nature research

Peer Review File

Manuscript Title: Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

This study by Lavezzo et al describes a combined outbreak investigation and intervention in a small village in Italy. Almost 80% of the population were targeted for two rounds of RT-PCR testing, one at the beginning of the lockdown period and one after ca. 2 weeks. The finding of asymptomatic individuals (41,1% in first, 44.8% in second survey) constitutes the first definitive assessment of the rate of asymptomatic infection in the literature. This figure is of enormous value as it informs estimates and decisions regarding the further dealing with the epidemic in many countries. Diverse and variable estimates of the asymptomatic fraction are currently being promoted, some of them based on unclear observations and indirect derivations from low laboratory testing rates, while the present assessment is based on close follow up in two subsequent tests of almost the entire population of a place, combined with repeated questionnaires. The figure will thus be a landmark observation (one can already see this from the attention the contribution attracts during the preprint phase). There is a lot of additional merit in the work, for instance in describing an increased susceptibility in the elderly, pointing at a decreased susceptibility in the young, and describing equal viral load irrespective of classification in the symptomatic or asymptomatic group. I have assessed all technical work related to RT-PCR detection and find no relevant issues. One aspect that could be improved is the coverage of antibody prevalence. If in any way possible (i.e., if blood samples were taken), authors should try to amend the study by the addition of serological test results. Even plain ELISA OD values would be of enormous value. However, I feel that the study is worthy of publication in a highly visible format due to its reference value for asymptomatic infection.

Referee #2 (Remarks to the Author):

The Vo experience provides a unique natural experiment about SARS-CoV-2 transmissibility in a closed population.

1. The definition of "asymptomatic" is too loose, ie absence of fever or cough or both. It is well established that there are many other relevant respiratory symptoms, ranging from anosmia to shortness of breath, as well as GI and other constitutional symptoms. The authors should revise their definition if they collected the relevant data, and if not, refrain from commenting on asymptomatic proportion altogether.
2. The SEIR model and the associated findings as summarised in Fig 3 are flawed because of the fundamental misclassification of asymptomatic vs symptomatic.
3. I should like to see data (other than age) comparing those successfully recruited into the population-based screens vs those missed, for both surveys; particularly on variables that could affect susceptibility and/or infectivity.
4. Quantitative PCR was done (vide Fig S5). Could the authors report the raw Ct values?
5. Clinical sampling details are missing. These are critically important to assess reliability and validity of laboratory results.
6. I would caution against inferring viral "clearance" by a single negative second test. This does not accord with accepted clinical guidance (two consecutive negative PCRs) or provides sufficiently meaningful new information from a viral dynamics perspective (eg recent Nature paper by Drosten's group).
7. Line 304-5 of Methods: "a normal distribution with mean and standard deviation equal to 1 day" – please justify.
8. The serial interval post-lockdown is implausibly long because confinement at home should have

intensified the contact between infector-infectee pairs within the household, thus shortening the interval. Please explain.
9. How was the Rt estimated? It is a non-trivial "art" and has many variations as detailed in the evolving literature. The few lines (311-6) of description are inadequate and in fact overly simplistic.
10. In fact, it would have been much more informative to formulate a household age-specific susceptibility model although I wonder if the paucity of cases might have precluded robustness in estimation.
11. In sum, the Vo dataset is invaluable but has not been fully, comprehensively and appropriately prosecuted in this paper.

Referee #3 (Remarks to the Author):

The manuscript details the viral transmission in the municipality of Vo' in Italy where the first known outbreak of SARS-CoV-2 infection occurred and the impact of a lock-down on transmission. Importantly, the authors provide data on the fraction of asymptomatic infections. The most interesting finding from the study is the apparent lower infection attack rate in the children although children were comparably exposed to sources of infection. The authors claim that there was no significant differene in the frequency of asymptomatic infection in different age groups. Up to now, it has been assumed that children and adults get infected in a comparable manner but that children have asymptomatic infections. In this report, the authors provide data which they claim suggests that children are inherently less liable to get infected and that there is an age gradient of susceptibility to getting infected. While this is an important finding, one cannot interpret this data without the age distribution of the underlying source population. It is essential that this is presented. Could incidence rates be presented?

It is important to comment on which results are generalisable to other towns or cities in Italy or elsewhere in the world, and which are unique to Vo or Italy?

The manuscript is needlessly long because of repetition in the results and discussion sections. This can be avoided.

The finding that approximately half of infections in a community are asymptomatic is in accord with previous reports that should be cited. (Gudbjartsson DF, et al. N Engl J Med. 2020 Apr 14. doi: 10.1056/NEJMoa2006100. [Epub ahead of print]

Specific comments:
1. Abstract -- "43.2% (95% CI 32.2-54.7%) of the confirmed SARS-CoV-2 infections detected across the two surveys were asymptomatic" -- how many of these were pre-symptomatic detections and how many were completely asymptomatic never showing any symptoms before or after their respiratory sample tested PCR positive?

2. Line 81. The first death in Italy and in this town was on 21st February. When was the first case detected?

3. Line 115 onwards: The authors state that the some of the patients who were asymptomatic in the first sampling remained asymptomatic at the second sampling. What about those asymptomatic at the second sampling? Are you sure that these individuals remained asymptomatic through their infection?

4. Line 136: "negative test at the first of second survey after a positive test in the first survey "? This is confusing?

5. Line 137: What is meant by at of recovery?

6. Line 191: What is meant by "exposed" to infection? Do you meant population infection rate of actual exposure to infection? If the latter, how was exposure defined and determined?

Lines 146-58 -- this is a series of anecdotes about the possible role of asymptomatic cases in transmission but it is not very compelling. Although you found similar viral shedding between symptomatic and asymptomatic cases this does not mean they have comparable infectiousness -- symptoms (cough, runny nose, etc) will promote transmission.

