

Investigation of a superspreading event preceding the largest meat processing plant-related SARS-Coronavirus 2 outbreak in Germany

Thomas Günther^{1§}, Manja Czech-Sioli², Daniela Indenbirken¹, Alexis Robitailles¹, Peter Tenhaken³, Martin Exner⁴, Matthias Ottinger⁵, Nicole Fischer^{2*}, Adam Grundhoff^{1§*}, Melanie M. Brinkmann^{6, 7*}

¹Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

²Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

³Health Office, Osnabrück, Germany

⁴Institute of Hygiene and Public Health, University of Bonn, Bonn, Germany

⁵Omikron Systems GmbH, Braunschweig, Germany

⁶Viral Immune Modulation Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany

⁷Institute of Genetics, Technische Universität Braunschweig, Braunschweig, Germany

*co-corresponding authors

§equal contribution

Corresponding author: Melanie M. Brinkmann
Institute of Genetics
Technische Universität Braunschweig
Inhoffenstrasse 7
38106 Braunschweig, Germany
Phone: +49-531-6181-3069
Email: m.brinkmann@tu-bs.de

Co-corresponding: Nicole Fischer
Adam Grundhoff

Number of Tables / Figures: 0/3

Number of Supplementary Tables / Figures: 4/2

Abstract (250 max)

Background

Here, we describe a multifactorial investigation of the events of a SARS-CoV-2 outbreak in the largest meat processing complex in Germany.

Methods

Timing of infection events, spatial relationship between workers in the meat processing plant, climate and ventilation conditions, sharing of living quarters and transport, and full viral genome sequences recovered from PCR-confirmed SARS-CoV-2 cases were analyzed.

Findings

Transmissions occurred in a confined area of a meat processing plant in which air is constantly recirculated and cooled to 10°C. Index case B1 transmitted the virus to co-workers in a radius of more than 8 meters during work-shifts on 3 consecutive days. Assessment of viral sequences shows that all cases share a set of eight single nucleotide mutations representing a novel sub-branch in the SARS-CoV-2 C20 clade. We identified the same set of mutations in samples collected in the time period between the initial infection cluster and a subsequent outbreak in the following month, with the largest number of confirmed SARS-CoV-2 positive cases in a meat processing facility reported so far.

Interpretation

Our results indicate climate conditions and airflow as factors that can promote efficient spread of SARS-CoV-2 via distances of more than 8 meters and provide insights into possible requirements for pandemic mitigation strategies in industrial workplace settings.

Funding

None.

Introduction

The first wave of SARS-CoV-2 infections peaked in Europe from March to mid of May 2020. Implementation of social and physical distancing measures resulted in declining infection numbers in most European countries. Currently, countries seek to implement alternative measures, for example infection management focused on hotspots, contact tracing and sentinel testing. Given this, it is important to immediately follow up on local infection clusters to prevent re-emergence of large-scale community transmission as seen during the first wave of SARS-CoV-2 infections.

Transmission of SARS-CoV-2 is thought to mainly occur via respiratory uptake of droplets¹ or aerosols. Aerosols are believed to be particularly important in cases where a single source transmits the virus to a large number of individuals, so-called super spreading events²⁻⁸. Whereas droplets typically travel no farther than 2 m, aerosols can stay in the air for prolonged periods of time and may deliver infectious viral particles substantially beyond 2m distances, especially in indoor settings with low fresh air exchange rates⁹⁻¹¹. Factors such as temperature, humidity and air circulation are thought to significantly influence stability and transport of droplets and aerosols and consequently transmission efficiency¹.

Meat processing plants have recently emerged as hotspots of SARS-CoV-2 around the world. This is thought to result not only from operational practices (e.g. close proximity of workers in the production line combined with physically demanding work that promotes heavy breathing), but also from sharing of housing and transportation that may facilitate viral transmission². The requirement to operate at low temperature in an environment with low air exchange rates is another factor that may promote spread of the virus among workers. However, direct scientific evidence for the nature of transmission events in a meat processing plant or the role of shared housing and transportation has not been reported yet.

Here, we report a transmission cluster in a German meat processing plant and provide data suggesting that environmental conditions promoted viral transmission from a single index case to more than 60% of co-workers within a distance of 8m. Viral sequence analyses revealed a previously unreported SARS-CoV-2 genotype that is not only shared by all individuals of the initial cluster, but also by samples collected shortly before a subsequent outbreak one month later, which represents the largest outbreak in a meat processing plant seen in Germany thus far.

Our findings indicate that a physical distance of 2 meters does not suffice to prevent transmission in environmental conditions such as those studied here; additional measures such as improved ventilation and airflow, installation of filtering devices or use of high-quality face masks are required to reduce the infection risk in these environments.

Research in context

Evidence before this study

SARS-CoV-2 outbreaks have recently been reported in several meat processing plants around the globe. As of July 5 2020, *PubMed* lists only a single original article on COVID-19 among workers in meat and poultry processing plants in 19 states of the United States. Eight original articles are listed in *PubMed* under the keywords superspreader/superspreading events and 377 articles include the term aerosols. None of these articles describe the infection events resulting in larger SARS-CoV-2 outbreaks in meat processing and similar food processing plants.

Added value of this study

We describe a cluster of SARS-CoV-2 transmission in a meat processing plant that originated from a worker who had self-reported previous contact with potentially SARS-CoV-2 infected persons from another meat processing plant. Analyzing housing and commuting parameters along with spatial and climate conditions in the work area, this study provides evidence that transmission occurred over a radius of at least 8 meters around the index case, within a work area where meat is processed at temperatures around 10°C. Our analysis of SARS-CoV-2 viral genome sequences identified a previously unreported virus variant with eight point mutations as the prototypical strain in this outbreak (hCoV-19/Germany/NRW-MPP-1/2020). Physical work and relatively low fresh air exchange rates together with continuous re-circulation of cooled air may have favored the transmission of SARS-CoV-2 in the investigated work setting.

Implications of all the available evidence

Common operational conditions in industrial meat processing plants promote the risk of SARS-CoV-2 superspreading events. Additional measures such as improved ventilation, optimized airflow management, installation of filtering or ultraviolet light devices or the use of high-quality face masks are required to reduce the infection risk in these environments.

Methods

On-site conditions

Work conditions were inspected during an on-site visit of the beef processing plant of MPP-R during operating hours. Information on housing, commuting and work places of the workers were provided by MPP-R.

Sample collection and SARS-CoV-2 RT-PCR

Oropharyngeal swap samples from workers in MPP-R were taken in the company's SARS-CoV-2 test center and analyzed by RT-qPCR in an accredited laboratory (Labor Kneißler GmbH & Co.KG, Burglengenfeld, Germany). Oropharyngeal swap samples from workers in MPP-D were taken by public health authorities in Osnabrück, Germany. 35 samples from MPP-R and two samples from MPP-D were sent to the University Medical Center Hamburg-Eppendorf for independent SARS-CoV-2 RT-qPCR confirmation and virus genome sequencing. For RT-qPCR, samples were mixed 1:1 with Roche PCR Media kit buffer (Roche, USA). SARS-CoV-2 qPCR was performed as described ^{12,13}.

Clinical samples from the University Medical Center Hamburg Eppendorf were processed according to protocols approved by Ethics Committee of the City of Hamburg (PV7306; WF026/13). The study and all measures taken to comply with current data protection and ethics regulations were registered with the ethics committee of the University of Bonn, North Rhine Westphalia, Germany, and agreement for publication within the framework of disease control, outbreak management and quality assurance was requested. The committee issued a statement of no objection to publish the study under reference number 337/20.

SARS-CoV-2 amplicon sequencing and bioinformatic analysis

Sample preparation for SARS-CoV-2 amplicon sequencing was performed as described ¹⁴ with modifications ¹⁵. Samples were sequenced on an Illumina MiSeq using 500cycle MiSeq v2 reagent kits (Illumina). All samples were sequenced twice (including independent cDNA synthesis and library preparation reactions) to exclude the possibility of variant frequencies resulting from random amplification artifacts. Except for sample B14 (in which one sequencing reaction was excluded due to insufficient quality), reported variant frequencies reflect the average values from independent replicates. Bioinformatic analysis was performed as described ¹⁵ with the following modifications: Input thresholds were set to at least 10 variant supporting reads with a minimum base quality of 30 (-C10 -q30). Only high confidence variants present in > 20% of reads in at least one individual sample were included and

annotated using ANNOVAR¹⁶. Minor frequency variants resulting in frame shift, stopgain or startloss were excluded.

