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PREDICTION OF POTENTIAL MUTATION OF CHICKEN CORONAVIRUS INTO FUTURE HUMAN CORONAVIRUS BASED ON SPIKE S1 GLYCOPROTEIN GENE

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ABSTRACT

The high mutation rates of the chicken coronavirus (IBV) cause economic threats to the poultry industry. However, the most dangerous situation is the likelihood of changing its sequences into human coronavirus (COVID-19-like virus). Therefore, in the present study we aimed to investigate the possibility of genetic mutation of IBV to COVID-19. Thus, the sequences of Spike (S1) Glycoprotein genes of both IBV and COVID-19 were aligned, analyzed and calculated to predict the possible changes that could happen in the sequences of S1. The results indicated that in the case of an independent function of probability of each cluster of S1 sequences, the potential mutation rate in the sequences of IBV to be as COVID-19 was equal to 1.87E-96. However, because the tendency for some sequence clusters of S1 gene was low or equal to zero, it is unattainable to mutate the chicken IBV into COVID-19 sequence. Furthermore, in case of the dependent function, the probability of assumed annual mutation to make IBV infectious for human may reach up to around 50% after about 260 years. As a conclusion, the mutating of chicken coronavirus into COVID-19-like virus is not impossible, but it might take a substantial period of time.

KEY WORDS: Chicken IBV, Coronavirus, COVID-19, Mutation, Statistical prediction.

INTRODUCTION

Based on the genetic characteristics, the coronavirinae family comprises four genera including Alpha-coronavirus, Beta-coronavirus, Gamma-coronavirus, and Delta-coronavirus. Amongst all RNA viruses, the RNA genome of the coronavirus is the largest ranging from (26 to 32 kb). In human, the 2019 novel coronavirus (2019-nCoV or SARS-CoV-2) has rapidly spread more than any other betacoronavirus (SARS-CoV and MERS-CoV). Nevertheless, the molecular evolution and mutation rate of this virus remained unclear. In late 2020 the COVID-19 pathogen has exposed to a number of mutations in several countries which made changes in its expression and impacts on the human body (WHO, 2020). Ji *et al*., (2020) suggested that the 2019-nCoV might seem to be a recombinant virus between the bat coronavirus and an unknown-origin coronavirus. It also indicated that the human 2019-CoV's genetic information is almost similar to the chicken coronavirus, while its codon usage is most similar bias to snakes. These findings reveal that a homologous recombination occurs in the 2019-nCoV might contribute in its cross-species transmission (Ji *et al*., 2020). In addition to humans, coronavirus can infect many different animal species, including chicken, bats, cattle, swine, horses, camels, dogs, rodents, rabbits, mink, snake, and other wildlife animals (WHO, 2003; Howley, 2013; MacLachlan and Dubovi, 2017). Structurally, the Alphacoronavirus group involves six members including human pathogens Cov229E and CoV-HKU1. While, the Betacoronavirus group includes the human pathogens CoV-OC43, SARS-CoV and MERS-CoV (Matoba *et al*., 2015; Myint, 1995; King *et al*., 2011; Lefkowitz *et al*., 2018). In poultry species, Infectious bronchitis virus (IBV) is the prototype species of the coronavirus family within the genus of Gamma coronavirus in the Nidovirales order, which is the type species of the coronavirus genus of the domestic chicken. It causes an acute and highly contagious disease which can cause substantial losses in the economy of the poultry industry worldwide

(Cavanagh 1997; Cavanagh and Naqi, 2003; Cavanagh 2005; Cook *et al*., 2012). IBV coronavirus is enveloped, non-segmented, single-stranded RNA's positive sense with the length around 27,608bp (NCBI Reference Sequence: NC_001451.1). The spike S1 protein of IB which is responsible for differentiation of IBV strains and acts as the prime aim of genotype description undergoes inhibitor of agglutination and stimulates neutralization of antibody. It has also a significant role in the attachment and entry of the virus into cells via cyanic acid receptors (Jackwood *et al*., 2012). However, for better designing of vaccination strategies to ensure greater protection against enzootic strains of IBV, it is important to well understand the epidemiological and changes that happen to the virus (Bourogâa, *et al*., 2014). A novel one, COVID-19, which has been first reported in China in December 2019, become a pandemic disease at 2020 (WHO, 2020). Because of the unknown behaviour of COVID-19 and similarity in the structure and pathogenesis of the majority of CoVs (St-Jean *et al*., 2004; Butler *et al*., 2006; Yuan *et al*., 2017), investigation of potential mutations in the other strains of viruses in coronavirinae family from other animals including avian species like chicken should be carried out and taken into account. Several variants and classic genotypes of IBV have been widely distributed in geographically different countries (De Wit *et al*., 2011). Some of these genotypes and serotypes have close relationships with some strains of vaccine, while some variants were found specific to their geographical locations (Bande *et al*., 2017). New variant strains are emerged due to the involvement of mutation and recombination processes in the variations of the genotype and phenotype of chicken IBV (Ennaji *et al.*, 2020). IBV and other coronaviruses are characterized by a high rate of mutation and a high rate of errors during the transcription of their genomes (Lai and Cavanagh, 1997). Therefore, the current study aimed to explore the possibility of genetic mutation of chicken's IBV to be COVID-19-like virus and its probable negative impact on the human being.

METHODOLOGY

The present study was conducted in Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq, at earlier period of 2021, where the epidemic COVID-19 has become more dangerous due to the consequent mutations; which may extended to other types of mutation or recombination with other pathogen strain in other organisms such as chicken. Accordingly, the aim of this investigation was to study the molecular comparison between chicken coronavirus and COVID-19 and the possibility of mutating IBV into the COVID-19-like pathogen. The current study focused on the comparison of Spike S1 gene sequence of both IBV and COVID-19, because it is considered the main entrance agent to infect the organism; and also the possibility of occurring mutations that may activate on IBV and /or recombinant with COVID-19 pathogen which may change the former into the later-like agent and consequently infecting the human body, using probability rules and statistical predicting model.

Similarity analysis

For sequence alignment, the complete sequences of the Spike S1 gene of some common available IBV strains and COVID-19 were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). Then, sequences of S1 of both coronaviruses (IBV and COVID-19) were aligned using the Geneious 8.0 software (Kearse *et al.*, 2012). The same software programs were used to compute the similarity correlations between sequences of the studied strains of coronaviruses.

Probability of mutation rate

Due to the unknown active sequence that may make the infection, so it was necessary to compute all possible probabilities of mutation rates of all sequence cluster of S1 gene (group of 10 successive nucleotides) sub-sequences. Some of such clusters have partial similarity with COVID-19 sequence. Therefore the probability of each cluster was calculated in order to compute the overall probability of mutation rate that may mutate the IBV into COVID-19-like pathogen. The data of alignment were analyzed in two trends; as multiplicative probability rule and as cumulative probability.

