Original Article

S1 gene sequence analysis of infectious bronchitis virus vaccinal strains (H120 & H52) and their embryo-passaged derivatives

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ABSTRACT

Avian infectious bronchitis is an acute and highly contagious disease that mainly causes respiratory symptoms in poultry. A number of serotypes and variants of the viral agent with poor cross-protection are the major problem to achieve desired immunity from vaccination. The S1 subunit of S glycoprotein (spike) is the major determinant of IBV so that a minor change in amino acid sequence of this protein, alters the virus strain. Therefore, characterization of the sequence of S1 gene is necessary to identify virus strains and their similarities with the vaccinal strains. In this research, the S1 sequence of H52 and H120 vaccinal strains of Razi Institute was fully characterized, and also the effect of serial passages in embryonated - eggs (5 passages beyond the master seed) on the S1 gene was investigated. The results showed that H120 and H52 strains of Razi Institute are 100% identical to the reference vaccine strains available in the GenBank. In addition, the H52 strain showed one amino acid substitution from the 3rd passage in which Glycine (G) was replaced by Valine (V) at position 118 making these passages exactly identical to the H120 strain while no change occurred for the H120 strain during these passages. Analysis of the original vaccinal strains which are widely administered in Iran, is definitely useful for prevention and control strategies against the circulating viruses. To identify the genetic change(s) responsible for attenuation of these strains during passages in embryonated-egg, characterization of other genes, especially those involved in replication is recommended.

Keywords: IBV, Vaccinal strain, S1 gene characterization, egg- passaged

INTRODUCTION

Avian infectious bronchitis is an acute, highly contagious and economically important disease of poultry with worldwide distribution. The viral agent mainly affects respiratory epithelium, making the birds susceptible to other infectious agents (Cavanagh, 2007). Infectious bronchitis virus (IBV) is classified in the genus *Gammacoronavirus* within the *Coronaviridae*. The virus, almost 120 nm in diameter, is an enveloped spherical to pleomorphic particle, which contains 20 nm spikes projecting from the outer surface of the particle (Masters and Perlman, 2013). Structural proteins are comprised of the Spike (S), membrane (M), nucleoprotein (N) glycoproteins and tiny amounts of small membrane protein (E) (Cavanagh, 1981; Sutou et al., 1988). The S glycoprotein consists of S1 and S2 proteins, S1 is anchored to the membrane by S2 and is responsible for attachment, entry and inducing hemagglutination inhibition and neutralizing antibodies. The S1 is about 520 amino acids comprising the hypervariable region (amino acids 38-387); minor changes in this area induces remarkable decline in protective immunity against heterologous serotypes and strains (Koch et al., 1990; Cavanagh et al., 1992; Kant et al., 1992). The variation in the S1 sequences of vaccinal strains has also been found within the same vaccine serotype produced by different companies, and even different batches by a single manufacturer (McKinley et al., 2008). Passages of vaccinal strains in embryonated eggs can also result in mutations in the IBV genome, for this reason only a limited number passages (up to 5) beyond the master seed is allowed for vaccine production by the World Organization for Animal Health (OIE) (http://www.oie.int/en/internationalstandard-setting/terrestrial-manual). This study was aimed to characterize the S1 gene of H52 and H120 vaccinal strains from Razi Institute and also evaluate the effect of five serial passages in emryonated-eggs on the entire length of S1 sequences.

MATERIALS AND METHODS

Virus preparation. The Master seeds of both strains (H120 and H52) were inoculated into the allantoic cavities of specific-pathogene free (SPF) chicken embryos. After two days of incubation at 37 °C, the allantoic fluid was harvested and inoculated into the new SPF eggs. The Subsequent passages were serially carried out until the 5th passage as above.

Viral RNA extraction. Viral RNA was extracted by using commercial viral High pure Viral Nucleic Acid kit (Roche; Germany) according to the manufacturer's instruction.