Results

We studied an outbreak in the largest meat processing plant in Germany, located in Rheda-Wiedenbrück, county of Gütersloh, state of North Rhine Westfalia (referred to as MPP-R in the following). MPP-R performs slaughter and fine processing as well as packaging of beef and pork. A second, independently operated processing plant specialized on sow deboning (MPP-D in the following) is located in Dissen (county of Osnabrück, state of Lower Saxony), approximately 30 km away from MPP-R. Due to occasional SARS-CoV-2 positive cases in the German meat industry, several state governments in Germany arranged SARS-CoV-2 PCR-based series testing of the entire staff of meat processing plants, including MPP-D and MPP-R, shortly before the events described in this study.

Series of events preceding the outbreak in MPP-R

As shown in Fig. 1A, government mandated series testing was performed over a five-day period in MPP-D and MPP-R. Test results were reported two days later (referred to as d0 in the following). 94 out of 279 tested MPP-D employees were found to be SARS-CoV-2 positive, suggesting an ongoing outbreak among MPP-D workers. In MPP-R, only four out of a total of 6,289 employees were found to be positive. None of the four cases was involved in meat processing and the cases were judged to likely be independent.

Two days later (Fig. 1B), two MPP-R workers from the early shift (referred to as cases B1 and B2 in the following) reported to the management of having had a brief contact with employees from MPP-D (D1 and D2 in the following) on d0, both of whom had received positive test results later that day (Fig. 1A). Cases B1 and B2 reported to have no symptoms.

SARS-CoV-2 outbreaks in MPP-R

B1 and B2 were tested in the company's test center three days after the encounter (Fig. 1B). Because the contact with MPP-D workers was not classified as high risk, both continued to work. On the fourth day following the encounter, B1 and B2 received positive test results. B1 and B2, as well as five workers with whom they had shared an apartment were quarantined. B1 and B2 were moved to a separate

apartment, whereas their flat mates remained in their original quarters. Eight days after the encounter, all remaining workers from the early shift (n=140) were tested. Test results available on day ten after the encounter found 18 early shift workers to be positive. All early shift workers were immediately quarantined thereafter. Follow-up testing performed during the following eight days identified another 11 positive cases among the already quarantined workers.

Following this outbreak, risk- and evidence-based screening performed by health authorities, general practitioners and the internal MPP-R test center identified increasing numbers (>110) of positive cases across different parts of the plant in the following weeks, suggesting an ongoing and more wide-spread second outbreak event. Indeed, subsequent serial testing by health authorities performed a month after the initial encounter identified more than 1,400 positive cases, constituting the largest outbreak in a German meat processing facility seen thus far.

Viral genotypes in the first outbreak

The timing of events suggested employees B1 and B2 as the most likely source(s) of the early MPP-R infection cluster. To further substantiate this hypothesis, we performed full viral genome sequencing of the 20 cases tested positive within ten days after the initial encounter between MPP-R and MPP-D workers. In Fig. 2A, we present a heat map showing positions and color-coded frequency values of nucleotide deviations from the Wuhan SARS-CoV-2 reference strain.

A total of eight exchanges are found with near 100% frequency across all samples. A search against 56,366 full length sequences available through GISAID¹⁷ identified six of these mutations to be commonly present in the 20C clade of SARS-CoV-2, a branch which accounts for approximately 17% of all SARS-CoV-2 sequences deposited in GISAID at the time of this writing. Interestingly, however, we did not find GISAID entries sharing the two remaining mutations (marked with asterisks in Fig. 2A; see Supplementary Table S1 for further details). Combined, the eight mutations therefore represent a novel sub-branch within the 20C clade that defines the prototypical viral genome signature of the infection cluster (submitted to GISAID, accession number 476705, strain id NRW-MPP-1). Whereas the B1 sequence is an exact prototype representative, we find an additional nucleotide exchange at 100% frequency (C7735T) in B2. The fact that this mutation is absent from the other samples rules out B2 as a possible source of the cluster with near certainty. Another six cases also exhibit a single additional nucleotide exchange that is not shared with any other sample.

Taken together, these observations suggest prototype virus transmission by B1 as the common source of infection in the cluster. Given the overall scarcity of non-prototypical nucleotide variants, the presence of additional exchanges most likely resulted from ongoing viral mutagenesis in a subset of newly infected individuals. However, the sequencing data alone cannot rule out the formal possibility that at least some of these variants represent independent infection events.

Potential transmission routes in the first outbreak

Given the above, we investigated potential transmission routes between the suspected index case B1 and the other employees within the cluster. The universal point of potential contact among all cases was work in the early shift of the beef processing plant. The shift comprises 147 individuals, most of whom work at fixed positions in a conveyor-belt processing line. The processing line occupies an elongated area approximately 32 meters (m) long and 8.5 m wide (see floor plan in supplementary Fig. S1A). Quarters of beef enter at one end of the line (referred to as proximal in the following) and are processed as they move in longitudinal direction across the room, until cuts are finally packaged near the far end of the line (referred to as distal in the following). Eight air conditioning units placed near the ceiling in the proximal half of the room constantly cool the air. Fans project the air in a lateral direction, either directly from frontal openings in the unit or via perforated hoses mounted underneath the ceiling (see schematics in Figs. S1A-C), effectively sectioning the room into zones in which air is perpetually re-circulated.

While data protection regulations do not allow us to indicate the precise position of the suspected index case, we can disclose that the individual occupied a fixed station within the proximal half of the room. Fig. 3A furthermore indicates the position of 86 employees relative to the suspected index case, along with test results and (where available) viral genotypes (see Table S1 for details). These 86 individuals include all employees with fixed work positions in the proximal half of the processing line (n=56), 22 employees with fixed work stations in adjoining areas, and estimated average location of 8 employees who typically move around the room during the shift (marked with an asterisk in Fig. 3A). While we do not have precise location information for the remaining 60 early shift workers (only one of whom tested positive on d20), all of these individuals occupied fixed stations within the distal half of the processing area.

The map in Fig. 3A immediately suggests a spatial relationship between the location of the suspected index case and the SARS-CoV2 positive workers. As shown in the distance matrix in Fig. 3B (see also

Supplementary Table S2), the probability for spatial overrepresentation of positive cases is highly significant and reaches a maximum (p-val 2.33E-05) within a radius of 8 m (referred to as 8m area hereafter; note that while the 8m maximum reflects statistical significance of overrepresentation, infection rates *per se* are higher in closer proximity to the index case).

In addition to work area locations, we were provided with information on apartments (n=11), bedrooms (n=16) and carpools (n=9) shared among workers from the early shift. In Fig. 3C, we show a statistical overrepresentation analysis of positive cases in shared units (see supplementary material for additional information). The 8m area around the index case is shown for comparison. Positive rates were statistically significant only for a single shared apartment and associated carpool (a1 and c3), and a shared bedroom (r5). The fact that 5 of 7 members in a1/c3, and 2 out of 3 members in r5 have fixed work stations within the 8m area (Supplementary Table S3), however, suggests that high infection rates in these units primarily reflect the number of group members who work in close proximity of the index case, rather than resulting from independent infection chains within the units themselves. This hypothesis is furthermore supported by a general positive correlation (average Pearson correlation coefficient $r=0.67$) between unit infection rate and percentage of unit members working in the 8m area (supplementary Figure S2). Hence, while some secondary infections may have occurred within apartments, bedrooms or carpools, our collective data strongly suggest that the majority of transmissions occurred within the beef processing facility, with case B1 being at the root of the cluster.