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

This study by Lavezzo et al describes a combined outbreak investigation and intervention in a small village in Italy. Almost 80% of the population were targeted for two rounds of RT-PCR testing, one at the beginning of the lockdown period and one after ca. 2 weeks. The finding of asymptomatic individuals (41,1% in first, 44.8% in second survey) constitutes the first definitive assessment of the rate of asymptomatic infection in the literature. This figure is of enormous value as it informs estimates and decisions regarding the further dealing with the epidemic in many countries. Diverse and variable estimates of the asymptomatic fraction are currently being promoted, some of them based on unclear observations and indirect derivations from low laboratory testing rates, while the present assessment is based on close follow up in two subsequent tests of almost the entire population of a place, combined with repeated questionnaires. The figure will thus be a landmark observation (one can already see this from the attention the contribution attracts during the preprint phase). There is a lot of additional merit in the work, for instance in describing an increased susceptibility in the elderly, pointing at a decreased susceptibility in the young, and describing equal viral load irrespective of classification in the symptomatic or asymptomatic group. I have assessed all technical work related to RT-PCR detection and find no relevant issues. One aspect that could be improved is the coverage of antibody prevalence. If in any way possible (i.e., if blood samples were taken), authors should try to amend the study by the addition of serological test results. Even plain ELISA OD values would be of enormous value. However, I feel that the study is worthy of publication in a highly visible format due to its reference value for asymptomatic infection.

We thank the reviewer for the positive feedback on the study. We fully agree that assessing seroprevalence is of paramount importance. Unfortunately, the ethical approval for this study was limited to the analysis of the swabs and blood samples were not collected at the time so we are unable to include the coverage of antibody prevalence in the manuscript.

Referee #2 (Remarks to the Author):

The Vo experience provides a unique natural experiment about SARS-CoV-2 transmissibility in a closed population.

1. The definition of "asymptomatic" is too loose, ie absence of fever or cough or both. It is well established that there are many other relevant respiratory symptoms, ranging from anosmia to shortness of breath, as well as GI and other constitutional symptoms. The authors should revise their definition if they collected the relevant data, and if not, refrain from commenting on asymptomatic proportion altogether.

We agree with the reviewer that this aspect deserves careful consideration.

In the original manuscript we defined symptomatic infections following the case definition reported by ECDC as of 2nd March 2020 (https://www.ecdc.europa.eu/en/case-definition-and-european-surveillance-human-infection-novel-coronavirus-2019-ncov) which included the sudden onset of at least one symptom among cough, fever, and shortness of breath. Our original definition also included hospitalized patients and patients providing a date of symptom onset, regardless of their symptoms.

As suggested by the reviewer, international guidelines for COVID-19 case definition have been recently updated (https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html) and now include a wider range of symptoms. We have thus revisited our definition of "symptomatic" infection accordingly and for clarity we have included the definition in the "Methods" section.

In the revised version of the manuscript, we used the following definition of symptomatic individual: a subject who required hospitalization and/or reported fever (yes/no or a temperature above 37 degrees Celsius) and/or cough and/or at least two of the following symptoms: sore throat, headache, diarrhoea, vomit, asthenia, muscle pain, joint pain, loss of taste or smell, shortness of breath. In the dataset, a single patient reported a temperature of 37.3, all other patients had > 37.5.

Importantly, our original definition of symptomatic and asymptomatic infections only comprised the occurrence of symptoms at the time of swab sampling. In the revised version of the manuscript we have accounted for follow up information on symptom occurrence after the time of swab sampling that we collected for each positive subject who did not show symptoms at the time of testing. All identified asymptomatic infections at the time of swab sampling were contacted by phone after the second survey (i.e. at the end of March 2020) for clinical follow up. This allows us to unequivocally allocate the study participants into 3 categories: Symptomatic (i.e. individuals testing positive and showing symptoms at the time of swab sampling), Pre-Symptomatic (i.e. individuals testing positive and not showing symptoms at the time of swab sampling but developing symptoms afterwards, as assessed by the follow up), Asymptomatic (i.e. individuals testing positive and not showing symptoms at the time of swab sampling nor developing symptoms afterwards, as assessed by the follow up).

Using these definitions, we found that:

- 6 subjects (IDs CuglAHUl, YETUlovd, zVOcHfvV, IrrnCJVN, aSTJZQjp, YzXbrhuJ) not showing symptoms at the time of swab sampling developed symptoms afterwards. This implies that 6 subjects classified as Asymptomatic in the original version of the manuscript are now classified as Pre-Symptomatic;
- 5 subjects (IDS zbzdjmyX, gEcKwzyS, shRGqSZM, heanXjZj, wNHAlivi) who were originally classified as Symptomatic are now classified as Asymptomatic. This is due to the fact that we had a date of symptoms onset in our records but no details on symptoms. We have contacted these individuals by phone asking details of the symptoms: one of them declared he was hospitalized due to a car accident, not for COVID-19, the other four confirmed that they had only a mild malaise.

We have updated all statistics in the manuscript according to the new definitions used and have included the follow up information in the data spreadsheet as well.

Summary of changes:

- In lines 46 – 48 of the Abstract, we edited the text as follows:

"Notably, 42.5% (95% CI 31.5-54.6%) of the confirmed SARS-CoV-2 infections detected across the two surveys were asymptomatic (i.e. did not have symptoms at the time of swab testing and did not develop symptoms afterwards)."

- In lines 132 – 135 of the Results section we added:
  "Notably, a total of 29 out of the 73 individuals (39.7%; 95% CI 28.5-51.9%) who tested positive at the first survey were asymptomatic (i.e. did not show symptoms at the time of swab sampling nor afterwards, see definition of symptomatic in the Methods section)."
- In lines 328 – 331 of the Methods section we added the definition of symptomatic:
  "**Definition of symptomatic**: a subject who required hospitalization and/or reported fever (yes/no or a temperature above 37 degrees Celsius) and/or cough and/or at least two of the following symptoms: sore throat, headache, diarrhoea, vomit, asthenia, muscle pain, joint pain, loss of taste or smell, shortness of breath."