Reverse transcription reaction. Reverse transcription (RT) was carried out using RevertAidTM First Strand cDNA synthesis Kit (Fermentas; Canada)

as follows: 6 μ l RNA and 1 μ l Random hexamer primer with 5 μ l DEPC-treated water heated at 65 °C for 5 minutes and cooled on ice. Then, 4 μ l 5X Reaction buffer, 1 μ l RibolockTM Rnase inhibitor (20u/ μ l), 2 μ l of 10 mM dNTP Mix, 1 μ l RevertAidTM M-MulV Reverse Transcriptase (200 u/ μ l) were added to the solution to final volume of 20 μ l. The mixture was put in a thermocycler at 25 °C for 5 minutes, followed by 42 °C for 60 minutes and 72 °C for 5 minutes.

Polymerase chain reaction (PCR). The PCR mixture contained 5 µl of 10 X buffers, 2 µl dNTP (0.2 mM of each), 6 µl MgSo4 (3mM), 7µl cDNA, 1 µl (20 pmol) of each primer, Forward (5'-TGAAAACTGAACAAAAGACA-3') and Reverse (5'-CATAACTAACATAAGGGCAA-3') flanking the whole S1 glycoprotein gene (known et al., 1993) and 2.75 units (1.1 µl) of *pfu* DNA polymerase (Fermentas; Canada) in a total volume of 50 µl. The PCR cycling program was started with initial denaturation at 95 °C for 2 minutes then followed by 37 cycles of 94 °C for 50 seconds, 47 °C for 60 seconds, 72 °C for 2 minutes and 40 seconds and, a final extension at 72 °C for 10 minutes. PCR products were run on agarose gel (1%). Samples were identified as positive based on the presence of the expected amplicon (1720 bp) with ethidium bromide staining.

Sequence analyzing. PCR products were purified using High Pure PCR Purification (Roche; Germany). Samples (50 μ l) containing 40 ng/ml were sent to Bioneer company (South Korea) for sequencing. The PCR products were sequenced in both directions using the above mentioned forward and reverse primers.

The obtained sequences were assembled by Geneious (4.8.3) software. The whole sequences from H52 and H120 passages were compared to the sequences available in the GenBank (http://Blast.ncbi.nlm.nih.gov/Blast.cgi) and were aligned together using Clustal W program (Thompson et al., 1994) and MEGA.5 software. The sequence of the first passage of H52 and H120 were deposited in the GenBank with accession number of KR605488 and KR605489, respectively.

Viral neutralization (VN) Test. Egg infectious dose (EID₅₀) was calculated using Kärber method (1931) for the H52 and H120 vaccinal strains and their embryo – passaged strains. Ten SPF chickens were inoculated by 1 dose ($10^{3.5}$ EID₅₀ viral particles) of each vaccinal strain via eye-drops. The chickens were monitored for 21 days and were bled 3 weeks after vaccination. The antibodies against IBV were measured by VN test according to the OIE protocol., 2001).

RESULTS

PCR amplification and sequence analyzing. The PCR product (1720 bp) was successfully amplified for all passages of both (H120 and H52) strains and observed on agarose gel.

Alignment of the entire length of the S1 gene from H52 strain during serial passages (1 to 5) in embryonated-egg showed that they are highly identical (99.8% nucleotide identity) with minor changes occurred from the third passage as follows and illustrated in Figure 1: Nucleotide No. 345 (T) in passages 1 and 2 changed to C in passages 3, 4 and 5, with no amino acid alteration.

- 1-Nucleotides No. 353 (G) in passage 1 and 2 changed to T in passage 3, 4 and 5. This change led to amino acid substitution of Glycine (118) to Valine.
- 2-Nucleotide No. 426 (G) in passage 1 and 2 changed to T in passages 3, 4 and 5, with no amino acid alteration.

Alignment of the all serial passages (1 to 5) of H120 strain revealed that they are absolutely identical (100 % nucleotide identity). Strikingly, the sequences of H120 passages were 100% identical to the 3rd to 5th passages of the H52 strain. Alignment of the full length of the S1 gene sequences of the H52 and H120 strains (1st passage) are depicted in Figure 2. The two S1 sequences and the encoded amino acids found in this study: (H52 passages 1 to 2) and [H52 (passages 3 to 5) - H120 (passages 1 to 5)] were also compared with the sequences available in the GenBank. The results showed that both of the sequences are 100% identical

to the reference vaccinal strains of IBV submitted in the GenBank (Table 1 and 2).