Viral genotypes in infection events before and after the first outbreak

The timeline in Fig. 1 suggests a potentially continuous transmission chain between the initial cluster in month 1 and the larger outbreak among MPP-R employees in month 2. We therefore determined viral genotypes in samples from 15 MPP-R employees collected during the early phase of the second outbreak. These included five samples from pork deboning workers who had tested positive on d19 (P1, P2) or d22 (P3, P4, P7), and ten samples from employees with various internal roles tested positive between d29 and d31 (O1-10). As shown in Fig. 2B, all samples exhibit the dominant signature mutations defining the prototypic sequence from the early infection cluster in month 1. Additional nucleotide variants with frequency values between ~20 and 100% were present in seven of the samples. Among the latter, two pairs (P2/O9, O3/O4) exhibited variant patterns which suggest that one of the employees had infected the other, or that both had acquired the virus from an individual not included in our sequencing regimen. Finally, we sought to evaluate whether the two hallmark mutations at position

6406 and 18972 may have emerged in the index case B1, or may have been already present in the ancestral virus. We therefore acquired samples from the two MPP-D workers (D1 and D2) who may have been in contact with B1 and B2 on d0. As shown in Figure 2C, both MPP-D workers share the prototype sequence seen in B1. Of note, D1 additionally exhibits the same mutation (C7735T) that differentiates the genotype in case B2. Hence, since D1 sequences show this mutation with a frequency of ~20%, it is possible that this individual may have been the common source of infection, passing on the prototypic sequence to case B1 and a variant genome to B2.

Discussion

Our results collectively point towards a superspreading event in the MPP-R beef processing plant that originated from a single employee. Our findings suggest that the facilities' environmental conditions, including low temperature, low air exchange rates, and constant air re-circularization, together with relatively close distance between workers and demanding physical work, created an unfavorable mix of factors promoting efficient aerosol transmission of SARS-CoV-2 particles. It is very likely that these or similar factors are also responsible for current worldwide ongoing outbreaks in other meat or fish processing facilities². The recurrent emergence of such outbreaks suggests that employees in meat or fish processing facilities need to be frequently and systematically screened to prevent future SARS-CoV-2 outbreaks. Furthermore, immediate action needs to be taken to quarantine all workers in a radius around an infected individual that may significantly extend beyond 2m. Importantly, while we observed transmission in a ~8m area, exact transmission distances are likely to vary substantially depending on facility layout and operation conditions. Additional studies are therefore required to determine the most important parameters which may be altered to lower infection risk, for example via optimization of airflow or ventilation conditions.

In contrast to work-related exposure, shared apartments, bedrooms, or carpools appear not to have played a major role in the initial outbreak described in this study. Nevertheless, later viral transmission within shared living quarters or work rides very well may have been a confounding factor in context of the second, larger outbreak occurring one month after the first outbreak. Our genotyping results are fully compatible with the hypothesis that this second outbreak was seeded by cases related to the initial cluster. We point out, however, that we have no information regarding the frequency of the NRW-MPP-1 genotype within the broader population. While the genotype had not been deposited in GISAID at the

time of our investigation, and while we thus far not have seen it in our own sequencing of approximately one thousand infected individuals from the Hamburg metropolitan area (Grundhoff and Fischer, unpublished), it is formally possible that NRW-MPP-1 may already have been more broadly distributed in the general population of the Gütersloh district at that time. In this context, it should also be noted that much of the production line workforce in meat processing facilities (including the majority of workers described here) is provided by external sub-contractors, potentially creating lines of transmission that interconnect facilities. It is therefore conceivable that NRW-MPP-1 is a genotype that may already have been particularly abundant among contractor employees. Given the large number of infected individuals in the second outbreak, it is likely that, by now, the NRW-MPP-1 genotype will have spread to the local population. It will therefore be difficult to retrospectively distinguish between the above possibilities. We therefore suggest that, in addition to frequent PCR testing across facilities, a subset of positive samples should be routinely subjected to viral genotyping to allow molecular tracing.

In conclusion, this study indicates that transmission of SARS-CoV-2 can occur over distances of at least 8m in confined spaces under conditions of relatively low air exchange rates and high rates of recirculated unfiltered air. The significance of this study is imminent for the meat and fish processing industry but might well reach beyond these industries, and points to the importance of air quality/flow in confined spaces to prevent future superspreading events.

Finally, we would like to point out important limitations of our study: Firstly, all data on workers, including work place location and sharing of apartments or transport, was provided by the employer (MPP-R). While the employer readily answered all our requests and we have no reason to doubt the accuracy or completeness of the provided information, we did not perform independent validation of this information. Secondly, while the authors performed a site visit, environmental conditions such as airflow direction or speed were only investigated qualitatively. Hence, while we believe this does not affect our major conclusions, our investigation should not be considered an epidemiological study.

Contributors

TG, AG, MO, NF, and MMB designed the study. TG, AG, ME, MO, NF, and MMB performed literature search; AG, MO, NF, and MMB wrote the manuscript; TG, MCS, DI, AG, ME, MO, NF, and MMB collected the data. TG, AR, and AG performed bioinformatic data analysis; TG, AG, MO, NF, and MMB generated the figures and tables.

Declaration of interests

We declare no competing interests.

Funding

Contributions by MMB were funded by the SMART BIOTECS alliance between the Technische Universität Braunschweig and the Leibniz Universität Hannover, an initiative supported by the Ministry of Science and Culture (MWK) of Lower Saxony, Germany and the Helmholtz Association.

The work of AG was funded, in part, by a COVID-19 grant provided by the Federal Ministry of Health.

The Heinrich Pette Institute, Leibniz Institute for Experimental Virology is supported by the Free and Hanseatic City of Hamburg and the Federal Ministry of Health.

References

1. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med* 2020; **382**(16): 1564-7.
2. Dyal JW, Grant MP, Broadwater K, et al. COVID-19 Among Workers in Meat and Poultry Processing Facilities - 19 States, April 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**(18).
3. On Kwok K, Hin Chan HH, Huang Y, et al. Inferring super-spreading from transmission clusters of COVID-19 in Hong Kong, Japan and Singapore. *J Hosp Infect* 2020.
4. Schwierzeck V, König JC, Kuhn J, et al. First reported nosocomial outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a pediatric dialysis unit. *Clin Infect Dis* 2020.
5. Xu XK, Liu XF, Wu Y, et al. Reconstruction of Transmission Pairs for novel Coronavirus Disease 2019 (COVID-19) in mainland China: Estimation of Super-spreading Events, Serial Interval, and Hazard of Infection. *Clin Infect Dis* 2020.
6. Yusef D, Hayajneh W, Awad S, et al. Large Outbreak of Coronavirus Disease among Wedding Attendees, Jordan. *Emerg Infect Dis* 2020; **26**(9).

7. Zhang S, Diao M, Yu W, Pei L, Lin Z, Chen D. Estimation of the reproductive number of novel coronavirus (COVID-19) and the probable outbreak size on the Diamond Princess cruise ship: A data-driven analysis. *Int J Infect Dis* 2020; **93**: 201-4.
8. Zhang Y, Li Y, Wang L, Li M, Zhou X. Evaluating Transmission Heterogeneity and Super-Spreading Event of COVID-19 in a Metropolis of China. *Int J Environ Res Public Health* 2020; **17**(10).
9. Asadi S, Wexler AS, Cappa CD, Barreda S, Bouvier NM, Ristenpart WD. Aerosol emission and superemission during human speech increase with voice loudness. *Sci Rep* 2019; **9**(1): 2348.
10. Asadi S, Wexler AS, Cappa CD, Barreda S, Bouvier NM, Ristenpart WD. Effect of voicing and articulation manner on aerosol particle emission during human speech. *PLoS One* 2020; **15**(1): e0227699.
11. Liu L, Li Y, Nielsen PV, Wei J, Jensen RL. Short-range airborne transmission of expiratory droplets between two people. *Indoor Air* 2017; **27**(2): 452-62.
12. Corman VM, Drosten C. Authors' response: SARS-CoV-2 detection by real-time RT-PCR. *Euro Surveill* 2020; **25**(21).
13. Pfefferle S, Reucher S, Norz D, Lutgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. *Euro Surveill* 2020; **25**(9).
14. J. Q. nCoV-2019 sequencing protocolv2. *articnetwork/ncov-2019*, [dxdoiorg/1017504/protocolsio/bbmuik6w](https://doi.org/10.17504/protocols.io/bbmuik6w) 2020.
15. Pfefferle S, Guenther T, Kobbe R, et al. Low and high infection dose transmission of SARS-CoV-2 in the first COVID-19 clusters in Northern Germany. *medRxiv* 2020.
16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010; **38**(16): e164.
17. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro Surveill* 2017; **22**(13).