Statistical analysis

MSO Excel was used in computation according to the multiplicative probability rule which assumed independent effect of each cluster (no interaction between clusters), where the base number (4) represents the number of bases (nucleotides: A, G, C, T) powered by the number of hetero-bases than COVOD-19 sequence, which was calculated for each cluster alignment. Therefore, a total of 174 clusters, were analyzed (the number of homo-nucleotides and number of hetero-nucleotides of IBV was

compared to COVID-19 sequence) and computed to illustrate potential mutation of IBV into COVID-19-like sequence. All statistical calculation was performed focused only on the possibility of potential changes of each hetero-nucleotide within S1 of IBV to be mutated into homo-nucleotide in the S1 of COVID-19-like pathogen. This means we expected that the potential mutation would be happened only in the hetero-nucleotides, while the homo-nucleotides between IBV and COVID-19 remain conserved. The main formula used for determining the probability was as follow:

$$p = 1/power$$

Where: p= Probability; power = $4^{\wedge Hetero}$ (4= No. of nucleotide (bases) as constant, Hetero = No. of hetero-bases that vary between IBV strains and COVID-19).

Furthermore, to analyze the correlation coefficients between possible similarity items for IBVs and COVID-19, the SPSS software (SPSS 2019) was used. SPSS was also used to predict the future infection possibility model for humans based on dependent probability rule which assumed that each cluster mutation may affect another cluster function (i.e. protein function); where the cumulative probability of events for all clusters was predicted based on supposing annual cluster mutating from IBV into COVID-19-like virus. Also, as for future predicting it may take in consideration the half interval period of all cluster mutations for virtual random mutations that may affect the similarity to expect the human infection probability. However, ARIMA model is a traditional time series procedure, used for predicting linear tendencies in stationary data (ARIMA is a non-stationary model enabling the differencing of the data series resulting from the model by changing first-difference of yt). ARIMA was applied according to the following model:

$$\Delta y_t = c + a1\,\Delta y_{t-1} + a2\,\Delta y_{t-2} + \cdots + ap\,\Delta y_{t-p} + \varepsilon_t - \theta1\,\varepsilon_{t-1} - \theta2\,\varepsilon_{t-2} - \cdots - \theta q\,\varepsilon_{t-q}$$

Where: $\Delta y_t = y_t - y_{t-1}$ = the change in actual observations at time t; εt is the random error at the same time t; c and a are ARIMA model's parameters. ARIMA model assumes that the error tends to be zero and the variance is constant ($(\varepsilon t) = 0$ and $(\varepsilon t) = \sigma 2$); and satisfies the sequence of independently and identically distributed (iid). Moreover, ARIMA is followed by (p, d, q); the p and q coefficients represent the order of the Autoregressive (AR) and Moving-Average (MA), respectively, and d represents the level of differencing (Chakraborty and Ghosh 2020).

RESULTS

Similarity

Following sequence alignment using Geneious 8.0 (Kearse *et al*., 2012), the pairwise percentage identities between COVID-19 and virus strains Ma5, 4/91 and H120 of chicken's IBV, were 43%, 44% and 46%, respectively. While, higher identical sites were found between strains of IBV virus itself with a similarity of 78% between Ma5 and 4/91; 78% between H120 and 4/91 and 99.8% between Ma5 and H120 Table 1.

Table (1): Similarity correlation relationships (%) between virus strains of IBVs and COVID-19

	COVID19	Ma5	4/91
Ma5	43		
4/91	44	78	
H120	46	99.80	78

Analysis of the probability of similarity

The contrast among IBV strains and COVID-19 sequence may start from the nucleotide 232 up to nucleotide 1970, which equal to 1739 bases as illustrated in Figures 1- (a, b, c, d, e, f and g). Figure 1-a, shows the start point of Alignments at nucleotide 232 which is adenine (A) that common for all studied strains. This nucleotide point has additional base (G) for COVID-19 compared to other three strains of IBV, so the last strains need to mutate twice (once for inserting guanine-G; and the other to alter the sequence of the four bases A, G, C and T, when be hetero), which has inverse probability of bases number-b (b= 4 bases) powered by the number of hetero-bases -t ($1/b^{\wedge t}$), such as at the first cluster that equal to $1/4^{\wedge 4}$ (0.00390625) mutation rate, and so on Figure 1-a.

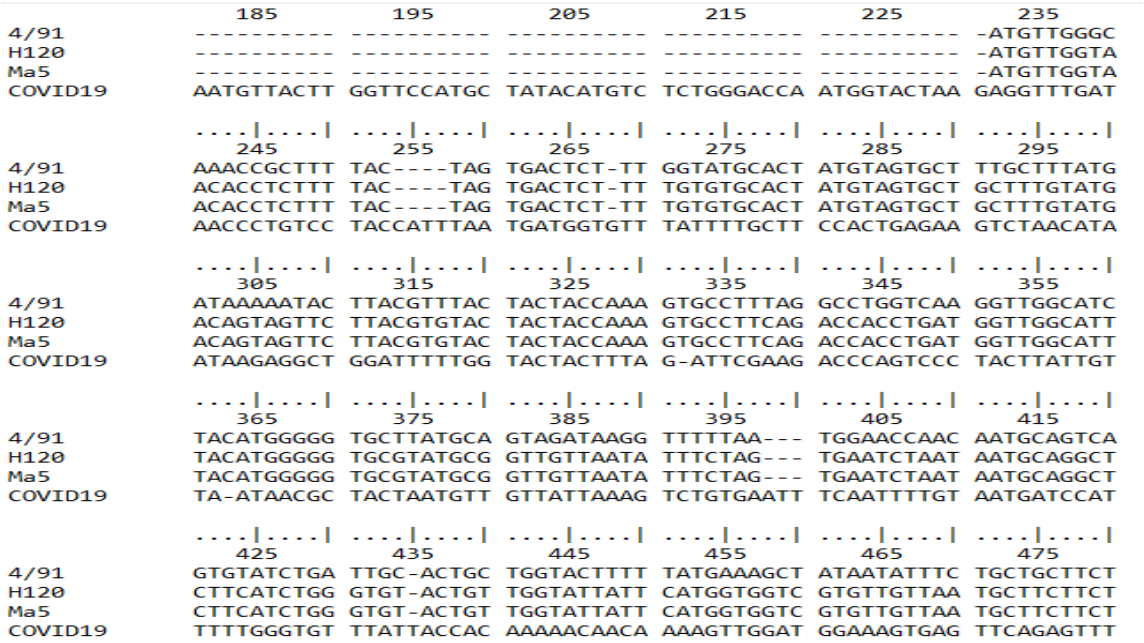

Fig. (1-a): First Partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

However, the probability of mutate rate changing IBV into COVID-19-like-virus from 25 sequence clusters of Figure 1- (a) is equal to 7.67046 E-93 as illustrated in Table 2. Fortunately, this numeric probability is very small. The same trend was conducted for the 23 sequence clusters alignment of Figure 1-b (the 585 cluster alignment was excepted) and so on for others. Thus, all other cluster alignments have been computed and illustrated in Table 3. Moreover, the probability of all clusters of sequences from the Figure 1-a, b, c, d, e, f and g are illustrated in Table 3.

