Original Research Paper

Microbiology

A COMPREHENSIVE SURVEY OF COVID–19 DETECTION USING CLOSED MOLECULAR TECHNIQUES IN THE URBAN CITY OF GUJARAT

Shivani Rupala*	Department Of Microbiology And Molecular Biology, Sanjeevani Metropolis, Rajkot, Gujarat, India. *Corresponding Author
Dr. Sonam Maniar	Head Of Department Of Microbiology And Molecular Biology, Dr. Bhatt Pathology Laboratory, Rajkot, Gujarat, India.
Gayatri Jadeja	Section Head Of Department Of Microbiology And Molecular Biology, Sanjeevani Metropolis Healthcare Ltd., Rajkot, Gujarat, India.
Ruchita Mer	Department Of Microbiology And Molecular Biology, Sanjeevani Metropolis, Rajkot, Gujarat, India.
Charmi Changela	Department Of Microbiology And Molecular Biology, Sanjeevani Metropolis, Rajkot, Gujarat, India.

ABSTRACT At the end of 2019 a novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing severe acute respiratory syndrome amplified globally from Wuhan, China. In March 2020 the World Health Organization (WHO) declared the SARS-Cov-2 virus a global pandemic. The disease is spread through inhalation or contact with infected droplets and the incubation period ranges from 2 to 14 days. The symptoms are usually fever, cough, sore throat, breathlessness, fatigue, weakness others. The disease is moderate in most people. It may continue to pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction. Many people are asymptomatic. Diagnosis is by the presentation of the virus in respiratory secretions by special molecular tests. Common laboratory findings include normal and or low white cell counts with elevated C-reactive protein (CRP). The computerized tomographic chest scan is usually abnormal even in those with asymptomatic or mild disease. Prevention leads to home isolation of suspected cases and those with mild illnesses and strict infection control measures at hospitals that include contact and droplet precautions. Due to the current review, we summarized and equally analyze the emergence and pathogenicity of COVID 19 infection and previous human coronavirus severe acute respiratory coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). In Particular, focus on public health impact, pathophysiology and clinical manifestation, diagnosis, case management.

**KEYWORDS :** Corona Virus, COVID-19, Origin, Outbreaks, Biomarkers, Symptoms, Pathogenesis, Pandemic.

INTRODUCTION

Coronaviruses belong to the Coronaviridae family. This virus shows a crown-like structure under an electron microscope due to the presence of glycoprotein spikes on the outer surface; thus, it was named a coronavirus. Coronaviruses are minute in size which is 65–125 nm in diameter and contain a single-stranded RNA as a nucleic material. The subgroups of the Coronaviruses family are alpha (α), beta (β), gamma (γ), and delta (d) coronavirus probably represents avian species. [1, 2, 3] In recent times, by the end of 2019, WHO was informed by the Chinese government about several cases of pneumonia with unfamiliar etiology. The coronavirus was originated from the Hunan seafood market in Wuhan city of China and rapidly infected more than 50 peoples. The live animals are frequently taken from the Hunan seafood market such as bats, frogs, snakes, birds, marmots, rabbits, and many others [4]. On 12 January 2020, the National Health Commission (NHC) of China released more details about the epidemic, suggested viral pneumonia [4]. From the sequence-based isolated analysis from the patients, the virus was identified as a novel coronavirus.

