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Coronavirus Host Divergence and Novel Coronavirus (Sars-CoV-2) Outbreak

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Abstract

SARS-CoV-2 (novel coronavirus, nCoV-2019) outbreak started in December 2019 in China has created a public health concern all around the world. Since infected patients transported out of China, the outbreak status was quickly changed into pandemic. Comparison of available genome sequences of the virus strains enlightened most questions as the cell receptors (ACE2) responsible for the virus tropism which determines possible organs and tissues to be affected by the virus as well as possible involvement of the age groups and host diversity. SARS-CoV-2, new member of Coronaviridae shares some clinical and epidemiological aspects similar to previous high pathogenic human coronavirus, SARS-CoV, existed in 2002. The most outstanding property of SARS-CoV-2 is high transmission rate (reproduction number, R0 ~ 3.58) between suspected and susceptible people. While bats are pointed as the original host and pangolins as an intermediate host, possibility of other species contribution is still unknown. According to recent data, reptiles i.e. snakes seem to be out of the group for possible intermediate hosts. Also there is no data supporting involvement of domestic animals even pets or food producing in the infection spectrum. This review summarizes the key findings of ongoing pandemia since the day disease existed. Molecular divergences now show that disease agent evolved into two types (S and L). Mutations and natural selections besides recombination will still continue to be the common feature of coronaviruses. Though implementation of common global measures and treatments other than rapid sharing the information will contribute prevention efforts and reducing the number of losses.

1. The Coronaviruses

Coronaviruses (CoVs) belong to *Orthocoronavirinae* subfamily in the virus family Coronaviridae. *Orthocoronavirinae* subfamily contains four genera *(Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus)* which all consisted of viruses that are important for medical and veterinary medicine. (1,2) Coronaviruses have been well known since mid-1960, and are generally related viruses targeting epitellial cells in respiratory and gastrointestinal tract. Alphacoronaviruses and betacoronaviruses usually infect various mammalian species but gamma and delta coronaviruses usually infect birds and fish. (3) Canine coronavirus, feline coronavirus and also commonly seen human coronaviruses 229E and NL63 belong to alphacoronavirus. More than 60 coronavirus species which infect bats have been detected where most of these viruses state in the betacoronavirus genus. Bovine coronavirus (BCoV) and some widely seen or pathogenic human coronaviruses like OC43, HKU1, SARS-CoV and MERS also take place in betacoronavirus (Figure 1). (1,2)

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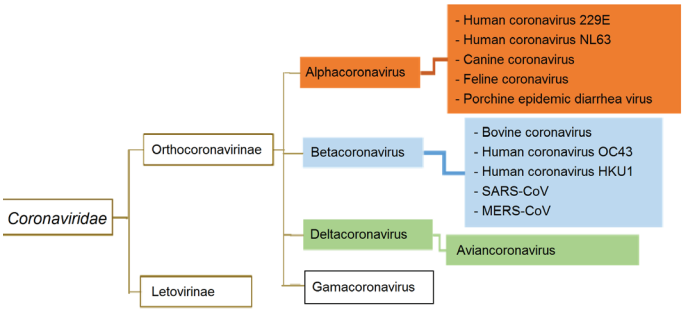

Figure 1. Classification status of important animal coronaviruses

CoV virions are in enveloped helically symmetric structure, contaning a single-stranded non-segmented RNA genome ranging between 26 to 32 kb which is the largest known RNA genome (Figure 2). (1) The CoVs genome comprises of four structural proteins; E (envelope protein), M (membrane protein), N (nucleocapsid protein), and S (spike protein). In adition, some betacoronoviruses have membrane-anchored HE (hemaglutinin-esterase) protein. The S glycoprotein, though to be strongly related to virus tropism, is associated with binding to cell surface receptors, fusion and cell entry. So, the S protein is the main target of neutralizating antibodies related to protective immunity. (1,2) Coronaviruses (CoV) have diverse host ranges in the nature and often can cross the host species barrier. (4) Possible genetic/antigenic changes in the S protein, not only in one unit but also in different domains of the viral genom are associated with tropism. Though cross host species interactions are correlated with the alterations and interactions between the different regions of S protein. (2) It is stated that, most of human coronaviruses are originated from bat coronaviruses with a direct or by intermediate host trans mission. (2)