Figure Legends

Figure 1: Timeline of events

(A) Series of events in MPP-R and MPP-D (boxes with solid and dashed outline, respectively) preceding the outbreak in MPP-R. The encounter between MPP-R and MPP-D workers which may have initiated the outbreak in MPP-R is shown in the grey box to the right. (B) Events in MPP-R during the outbreak in month 1. The three consecutive days during which the index case worked in the early shift and thus work-related exposure may have occurred are highlighted in blue. (C) Events in MPP-R during month 2. Risk- & evidence-based sampling during month 2 was performed by health authorities, general practitioners, as well as the internal test center from MPP-R. While we do not have exact number of total cases for this time period, minimum incidence numbers shown above the timeline (>110) is based on information provided in official reports from the local health authorities (<https://www.kreisguetersloh.de/aktuelles/corona/pressemitteilungen-coronavirus/>). Boxes below the timeline mark positive cases from internal MPP-R testing that were subjected to viral genotyping.

Saturdays, Sundays and holidays are shown as grey numbers across all panels.

Figure 2: SARS-CoV-2 genotypes

The heat map shows the position (left to right), identity (top row) and frequency (color code) of variant nucleotide positions detected by SARS-CoV-2 full genome amplicon sequencing in (A) 20 samples of MPP-R workers tested positive in month 1, (B) 15 samples of MPP-R workers tested positive in month 2, and (C) two workers from MPP-D who may have transmitted the virus to cases B1 and/or B2. Individual collection dates are shown to the right of each sample. Variant sequences are given relative to the Wuhan reference strain NC_045512. The two silent mutations which define the prototype of the investigated outbreak are marked with an asterisk. Frequencies below 100% mean that only a fraction of viral genomes show nucleotide variations, indicating the presence of viral intra-host sub-populations. White rectangles denote nucleotide positions at which sequencing coverage was insufficient to perform variant calling. For nucleotide positions in coding regions, the corresponding viral ORF(s) are shown above the variant position. Variants without such information are located in non-coding regions. Absolute values for variant frequencies and amino acid changes associated with nucleotide variants, along with identifiers of entries which were submitted to GISAID are provided in Supplementary Table S1.

Figure 3: Workplace location and infection events in the beef processing plant

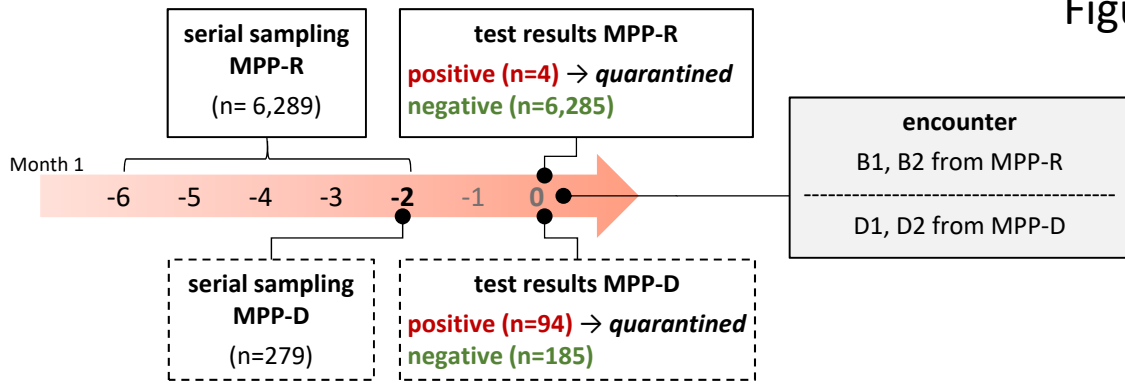
(A) Distance (in meters) of PCR-tested workers from the suspected index case B1 at the workplace. For workers without fixed position in the beef processing plant (marked with an asterisk) coordinates indicate estimated average location during the early shift. Squares and diamonds denote prototype or variant SARS-CoV-2 genotypes, respectively. Filled blue circles denote cases for which viral genomes were not sequenced (i.e., workers tested positive after d8). Positive test dates and genotypes are given in Supplementary Table S1.

(B) Top panel: Observed accumulated percentage of positive cases (*red line*) within the indicated distance from the suspected index case. The gray dashed line shows the average infection rate that would be expected for a random spatial distribution of positive cases. *Bottom panel:* $-\log_{10}$ p-value for the frequency of accumulated positive cases within the given distance being significantly higher than expected based on a random spatial distribution of positive cases (see Supplementary methods and Table S2 for numeric values and further information on p-value calculation). Only employees with fixed work positions were included in the calculation.

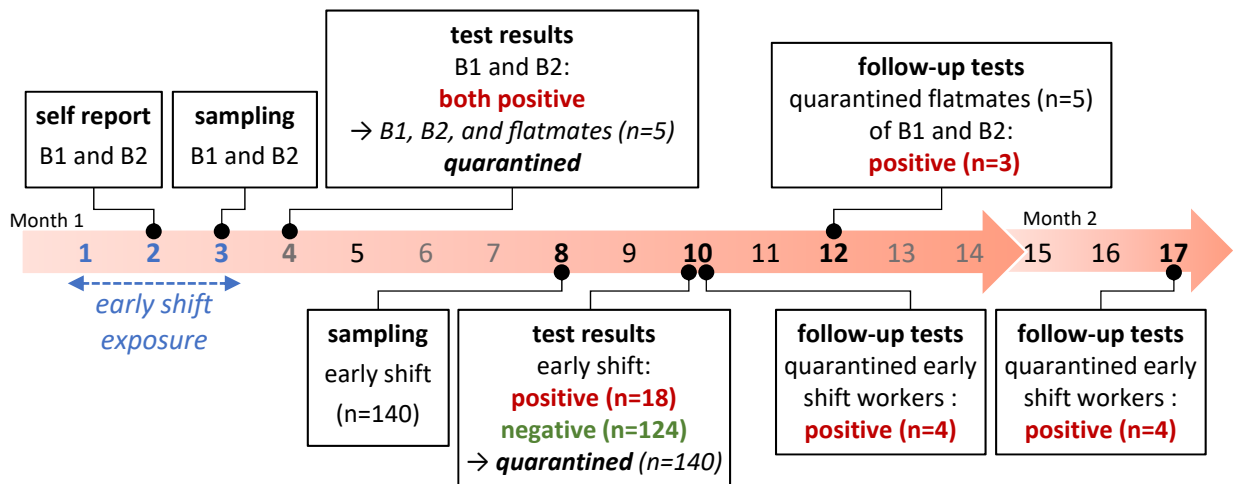
(C) Values on the x-axis show infection rates among members of shared apartments, bedrooms or carpools. Values on the y-axis reflect $-\log_{10}$ p-values for the hypothesis that the infection rate within a given unit is higher than expected based on a random distribution of positive cases among all workers sharing one or more unit (see Supplementary methods and Table S3 for numeric values and further information on p-value calculation). Infection rates and p-values associated with the 8m work area around the index case (see panels A and B) are shown for comparison. Bubble sizes indicate the total number of individuals within each unit or area. All data points with significant p-values (≤ 0.05) are labeled with unit or area id, positive and total number of associated individuals, and p-value