VIRAL LIFE CYCLE AND HOST CELL INVASION

The virus is transmitted by respiratory droplets and aerosols from person to person. Once inside the body, the virus is attached to host receptors and enters host cells through endocytosis or membrane fusion. The Coronaviruses are made up of four structural proteins, namely, the spike (S), membrane (M), envelop (E) and nucleocapsid (N) proteins.[5,6] The S protein is seen to be protruding from the viral surface and is the most crucial step for host attachment and penetration. This protein is composed of two functional subunits (S1 and S2), among which S1 is responsible for binding to the host cell receptor and the S2 subunit plays a role in the fusion of viral and host cellular membranes.[5] ACE-2 has been identified as a functional receptor for SARS-CoV and is highly expressed on the pulmonary epithelial cells.[7] It is through this host receptor that the S protein binds initially to start the host cell invasion by the virus.[8,9] After binding of SARS-CoV- 2 to the ACE-2, the S protein undergoes activation through a two-step protease cleavage: the first one for priming at the S1/S2 cleavage site and the second cleavage for activation at a position adjacent to a fusion peptide within the S2 subunit.[10,11] The initial cleavage stabilizes the S2 subunit at the attachment site and the subsequent cleavage presumably activates the S protein causing conformational changes leading to viral and host cell membrane fusion.[12] Post membrane fusion, the virus enters the pulmonary alveolar epithelial cells and the viral contents are released inside. Now inside the host cell, the virus undergoes replication and formation of a negative-strand RNA by the pre-existing single-strand positive RNA through RNA polymerase activity (transcription). This newly formed negative-strand RNA serves to produce new strands of positive RNAs which then go on to synthesize new proteins in the cell cytoplasm (translation).[13,14] The viral N protein bind to the new genomic RNA and the M protein.[5]
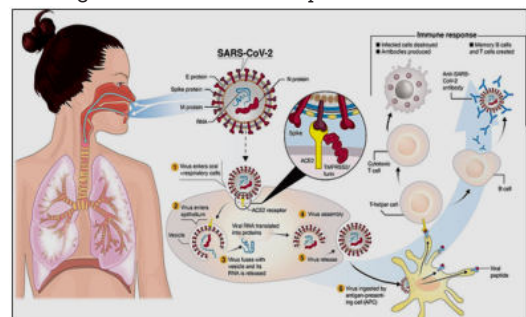
Figure 1: Transmission and Life Cycle of SARS – CoV/COVID-19 [15]

MODES OF TRANSMISSION OF VIRUS CAUSING COVID-19

COVID-19 virus is primarily spread from person to person via respiratory droplet transmission, which occurs when a person is in close contact with someone who is symptomatic such as coughing and sneezing. This occurs through the exposure of the mucosal surfaces of the host, that is, eyes, nose, and mouth, to the incoming infective respiratory droplets. [16, 17, 18] Transmission of the virus may also occur through fomites used by or used on the infected individual such as bed sheets, blankets, kitchen utensils, thermometers, and stethoscopes. Airborne transmission has not been reported for COVID-19, except in specific circumstances in which procedures that generate aerosols are performed, that is, endotracheal intubation, bronchoscopy, open suctioning, nebulization with oxygen, bronchodilators or steroids, bag and mask ventilation before intubation, tracheostomy, and cardiopulmonary resuscitation.[17,18, 19]

The incubation period of COVID-19, which is the period from exposure to the virus to symptom onset, is 5–6 days but can be up to 14 days. During this period, also known as the 'pre symptomatic' period, the infected individuals can be contagious and transmit the virus to healthy individuals in the population.[20] The most common symptoms fever, body aches, breathlessness, malaise, and dry cough, although patients may present with asymptomatic, mild, moderate, or severe disease.[21,22] Some patients may also present with gastrointestinal symptoms such as abdominal pain, vomiting, and loose stools [23,24]
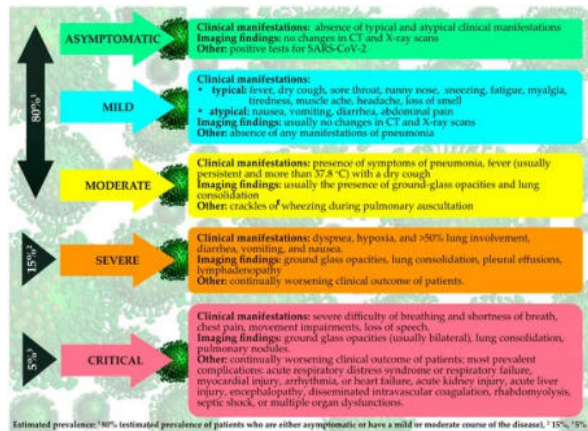

Figure 2: Clinical Layout of COVID 19 Diseases [25]

DIAGNOSTIC TECHNIQUES TO DETECT THE COVID-19 DISEASE

For patients with suspected infection, performing real-time fluorescence (RT-PCR) to detect the positive nucleic acid of SARS-CoV-2 in the upper respiratory tract via nasopharyngeal and Oropharyngeal swab. After being stored and 4.C, the samples are sent to the laboratory where amplification of the viral genetic material is done through a reverse transcription process. [5] This involves the synthesis of a double-stranded DNA molecule from existing viral RNA by either reverse transcription PCR or real-time RT-PCR. [26, 27]Finally, the conserved portion of the SARS CoV-2 genetic code is identified on the amplified genetic material. [5]