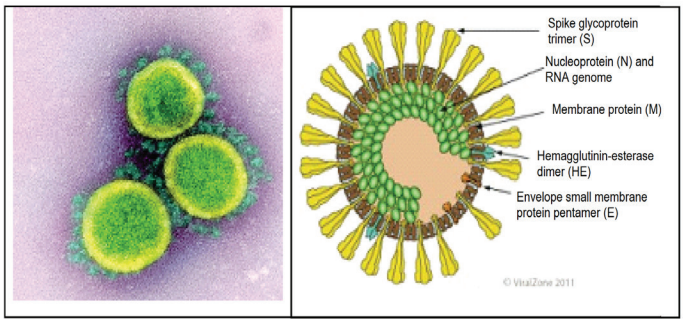

Figure 2. Electron microscobic image of SARS-CoV-2 (left) and schematic diagram for coronavirus stucture (right).(5,6)

2. Recent emergence of zoonotic coronaviruses

In 2002, Severe Acute Respiratory Syndrome virus (SARS-CoV) had an epidemic importance which started in South of China. (7) The virus mainly passed through horseshoe bats (Rhinolophus sinicus) and intermediate hosts were exerted as civet cats *(Paguma larvata)*, raccoon dogs *(Nyctereutes procyonoides)* and Chinese ferret badgers *(Melogale moschata).* (8,9) SARS-CoV led to approximately 800 human deaths, 8098 confirmed cases and had global effect with a fatality rate of 9.6%. The basic reproduction number (R_0) for SARS coronavirus (now called SARS-CoV-1) was between 2.7-3.4. (7,10,11) After a decade of period (from 2012 to 2013), a new zoonotic coronavirus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) existed in the Middle East, Europe and Africa. MERS-CoV had caused 2494 confirmed human cases and 858 deaths in 27 countries. (7) Pronounced fatality rate was 34.4% (7) which was 3.6 times higher than SARS-CoV-1 while the R_0 value was moderately low (0.8 – 1.3) which led to a slow dissemination among human populations. (11) MERS-CoV also had a zoonotic transmission evaluation; originated from Pipistrellus bat-CoV HKU5 (3,12) but dromedary (Arabian) camels were determined as the intermediate host. (13) Besides these viruses detected in the last decade, phylogenetic analysis showed possible relation of HCoV-OC43 with another animal coronavirus bovine coronavirus (BCoV). (14)

3.Human coronaviruses and epidemiology of SARS-CoV-2

So far, six known human coronaviruses were classified with their pathogenicity. Human coronavirus HCoV-229E, HKU1, OC43 and NL63 defined as low pathogenic and cause mild respiratory diseases, while high pathogenic human coronaviruses including SARS-CoV-1 and MERS-CoV, which cause fatal pneumonia. (13) Recently, in December 2019, a respiratory illness was identified in Wuhan, China. In a one month period, World Health Organization (WHO) has declarated global emergency, named the disease as COVID-19 and causative agent as SARS-CoV-2 based on the genetic relationship to previous virus obtained in SARS cases. (15) Soon after, virus was classified in *Sarbecovirus* subgenus. According to whole genom analysis this virus was not closely similar to SARS-CoV-1(79%) and MERS-CoV (50%) (16) (Figure 3), but prevalence of novel coronavirus cases were uncomparably higher than SARS-CoV-1 outbreak occured between 2002 and 2003 (Table 1). SARS-CoV-2 infection spreaded across the world and infected cases rose dramatically (Figure 4, 5, 6). According to the 63th report of WHO (23 March 2020) 332930 individuals have been infected with 14510 deaths and the disease has spread to 193 country or territories (Table 2) (17). Entirely most of the cases were from China with 81601 patients, while starting in Italy the Europe forged ahead. Travel related cases had a major role in global spread and there are 251329 cases outside the China; mostly in Italy, Iran and Spain about 59138 , 21638 and 28572 cases respectively. It has a reported fatality

rate of 4.2 % at global scale but, as the spread of the infection is closely related to local biosecurity involvement, the fatalily rate also changes by country. Currently, the estimated fatality rate is 4% in China and 4.3% outside China, extreme fatality is also possible as in Italy (9.2% according to WHO- Situation report 63). (17) Besides, most of the fatality rates were detected in male and elder patients. (18)

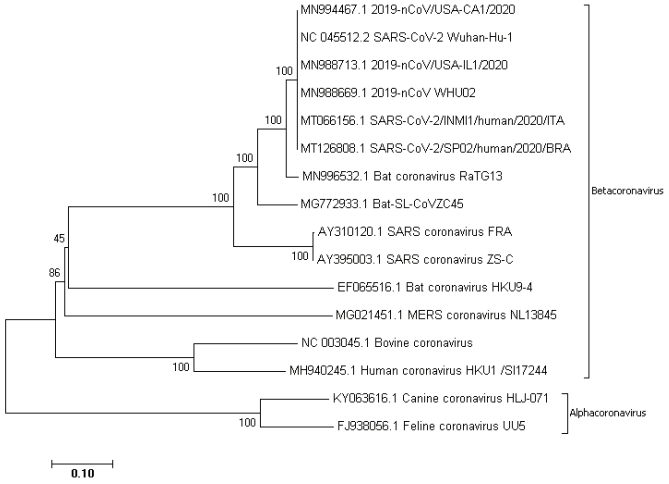

Figure 3. Phylogenetic analysis based on an alignment of the whole genome sequences of novel coronavirus and some other representative coronaviruses.