Figure 1

A



B



C

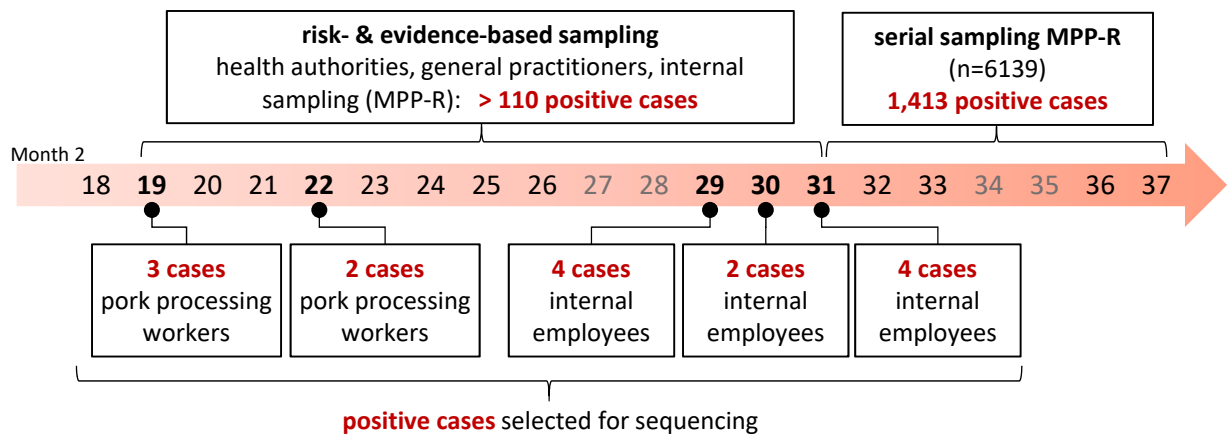


Figure 2

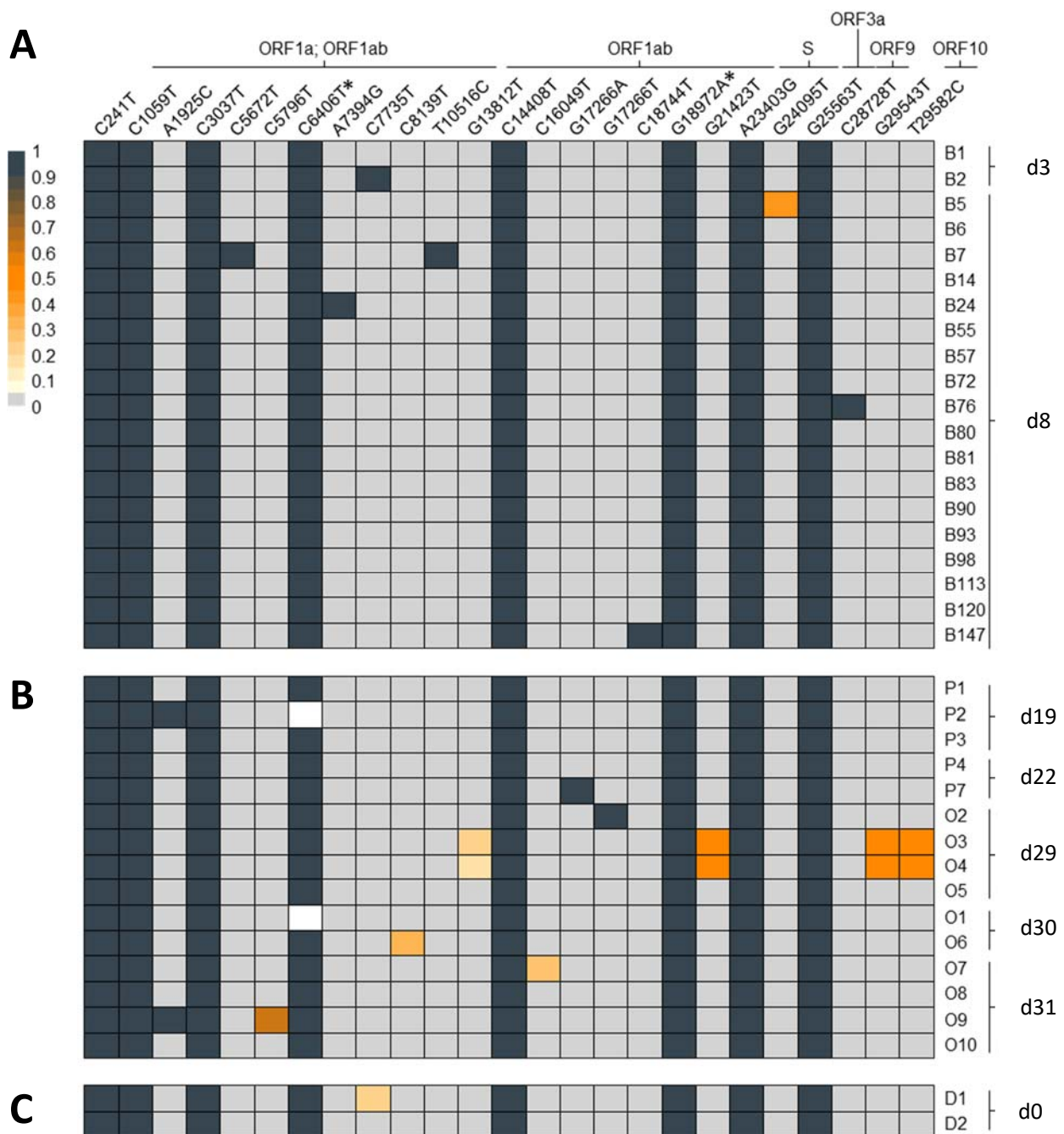
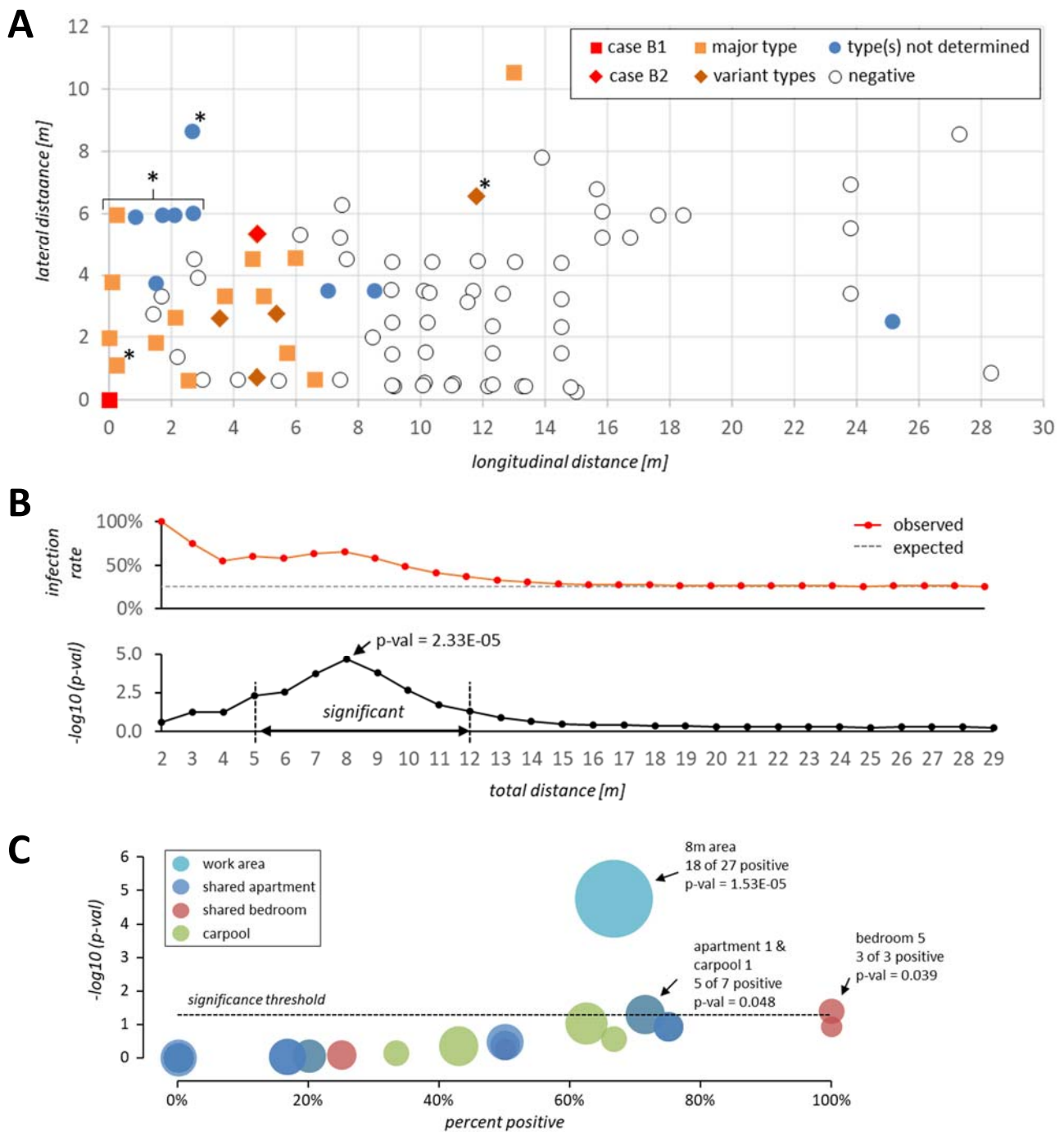


Figure 3



Investigation of a superspreading event preceding the largest meat processing plant-related SARS-Coronavirus 2 outbreak in Germany

Thomas Günther^{1§}, Manja Czech-Sioli², Daniela Indenbirken¹, Alexis Robitailles¹, Peter Tenhaken³, Martin Exner⁴, Matthias Ottinger⁵, Nicole Fischer^{2*}, Adam Grundhoff^{1§*}, Melanie M. Brinkmann^{6*}

Supplementary online material:

Supplementary Tables:

Supplementary Table S1: Positive test result dates and viral genotypes among MPP-R workers

Supplementary Table S2: Infection rates among early shift beef processing workers in relation to workplace distance from the index case.