The test is recommended to be repeated for verification in cases of a E gene was positive test and again to confirmation by RDRP and ORF1A gene for clearance of viral in COVID 19 positive cases. [28, 29]

In patients with COVID-19, the white blood cell (WBC) count can vary. Leukopenia, leukocytosis, and lymphopenia have been reported, although lymphopenia occurs most commonly. Elevated lactate dehydrogenase and ferritin levels are common [30, 31] High D-dimer levels and more severe lymphopenia have been associated with mortality. Imaging findings—Chest computed tomography (CT) in patients with COVID-19 most commonly demonstrates ground-glass specification with or without developed abnormalities, consistent with viral pneumonia. [32, 33]

MATERIALS AND METHODS

This experiential study was carried out in the molecular biology department. A total of 2400 Patients of all ages and both sexes were included in the study. The ICMR guidelines were followed for selecting the patient population. SARS-CoV-2 positive and negative nasopharyngeal and Oropharyngeal archived samples in viral transport medium (VTM). nasopharyngeal and Oropharyngeal swabs in VTM from patients with H1N1 and severe acute respiratory illness (SARI) and blood samples from SARS-CoV-2 positive and negative cases were used for validating the Truenat screening (Beta CoV) and confirmatory (SARS-CoV-2) OR COVID-19 duplex assays. This COVID-19 duplex test kit detects the E gene and ORF1A gene of the virus.

Evaluation of analytical sensitivity of the test: Aliquot of one VTM sample with low Ct values for envelope protein (E) gene and RNA-dependent RNA polymerase (RDRP) was used for extraction by Trueprep Auto (as per manufacturer's protocol) and also by manual RNA extraction kit. Ribonuclease P (RNase P), a human constitutive gene, was used as an internal control for all the assays. RNA extracted from the samples was diluted 10-fold from 10^{-1} (dilution 1) to 10^{-6} (dilution 6). Six dilutions (1:10) were made from both Trueprep elute. These were run in parallel in TaqMan rRT-PCR using ICMR-NIV protocol, considered as the reference standard, and Truenat assays

TrueNat is a chip-based, battery-operated RT- PCR kit. Earlier, it could only identify the E-gene in the SARS-CoV-2 virus that causes COVID-19. E-gene helps the virus build a spherical envelope around it. But the current devices are now equipped to detect the RDRP enzyme found in the virus RNA. Therefore, ICMR has ruled that these tests can be treated as a confirmation for the presence of the COVID-19 virus.

A clinical detail for each patient was recorded in our test requisition form and approved for the study. Date of onset symptoms, symptoms status, other medical conditions, and the patient was hospitalized or not, date of the hospitalization, purpose of the test, travel history, past contact of positive patient, COVID-19 vaccine received, type of vaccine, their area of the belonging region was also recorded.

RESULTS
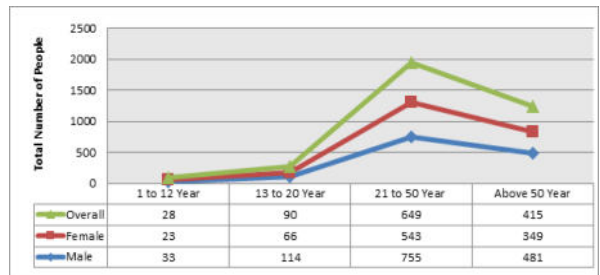
GRAPH: - 1 COVID-19 CASES AMONG MALES & FEMALES ACROSS DIFFERENT AGE CATEGORIES

The SARS-CoV-2 virus being a novel virus can infect the human race irrespective of age categories and gender. This observational study was conducted in the molecular biology department in our diagnostic laboratory in a patient of all ages and both sexes registered for the SARSCoV2 RT-PCR test. The patient was divided into 1 to 12 years, 13 to 20 years, 21 to 50 years, and >50 years age group. There were 1373