Properties	SARS-CoV-1	SARS-CoV-2
Original host	*Rhinolophus sinicus*	*Rhinolophus affinis* (Possible)
İntermediate host	Civet cats	Pangolin(Possible)
Species pathogen	β Coronavirus	β Coronavirus
Total genome lenght	29,751	29903
Case fatality rate	9.6%	4.2%
Propagation mode	Droplets or close contacts	Droplets or close contacts
Diagnostic methods	RT-PCR, rRT-PCR, RT-LAMP, rRT-LAMP	RT-PCR, rRT-PCR, RT-LAMP, rRT-LAMP

Table 1. Comparison of SARS-CoV-1 and SARS-CoV-2 properties.

*Table was constituted according to the data described. (15,17,19)

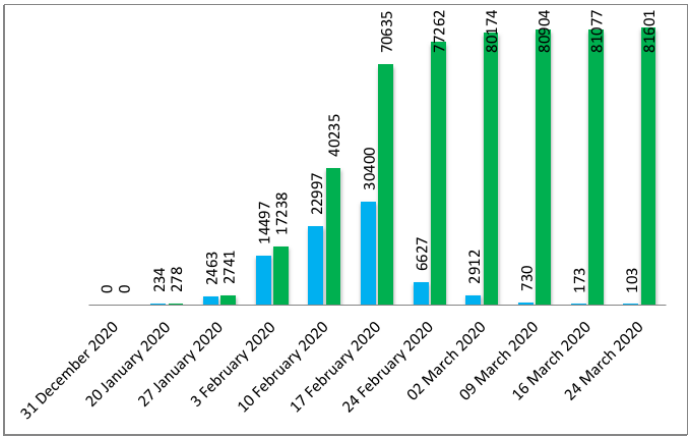

Figure 4. Temporal comparison of case distribution detected in China between 31 December 2019 – 24 March 2020 (Who situation reports); Blue line: new cases, Green line: Cumulative case numbers.

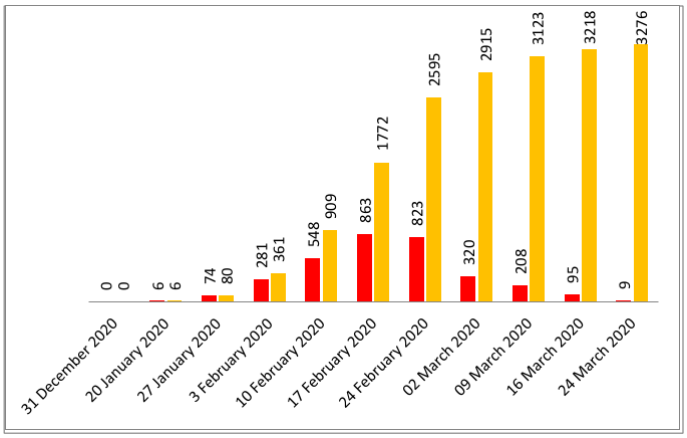

Figure 5. Temporal comparison of death case distribution detected in China between 31 December 2019 – 24 March 2020 (Who situation reports); Red line: New cases, Yellow line: Cumulative death cases.

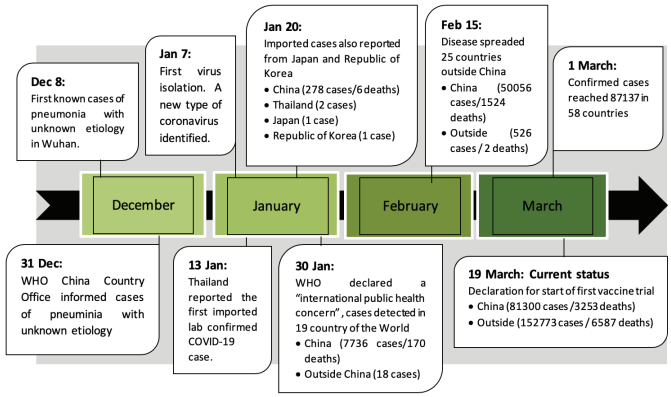

Figure 6. Timeline for COVID-19 pandemia, 2020

Region	Confirmed case number	Death case number
Western Pacific Region	95637	3473
European Region	171424	8743
South-East Asia Region	1776	58
Eastern Mediterranean Region	25375	1741
Region of Americas	37016	465
African Region	990	23
Globaly	332930	14510