Supplementary Table S3: Infection rates across apartments, bedrooms and carpools shared by early shift beef processing workers.

Supplementary Table S4: Strains in GISAID containing one of the two single nucleotide mutations C6406T and G18972A.

Supplementary Figures:

Supplementary Figure S1: Schematic layout and air-flow conditions of beef processing plant in MPP-R.

Supplementary Figure S2: Positive correlation between infection rates and work location in shared apartments, bedrooms and carpools.

Supplementary material and methods:

Statistical analysis

P-values in Fig. 3B and Supplementary Table S2 indicate the cumulative probability of infection rates among workers with fixed stations in the indicated distance ranges being equal or higher than observed, under the null hypothesis that the probability of any given individual being positive is independent of spatial location and reflects overall positive rates among workers with fixed stations around the index case (20 out of 78 = 25.6%; see Supplementary Table S2). Similarly, for each shared unit in Fig. 3C and Supplementary Table

S3 we calculated p-values for infection frequencies being equal or higher than observed among all individuals who share one or more unit (22 out of 65 = 33.8%; please note that due to data protection regulations we cannot reveal which worker IDs belong to a shared unit).

Cumulative probability mass values were calculated using the BINOM.DIST.RANGE function from Microsoft Excel for Microsoft 365 MSO (v16.0.12827.20328) with the following input values: probability s : average infection rate among workers with fixed stations (0.256) or workers sharing one or more unit (0.338), minimum number of successes n : observed number of positive workers in distance range or shared unit, trials t and maximum number of successes $n2$: total number of workers in distance range or shared unit

Comparison of viral genotypes with GISAID database entries

We performed a blast search of the prototypical NRW-MPP-1 genotype identified in this study against all 56,366 sequences deposited in GISAID as of July 6 2020. None of the entries contained the combination of the two nucleotide variants G18972A and C6406T that are shared across all samples investigated in our study. As shown in Supplementary Table S4, a very limited number (23 out of 56,366 sequences) contain one of the two mutations. Two samples from the US (collected on the same date as B1 and B2) also carry the variant C6406T, but additionally exhibit another 7 and 8 mutations. These samples clearly belong to a different sub-branch of clade 20C defined by a previously introduced mutation at position 27964. 21 samples from the UK also contain one of the two mutations, but belong to the separate clade 20B. The occurrence of these variants in different clade identifies them as homoplasies and suggests that these isolates are not closely related to the NRW-MPP-1 genotype.

Description of housing conditions, work area conditions, and working conditions

Housing conditions: Many of the workers share apartments and those usually commute together to their workplace in vans organized by the company. The company provided us with anonymized information about the housing situation of the workers regarding information on shared apartments, bedrooms and carpools. The largest housing unit encompassed seven workers for the initial outbreak in month 1 in the beef processing plant (see Supplementary Table S3; note that due to data protection regulations we cannot reveal which workers belong to individual shared units). In addition, we collected information about the work

area and the working conditions during our on-site visit. During that visit, we visited the beef processing plant during operating hours accompanied by technical staff from MPP-R.

Work area conditions: The plant comprises separate areas in which slaughtering and meat processing is performed. While slaughtering takes place at ambient temperatures with higher air exchange rates, beef and pork processing are performed in rooms cooled to approximately 10°C with a high proportion of recirculated cooled air. The beef processing plant has a size of 2,800 m² and is 6.1 m high (Supplementary Figure S1A-B). The entire room and the production line are cleaned and disinfected daily according to food hygiene regulations in Germany. On the day of the on-site visit, the temperature in area 1 and 2 in the beef processing plant ranged between 9,5-10,7°C and between 5.4-8.7°C in area 3. Relative humidity was measured to be 34% right below the cooling fans in area 1 and 68% in the remaining part of area 1, and between 67-71% in areas 2 and 3 (Supplementary Figure S1A).

Cooling fans are cooling recirculated air without filters (C1-8). C3-8 are connected to a perforated hose directed towards area 3 whereas C1 and C2 lack a hose. C1 and C2 turn on only when temperatures rise above 10°C. Cooled air is expelled through the hall up to approximately 12 meters. Cooling fans 3-8 are operating permanently and are expelling cooled air through attached perforated hoses. The air exchange rate for the entire beef processing plant is <1. This means that it takes more than one hour to have the air replaced by fresh air. Specification of the cooling fans is as follows: Manufacturer: Guenther AG & Co. KG, Fuerstenfeldbruck, Germany, Model: S-GGHF 50Hz, Type 050.1E/17-AS, capacity 18,6 kW, airflow 6440 m³/h, air throw 37 m, dimensions: Length 1363 mm, Height 747 mm, Depth 713 mm.

Working conditions: The workers in the beef processing plant that are working on the platform (proximal side) and the connected processing line (starting in area 1 and ending in the middle of area 2) are trained for specific cuts and therefore have fixed work places (Supplementary Figure S1). Hence, workers could be traced in detail during their working hours. While 5-6 workers handle the beef quarters entering the plant on the platform and prepare them for cutting, the quarters are then translocated onto three conveyor-belt processing lines where 24-25 workers separate the meat from the bones. Next, finer cuts are performed (shearing) by 26-27 workers. Towards the distal part of the plant as well as in area 3, the beef is packed into vacuum packaging (Supplementary Figure S1B). While the production line workers have fixed workplaces, the supervisory staff has flexible workplaces and commutes within the beef processing plant.

Shifts in the beef processing plant change once per day. The staff for early and a late shifts are provided by two independent sub-contractors and hence no staff is exchanged between the shifts. A shift has two 30 min breaks and one break of one hour. During breaks, the workers from a shift visit the canteen. Workers do not have fixed seats in the canteen. Supervisory staff does not spend the break times together with the production line workers. The supervisors do not share housing or transport facilities with the production line workers.

Measures implemented by MPP-R during SARS-CoV-2 pandemic

With the onset of the SARS-CoV-2 pandemic, additional preventive measures for the production staff were imposed by MPP-R. The company adhered to the recommendations of the relevant occupational Health and safety guidelines (BGN "Ergänzung der Gefährdungsbeurteilung im Sinne des SARS-CoV-Arbeitsschutzstandards Branche Fleischwirtschaft"). Additional measures were developed and implemented by the company. Hygiene regulations like hand hygiene and one-way traffic in hallways were reinforced, and an internal multi-lingual information campaign was enrolled to raise awareness for prevention and self-detection of early COVID-19 symptoms. A body temperature thermo scanner was set up to check all employees' body temperature entering the building. Workers have been made aware of the company's SARS-CoV-2 test center and were motivated to report any events where they see themselves being at risk. Specific work place assessments were performed to decipher possibilities to extend distances between workers. Simple one-layer face masks were made compulsory. Regulations were in place to prohibit rotation between working places for the workers. Measures in the canteen were imposed to reduce physical contact and to enforce that workers would spend their break times exclusively with workers from their own shift. Since the outbreak of the pandemic, the company managed to prevent intra-company infection chains until the event described in this paper. The implementation of the measures were audited in month 1 by unannounced inspections of the Occupational Health and Safety Experts of the competent authority and by the Occupational Health and Safety experts of the "Berufsgenossenschaft Nahrungsmittel und Gastgewerbe". The company had set up their own test center for PCR-based SARS-CoV-2 testing in month 1. In the SARS-CoV-2 test center trained staff takes oropharyngeal swap samples from workers and other staff. The samples were analysed by RT-qPCR in an accredited laboratory (Labor Kneißler GmbH & Co.KG, Burglengenfeld, Germany). Staff was tested based on self-reported symptoms, possible contacts to

other infected persons, returning to work after more than 96 h absence from work, or based on risk-assessment of possible work place contacts.

