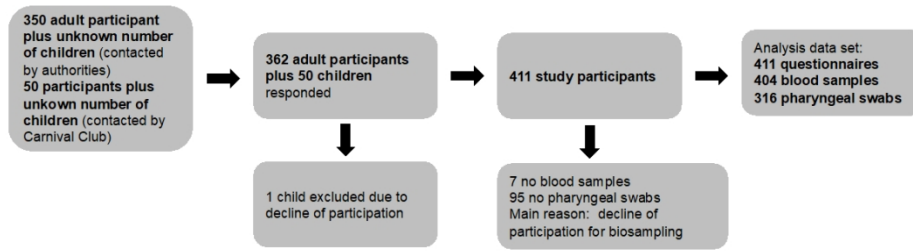


Fig. 1: Study participants. Of the 400 people contacted originally (left) 362 adults and 49 children agreed to enroll in the study. An overview of the number of samples collected is given on the right. Downstream sample processing included centrifugation of blood samples for plasma collection (SARS-CoV-2 ELISAs), and viral RNA extraction from swab samples (SARS-CoV-2 RT-PCR).

342x107mm (96 x 96 DPI)

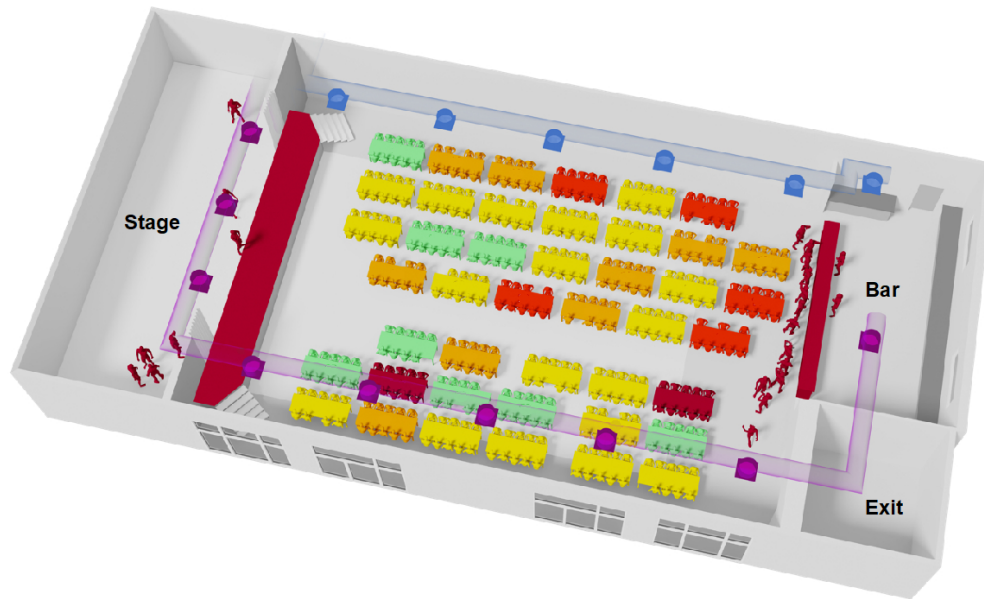


Fig. 2: Reconstructed 3D-Model of the venue hall. Self-administered questionnaires included questions about main seating-position of the participants during the evening event as specifying table and seat with the help of a schematic seating plan. Metric room coordinates for all tables, seats, and ventilation-points were assessed and the seating was reconstructed from pictures taken during the event. Therefore, the location of the stage, the bar, the exit as well as the tables and the air-inlets/outlets were reconstructed in a 3D-Model. The original external dimensions of the building were 27m x 13.20m x 4.20m. Tables, where more than 7 infected individuals have stayed are colored in dark red, this includes the stage and bar as well. Air-inlets are colored in violet and the air-outlets in blue. Infected participants had been seated mostly at tables close to the bar, the bar itself and on stage. One table with 8 out of 11 infected people, was located far away from the bar at the other side of the hall and close to an air inlet. The group sitting on stage showed as well high numbers of infection (18 infected out of 24). Greater proximity to air outlets seems to be associated with increased risk of infection with a crude OR=1.39 [0.86; 2.25].

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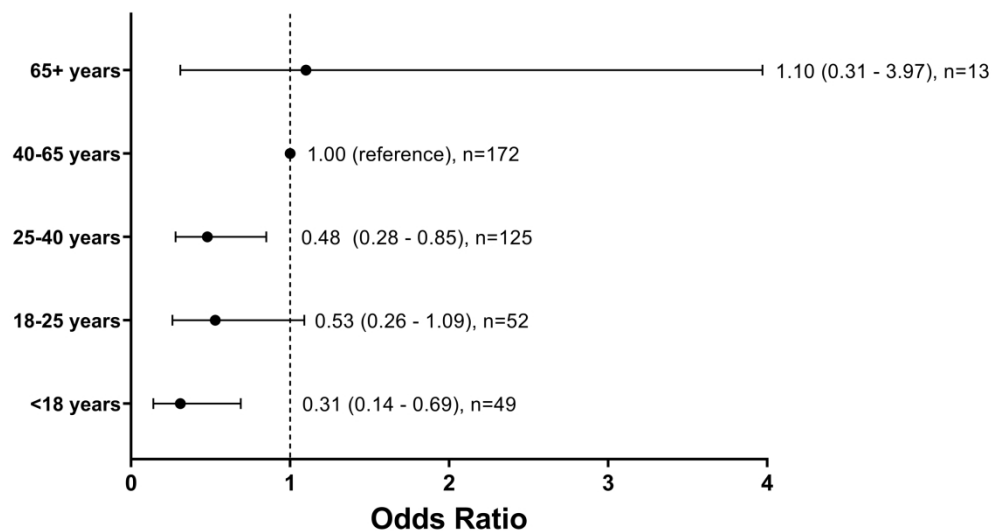


Fig. 3 Odds-Ratio for the likelihood of SARS-CoV-2 infection by age groups. Participants were divided into age groups of 8, 15, or 25 years, participants younger than 18 or older than 65 years. Participants were considered to have been infected during the event if they were SARS-CoV-2 antibody positive (ELISA).

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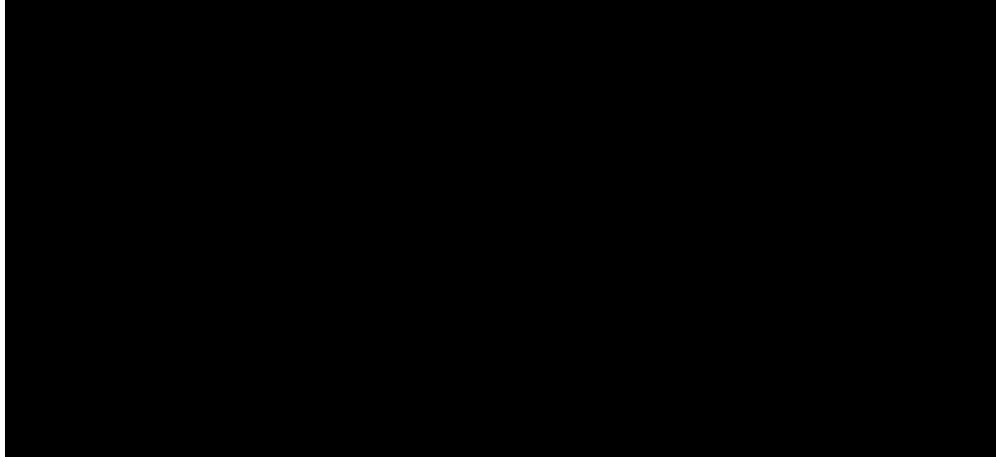


Fig. 4: Odds ratios for the association of SARS-CoV-2 infection with specific activities of the participants and their location in the venue relative to ventilation shafts. The model was additionally adjusted for age, sex, duration of attendance, participation in multiple activities, and cumulative proximity to other infected persons, and common household.

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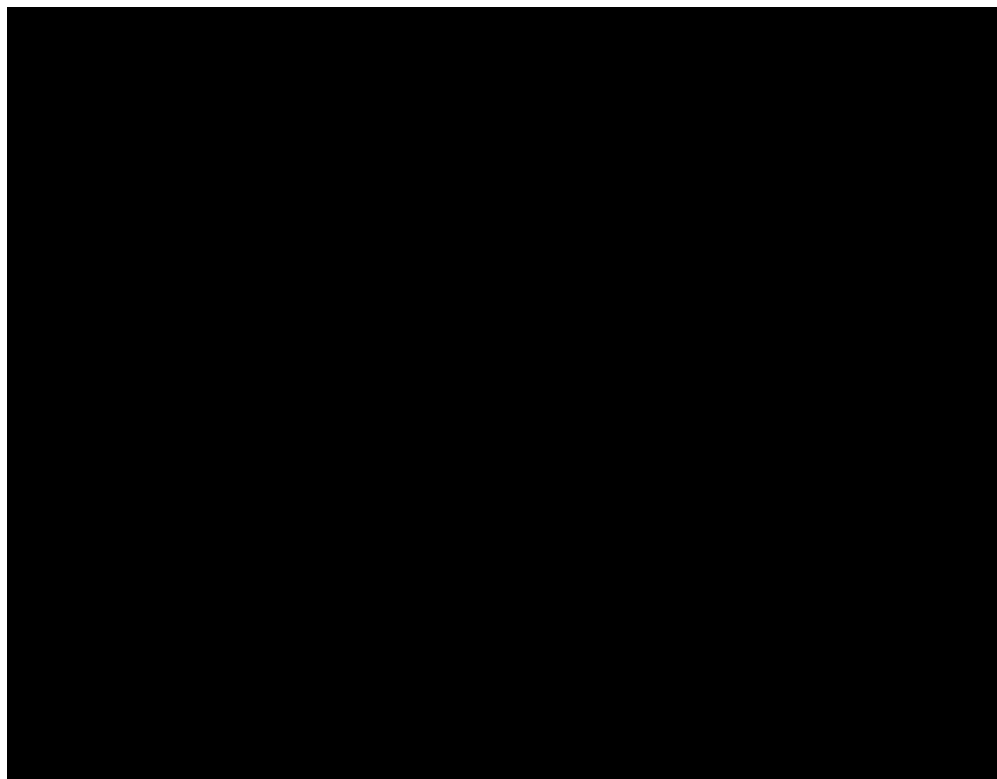


Fig. 5: Odds ratios for symptoms of SARS-CoV-2 antibody-positive participants in the 14 days following the super spreading event. The information on symptoms was derived from the self-administered questionnaire, which was filled out on the day of sample collection. Odds ratio estimates (OR) are shown with confidence intervals.

