Kappensitzung 2020 – das erste SARS-CoV-2 Superspreading-Event in Deutschland

Eine epidemiologische Querschnittsstudie

Inaugural-Dissertation

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Abkürzungsverzeichnis

SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SARS-CoV-1	Severe acute respiratory syndrome coronavirus type 1
PCR	Polymerase chain reaction
Covid-19	Coronavirus disease 2019
UTM	Universal transport medium
IgA	Immunglobulin A
IgG	Immunglobulin G
DNA	Desoxyribonuclein acid
RNA	Ribonuclein acid
EDTA	Ethylendiamintetraacetat
ELISA	Enzyme-linked Immunosorbent Assay
RT-PCR	Reverse transcription polymerase chain reaction
E-Gen	Envelope Gen
RdRP-Gen	RNA dependent RNA-Polymerase Gen
PFU	Plaque forming units
MEM	Minimum essential medium
ISO	Internationale Organization für Standardisierung
ePM 2,5	Efficiency Particulate Matter 2,5 micrometer

1. Deutsche Zusammenfassung

1.1. Einleitung und Fragestellung

1.1.1. Einleitung

Das SARS-CoV-2-Virus ist Ende 2019 erstmals aufgetreten und hat eine Pandemie ausgelöst, welche die Gesundheit der Menschheit bedroht und das öffentliche Leben in großen Teilen der Welt zeitweise zum Erliegen brachte (Chakraborty et al., 2020).

Das Covid-19 auslösende Virus ist, ähnlich wie bereits SARS-CoV-1 (De Serres et al., 2013), durch seine Überdispersion (Lloyd-Smith et al., 2005), und hohe Virulenz, charakterisiert und hat somit Eigenschaften, die die pandemische Ausbreitung begünstigen. Diese Eigenschaften sorgen dafür, dass Superspreading Events die Dynamik der Pandemie entscheidend mitbestimmen können.

Der erste große Ausbruch von Covid-19 in Deutschland ereignete sich im Kreis Heinsberg (RKI, Täglicher Lagebericht zu Covid 19, 8.3.2020). Eine Karnevalsveranstaltung, genannt „Kappensitzung“, am 15.02.2020 konnte durch das ortsständige Gesundheitsamt als Event mit vielen Ansteckungen identifiziert werden.

Dieses potenzielle Superspreading Event bot eine einzigartige Chance derartige Ausbruchsphänomene zu untersuchen, da sich dort ein großer Teil der Feiernden infizierte und ein sehr großer Anteil der Besucher als Studienprobanden gewonnen werden konnten. Das Ziel der Studie sowie dieser Dissertation ist es individuelle als auch umweltbedingte Risikofaktoren für eine Infektion mit Covid-19 im Rahmen eines Superspreading Events zu untersuchen. Zusätzlich soll der Einfluss der Lüftung auf die Verteilung der Infizierten im Raum untersucht werden.

Zuvor veröffentlichte Berichte zu Superspreading Events, während der Covid-19 Pandemie befassen sich zu einem großen Anteil mit Ausbrüchen in Gesundheitseinrichtungen. Nur wenige Meta-Analysen fassen Veröffentlichungen zum Thema Superspreading Events zusammen. Die Studien zeichnen sich durch geringe Anzahl an Infizierten (meist unter 100), durch fehlende Daten und einen starken Reporting Bias aus (Leclerc et al., 2020). Es ist keine Studie bekannt, die das individuelle Verhalten

der Teilnehmer, die Belüftungssituation sowie die Rolle von Minderjährigen während eines Superspreading Events untersucht.

1.1.2. Fragestellung

Diese Dissertation untersucht die oben genannte Veranstaltung und versucht die Voraussetzung, Dynamiken und die Ergebnisse des Infektionsgeschehens darzustellen.

Die untersuchten Hypothesen dieser Dissertation lauten:

Die untersuchte Veranstaltung ist ein Superspreading Event. Es zeigen sich viele Teilnehmer während der Untersuchung seropositiv.

Es lässt sich ein Zusammenhang zwischen dem Verhalten der Teilnehmer und der Wahrscheinlichkeit einer Infektion zeigen.

Der räumliche Aufenthaltsort von Teilnehmern während der Veranstaltung im Bezug zum Belüftungssystem zeigt einen Zusammenhang mit der Wahrscheinlichkeit einer Infektion.

Es zeigt sich ein Zusammenhang zwischen individuellen, demographischen Eigenschaften von Teilnehmern und der Wahrscheinlichkeit einer Infektion zeigen.

1.1.3. Ethikvotum, Informed Consent und DRKS

Die Ethikkommission des Universitätsklinikums Bonn erteilte ein positives Ethikvotum mit der Kennnummer 085/20. Alle Teilnehmer erhielten detaillierte Information zur geplanten Studie und unterschrieben, dass die Blut- und Speichelproben für die Forschung genutzt werden dürfen. Für Kinder sowie für Jugendliche wurde ein angepasstes Informationsschreiben erstellt. Alle Dokumente wurden in Zusammenarbeit mit der Studienzentrale der Universität Bonn erstellt und durch die Ethikkommission genehmigt. Die Studienteilnahme erfolgte somit mit „informed consent“ aller Studienteilnehmer. Die Studie wurde im Deutschen Register Klinischer Studien registriert (www.drks.de, Identifikationsnummer DRKS00021306).

1.2. Material und Methoden

Alle bekannten erwachsenen Teilnehmer der Kappensitzung wurden eingeladen an der Studie teilzunehmen und Familienangehörige, inklusive Minderjähriger, mitzubringen, falls auch diese an der Veranstaltung teilgenommen haben. Von den etwa 450 Teilnehmern konnten 411 als Probanden gewonnen werden, was einer Teilnahmequote von 91,3 % entspricht. Die Probanden wurden gebeten Blutentnahmen (EDTA (Sarstedt)) und einen Rachenabstrich (FLOQSwabs (Copan)) durchführen zu lassen sowie einen Fragebogen zum demografischen Hintergrund, Erkältungssymptomen zu verschiedenen Zeitpunkten sowie Verhalten, Aufenthaltsdauer und Aufenthaltsort während der Veranstaltung anzugeben.

Die Bestimmung der Anti-SARS-CoV-2 Antikörper Immunglobulin-G und Immunglobulin-A wurde mittels ELISA durchgeführt. Ein Verhältnis von Extinktion der Probe zu Extinktion der Kalibrierprobe (Ratio) von 0,8 oder höher galt als positives Ergebnis (Herstellerangabe).

Die Rachenabstriche wurden zur RT-PCR Testung genutzt. Durch real time RT-PCR wurde nach zwei viralen Zielgenen (E und RdRP) gesucht.

Für das E-Gen wurde als Primer E_Sarbeco_F1 und R sowie probe E_Sarbeco_P1 genutzt. Für das RdRP-Gen: RdRP_SARSr_F, und R, sowie probe RdRP_SARSr-P2 (Corman et at., 2020). Eine interne Kontrolle der RNA-Extraktion, der reversen Transkription und Amplifikation wurde zusätzlich für jede Probe durchgeführt (innuDETECT Internal Control RNA Assay, Analytik Jena #845-ID-0007100). Eine Amplifikation in beiden Reaktionen zeigte ein positives Resultat an.

Zuletzt wurde die Neutralisationsfähigkeit der Antikörper getestet. Die inaktivierten Proben wurden mit OptiPROTMSFM (Gibco) verdünnt und 120ml jedes verdünnten Plasmas wurde mit 80 PFUs für SARS-CoV-2 und 120ml OptiPRO SFM (Gibco) Zellkulturmedium vermischt und in $1{,}25 \times 10^5$ Vero E6 cells/wells angesiedelt. Nach Entfernung des Inokulum wurde Carboxymethylcellulose (Sigma) und 2xMEM (Biochrom) zugesetzt. Nach dreitägiger Inkubationszeit wurden die Platten mit 6 % Formaldehydlösung fixiert und mit 1 % Kristallviolett und 20 % Ethanol gefärbt wodurch die Plaquebildung sichtbar gemacht wurde. Der neutralisierende Titer wurde als Kehrwert der Serumverdünnung, welche zu

50 % Neutralisation des Ausgangsvirus (NT50) führte, berechnet. Dies zeigt sich durch eine Reduktion der Plaques.

Die Raumkoordinaten der Tische und Bänke wurden durch Fotos und Videos vom Event rekonstruiert. Die Koordinaten der Bar, der Bühne und der Lüftungsschächte wurde bei einer Ortsbegehung erstellt. Durch die Angabe des Aufenthaltsortes während der Events wurde jedem Teilnehmer eine Koordinate zugeordnet. Bei mehreren Angaben wurden einer Person mehrere Koordinaten zugeordnet und diese gleich gewichtet.

Für jede Person wurde der inverse Abstand zu den Lüftungschächten der Zuluft und Abluft berechnet. Zusätzlich wurde der inverse Abstand aller und der inverse Abstand der infizierten Personen zu jeder einzelnen Person berechnet und einen oder mehrere potenzielle Superspreader zu entdecken.

Die statistische Auswertung wurde mit SAS 9.4 durchgeführt. Die möglichen Zusammenhänge zwischen den erhobenen Informationen und dem Status der Infektion wurde mittels logistischer Regression berechnet. Die Ergebnisse wurden für die möglichen Störfaktoren Alter, Geschlecht und Dauer der Teilnahme bereinigt. Zur Darstellung des Zusammengangs wurden Odds Ratios mit einem 95 % Konfidenzintervall angegeben. Da es sich um ein spezifisches Event mit einer eingeschränkten Anzahl an Individuen handelt wurde davon abgesehen p-Werte anzugeben.