Supplementary Table S2: Infection rates among early shift beef processing workers in relation to workplace distance from the index case.

distance	count ^a	positive		infection rate	p-value ^c
		count ^b			
1	0	0		n.a.	n.a.
2	1	1		100.0%	0.256410
3	4	3		75.0%	0.054464
4	9	5		55.6%	0.054091
5	15	9		60.0%	0.005040
6	19	11		57.9%	0.002848
7	22	14		63.6%	0.000193
8	26	17		65.4%	0.000023
9	29	17		58.6%	0.000171
10	37	18		48.6%	0.002173
11	44	18		40.9%	0.019181
12	48	18		37.5%	0.046932
13	54	18		33.3%	0.128565
14	58	18		31.0%	0.212003
15	62	18		29.0%	0.314127
16	65	18		27.7%	0.398091
17	68	19		27.9%	0.376061
18	70	19		27.1%	0.431501
19	71	19		26.8%	0.459332
20	72	19		26.4%	0.487055
21	72	19		26.4%	0.487055
22	72	19		26.4%	0.487055
23	72	19		26.4%	0.487055
24	72	19		26.4%	0.487055
25	75	19		25.3%	0.568323
26	76	20		26.3%	0.490084
27	76	20		26.3%	0.490084
28	76	20		26.3%	0.490084
29	78	20		25.6%	0.543238

a,b cumulative counts (excluding the index case) of workers with fixed work stations the indicated distance from the index case
c p-value for the hypothesis that the infection rate within the given range is higher than expected by chance

Supplementary Table S3: Infection rates across apartments, bedrooms and carpools shared by early shift beef processing workers.

unit id	unit type	unit members				unit members in 8 m area	
		total count ^a	positive count ^b	infection rate	p-value ^c	count ^d	percentage ^e
a1	apartment	7	5	71.4%	0.037	5	71.4%
a2	apartment	4	3	75.0%	0.098	4	100.0%
a3	apartment	4	2	50.0%	0.379	2	50.0%
a4	apartment	6	1	16.7%	0.899	1	16.7%
a5	apartment	6	3	50.0%	0.289	2	33.3%
a6	apartment	5	1	20.0%	0.852	1	20.0%
a7	apartment	6	1	16.7%	0.899	1	16.7%
a8	apartment	4	0	0.0%	1.000	2	50.0%
a9	apartment	4	3	75.0%	0.098	3	75.0%
a10	apartment	4	0	0.0%	1.000	1	25.0%
a11	apartment	6	0	0.0%	1.000	1	16.7%
r1	bedroom	2	0	0.0%	1.000	0	0.0%
r2	bedroom	2	0	0.0%	1.000	0	0.0%
r3	bedroom	2	0	0.0%	1.000	0	0.0%
r4	bedroom	2	1	50.0%	0.534	1	50.0%
r5	bedroom	3	3	100.0%	0.032	2	66.7%
r6	bedroom	2	1	50.0%	0.534	2	100.0%
r7	bedroom	4	1	25.0%	0.783	0	0.0%
r8	bedroom	2	0	0.0%	1.000	1	50.0%
r9	bedroom	2	1	50.0%	0.534	1	50.0%
r10	bedroom	2	0	0.0%	1.000	1	50.0%
r11	bedroom	2	0	0.0%	1.000	1	50.0%
r12	bedroom	2	2	100.0%	0.101	2	100.0%
r13	bedroom	2	0	0.0%	1.000	1	50.0%
r14	bedroom	2	0	0.0%	1.000	0	0.0%
r15	bedroom	2	0	0.0%	1.000	0	0.0%
r16	bedroom	2	0	0.0%	1.000	1	50.0%
c1	carpool	7	5	71.4%	0.037	5	71.4%
c2	carpool	4	0	0.0%	1.000	1	25.0%
c3	carpool	8	5	62.5%	0.073	6	75.0%
c4	carpool	7	3	42.9%	0.393	6	85.7%
c5	carpool	5	1	20.0%	0.852	1	20.0%
c6	carpool	4	3	75.0%	0.098	3	75.0%
c7	carpool	2	0	0.0%	1.000	1	50.0%
c8	carpool	3	2	66.7%	0.238	1	33.3%
c9	carpool	3	1	33.3%	0.682	2	66.7%

a count of all members in a given unit

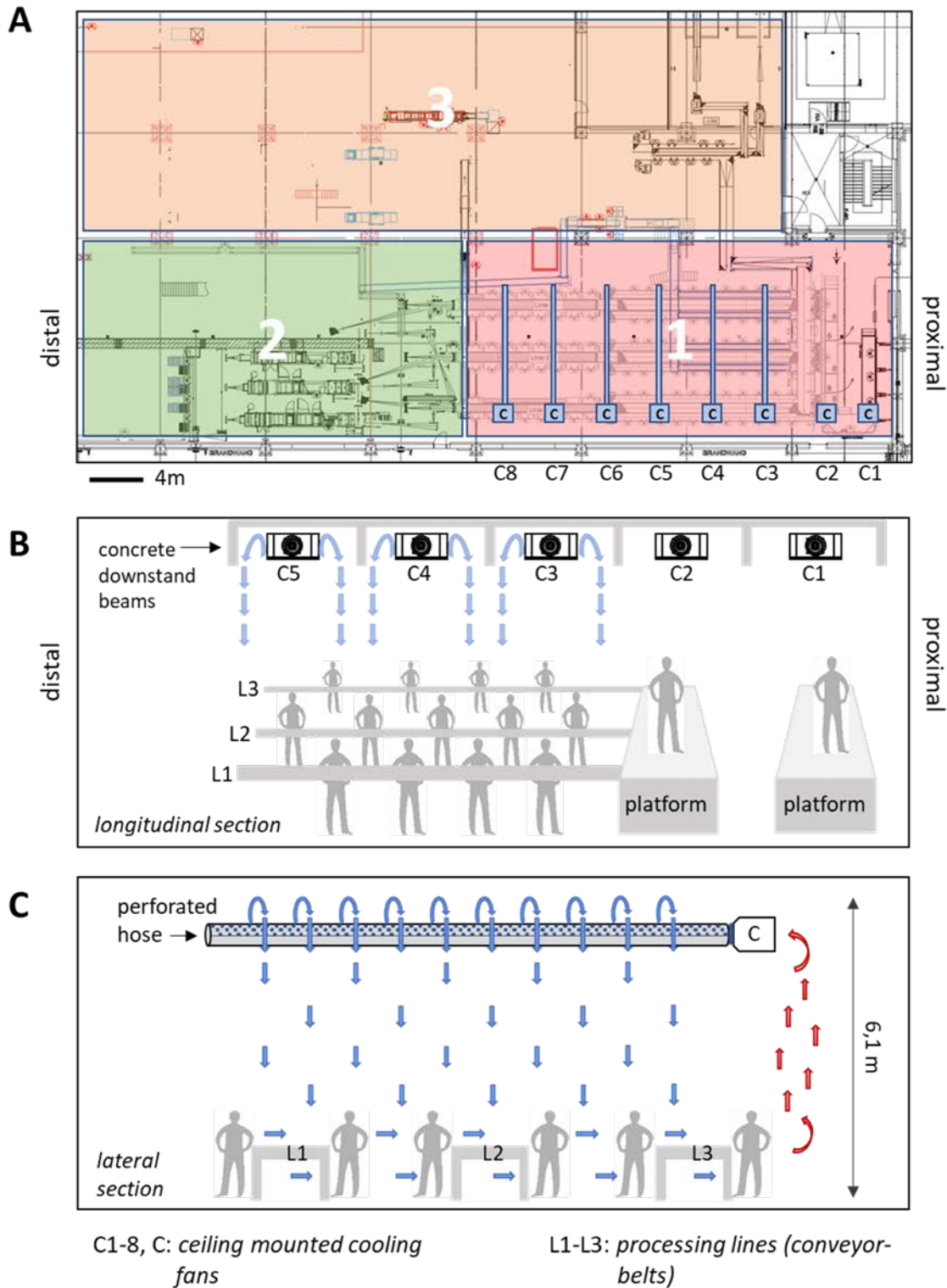
b count of infected members in a given unit

c p-value for the hypothesis that the infection rate within the given unit is higher than expected by chance

d,e count (b) and percentage (c) of unit members with fixed work stations within a maximum distance of 8 meters from the index case

Supplementary able S4: Strains in GISAID containing one of the two single nucleotide mutations C6406T and G18972A.