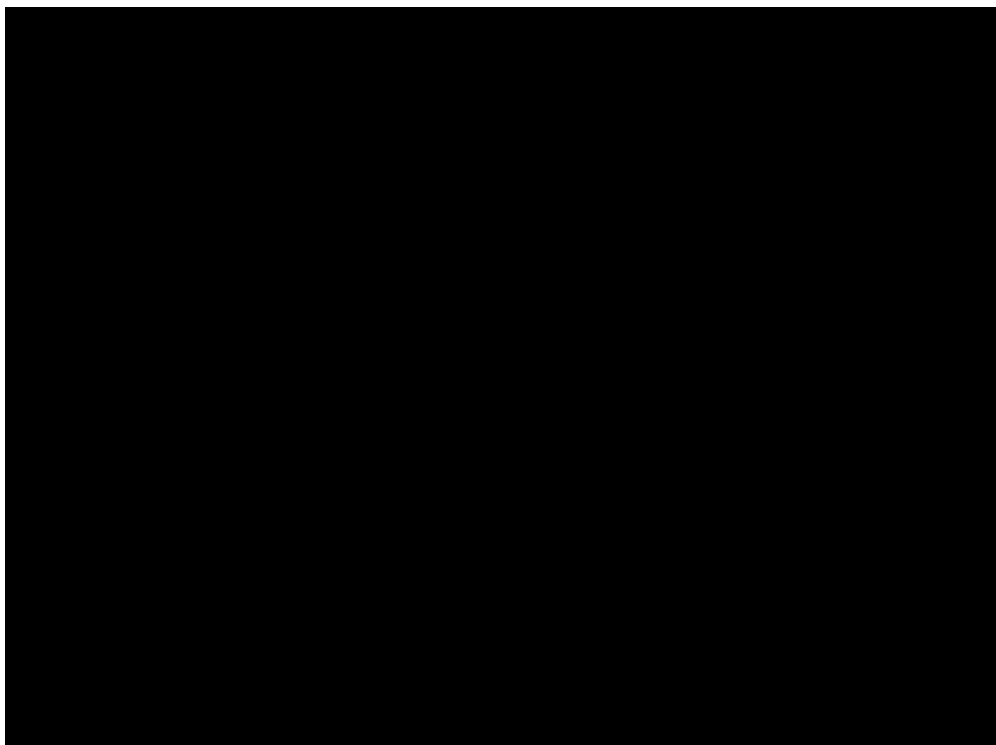
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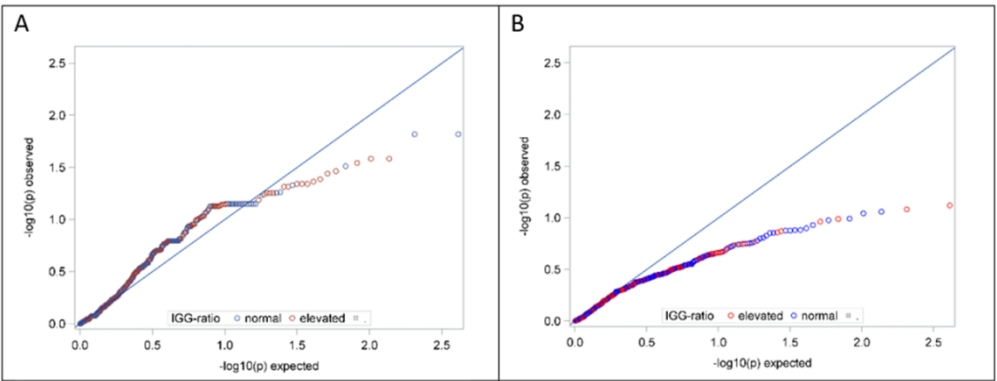
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| | OR | 95 % confidence interval | | p-value |
|---|------|--------------------------|------|---------|
| Proximity of infected persons [sum 1/m] | 0.99 | 0.98 | 1.01 | 0.43 |
| Adjusted a) | 1 | 0.98 | 1.02 | 0.96 |
| Mutually adjusted b) | 0.99 | 0.97 | 1.01 | 0.57 |
| Mutually adjusted c) | 0.99 | 0.97 | 1.02 | 0.65 |
| Alternative consideration in distance-bands | | | | |
| Infected persons within ≤ 1.5 m [count] | 1.01 | 0.96 | 1.07 | 0.68 |
| Infected persons in 1.5 - ≤ 3 m [count] | 0.96 | 0.92 | 1 | 0.04 |
| Infected persons in 3 - ≤ 4.5 m [count] | 1.03 | 1 | 1.06 | 0.02 |
| Infected persons within ≤ 1.5 m [count] adjusted a) | 1.03 | 0.97 | 1.1 | 0.37 |
| Infected persons in 1.5 - ≤ 3 m [count] | 0.96 | 0.92 | 1.01 | 0.11 |
| Infected persons in 3 - ≤ 4.5 m [count] | 1.03 | 1 | 1.06 | 0.08 |
| Infected persons within ≤ 1.5 m [count] mutually adjusted b) | 1.01 | 0.95 | 1.07 | 0.73 |
| Infected persons in 1.5 - ≤ 3 m [count] | 0.98 | 0.94 | 1.02 | 0.36 |
| Infected persons in 3 - ≤ 4.5 m [count] | 1.05 | 1.02 | 1.08 | 0.001 |
| Infected persons within ≤ 1.5 m [count] mutually adjusted c) | 1.02 | 0.95 | 1.09 | 0.64 |
| Infected persons in 1.5 - ≤ 3 m [count] | 0.98 | 0.93 | 1.03 | 0.36 |
| Infected persons in 3 - ≤ 4.5 m [count] | 1.04 | 1 | 1.07 | 0.04 |

Supplementary table 1: Estimated relative risk of SARS-CoV-2 infection (IGG-positive) from logistic regression on summary measures of spatial proximity between participants in terms of odds ratio estimates (OR) with confidence interval and p-values. a) adjusted for sex, age, common household and duration. b) multivariate analysis, mutually adjusted for distance to ventilation system, participation in (multiple) performances, going out of doors during the intermission, and participating in the grand finale. c) multivariate analysis, mutually adjusted for distance to ventilation system, participation in (multiple) performances, going out of doors during the intermission, and participating in the grand finale and adjusted for sex, age, common household and duration.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

| | | | Page Number |
|---------------------------|---------------------|---|-------------|
| Title and abstract | | | |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| Abstract | #1b | Provide in the abstract an informative and balanced summary of what was done and what was found | 2-3 |
| Introduction | | | |
| Background / rationale | #2 | Explain the scientific background and rationale for the investigation being reported | 4-5 |
| Objectives | #3 | State specific objectives, including any prespecified hypotheses | 2 |
| Methods | | | |
| Study design | #4 | Present key elements of study design early in the paper | 5 |
| Setting | #5 | Describe the setting, locations, and relevant dates, including periods | 7 |

| | | | |
|----|----------------------|---|-----|
| | | of recruitment, exposure, follow-up, and data collection | |
| 1 | | | |
| 2 | Eligibility criteria | #6a Give the eligibility criteria, and the sources and methods of selection | 5 |
| 3 | | of participants. Describe methods of follow-up. | |
| 4 | | | |
| 5 | | | |
| 6 | Eligibility criteria | #6b For matched studies, give matching criteria and number of exposed | 5 |
| 7 | | and unexposed | |
| 8 | | | |
| 9 | | | |
| 10 | Variables | #7 Clearly define all outcomes, exposures, predictors, potential | 5 |
| 11 | | confounders, and effect modifiers. Give diagnostic criteria, if | |
| 12 | | applicable | |
| 13 | | | |
| 14 | | | |
| 15 | Data sources / | #8 For each variable of interest give sources of data and details of | 7 |
| 16 | measurement | methods of assessment (measurement). Describe comparability of | |
| 17 | | assessment methods if there is more than one group. Give information | |
| 18 | | separately for for exposed and unexposed groups if applicable. | |
| 19 | | | |
| 20 | | | |
| 21 | | | |
| 22 | Bias | #9 Describe any efforts to address potential sources of bias | 4 |
| 23 | | | |
| 24 | Study size | #10 Explain how the study size was arrived at | 5 |
| 25 | | | |
| 26 | | | |
| 27 | Quantitative | #11 Explain how quantitative variables were handled in the analyses. If | 5-6 |
| 28 | variables | applicable, describe which groupings were chosen, and why | |
| 29 | | | |
| 30 | | | |
| 31 | Statistical | #12a Describe all statistical methods, including those used to control for | |
| 32 | methods | confounding | |
| 33 | | | |
| 34 | 7-8 | | |
| 35 | | | |
| 36 | | | |
| 37 | Statistical | #12b Describe any methods used to examine subgroups and interactions | 7-8 |
| 38 | methods | | |
| 39 | | | |
| 40 | | | |
| 41 | Statistical | #12c Explain how missing data were addressed | 8 |
| 42 | methods | | |
| 43 | | | |
| 44 | | | |
| 45 | Statistical | #12d If applicable, explain how loss to follow-up was addressed | 8 |
| 46 | methods | | |
| 47 | | | |
| 48 | | | |
| 49 | Statistical | #12e Describe any sensitivity analyses | |
| 50 | methods | | |
| 51 | | | |
| 52 | 8 | | |
| 53 | | | |
| 54 | Results | | |
| 55 | | | |
| 56 | | | |
| 57 | Participants | #13a Report numbers of individuals at each stage of study—eg numbers | 8 |
| 58 | | potentially eligible, examined for eligibility, confirmed eligible, | |
| 59 | | | |
| 60 | | | |

included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.