1.3. Ergebnisse

Unsere Studie erreichte mit 411 von 450 Teilnehmer eine Teilnahmequote von 91,3 % (**Abb. 1**). Von den 404 Blutproben wiesen 186 ein positives Ergebnis für IgG und 161 ein positives Ergebnis für IgA auf. Die bereits durch Streeck et al. gezeigte geringere Spezifität von IgA wurde dies nicht weiter berücksichtigt (Streeck et al., 2020).

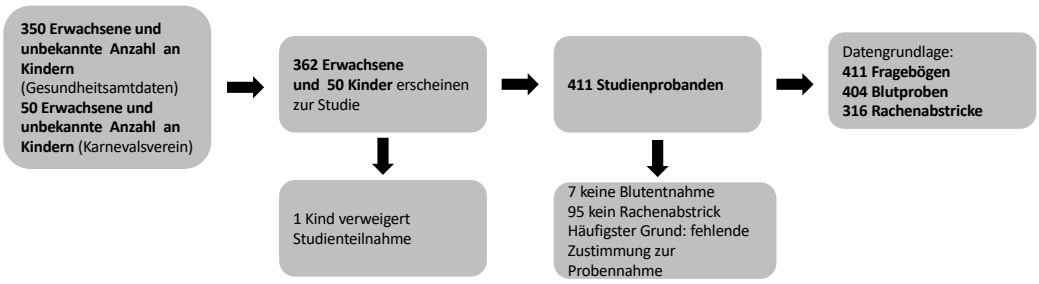

Abb. 1: Studienprobanden. Von 400 kontaktierten Personen erschienen 362 Erwachsene und 49 Kinder zur Studie. Der gesamte Datensatz, der zur Auswertung genutzt wurde, ist rechts zu sehen. (Modifiziert nach Wessendorf et al, 2021)

Die Quote an seropositiven Probanden lag also bei 46,0 % ((95 % CI: [41,2 %; 51,0 %]). Die gemeldeten Fälle der Gemeinde gaben eine Infektionsquote von 3,1% an und wir schätzten die Infektionsrate in der gesamten Bevölkerung der Gemeinde auf 15,5 % (95 % CI: [12,3 %; 19,00 %]) (Streeck et al., 2020). Alter und Bildungsstand waren breit gefächert (**Tab. 1**). Die RT-PCR ergab 19 positive Fälle.

Tab. 1: Demographische Daten und Informationen über Probanden dargestellt nach positivem und negativem Ergebnis der serologischen Testung (Modifiziert nach Wessendorf et al, 2021)

	Nicht infiziert N %	Infiziert N %
Gesamt	218	186
weiblich	114 (52 %)	89 (48 %)
Alter		
<18 Jahre	31 (14 %)	15 (8 %)
18-24 Jahre	30 (14 %)	20 (11 %)
25-39 Jahre	81 (37 %)	43 (23 %)
40-64 Jahre	71 (33 %)	100 (54 %)
65+ Jahre	5 (2 %)	8 (4 %)
BMI (kg/m²) (Std Abw)	24.3 (5.12)	26.2 (5.16)
teilnehmende Haushaltsmitglieder (Std Abw)	2.1 (1.12)	2.4 (1.16)
Höchster Bildungsabschluss		
keiner	27 (13 %)	13 (7 %)
Hauptschule	27 (13 %)	23 (13 %)
Realschule	55 (26 %)	71 (39 %)
Abitur/Fachabitur	54 (25 %)	34 (18 %)
Universität, Fachhochschule	52 (24 %)	43 (23 %)
Dauer des Aufenthalts [h] (Std Abw)	4.7 (2.06)	5.8 (1.85)
Servicemitarbeiter	4 (2 %)	22 (12 %)
auf der Bühne	80 (37 %)	62 (34 %)
Teil des 11er Rat	6 (3 %)	18 (10 %)
auf der Bühne während "Finale"	26 (12 %)	48 (26 %)
Verhalten während der Pause		
drinnen	73 (36 %)	85 (48 %)
draußen	114 (55 %)	72 (39 %)
Alkoholkonsum [Getränke] (Std Abw)	11.3 (7.76)	12.2 (7.40)
ehemaliger Raucher	34 (16 %)	45 (24 %)
Raucher (>10 Zigaretten pro Tag)	54 (25 %)	23 (12 %)
mindestens Eine Vorerkrankung	29 (13 %)	28 (15 %)
durchschn. Abstand zu Anderen [m] (Std Abw)	9.2 (1.68)	9.1 (1.70)
Abstand zu Zuluft [m] (Std Abw)	6.1 (3.22)	6.0 (3.30)
Abstand zu Abluft [m] (Std Abw)	4.8 (2.94)	5.1 (2.87)

Es konnte kein Zusammenhang zwischen Geschlecht und Infektionsrisiko festgestellt werden. Auch bestehende Vorerkrankungen erhöhten das Risiko nicht, allerdings war die Anzahl an Probanden mit Vorerkrankungen sehr gering, wodurch die Aussagekraft hierfür geschmälert wird.

Eine Assoziation zwischen Alter und Risiko für eine Infektion, während eines Superspreading Events konnte gezeigt werden (**Abb. 2**). Der Effekt blieb bei Adjustierung für Dauer der Teilnahme, Geschlecht und gemeinsame Haushaltszugehörigkeit erhalten. Zusammenfassend lassen sich eine Erhöhung um 28 % pro Dekade an Lebensjahren erkennen.

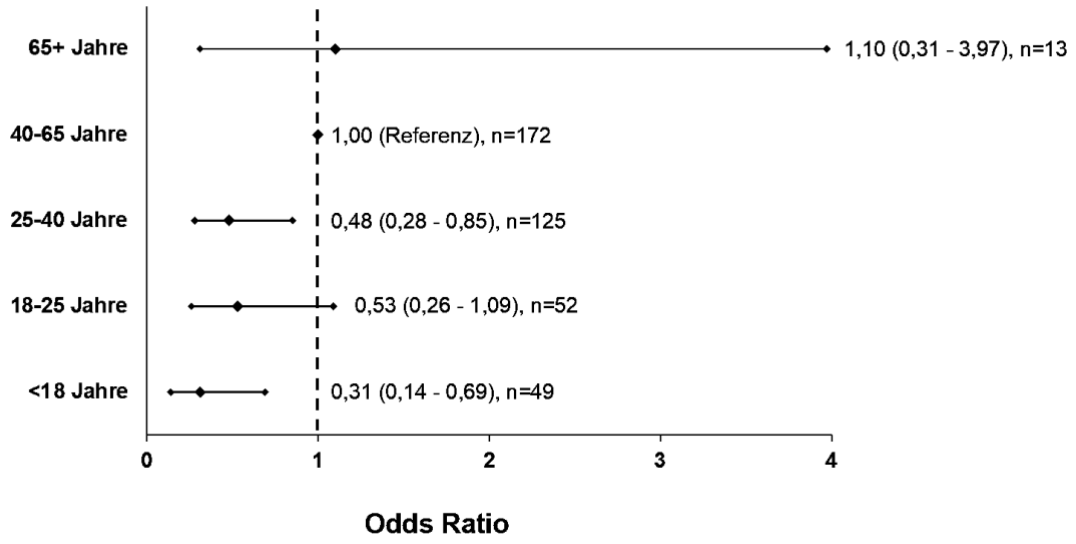

Abb. 2: Odds Ratio für Wahrscheinlichkeit einer Infektion in verschiedenen Altersgruppen. Einteilung der Probanden in verschiedene Altersgruppen in Kategorien: Kinder, junge Erwachsene, Erwachsene, ältere Erwachsene und Senioren. Als Referenz wurde das Infektionsrisiko für ältere Erwachsene genutzt. (Modifiziert nach Wessendorf et al, 2021)

Ein Schwerpunkt der Studie ist der Zusammenhang zwischen Infektionsgeschehen und der Lüftung. Die Lüftungsanlage nutzt F7-Filter (ISO ePM 2,5), hat einen Luftvolumenstrom von 7500 m3/h und nutzt 25 % Frischluft. Über Schächte (**Abb. 3**) wird Luft aus dem Raum gesaugt (lila) und zurück in den Raum geleitet (blau). Durch Drosselklappen wird der Luftstrom gleichmäßig auf die Schächte verteilt. Zusammenfassend zeigen sich Tische in der Nähe der rückführenden Schächte mit mehr Infektionen. Außerdem zeigen sich viele Infektionen im Bereich der Bar und der Bühne.