2. The SEIR model and the associated findings as summarised in Fig 3 are flawed because of the fundamental misclassification of asymptomatic vs symptomatic.

As discussed in our previous answer, clinical follow up and the revised definitions used allowed us to divide the infected population into 3 well-defined classes: Symptomatic subjects (i.e. testing positive and showing symptoms at the time of swab sampling); Asymptomatic subjects (i.e. testing positive and not showing symptoms at the time of swab sampling nor developing symptoms afterwards); Pre-Symptomatic subjects (i.e. individuals testing positive and not showing symptoms at the time of swab sampling but developing symptoms afterwards).

The model has been designed to reflect the pathway of infection and symptom development and capture the prevalence of pre-symptomatic SARS-CoV-2 infection on top of the symptomatic and asymptomatic infection prevalence at the two surveys. Please find below a detailed description of the modified model structure.

Summary of changes:

- In lines 407 – 452 we edited the text as follows:
  "The flow diagram of the model is given in **Extended Data Figure 5**. We assumed that the population of Vo' was fully susceptible to SARS-CoV-2 (S compartment) at the start of the epidemic. Upon infection, infected subjects incubate the virus (E compartment) and have undetectable viraemia for an average of $1/\nu$ days before entering a stage (TP compartment) that lasts an average of $1/\delta$ days, in which subjects show no symptoms and have detectable viraemia. We assume that a proportion p of the infected population remains asymptomatic throughout the whole course of the infection (I_A compartment) and that the remaining proportion $1-p$ develops symptoms (I_S compartment). We assume that symptomatic (I_S), asymptomatic (I_A) and pre-symptomatic ((1-p)TP) subjects contribute to the onward transmission of SARS-CoV-2 and that symptomatic () and asymptomatic () subjects transmit the virus for an average of $1/\gamma$ days. The average duration of the infectious period is given by $1/\delta + 1/\gamma$. We further assume that the virus can be detected by swab testing beyond the duration of the infectious period; this assumption is compatible with the hypothesis that transmission occurs for viral loads above a certain threshold but the diagnostic test can detect the presence of virus below the threshold for transmission. Compartments TP_S and TP_A respectively represent symptomatic and asymptomatic subjects who are no longer infectious but have a detectable viral load, and hence test positive. Eventually, the viral load of all infections decreases below detection and subjects move into a test negative (TN)

compartment. We assume a step change in the reproduction number on the day that lockdown started. Before the implementation of quarantine the reproduction number is given by $R_0^1 = \frac{\beta}{\gamma}$ and we assume that it drops to $R^2 = w\, R_0^1$ after the start of the lockdown, where $1 - w$ represents the percent reduction in R_0^1 due to the intervention. We let T_i denote the number of subjects swabbed on survey i ($i = 1,2$) and let P_{Ai}, P_{Pi} and P_{Si} respectively denote the number of swabs testing positive among asymptomatic, pre-symptomatic (i.e. those showing no symptoms at the time of testing but developing symptoms afterwards) and symptomatic subjects, respectively. We assume that the number of positive swabs among symptomatic, pre-symptomatic and asymptomatic infections on survey i follows a binomial distribution with parameters T_i and π_{Xi}, where π_{Xi} represents the probability of testing positive on survey i for class X (= A,S). For symptomatic subjects, π_{Si} is given by $\pi_{Si} = \frac{I_S(t_i)+TP_S(t_i)}{N}$, for asymptomatic subjects π_{Ai} it is given by $\pi_{Ai} = \frac{pTP(t_i)+I_A(t_i)+TP_A(t_i)}{N}$ and for pre-symptomatic subjects π_{Pi} is given by $\pi_{Pi} = \frac{(1-p)TP(t_i)}{N}$, assuming perfect diagnostic sensitivity and specificity. The likelihood of the model is given by the product of the binomial distributions for symptomatic, pre-symptomatic and asymptomatic subjects at times t_i, $i = 1,2$. Inference was conducted in a Bayesian framework, using the Metropolis-Hastings Markov Chain Monte Carlo (MCMC) method with uniform prior distributions[24]. We fixed the average generation time (equal to $1/\nu + 1/\delta + 1/\gamma$) to 7 days[20] and let the model infer $1/\nu$ and $1/\gamma$. We explored the following values of R_0^1: 2.1, 2.4, 2.7, which are compatible with a doubling time of 3-4 days, as observed in Vo' and elsewhere in the Veneto region. We assumed that seeding of the infection occurred on 4 February 2020. We explored different scenarios on the average duration of viral detectability beyond the infectious period and fixed $1/\sigma$ to be 2, 4, 6, 8, 10 and 12 days. We estimate the number of infections introduced in the population from elsewhere at time t_0 (4 February 2020), the proportion of asymptomatic infections p, the average durations $1/\nu$, $1/\delta$ and $1/\gamma$ and the percent reduction in R_0^1 due to the interventions $(1 - w)100\%$. "

3. I should like to see data (other than age) comparing those successfully recruited into the population-based screens vs those missed, for both surveys; particularly on variables that could affect susceptibility and/or infectivity.

We have provided a statistical comparison of the age distribution of the recruited versus non-recruited population in Extended Data Figure 1 (panels c and d). The non-recruited population was calculated by subtracting the number of study participants in each age-group from the publicly available data on the age-structure of the Vo' population. We find that the age-distribution of the recruited versus non-recruited populations are statistically different in both the first (p-value < 0.001, Fisher's exact test) and second (p-value < 0.001, Fisher's exact test) survey. However, the age-distribution of the recruited population in the first and second surveys did not vary (Extended Data Figure 1a), so all comparisons between the first and second survey remain valid.