Viral neutralization. The neutralizing antibodies elicited against the all serial passages of H52 and H120 strains efficiently and consistently neutralized the homologous IBV Massachusetts serotype.

DISCUSSION

IBV, as a member of the Coronaviridae, has high capacity for genetic change occurring through point mutation, insertion, deletion and genetic recombination (Thor et al., 2011; Jackwood et al., 2012). These mechanisms of genetic diversities lead to emergence of new IBV serotypes and variants which complicate designing appropriate control strategies using the most homologous vaccine (Lee and Jackwood, 2001; Jackwood et al., 2010). The evolution of IBV is also influenced by the application of multiple vaccinal strains, population density and host immune status (Lee and Jackwood, 2001). It is, therefore, an obvious requirement for monitoring and characterization of circulating viruses in the field as well as assessment of the effectiveness of vaccines used against these viruses (Cavanagh, 2007). Furthermore, molecular characterization is now considered as an essential component of vaccine evaluation for vaccine manufacturers in the world (www.oie.int/en/ international-standard- setting/terrestrial-manual). The H52 and H120 vaccinal strains have been produced and administered in Iran for a long time while molecular characterization of these strain was largely expected. In the first step, we attempted to characterize the full length of the S1 gene of these vaccinal strains as a major determinant of the IBV. This aim was successfully achieved, emphasizing that the H52 and H120 strains manufactured in Razi institute are absolutely identical to the reference vaccinal strains submitted to the GenBank. These results, together with the VN test findings approve the certainty of these strains and also justify their application against the homologous serotype and variant in the field.

Isolate name	Accession number	Isolated from
Infectious bronchitis virus isolate MassD/Cvial1 S1 protein (S1) gene	EU283085.1	Vaccine vial
Infectious bronchitis virus strain H120 spike glycoprotein (S) gene	KF188436.1	Vaccine vial
Infectious bronchitis virus isolate UFMG/PM1 spike glycoprotein S1 subunit gene	JX182773.1	Chicken
Infectious bronchitis virus isolate Mass/Avial1 S1 protein (S1) gene	EU283073.1	Vaccine vial
Avian infectious bronchitis virus strain Ma5 spike glycoprotein S1 subunit precursor (S1) gene	AY561713.1	Vaccine vial
Infectious bronchitis virus strain ck/CH/LSD/110726	KJ425512.1	Chicken
Infectious bronchitis virus strain ck/CH/LJL/121059	KJ425509.1	Chicken
Infectious bronchitis virus strain ck/CH/LHLJ/110310	KJ425505.1	Chicken
Infectious bronchitis virus strain ck/CH/LHB/131142	KJ425501.1	Chicken
Infectious bronchitis virus strain ck/CH/LHB/131118	KJ425499.1	Chicken

Table 1. Ten sequences in the GenBank most identical the H52 Strain of Razi Institute

Isolate name	Accession number	Isolated from
Avian infectious bronchitis virus (strain H120) peplomeric protein gene encoding the S1 and S2 subunits	M21970.1	Vaccine vial
Infectious bronchitis virus strain ck/CH/LSD/121228	KJ435285.1	Chicken
Infectious bronchitis virus strain ck/CH/LHLJ/131216	KJ425507.1	Chicken
Infectious bronchitis virus strain ck/CH/LDL/101212	JF828981.1	Chicken
Infectious bronchitis virus isolate IR/17/00 S1 spike glycoprotein gene	HQ 842709.1	Chicken
Infectious bronchitis virus strain ck/CH/LDL/101212 S1 protein (S1) gene	JF330848.1	Chicken
Infectious bronchitis virus isolate THA320352 spike glycoprotein S1 subunit (S1) gene	GQ885138.1	Chicken
Infectious bronchitis virus strain ck/CH/LHLJ/111050	KJ425506.1	Chicken
Infectious bronchitis virus strain ck/CH/LHB/131142	KJ425501.1	Chicken
Infectious bronchitis virus strain ck/CH/LHLJ/111050 S1 protein (S1) gene	JQ739315.1	Chicken