The correlation coefficients between the items of mutation rate probability (Table 4) showed logically that the association between the numbers of hetero-bases of each cluster and the known bases number (4) powered by such hetero-bases number was positive (0.572) and highly significant (p<0.01); also, the correlation coefficient between the numbers of hetero-bases of each cluster and the probability of mutated IBV into like-COVID-19 sequence was negative (-0.542) and highly significant (p<0.01); this mean that each increasing in hetero-base number in the cluster reduces significantly the probability of mutating rate. However, the correlation coefficients between the other studied items were insignificant (p>0.05) as shown in Table 4.

Table (2): Calculation of hetero-nucleotides and computing the probability of mutation rate for the first 25 clusters alignment

Cluster No	Alignments	Total bases	Homo	Hetero	Power (4^{Hetero})	Probability (1/Power)	Cumulative Multiplicative probability
1	235	9	5	4	256	0.00390625	0.00390625
2	245	10	4	6	4096	0.000244141	9.53674E-07
3	255	6	5	1	4	0.25	2.38419E-07
4	265	10	6	4	256	0.00390625	9.31323E-10
5	275	10	3	7	16384	6.10352E-05	5.68434E-14
6	285	10	1	9	262144	3.8147E-06	2.1684E-19
7	295	10	3	7	16384	6.10352E-05	1.32349E-23
8	305	10	4	6	4096	0.000244141	3.23117E-27
9	315	10	0	10	1048576	9.53674E-07	3.08149E-33
10	325	10	7	3	64	0.015625	4.81482E-35

11	335	10	3	7	16384	6.10352E-05	2.93874E-39
12	345	10	3	7	16384	6.10352E-05	1.79366E-43
13	355	10	2	8	65536	1.52588E-05	2.73691E-48
14	365	10	5	5	1024	0.000976563	2.67276E-51
15	375	10	4	6	4096	0.000244141	6.5253E-55
16	385	10	7	3	64	0.015625	1.01958E-56
17	395	10	3	7	16384	6.10352E-05	6.22302E-61
18	405	10	5	5	1024	0.000976563	6.07716E-64
19	415	10	5	5	1024	0.000976563	5.93473E-67
20	425	10	3	7	16384	6.10352E-05	3.62227E-71
21	435	10	4	6	4096	0.000244141	8.84344E-75
22	445	10	1	9	262144	3.8147E-06	3.3735E-80
23	455	10	4	6	4096	0.000244141	8.23609E-84
24	465	10	2	8	65536	1.52588E-05	1.25673E-88
25	475	10	3	7	16384	6.10352E-05	**7.67046E-93**

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    485        495        505        515        525        535
4/91             GTAGCCATG- --ACAGTACC ACCTGCTGGT ATGTCTT--- GGTCAGTTTC ACAG--TTTT
H120             ATAGCTATG- --ACGGCACC GTCATCAGGT ATGGCTT--- GGTCTAGCAG TCAG--TTTT
Ma5              ATAGCTATG- --ACGGCACC GTCATCAGGT ATGGCTT--- GGTCTAGCAG TCAG--TTTT
COVID19          ATTCTAGTGC GAATAATTGC ACTTTTGAAT ATGTCTCTCA GCCTTTTCTT ATGGACCTTG

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    545        555        565        575        585        595
4/91             GTACAGCTCA TTGTAACTTC TCAGACTTTA CAGTGTTTGT ----------T ACGCATTGTT
H120             GTACTGCATA CTGTAACTTT TCAGATACTA CAGTGTTTGT ----------T ACACATTGTT
Ma5              GTACTGCATA CTGTAACTTT TCAGATACTA CAGTGTTTGT ----------T ACACATTGTT
COVID19          AAGGAAAACA GGGTAATTTC AAAAATCTTA GGGAATTTGT GTTTAAGAAT ATTGATGGTT

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    605        615        625        635        645        655
4/91             TTAAAAGTCA ACAAGGTAGT TGTCCATTGA CAGGTATGAT TCCTCAGAAT CATATTCGTA
H120             ACAAA----- -CATGTTGGG TGTCCTATAA CTGGCATGCT TCAACAGCAT TCTATACGTG
Ma5              ATAAA----- -CATGGTGGG TGTCCTATAA CTGGCATGCT TCAACAGCAT TCTATACGTG
COVID19          ATTTTAAAAT ATATTCTAAG CACACGCCTA TTAATTTAGT GCGTGATCTC CCTCAGGGTT

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    665        675        685        695        705        715
4/91             TTTCTGCTAT GAGATCTGGA TTTTTGTTTT ATAATTTAAC AGTTAGCGTA TCTAAATACC
H120             TTTCTGCTAT GAAAAATGGC CAGCTTTTTT ATAATTTAAC AGTTAGTGTA GCTAAGTACC
Ma5              TTTCTGCTAT GAAAAATGGC CAGCTTTTCT ATAATTTAAC AGTTAGTGTA GCTAAGTACC
COVID19          TTTCGGCTTT AGAACC--AT TGGTAGATTT GCCAATAGGT A-TTAACATC ACTAGGTTTC
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Fig. (1-b):. Second partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   725        735        745        755        765        775
4/91            CTAAATTTAA ATCGCTTCAA TGTGTTGGCA ATTCTACATC TG------TC TATTTAAATG
H120            CTACTTTTAA ATCATTTCAG TGTGTTAATA ATTTAACATC CG------TA TATTTAAATG
Ma5             CTACTTTTAA ATCATTTCAG TGTGTTAATA ATTTAACATC CG------TA TATTTAAATG
COVID19         --AAACTTTA CTTGCTTTA- CATAGAAGTT ATTTGACTCC TGGTGATTCT TCTTCAGGTT