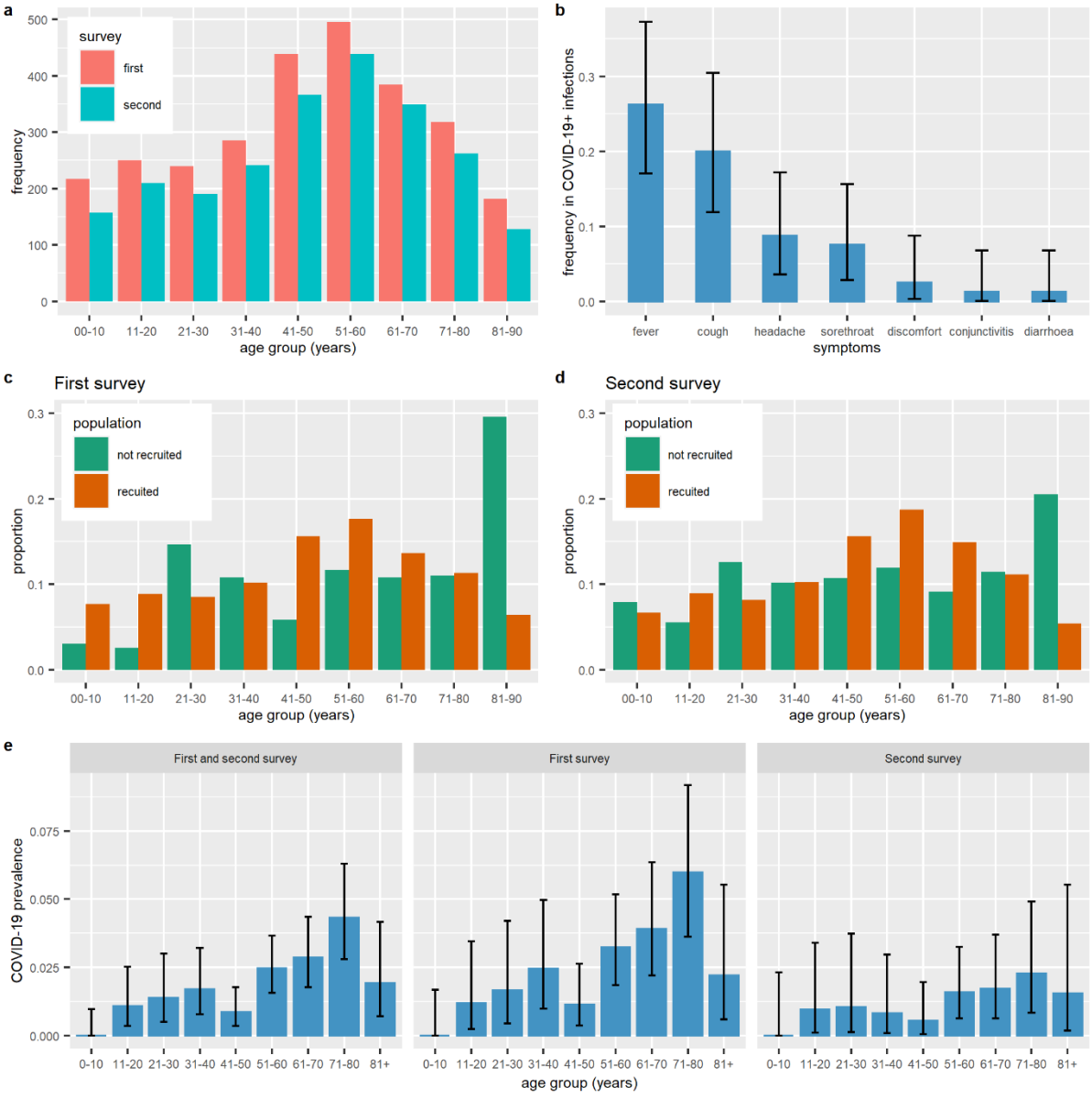
We could not conduct the other comparisons requested by the reviewer as the information needed is classified as sensitive and the ethical approval does not allow to collect information for the subjects who do not voluntary participate in the study.

Summary of changes:

- In lines 118 – 122 we added the following:
  "Statistical analysis showed that while the recruited and non-recruited populations are different in terms of age distribution (p-values < 0.001 for the first and second surveys,

Fisher's exact test), there was no statistically significant bias in the composition of the different age groups enrolled in the two surveys (p-value = 0.31, Exact Wilcoxon-Mann-Whitney Test) (**Extended Data Figure 1**)."

- Extended Data Figure 1 has been formatted as shown below:



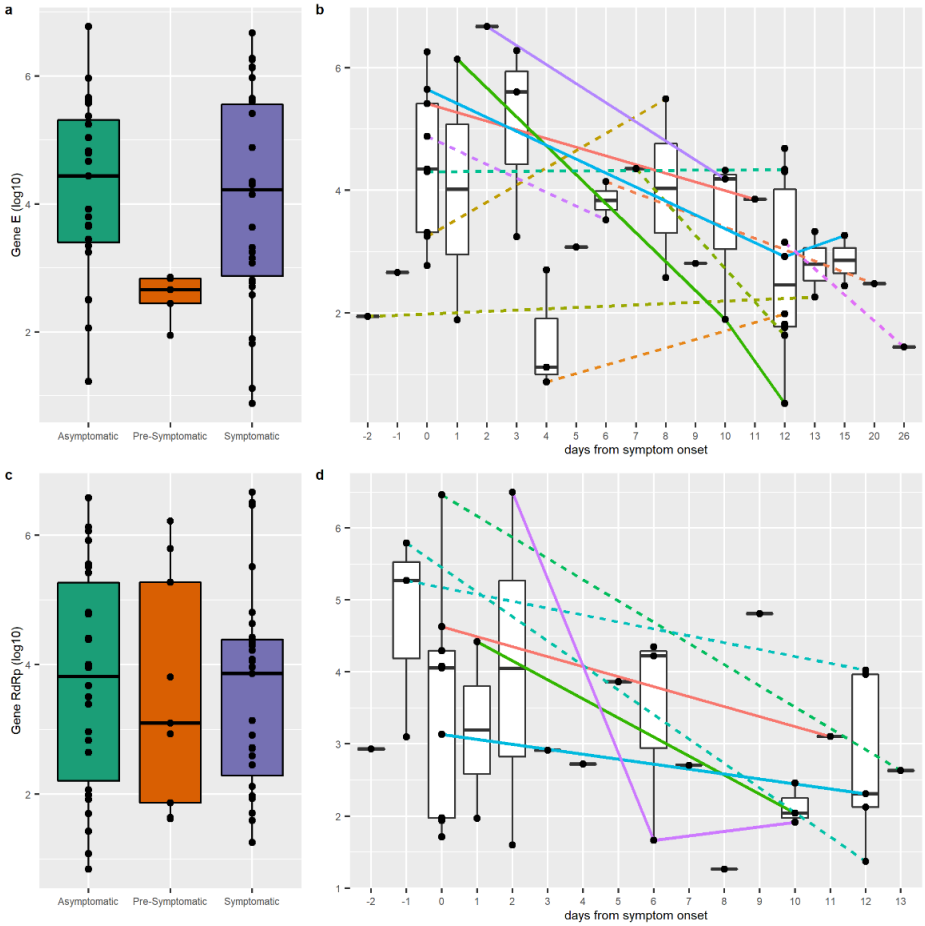
4. Quantitative PCR was done (vide Fig S5). Could the authors report the raw Ct values?

The raw Ct values were already reported in the supplementary excel file ("Ct values" columns in sheet named RT-PCR_comparison and RT-PCR_DATA spreadsheets). We have added a comment in the text to highlight the availability of these data.

In the revised version of the dataset we present the quantitative PCR results (both in terms of genome equivalent copies per ml) for the three Symptomatic, Asymptomatic and Pre-Symptomatic categories, as discussed in our response to question 1.

Summary of changes:

- In lines 371 – 372 we added:
  "Both viral load genome equivalents and raw Ct data are provided in the dataset."
- In lines 182 – 189 we edited the text as follows:
  "The analysis of viral genome equivalents inferred from Ct (cycle threshold) data from real-time reverse-transcription PCR (RT-PCR) assays indicated that asymptomatic and symptomatic individuals did not differ when compared for viral PCR template recovered in the nasopharyngeal swabs (p-values 0.62 and 0.74 for gene E and gene RdRp, respectively; Exact Wilcoxon-Mann-Whitney) (**Extended Data Figure 3**). We find that the viral load tends to peak around the day of symptom onset and for most of the subjects tends to decline after symptom onset (**Extended Data Figure 3**)."
- Extended Data Figure 3 now includes the distribution of the viral load by day since symptom onset, and trends in the viral load for subjects with sequential measurement for either gene (dashed lines) or both genes (solid lines):



5. Clinical sampling details are missing. These are critically important to assess reliability and validity of laboratory results.

We have now added clinical sampling details in the "Methods" section, as detailed below.

Summary of changes:

- In the Methods section, lines 332 – 344 we added the following:
  "Upper respiratory tract samples were collected by healthcare professionals with a single flocked tapered swab used for the oropharynx then nasal mid-turbinates and immediately

put into a sterile tube containing transport medium (eSwab®, Copan Italia Spa, Brescia, Italy). Sampling was performed according Centers for Disease Control and Prevention (CDC) guidelines[19]. Briefly, for oropharyngeal sampling, the swab was inserted into the posterior pharynx and tonsillar areas and rubbed over both tonsillar pillars and posterior oropharynx, avoiding touching the tongue, teeth, and gums; for deep nasal sampling, the swab was inserted into both nostrils for about 2 cm while gently rotating against the nasal wall several times. Samples were stored at 2-8 °C until testing, which was performed within 72 hours from collection. As a measure of the correct execution of the sampling, each PCR contains an internal control designed to amplify the human RNase P housekeeping gene. Reactions that failed to show the internal positive control were classified as invalid and repeated."

6. I would caution against inferring viral "clearance" by a single negative second test. This does not accord with accepted clinical guidance (two consecutive negative PCRs) or provides sufficiently meaningful new information from a viral dynamics perspective (e.g. recent Nature paper by Drosten's group).

We agree with the reviewer that clearance is best defined using two consecutive negative PCRs.

In the dataset we reported the results of the swabs collected in the first and second surveys only. However, all subject testing positive were independently monitored by the local health surveillance officer and all individuals who turned negative in the second survey were confirmed negative in the consecutive additional test. We have added this information in the text.

Summary of changes:

- In lines 150 – 151 we have added the following:
  "For all infections identified in the study, clearance was confirmed by an additional negative test conducted independently by the local health authority (data not shown)."

7. Line 304-5 of Methods: "a normal distribution with mean and standard deviation equal to 1 day" – please justify.

We have revised the algorithm used to estimate the serial interval and have removed this assumption. The algorithm used is detailed in Supplementary Information Text S1.

Summary of changes:

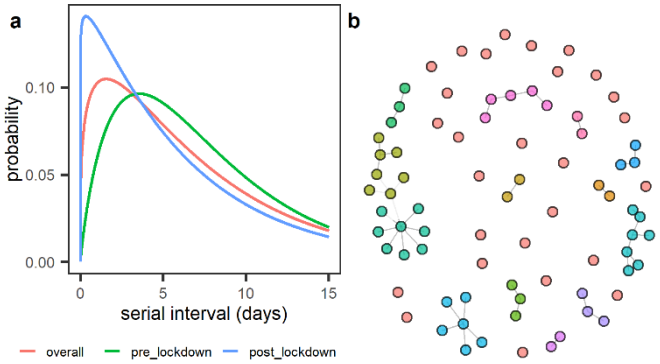
- We have revised the **Reconstructing transmission chains** section of the Methods and removed "a normal distribution with mean and standard deviation equal to 1 day".
- We have added the algorithm used to estimate the serial interval in the Supplementary Information Text S1.