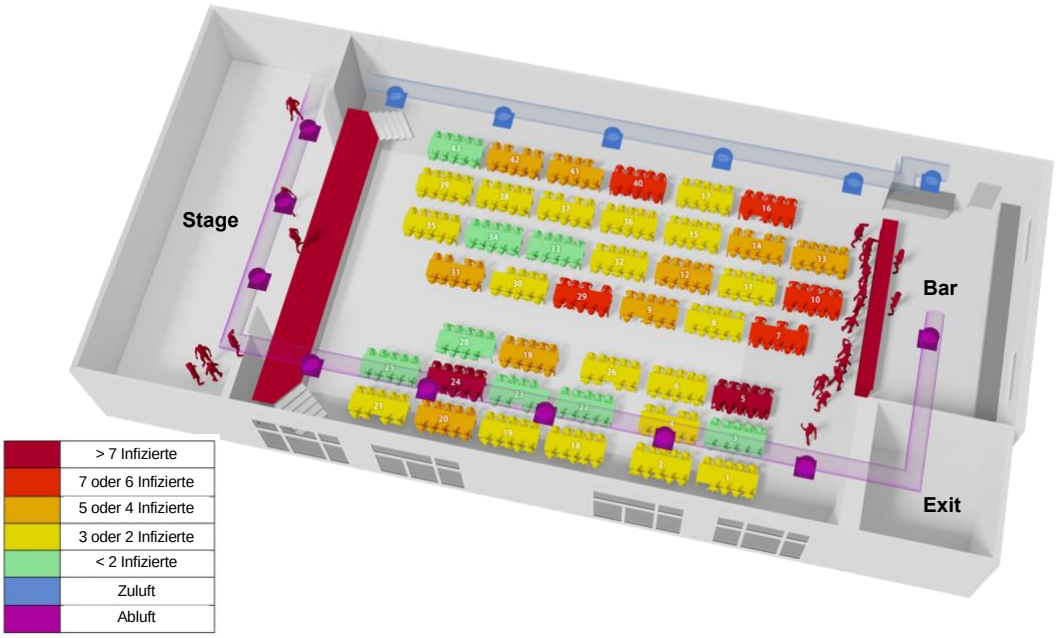

Abb. 3: **3D-Modell des Veranstaltungssaals** (27m x 13,2m, x 4,2m), die Zuluftschächte (blau) und Abluftschächte (lila) sind eingezeichnet. Die Färbung der Tische gibt die absolute Anzahl an Infizierten pro Tisch an. Im Bereich der Bühne sowie der Bar zeigten sich viele Infektionen. (Modifiziert nach Wessendorf et al, 2021)

Es lässt sich ein Zusammenhang zwischen Nähe zu Abluftschächten und Infektionsrisiko darstellen (**Abb. 4**).

Bei Rauchern (mindestens 10 Zigaretten pro Tag) zeigte sich ein geringeres Risiko für eine Infektion (OR: 0,32 [0,12; 0,81]).

Wir untersuchten ob Verhaltensweisen während des Events die das Infektionsrisiko beeinflussen. Probanden, welche die Pause des Events im Freien verbrachten, hatten ein geringeres Risiko (OR: 0,55 [0,33; 0,91]). Die Teilnahme an Bühnenaufführungen beeinflusste das Risiko nur gering (OR pro Aufführung: 1,08 [0,91; 1,27]), während jedoch die Teilnahme an der letzten Aufführung mit lautem Gesang und vielen Menschen auf der Bühne das Risiko erhöhte (OR: 1,41 [0,65; 3,02]). Für jede Stunde, welche die Probanden auf der Veranstaltung verbrachten, erhöhte das Risiko für eine Infektion um 32 % (OR pro Stunde: 1,32 [1,16; 1,9]).

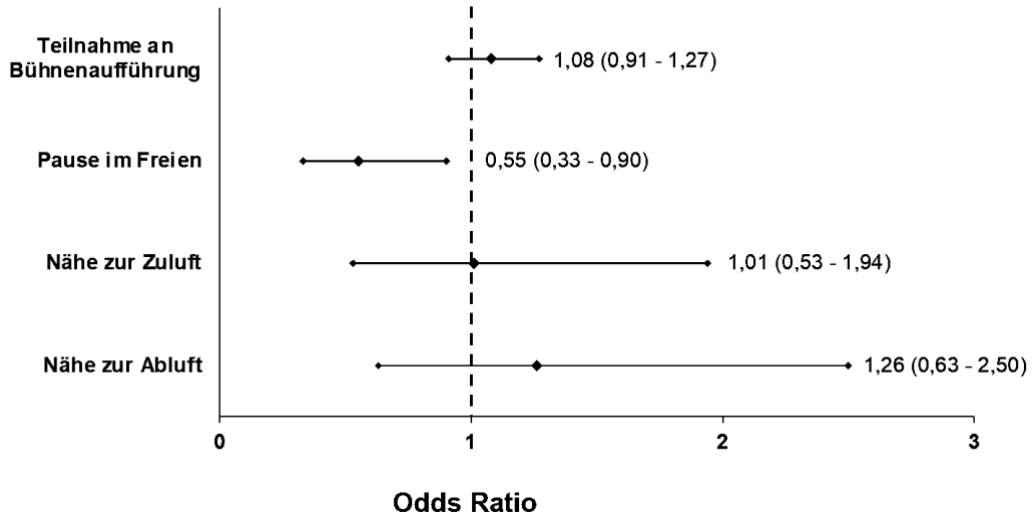

Abb. 4: Odds Ratio für eine Infektion mit SARS-CoV-2 nach Aktivität und Nähe zu Lüftungsschächten Risiko einer Infektion für Probanden, die an einer Aufführung auf der Bühne teilnahmen, die die Pause im Freien verbrachten und das Risiko für Probanden die näher an der Zuluft bzw. an der Abluft positioniert waren. (Modifiziert nach Wessendorf et al, 2021)

Die Odds Ratios für die Symptome, welche sich in den 14 Tagen nach der Veranstaltung bei den Probanden zeigten, wurden berechnet (**Abb. 5**). Der Verlust von Geruchs- (OR: 8,78 [4,81; 16,02]) und Geschmacksverlust (OR: 10,09 [5,13; 19,88]) zeigte die stärkste Assoziation zur Seropositivität.

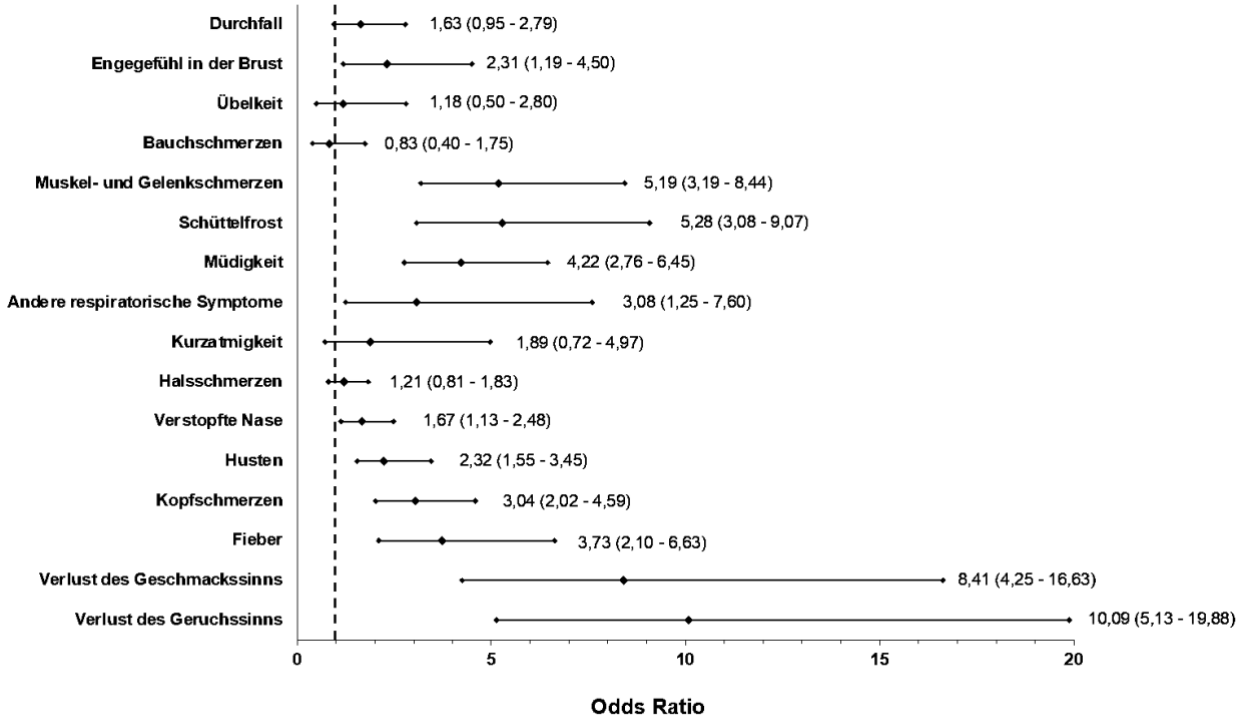

Abb. 5: Odds Ratio für Symptome in den 14 Tagen nach dem Super-Spreading-Event. Ein hoher Odds Ratio zeigt an, dass ein Symptom mit einer Infektion mit SARS-CoV-2 assoziiert ist. (Modifiziert nach Wessendorf et al, 2021)

Der Versuch einen oder mehrere Probanden als Superspreader zu identifizieren, durch Plot-Analyse der p-Werte für die Summe der inversen Abstände zu infizierten Probanden (**Abb. 6**), sowie durch die Anzahl an infizierten Probanden in bestimmten Abstandskategorien (**Tab. 2**), ergab kein Ergebnis.