 Table 2. Ten sequences in the GenBank most identical the H120 Strain of Razi Institute

	310	320	330	340	350
Consensus	TACTGTAACT	TTTCAGATAC	TACAGIGITT	GTTACACATT	GTTACAAACA
Identity					
1. H52-P1	TACTGTAACT	TTTCAGATAC	TACAGTGTTT	GTTACACATT	GTTAMAAACA
Frame 1	Y C N	F S D T	T V F	V T H	C Y K H
2. H52-P2	TACTGTAACT	TTTCAGATAC	TACAGTGTTT	GTTACACATT	GTTAMAAACA
Frame 1	Y O N	F S D T	T V F	V T H	CYKH
3. H52-P3	TACTGTAACT	TTTCAGATAC	TACAGTGTTT	GTTACACATT	GTTACAAACA
Frame 1	Y C N	F S D T	T V F	V T H	C Y K H
4. H52-P4	TACTGTAACT	TTTCAGATAC	TACAGTGTTT	GTTACACATT	GTTACAAACA
Frame 1	Y C N	F S D T	T V F	V T H	C Y K H
5. H52-P5	TACTGTAACT	TTTCAGATAC	TACAGTGTTT	GTTACACATT	GTTACAAACA
Frame 1	Y C N	F S D T	T V F	V T H	C Y K H
	360	370	380	390	400
Consensus Frame 1 Identity		P I T	GCATGCTTCA G M L Q	ACAGCATTCT QHS	ATACGTGTTT I R V
1. H52-P1	TG <mark>G</mark> TGGGTGT	CCTATAACTG	GCATGCTTCA	ACAGCATTCT	ATACGTGTTT
Frame 1	G G C	P I T	G M L Q	Q H S	I R V
2. H52-P2	TGGTGGGTGT	CCTATAACTG	GCATGCTTCA	ACAGCATTCT	A TACG TG TTT
Frame 1	G G C	P I T	G M L Q	Q H S	I R V
3. H52-P3	TGTTGGGTGT	CCTATAACTG	$\begin{smallmatrix} GCATGCTTCA\\ G & M & L & \mathbb{Q} \end{smallmatrix}$	ACAGCATTCT	A TACG TG TTT
Frame 1	V G C	P I T		Q H S	I R V
4. H52-P4	TGTTGGGTGT	CCTATAACTG	GCATGCTTCA	ACAGCATTCT	A TACGTGTTT
Frame 1	V G C	PIT	GML Q	Q H S	I R V
5. H52-P5	TGTTGGGTGT	CCTATAACTG	$\begin{smallmatrix} GCATGCTTCA\\ G & M & L & \bigcirc \end{smallmatrix}$	ACAGCATTCT	ATACGTGTTT
Frame 1	V G C	P I T		Q H S	I R V
	410	420	430	440	450
Consensus Frame 1 Identity	CTGCTATGAA S A M K	N G Q	CTTTTTTATA L F Y	N L T V	TAGTGTAGCT SV A
1. H52-P1	CTGCTATGAA	AAATGGCCAG	CTTTTCTATA	ATTTAACAGT	TAGTGTAGCT
Frame 1	S A M K	N G Q	L F Y	N L T V	S V A
2. H52-P2 Frame 1	CTGCTATGAA S A M K	AAATGGCCAG	CTTTTCTATA L F Y	$\begin{smallmatrix} A \ T \ T \ T \ A \ A \ C \ A \ G \ T \\ \mathbb{N} L T V \\ \end{smallmatrix}$	TAGTGTAGCT SVA
3. H52-P3	C TGC TA TGAA	AAATGGCCAG	C TTTTTTATA	ATTTAACAGT	TAGTGTAGCT
Frame 1	S A M K	N G Q	L F Y	N L T V	S V A
4. H52-P4	C TGC TA TGAA	AAATGGCCAG	$\begin{smallmatrix} C & T & T & T & T & T & T & T \\ L & F & Y \\ \end{smallmatrix}$	ATTTAACAGT	TAGTGTAGCT
Frame 1	S A M K	N G Q		N L T V	SVA
5. H52-P5	CTGCTATGAA	AAATGGCCAG	CTTTTTTATA	ATTTAACAGT	TAGTGTAGCT
Frame 1	S A M K	N G Q	L F Y	N L T V	S V A

Figure 1. The region of the H52 strain illustrating the nucleotide and amino acid changes occurred from the third passages.