(58%) males and 981 (41%) females. Maximum patient were in 21 to 50 years of age group (n=1298) followed by 1 to 12 years (n=56), 13 to 20 years (n= 56), >50 years (n= 830). There were more males as compared to females in all age groups. The researchers consider higher rates of smoking, lower handwashing rates, prior respiratory conditions, biological difference between sexes as a driving force for higher infection and mortality among males [34] Hormonal response elements like putative androgen response elements (AREs) and estrogen response elements (ORE) produces several innate immunity responses through a genetic mechanism which results in dimorphic innate immunity. Several studies emphasized the higher susceptibility of males to viral infection and produce lower antibodies than women. [35]
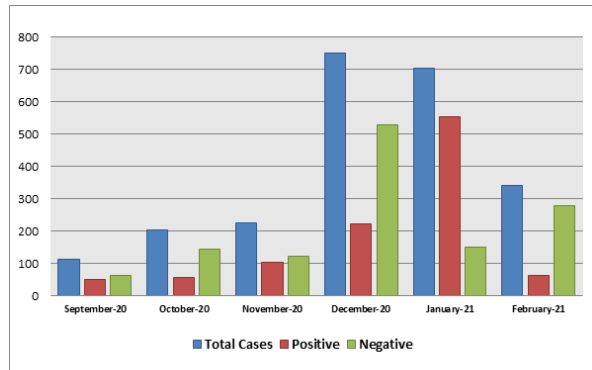
GRAPH: - 2 TOTAL NUMBER OF CASES IN URBAN AREA OF GUJARAT

The bar chart shows the total number of confirmed COVID-19 cases across the urban city of Gujarat along with the total number of positive as well as negative cases from September 2020 to February 2021. The highest number of cases in December and January it's approximately 750 and 700 respectively. However, the least number of cases in September, October, November, and February. One of the most two reasons for this is, the person came in contact with the positive patient and another reason is they have a travel history.

The risks of getting COVID-19 are higher in crowded and inadequately ventilated spaces where infected people spend a long time together nearby. These environments are where the virus appears to spread by respiratory droplets or aerosols more efficiently, so taking precautions is even more important.
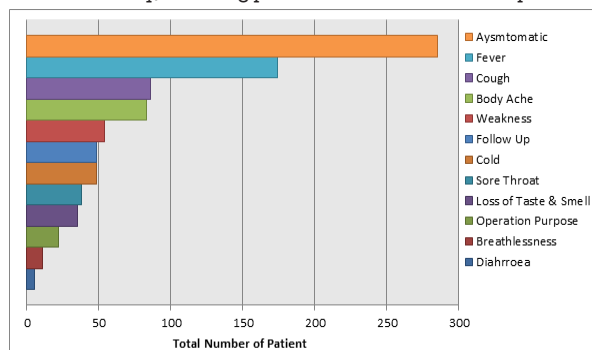
GRAPH: - 3 VARIOUS FACTORS FOR THE OUTBREAK

The total mortality rate of an epidemic can be high even if the symptoms for the vast majority are mild. For the majority of people, symptoms are mild or asymptomatic and in some cases similar to the common flu. An epidemic of the same disease can cause a very large number of deaths, as we discuss here, the symptoms of COVID-19 can be very serious in many cases. Many of these patients need treatment in intensive care units (ICUs). The world health organization (WHO) reports that "about a quarter of severe and critical cases have to need mechanical ventilation" (Koh 2020).

COVID-19 leads to several symptoms, but from what is known currently some symptoms are much more common than others. The WHO described the symptoms of 55,924 laboratory-confirmed cases of COVID-19 in China in the time up to February 20 (Kizito & Semwanga 2020). As our data reflects that the common symptoms are fever and dry cough as well as body ache, also many people have shortness of breath, weakness, cold and some have diarrhea. Although anosmia seems to be a common finding among COVID-19 patients, their loss of smell directly affects daily activities related to the olfactory function is also a strong predictor of COVID-19 diagnosis, which is the primary symptom of COVID-19 infection. However, patients who are scheduled for surgery should *always* be assumed to be potential carriers of the virus throughout their hospital stay, even if they pass the pre-assessment array including normal temperature, no history of exposure or travel from another country or state, and no respiratory symptoms. Patients are "screened" with the gold-standard polymerase chain reaction (PCR) test 24 hours before the surgery. If patients get positive results in the PCR test, they are isolated, they have to follow the national COVID-19 public health protocols and also for exposed staff, and the surgery is postponed. If they get a negative result in the PCR but are positive for the antibody, they will not require further testing during their hospital stay. If patients show negative results in the PCR test, they are tested with PCR every week during their stay and always undergo an "exit test PCR" on discharge.

DISCUSSION

It is now well established that SARS CoV2 can be transmitted from one person to another through direct contact or through aerosols. It is estimated that an average of three persons is infected by an infected SARSCoV 2 patient with a reproductive rate of approximately 3.28. [36] The symptoms of SARS-CoV 2 resemble those of flu or common cold and the range of infection varies from asymptomatic individuals to individuals who present with fever, cough, cold, loss of smell sensation, diarrhea, and fatigue. (Sadhna and Hawaldar et al.)