| | | | | |
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| 5 | Participants | #13b | Give reasons for non-participation at each stage | 8 |
| 6 | | | | |
| 7 | Participants | #13c | Consider use of a flow diagram | |
| 8 | | | | |
| 9 | | | | |
| 10 | 8 | | | |
| 11 | | | | |
| 12 | Descriptive data | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. | 8 |
| 13 | | | | |
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| 19 | Descriptive data | #14b | Indicate number of participants with missing data for each variable of interest | |
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| 24 | | | | |
| 25 | Descriptive data | #14c | Summarise follow-up time (eg, average and total amount) | |
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| 30 | Outcome data | #15 | Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable. | |
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| 38 | Main results | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 8-9 |
| 39 | | | | |
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| 44 | Main results | #16b | Report category boundaries when continuous variables were categorized | 8-9 |
| 45 | | | | |
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| 48 | Main results | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| 49 | | | | |
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| 52 | 8-9 | | | |
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| 54 | Other analyses | #17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10-11 |
| 55 | | | | |
| 56 | | | | |
| 57 | | | | |
| 58 | Discussion | | | |
| 59 | | | | |
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|----|--------------------|---------------------|--|-------|
| 1 | Key results | #18 | Summarise key results with reference to study objectives | 12 |
| 2 | | | | |
| 3 | Limitations | #19 | Discuss limitations of the study, taking into account sources of | 12 |
| 4 | | | potential bias or imprecision. Discuss both direction and magnitude of | |
| 5 | | | any potential bias. | |
| 6 | | | | |
| 7 | | | | |
| 8 | Interpretation | #20 | Give a cautious overall interpretation considering objectives, | 11-12 |
| 9 | | | limitations, multiplicity of analyses, results from similar studies, and | |
| 10 | | | other relevant evidence. | |
| 11 | | | | |
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| 13 | Generalisability | #21 | Discuss the generalisability (external validity) of the study results | 12 |
| 14 | | | | |
| 15 | | | | |
| 16 | Other | | | |
| 17 | Information | | | |
| 18 | | | | |
| 19 | | | | |
| 20 | Funding | #22 | Give the source of funding and the role of the funders for the present | 15 |
| 21 | | | study and, if applicable, for the original study on which the present | |
| 22 | | | article is based | |
| 23 | | | | |
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BMJ Open

Dynamics, outcomes, and prerequisites of the first SARS-CoV-2 superspreading event in Germany, in February 2020: a cross-sectional epidemiological study

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Dynamics, outcomes, and prerequisites of the first SARS-CoV-2 superspreading event in Germany, in February 2020: a cross-sectional epidemiological study

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Word Count: 4,581

Keywords: SARS-CoV-2, COVID-19, superspreading, cross-sectional study, ventilation

Abstract

Objectives

The first German SARS-CoV-2 outbreak was a superspreading event in Gangelt, North Rhine-Westphalia during indoor carnival festivities called “Kappensitzung” (15th of February 2020). We determined SARS-CoV-2 RT-PCR positivity rate, SARS-CoV-2-specific antibodies, and analyzed the conditions and dynamics of superspreading, including ventilation, setting dimensions, distance from infected persons and behavioral patterns.

Design

In a cross-sectional epidemiological study (51 days post-event), participants were asked to give blood, pharyngeal swabs and complete self-administered questionnaires.

Setting

1
2
3 42 The SARS-CoV-2 superspreading event took place during festivities in the small
4 43 community of Gangelt in February 2020. This 5 h event included 450 people (6-79
5 44 years of age) in a building of 27m x 13.20m x 4.20m.

8 45 **Participants**

9 46 Out of 450 event participants, 411 volunteered to participate in this study.

10 47 **Primary and Secondary Outcome Measures**

11 48 Primary outcome: infection status (determined by IgG ELISA). Secondary outcome:
12 49 symptoms (determined by questionnaire).

13 50 **Results**

14 51 Overall, 46% (n=186/404) of participants had been infected, and their spatial
15 52 distribution was associated with proximity to the ventilation system (OR 1.39, 95% KI
16 53 [0.86; 2.25]). Risk of infection was highly associated with age: children (OR: 0.33
17 54 [0.267; 0.414]) and young adults (age 18-25) had a lower risk of infection than older
18 55 participants (average risk increase of 28% per 10 year). Behavioral differences were
19 56 also risk-associated including time spent outside (OR: 0.55 [0.33; 0.91]) or smoking
20 57 (OR: 0.32 [0.124; 0.81]).

21 58 **Conclusions**

22 59 Our findings underline the importance of proper indoor ventilation for future events.
23 60 Lower susceptibility of children/young adults indicates their limited involvement in
24 61 superspreading.

25 62 26 63 27 64 **Strengths and limitations of this study**

- 28 65
- 29 66 • The setting and the participant group are extremely well-defined.
 - 30 67 • Participants were invited on the basis of one criterion, namely their presence at
31 68 the superspreading event; there was no other preselection/bias in the study
32 69 enrollment and the participation rate was high (91% of those invited).
 - 33 70 • The study was conducted 51 days after the event, so it is possible that
34 71 participants could have become infected unrelated to the event.
 - 35 72 • The number of index cases is unknown.
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75 Introduction

76 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly
77 transmissible and pathogenic RNA virus that emerged in late 2019 and has caused a
78 pandemic threatening human health and public safety worldwide.¹ While factors
79 shaping the dynamics of a pandemic are multifactorial, virulence and reproductive
80 number are important properties of a virus.² For SARS-CoV-2 there is a substantial
81 over-dispersion of the secondary infection distribution (individual R_0) for an individual
82 infected with SARS-CoV-2. An over-dispersed R_0 means that most infected people
83 do not transmit (individual $R_0 = 0$) while a minority of infected people are super-
84 spreaders (individual $R_0 > 5$). Superspreading has been observed for many infectious
85 pathogens, such as measles or SARS.³ During the SARS pandemic in 2003 a
86 superspreading event was defined as one infected person infecting eight others.⁴ For
87 SARS-CoV-2 it has been estimated that 80% of the infections are caused by 10% of
88 infected individuals highlighting the importance of the cluster factor (k).² In Germany
89 an indoor carnival event in the beginning of 2020 is considered as the first major
90 outbreak in a German city and was considered a hotspot during the beginning of the
91 pandemic in Germany.⁵ Other SARS-CoV-2 superspreading events worldwide have
92 been linked to indoor gatherings with close proximity of individuals.⁶ Nevertheless,
93 most of the reported superspreading events had less than 100 cases and the reports
94 are limited by missing data or a reporting bias.⁶

95
96 Here, we closely examined the prerequisite of a unique super-spreading event in
97 Germany during the SARS-CoV-2 pandemic, where nearly half of the participants
98 became infected including children. We systematically analyzed infection rate,
99 potential individual, and environmental risk factors for infection as well as the role of
100 the ventilation system.

102 Materials and methods

104 Study design and sampling

105 This cross-sectional epidemiological study was conducted 51 days after a carnival
106 celebration in the beginning of 2020. Eleven days after the event authorities sent all
107 known participants into quarantine after testing 38 out of 99 individuals PCR-positive.

1
2
3 108 All adults known to have attended the event were invited to participate in the study.
4
5 109 About 450 persons attended the event of which 411 participated in the study (**figure**
6
7 110 **1**, participation rate 91.3%). All study participants provided written informed consent
8
9 111 before enrolment. The Ethics Committee of the Medical Faculty of the University of
10
11 112 Bonn approved the study (approval number 085/20).

12 113 Self-administered questionnaires included questions about demographic background,
13
14 114 symptoms of viral infection as well as detailed information about the behavior during
15
16 115 the event. Participants' arrival and exit times were assessed in 1-hour categories.
17
18 116 Study participants were asked to provide blood specimens and pharyngeal swabs for
19
20 117 further analysis. The local health department supplied data on hospitalizations and
21
22 118 fatalities in our cohort (manuscript submitted elsewhere).

23 119

24 120 Patient and public involvement

25 121 This study was designed in close collaboration with both the local health department
26
27 122 of Heinsberg and the 'Council of 11' of Gangelt, the organizers of the event described
28
29 123 herein. The organizers as well as the city's head councilman were also involved in
30
31 124 recruitment by appealing to the local population to participate in the study. Since the
32
33 125 community of Gangelt was the center of the first German outbreak of SARS-CoV-2,
34
35 126 there was a great interest from the local public to participate in this study to help
36
37 127 understand this new virus and to gain access to early testing. Accordingly, the Ministry
38
39 128 of Labor, Health, and Social Affairs of the state government funded this study. In turn,
40
41 129 as a service to the public we informed each participant of their PCR and ELISA result
42
43 130 via letter and offered a phone hotline for questions about the results.

44 131

45 132 Spatial information and description of the event

46 133 The event took place on February 15th, 2020 and consisted of speeches, dance, and
47
48 134 music performances for a total of five hours, with one large intermission. It was a
49
50 135 ticketed event, where ticket sale was open to the public. Most of the participants were
51
52 136 inhabitants of Gangelt. It was hosted at a small community center (320 square meters)
53
54 137 in a single open space with a bar in the front close to the entrance and a stage at the
55
56 138 back. The tables, each with two benches, were arranged in two blocks with a center
57
58 139 aisle towards the stage. Alcoholic and nonalcoholic drinks were served in glasses and
59
60 140 a food truck was located outside in front of the venue. While most participants (about
141 450 people, 1.4 individuals per square meter) were sitting in the hall, a committee of

1
2
3 142 eleven individuals hosting the event were sitting on stage. The eleven people on stage
4
5 143 switched after a break.