Table 2. Current Global distribution of SARS-CoV-2 (21 March 2020). (17)

The basic reproduction number (R_0), is a term which defines the number of people can be infected by one person who is infected and shares the virus. If the R_0 is less than 1 the outbreak will disappear in a responsible period. But, if the it is over 1 ($R_0 \geq 1$) then the virus will spread in population. Thus the higher R_0 value represents the higher dissemination rate. For SARS-CoV-2 while the estimated R_0 value was 2.2 (20),calculated (occurred) value is 3.58 from person to person and 2.3 from reservoir host to person with a simulating model which identifies epidemic growth rate. (11) Current strategies are focused on biosecurity and isolation as preventive measure to decrease the R_0 value.

Recent studies suggests that the transmission came forward from respiratory droplets and direct contact. (18) Due to the rapid transmission between infected and non-infected individuals, asymptomatic patients are suggested as to be the main source for human cases. (21) Incubation period of this highly contagious coronavirus was estimated between 2 and 14 days (average 4-8) and survalience time in the air is determined as 2 hours. (22,23)

SARS-CoV-2 was found more stable on plastic and stainless steel than on copper and cardboard. Infectious virus titer of $10^{5.25}$ $TCID_{50}$ had decreased to $10^{3.25}$ to $10^{2.7}$ $TCID_{50}$ and had remained viable for 3 hours. In addition, the half-life of SARS-CoV-2 had detected with median estimates of approximately 1.1 to 1.2 hours in aerosols, 5.6 hours on stainless steel and 6.8 hours on plastic. Depending on the inoculum, as including proteinous sources, shed virus can remain viable and infectious on surfaces up to days. Although it was stated that the variations between the experiments and the other effective factors like amount of viral load can cause standart deviations in the results, mentioned study points out that those of results may also indicate possible transmission routes by aerosol or fomites of SARS-CoV-2. (24) According to comperative data, different human coronavirus strains can remain infectious on different types of materials starting from 2 hours up to 9 days. Besides it was announced that related coronaviruses can be inactivated by ethanol (71%) , hydrogen peroxide (0.5%), and sodium hypochlorite (0.1%) in 1 minute. (25)

4. Cellular receptors for virus entry

For cross-species transmission, cellular tropism which atributes to host cell susceptibility, permissivity of the host cell and accesibility of the susceptible host cell and innate immune response plays crucial roles. (2) For coronaviruses, adaptation to a new host is mainly associated with the structer and function of glycoprotein S. Molecular studies showed that glycoprotein S can bind to the host receptors with their receptor binding domains (RBDs). Although its mechanism has not yet been discovered, both HCoV-NL63 (alphacoronacirus) and SARS-CoV (betacoronavirus) use the angiotensin-converting enzyme-2 (ACE2) molecule as a host cell receptor. (2,26) It is reported that the SARS-CoV-2 also uses the ACE2 receptor associated with the coronavirus spike (S) glycoproteins, similar to SARS-CoV-1. (27) Afterwards, scientists focused on the structure of ACE2 cell receptor, to investigate potential routes, finding intermediate or resistent hosts for SARS-CoV-2 infections.

In addition to the alveolar cells of the lung, upper oesophagus, ileum, colon, kidney, bladder, urothelial and myocardial cells; a high ACE2 expression is also observed in epithelial cells of tongue and claimed to contain high risk route of the infection. (28) From another study based on online data bases, high expression of ACE2 in renal tubular cells exhibited and indicated as a causative reason of abnormal renal function which was found about 10% of the patients infected with SARS-CoV-2 in addition to respiratory system damage. (29) At the same study high expression of ACE2 was also found in testicular cells and suggested that SARS-CoV-2 may have a pottenital of testicular damage. (29) Moreover ACE2 receptor expression was also recognized in human cornea and conjunctival tissues, which is correlated with the conjunctivitis identified in COVID-19 patients. But the exact relation of SARS-CoV-2 and ocular infections are still an issue that has to be clarified. (30)

5. Possible animal source for SARS-CoV-2

The existence of novel coronavirus is postulated to be linked with Huanan seafood Wholesale Market. (22) Although in early stages of the outbreak; before human to human transmission was confirmed (31), it was suggested that babboo rats, raccons or snakes which are sold in the market could be the origin of the virus. (20) Following whole genome sequencing which showed 96.2% similarty, the SARS-CoV-2 shown to be corelated to BatCoV RatG13 of bats (SARS-like bat coronavirus). (20,32) Although the main host had been indicated, questions remained if there is an intermediate host between bats and human as it was case for SARS-CoV and MERS-CoV.