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   785        795        805        815        825        835
4/91            GTGATCTTGT TTTCACTTCT AATGAAACAA CTCACGTTAC GGGTGCAGGC GTTTATTTTA
H120            GTGATCTTGT TTACACCTCT AATGAGACCA CAGATGTTAC ATCTGCAGGT GTTTATTTTA
Ma5             GTGATCTTGT TTACACCTCT AATGCGACCA CAGATGTTAC ATCTGCAGGT GTTTATTTTA
COVID19         GGACAGCTGG TGCTGCAGCT TATTATGTGG GTTATCTTCA ACCTAGGACT TTTCTATTAA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   845        855        865        875        885        895
4/91            AAAGTGGTGG GCCTGTAACT TATAAAGTTA TGAAAGAAGT TAAAGCCCTA GCCTACTTTA
H120            AAGCTGGTGG ACCTATAACT TATAAAGTTA TGAGAGAAGT TAGAGCCCTG GCTTATTTTG
Ma5             AAGCTGGTGG ACCTATAACT TATAAAGTTA TGAGAGAAGT TAGAGCCCTG GCTTATTTTG
COVID19         AATATAATGA AAATGGAACC ATTACAG--A TGCTGTAGAC TGTGCACTTG ACCCTCTCTC

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   905        915        925        935        945        955
4/91            TTAATGGTAC CGCACA---A GAGGTTATTT TATGTGATAA CTCACCTAGA GGTTTGCTTG
H120            TTAATGGTAC TGCACA---A GATGTTATTT TGTGTGATGG GTCACCTAGA GGCTTGTTAG
Ma5             TTAATGGTAC TGCACA---A GATGTTATTT TGTGTGATGG GTCACCTAGA GGCTTGTTAG
COVID19         AGAAACAAAG TGTACGTTGA AATCCTTCAC TGTAGAAAAA GGAATCTATC AAACTTCTAA
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Fig. (1-c): Third partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   965        975        985        995       1005       1015
4/91            CAT------- --GTCAGTAT AAC--ACTGG TAATTTTTCA GATG------ GATTCTACCC
H120            CAT------- --GCCAGTAT AAT--ACTGG CAATTTTTCA GATG------ GCTTTTATCC
Ma5             CAT------- --GCCAGTAT AAT--ACTGG CAATTTTTCA GATG------ GCTTTTATCC
COVID19         CTTTAGAGTC CAACCAACAG AATCTATTGT TAGATTTCCT AATATTACAA ACTTGTGCCC

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1025       1035       1045       1055       1065       1075
4/91            TTTTACTAAT TCTTCTTTAG TTAAGGATAG GTTTAT-TGT ATATCGAGAA AGTAGCACTA
H120            TTTTACTAAT AGTAGTTTAG TTAAGCAGAA GTTTAT-TGT CTATCGTGAA AATAGTGTTA
Ma5             TTTTACTAAT AGTAGTTTAG TTAAGCAGAA GTTTAT-TGT CTATCGTGAA AATAGTGTTA
COVID19         TTTTGGTGA- AGTTTTTAAC GCCACCAGAT TTGCATCTGT TTATGCTTGG AACAGGAAGA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1085       1095       1105       1115       1125       1135
4/91            ACACTA-CTT TAGAGTTAAC TAATTTCACT TTTACTAATG TAAGTAATGC TTCTCCTAAT
H120            ATACTA-CTT TTACGTTACA CAATTTCACT TTTCATAATG AGACTGGCGC CAACCCAAAT
Ma5             ATACTA-CTT TTACGTTACA CAATTTCACT TTTCATAATG AGACTGGCGC CAACCCAAAT
COVID19         GAATCAGCAA CTGTGTTGCT GATTATTCTG TCCTATATAA TTCCGCATCA TTTTCCACTT

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1145       1155       1165       1175       1185       1195
4/91            TCAGGTGGCG TTGATACTTT CCAATTATAT CAAACACATA CTGCTCAG-- GATGGTTAT-
H120            CCTAGTGGTG TCCAGAATAT TCAAACTTAC CAAACACAAA CAGCTCAG-- AGTGGTTAT-
Ma5             CCTAGTGGTG TCCAGAATAT TCAAACTTAC CAAACACAAA CAGCTCAG-- AGTGGTTAT-
COVID19         TTAAGTGTTA TGGAGTGTCT CCTACTAAAT TAAATGATCT CTGCTTTACT AATGTCTATG
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Fig. (1-d): Fourth partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19**)**