Consensus Frame 1	1 10 ATGTTGGTAACA M L V T	20 CCTCTTTACTA P L L L	30 GTGACTCTTTTG V T L L	40 TGTGCACTATGT C A L C	50 60 AGTGCTGCTTTG S A A L
H52-P1 Frame 1	1 10 ATGTTGGTAACA M L V T	CCTCTTTTACTA P L L L	GTGACTCTTTTG V T L L	40 TGTGCACTATGT C A L C	50 60 AGTGCTGCTTTG S A A L
H120-P1 Frame 1	ATGTTGGTAACA M L V T	CCTCTTTTACTA P L L L	GTGACTCTTTTG V T L L	49 TGTGCACTATGT C A L C	AGTGCTGCTTTG S A A L
Consensus Frame 1	70 TATGACAGTAGT Y D S S	S Y Y Y	90 TACTACCAAAGT Y Y O S	100 GCCTTCAGACCA A F R P	110 120 CCTGATGGTTGG PDGW
H52-P1 Frame 1	70 TATGACAGTÁGT Y D S S	TCTTACGTGTAC S Y V Y	90 TACTACCAAAGT Y Y Q S	100 GCCTTCAGACCA A F R P	110 120 CCTGATGGTTGG P D G W
H120-P1 Frame 1	70 TATGACAGTAGT Y D S S	TCTTACGTGTAC S Y V Y	90 TACTACCAAAGT Y Y Q S	100 GCCTTCAGACCA A F R P	110 120 CCTGATGGTTGG P D G W
Consensus Frame 1	130 CATTTACATGGG H L H G	140 GGTGCGTATGCG G A Y A	150 GTTGTTAATATT VVNI	160 TCTAGTGAATCT SSES	170 180 AATAATGCAGGC N N A G
H52-P1 Frame 1	130 CATTTACATGGG H L H G	GGTGCGTATGCG G A Y A	150 GTTGTTAATATT V V N I	160 TCTAGTGAATCT S S E S	170 180 AATAATGCAGGC N N A G
H120-P1 Frame 1	130 CATTTACATGGG H L H G	GGTGCGTATGCG G A Y A	GTTGTTAATATT V V N I	160 TCTAGTGAATCT S S E S	170 180 AATAATGCAGGC N N A G
Consensus Frame 1	190 TCTTCATCTGGG SSSSG	200 TGTACTGTTGGT C T V G	210 ATTATTCATGGT I I H G	220 GGTCGTGTTGTT GRVV	230 240 AATGCTTCTTCT NASS
H52-P1 Frame 1	190 TCTTCATCTGGG S S S G	TGTACTGTTGGT C T V G	210 ATTATTCATGGT I I H G	220 GGTCGTGTTGTT G R V V	230 240 AATGCTTCTTCT N A S S
H120-P1 Frame 1	190 TCTTCATCTGGG S S S G	200 TGTACTGTTGGT C T V G	210 ATTATTCATGGT I H G	220 GGTCGTGTTGTT G R V V	230 240 AATGCTTCTTCT N A S S
Consensus Frame 1	250 ATAGCTATGACG I A M T	260 GCACCGTCATCA A P S S	270 GGTATGGCTTGG G M A W	280 TCTAGCAGTCAG SSSSO	290 300 TTTTGTACTGCA FCTA
H52-P1 Frame 1	250 ATAGCTATGACG I A M T	GCACCGTCATCA A P S S	270 GGTATGGCTTGG G M A W	280 TCTAGCAGTCAG S S S Q	290 300 TTTTGTACTGCA F C T A