Sequence and structural analyses of ACE2 among human, non-human primates (gibbon, green monkey, macaque, orangutan and chimpanzee), domestic animals (cat, dog, bovine, sheep, goat, swine, horse and chicken), wild animals (ferret, civetand chinese horseshoe bat) and rodents (Mouse and rat) suggest that SARS-CoV-2 may not infect chicken, while non-human primates may be intermediate host for transmission. (33) Analysis with binding model of S protein (RBD region) and ACE2 showed that pangolins, snacks and turtles may act as the potential intermediate hosts. (13) But in a later study, intermediate host was suggested more likely to be a warm-blood vertebrate, than snakes.(34) In a recent study (35) one coronavirus isolate detected from Malayan pangolins exhibited a high sequence identity (100 % in E gene, 98.2% in M gene, 96.7% in N gene and 90.4% in S gene,hence it was suggested that SARS-CoV-2 should be arised as a result of recombination between Pangolin-CoV-like virus and Bat-CoV-RatG13-like virus. Besides, nasal and oral swab samples from a dog kept in the same house with a confirmed COVID-19 patient were tested weak (doubtful) positive for SARS-CoV-2. (35) No specific clinical signs were observed in the suspected dog. Thus, it could be speculated for this dog to receive the virus by aerosols from the owner during the high level of virus shedding period.

6. Susceptibility of Asian people

Both SARS-CoV-1 and SARS-CoV-2 epidemics spread out from China, East Asian were suspected to be more susceptible to the coronaviruses. Though; ACE2 expression had also under debated for the demographic predictions of SARS-CoV-2 infections. In a study which focused on single-cell RNA sequencing (RNAseq) analysis, it was pronounced higher expression at Asian males and suggested ACE2 gene polymorphism could be the reason for higher expression levels of ACE2 in East Asian. But this study, had not exhibited a genetic support of Coronavirus S protein resistant mutants in different populations. (37) Contrary to that results, another study investigated genetic variation data of ACE2, retrieved from the 1000 Genome; showed that average expression level of ACE2 in Asian is not significantly different from African or European ancestry. (7) A positive correlation between receptor expression and increasing age (middle or older age adults) was demonstrated in the same study (7) which describes higher detected susceptibility at elderly people.

7. Fundementals of diagnosis

Alongside the genomic sequence and receptor modeling studies, isolation of SARS-CoV-2 contributes to development of serological and rapid diagnostic tests. Mostly SARS-CoV-2 studies applied by using VeroE6, Huh7, or human airway epithelial cells. (16,39) SARS-CoV-2 propogation differences in cell types were investigated. Human adenocarcinoma cells (A549), human liver cells (HUH7.0), human embryonic kidney cells (HEK-293T), Vero E6, Vero CCL81 and big brown bat kidney cell lines (EFK3B) were used to compare virus isolation at nasopharyngeal (NP) and oropharyngeal (OP) swab specimens from a COVID-19 confirmed patient. Higher replication titer of SARS-CoV-2 was shown on VeroE6 and VeroCCL81 cell lines with CPE detection at 2nd day (d) postinfection (p.i.). (40) In another study which compares different cell lines in terms of proteolytical activation by TMPRSS2 (Transmembrane protease serine 2, enzyme that is related with coronavirus S proteins cleavage at the S1/S2 cleavage site in infected cells); revailed ~10-fold higher expression of TMPRSS2 messenger RNA in VeroE6/TMPRSS2 cells compared to normal human lung tissue and other human cell lines. Detected CPEs were described as detachment/floating and syncytium formation 2 or 3 d p.i. (Figure 7), and VeroE6/TMPRSS2 cells were determined highly susceptible for SARS-CoV-2 isolation than Vero, Calu-3, and A549 cell. (41)

Genomic sequences of SARS-CoV-2 isolates obtained from different human patients shared 99.9% identity, indicating low rate of mutational changes in human hosts at least at entire part of the outbreak. It is also though that the occurrence of the virus, was not mosaic consisting and could be from one

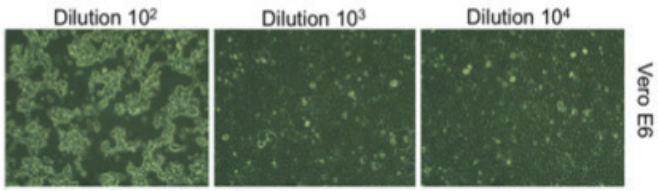

Figure 7. SARS-CoV-2 propagation on VeroE6 cell culture. (40)

source. (16,39) Recently, SARS-CoV-2 strains evolved into two types (L ans S types) by demonstration of two SNPs with population genetic analysis. (42) According to evolutionary analyses it is suggested that S type is more ancient and L type is much prevalent and agressive. But if L type evolved from S type in human or in the intermediate host is currently unknown.