Recently ICMR approved the use of an indigenous Truenat RTPCR system(MolBio diagnostics) for diagnosis of SARS CoV 2 in India. It is a disposable, temperature stable, chip-based RT PCR test that is based on TaqMan chemistry and uses the E gene from Sarbecovirus for screening of infected individuals followed by a confirmatory test using the RdRP gene of SARS CoV 2. Corman et al. found that SARS-related viral genomes consist mainly of RdRP (RNA dependent RNA polymerase) gene in the open reading frame of the ORF 1ab region, the E gene (envelope protein gene), and the N gene (nucleocapsid protein gene). They observed that the analytical sensitivity of RdRP and genes was very high with a detection limit of 3.6 and 3.9 copies per reaction as compared to the N gene whose analytical sensitivity was found to be about 8.3 copies per reaction.

In Truenat RT PCR, the E gene detects the numerous Coronaviruses including SARS CoV2 while the RdRP gene only detects SARS CoV 2 which is used as a confirmatory test. The turnaround time is very fast with high reproducibility and lower chances of error.

They observed sensitivity of 81.8%by RdRP assay for detection of SARS CoV2. We observed sensitivity of 96.5%. The negative results may be because RNA being less stable than DNA, transportation and storage conditions may affect the results, and the risk of false-negative RT-PCR result increases.

To the best of our knowledge, this is the first study on the Truenat RT-PCR in a diagnostic center in the urban city of Gujarat

CONCLUSION

The coronavirus pandemic is spreading progressively. The

number of new cases diagnosed daily is drastically increased across the globe. The COVID-19 is now an international health emergency which causes significant morbidity and mortality rate. This disease is transmitted via closed contact from person to person. The patient may be completely asymptomatic with a positive swab test, may present with mild illness. This study represented a comprehensive analysis of the COVID-19 outbreak situation in the urban city of Gujarat. The cases are rising very fast and need to control across the globe. There are six different aspects covered up in this study such as the common symptoms of SARS CoV-2 that are observed, age-wise spread to observed which age group is more affected the most with gender. It correlated to presenting the growth trends of infected cases in the urban city. The current study implemented various techniques for the detection of coronavirus.

Molecular methods are the gold standard method for diagnosis of SARS CoV 2 infection as these methods target and identify specific genes of the virus as compared to CT scan and other diagnostic modalities The current pandemic of SARS CoV 2 is showing an upward trend and the primary aim of WHO and the governments all across the globe is in enhancing the testing strategy. Truenat RTPCR is one such promising indigenous equipment that is cost-effective and can be used to diagnose Corona infection in small laboratories and community centers with minimum infrastructure. This will help in reducing the burden on government laboratories, various states of India, researchers, scientists, and students. It was also favorable for the control of COVID-10 outspread in their respective regions.