6 144 Metric room coordinates (length and width [m]) for areas, tables, benches and
7
8 145 ventilation shafts were assessed via measurements, seating plan and photos from the
9
10 146 event. Persons providing multiple positions were considered as spending an equal
11
12 147 amount of time on different positions. When exact seating was unclear and information
13
14 148 was available on table or greater area localisation (bar, stage), average coordinate
15
16 149 values were used. On the grounds of these coordinates, we calculated pairwise metric
17
18 150 distances between all persons and distances to closest inlet and outlet airshafts. For
19
20 151 all persons their pairwise inverse distances were summarized as mean inverse
21
22 152 distance. Inverse metric distances to persons or airshafts were regarded as
23
24 153 representing infectious potential through local proximity, and inverse distances were
25
26 154 capped at 2.5 (the inverse of the width of a seat of 0.4 m). Alternatively, we counted
27
28 155 all infected persons within adjacent rings of 1.5 m width around each participant as a
29
30 156 measure of crowdedness and infectious potential.

31 157

31 158 Pharyngeal swab and blood preparation

32 159 Pharyngeal swabs of participants were performed with FLOQSwabs (Copan) and
33
34 160 immediately stored in UTM RT-mini tubes containing UTM Viral Stabilization Media
35
36 161 (Copan) at 4 °C. Venous blood was drawn into EDTA tubes (Sarstedt) per participant
37
38 162 and was transported to the laboratory at the University Hospital Bonn.

39 163 Anti-SARS-CoV-2 ELISA

40 164 Anti-SARS-CoV-2 IgA and IgGs were determined using enzyme-linked
41
42 165 immunosorbent assays (ELISA) on the EUROIMMUN Analyzer I platform (EI 2606-
43
44 166 9601 A, and EI2606-9601 G, respectively).⁵ A result was considered positive when a
45
46 167 ratio (extinction of sample/extinction of calibrator) of 0.8 or higher was reached. The
47
48 168 guidelines of the German Medical Association (RiliBÄK) were abided by, including
49
50 169 internal and external quality controls.

51 170

53 171 Reverse transcription polymerase chain reaction (RT-PCR)

54 172 Viral RNA was extracted from each 300µl swab sample via the chemagic Viral 300
55
56 173 assay (according to manufacturer's instructions) on the Perkin Elmer chemagic™
57
58 174 Prime™ instrument platform. The presence of two viral target genes (E and RdRP)
59
60 175 was assessed in each sample by real time RT-PCR (SuperScript™III One-Step RT-

1
2
3 176 PCR System with Platinum™ TaqDNA Polymerase, Thermo Fisher). The following
4
5 177 primers were used, for E gene: E_Sarbeco_F1 and R, and probe E_Sarbeco_P1, for
6
7 178 RdRP gene: RdRP_SARSr_F, and R, and probe RdRP_SARSr-P2.⁷ In addition, an
8
9 179 internal control for RNA extraction, reverse transcription, and amplification was applied
10
11 180 to each sample (innuDETECT Internal Control RNA Assay, Analytik Jena #845-ID-
12
13 181 0007100). If amplification occurred in both virus-specific reactions samples were
14
15 182 considered positive.
16

17 183

18 184 SARS-CoV-2 neutralization assay

19 185 A plaque reduction neutralization test was used to determine SARS-CoV-2
20
21 186 neutralization capacity as previously described.⁵ Briefly, plasma samples were heat-
22
23 187 inactivated and supernatant transferred to a new tube and serially two-fold diluted in
24
25 188 OptiPRO™SFM (Gibco). 120 µL of each plasma dilution was mixed with 80 plaque-
26
27 189 forming units (PFU) of SARS-CoV-2 in 120 µL OptiPRO™SFM cell culture medium
28
29 190 and used to infect Vero E6 cells (1.25x10⁵ cells/well seeded into 24-well plates 24 h
30
31 191 before). Subsequently, the inoculum was removed and cells were overlaid with a
32
33 192 mixture of carboxymethylcellulose (Sigma) and 2xMEM (Biochrom). Following 3-day
34
35 193 incubation, the overlay was removed and the 24-well plates were fixed using a 6% (v/v)
36
37 194 formaldehyde solution and stained with 1% (w/v) crystal violet in 20% ethanol revealing
38
39 195 the formation of plaques. Finally, the neutralizing titers were calculated as the
40
41 196 reciprocal of serum dilutions resulting in neutralization of 50% input virus (NT50), read
42
43 197 out as reduction in the number of plaques.
44

45 198

46 199 Data management and quality control

47 200 The Clinical Study Core Unit of the Study Center Bonn (SZB) supported the study by
48
49 201 outlining the study protocol and developing the informed consent form as well as
50
51 202 participants information sheets with respect to data management and quality control.
52
53 203 The data were gathered on paper-based Case Report Forms (pCRF). Data was
54
55 204 entered as double-data-entry into the REDCap study database programmed and
56
57 205 hosted by SZB. Study personnel was trained by experienced members of the SZB. A
58
59 206 quality manager was on site to support the study team. Monitoring of trial data and
60
207 informed consent forms was performed according to the monitoring plan by qualified
208
209 SZB staff. The ethics committee of the Medical Faculty of the University of Bonn was
involved and approved the study (reference no. 085/20)

210

211 Statistical analysis

212 Associations between positive infection status (defined as an IgG ratio ≥ 0.8), and
213 exposure variables were analysed via logistic regression models. Exposure variables
214 were included crudely, and adjusted for the potential confounding factors age, sex, and
215 duration of attendance as fixed effects. To correct for common household effects a
216 random effects model was used. We present odds ratios with 95% confidence
217 intervals. Because we present data on a single specific event among a limited number
218 of participants, we completely refrain from presenting p-values. All analyses were done
219 with SAS 9.4.

220

221 Results

222 411 out of an estimated 450 participants of the event responded to our study invitation,
223 resulting in a response rate of 91.3%. 404 individuals provided plasma samples and
224 316 pharyngeal swabs (**figure 1**). Genders were represented equally among all 404
225 participants (n= 201/404, 50% were male) with a broad range in age (6-79 years,
226 median age 36 years) and level of education (**table 1**). 297 individuals were residents
227 of the community the event took place in, 103 lived in other parts of the county, and 11
228 were external visitors. In total five participants of the event were hospitalized and one
229 participant subsequently died.

230

231 Overall, 186 out of 404 individuals tested seropositive for IgG- and 161 for IgA-
232 antibodies (**suppl. figure 1**). To confirm seropositivity we performed a plaque
233 reduction neutralization assay (**suppl. figure 2**) demonstrating neutralizing activity
234 against SARS-CoV-2 of their respective antibody responses. Given the low specificity
235 of the IgA assay, IgA seropositivity was not further considered.⁵ 19 participants tested
236 positive in RT-PCR; these were considered infected during the superspreading event
237 only if they were also IgG-positive (this was the case with 16 out of the 19 participants).

238

239 Overall, we found that (n= 186/404) 46.0% (95% CI: [41.2%; 51.0%]) tested
240 seropositive who attended the event, which was significantly higher than the overall
241 estimated infection rate in the same community at large at that time. Indeed, officially
242 3.1% of the community were reported as positive cases at that time, but we estimated
243 the infection rate to be 15.5% (95% CI:[12.3%; 19.0%])⁵ for the community. Taken

1
2
3 244 together, an estimated 46% of participants became infected during a single
4
5 245 superspreading event.

6 246
7 247 No association between the gender of participants and risk of infection was found ((OR:
8 248 1.01 [0.65; 1.58]) for women). On average infected individuals had a higher body mass
9 249 index (26.2kg/m² compared to 24.3kg/m² for uninfected individuals). Infected
10 250 participants were more likely to be clustered living in the same household (**table 1**).
11 251 Having at least one comorbidity, including lung disease (n= 11/26, 42.3%),
12 252 cardiovascular disease (n= 8/15, 53.3%), neurological disease (n= 1/6, 16.7%), cancer
13 253 (n= 7/12) (58.3%) or diabetes (n= 4/5, 80%), did not increase the risk of infection (OR:
14 254 0.64 [0.33; 1.26]). We next assessed whether age influenced the risk of infection at the
15 255 event, considering gender, duration of attendance and common household as
16 256 covariates. Comparison across age-categories showed a lower risk for children (OR:
17 257 0.31 [0.14; 0.69]), and also for young adults (18-25 years, OR: 0.53 [0.26; 1.09]) as
18 258 well as adults between 25 and 40 years (OR: 0.48 [0.28; 0.85]) in comparison to older
19 259 adults (40 to 65 years) (OR: 1, reference), while seniors had a slightly higher risk (older
20 260 than 65 years, OR: 1.1 [0.31; 3.97]) (**figure 2**). Our data suggests that an additional 10
21 261 years of age were on average associated with 28% increased risk of infection (OR:
22 262 1.28 [1.10; 1.48]).

23 263 To understand the spreading dynamics of SARS-CoV-2 during the event, we first
24 264 performed a detailed analysis of potential risk factors and social behavior. We first
25 265 analyzed whether the ventilation system influenced the distribution of SARS-CoV-2
26 266 infected individuals. It is important to state that the system's air flow consisted of 75%
27 267 used and 25% fresh air. The air flow can be described as clockwise. The air system
28 268 uses vents along one side of the venue and on stage to take in air (**figure 3**, air inlets
29 269 purple). After 25% of fresh air has been added and the air has been filtered, vents
30 270 along the other side of the venue return the air into the room (**figure 3**, air outlets blue).
31 271 All ventilation points received the same amount of air due to throttle valves. For noise
32 272 protection reasons windows remained closed. The air-system used F7-Filters (ISO
33 273 ePM $\geq 2,5$) and had an air volume flow of 7500 m³/h.