For the early and rapid detection of infected patients, quantitative real-time polymerase chain reaction (qRT-PCR) assays were evaluated. (43) Molecular diagnosis of novel coronavirus mostly based on N, E, ORF1ab, ORF1b-nsp14, and S genes or multiple targets for Pan-coronavirus detection. (44)

The virus has been detected in bronchoalveolar-lavage, saliva, sputum, nasopharyngeal swabs, and throat. (45,46,47,48) Peaked viral load at throat swab and sputum was reported at 5-6 days after onset of the symptoms. (49) Beside the respiratory tract samples; urine, stool and anal swabs were also found positive with qRT-PCR. (49,50) Though it is estimated that SARS-CoV-2 can invade into urinary, hematological and digestive system other than respiratory system. (50) This recomendation matchs up with ACE2 expression researchs of ileum, colon, kidney and bladder. (28,29) Interestingly; in a patient, viral load was higher in anal swab than in oropharyngeal swab. (50) SARS-CoV-2 detected by RT-PCR in tear and conjunctival secretions from positive patients (51,52). Also in rhesus macaques, COVID-19 infection was generated by conjunctival inoculation to investigate efficiency of ocular transmission. (53) But virus positivity rate in tears and conjunctival samples is very low contrast to other samples. Even though it is still recommended to take precautions to avoid ocular transmission, the chance of entry by ocular tissues as well as ocular infection rate is not certain. (54)

8. Antivirals

Since the start of SARS-CoV-2 outbreak, studies have focussed on antivirals and vaccine development. Different antivirals are being tested in vitro and in animal models according to the experiences obtained from other viral infection, as well as previous outbreak of SARS-CoV, mainly because SARS-CoV-1 and SARS-CoV-2 commonly use ACE2 as cell receptor. But referring previous studies, for complete protection, it is pronunced that serum neutralizing antibodies could be insufficient alone. Hence, although more detailed data are required, stimulation both of neutralizing antibody response and T cell

response is recomended for novel vaccine formulations. (55) Among many recommended antiviral drug candidates; ACE2-based peptide, 3CLpro inhibitor, novel vinylsulfone protease inhibitor, remdesivir and chloroquine revealed as effective in the control of CoVID-19. (18) Articles and practices are standing on three drugs; Remdesivir, Favipiravir and chloroquine. (18,56,57) Remdesivir and chloroquine found highly effective in the control of SARS-CoV-2 in vitro (57), and also significant improvement in chest imagining and shorter viral clearance had recognized by using Favipiravir (56). But still there has been no proven effective treatment or vaccine when this paper is prepared.

9. Conclutions

According to WHO's declaration, it is the sixth publich health concern after H1N1, polio, Ebola in West Africa, Zika and Ebola in the Democratic Republic of Congo. (18) Hopefully, in a study which investigates relation between temperature/ humidity and doubling time of cases; positive correlation with temperature and inversely with humidity was indicated. Though; reduced aggression of SARS-CoV-2 at spring and summer in the north hemisphere was suggested. (58) But as it is emphasized before, zoonotic character was an evalutionary process of coronaviruses and it will also occur in the future. (2) Current novel coronavirus outbreak was unpredicted but luckly sequence data, clinical manifestations and case rates defined and reported immediately. Despite global cooperation, there are many issues stays unclear. But the outcomes enriched from this pandemia will contribute to all the countries for following /other viral epidemics in the future. Conclutions on biodefence /biowoepon potentional of SARS-CoV-2 outbreak widely discussable on political and some other fields is not available to be verified.

References

1. Payne S. Family Coronaviridae. In: Payne S. eds. Viruses (1st ed). United Kingdom; Academic Press;2017:149-158.

2. Hulswit RJ, de Haan CA, Bosch BJ. Coronavirus Spike Protein and Tropism Changes. Adv Virus Res. 2016(96):29-57.

3. Chan JF, Lau SK, Woo PC. The emerging novel Middle East respiratory syndrome coronavirus: the "knowns" and "unknowns". J Formos Med Assoc. 2013;112:372-381.