REFERENCES
1. Zhong N, Zheng B, Li Y, Poon L, Xie Z, Chan K, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. The Lancet 2003; 362(9393):1353–8.
2. Perlman, S.; Netland, J. Coronaviruses post-SARS: Update on replication and pathogenesis. Nat. Rev. Microbiol. 2009, 7, 439–450. [CrossRef] [PubMed]
3. Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet 2020, 395, 565–574. [CrossRef]
4. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. The Lancet 2020.
5. Cascella M, Rajnik M, Cuomo A, et al., Features, evaluation and treatment coronavirus (COVID-19). Stat pearls [internet]. Treasure Island (FL): Stat Pearls Publishing, Jan2020.
6. Bosch BJ, van der Zee R, de Haan CA, et al. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol 2003;77:8801–11.
7. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.
8. Chen Y, Guo Y, Pan Y, et al. Structure analysis of the receptor binding of 2019-nCoV[published online ahead of print, 2020 Feb 17]. Biochem Biophys Res Commun 2020;525:135–40.
9. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B beta coronaviruses. Nat Microbiol 2020;5:562–9
10. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020;11:1620.
11. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci U S A 2009;106:5871–6.
12. Belouzard S, Millet JK, Licitra BN, et al. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 2012;4:1011–33.
13. Available https:// www. wikidoc. org/ index. php/ File: Coronavirus replication. png (accessed 10 Jun 2020)
14. Yang N, Shen HM. Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19. Int J Biol Sci 2020;16:1724–31.
15. Colin D. Funk, Craig Laferrière, and Ali Ardakani - Funk CD, Laferrière C y Ardakani A (2020) Una instantánea de la carrera mundial de vacunas dirigidas contra el SARS-CoV-2 y la pandemia COVID-19. Parte delantera. Pharmacol. 11:937. Funk CD, Laferrière C and Ardakani A (2020) A Snapshot of the Global Race for Vaccines Targeting SARS-CoV-2 and the COVID-19 Pandemic. Front. Pharmacol.
16. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020;382:1199–207.
17. Available https://www.who.int/news-room/commentaries/detail/modes-oftransmission- of-virus-causing-COVID-19-implications-for-ipc-precautionrecommendations (accessed 10 Jun 2020)
18. Burke RM, Midgley CM, Dratch A, et al. Active monitoring of persons exposed to patients with confirmed COVID-19: United States, January–February 2020. MMWR Morb Mortal Wkly Rep 2020.
19. Ong SW, Tan YK, Chia PY, et al. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 2020;323:1610.
20. Available https:// www. who. int/ docs/ default source/ coronaviruse/ situationreports/20200402-sitrep-73-COVID 19.pdf?sfvrsn=5ae25bc7_6 (accessed 10 Jun 2020)
21. Donnelly CA, Ghani AC, Leung GM, et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in hong kong. Lancet 2003;361:1761–6.
22. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 2020;323:1488–94.
23. Cheung KS, Hung IF, Chan PP, et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the hong kong cohort and systematic review and meta-analysis. Gastroenterology 2020;
24. Available https://www.webmd.com/lung/COVID19-digestive-symptoms (accessed 15 Jun 2020)
25. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledgehttps://www.mdpi.com/2077-0383/9/6/1753/htm
26. Bhadra S, Jiang YS, Kumar MR, et al. Real-time sequence-validated loop-mediated isothermal amplification assays for detection of Middle East respiratory syndrome coronavirus (MERS-CoV). PLoS One 2015;10:e0123126.
27. Chan JF, Choi GK, Tsang AK, et al. Development and evaluation of novel real-time reverse transcription-PCR assays with locked nucleic acid probes targeting leader sequences of human-pathogenic Coronaviruses. J Clin Microbiol 2015;53:2722–6.
28. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020;9:386–9.
29. 46 Fang Y, Zhang H, Xie J, et al. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology 2020.
30. Lagier, J.C.; Colson, P.; Tissot Dupont, H.; Salomon, J.; Doudier, B.; Aubry, C.; Gouriet, F.; Baron, S.; Dudouet, P.; Flores, R.; et al. Testing the repatriated for SARS-Cov2: Should laboratory-based quarantine replace traditional quarantine? Travel Med. Infect. Dis. **2020**. [CrossRef]
31. Won, J.; Lee, S.; Park, M.; Kim, T.Y.; Park, M.G.; Choi, B.Y.; Kim, D.; Chang, H.; Kim, V.N.; Lee, C.J.; et al. Development of a laboratory-safe and low-cost detection protocol for SARS-CoV-2 of the coronavirus disease 2019 (COVID-19). Exp. Neurobiol. **2020**. [CrossRef]
32. Ai, T.; Yang, Z.; Hou, H.; Zhan, C.; Chen, C.; Lv, W.; Tao, Q.; Sun, Z.; Xia, L. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases. Radiology **2020**. [CrossRef]
33. Bai, H.X.; Hsieh, B.; Xiong, Z.; Halsey, K.; Choi, J.W.; Tran, T.M.L.; Pan, I.; Shi, L.B.; Wang, D.C.; Mei, J.; et al. Performance of radiologists in di_erentiating COVID-19 from viral pneumonia on chest CT. Radiology **2020**. [CrossRef]
34. Betron M, Gottert A, Pulerwitz J, Shattuck D, Stevanovic-Fenn N. Men and COVID-19: Adding a gender lens. Global Public Health. 2020;15:1090-2. doi: 10.1080/17441692.2020.1769702. PubMed PMID: 32436422.
35. Hannah MF, Bajic VB, Klein SL. Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. Brain, Behavior, and Immunity. 2008;22:503-16. doi: 10.1016/j.bbi.2007.10.005. PubMed PMID: 18053684.
36. Cohen J. "Wuhan seafood market may not be source of novel virus spreading globally". ScienceMag American Association for the Advancement of Science. (AAAS).