EMBO Molecular Medicine

SARS-CoV-2 outbreak investigation in a German meat processing plant

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 15th Sep 2020

15th Sep 2020

Dear Prof. Brinkmann,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine and for your patience while we retrieved the last report. We have now finally received the enclosed two reports on your article and as you will see, both referees are supportive of publication. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

Please address the minor comments from both referees and we would encourage you to add a phylogenetic tree as suggested by referee 1.

Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses to their comments (as Word file).

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

This is a really nicely executed study on an outbreak of SARS Coronavirus 2 in a German meat processing plant. The work is meticulous and really nicely executed. The paper is clear and well written and, in a world, flooded with epidemiological reports of COVID outbreaks this is one of the best. The only real minor issue is the some of the phrasing tend to lead the reader and I would prefer that the authors have a more impartial tone and allow the reader to develop the concepts within the paper. Whilst I agree this is a super shedding event, I think the title could be adapt to more representative of the study. Which is understanding an major outbreak of SARS Coronavirus 2 in a closed system, where super shedding may be the key mechanism. Additionally, although it may seem unnecessary, I think the more "genomic" of us would like to see a phylogenetic tree to better visualise this outbreak into the broader context of the viral diversity (this can be incorporated into figure 2). Other than that, excellent piece of work and provides a really unique insight into the disease epidemiology. Well done!

Referee #2 (Remarks for Author):

Review
EMM-2020-13296 "Investigation of a superspreading event preceding a large meat processing plant-related SARS-Coronavirus 2 outbreak in Germany"
The work describes a large multifactorial investigation of an outbreak in the largest meat processing complex in Germany. Analysis is done with data on timing of infection events, spatial relationships between workers, climate and ventilation, living quarters sharing and full viral genome sequences.
Very thoroughly depicted and explained time lines of events. Reasonable analysis, discussion and conclusions of the D1, D2, B1 and B2 viral genetic data. Very well described spatial correlations and risk analysis.
Comments:
Please clarify if only the positive workers from plat D were quarantined, as it appears form Figure 1A. Did the 185 negative workers continue plant operations?
What is the difference between pork processing workers and internal employees (Figure 1C)
Figure 1C. The information from the official reports (from the hyperlink) of the local health authorities appears to add up to more than 200 cases over the state period, please clarify or correct the >110 positive cases count.
Please add a sentence in the amplicon sequencing and bioinformatics analysis section of Materials and Methods to define what was achieved as average NGS coverage and what was considered insufficient coverage (as pointed out to be the reason for the white boxes in Figure 2B).

Requested final amendments (EMBO MM – Celine Carret)

Please address the minor comments from both referees and we would encourage you to add a phylogenetic tree as suggested by referee 1.

We have addressed the minor comments from both referees (see below) and have added a phylogenetic tree as supplementary figure S3. We would like to use this opportunity to thank both reviewers for their time to review our manuscript and for their insightful comments.

******* Reviewer's comments *******

Referee #1 (Remarks for Author):

This is a really nicely executed study on an outbreak of SARS Coronavirus 2 in a German meat processing plant. The work is meticulous and really nicely executed. The paper is clear and well written and, in a world, flooded with epidemiological reports of COVID outbreaks this is one of the best. The only real minor issue is the some of the phrasing tend to lead the reader and I would prefer that the authors have a more impartial tone and allow the reader to develop the concepts within the paper. Whilst I agree this is a super shedding event, I think the title could be adapt to more representative of the study. Which is understanding an major outbreak of SARS Coronavirus 2 in a closed system, where super shedding may be the key mechanism. Additionally, although it may seem unnecessary, I think the more "genomic" of us would like to see a phylogenetic tree to better visualise this outbreak into the broader context of the viral diversity (this can be incorporated into figure 2). Other than that, excellent piece of work and provides a really unique insight into the disease epidemiology. Well done!

Thank you very much for your appreciation of our work and your time for reviewing it.
We have adjusted the wording of some paragraphs as per your suggestion and would like to propose the following new title:

SARS-CoV-2 outbreak investigation in a German meat processing plant

As suggested, we have also included a phylogenetic tree as supplementary figure S3.