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1205       1215       1225       1235       1245       1255
4/91            ---------- TATAATTTTA ATTTATCATT TCTGA---GT AGTTTTGTG- -----TATAA
H120            ---------- TATAATTTTA ATTTTTCCTT TCTGA---GT AGTTTTGTT- -----TATAA
Ma5             ---------- TATAATTTTA ATTTTTCCTT TCTGA---GT AGTTTTGTT- -----TATAA
COVID19         CAGATTCATT TGTAATTAGA GGTGATGAAG TCAGACAAAT CGCTCCAGGG CAAACTGGAA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1265       1275       1285       1295       1305       1315
4/91            ACCAT-CTGA TTTTATGTAT G--------- --GGTCATAC CACCCAAATT GTAATTTTAG
H120            GGAGT-CTAA TTTTATGTAT G--------- --GATCTTAT CACCCAAGTT GTAATTTTAG
Ma5             GGAGT-CTAA TTTTATGTAT G--------- --GATCTTAT CACCCAAGTT GTAATTTTAG
COVID19         AGATTGCTGA TTATAATTAT AAATTACCAG ATGATTTTAC AGGCTGCGTT ATAGCTTGGA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1325       1335       1345       1355       1365       1375
4/91            ACCAGAG-AA TATTAATAAT --GGCTTATG GTTTAATTCA TTATCTGTGT CACTTACTTA
H120            ACTAGAA-AC TATTAATAAT --GGTTTGTG GTTTAATTCA CTTTCAGTTT CAATTGCTTA
Ma5             ACTAGAA-AC TATTAATAAT --GGTTTGTG GTTTAATTCA CTTTCAGTTT CAATTGCTTA
COVID19         ATTCTAACAA TCTTGATTCT AAGGTTGGTG GTAATTATAA TTACCTGTAT AGATTGTTTA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1385       1395       1405       1415       1425       1435
4/91            CGGACCCA-- -TTCAAGGTG GTTGTAAGCA ATCTGTTTTT AGTAATAAAG CAACTTGTTG
H120            CGGTCCTC-- -TTCAAGGTG GTTGCAAGCA ATCTGTCTTT AGTGGTAGAG CAACCTGTTG
Ma5             CGGTCCTC-- -TTCAAGGTG GTTGCAAGCA ATCTGTCTTT AGTGGTAGAG CAACCTGTTG
COVID19         GGAAGTCTAA TCTCAAACCT TTTGAGAGAG ATATTTCAAC TGAAATCTAT CAGGCCGGTA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1445       1455       1465       1475       1485       1495
4/91            CTATGCTTAT TCTTA----C CGAGGTCCTA CTAGATGTAA GGGTGTTTAT AGAGGGGAGC
H120            TTATGCTTAC TCATA----T GGAGGTCCTT TGCTGTGTAA AGGTGTTTAT TCAGGTGAGT
Ma5             TTATGCTTAC TCATA----T GGAGGTCCTT TGCTGTGTAA AGGTGTTTAT TCAGGTGAGT
COVID19         GCACACCTTG TAATGGTGTT GAAGGTTTTA ATTGTTACTT TCCTTTACAA TCATATGGTT
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Fig. (1-e): Fifth partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1505       1515       1525       1535       1545       1555
4/91            TAACGCAATA CTTTGAATGT GGACTTCTAG TTTATGTAAC TAAGAGTGAT GGCTCTCGTA
H120            TAGATCATAA TTTTGAATGT GGACTGTTAG TTTATGTTAC TAAGAGCGGT GGCTCTCGTA
Ma5             TAGATCATAA TTTTGAATGT GGACTGTTAG TTTATGTTAC TAAGAGCGGT GGCTCTCGTA
COVID19         TCCAAC-CCA CTAATGGTGT TGGTTACCAA CCATACAGAG TAGTAGTACT TTCTTTTGAA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1565       1575       1585       1595       1605       1615
4/91            TACAAACTAG AAGTGAACCA CTGGTGTTAA CTCAATATAA TTATAACAAC ATTACTTTAA
H120            TACAAACAGC CACTGAACCG CCAGTTATAA CTCAACACAA TTATAATAAT ATTACTTTAA
Ma5             TACAAACAGC CACTGAACCG CCAGTTATAA CTCAACACAA TTATAATAAT ATTACTTTAA
COVID19         CTTCTACATG CACC-AGCAA CTGTTTGTGG ACCTAAA-AA GTCTACTAAT TTGGTTAAAA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1625       1635       1645       1655       1665       1675
4/91            ATAAGTGTGT TGAGTATAAT ATATATGGTA GAGTTGGTCA AGGTTTTATT ACTAA-TGTA
H120            ATACTTGTGT TGATTATAAT ATATATGGCA GAACTGGCCA AGGTTTTATT ACTAA-TGTA
Ma5             ATACTTGTGT TGATTATAAT ATATATGGCA GAACTGGCCA AGGTTTTATT ACTAA-TGTA
COVID19         ACAAATGTGT CAATTTCAAC TTCAATGGTT TAACAGGCAC AGGTGTTCTT ACTGAGTCTA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1685       1695       1705       1715       1725       1735
4/91            ACTGAAGCAA CTGCTAATTA TAGTTATCTA GCAGATGGTG GTTTAGCTAT TTTAGATACT
H120            ACCGACTCAG CTGTTAGTTA TAATTATCTA GCAGACGCAG GTTTGGCTAT TTTAGATACA
Ma5             ACCGACTCAG CTGTTAGTTA TAATTATCTA GCAGACGCAG GTTTGGCTAT TTTAGATACA
COVID19         ACAAAAAGTT TCTGCCTTTC CAACAATTTG GCAGAGACAT TGCTGACACT ACTGATGCTG

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1745       1755       1765       1775       1785       1795
4/91            TCAG-GAGCC ATAGACATAT TTGTTGTTCG AGGTGC---- ATATGGTCTT AATTATTATA
H120            TCTG-GTTCC ATAGACATCT TTGTCGTACA AAGTGA---- ATATGGTCTT AATTATTATA
Ma5             TCTG-GTTCC ATAGACATCT TTGTCGTACA AAGTGA---- ATATGGTCTT AATTATTATA
COVID19         TCCGTGATCC ACAGACACTT GAGATTCTTG ACATTACACC ATGTTCTTTT GGTGGTGTCA
```

Fig. (1-f): Sixth partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1805       1815       1825       1835       1845       1855
4/91            AGGTTA--AT CCCTGTGA-A GATGTTAACC AACAGTTTGT AGTGTCT--- ---GGTGGCA
H120            AGGTTA--AC CCTTGCGA-A GATGTCAACC AGCAGTTTGT AGTTTCT--- ---GGTGGTA
Ma5             AGGTTA--AC CCTTGCGA-A GATGTCAACC AGCAGTTTGT AGTTTCT--- ---GGTGGTA
COVID19         GTGTTATAAC ACCAGGAACA AATACTTCTA ACCAGGTTGC TGTTCTTTAT CAGGATGTTA

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1865       1875       1885       1895       1905       1915
4/91            ATTTAGTTGG CATTCTTACA TCTCATAATG -AAACAGATT CTGAATTTAT TGA-GAACCA
H120            AATTAGTAGG TATTCTTACT TCACGTAATG -AGACTGGTT CCCAGCTTCT TGA-GAATCA
Ma5             AATTAGTAGG TATTCTTACT TCACGTAATG -AGACTGGTT CCCAGCTTCT TGA-GAATCA
COVID19         ACTGCACAGA AGTCCCTGTT GCTATTCATG CAGATCAACT TACTCCTACT TGGCGTGTTT

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   1925       1935       1945       1955       1965       1975
4/91            GTTTTACA-- -TCAAAC--- TCACTAACG- GAACACGTCG CTCTAGACGT ----------
H120            GTTTTACA-- -TCAAAA--- TCACTAATG- GAACACGTCG TTTTAGACGT ----------
Ma5             GTTTTACA-- -TCAAAA--- TCACTAATG- GAACACGTCG TTTTAGACG- ----------
COVID19         ATTCTACAGG TTCTAATGTT TTTCAAACAC GTGCAGGCTG TTTAATAGGG GCTGAACATG
```

Fig. (1-g):. Seventh partial Alignments for comparison among the studied strains of coronavirus (IBV strains vs. COVID-19)

Table 3. Cumulative Mutation rate probabilities of all sequence clusters alignment

Figure 1-	Sequence cluster No.	Mean of Cluster's Mutation rate	Cumulative Multiplicative probability
a	25	0.255465126	7.67046E-93
b	23	0.003806591	1.4028E-191
c	24	0.000842571	1.6418E-288
d	24	0.001214504	8.9003E-308 to 0
e	29	0.002799511	0
f	30	0.003920174	0
g	17	0.001889397	0
Total	172	0.016112496	**1.87E-96**

Table 4. Correlation coefficients between mutation rate items and probability to mutate