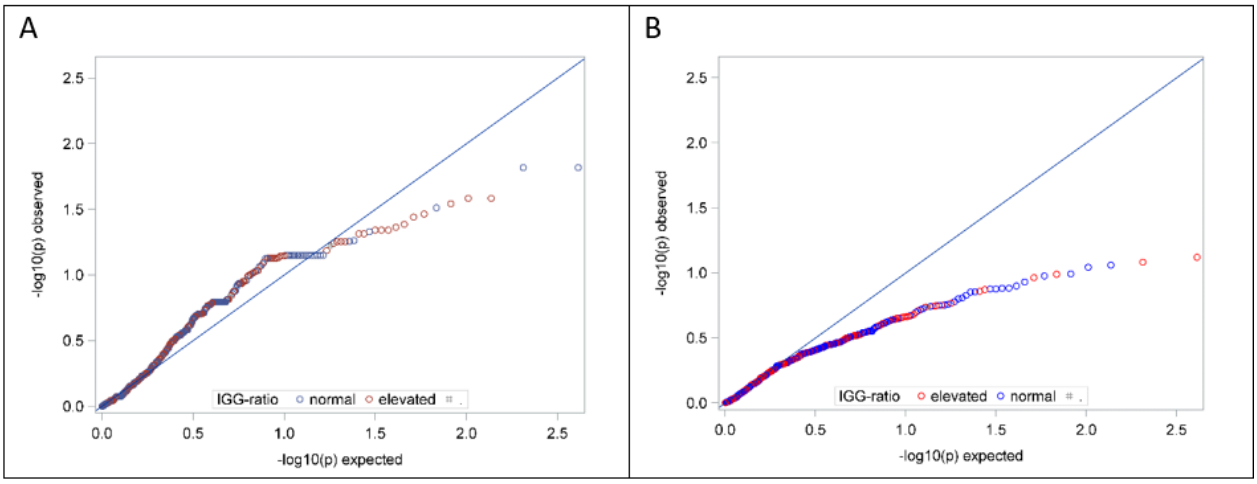

Abb. 6: Quantile Plot Analyse der p- Werte für invertierten Abstände zu jedem Probanden als Risikofaktor für eine Infektion. Probanden die als Superspreader in Betracht kommen, würden oberhalb der Diagonalen erscheinen. In A wurden die Rohdaten aufgetragen, In B wird für Alter, Geschlecht, gemeinsame Haushaltszugehörigkeit und Dauer der Teilnahme adjustiert. Die Analyse ergab keinen Anhalt dafür einen oder mehrere Probanden als Superspreader zu identifizieren. (Wessendorf et al, 2021)

Tab. 2: Odds Ratio für das Risiko einer Infektion nach Summe des inversen Abstands zu Infizierter Personen sowie nach Anzahl von Infizierten in Abstandskategorien.

Adjustiert nach a) Geschlecht, Alter, gemeinsame Haushaltszugehörigkeit, und Dauer der Teilnahme, b) für Distanz zu Lüftungschächten, Teilnahme an Aufführungen, Verhalten während der Pause sowie Teilnahme an „Finale“, c) alle Faktoren aus a) und b). Die Adjustierung wurde durchgeführt, um stark beeinflussende Variablen zu erkennen. Die Analyse ergab kein Ergebnis, welches einen oder mehrere Superspreader identifiziert. (Modifiziert nach Wessendorf et al, 2021)

		OR	95 % Konfidenz interval		p-Werte
Abstand zu Infizierter Person [Summe 1/m]		**0,99**	0,98	1,01	0,43
	adjustiert a)	**1**	0,98	1,02	0,957
	mehrfach adjustiert b)	**0,99**	0,97	1,01	0,571
	mehrfach adjustiert c)	**0,99**	0,97	1,02	0,646
Alternative Darstellung in Abstandskategorien					
Infizierte Person ≤1.5m		**1,01**	0,96	1,07	0,681
Infizierte Person 1.5 - ≤3m		**0,96**	0,92	1	0,043
Infizierte Person 3 - ≤4.5m		**1,03**	1	1,06	0,023
Infizierte Person ≤1.5m	adjustiert a)	**1,03**	0,97	1,1	0,366
Infizierte Person 1.5 - ≤3m		**0,96**	0,92	1,01	0,113
Infizierte Person 3 - ≤4.5m		**1,03**	1	1,06	0,083
Infizierte Person ≤1.5m	adjustiert b)	**1,01**	0,95	1,07	0,734
Infizierte Person 1.5 - ≤3m		**0,98**	0,94	1,02	0,359
Infizierte Person 3 - ≤4.5m		**1,05**	1,02	1,08	0,001
Infizierte Person ≤1.5m	adjustiert c)	**1,02**	0,95	1,09	0,638
Infizierte Person 1.5 - ≤3m		**0,98**	0,93	1,03	0,363
Infizierte Person 3 - ≤4.5m		**1,04**	1	1,07	0,041

Die serologische Antikörper Untersuchung ergab eine Korrelation von Werten von IgA-Ratio und IgG-Ratio (**Abb. 7**). Die Fähigkeit zur Neutralisation von SARS-CoV-2 und der IgG-Ratio zeigten auch Korrelation (**Abb. 8**).

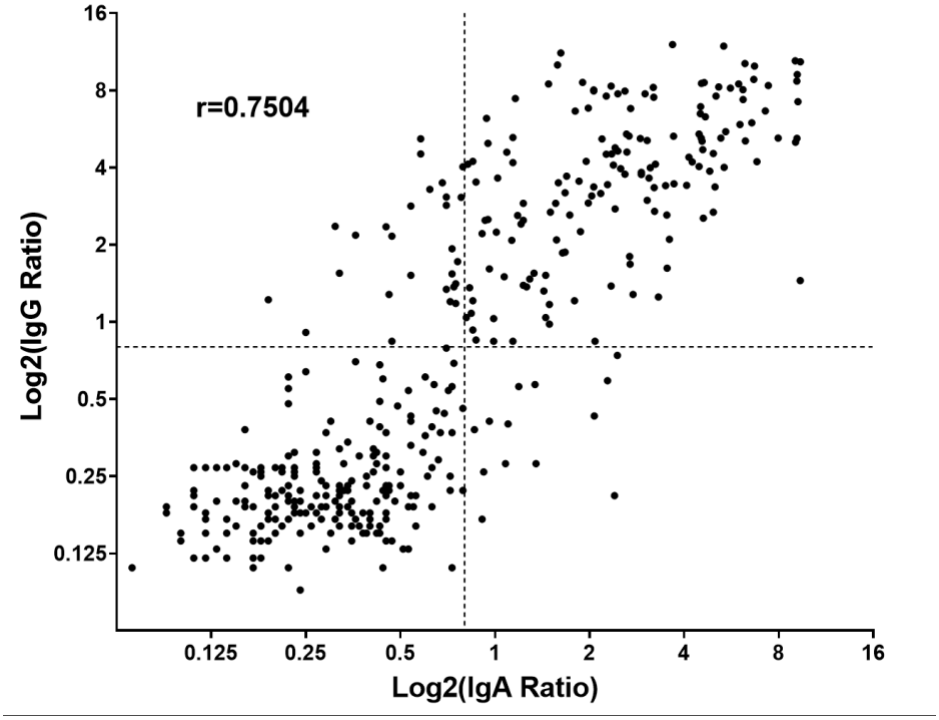

Abb. 7: Korrelation von SARS-CoV-2 Euroimmun ELISA Ergebnissen für IgA-Ratio und IgG-Ratio für jeden Probanden. Die Korrelation von IgA und IgG für einen Probanden zeigten sich signifikant (r: Pearson Koeffizient, p<0.0001, 95 % CI, 0.7043 bis 0.7902). Die gestrichelten Linien zeigen den Wert an, ab dem ein Ergebniss als positiv betrachtet wurde. (Wessendorf et al, 2021)

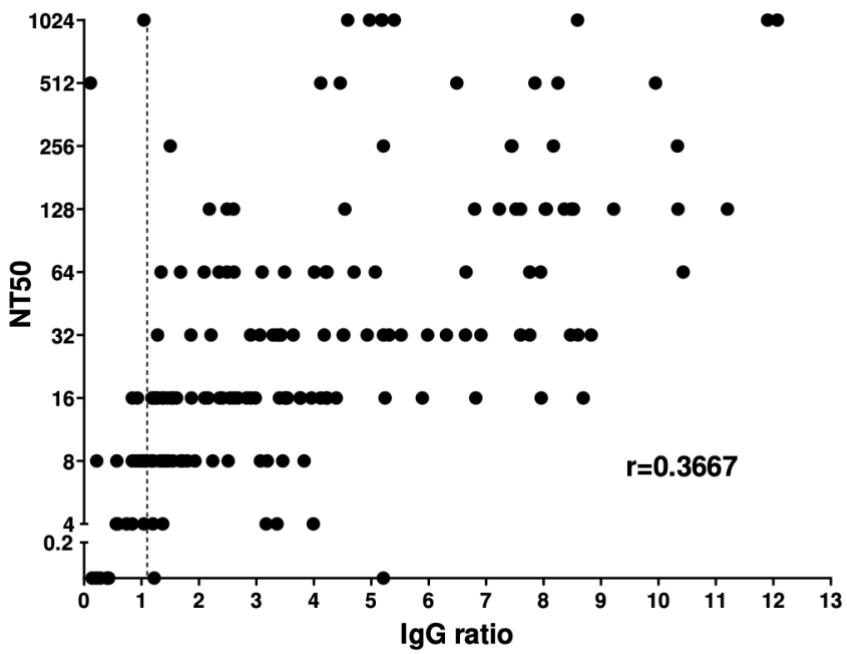

Abb. 8: Korrelation von der Fähigkeit der Plasmaneutralization und dem SARS-CoV-2 Euroimmun ELISA Ergebniss für IgG für jeden Probanden. Die Korrelation zeigt sich signifikant r: Pearson Koeffizient, p<0.0001, 95 % CI, 0.2275 bis 0.4192). Proben mit negativem Ergebnis in der Neutralisation wurden als 0.1 gesetzt, um auf der logarithmischen Achse zu erscheinen. (Wessendorf et al, 2021)

1.4. Diskussion

Die hohe Rate an seropositiven Probanden legt nahe, dass es sich bei der Kappensitzung um ein Superspreading Event handelt. Es ist davon auszugehen, dass ein großer Teil der seropositiven Probanden sich während des Events ansteckte, da die Teilnehmer 11 Tage nach dem Event in Quarantäne geschickt wurden und wenig später ein Lockdown in Heinsberg verhängt wurde.