4. Chan JF, To KK, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 2013, 21(10):544-55.

5. National Institute of Allergy and Infectious Disease. New Images of Novel Coronavirus SARS-CoV-2 Now Available. 2020; https://www.niaid.nih.gov/news-events/novel-coronavirus-sarscov2-images

6. Viralzone. Coronaviridae. Aviable in 2020; https://viralzone.expasy.org/30?outline=all_by_species

7. Han Q, Lin Q, Jin S, You L. Recent insights into 2019-nCoV: a brief but comprehensive review. J Infect. 2020; S0163-4453(20)30087-6. doi: 10.1016/j.jinf.2020.02.010.

8. Li W, Wong SK, Li F, Kuhn JH, Huang IC, Choe H et al. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. J Virol. 2006;80(9):4211–4219.

9. Lau SK, Woo PCY, Li KSM, Huang Y, Tsoi H, Wong BHLet al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. PNAS. 2005;102:14040-14045.

10. Cheng VCC, Lau SKP, Woo PCY, Yuen KY. Severe Acute Respiratory Syndrome Coronavirus as an Agent of Emerging and Reemerging Infection. Clin Microbiol Rev. 2007;20(4):660-694.

11. Chen TM, Rui J, Wang QP, Zhao ZY, Cui JA, Yin L. A mathematical model for simulating the phase-based transmissibility of a novel coronavirus. Infect Dis Poverty. 2020; https://doi.org/10.1186/s40249-020-00640-3

12. Woo PCY, Lau SKP, Li KSM, Tsang AKL Yuen K. Genetic relatedness of novel human group C betacoronavirus to Tylonyeteris bat coronavirus HKU4 and Pipistrellus bat coronavirus HKU5. Emerg Microbes Infec. 2012; 1e35. doi:10.1038/emi.2012.45

13. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. J Med Virol. 2020; doi: 10.1002/jmv.25726.

14. Vijgen L, Keyaerts E, Lemey P, Maes P, Van Reeth K, Nauwynck H, et al., Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. J Virol. 2006; 80 (14):7270–7274. http://dx.doi.org/10.1128/JVI.02675-05.

15. WHO Coronavirus disease 2019 (COVID-19), Situation Report – 52 https://www.who.int/docs/default-source/coronaviruse/20200312-sitrep-52-covid-19.pdf?sfvrsn=e2bfc9c0_2

16. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574.

17. WHO Coronavirus disease 2019 (COVID-19), Situation Report – 63 https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200323-sitrep-63-covid-19.pdf?sfvrsn=2176eb7a_2

18. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents. 2020;105924. doi: 10.1016/j.ijantimicag.2020.105924.

19. Xu J, Zhao S, Teng T, Abdalla AE, Zhu W, Xie L et al. Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. Viruses. 2020;12(2). pii: E244. doi: 10.3390/v12020244.

20. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. N Engl J Med. 2020; 10.1056/NEJMoa2001316.

21. Biscayart C, Angeleri P, Lloveras S, Chaves TDSS, Schlagenhauf P, Rodríguez-Morales AJ. The next big threat to global health? 2019 novel coronavirus (2019-nCoV): What advice can we give to travellers? - Interim recommendations January 2020, from the Latin-American society for Travel Medicine (SLAMVI). Travel Med Infect Dis. 2020; 33:101567.

22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497-506.

23. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-513.

24. van Doremalen N, Morris DH, Holbrook MG, Holbrook MG, Gamble A, Williamson BN et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020; https://dx.doi.org/10.1056/NEJMc2004973

25. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents J Hosp Infect. 2020;104: 246-251.

26. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003; 426(6965): 450-4.

27. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020; doi: 10.1038/s41564-020-0688-y.

28. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020; 12(8): 1-5.

29. Fan C, Li K, Ding Y, Lu W, Wang J. ACE2 expression in kidney and testis may cause kidney and testis damage after 2019-nCoV infection. 2020; https://doi.org/10.1101/2020.02.12.20022418.

30. Zhang X, Chen X, Chen L, Deng C, Zou X, Liu W et al. The infection evidence of SARS-COV-2 in ocular surface a single-center cross-sectional study. 2020; doi: https://doi.org/10.1101/2020.02.26.2002793.

31. Wang R, Zhang X, Irwin DM, Shen Y. Emergence of SARS-like coronavirus poses new challenge in China. J Infect. 2020;80(3):350-371.

32. Paraskevisa D, Kostakia EG, Magiorkinisa G, Panayiotakopoulosb G, Sourvinosc G, Tsiodrasd S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol. 2020;79:104212.