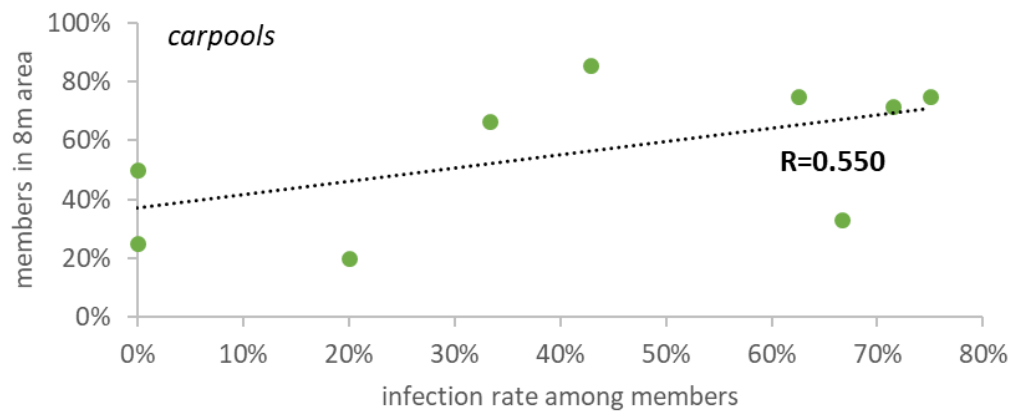
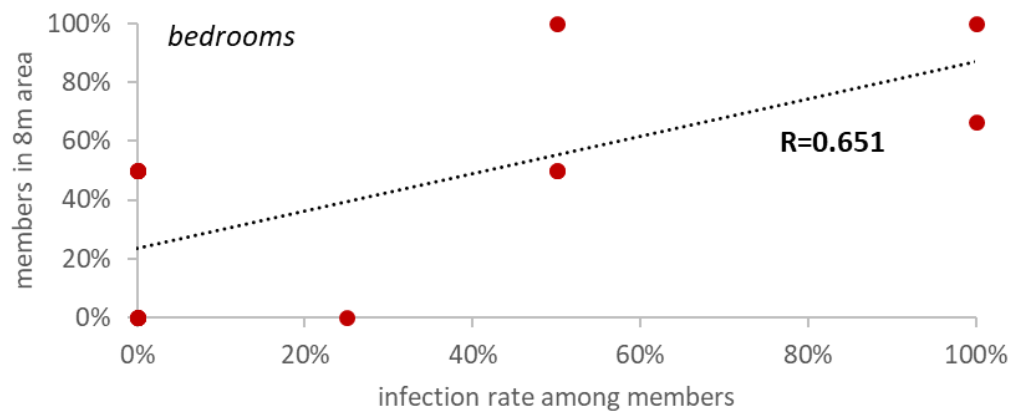
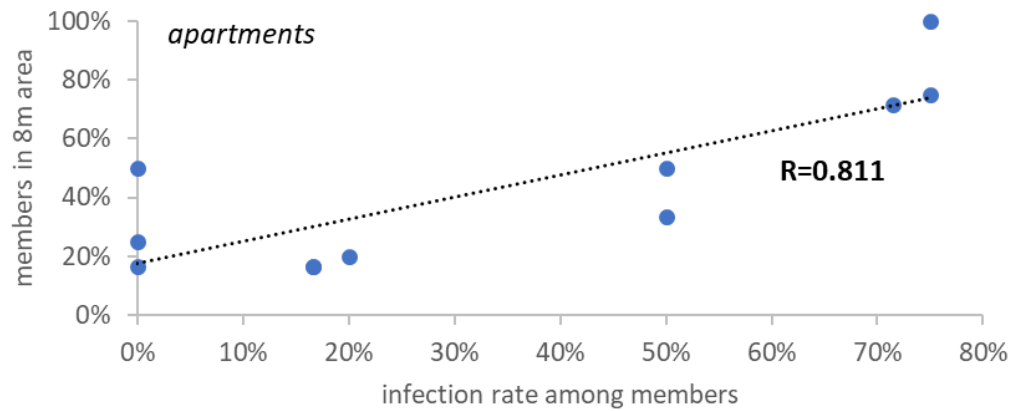
strain	gisaid accession	date	country	division	clade	C6406T	C18972A	date submitted
England/CAMB-782FE/2020	EPI_ISL_433715	2020-04-04	UK	England	20B	no	yes	2020-04-29
Wales/PHWC-15D09A/2020	EPI_ISL_472664	2020-05-03	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-15D188/2020	EPI_ISL_472676	2020-04-19	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-15DC78/2020	EPI_ISL_472768	2020-05-13	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-16093F/2020	EPI_ISL_472995	2020-05-22	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-16344A/2020	EPI_ISL_473296	2020-05-06	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-163592/2020	EPI_ISL_473965	2020-04-24	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-163BEB/2020	EPI_ISL_474055	2020-05-09	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-163E4C/2020	EPI_ISL_474091	2020-05-06	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-163F67/2020	EPI_ISL_474107	2020-04-22	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-164F1B/2020	EPI_ISL_479398	2020-05-18	UK	Wales	20B	yes	no	2020-06-30
Wales/PHWC-2ACA2/2020	EPI_ISL_445688	2020-04-06	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-2E312/2020	EPI_ISL_446088	2020-04-16	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-31B6F/2020	EPI_ISL_474326	2020-04-11	UK	Wales	20B	no	yes	2020-06-23
Wales/PHWC-31BE7/2020	EPI_ISL_446542	2020-04-11	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-323C0/2020	EPI_ISL_446641	2020-04-08	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-33101/2020	EPI_ISL_446739	2020-04-14	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-34267/2020	EPI_ISL_446892	2020-04-18	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-34926/2020	EPI_ISL_446995	2020-04-18	UK	Wales	20B	yes	no	2020-05-16
Wales/PHWC-35A7D/2020	EPI_ISL_474519	2020-04-25	UK	Wales	20B	yes	no	2020-06-23
Wales/PHWC-369E8/2020	EPI_ISL_474744	2020-04-24	UK	Wales	20B	yes	no	2020-06-23
USA/CA-CZB-1320/2020	EPI_ISL_468354	2020-05-20	USA	California	20C	yes	no	2020-06-16
USA/CA-CZB-1322/2020	EPI_ISL_468355	2020-05-20	USA	California	20C	yes	no	2020-06-16



Supplementary Fig. S1: Schematic layout and air-flow conditions of beef processing plant in MPP-R.

(A) Schematic layout of the beef processing plant. Beef halves enter the processing plant on the proximal side (area 1, red). In the section cooled by ceiling mounted cooling fans 1 (C1) and 2 (C2), beef halves are cut in quarters. Quarters are then translocated onto three processing lines (conveyor-belts, L1-3). L1-3 are

moving in proximal-distal direction. Beef quarters are further processed in the section of cooling fans C3-5 (deboning), and finer cuts (shearing) are performed in section of cooling fans C6-8. Towards the distal part of the plant (area 2, green), the beef is packed into consumer packaging and packaging is sealed. Area 3 (orange) is used for weighing and packaging consumer units into boxes. Boxes are placed on pallets for shipping. Index case B1 worked in area 1 (red). Air conditioning units in area 1 are cooling recirculated air without filters (C1-8). C3-8 are connected to a perforated hose directed towards area 3 whereas C1 and C2 lack a hose. The air exchange rate value for the entire beef processing plant is <1 (i.e., it takes more than one hour to replace the air). **(B)** Schematic longitudinal section in distal-proximal direction of area 1. Beef halves are cut on platforms in the section cooled by cooling fans C1 and C2. C1 and C2 are operating without hose and turn on only when temperatures rise above 10°C . Cooled air is expelled through the hall up to a lateral distance of approximately 12 meters. Cooling fans 3-8 are operating permanently and are expelling cooled air through attached perforated hoses. **(C)** Schematic cross-section in the axis of one cooling fan/perforated hose. Cooling fan is expelling cooled, recirculated, unfiltered air into attached hoses. Hoses are perforated in the upper half thereby expelling air towards the ceiling. Concrete down stand beams are guiding the cool air downwards. The resulting air flow is resembling a laminar flow of cooled air from ceiling to the working area at the level of the conveyor-belt processing lines.



Supplementary Fig. S2: Positive correlation between infection rates and work location in shared apartments, bedrooms and carpools.

For each apartment (top panel), bedroom (center panel) or carpool (bottom panel), plots show the percentage of positive unit members (x-axis) and the percentage unit members with fixed work stations in an 8 m area around the index case (y-axis). Individual values are given in Supplementary Table S4. Pearson correlation coefficients (R) are shown next to linear regression curves (dotted lines).