Da SARS-CoV-2 in Aerosolen überleben kann und weiterhin infektiös sein kann (Lednicky et al., 2020; Santarpia et al., 2020; Günther et al., 2020) und vorherige Studien eine gewisse Assoziation zwischen Belüftung und Infektionsgeschehen erkennen ließen (Pokora et al., 2021; Nazarenko 2020) erscheint unser Resultat zum Einfluss der Lüftung auf die Verteilung an Infiziertem im Raum plausibel. Die Filter der Lüftung haben aufgrund ihrer Norm keinen Einfluss auf die Verbreitung des Virus und

der Frischluft Anteil ist zu gering, um die Aerosolbelastung maßgeblich zu lindern. Die baulichen Gegebenheiten und die Resultate der Studie untermauern die Relevanz von angemessenen Belüftungssystemen für Veranstaltungen in Innenräumen. Das stark verringerte Risiko für Probanden, welche die Pause im Freien verbrachten, unterstreicht die Relevanz der suffizienten Lüftung zur Aerosolminderung.

Es zeigt sich keine Assoziation zwischen dem Konsum von Alkohol während eines Superspreading Events um dem Risiko sich zu infizieren. Dieses Resultat unterstützt ein Alkoholverbot als Maßnahme zur Verringerung der Infektionen nicht. Bei Rauchern sind schwerere Verläufe von Covid-19 zu erwarten und Raucher benötigen öfter intensivmedizinische Behandlungen (Vardavas und Nikitara, 2020; Engin et at., 2020). Israel et al (2020) beschreiben eine geringeres Infektionsrisiko für Raucher. Eine Metaanalyse (Grundy et al., 2020) beschreibt jedoch eine geringere Wahrscheinlichkeit positiv auf das Virus getestet zu werden und unsere Studienergebnisse unterstützen diese Aussage. Der mögliche biochemische Zusammenhang, beispielsweise über den nikotinische Acetycholinrezeptor (Changeux et al., 2020), kann jedoch in Zukunft gegebenenfalls als therapeutische oder prophylaktische Option zur Bekämpfung des Virus genutzt werden (Paleiron et al. 2021).

Unsere Resultate legen nahe, dass Kinder und junge Erwachsene ein niedrigeres Risiko haben sich während eines Superspreading Events zu infizieren. Bereits untersucht wurde das Gesamtrisiko für Kinder eine Infektion zu erleiden (Li et at., 2020). Nun konnte dieser Trend erstmals für ein Superspreading Event gezeigt werden. Dieses Resultat legt nah, das die limitierte Rolle von Kindern, Jugendlichen und jungen Erwachsenen während Veranstaltungen im Bezug auf Maßnahmen zur Eindämmung der Pandemie berücksichtigt werden sollten.

1.5. Zusammenfassung

Der Beginn der COVID-19-Pandemie war geprägt von Superspreading-Ereignissen einschließlich großflächiger Ausbrüche. In Deutschland war der erste SARS-CoV-2-Ausbruch ein Superspreading-Ereignis in einem ländlichen Gebiet in Heinsberg im Februar 2020.

51 Tage nach dem Ereignis wurden alle bekannten Teilnehmer gebeten, Blutproben und Rachenabstriche abzugeben und einen selbst ausgefüllten Fragebogen zu beantworten. Metrische Raumkoordinaten für alle Tische, Sitzplätze und Lüftungspunkte wurden erfasst.

Wir analysierten die Infektionsraten aller 411 Teilnehmer und das Infektionsrisiko in Abhängigkeit von verschiedenen Faktoren wie Alter, Alkoholkonsum und Belüftungssystem. Insgesamt hatten sich 46 % (n=186/404) der Teilnehmer infiziert. Wir zeigen, dass die räumliche Verteilung der infizierten Teilnehmer mit der Nähe zum Belüftungssystem zusammenhing**.** Interessanterweise war das Infektionsrisiko stark mit dem Alter assoziiert, wobei Kinder und junge Erwachsene ein geringeres Infektionsrisiko hatten als ältere Teilnehmer, was zu einem durchschnittlichen Anstieg des Infektionsrisikos um 28 % pro 10 Jahre Altersunterschied führte. Auch Verhaltensunterschiede wirkten sich auf das Infektionsrisiko aus, darunter der Aufenthalt im Freien oder das Rauchen**.**

Unsere Ergebnisse unterstreichen die Bedeutung einer angemessenen Innenraumlüftung für künftige Veranstaltungen. Die geringere Anfälligkeit von Kindern und jungen Erwachsenen deutet darauf hin, dass sie nur in begrenztem Umfang an Superspreading-Ereignissen beteiligt sind.

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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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ABSTRACT

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

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

Setting The SARS-CoV-2 superspreading event took place during festivities in the small community of Gangelt in February 2020. This 5-hour event included 450 people (6–79 years of age) in a building of 27 m × 13.20 m × 4.20 m.

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

Primary and secondary outcome measures Primary outcome: infection status (determined by IgG ELISA). Secondary outcome: symptoms (determined by questionnaire).

Results Overall, 46% (n=186/404) of participants had been infected, and their spatial distribution was associated with proximity to the ventilation system (OR 1.39, 95% CI 0.86 to 2.25). Risk of infection was highly associated with age: children (OR 0.33, 95% CI 0.267 to 0.414) and young adults (age 18–25 years) had a lower risk of infection than older participants (average risk increase of 28% per 10 years). Behavioural differences were also risk associated including time spent outside (OR 0.55, (95% CI 0.33 to 0.91) or smoking (OR 0.32, 95% CI 0.124 to 0.81).

Conclusions Our findings underline the importance of proper indoor ventilation for future events. Lower susceptibility of children/young adults indicates their limited involvement in superspreading.

Strengths and limitations of this study

⇒ The setting and the participant group are extremely well defined.
⇒ Participants were invited on the basis of one criterion, namely their presence at the superspreading event; there was no other preselection/bias in the study enrolment, and the participation rate was high (91% of those invited).
⇒ The study was conducted 51 days after the event, so it is possible that participants could have become infected unrelated to the event.
⇒ The number of index cases is unknown.

INTRODUCTION

SARS-CoV-2 is a highly transmissible and pathogenic RNA virus that emerged in late 2019 and has caused a pandemic threatening human health and public safety worldwide.[1] While factors shaping the dynamics of a pandemic are multifactorial, virulence and reproductive number are important properties of a virus.[2] For SARS-CoV-2, there is a substantial overdispersion of the secondary infection distribution (individual R0) for an individual infected with SARS-CoV-2.[2] An overdispersed R0 means that most infected people do not transmit (individual R0=0), while a minority of infected people are superspreaders (individual R0 >5). Superspreading has been observed for many infectious pathogens, such as measles or SARS.[3] During the SARS pandemic in 2003, a superspreading event was defined as one infected person infecting eight others.[4] For SARS-CoV-2, it has been estimated that 80% of the infections are caused by 10% of infected individuals highlighting the importance of the cluster factor (k).[2] In Germany, an indoor carnival event in the beginning of 2020 is considered as the first major outbreak in a German city and was considered a hotspot during the beginning of the pandemic in Germany.[5] Other SARS-CoV-2 superspreading events worldwide have been linked to indoor gatherings with close proximity of individuals.[6] Nevertheless, most

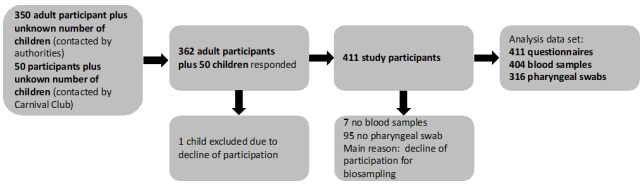

Figure 1 Study participants. Enrolment and flow of participants through the study. Downstream sample processing included centrifugation of blood samples for plasma collection (SARS-CoV-2 ELISAs) and viral RNA extraction from swab samples (SARS-CoV-2 RT-PCR).

of the reported superspreading events had less than 100 cases, and the reports are limited by missing data or a reporting bias.[6]

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

MATERIALS AND METHODS

Study design and sampling

This cross-sectional epidemiological study was conducted 51 days after a carnival celebration in the beginning of 2020. Eleven days after, the event authorities sent all known participants into quarantine after testing 38 out of 99 individuals PCR positive. All adults known to have attended the event were invited to participate in the study. About 450 persons attended the event of which 411 participated in the study (figure 1, participation rate 91.3%). All study participants provided written informed consent before enrolment.

Self-administered questionnaires included questions about demographic background, symptoms of viral infection as well as detailed information about the behaviour during the event. Participants' arrival and exit times were assessed in 1-hour categories. Study participants were asked to provide blood specimens and pharyngeal swabs for further analysis. The local health department supplied data on hospitalisations and fatalities in our cohort (manuscript submitted elsewhere).

Patient and public involvement

This study was designed in close collaboration with both the local health department of Heinsberg and the 'Council of 11' of Gangelt, the organisers of the event described herein. The organisers as well as the city's head councilman were also involved in recruitment by appealing to the local population to participate in the study. Since the community of Gangelt was the centre of the first German outbreak of SARS-CoV-2, there was a great interest from the local public to participate in this study to help understand this new virus and to gain access to early testing. Accordingly, the Ministry of Labor, Health, and Social Affairs of the state government funded this study. In turn, as a service to the public, we informed each participant of their PCR and ELISA result via letter and offered a phone hotline for questions about the results.