H120-P1 Frame 1	250 ATAGCTATGACG I A M T	GCACCGTCATCA A P S S	270 GGTATGGCTTGG G M A W	280 TCTAGCAGTCAG S S S Q	290 300 TTTTGTACTGCA F C T A
Consensus Frame 1	310 TACTGTAACTTT Y C N F	320 TCAGATACTACA S D T T	330 GTGTTTGTTACA V F V T	340 CATTGTTAYAAA H C Y K	350 360 CATGKTGGGTGT H ? G C
H52-P1 Frame 1	310 TACTGTAACTTT Y C N F	320 TCAGATACTACA S D T T	330 GIGIIIGIIACA V F V T	340 CATTGTTA <mark>T</mark> AAA H C Y K	350 360 CATG <mark>G</mark> TGGGTGT H G G C
H120-P1 Frame 1	310 TACTGTAACTTT Y C N F	320 TCAGATACTACA S D T T	330 GTGTTTGTTACA V F V T	340 Catigita <mark>c</mark> aaa H C Y K	350 360 CATG <mark>T</mark> TGGGTGT H V G C
Consensus Frame 1	370 CCTATAACTGGC PITG	380 ATGCTTCAACAG M L Q Q	390 CATTCTATACGT H S I R	400 GTTTCTGCTATG VSAM	410 420 AAAAATGGCCAG K N G Q
H52-P1 Frame 1	370 CCTATAACTGGC P I T G	380 ATGCTTCAACAG M L Q Q	390 CATTCTATACGT H S I R	400 GTTTCTGCTATG V S A M	410 420 AAAAATGGCCAG K N G Q
H120-P1 Frame 1	370 CCTATAACTGGC P I T G	380 ATGCTTCAACAG M L Q Q	390 CATTCTATACGT H S I R	400 GTTTCTGCTATG V S A M	410 420 AAAAATGGCCAG K N G Q
Consensus Frame 1	430 CTTTTYTATAAT L F Y N	440 TTAACAGTTAGT L T V S	450 GTAGCTAAGTAC VAKY	460 CCTACTTTTAAA PTFK	470 480
H52-P1 Frame 1	430 CTTTTCTATAAT L F Y N	440 TTAACAGTTAGT L T V S	450 GTAGCTAAGTAC V A K Y	460 CCTACTTTTAAA P T F K	470 480 TCATTTCAGTGT S F Q C
H120-P1 Frame 1	430 CTTTTTTTATAAT L F Y N	440 TTAACAGTTAGT L T V S	450 GTAGCTAAGTAC V A K Y	460 CCTACTTTTAAA P T F K	470 480 TCATTTCAGTGT S F Q C
Consensus Frame 1	490 GTTAATAATTTA VNNL	500 ACATCCGTATAT TSVY	510 TTAAATGGTGAT LNGD	520 CTTGTTTACACC L V Y T	530 540 TCTAATGAGACC SNET
H52-P1 Frame 1	490 GTTAATAATTTA V N N L	ACATCCGTATAT T S V Y	510 TTAAATGGTGAT L N G D	520 CTTGTTTACACC L V Y T	530 540 TCTAATGAGACC S N E T
H120-P1 Frame 1	490 GTTAATAATTTA V N L	ACATCCGTATAT T S V Y	510 TTAAATGGTGAT L N G D	520 CTTGTTTACACC L V Y T	530 540 TCTAATGAGACC S N E T