8. The serial interval post-lockdown is implausibly long because confinement at home should have intensified the contact between infector-infectee pairs within the household, thus shortening the interval. Please explain.

We thank the reviewer for their valuable inputs. We have revised the algorithm used for the serial interval estimation and estimate the serial interval over the whole study period [mean 6.8 days (95% CI 5.9--9.1)], along with the serial interval before and after the lockdown. We find indeed that the serial interval post-lockdown [mean 6.2 days (95% CI: 2.6-10.7)] shortened compared to the serial interval pre-lockdown [mean 7.6 days (95% CI: 6.4-8.7)]. We edited the text accordingly and provide the algorithm used in Supplementary Text S1 along with the code https://github.com/ncov-ic/SEIR_Covid_Vo.

Summary of changes:

- In the Abstract, lines 48 – 49, we included the overall serial interval estimate:
  "The mean serial interval was 7.2 days (95% CI 5.9-9.6)."
- In lines 206 – 210 we revised the text as follows:
  "From the inferred transmission pairs, we estimated a serial interval distribution over the whole study period with mean 7.2 days (95% CI 5.9-9.6). We found that the lockdown reduced the serial interval from a mean of 7.6 days (95% CI: 6.4-8.7) before the lockdown to a mean of 6.2 days (95% CI: 2.6-10.7) after the lockdown."
- In lines 377 – 395 we revised the text as follows:
  "We provide the algorithms used to infer the serial interval (the time from symptom onset of the infector to symptom onset of the infectee) distribution and the effective reproduction number (the average number of secondary infections generated by the identified infectors) in Supplementary Information Text S1 and S2, respectively. Briefly, we inferred the date of symptom onset for the subjects testing positive but with missing onset date from the observed time-lags from symptoms onset to confirmation (for the subjects testing positive at multiple sampling times, we used the first sampling time). We then used the observed and inferred dates of symptom onset alongside the contact information to infer transmission pairs within the sampled population. In turn, reconstructed transmission pairs were used to characterise the serial interval in the whole study period as well as during the pre- and post-lockdown periods."
- We show the serial interval distributions in Extended Data Figure 4:



9. How was the Rt estimated? It is a non-trivial "art" and has many variations as detailed in the evolving literature. The few lines (311-6) of description are inadequate and in fact overly simplistic.

We have included the algorithm used to estimate Rt in Supplementary Information Text S2.

In a nutshell, we iteratively infer transmission pairs from the reported contacts and the estimated serial interval distribution and then calculate the reproduction number as the average number of infections generated by the observed or imputed infectors. For infectees who report contacts with positive subjects, the infector was randomly sampled among the named positive contacts. For infectees who do not report contacts with positive subjects we infer the infector by sampling from the serial interval distribution. Please refer to Supplementary Information Text S2 for a detailed description or the algorithm used.

We provide R estimates for the whole study period as well as for the pre- and post-lockdown periods. The code is available at https://github.com/ncov-ic/SEIR_Covid_Vo.

Summary of changes:

- We have revised lines 373 – 403 of the Methods section as follows:
  "**Reconstructing transmission chains:** We used data on contacts traced within the community and on household contacts derived from household composition data (reported in the dataset) to impute chains of transmission and transmission clusters. We used the R package epicontacts[22,23] to visualise the reconstructed transmission chains. We provide the algorithms used to infer the serial interval (the time from symptom onset of the infector to symptom onset of the infectee) distribution and the effective reproduction number (the average number of secondary infections generated by the identified infectors) in Supplementary Information Text S1 and S2, respectively. Briefly, we inferred the date of symptom onset for the subjects testing positive but with missing onset date from the observed time-lags from symptoms onset to confirmation (for the subjects testing positive at multiple sampling times, we used the first sampling time). We then used the observed and inferred dates of symptom onset alongside the contact information to infer transmission pairs within the sampled population. In turn, reconstructed transmission pairs were used to characterise the serial interval in the whole study period as well as during the pre- and post-lockdown periods. Central effective reproduction number estimates were calculated as the average number of secondary infections generated by observed or imputed infectors, having assigned the infector stochastically when more than one or no potential infectors were identified. The 95% confidence intervals were estimated by bootstrapping. All details are provided in Supplementary Information Text S1 and S2."
- We have included the algorithm used to estimate the reproduction number in Supplementary Information Text S2, along with the code implementing the algorithm that is available at https://github.com/ncov-ic/SEIR_Covid_Vo.

10. In fact, it would have been much more informative to formulate a household age-specific susceptibility model although I wonder if the paucity of cases might have precluded robustness in estimation.

We thank the reviewer for their suggestion. There are different age-specific hypotheses that it would be interesting to explore, including clinical development and potentially infectiousness, on top of susceptibility. Unfortunately, a household age-specific susceptibility model for Vo' would require a larger number of positive cases to be resolved, there is not enough statistical power in the data.

11. In sum, the Vo dataset is invaluable but has not been fully, comprehensively and appropriately prosecuted in this paper.

We agree that the Vo dataset is invaluable, and it will be a useful resource for future research. Using new clinical follow up data, we have consolidated the definitions of symptomatic, asymptomatic and pre-symptomatic infections and revised all results accordingly. To the best of our knowledge, this study is among the few systematic ones to report the prevalence of pre-symptomatic, symptomatic and asymptomatic infection in the general population at sequential time points and the revised manuscript includes a comprehensive analysis and modelling of the data collected in the study.