Spatial information and description of the event

The event took place on 15 February 2020 and consisted of speeches, dance and music performances for a total of 5 hours, with one large intermission. It was a ticketed event, where ticket sale was open to the public. Most of the participants were inhabitants of Gangelt. It was hosted at a small community centre (320 square metres) in a single open space with a bar in the front close to the entrance and a stage at the back. The tables, each with two benches, were arranged in two blocks with a centre aisle towards the stage. Alcoholic and non-alcoholic drinks were served in glasses, and a food truck was located outside in front of the venue. While most participants (about 450 people, 1.4 individuals per square metre) were sitting in the hall, a committee of eleven individuals hosting the event were sitting on stage. The eleven people on stage switched after a break.

Metric room coordinates (length and width (m)) for areas, tables, benches and ventilation shafts were assessed via measurements, seating plan and photos from the event. Persons providing multiple positions were considered as spending an equal amount of time on different positions. When exact seating was unclear and information was available on table or greater area localisation (bar abd stage), average coordinate values were used. On the grounds of these coordinates, we calculated pairwise metric distances between all persons and distances to closest inlet and outlet airshafts. For all persons, their pairwise inverse distances were summarised as mean inverse distance. Inverse metric distances to persons or airshafts were regarded as representing infectious potential through local proximity, and inverse distances were capped at 2.5 (the inverse of the width of a seat of 0.4 m). Alternatively, we counted all infected persons within adjacent rings of 1.5 m width around each participant as a measure of crowdedness and infectious potential.

Pharyngeal swab and blood preparation

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

Anti-SARS-CoV-2 ELISA

Anti-SARS-CoV-2 IgA and IgGs were determined using ELISA on the EUROIMMUN Analyzer I platform (EI 2606–9601 A, and EI2606-9601 G, respectively).[5] A result was considered positive when a ratio (extinction of sample/extinction of calibrator) of 0.8 or higher was reached. The guidelines of the German Medical

Association (RiliBÄK) were abided by, including internal and external quality controls.

Reverse transcription PCR (RT-PCR)

Viral RNA was extracted from each 300 µL swab sample via the chemagic Viral 300 assay (according to manufacturer's instructions) on the Perkin Elmer chemagic Prime instrument platform. The presence of two viral target genes (E and RdRP) was assessed in each sample by real time RT-PCR (SuperScriptIII One-Step RT-PCR System with Platinum TaqDNA Polymerase, Thermo Fisher). The following primers were used: for E gene: E_Sarbeco_F1 and R, and probe E_Sarbeco_P1; for RdRP gene: RdRP_SARSr_F and R and probe RdRP_SARSr-P2.[7] In addition, an internal control for RNA extraction, reverse transcription and amplification was applied to each sample (innuDETECT Internal Control RNA Assay, Analytik Jena #845-ID-0007100). If amplification occurred in both virus-specific reactions, samples were considered positive.

SARS-CoV-2 neutralisation assay

A plaque reduction neutralisation test was used to determine SARS-CoV-2 neutralisation capacity as previously described.[5] Briefly, plasma samples were heat inactivated, and supernatant was transferred to a new tube and serially twofold diluted in OptiPRO SFM (Gibco). One hundred twenty microlitres of each plasma dilution was mixed with 80 plaque-forming units of SARS-CoV-2 in 120 µL OptiPRO SFM cell culture medium and used to infect Vero E6 cells (1.25×10^5 cells/well seeded into 24-well plates 24 hours before). Subsequently, the inoculum was removed, and cells were overlayed with a mixture of carboxymethylcellulose (Sigma) and 2× MEM (Biochrom). Following 3-day incubation, the overlay was removed, and the 24-well plates were fixed using a 6% (v/v) formaldehyde solution and stained with 1% (w/v) crystal violet in 20% ethanol revealing the formation of plaques. Finally, the neutralising titres were calculated as the reciprocal of serum dilutions resulting in neutralisation of 50% input virus (NT50), read out as reduction in the number of plaques.

Data management and quality control

The Clinical Study Core Unit of the Study Center Bonn (SZB) supported the study by outlining the study protocol and developing the informed consent form as well as participants information sheets with respect to data management and quality control. The data were gathered on paper-based case report forms. Data were entered as double data entry into the REDCAp study database programmed and hosted by SZB. Study personnel was trained by experienced members of the SZB. A quality manager was on site to support the study team. Monitoring of trial data and informed consent forms was performed according to the monitoring plan by qualified SZB staff.

Statistical analysis

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

RESULTS

Four hundred and eleven out of an estimated 450 participants of the event responded to our study invitation, resulting in a response rate of 91.3%. 404 individuals provided plasma samples and 316 pharyngeal swabs (figure 1). Genders were represented equally among all 404 participants (n=201/404, 50% were male) with a broad range in age (6–79 years, median age 36 years) and level of education (table 1). Two hundred and ninety-seven individuals were residents of the community the event took place in, 103 lived in other parts of the county and 11 were external visitors. In total, five participants of the event were hospitalised, and one participant subsequently died.

Overall, 186 out of 404 individuals tested seropositive for IgG and 161 for IgA antibodies (online supplemental figure 1). To confirm seropositivity, we performed a plaque reduction neutralisation assay (online supplemental figure 2) demonstrating neutralising activity against SARS-CoV-2 of their respective antibody responses. Given the low specificity of the IgA assay, IgA seropositivity was not further considered.[5] Nineteen participants tested positive in RT-PCR; these were considered infected during the superspreading event only if they were also IgG positive (this was the case with 16 out of the 19 participants).

Overall, we found that (n=186/404) 46.0% (95% CI 41.2% to 51.0%) tested seropositive who attended the event, which was significantly higher than the overall estimated infection rate in the same community at large at that time. Indeed, officially 3.1% of the community were reported as positive cases at that time, but we estimated the infection rate to be 15.5% (95% CI 12.3% to 19.0%)[5] for the community. Taken together, an estimated 46% of participants became infected during a single superspreading event.

No association between the gender of participants and risk of infection was found ((OR 1.01, 95% CI 0.65 to 1.58) for women). On average infected individuals had a higher body mass index (26·2 kg/m² compared with 24.3 kg/m² for uninfected individuals). Infected participants were more likely to be clustered living in the same household (table 1). Having at least one comorbidity, including lung disease (n=11/26, 42.3%), cardiovascular disease (n=8/15, 53.3%), neurological disease (n=1/6, 16.7%),

Table 1 Distribution of demographic factors and exposure information of interest among study participants who tested positive or negative in serology test of SARS-CoV-2 infection

	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) (SD)	24.3 (5.12)	26.2 (5.16)
Participating household member (SD)	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 (hour) (SD)	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)
Behaviour during break:		
Remaining seated	73 (36)	85 (48)
Going outside	114 (55)	72 (39)
Alcohol consumption (drink) (SD)	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)
Average distance to other participants (m) (SD)	9.2 (1.68)	9.1 (1.70)
Distance to air inlet (m) (SD)	6.1 (3.22)	6.0 (3.30)
Distance to air outlet (m) (SD)	4.8 (2.94)	5.1 (2.87)

*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.
BMI, body mass index.

cancer (n=7/12) (58.3%) or diabetes (n=4/5, 80%), did not increase the risk of infection (OR 0.64, 95% CI 0.33 to 1.26). We next assessed whether age influenced the risk of infection at the event, considering gender, duration of attendance and common household as covariates. Comparison across age categories showed a lower risk for children (OR 0.31, 95% CI 0.14 to 0.69), and also for young adults (18–25 years, OR 0.53, 95% CI 0.26 to 1.09) as well as adults between 25 and 40 years (OR 0.48, 95% CI 0.28 to 0.85) in comparison to older adults (40–65 years)

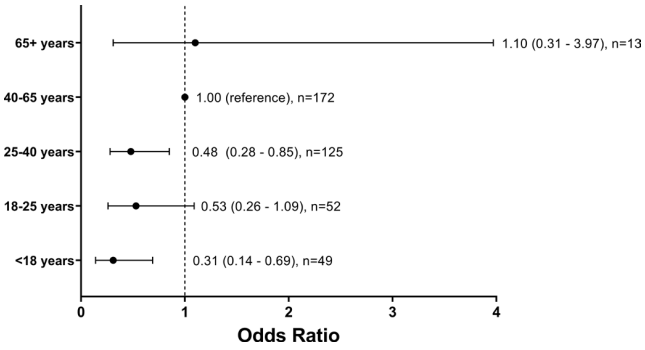

Figure 2 ORs for the likelihood of SARS-CoV-2 infection by age groups. Participants were divided into age groups of 8, 15 or 25 years, participants younger than 18 years or older than 65 years. Participants were considered to have been infected during the event if they were SARS-CoV-2 antibody positive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

(OR 1, reference), while seniors had a slightly higher risk (older than 65 years, OR 1.1, 95% CI 0.31 to 3.97) (figure 2). Our data suggest that an additional 10 years of age were on average associated with 28% increased risk of infection (OR 1.28, 95% CI 1.10 to 1.48).