		Power ($4^{\wedge Hetero}$)	Probability (1/ Power)	Cumulative Multiplicative probability
Hetero number	Pearson Correlation	**.572****	**-.542****	**-.049**
	Sig. (2-tailed)	.000	.000	.518
	N	174	174	174
Power ($4^{\wedge Hetero}$)	Pearson Correlation	1	**-.109**	**-.026**
	Sig. (2-tailed)		.152	.735
	N	174	174	174
Probability (1/ Power)	Pearson Correlation	-.109	1	**-.014**
	Sig. (2-tailed)	.152		.852
	N	174	174	174

**= significant at ($p<0.01$)

Future prediction

Irrespective to the findings of multiplicative probability, if the spontaneous mutation occurred randomly for just one cluster annually (hetero-bases of IBV convert into COVID-19-like sequence), thus for the upcoming successive years, and for probable mutations taken as accumulative probability rate, then during the next 174 years (up to the year 2194) the probability of mutation rate will be about 0.415 Figure 2. Hence, and for predicting the highest probable event during the half period of the studied years (equal to the numbers of common clusters for random mutations), and during the upcoming 87 additional years (up to the year 2281), the probability of mutation rate will become about 0.475 Figure 2. The ARIMA model of predicting mutation rate for the future indicating that the probability may reach the half of happening event as an upper critical level of forecasting Figure 2. However, the ARIM model's fit and description are presented in Table 5; where the ARIMA type resulted in (0,1,0) model, that is mean the model is a random walk without autoregressive (p) and moving average (q) estimates and with a level of differencing (d) equal 1(optimum walk model). Also, the stationary R-squared is high and equal to (0.975) with absolute maximum value of R-square Table 5. The model statistics of ARIMA prediction are shown in Table 6. The results of the Ljung-Box indicating a non-significant (p>0.05) effect of the model; which may give hope to no more mutating in the future as opposite as expected.

Table (5): Model fit and description

Model Fit and Description			
Fit Statistic	**Mean**	**Model ID**	**Model Type**
Stationary R-squared	.975	(Cumulative_Prob)	ARIMA(0,1,0)
R-squared	1.000		
RMSE	.001		
MAPE	1.700		
MaxAPE	59.045		
MAE	.001		
MaxAE	.003		
Normalized BIC	-12.951		

Table 6. Model statistics of ARIMA prediction

Model Statistics						
Model	Number of Predictors	Model Fit statistics	Ljung-Box Q(18)			Number of Outliers
		Stationary R-squared	Statistics	DF	Sig.	
Cumulative_Prob-Model_1	0	.975	16.708	18	.543	13

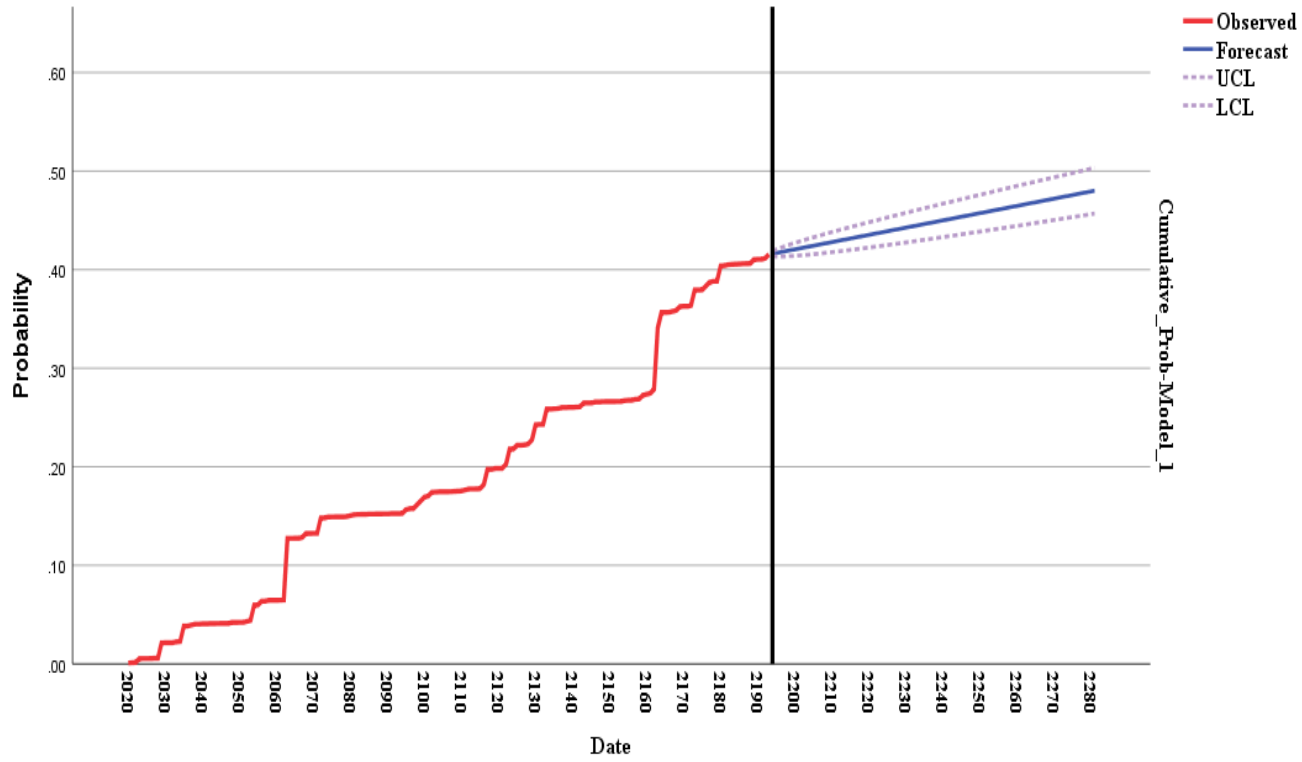

Fig. (2): Prediction mutating rate of IBV into COVID-19-like virus in the future

DISCUSSION

All viruses (including chicken IBV) naturally mutate over time. Previous studies indicated that there were huge changes in genetic diversity and genotypes of IBV strains over the past decade, and thus the presence of multiple variants of IBV and the increasing of its genotypes has caused serious challenges for suppressing IBV through vaccination (Fan *et al*., 2019). Chicken IBV like other coronaviruses, has high rate of mutation and recombination which cause genetic variation through rapid replication (Ennaji *et al*., 2020). Furthermore, high rate of errors and limited capability of proofreading of the viral RNA polymerase produced different types of mutations such as substitutions, insertions and deletions (Lai and Cavanagh 1997; Umar *et al*., 2016). Although huge investigations on mutation cases in both pathogens IBV and COVID-19 have been reported, prediction of potential mutation of IBV virus into future COVID-19-like viruses has not been indicated yet (Wang and Huang 2020; Cavanagh 2005; Lin and Chen 2017; Fischer *et al*., 2020; Ji *et al*., 2020; Baig *et al*., 2020).