34 274
35 275 Most tables located close to the air-inlets and showed no or only few infections (**figure**
36 276 **3**, green) also most surrounding tables showed low numbers of infection (**figure 3**,
37 277 yellow). Tables close to the air-outlets show high (4 or 5 infected per table) and very
38 278 high (6 or 7 infected per table) numbers of infected individuals. Infected participants

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3 279 had been seated mostly at tables close to the bar, at the bar, or on stage. One table
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5 280 with 8 out of 11 infected people, was located far away from the bar at the other side of
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7 281 the hall and close to an air inlet. The group sitting on stage showed high numbers of
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9 282 infection (18 infected out of 24, **table 1**). Of note is that the overall number of
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11 283 participants per table was not equal for all tables. Greater proximity to air outlets was
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13 284 associated with increased risk of infection with a crude OR=1.39 [0.86; 2.25]. This
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15 285 association remained stable and was hardly attenuated from adjustment for proximity
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17 286 to air inlet, age, gender, duration of attendance, proximity to other infected persons,
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19 287 stage-activity and going outside during the intermission (**figure 4**, multiple adjusted
20
21 288 OR=1.26 [0.63; 2.50]). A similar apparent effect for proximity to air inlets (crude
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23 289 OR=1.17 [0.72; 1.89]) disappeared when duration of attendance was added to the
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25 290 model (**figure 4**, multiple adjusted OR=1.01 [0.53; 1.94]). Overall, however, we found
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27 291 the increased risk for individuals located closer to the air outlet remarkably persistent
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29 292 (**figure 4**).

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30 294 We further studied the sum of the inverse distance to all infected participants as a
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32 295 measure of proximity to either one common virus source or mutual infection. However,
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34 296 there was no evidence for increased risk of infection from greater proximity to other
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36 297 infected persons (**suppl. table 1**). Furthermore, we found no evidence for a single
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38 298 person being the source of the infection from the quantile-plot of p-values from 401
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40 299 analyses conducted separately for each participant as potential source of infection
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42 300 (**suppl. figure 3**).

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43 302 To understand the association of risk with behavior patterns we next investigated the
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45 303 influence of several factors on SARS-CoV-2 infection including time spent outside,
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47 304 smoking, performing on stage and participation during the final act ("Finale") for 30
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49 305 minutes. Results were all adjusted for age, sex, common household, and duration of
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51 306 attendance. Participation in multiple performances was associated with slightly
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53 307 increased risk of infection (OR per performance: 1.08 [0.91; 1.27]), results
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55 308 for participation in the last "Finale" were stronger (OR: 1.41 [0.65; 3.02]), although
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57 309 neither was significant (**figure 4**). Duration of attendance was persistently and strongly
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59 310 associated with an increased infection risk of 32% with each additional hour spent at
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311 the party (OR per hour: 1.32 [1.16; 1.49]). All other analyses were adjusted for this
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variable as potential confounding factor.

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5 314 We next determined the level of alcohol consumption as number of drinks (high-proof
6 315 liquor or beer) and did not observe any influence for the amount of alcohol consumption
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8 316 on the risk of becoming infected (OR per drink: 1.00 [0.96; 1.05]). Furthermore,
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10 317 participants who spent the break outside were less likely to be infected (OR: 0.55 [0.33;
11 318 0.91]) compared to individuals who spent the break inside the venue hall (**figure 4**).
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13 319 Interestingly, however, when we determined the impact of being regular smoker
14 320 (defined as smoking of at least 10 cigarettes a day) on the risk of SARS-CoV-2 infection
15 321 we observed a reduced risk of infection (OR: 0.32 [0.12; 0.81]) even after adjustment
16 322 for “time spent outside”. Taken together, our results demonstrated that the duration of
17 323 attendance at the carnival party correlated with an increased risk of infection, but the
18 324 number of alcoholic drinks was not associated with infection risk, while regular smoking
19 325 and spending the break of the event outside showed a negative correlation with the
20 326 risk of infection.
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29 328 We next stratified seropositive individuals by their reported symptoms. Odds-ratios for
30 329 each symptom were calculated for the timespan of 14 days following the event (**figure**
31 330 **5**). We identified that loss of smell (OR: 8.78 [4.81; 16.02]) and taste (OR: 10.09 [5.13;
32 331 19.88]) exhibited the strongest association with SARS-CoV-2 infection. Other
33 332 symptoms which were strongly associated with COVID-19 were: sweats and chills
34 333 (OR: 5.28 [3.08; 9.07]), muscle and joint ache (OR: 5.19 [3.19; 8.44]), fatigue (OR:
35 334 4.22 [2.76; 6.45]) and fever (OR: 3.73 [2.10; 6.63]) (**figure 5**). Importantly, 15.1%
36 335 (28/186) of the infected individuals reported no symptoms at all in a period of 14 days
37 336 after the event. The rate of asymptomatic infections of participants of the event was
38 337 lower than generally observed in the community where the event took place (36%).⁵
39 338 Overall, there was a lower proportion of asymptomatic cases among individuals
40 339 infected after the event compared to members of the community, while loss of smell
41 340 and taste showed the strongest association with an infection.
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54 342 **Discussion**

55 343 The high overdispersion characteristics of SARS-CoV2 and its ability to be transmitted
56 344 via aerosols under certain conditions are one of the main reasons that the beginning
57 345 of the SARS-CoV2 pandemic was shaped by superspreading events.^{8,9} Germany`s

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3 346 first superspreading event was an indoor carnival event in the beginning of 2020 in a
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5 347 rural community. In this naturally occurring experiment, we found that nearly half of the
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7 348 participants became infected and determined multiple prerequisites for superspreading
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9 349 and risk factors for becoming infected. While our study population is not a
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11 350 representative sample of the general population the event may be regarded as
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13 351 exemplary for similar party occasions and may help reduce the number of those
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15 352 infected in the future. At the time of the event described herein SARS-CoV-2 had not
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17 353 diversified yet, but ever since many variants of the virus have arisen and have taken
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19 354 turns dominating the global pandemic. Therefore, the results shown here need to be
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21 355 viewed as qualified in describing a superspreading event under the circumstances in
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23 356 the beginning of the pandemic. However, they help us to understand infection
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25 357 dynamics and requisites for infection with this virus family, ultimately giving a frame of
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27 358 reference for similar studies conducted throughout the alpha, delta, and omicron waves
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29 359 of the COVID-19 pandemic.

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33 361 An important factor associated with infection risk was the ventilation system and the
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35 362 individual proximity to the ventilation outlets. Individuals close to the air-outlets that
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37 363 contained air with low amount of fresh air had the highest infection risk compared to
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39 364 those close to the air-inlets. This was particularly interesting, because we did not see
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41 365 any increased risk of infection from greater proximity to other infected persons, which
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43 366 indicates that ventilation was perhaps more important than physical proximity. Our
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45 367 findings are in line with previous studies that demonstrated SARS-CoV-2 to be able to
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47 368 become air-borne under certain conditions and that the ventilation system can have an
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49 369 influence on virus spread.^{10,11,12} The air filters in the venue were not capable of
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51 370 intercepting virus particles supporting the notion on the importance of proper indoor
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53 371 ventilation systems.^{13,14} Indeed, spending the break of the event outside decreased
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55 372 the possibility of infection underscoring the benefit of proper ventilation or fresh air to
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57 373 lower the amount of aerosols. Due to the nature of the event, the spatial distribution of
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59 374 the participants was not fixed throughout the evening, and not perfectly recapitulated,
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375 so this information carries some error. However, allowing for multiple positions per
376 person we used all available information. Assuming further error in the spatial data to
377 be random, this might lead to a dilution of effects, i.e. true associations may remain
378 undetected. Complementary analyses including e.g. the persons' functions during the
379 event show consistent results, so we see no evidence suggesting bias in our findings.

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3 380 Nevertheless, the infection-rate might be overestimated as the study was conducted
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5 381 51 days after the event as participants could have become infected not related to the
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7 382 event. However, this weakness is limited by the official shut down of the community
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9 383 shortly after the event: A detailed timeline of the containment measures put in place
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11 384 after the superspreading event is included in Streeck et al.⁵. Briefly, a strict home
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13 385 quarantine for all attendees of the carnival event was imposed after 38 out of 99
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15 386 participants tested positive for SARS-CoV-2. In addition, 13 days after the event the
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17 387 town went into full lockdown, including the closing of schools, childcare and outpatient
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19 388 care facilities, and restrictions of public access to the town. These concerted
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21 390 containment measures proved so effective that the peak of new infections in the
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23 391 community was already reached 27 days after the event.

