PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (REAP-1)
AUTHORS	Leber, Werner; Lammel, Oliver; Redlberger-Fritz, Monika; Mustafa-Korninger, Maria Elisabeth; Glehr, Reingard Christina; Camp, Jeremy; Agerer, Benedikt; Lercher, Alexander; Popa, Alexandra; Genger, Jakob-Wendelin; Penz, Thomas; Aberle, Stephan; Bock, Christoph; Bergthaler, Andreas; Stiasny, Karin; Hochstrasser, Eva-Maria; Hoellinger, Christian; Siebenhofer, Andrea; Griffiths, Chris; Panovska-Griffiths, Jasmina

VERSION 1 – REVIEW

REVIEWER	Maltezou, Helena C. Aristotle Univ Thessaloniki
REVIEW RETURNED	01-Nov-2020

GENERAL COMMENTS	This is an interesting article. Please find below my comments: 1. Introduction: please update the number of cases of SARS-CoV-2 globally. 2. Same for cases in Austria. 3. You may want to use the following article on the discussion about transmission and attacke rates: Transmission dynamics of SARS-CoV-2 in families with children in Greece: a study of 23 clusters. Journal of Medical Virology 2020 (pubmed) 4. Methods and Results: You mention nothing about asymptomatic cases. Did you test any asymptomatic person with a history of exposure-contact in the context of teh outbreak? 5. Methods; you describe the outcomes of your study in a specific section (Outcomes measures). However, you repeat them in the Statistical analysis section. please o not duplicate.

REVIEWER	Mullol i Miret, Joaquim Hospital Clinic de Barcelona, Unitat de Rinologia i Clínica de l'Olfacte, Servei d'Otorinolaringologia During the last 5 years, Joaquim Mullol has been member of national and international scientific advisory boards (consulting), received fees for lectures, or grants for research projects from: Allakos, ALK-Abelló, AstraZeneca, Genentech-Roche, GSK, Hartington Pharmaceuticals, Menarini, Mitsubishi-Tanabe, MSD, Mylan-MEDA Pharma, Novartis, Procter & Gamble, Sanofi-Genzyme & Regeneron, UCB Pharma, and Uriach Group.
REVIEW RETURNED	22-Nov-2020

GENERAL COMMENTS	This manuscript by Werner Lebner and coworkers presents a COVID-19 surveillance sentinel study done in an Austrian Primary

	Care setting in patients with flu-like symptoms. Although well performed and potentially of scientific interest in the early stages of the COVID-19 outbreak I consider this study is not anymore of interest, at least at international level, due to the following: 1st) The “low number” of studied patients. To date, there are a number of studies that have had similar conclusions already published and with larger cohorts. 2nd) The lack of study of “asymptomatic patients” (found to be very important for surveillance) constitutes an important limitation not only in the study design (not well know at that time) but also in the manuscript discussion. 3rd) The “assessment of smell and taste” in the early studies have been proved to be wrong or at least unclear and confusing since no quantitative analysis (no smell tests, no VAS scores) were done. In addition, no clear definitions of taste (usually confused with flavor which mainly depends on smell while real taste only assesses sweet, salted, acidic/sour, bitter, and umami). In this study no clear definitions of smell and taste are given nor how these senses were analyzed. 4th) Many references, although from 2020, are from the earlier stages of the disease. Concerning to smell and taste there are a number of more recent studies than those cited as well as good reviews and meta-analysis. This reviewer understands the effort of the authors but the information provided in this manuscript doesn’t give new insights in the scientific knowledge of COVID-19, at least at international level. Neither concerning to surveillance recommendations nor to specific outcomes such as loss of smell and taste. I consider that this manuscript could represent an excellent publication for a local journal.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer: 1
Dr. Helena C. Maltezou, Aristotle Univ Thessaloniki

We would like to thank the reviewers for their thorough and critical appraisal of our manuscript. The points made will help us clarify both purpose, context and scope of our study, hopefully alleviating the concerns raised.

1. We have updated the SARS-CoV-2 cases globally.

2. We have done the same for Austria.

3. Thank you for referring to the Greek study, which shows low infection rates among children, which is consistent with our study where no children (<18 years) tested RT-qPCR positive.

Page 13, Lines 429-432: We have added the following sentence to the Discussion: “In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any

infected children may have remained asymptomatic or did not attend the practice because of mild disease.{Maltezou, 2020 #2769}"

4. No, we did not include asymptomatic people as we were explicitly interested in the patients presenting with symptoms of flu-like illness to general practice. Flu-like illness is one of the most common causes of GP consultation, particularly during winter and spring season. Evidence on RT-qPCR performance and Covid-19-specific presentation in primary care are still lacking. Our study addresses some of these gaps in the literature.

Page 5, Line 162: We have added the following sentence to Methods, Section: "Asymptomatic people were excluded from this study."

5. We have removed the duplication, by merging the sections on Outcome Measures and Statistical Analysis into a single Section.

Response to Reviewer: 2
Prof. Joaquim Mullol i Miret, Hospital Clinic de Barcelona

1. We appreciate the reviewer's concern about the relatively "low number" of patients included and understand that the issue of possible bias in this. However, we note that a small sample size requires high study precision. We, therefore, assured we had reduced occurrence of any study bias as follows: All patients calling the practice with flu-like symptoms were triaged to receive a same day appointment with the GP, allowing selection of symptomatic patients only (reduced sampling bias); all swab samples were sent to the laboratory in Vienna using established couriers services and RT-qPCR results were reported as per the standard Austrian Influenza Surveillance Network protocol (reduced attrition bias); and data were collected prospectively using a standardised clinical questionnaire developed in collaboration with the University of Vienna (reduced recall bias). Furthermore, to reduce the bias of false reactive/non-reactive results, we combined RT-qPCR results with five different ELISA platforms and neutralising antibody. Although some studies have been published from community testing centres, to our knowledge, no study has reported the implementation of RT-qPCR testing in general practice using a national clinical pathway protocol. Indeed, despite reporting results on a small number of patients, our study is incredibly valuable both for national and international audiences.