To understand the spreading dynamics of SARS-CoV-2 during the event, we first performed a detailed analysis of potential risk factors and social behaviour. We first analysed whether the ventilation system influenced the distribution of SARS-CoV-2 infected individuals. It is important to state that the system's air flow consisted of 75% used and 25% fresh air. The air flow can be described as clockwise. The air system uses vents along one side of the venue and on stage to take in air (figure 3, air inlets purple). After 25% of fresh air has been added and the air has been filtered, vents along the other side of the venue return the air into the room (figure 3, air outlets blue). All ventilation points received the same amount of air due to throttle valves. For noise protection reasons windows remained closed. The air system used F7-Filters (ISO ePM ≥2.5) and had an air volume flow of 7500 m^3/hour.

Most tables located close to the air inlets and showed no or only few infections (figure 3, green) also most surrounding tables showed low numbers of infection (figure 3, yellow). Tables close to the air outlets show high (four or five infected per table) and very high (six or seven infected per table) numbers of infected individuals. Infected participants had been seated mostly at tables close to the bar, at the bar or 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 high numbers of infection (18 infected out of 24, table 1). Of note is that the overall number of participants per table was not equal for all tables. Greater proximity to air outlets was associated with increased risk of infection with a crude OR=1.39, 95% CI 0.86 to 2.25. This association remained stable and was hardly attenuated from adjustment for proximity to air inlet, age, gender, duration of attendance, proximity to other infected persons, stage activity and going outside

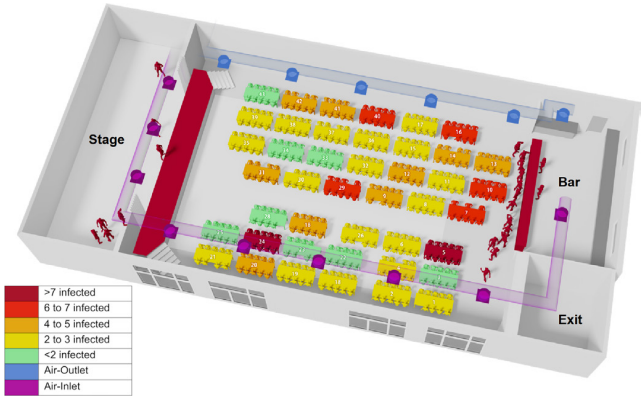

Figure 3 Reconstructed three-dimensional (3D) model of the venue Hall. The venue was a single open space with a stage on one end and a bar as well as the exit on the opposite end. distribution of tables and seating was as indicated by table and chairs symbols. Please note that the people pictured are 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 × 13.20m × 4.20 m. Tables, where more than seven infected individuals have stayed, are coloured in dark red; this includes the stage and bar as well. Air inlets are coloured in violet, and the air outlets are coloured 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).

during the intermission (figure 4, multiple adjusted OR=1.26, 95% CI 0.63 to 2.50). A similar apparent effect for proximity to air inlets (crude OR=1.17, 95% CI 0.72 to 1.89)) disappeared when duration of attendance was added to the model (figure 4, multiple adjusted OR=1.01, 95% CI 0.53 to 1.94). Overall, however, we found the increased risk for individuals located closer to the air outlet remarkably persistent (figure 4).

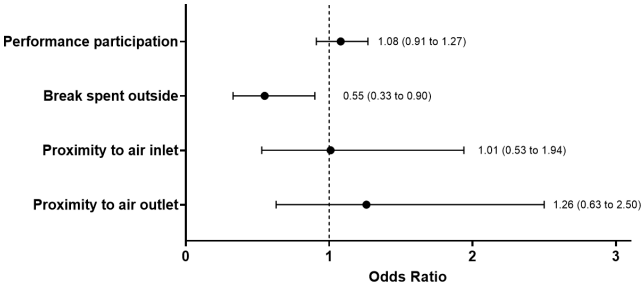

Figure 4 ORs for the association of SARS-CoV-2 infection with specific activities of the participants and their location in the venue relative to ventilation shafts. The model was additionally adjusted for age, sex, duration of attendance, participation in multiple activities and cumulative proximity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

We further studied the sum of the inverse distance to all infected participants as a measure of proximity to either one common virus source or mutual infection. However, there was no evidence for increased risk of infection from greater proximity to other infected persons (online supplemental table 1). Furthermore, we found no evidence for a single person being the source of the infection from the quantile plot of p values from 401 analyses conducted separately for each participant as potential source of infection (online supplemental figure 3).

To understand the association of risk with behaviour patterns, we next investigated the influence of several factors on SARS-CoV-2 infection including time spent outside, smoking, performing on stage and participation during the final act ('Finale') for 30 min. Results were all adjusted for age, sex, common household and duration of attendance. Participation in multiple performances was associated with slightly increased risk of infection (OR per performance: 1.08, 95% CI 0.91 to 1.27), results for participation in the last 'Finale' were stronger (OR 1.41, 95% CI 0.65 to 3.02), although neither was significant (figure 4). Duration of attendance was persistently and strongly associated with an increased infection risk of 32% with each additional hour spent at the party (OR per hour: 1.32, 95% CI 1.16 to 1.49). All other analyses were adjusted for this variable as potential confounding factor.

We next determined the level of alcohol consumption as number of drinks (high-proof liquor or beer) and did not observe any influence for the amount of alcohol consumption on the risk of becoming infected (OR per drink: 1.00, 95% CI 0.96 to 1.05). Furthermore, participants who spent the break outside were less likely to be infected (OR 0.55, 95% CI 0.33 to 0.91) compared with individuals who spent the break inside the venue hall (figure 4). Interestingly, however, when we determined the impact of being regular smoker (defined as smoking of at least 10 cigarettes a day) on the risk of SARS-CoV-2 infection, we observed a reduced risk of infection (OR 0.32, 95% CI 0.12 to 0.81) even after adjustment for 'time spent outside'. Taken together, our results demonstrated that the duration of attendance at the carnival party correlated with an increased risk of infection, but the number of alcoholic drinks was not associated with infection risk, while regular smoking and spending the break of the event outside showed a negative correlation with the risk of infection.

We next stratified seropositive individuals by their reported symptoms. ORs for each symptom were calculated for the timespan of 14 days following the event (figure 5). We identified that loss of smell (OR 8.78, 95% CI 4.81 to 16.02) and taste (OR, 95% CI 10.09, 95% CI 5.13 to 19.88) exhibited the strongest association with SARS-CoV-2 infection. Other symptoms which were strongly associated with COVID-19 were: sweats and chills (OR 5.28, 95% CI 3.08 to 9.07), muscle and joint ache (OR 5.19, 95% CI 3.19 to

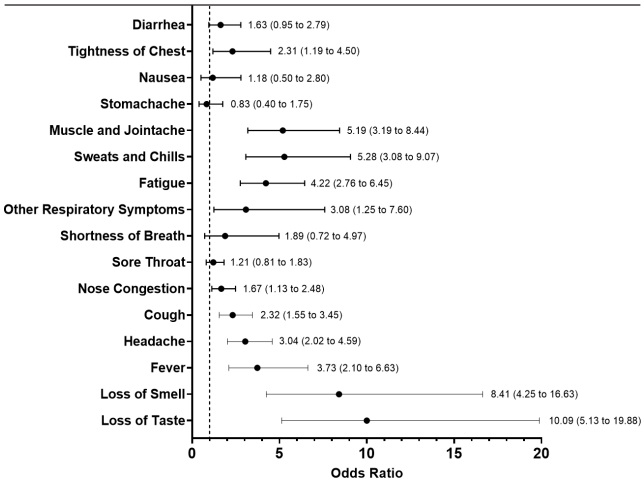

Figure 5 ORs for symptoms of SARS-CoV-2 antibody positive participants in the 14 days following the super spreading event. The information on symptoms was derived from the self-administered questionnaire, which was filled out on the day of sample collection. OR estimates are shown with CIs.

8.44), fatigue (OR 4.22, 95% CI 2.76 to 6.45) and fever (OR 3.73, 95% CI 2.10 to 6.63) (figure 5). Importantly, 15.1% (28/186) of the infected individuals reported no symptoms at all in a period of 14 days after the event. The rate of asymptomatic infections of participants of the event was lower than generally observed in the community where the event took place (36%).[5] Overall, there was a lower proportion of asymptomatic cases among individuals infected after the event compared with members of the community, while loss of smell and taste showed the strongest association with an infection.

DISCUSSION

The high overdispersion characteristics of SARS-CoV2 and its ability to be transmitted via aerosols under certain conditions are one of the main reasons that the beginning of the SARS-CoV2 pandemic was shaped by superspreading events.[8 9] Germany's first superspreading event was an indoor carnival event in the beginning of 2020 in a rural community. In this naturally occurring experiment, we found that nearly half of the participants became infected and determined multiple prerequisites for superspreading and risk factors for becoming infected. While our study population is not a representative sample of the general population, the event may be regarded as exemplary for similar party occasions and may help reduce the number of those infected in the future. At the time of the event described herein SARS-CoV-2 had not diversified yet, but ever since many variants of the virus have arisen and have taken turns dominating the global pandemic. Therefore, the results shown here need to be viewed as qualified in describing a superspreading event under the circumstances in the beginning of the pandemic. However, they help us to understand infection dynamics and requisites for infection with this virus family, ultimately giving a frame of reference for similar studies conducted throughout the alpha, delta and omicron waves of the COVID-19 pandemic.