24 392 The consumption of alcoholic drinks did not increase the risk of infection. While it has
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26 393 been assumed that the alcoholic effect of decreased social inhibition may increase
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28 394 likelihood of infection, we did not find any evidence for this association questioning
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30 395 measures of a ban on alcohol to reduce numbers of infected. It is known that current
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32 396 and former smokers disproportionately suffer from severe COVID-19 and their
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34 397 numbers are relatively increased among those patients that need intensive care
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36 398 treatment compared to non-smokers^{15,16}. However, it has been previously speculated
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38 400 that the risk of infection is lower for smokers.¹⁷ Furthermore, a meta-analysis of seven
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40 401 studies suggests that smokers have a reduced risk of testing positive for SARS-CoV-
41
42 402 2.¹⁸ Interestingly, we also observed that regular smoking lowered the risk of infection.
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44 403 The association might for example be explained by a role of the nicotinic acetylcholine
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46 404 receptor (nAChR).¹⁹ Because other viruses, such as rabies virus, have been known to
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48 405 bind nAChRs, it was hypothesized recently, that SARS-CoV-2 spike protein might bind
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50 406 nAChRs as a coreceptor for infection.^{20,21} Indeed, *in silico* molecular docking
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52 407 simulations predicted binding of spike to nAChRs.²² If this interaction proves to be of
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54 408 advantage to the virus, then nicotine or its derivatives which bind nAChRs could
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56 409 compete with SARS-CoV-2 for binding and thereby reduce interactions of the virus
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58 410 with its target cells. Currently, at least one prospective observational study is being
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60 411 undertaken on the effects of smoking on COVID-19 infection rates, including a smoking
cessation control group on nicotine substitutes.²³ While we strongly advise that
smoking should not be considered as a protective habit to prevent risk of infection, this

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3 413 knowledge may lead to the investigation of a therapeutic or prophylactic treatment on
4 414 the basis of this molecular target.²⁴

6 415 Our results indicate a trend that younger people are less likely to be infected compared
7 416 to older age groups. This trend is strongest for people under 18 but levels out over 40
8 417 years of age. The risk of infection for children in superspreading events has not been
9 418 investigated but the overall risk for infection in children seems to be lower than for
10 419 adults as a systematic review and its recent update reported, which is further supported
11 420 by our findings.^{25,26} Considering the risk of infection with SARS-CoV-2 in general
12 421 however, in a meta-analysis Madewell et al. conclude that the secondary attack rate in
13 422 households is lower to children contacts than to adult contacts²⁷. Many primary articles
14 423 and meta-analyses point out the confounding effect of SARS-CoV-2 infections being
15 424 mostly asymptomatic in young children has on the identification of children as index
16 425 persons. To some extent, this problem could be avoided in our study since all
17 426 participants of the event were invited to take part, regardless of age. As all individuals
18 427 were exposed at the same event and time our study is a very suitable model for the
19 428 previously described notion, that children are less likely to become infected. Indeed, a
20 429 recently published meta-analysis by Viner et al. showed a low susceptibility for children
21 430 and adolescents (OR of 0.56 (95% CI, 0.37-0.85)) which strongly supports our findings
22 431 of a lower risk of infection in that age group, which is even lower in our study²⁸. Our
23 432 finding supports the previously shown minor influence on the spreading of the virus by
24 433 children. The finding that for every 10 additional years of age the risk of infection
25 434 increases during an event indicates that younger people and their limited role should
26 435 be considered when measures to contain the pandemic are implemented. It should be
27 436 mentioned that although children had similar exposure compared to adults and
28 437 probably spent even less time outside the venue hall, the behaviors of children may be
29 438 different compared to adults. Therefore, we cannot exclude that our findings of lower
30 439 seroprevalence in children might be biased by factors very specific to this particular
31 440 event. Taken together, we demonstrate important risk factors for infection during a
32 441 superspreading event, which helps to understand transmission dynamics in order to
33 442 improve comprehensive public health preparedness measures, including mandatory
34 443 ventilation during indoor events and age-adjusted measures according to different risk
35 444 of infection.

58 445 As to the strengths and limitations of this study, the participant group is extremely well-
59 446 defined and there was no bias or preselection during enrollment as there was only one

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3 447 criteria for invitation, namely presence at the event. Because of the time between the
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5 448 event and the study it is possible that participants were infected unrelated to the event,
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7 449 but the official shut down of the community limits this risk. The number of index cases
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9 450 during the event is not known and it is possible that a high number of individuals were
10
11 451 already infectious. In addition, the identification of a past SARS-CoV-2 infection via
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13 452 serological test is not perfect and according to the manufacturer their IgG detection is
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15 453 94.4 % sensitive (on samples collected >10 days after beginning of symptoms or direct
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17 454 detection of virus) and 99.0 % specific (for a ratio ≥ 0.8). For our infection rate analysis
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19 455 this predicts 2 false positives and 10 false negative IgG results. However, when field-
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21 456 tested by the UK National Health Service (NHS) the same assay showed 74.7 %
22
23 457 sensitivity (62 false-negatives in our data set) and the same specificity of 99.0 %.

24 458

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496 **Contributors**

497 L.W., E.R., B.S. and H.S. wrote the manuscript. L.W., R.M.S., and E.R. organized and
498 ran the testing center. M.E. inspected the event venue and examined ventilation and
499 air filtration systems. ER and BS organized and performed sample processing,
500 experiments, analyses, and corrected the manuscript. N.L. and A.H. performed
501 statistical analysis. M.C., C.F., and A.K. monitored the study. M.E., K.H.J. and H.S.
502 oversaw the study and corrected the manuscript.

504 **Data availability statement**

505 All data from the study will be made available upon reasonable request.

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511 **Competing interests**

512 The authors declare no conflict of interest.

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581

582 Tables

583

| | Not infected N% | Infected N% |
|--|--------------------|----------------|
| Total number | 218 | 186 |
| Female | 114 (52%) | 89 (48%) |
| Age: | | |
| <18 years | 31 (14%) | 15 (8%) |
| 18- 24 years | 30 (14%) | 20 (11%) |
| 25-39 years | 81 (37%) | 43 (23%) |
| 40-64 years | 71 (33%) | 100 (54%) |
| 65+ years | 5 (2%) | 8 (4%) |
| BMI [kg/m ²] (Std Dev) | 24.3 (5.12) | 26.2 (5.16) |
| Participating household member (Std Dev) | 2.1 (1.12) | 2.4 (1.16) |
| Highest level of formal education: | | |
| None | 27 (13%) | 13 (7%) |
| lower secondary school | 27 (13%) | 23 (13%) |
| secondary school | 55 (26%) | 71 (39%) |
| higher education entrance qualification | 54 (25%) | 34 (18%) |
| (technical) university degree | 52 (24%) | 43 (23%) |
| Duration of attendance [h] (Std Dev) | 4.7 (2.06) | 5.8 (1.85) |
| Service team | 4 (2%) | 22 (12%) |
| On stage during event | 80 (37%) | 62 (34%) |
| Member of „Council of 11“ | 6 (3%) | 18 (10%) |
| On stage during “Finale” | 26 (12%) | 48 (26%) |
| Behavior during break: | | |
| Remaining seated | 73 (36%) | 85 (48%) |

| | Going outside | 114 (55%) | 72 (39%) |
|---|---------------|-------------|-------------|
| Alcohol consumption [drink] (Std Dev) | | 11.3 (7.76) | 12.2 (7.40) |
| Former smoker | | 34 (16%) | 45 (24%) |
| Active smoker (≥ 10 cigarettes/day) | | 54 (25%) | 23 (12%) |
| At least one comorbidity | | 29 (13%) | 28 (15%) |
| Avg. distance to other participants [m] (Std Dev) | | 9.2 (1.68) | 9.1 (1.70) |
| Distance to air inlet [m] (Std Dev) | | 6.1 (3.22) | 6.0 (3.30) |
| Distance to air outlet [m] (Std Dev) | | 4.8 (2.94) | 5.1 (2.87) |

Table 1: Distribution of demographic factors and exposure information of interest among study participants who tested positive or negative in serology test of SARS-Cov2-infection

`Council of 11´ stands for the hosts of the events located on stage (personnel switched during the break).

`Finale´ describes the final presentation of the event with all performers on stage.

Figure Legends

Figure 1: Study participants

Enrollment and flow of participants through the study. Downstream sample processing included centrifugation of blood samples for plasma collection (SARS-CoV-2 ELISAs), and viral RNA extraction from swab samples (SARS-CoV-2 RT-PCR).

Figure 2: Odds ratios for the likelihood of SARS-CoV-2 infection by age groups

Participants were divided into age groups of 8, 15, or 25 years, participants younger than 18 or older than 65 years. Participants were considered to have been infected during the event if they were SARS-CoV-2 antibody positive (ELISA).

Figure 3: Reconstructed 3D-Model of the venue hall

The venue was a single open space with a stage on one end and a bar as well as the exit on the opposite end. Distribution of tables and seating was as indicated by table and chairs symbols. Please note that the people pictured are illustrative and do not represent individual participants. Self-administered questionnaires included questions about main seating-position of the participants during the evening event as specifying table and seat with the help of a schematic seating plan. Metric room coordinates for all tables, seats, and ventilation-points were assessed and the seating was reconstructed from pictures taken during the event. Therefore, the location of the stage, the bar, the exit as well as the tables and the air-inlets/outlets were reconstructed in a 3D-Model. The original external dimensions of the building were 27m x 13.20m x 4.20m. Tables, where more than 7 infected individuals have stayed are colored in dark red, this includes the stage and bar as well. Air-inlets are colored in violet and the air-outlets in blue. Infected participants had been seated mostly at tables close to the bar, the bar itself and on stage. One table with 8 out of 11 infected people, was located far away from the bar at the other side of the hall and close to an air inlet. The group sitting on stage showed as well high numbers of infection (18 infected out of 24).

Figure 4: Odds ratios for the association of SARS-CoV-2 infection with specific activities of the participants and their location in the venue relative to ventilation shafts

The model was additionally adjusted for age, sex, duration of attendance, participation in multiple activities, and cumulative proximity to other infected persons, and common household.

Figure 5: Odds ratios for symptoms of SARS-CoV-2 antibody-positive participants in the 14 days following the super spreading event

The information on symptoms was derived from the self-administered questionnaire, which was filled out on the day of sample collection. Odds ratio estimates (OR) are shown with confidence intervals.

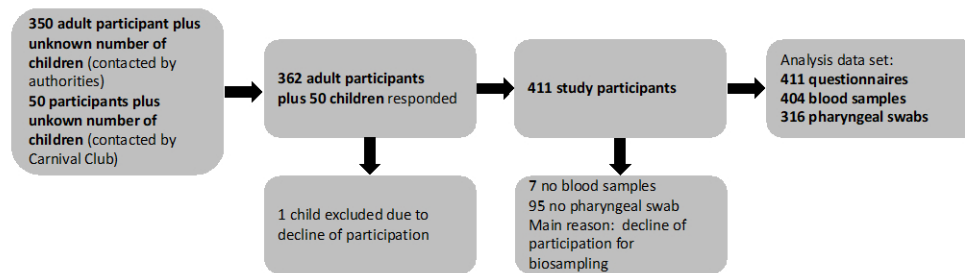


Fig. 1: Study participants. Enrollment and flow of participants through the study. Downstream sample processing included centrifugation of blood samples for plasma collection (SARS-CoV-2 ELISAs), and viral RNA extraction from swab samples (SARS-CoV-2 RT-PCR).