Referee #3 (Remarks to the Author):

The manuscript details the viral transmission in the municipality of Vo' in Italy where the first known outbreak of SARS-CoV-2 infection occurred and the impact of a lock-down on transmission. Importantly, the authors provide data on the fraction of asymptomatic infections. The most interesting finding from the study is the apparent lower infection attack rate in the children

although children were comparably exposed to sources of infection. The authors claim that there was no significant difference in the frequency of asymptomatic infection in different age groups. Up to now, it has been assumed that children and adults get infected in a comparable manner but that children have asymptomatic infections. In this report, the authors provide data which they claim suggests that children are inherently less liable to get infected and that there is an age gradient of susceptibility to getting infected. While this is an important finding, one cannot interpret this data without the age distribution of the underlying source population. It is essential that this is presented. Could incidence rates be presented?

The age-distribution of the resident population of Vo and of the population recruited at the first and second survey are provided in Extended Data Table 1. The age-distribution of the PCR positive infections, together with the age-stratified prevalence of SARS-CoV-2 infection and the age-stratified incidence observed in the second survey are provided in Table 2.

We further tested the null hypothesis that the ratio of symptomatic to asymptomatic infection is constant in each age group and confirm that we did not observe significant differences in the frequency of asymptomatic infection in the different age groups (p-value = 0.96, Fisher's exact test).

Summary of changes:

- In lines 246 – 248 we edited the text as follows:
  "Among confirmed SARS-CoV-2 infections, we did not observe significant differences in the frequency of asymptomatic infection between age groups (**Figure S10,** p-value = 0.96, Fisher's exact test)."

It is important to comment on which results are generalisable to other towns or cities in Italy or elsewhere in the world, and which are unique to Vo or Italy?

We have added a comment on the generalisability of our results in the discussion section.

Summary of changes:

- In lines 108 – 111 we included the following:
  "The experience of Vo' shows that despite the silent and widespread transmission of SARS-CoV-2, transmission can be controlled and represents a model for the systematic suppression of viral outbreaks under similar epidemiological and demographic conditions."
- In lines 301 – 311 we included the following:
  "This study has informed the policy adopted by the Veneto Region, where swabs are available to all contacts of positive symptomatic cases. This testing and tracing approach has had a tremendous impact on the course of the epidemic in Veneto compared to other Italian regions. In this context, the control strategy applied to Vo' serves as a model to suppress SARS-CoV-2 transmission across spatial scales. Enhanced community surveillance, the early detection of SARS-CoV-2 transmission and the timely implementation of interventions are key to control COVID-19 and reduce its substantial public health, economic and societal burden worldwide."

The manuscript is needlessly long because of repetition in the results and discussion sections. This can be avoided.

We have extensively revised and shortened the text, avoiding repetitions as also recommended by the editor. Please refer to the revised manuscript in tracked changes for all deletions.

The finding that approximately half of infections in a community are asymptomatic is in accord with previous reports that should be cited. (Gudbjartsson DF, et al. N Engl J Med. 2020 Apr 14. doi: 10.1056/NEJMoa2006100. [Epub ahead of print]

Thank you for the suggestion, we have now cited Gudbjartsson DF, et al., and commented on the similarity of our results in terms of proportion of asymptomatic infection as well as in the observed rates of infection in children**.**

Summary of changes:

- In lines 244 – 246 we added the following:
  "Our finding that 42.5% (95% CI 31.5-54.6%) of all confirmed SARS-CoV-2 infections across the two surveys were asymptomatic are in accordance with other population surveys[13]."
- In lines 251 – 258 we added the following:
  "We found that none of the children under 10 years of age who took part in the study tested positive for SARS-CoV-2 infection at either survey, despite at least 13 of them living together with infected family members (**Extended Data Table 3**). This agrees with a recent study conducted in Iceland[13] and is particularly intriguing given the very high observed odd ratio for adults to become infected when living together with SARS-CoV-2 positive family members. However, this result does not mean that children cannot be infected by SARS-CoV-2 but suggests that children may be less susceptible than adults. The pathogenesis of SARS-CoV-2 in young children is not well understood[16]."

Specific comments:

1. Abstract -- "43.2% (95% CI 32.2-54.7%) of the confirmed SARS-CoV-2 infections detected across the two surveys were asymptomatic" -- how many of these were pre-symptomatic detections and how many were completely asymptomatic never showing any symptoms before or after their respiratory sample tested PCR positive?

The figure in the abstract referred to the overall proportion of asymptomatic infection at the time of sampling. We have now revisited the figures using clinical follow up information on all asymptomatic infections. For details please see our response to question 1 of Referee #2.

We found that, across the two surveys, of the confirmed SARS-CoV-2 infections 42.5% (95% CI 31.5-54.6%) were asymptomatic (at sampling and afterwards).

Summary of changes:

- In lines 46 – 48 we have edited the text as follow:
  "Notably, 42.5% (95% CI 31.5-54.6%) of the confirmed SARS-CoV-2 infections detected across the two surveys were asymptomatic (i.e. did not have symptoms at the time of swab testing and did not develop symptoms afterwards)."