33. Li R, Qiao S, Zhang G. Analysis of angiotensin-converting enzyme 2 (ACE2) from different species sheds some light on cross-species receptor usage of a novel coronavirus 2019-nCoV. J Infect. 2020; https://doi.org/10.1016/j.jinf.2020.02.013

34. Zhang C, Zheng W, Huang X, Bell EW, Zhou X, Zhang Y. Protein structure and sequence re-analysis of 2019-nCoV genome does not indicate snakes as its intermediate host or the unique similarity between its spike protein insertions and HIV-1. 2020; doi: 10.1101/2020.02.04.933135.

35. Xiao K, Zhai J, Feng Y, Zhou N, Zhang X, Zou JJ et al. Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. 2020; doi: https://doi.org/10.1101/2020.02.17.951335.

36. OIE Follow-up report No. 1; 07/03/2020, COVID-19-Honkong https://www.oie.int/wahis_2/public/wahid.php/

Reviewreport/Review?page_refer=MapFullEventReport&reportid=33455 aviability;17.03.2020

37. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov. 2020;6:11.

38. Chen Y, Shan K, Qian W. Asians and other races express similar levels of and share the same genetic polymorphisms of the SARS-CoV-2 cell-entry receptor. 2020; doi:10.20944/preprints202002.0258.v1.

39. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; doi: 10.1038/s41586-020-2012-7.

40. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J et al. Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with 2019 Novel Coronavirus Disease, United States. J Emerg Infect Dis. 2020; 26(6), https://doi.org/10.3201/eid2606.200516

41. Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. P Natl Acad Sci Usa. 2020; 202002589; doi: 10.1073/pnas.2002589117

42. Tang X, Wu C, Li X, Song Y, YaoX, Wu X et al. On the origin and continuing evolution of SARS-CoV-2, Natl Sci Rev. 2020; nwaa036, https://doi.org/10.1093/nsr/nwaa036

43. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clin Chem. 2020; pii: hvaa029. doi: 10.1093/clinchem/hvaa029.

44. WHO- Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

45. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733. doi: 10.1056/NEJMoa2001017.

46. To KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, Chan JMC et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis. 2020; doi: 10.1093/cid/ciaa149.

47. Lin X, Gong Z, Xiao Z, Xiong J, Fan B, Liu J. Novel Coronavirus Pneumonia Outbreak in 2019: Computed Tomographic Findings in Two Cases. Korean J Radiol. 2020; 21(3):365-368.

48. Bastola A, Sah R, Rodriguez-Morales AJ, Lal BK, Jha R, Ojha et al. The first 2019 novel coronavirus case in Nepal. Lancet Infect Dis. 2020;20(3):279-280.

49. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis. 2020; doi: 10.1016/S1473-3099(20)30113-4.

50. Peng L, Liu J, Xu W, Luo Q, Deng K, Bingliang L et al. 2019 Novel Coronavirus can be detected in urine, blood, anal swabs and oropharyngeal swabs samples. 2020; https://doi.org/10.1101/2020.02.21.20026179.

51. Jianhua Xia MM, Jianping Tong MD, Mengyun Liu MM, Ye Shen MD. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. 2020; https://doi.org/10.1002/jmv.25725.

52. Zhou Y, Zeng Y, Tong T, Chen C. Ophthalmologic evidence against the interpersonal transmission of 2019 novel coronavirus through conjunctiva. 2020, https://doi.org/10.1101/2020.02.11.20021956.

53. Deng W, Bao L, Gao H, Xiang Z, Qu Y, Son Z et al. Rhesus macaques can be effectively infected with SARS-CoV-2 via ocular conjunctival route. 2020; doi: https://doi.org/10.1101/2020.03.13.990036.

54. Sun CH, Wang Y, Liu G, Liu Z. Role of the Eye in Transmitting Human Coronavirus: What We Know and What We Do Not Know. 2020; doi: 10.20944/preprints202003.0271.v2 (Preprint).

55. Poland GA. Another coronavirus, another epidemic, another warning. Vaccine. 2020; 38(10):v-vi. doi: 10.1016/j.vaccine.2020.02.039.

56. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. 2020;https://doi.org/10.1016/j.eng.2020.03.007

57. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M et al. Remdesivir and chloroquine effectively inhibit the recent emerged novel coronavirus (2019-nCoV) invitro. Cell Res. 2020;30(3):269-271.

58. Oliveiros B, Caramelo L, Ferreira NC, Caramelo F. Role of

temperature and humidity in the modulation of the doubling time of COVID-19 cases. 2020; doi: https://doi.org/10.1101/2020.03.05.20031872.