338x123mm (78 x 78 DPI)

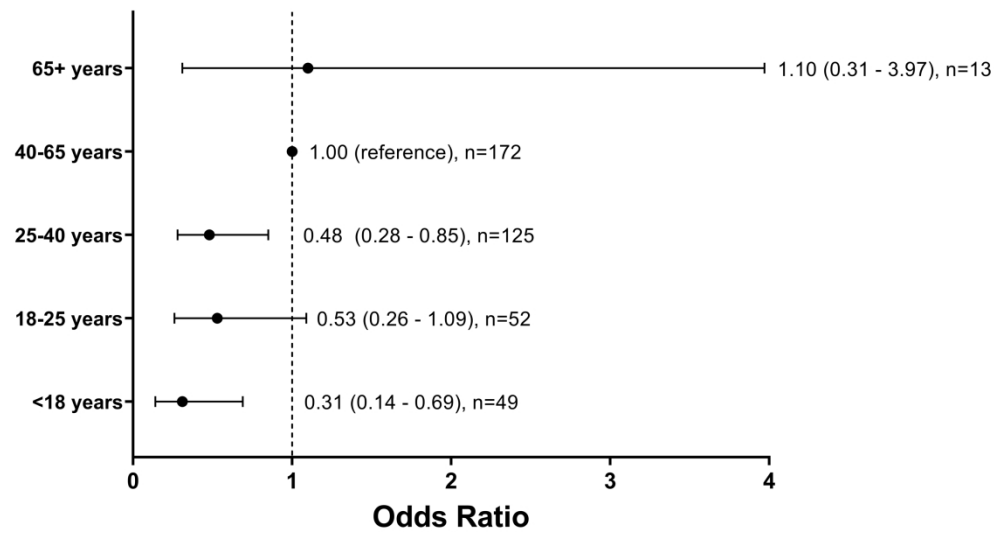


Fig. 2 Odds-Ratio for the likelihood of SARS-CoV-2 infection by age groups. Participants were divided into age groups of 8, 15, or 25 years, participants younger than 18 or older than 65 years. Participants were considered to have been infected during the event if they were SARS-CoV-2 antibody positive (ELISA).

283x160mm (300 x 300 DPI)

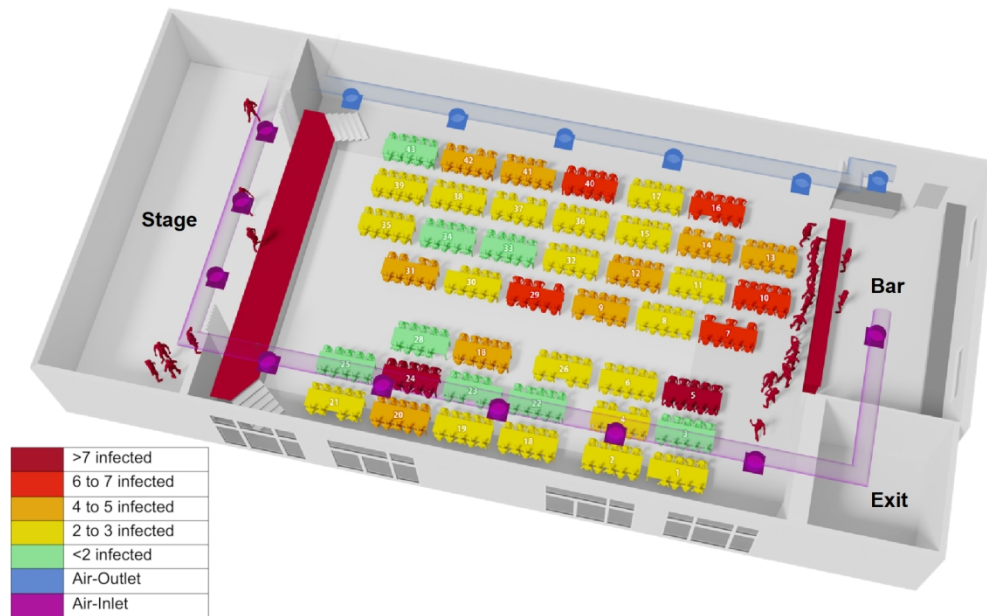


Fig. 3: Reconstructed 3D-Model of the venue hall. The venue was a single open space with a stage on one end and a bar as well as the exit on the opposite end. Distribution of tables and seating was as indicated by table and chairs symbols. Please note that the people pictured are illustrative and do not represent individual participants. Self-administered questionnaires included questions about main seating-position of the participants during the evening event as specifying table and seat with the help of a schematic seating plan. Metric room coordinates for all tables, seats, and ventilation-points were assessed and the seating was reconstructed from pictures taken during the event. Therefore, the location of the stage, the bar, the exit as well as the tables and the air-inlets/outlets were reconstructed in a 3D-Model. The original external dimensions of the building were 27m x 13.20m x 4.20m. Tables, where more than 7 infected individuals have stayed are colored in dark red, this includes the stage and bar as well. Air-inlets are colored in violet and the air-outlets in blue. Infected participants had been seated mostly at tables close to the bar, the bar itself and on stage. One table with 8 out of 11 infected people, was located far away from the bar at the other side of the hall and close to an air inlet. The group sitting on stage showed as well high numbers of infection (18 infected out of 24).

155x96mm (300 x 300 DPI)

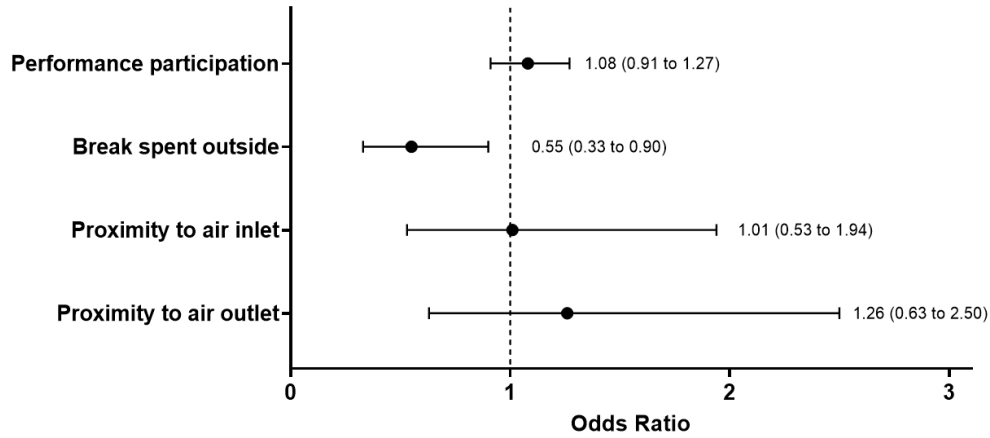


Fig. 4: Odds ratios for the association of SARS-CoV-2 infection with specific activities of the participants and their location in the venue relative to ventilation shafts. The model was additionally adjusted for age, sex, duration of attendance, participation in multiple activities, and cumulative proximity to other infected persons, and common household.

259x115mm (120 x 120 DPI)

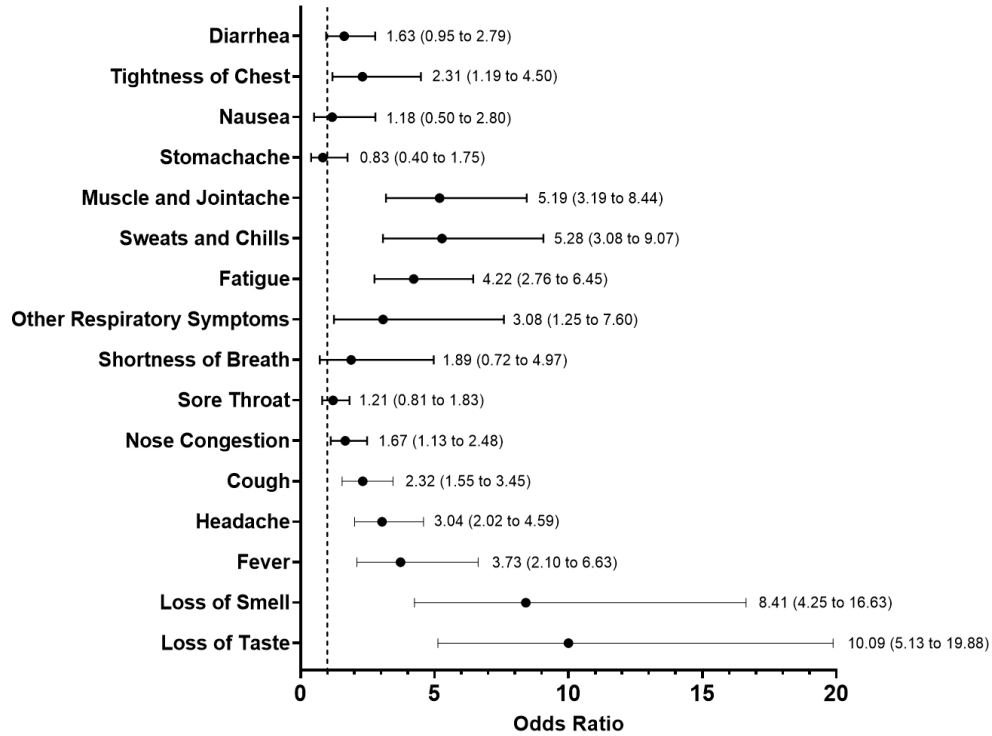
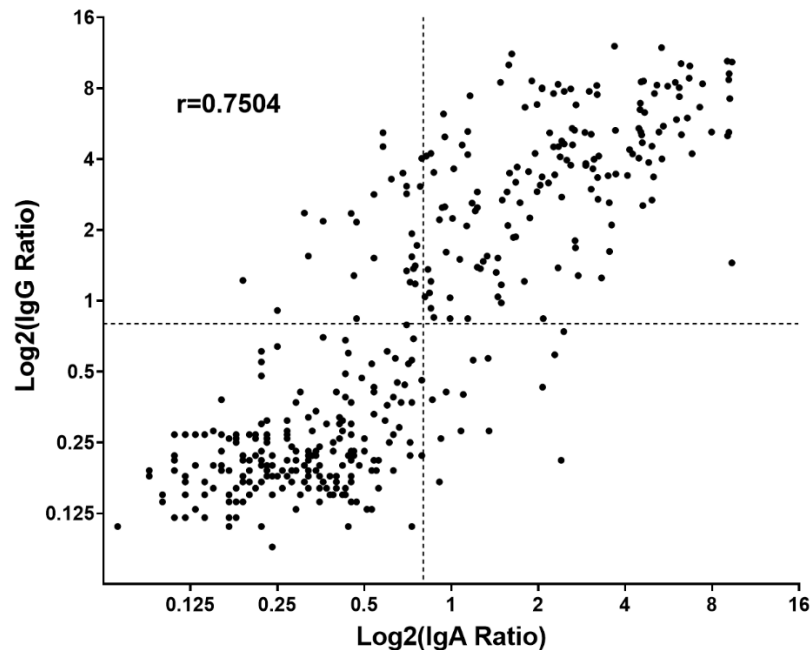