Referee #2 (Remarks for Author):

Review
EMM-2020-13296 "Investigation of a superspreading event preceding a large meat processing plant-related SARS-Coronavirus 2 outbreak in Germany"
The work describes a large multifactorial investigation of an outbreak in the largest meat processing complex in Germany. Analysis is done with data on timing of infection events, spatial relationships between workers, climate and ventilation, living quarters sharing and full viral genome sequences. Very thoroughly depicted and explained time lines of events. Reasonable analysis, discussion and conclusions of the D1, D2, B1 and B2 viral genetic data. Very well described spatial correlations and risk analysis.
Comments:
Please clarify if only the positive workers from plat D were quarantined, as it appears form Figure 1A. Did the 185 negative workers continue plant operations?

Of the 185 employees with negative test results, all who were working in meat processing halls (i.e. in areas handling meat - deboning, cutting, packaging) were quarantined, whereas employees that were not working in the meat processing halls such as administrative staff or security staff were not quarantined. We have added this information to the legend of Figure 1A.

What is the difference between pork processing workers and internal employees (Figure 1C) Figure 1C.

Pork processing workers are working at the conveyor belt, where they are deboning and cutting meat. The internal employees were working in other areas of the plant such as the convenience food section, technical operation, or occupational safety.
We have clarified this in the legend of Figure 1C.

The information from the official reports (from the hyperlink) of the local health authorities appears to add up to more than 200 cases over the state period, please clarify or correct the >110 positive cases count.

The number of infected persons refers to the official report of June 17 2020 from which it can be read that at this time, 114 SARS-CoV-2 infected persons were confirmed (Kreis Gütersloh/District of Gütersloh), with only a few persons in the general population who had no relation to MPP-R. An exact number of the few persons in the general population cannot be deduced from the report dated 17th of June and previous reports.

For this reason we give an approximate number of >110.

Please add a sentence in the amplicon sequencing and bioinformatics analysis section of Materials and Methods to define what was achieved as average NGS coverage and what was considered insufficient coverage (as pointed out to be the reason for the white boxes in Figure 2B).

We thank the reviewer for this valuable suggestion and added a table, appendix Table SVI, in which we provide all Amplicon Sequencing statistics, including read count, average coverage, stdev and percentile covered.
White boxes indeed denote nucleotides positions without coverage. We changed the sentence in Figure legend 2B, now reading:

- *White rectangles denote nucleotide positions which were not covered by amplicon-seq reads within the respective sample.*

1st Revision - Editorial Decision 25th Sep 2020

The authors performed the requested changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Prof Dr Melanie Brinkmann; Prof Dr Nicole Fischer; Prof Dr Adam
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2020-13296

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample size was determined by the number of infected/positively tested workers. The number of samples is comprehensive enough to support a statistical analysis of the results
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	not applicable
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	not applicable; no samples were excluded
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	We included all samples available through the test center of the meat processing plant.
For animal studies, include a statement about randomization even if no randomization was used.	not applicable
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The information that included information on test results and personal data has been pseudonymized at the source.
4.b. For animal studies, include a statement about blinding even if no blinding was done	not applicable
5. For every figure, are statistical tests justified as appropriate?	yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, tests are appropriate: No a priori assumptions were made; we performed binomial tests to investigate if the distribution of positive cases follows a binomial/normal distribution (e.g., with regard to spatial distribution around the suspected index case). The test method is described in detail in the Material and Methods section.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	not applicable - we only performed binomial tests and did not compare groups of data
Is the variance similar between the groups that are being statistically compared?	not applicable - we only performed binomial tests and did not compare groups of data

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	not applicable
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	not applicable

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	not applicable
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	not applicable
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	not applicable

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	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
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Informed consent was obtained from all persons by the operator of the meat processing plant.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	not applicable
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	not applicable
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	not applicable
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	not applicable

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Viral sequences were submitted to GISAID (www.gisaid.org) as well as ENA (www.ebi.ac.uk/ena/browser/home). ENA Accession Numbers and GISAID identifiers for individual sequence are provided in the Data Availability section and in Appendix Table SI.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	See above: all sequences have been deposited in public databases.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	not applicable to the study
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	not applicable to the study

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	not applicable