Previous studies used Spike S1 protein as a marker to track and analyze mutation as it mediates infection of 2019-nCoV in the mammalian cells and is also the target of vaccine strategies (Korber *et al*., 2020). The S protein also mediates virus attachment and membrane fusibility during contagion (King *et al*., 2011). Therefore, in the present study, data were analyzed based on the sequences of S1 gene. Through comprehensive analysis of the available sequences of S1 gene of 2019-nCoV and IBV strains, we have tracked only hetero-nucleotide bases between both strains (Chicken IBV and Human 2019-nCoV). This study indicated that the average of similarity correlation (pairwise percentage identity) of S1 gene sequences between COVID-19 and IBV strains is relatively high about 43% to 46% Table1. Based on the S1 glycoprotein sequences, 20% to 25% variations between serotypes of chicken IBV were found and sometimes such variations can be as high as 50%, which impacts the cross-protection against viral strains (Cavanagh *et al.*, 1992). Correspondingly, our analysis of S1 alignment also found high identical sites between strains of IBV virus itself with similarity 78% to 99.8% Table 1. Lately, phylogenetic analysis based on investigation of S1 gene of IBV has recognized more than 30 distinct lineages and six different genotypes of virus which are present in different geographical locations (De Wit *et al.*, 2011; Valastro *et al.*, 2016). This indicates that the potential mutation in chicken IBV over time could cause the S1 gene of IBV to be mutated

into COVID-19-like virus in its behaviour (infection) in the future.

The virtual changes in S1 of IBV toward COVID-19-like virus Figures 1-a, b, c, d, e, f and g is built upon the random mutations that occur each moment and also the gene expression that is adequate to the common environmental factors which encourage such converting. In order to adapt to a large pool of species, coronaviruses undergo rapid mutation and recombination (Wu *et al*., 2020). Nonetheless, on the other hand, the huge numbers of nucleotides and clusters in RNA sequence of IBV S1 gene or in its whole genome may not permit to conversion it within a short time into COVID-10- like pathogen. However, time as an active factor affecting the biological variables, may change S1 gene sequence into COVID-19-like sequences during long life intervals via slowly and silent mutations. Phylogenetic analysis indicated that the IBV is closer to COVID-19 than that of both bat and snake (Ji *et al*., 2020). However, the possibility of mutating S1 gene of IBV into COVID-19-like virus (Tables 2 and 3) is related to two main causes: the first is a random mutation that may identify; and the second is the time of mutating; in addition to the gene part of mutating, where the mutation may occur at S1 gene, but not at the exact level of sequence that make infection. Therefore, the possibility of infection will remain as part of probability event. Due to important biological properties and the high variability of the S1 antigenic evolution in IBV which has been mostly related to variations in the sequence of the S1 glycoprotein, thus different antigenic variants, serotypes and subtypes of IBV are thought to be produced by point mutations, deletions, insertions, or RNA recombination of S1 gene (Wang and Huang 2000).

Moreover, the current result Table 4 indicated that the correlation coefficient between hetero-bases numbers within each cluster and the probability of mutating IBV into a like-COVID-19 sequence is highly significant (p<0.01), that is referred to converting percentage by about 54 %, when only the hetero-bases changed into homo-bases in IBV sequence. Also Figure 2, illustrating that the predicting of mutating is not absolutely impossible, but need more time to change (at least 260 years to be around the half proportion as the probability of infection). Nonetheless, the present findings insure that the probability of mutating the whole S1 gene sequence of IBV into COVID-19-like virus is relatively impossible from the molecular point of view, but from random mutation across long time aspect, it remain a chance to be happening event, which will be a disaster on the human life. Similarly, according to molecular studies, only a few changes in the amino acid composition in the S1 part of the virus spike protein can cause to emerge a new variant or serotype, while most of the other parts of viral genomes remain unchanged (Cavanagh, 2007). This could be due to recombination as a result of mixed infections, immunological pressure and a reduction of dominant serotypes as a result of the widespread use of vaccines (Lee, 2002; Liu *et al*., 2006). Hence, we can point out that even small amount of mutations in the S1 of chicken IBV to be like sequences of S1 of COVID-19 virus could be enough to cause infection in different organisms including humans. Thus, here we reported the prediction of potential mutation in chicken IBV. Hussen (2020) was forecasted a relatively increased trend of infected cases of COVOD-19 during 2020. Results of our study fill a gap in tracking and predicting the potential mutation of chicken IBV into COVID-19-like viruses.

CONCLUSION

Based on the calculation and statistical analysis of hetero-nucleotides of S1 gene of IBV and COVID-19, the correlation coefficients between mutation rate items and the probability and prediction of potential random mutation rate, it could be concluded that the possibility of potential mutation of the chicken IBV into COVID-19-like virus and the emergence of variants of IBV to be like COVID-19 virus is statistically not absolutely impossible, but it might require a considerable amount of time.

Declaration

We declare that we have no competed financial, interests or personal relationships that might have appeared to affect the work of this paper.

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REFERENCES

Baig, A.M., Khaleeq, A., Ali, U., and Syeda, H. (2020). Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. ACS Chem. Neurosci. 11: 995-998.

Bande, F., Arshad, S.S., Omar, A.R., Hair-Bejo, M., Mahmuda, A., and Nair, V. (2017). Global distributions and strain diversity of avian infectious bronchitis virus: a review. Anim. Heal. Res. Rev. 18: 70-83.

Bourogâa, H., Larbi, I., Miled, K., Hellal, Y.K., Hassen, J., Behi, I., Nsiri, J., and Ghram, A. (2014). Evaluation of protection conferred by a vaccination program based on the H120 and CR88 commercial vaccines against a field variant of avian infectious bronchitis virus. J. Appl. Poult. Res. 23: 156-164.

Butler, N., Pewe, L., Trandem, K., and Perlman, S. (2006). Murine encephalitis caused by HCoV-OC43, a human coronavirus with broad species specificity, is partly immune-mediated. Virology. 347: 410-421.

Cavanagh, D. (2005). Coronaviruses in poultry and other birds. Av. Pathol. 34: 439-448.

Cavanagh, D. (2007). Coronavirus avian infectious bronchitis virus. Vet. Res 38: 281-297.

Cavanagh, D. and Naqi, S. (2003). Infectious bronchitis. Dis. poult. 11:101-119.

Cavanagh, D., Davis, P.J., Cook, J.K., Li, D., Kant, A., and Koch, G. (1992). Location of the amino acid differences in the S1 spike glycoprotein subunit of closely related serotypes of infectious bronchitis virus. Av. pathol. 21: 33-43.