2. We acknowledge your point regarding asymptomatic patients. However, our study was designed to exclusively investigate people presenting with symptoms to general practice. During winter and spring season, people presenting with flu-like symptoms form a majority of general practice consultation. Therefore, data on RT-qPCR test performance and any COVID-19 symptoms specific to this setting are urgently needed to inform care. Hence, although asymptomatic infection is an important contributor to viral spread, asymptomatic people were excluded from the study. For clarity, we added the following sentences to the study

Page 5, Line 162: "Asymptomatic people were excluded from this study".

Page 3-4, Lines 101-108: "Asymptomatic infection is common (17 %, range 4-41%), but the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.{Sayampanathan, 2021 #2773;Byambasuren, 2020 #2770;Bi, 2020 #2614}"

Page 13, Lines 459 to 462: "Although asymptomatic infection is common,{Byambasuren, 2020 #2764} asymptomatic people were excluded from this study as we were focusing on symptom-driven

presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further."

3. We accept that lack of an objective tool such as the VAS score to assess olfactory and gustatory dysfunction is a major omission in this study. Instead patients were asked about change or loss of taste/smell using a standard questionnaire. To acknowledge this limitation, we have added the following sentence to the manuscript.

Page 13, Lines 457 – 459: "However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),{Sung, 2018 #2766;Rojas-Lechuga, 2020 #2765} but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias."

4. We agree, since submission of this study to the BMJ Open on September 30, 2020, additional important papers have been published.

We, therefore, added the following additional sentences and references to the manuscript:

Page 3-4, Lines 101-108: "Asymptomatic infection is common (17 %, range 4-41%), but the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.{Sayampanathan, 2021 #2773;Byambasuren, 2020 #2770;Bi, 2020 #2614}"

Page 12, Line 414 - 415: Reference (1) has been replaced with the following references: {Aziz, 2020 #2778;von Bartheld, 2020 #2776}.

Reference 13 from our group is a bioRxiv preprint, which has been replaced with its publication in Science Translational Medicine{Popa, 2020 #2768}

We would also like to response to the reviewer's final concerns regarding lack of "new insights in the scientific knowledge of Covid-19" and publication in an international journal. Acknowledging the study limitations, to which we have added based on reviewer's comments, we strongly believe that our paper adds important knowledge to the body of evidence for the following reasons:

Firstly, many countries, including the UK and USA, excluded general practice from a centralised test and trace system. However, as stated in our manuscript (Page 4, Lines 126-128), the European Centre for Disease Prevention and Control (ECDC) recommended inclusion of primary care for COVID-19 surveillance{(ECDC), 2020 #2672} and integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection.18" Given the delay and complexity in delivery of a centralised system, people are increasingly calling for a more localised approach. By focusing on symptomatic infection, we show that general practice can play an essential role in rapid and accurate detection of Covid-19. More than half (16/29, 55%) of the people who tested RT-qPCR reactive in the local COVID-19 cluster were detected at the study practice, including the first and the last case. This shows that testing in general practice can contribute to termination of a local outbreak and demonstrates effective implementation of ECDC policy at local level.

Secondly, our study builds on an established protocol (including clinical care and sample pathway, and an integrated national notification system) for National influenza surveillance in Austria. Similar systems have also been implemented in most European countries (https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/facts-sentinel-surveillance). The current Austrian surveillance protocol was established in 2000 and although conducted at a single practice, our study represents 91 other testing primary care and paediatric

testing sites across the country during the first wave of COVID-19. Our findings have led to two major policy changes in Austria: 1) expansion of the number of national surveillance practices from 91 to 231 sites to offer combined influenza and COVID-19 RT-qPCR testing and 2) introduction of lateral flow antigen testing in health care settings, including general practice, on the 22nd of October 2020, acknowledging the positive contribution of primary care in the control and prevention of COVID-19. We are currently evaluating implementation of SARS-CoV-2 lateral flow antigen testing across a network of 20 general practices in Austria (REAP-2 study). This new intervention has been informed by the protocol described in our manuscript (REAP-1) and has been implemented in response to the national policy change. This protocol forms the baseline for a series of follow up studies conducted at scale during the second wave, which we are planning to submit for peer review in the near future.

Finally, our study is also part of a wider group of researchers using genomic epidemiology to characterise the link between local outbreaks and the international spread of the virus. {Popa, 2020 #2768} As a result, we identified the presence of three different viral strains including 11/13 viral genes carrying the G614D mutation (clades G and GR). This mutation has recently been associated with both higher rates of upper respiratory tract infection and higher transmissibility.{Plante, 2020 #2767}

In summary, our study characterises a local COVID-19 outbreak using clinical, laboratory and genomic data. We describe a clinical protocol for implementation of SARS-CoV-2 testing in general practice either as a stand-alone intervention or as an add-on to an existing national influenza testing and surveillance system. It demonstrates variation in clinical presentation of people presenting with COVID-19 to general practice as well as variation in the presence of viral strains. The first case infected with the new British SARS-CoV-2 variant (B1.1.7) was recently detected at a sentinel practice, highlighting the important role of general practice in national infection surveillance.

We therefore feel it hugely important to share our findings with the international research community.