An important factor associated with infection risk was the ventilation system and the individual proximity to the ventilation outlets. Individuals close to the air outlets that contained air with low amount of fresh air had the highest infection risk compared with those close to the air inlets. This was particularly interesting, because we did not see any increased risk of infection from greater proximity to other infected persons, which indicates that ventilation was perhaps more important than physical proximity. Our findings are in line with previous studies that demonstrated SARS-CoV-2 to be able to become airborne under certain conditions and that the ventilation system can have an influence on virus spread.[10–12] The air filters in the venue were not capable of intercepting virus particles supporting the notion on the importance of proper indoor ventilation systems.[13 14] Indeed, spending the break of the event outside decreased the possibility of infection underscoring the benefit of proper ventilation or fresh air to lower the amount of aerosols. Due to the nature of the event, the spatial distribution of the participants was not fixed throughout the evening and not perfectly recapitulated, so this information carries some error. However, allowing for multiple positions per person, we used all available information. Assuming further error in the spatial data to be random, this might lead to a dilution of effects; that is, true associations may remain undetected. Complementary analyses including, for example, the persons' functions during the event show consistent results, so we see no evidence suggesting bias in our findings. Nevertheless, the infection rate might be overestimated as the study was conducted 51 days after the event as participants could have become infected not related to the event. However, this weakness is limited by the official shut down of the community shortly after the event: a detailed timeline of the containment measures put in place after the superspreading event is included in Streeck *et al*.[5] Briefly, a strict home quarantine for all attendees of the carnival event was imposed after 38 out of 99 participants tested positive for SARS-CoV-2. In addition, 13 days after the event, the town went into full lockdown, including the closing of schools, childcare and outpatient care facilities and restrictions of public access to the town. These concerted containment measures proved so effective that the peak of new infections in the community was already reached 27 days after the event.

The consumption of alcoholic drinks did not increase the risk of infection. While it has been assumed that the alcoholic effect of decreased social inhibition may increase likelihood of infection, we did not find any evidence for this association questioning measures of a ban on alcohol to reduce numbers of infected. It is known that current and former smokers disproportionately suffer from severe COVID-19, and their numbers are relatively increased among those patients that need

intensive care treatment compared with non-smokers.[15 16] However, it has been previously speculated that the risk of infection is lower for smokers.[17] Furthermore, a meta-analysis of seven studies suggests that smokers have a reduced risk of testing positive for SARS-CoV-2.[18] Interestingly, we also observed that regular smoking lowered the risk of infection. The association might, for example, be explained by a role of the nicotinic acetylcholine receptor (nAChR).[19] Because other viruses, such as rabies virus, have been known to bind nAChRs, it was hypothesised recently that SARS-CoV-2 spike protein might bind nAChRs as a coreceptor for infection.[20 21] Indeed, in silico molecular docking simulations predicted binding of spike to nAChRs.[22] If this interaction proves to be of advantage to the virus, then nicotine or its derivatives that bind nAChRs could compete with SARS-CoV-2 for binding and thereby reduce interactions of the virus with its target cells. Currently, at least one prospective observational study is being 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 knowledge may lead to the investigation of a therapeutic or prophylactic treatment on the basis of this molecular target.[24]

Our results indicate a trend that younger people are less likely to be infected compared with older age groups. This trend is strongest for people under 18 years but levels out over 40 years of age. The risk of infection for children in superspreading events has not been investigated, but the overall risk for infection in children seems to be lower than for adults as a systematic review and its recent update reported, which is further supported by our findings.[25 26] Considering the risk of infection with SARS-CoV-2 in general, however, in a meta-analysis, Madewell *et al*[27] conclude that the secondary attack rate in households is lower to children contacts than to adult contacts. Many primary articles and meta-analyses point out the confounding effect of SARS-CoV-2 infections being mostly asymptomatic in young children has on the identification of children as index persons. To some extent, this problem could be avoided in our study since all participants of the event were invited to take part, regardless of age. As all individuals were exposed at the same event and time, our study is a very suitable model for the previously described notion that children are less likely to become infected. Indeed, a recently published meta-analysis by Viner *et al* showed a low susceptibility for children and adolescents (OR of 0.56, 95% CI 0.37 to 0.85)), which strongly supports our findings of a lower risk of infection in that age group, which is even lower in our study.[28] Our finding supports the previously shown minor influence on the spreading of the virus by children. The finding that for every 10 additional years of age the risk of infection increases during an event indicates that younger people and their limited role should be considered when measures to contain the pandemic are implemented. It should be mentioned that although children had similar exposure compared with adults and probably spent even less time outside the venue hall, the behaviours of children may be different compared with adults. Therefore, we cannot exclude that our findings of lower seroprevalence in children might be biased by factors very specific to this particular event. Taken together, we demonstrate important risk factors for infection during a superspreading event, which helps to understand transmission dynamics in order to improve comprehensive public health preparedness measures, including mandatory ventilation during indoor events and age-adjusted measures according to different risk of infection.

As to the strengths and limitations of this study, the participant group is extremely well defined, and there was no bias or preselection during enrolment as there was only one criteria for invitation, namely presence at the event. Because of the time between the event and the study, it is possible that participants were infected unrelated to the event, but the official shut down of the community limits this risk. The number of index cases during the event is not known, and it is possible that a high number of individuals were already infectious. In addition, the identification of a past SARS-CoV-2 infection via serological test is not perfect, and according to the manufacturer, their IgG detection is 94.4% sensitive (on samples collected >10 days after beginning of symptoms or direct detection of virus) and 99.0% specific (for a ratio ≥0.8). For our infection rate analysis, this predicts 2 false positives and 10 false negative IgG results. However, when field tested by the UK National Health Service, the same assay showed 74.7% sensitivity (62 false-negatives in our data set) and the same specificity of 99.0%.

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Contributors LW, ER, BS and HS wrote the manuscript. LW, RMS and ER organised and ran the testing center. ME inspected the event venue and examined ventilation and air filtration systems. ER and BS organised and performed sample processing, experiments, analyses and corrected the manuscript. NL and AH performed statistical analysis. MC, CF and AK monitored the study. ME, K-HJ and HS oversaw the study and corrected the manuscript. HS acts as a guarantor for this work.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval The Ethics Committee of the Medical Faculty of the University of Bonn, Germany approved the study (reference number 085/20). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data are available upon reasonable request.

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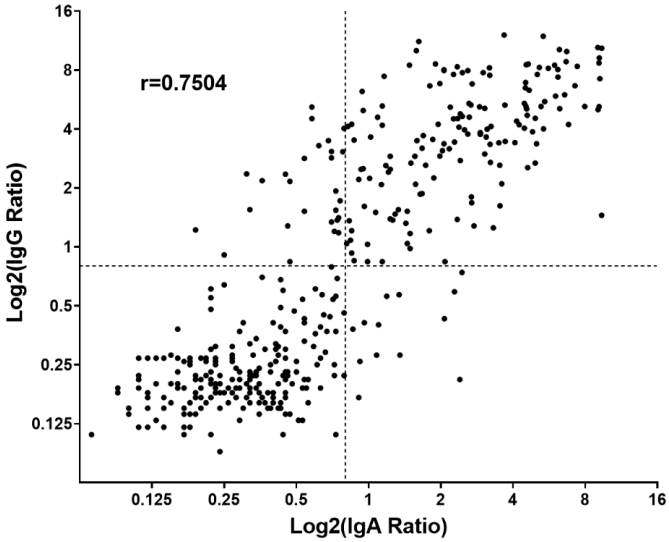
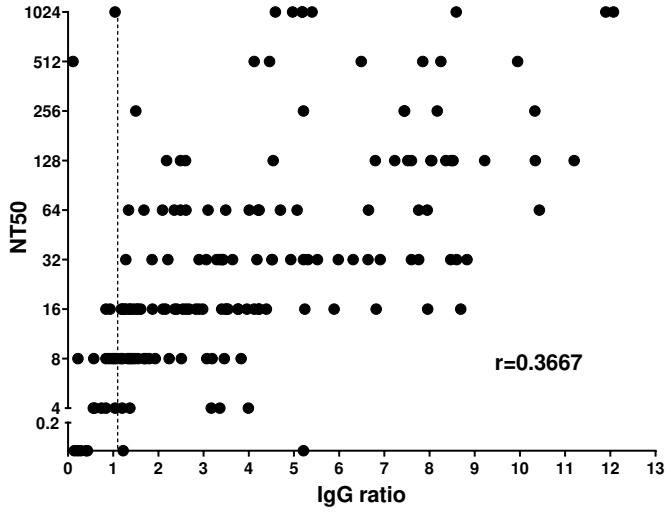
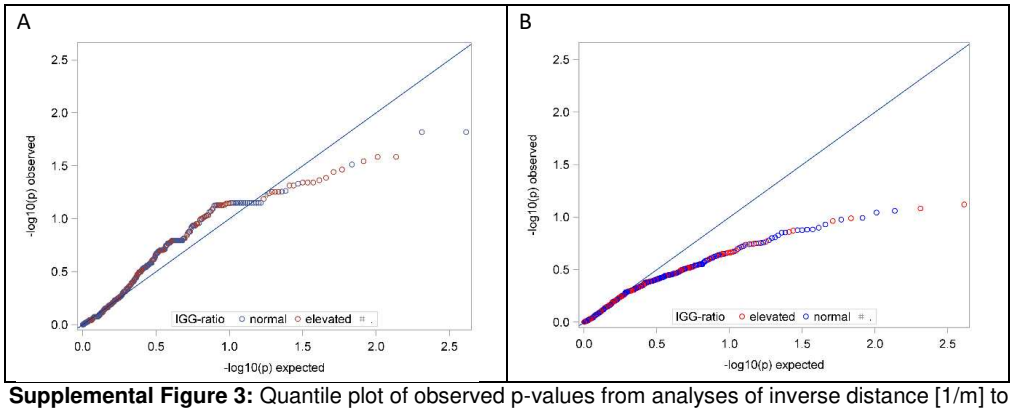
Supplementary Material



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.

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