2. Line 81. The first death in Italy and in this town was on 21st February. When was the first case detected?

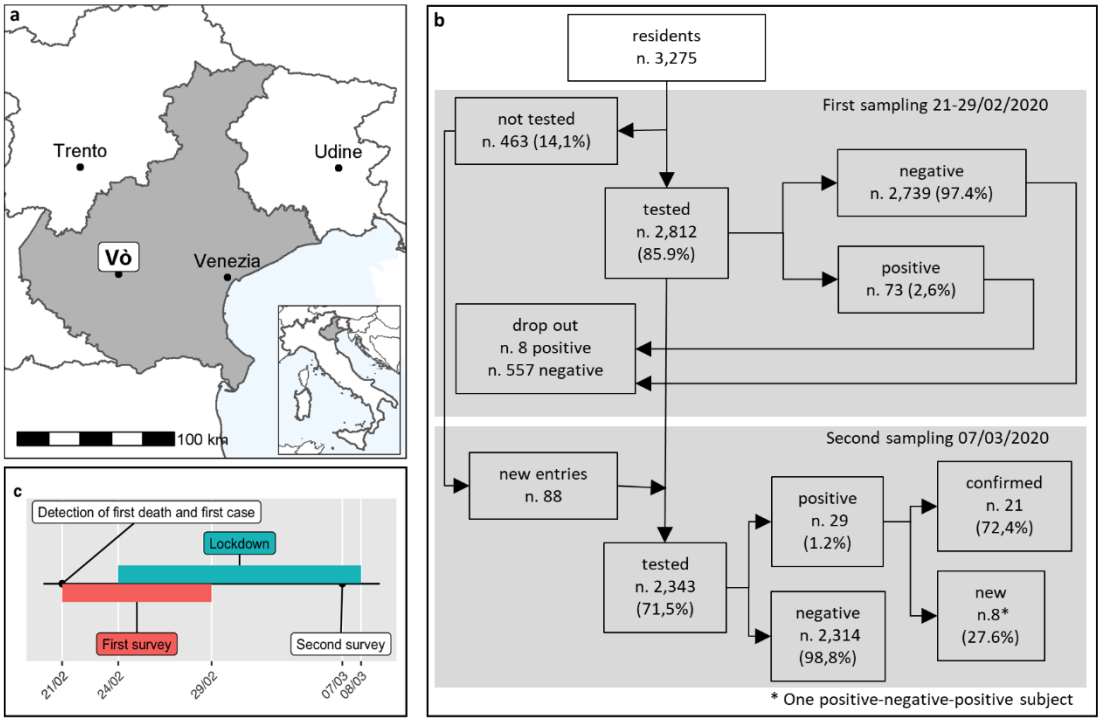
In Vo' the first case was detected on 21st February 2020, on the same date of the first death. We have included this information in the text and in Figure 1c.

Summary of changes:

- In lines 315 – 319 we write:
  "Upon the detection of SARS-CoV-2 in a deceased resident of Vo' on 21 February, the same day where the first COVID-19 case was detected in Vo' and one day after the first locally

acquired COVID-19 infection was identified in Italy, we conducted an epidemiological study to investigate the prevalence of SARS-CoV-2 infection in the population."

- We have included a timeline of the key events in Figure 1c:



3. Line 115 onwards: The authors state that the some of the patients who were asymptomatic in the first sampling remained asymptomatic at the second sampling. What about those asymptomatic at the second sampling? Are you sure that these individuals remained asymptomatic through their infection?

The reviewer raises a valid point. All identified asymptomatic infections at the second survey were contacted by phone at the end of March 2020 for clinical follow up, which allowed us to confirm whether these individuals remained asymptomatic throughout their infection or developed symptoms afterwards. For details please see our response to question 1 of Referee #2.

4. Line 136: "negative test at the first of second survey after a positive test in the first survey"? This is confusing?

We have now edited the sentence as follows: "had a negative test after a previous positive result at the first survey". Thank you for brining this to our attention.

5. Line 137: What is meant by of recovery?

We used "recovery" in place of "viral clearance", i.e. having two negative tests after a positive test at the first or second survey. For clarity, we have removed the word "recovery".

Summary of changes:

- We modified lines 157 – 162 as follows:
  "In particular, 61.4% (95% CI 45.5-75.6%) of symptomatic and 55.2% (95% CI 35.7-73.6%) of asymptomatic SARS-CoV-2 infections cleared the virus during the study period (i.e. had a negative test after a positive result at the first survey); the highest rate (100%) was observed in the age groups of symptomatic 31-40 and 41-50 year olds (**Extended Data Table 2**)."

6. Line 191: What is meant by "exposed" to infection? Do you meant population infection rate of actual exposure to infection? If the latter, how was exposure defined and determined?

By "exposed" we simply meant "infected". The proportion of population infected (also known as the attack rate) was estimated from the fit of the compartmental model described in Extended Data Figure 5 to the data. We sampled 100 realisations from the posterior distribution of the model parameters and run the model until epidemic extinction. The proportion of population infected was estimated as the percent reduction of the population in the S (susceptible) compartment at the end of the epidemic compared to the start of the epidemic.

Summary of changes:

- In lines 227 – 230 we edited the text as follows:
  "The model suggests that up to 93.2% (95% CrI 91.0-94.9%) of the population would have been infected in the absence of interventions and that with the lockdown, 5.4% (95% CrI 4.2-7.1%) of the population of Vo' was infected by SARS-CoV-2(**Figure 3**). These estimates are in line with the attack rates recently estimated for the Veneto region[11]."

Lines 146-58 -- this is a series of anecdotes about the possible role of asymptomatic cases in transmission but it is not very compelling. Although you found similar viral shedding between symptomatic and asymptomatic cases this does not mean they have comparable infectiousness -- symptoms (cough, runny nose, etc) will promote transmission.

We agree that symptomatic subjects could shed more than asymptomatic subjects. We revised the text as suggested and moved the description of the contact tracing and of the family cluster in Supplementary Text S3.

Summary of changes:

- In lines 270 – 281 we have edited the text as follows:

"It remains to be determined the extent to which symptoms may promote viral shedding but the decreasing trend in viral load post symptom onset suggests that pre-symptomatic transmission may play an important role[18]."

Reviewer Reports on the First Revision:

Referee #2 (Remarks to the Author):

The authors have addressed most of my technical comments satisfactorily.

Referee #3 (Remarks to the Author):

The authors have adequately addressed the comments of the reviewers. No further comments.