Cavanagh, S. (1997). Content analysis: concepts, methods and applications. Nurse researcher 4: 5-16.

Chakraborty, T., and Ghosh, I. (2020). Real-time forecasts and risk assessment of novel coronavirus (COVID-19) cases: A data-driven analysis. Chaos, Solitons & Fractals 135: 109850.

Cook, J.K., Jackwood, M. and Jones, R.C. (2012). The long view: 40 years of infectious bronchitis research. Avian Pathol. 41: 239-250.

De Wit, J.J., Cook, J.K., and Van der Heijden, H.M. (2011). Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian pathol. 40: 223-235.

Ennaji, Y., Khataby, K., and Ennaji, M.M. (2020). Infectious bronchitis virus in poultry: Molecular epidemiology and factors leading to the emergence and reemergence of novel strains of infectious bronchitis virus. In Emerging and Reemerging Viral Pathogens (pp. 31-44). Academic Press.

Fan, W., Tang, N., Dong, Z., Chen, J., Zhang, W., Zhao, C., He, Y., Li, M., Wu, C., Wei, T., and Huang, T. (2019). Genetic analysis of avian coronavirus infectious bronchitis virus in yellow chickens in Southern China over the past decade: revealing the changes of genetic diversity, dominant genotypes, and selection pressure. Viruses. 11: 898.

Fischer, S., Klosterhalfen, D., Kump, F.W.S., and Casteel, M. (2020). Research Note: First evidence of infectious bronchitis virus Middle-East GI-23 lineage (Var2-like) in Germany. Poult. sci. 99: 797-800.

Howley, DMKP. (2013). Fields Virology. I. Lippincott Williams & Wilkins: 830P.

Hussen, S H. (2020). Forecasting of COVID-19 Cases in Kurdistan Region Using Some Statistical Models. Acad. J. Appl. Math. Sci. 6: 172-180.

Jackwood, M.W., Hall, D., and Handel, A. (2012). Molecular evolution and emergence of avian gammacoronaviruses. Infect. Gen. Evol. 12: 1305-1311.

Ji, W., Wang, W., Zhao, X., Zai, J., and Li, X. (2020). Cross-species transmission of the newly identified coronavirus 2019-nCoV. J. Med. Virol. 92: 433-440.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., and Thierer, T. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28: 1647-1649.

King, A.M., Lefkowitz, E., Adams, M.J., and Carstens, E.B. (2011). Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses (Vol. 9). Elsevier.

Korber, B., Fischer, W., Gnanakaran, S.G., Yoon, H., Theiler, J., Abfalterer, W., Foley, B., Giorgi, E.E., Bhattacharya, T., Parker, M.D., and Partridge, D.G. (2020). Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2. BioRxiv.

Lai, M.M., and Cavanagh, D. (1997). The molecular biology of coronaviruses. Adv. Virus Res. 48: 1-100.

Lee, C.W. (2002). Evolution of avian infectious bronchitis virus: genetic drift and recombination. Kor. J. Vet. Serv. 25: 97-103.

Lefkowitz, E.J., Dempsey, D.M., Hendrickson, R.C., Orton, R.J., Siddell, S.G., and Smith, D.B. (2018). Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). Nucl. ac. res. 46: D708-D717.

Lin, S.Y., and Chen, H.W. (2017). Infectious bronchitis virus variants: molecular analysis and pathogenicity investigation. International journal of molecular sciences. 18: 2030.

Liu, S.W., Zhang, Q.X., Chen, J.D., Han, Z.X., Liu, X., Feng, L., Shao, Y.H., Rong, J.G., Kong, X.G., and Tong, G.Z., 2006. Genetic diversity of avian infectious bronchitis coronavirus

strains isolated in China between 1995 and 2004. Arch. Virol. 151: 1133-1148.

MacLachlan N.J., and Dubovi, E.J. (2017). Fenner's veterinary virology, 5th ed. Academic Press, New York, NY.

Matoba, Y., Abiko, C., Ikeda, T., Aoki, Y., Suzuki, Y., Yahagi, K., Matsuzaki, Y., Itagaki, T., Katsushima, F., Katsushima, Y., and Mizuta, K. (2015). Detection of the human coronavirus 229E, HKU1, NL63, and OC43 between 2010 and 2013 in Yamagata, Japan. Jap. J. Infect. Dis. 68: 138-141.

Myint, S.H. (1995). Human coronavirus infections. In The Coronaviridae (pp. 389-401). Springer, Boston, MA.

SPSS, Inc. (2019). Statistical Package for Social Sciences for Windows Graduate Pack Advanced Version. Version 26.0, SPSS Inc., IBM publications.

St-Jean, J.R., Jacomy, H., Desforges, M., Vabret, A., Freymuth, F., and Talbot, P.J. (2004). Human respiratory coronavirus OC43: genetic stability and neuroinvasion. J. Virol. 78: 8824-8834.

Umar, S., Shah, M.A.A., Munir, M.T., Ahsan, U. and Kaboudi, K. (2016). Infectious bronchitis virus: evolution and vaccination. Wor Poult. Sci. J. 72: 49-60.

Valastro, V., Holmes, E.C., Britton, P., Fusaro, A., Jackwood, M.W., Cattoli, G., and Monne, I. (2016). S1 gene-based phylogeny of infectious bronchitis virus: an attempt to harmonize virus classification. Infect. Gen. Evol. 39: 349-364.

Wang, C.H., and Huang, Y.C. (2000). Relationship between serotypes and genotypes based on the hypervariable region of the S1 gene of infectious bronchitis virus. Arch. Virol. 145: 291-300.

World Health Organization (2003). Consensus document on the epidemiology of severe acute respiratory syndrome (SARS) (No. WHO/CDS/CSR/GAR/2003.11). World Health Organization.

World Health Organization (2020). Coronavirus disease 2019 (COVID-19) strategic preparedness and response plan: Accelerating readiness in the Eastern Mediterranean Region: February 2020 (No. WHO-EM/CSR/260/E). World Health Organization. Regional Office for the Eastern Mediterranean.

Wu, A., Niu, P., Wang, L., Zhou, H., Zhao, X., Wang, W., Wang, J., Ji, C., Ding, X., Wang, X., and Lu, R. (2020). Mutations, recombination and insertion in the evolution of 2019-nCoV. BioRxiv.

Yuan, Y., Cao, D., Zhang, Y., Ma, J., Qi, J., Wang, Q., Lu, G., Wu, Y., Yan, J., Shi, Y., and Zhang, X. (2017). Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat. commun. 8: 1-9.