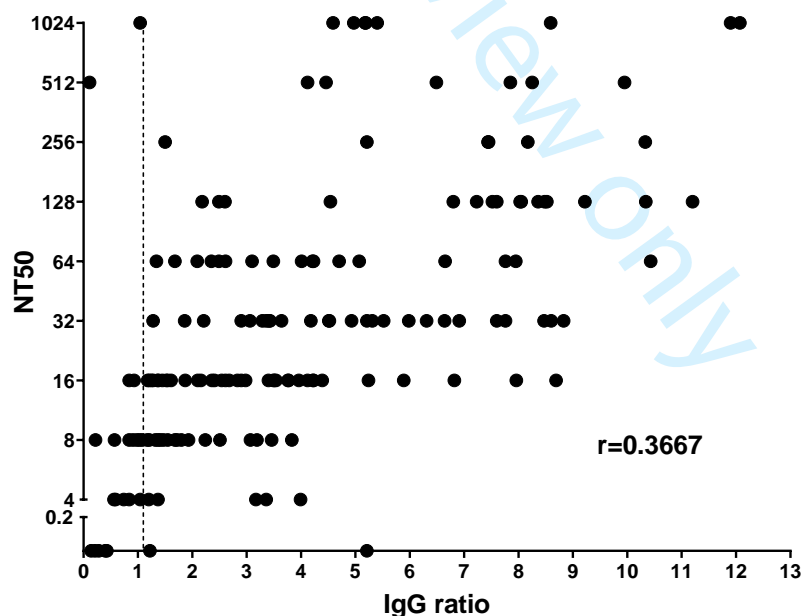
Fig. 5: Odds ratios for symptoms of SARS-CoV-2 antibody-positive participants in the 14 days following the super spreading event. The information on symptoms was derived from the self-administered questionnaire, which was filled out on the day of sample collection. Odds ratio estimates (OR) are shown with confidence intervals.

280x208mm (120 x 120 DPI)

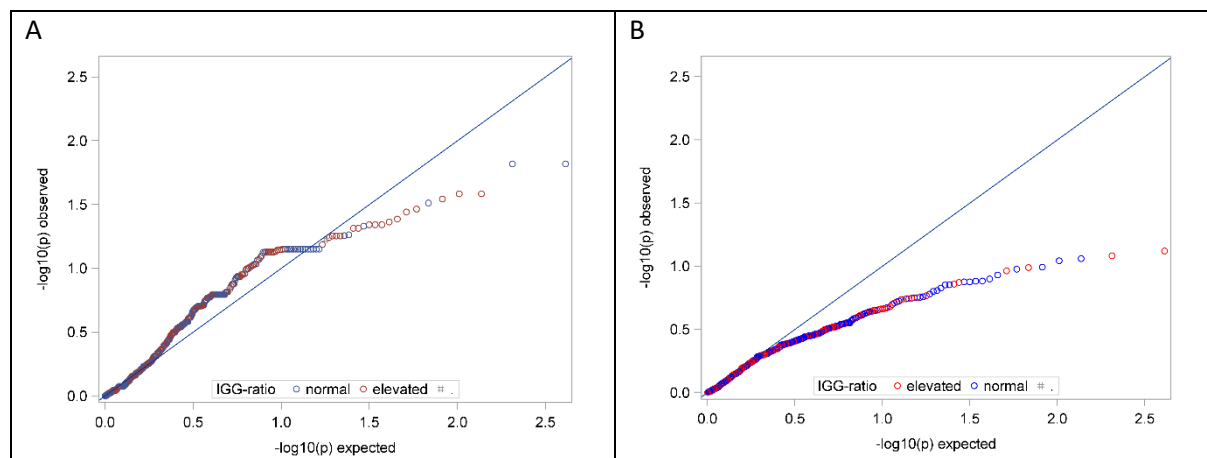
Supplementary Material



Suppl. Fig. 1: Correlation of SARS-CoV-2 Euroimmun ELISA results for IgA and IgG. The correlation of IgA levels to IgG levels in the same person was significant (r : Pearson coefficient, $p < 0.0001$, 95 % CI, 0.7043 to 0.7902). The dotted lines mark the ratios above which each ELISA result is considered positive.



Suppl. Fig. 2: Correlation of plasma neutralization capacity and IgG ELISA results (Euroimmun) from each donor. The dotted line marks the ratio above which the ELISA result is considered positive. The correlation coefficient (Pearson) was 0.3667 (95 % CI, 0.2275 to 0.4192, $p < 0.0001$). Samples with a negative result in the neutralization assay were set as 0.1 here so as to appear on the logarithmic axis.



Supplemental Figure 3: Quantile plot of observed p-values from analyses of inverse distance [1/m] to single specific study participants as risk factor for corona-virus infection. In case of no association, the ordered log-transformed p-values are expected to lie on, or below the diagonal. Panel A: results from crude analyses, Panel B: analyses were adjusted for age, sex, common household and duration of attendance.

| | OR | 95% confidence interval | | p-value |
|--|-------------|-------------------------|------|---------|
| proximity to infected persons [sum 1/m] | 0,99 | 0,98 | 1,01 | 0,430 |
| adjusted a) | 1,00 | 0,98 | 1,02 | 0,957 |
| mutually adjusted b) | 0,99 | 0,97 | 1,01 | 0,571 |
| mutually adjusted c) | 0,99 | 0,97 | 1,02 | 0,646 |
| Alternative consideration in distance-bands | | | | |
| Infected persons within ≤1.5m [count] | 1,01 | 0,96 | 1,07 | 0,681 |
| Infected persons in 1.5 - ≤3m [count] | 0,96 | 0,92 | 1,00 | 0,043 |
| Infected persons in 3 - ≤4.5m [count] | 1,03 | 1,00 | 1,06 | 0,023 |
| Infected within ≤1.5m [count] adjusted a) | 1,03 | 0,97 | 1,10 | 0,366 |
| Infected in 1.5 - ≤3m [count] | 0,96 | 0,92 | 1,01 | 0,113 |
| Infected in 3 - ≤4.5m [count] | 1,03 | 1,00 | 1,06 | 0,083 |
| Infected within ≤1.5m [count] mutually adjusted b) | 1,01 | 0,95 | 1,07 | 0,734 |
| Infected in 1.5 - ≤3m [count] | 0,98 | 0,94 | 1,02 | 0,359 |
| Infected in 3 - ≤4.5m [count] | 1,05 | 1,02 | 1,08 | 0,001 |
| Infected within ≤1.5m [count] mutually adjusted c) | 1,02 | 0,95 | 1,09 | 0,638 |
| Infected in 1.5 - ≤3m [count] | 0,98 | 0,93 | 1,03 | 0,363 |
| Infected in 3 - ≤4.5m [count] | 1,04 | 1,00 | 1,07 | 0,041 |

Supplementary table 1: Estimated relative risk of SARS-CoV-2 infection (IGG-positive) from logistic regression on summary measures of spatial proximity between participants in terms of odds ratio estimates (OR) with confidence interval and p-values. a) adjusted for sex, age, common household and duration. b) multivariate analysis, mutually adjusted for distance to ventilation system, participation in (multiple) performances, going out of doors during the intermission, and participating in the grand finale. c) multivariate analysis, mutually adjusted for distance to ventilation system, participation in (multiple) performances, going out of doors during the intermission, and participating in the grand finale and adjusted for sex, age, common household and duration.

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

| | Item No | Recommendation | Page No |
|------------------------------|---------|--|---------|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| Introduction | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 3 |
| Methods | | | |
| Study design | 4 | Present key elements of study design early in the paper | 3,4 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 3-7 |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants | 3,4 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 2 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 4 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 3 |
| Study size | 10 | Explain how the study size was arrived at | 3 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 4 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 4 |
| | | (b) Describe any methods used to examine subgroups and interactions | 5 |
| | | (c) Explain how missing data were addressed | 4 |
| | | (d) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (e) Describe any sensitivity analyses | 14 |
| Results | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 19 |
| | | (b) Give reasons for non-participation at each stage | 19 |
| | | (c) Consider use of a flow diagram | 19 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 7 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 7 |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 7,8 |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 9,10 |

| | | | |
|--------------------------|----|--|-------|
| | | (b) Report category boundaries when continuous variables were categorized | 8,10 |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 9-11 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11-13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 14 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13,14 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 11 |
| Other information | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 16 |

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.