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Consensus	550	560	570		590 600
H52-P1 Frame 1	550 ACAGATGTTACA T D V T	560 TCTGCAGGTGTT	570 TATTTTAAAGCT Y F K A	580 GGTGGACCTATA G G P T	590 600 ACTTATAAAGTT
H120-P1 Frame 1	550 ACAGATGTTACA T D V T	560 TCTGCAGGTGTT S A G V	570 TATTTTAAAGCT Y F K A	580 GGTGGACCTATA G G P I	590 ACTTATAAAGTT T Y K V
Consensus Frame 1	610 A <mark>TGAGAGAAGTT</mark> M R E V	620 AGAGCCC <mark>T</mark> GGC <mark>T</mark> R A L A	630 TATTTGTTAAT Y F V N	640 GGTACTGCACAA GTAQ	650 660 GATGTTATTTG D V I L
H52-P1 Frame 1	610 ATGAGAGAAGTT M R E V	AGAGCCCTGGCT R A L A	630 TATTTTGTTAAT Y F V N	640 GGTACTGCACAA G T A Q	650 660 GATGTTATTTTG D V I L
H120-P1 Frame 1	610 ATGAGAGAAGTT M R E V	AGAGCCCTGGCT R A L A	630 TATTTTGTTAAT Y F V N	640 GGTACTGCACAA G T A Q	650 660 GATGTTATTTTG D V I L
Consensus Frame 1	670 TGTGATGGGTCA C D G S	680 CCTAGAGGCTTG PRGL	690 TTAGCATGCCAG L A C Q	700 TATAATACTGGC Y N T G	710 720 AATTTTTCAGAT N F S D
H52-P1 Frame 1	670 TGTGATGGGTCA C D G S	680 CCTAGAGGCTTG P R G L	690 TTAGCATGCCAG L A C Q	700 TATAATACIGGC Y N T G	710 720 AATTTTTCAGAT N F S D
H120-P1 Frame 1	670 TGTGATGGGTCA C D G S	680 CCTAGAGGCTTG P R G L	690 TTAGCATGCCAG L A C Q	700 TATAATACTGGC Y N T G	710 720 AATTTTTCAGAT N F S D
Consensus Frame 1	730 GGCTTTTATCCT G F Y P	740 TTTACTAATAGT F T N S	750 AGTTTAGTTAAG SLVK	760 CAGAAG <mark>TTT</mark> ATT Q K F I	770 780 GTCTATCGTGAA VYRE
H52-P1 Frame 1	730 GGCTTTTATCCT G F Y P	740 TTTACTAATAGT F T N S	750 AGTTTAGTTAAG S L V K	760 CAGAAGTTTATT Q K F I	770 780 GTCTATCGTGAA V Y R E
H120-P1 Frame 1	730 GGCTTTTATCCT G F Y P	740 TTTACTAATAGT F T N S	750 AGTTTAGTTAAG S L V K	760 CAGAAGTTTATT Q K F I	770 780 GTCTATCGTGAA V Y R E
Consensus Frame 1	790 AATAGTGTTAAT NSVN	ACTACTTTTACC T T F T	810 TTACACAATTTC L H N F	820 ACTTTTCATAAT T F H N	830 840 GAGACTGGCGCC E T G A
H52-P1 Frame 1	790 AATAGTGTTAAT N S V N	ACTACTTTTACG T T F T	810 TTACACAATTTC L H N F	820 ACTTTTCATAAT T F H N	830 840 GAGACTGGCGCC E T G A
H120-P1 Frame 1	790 AATAGTGTTAAT N S V N	ACTACTTTTACG T T F T	810 TTACACAATTTC L H N F	820 ACTTTTCATAAT T F H N	830 840 GAGACTGGCGCC E T G A
Consensus Frame 1	850 AACCCAAATCCT N P N P	860 AGTGGTCCAG SGVQ	870 AATATTCAAACT NIQT	880 TACCAAACACAA Y Q T Q	890 900 ACAGCTCAGAGT T A Q S
H52-P1 Frame 1	AACCCAAATCCT N P N P	AGTGGTGTCCAG S G V Q	870 AATATTCAAACT N I Q T	880 Taccaaacacaa Y Q T Q	890 900 ACAGCTCAGAGT T A Q S
H120-P1 Frame 1	850 AACCCAAATCCT N P N P	860 AGTGGTGTCCAG S G V Q	870 AATATTCAAACT N I Q T	880 Taccaaacacaa y Q T Q	890 900 ACAGCTCAGAGT T A Q S
Consensus Frame 1	910 GGTTATTATAAT GYYN	920 TTTAATTTTTCC F N F S	930 TTTCTGAGTAGT FLSS	940 TTTGTTTATAAG F V Y K	950 960 GAG <mark>TCTAATTTT</mark> ESN F
H52-P1 Frame 1	910 GGTTATTATAAT G Y Y N	920 TTTAATTTTTCC F N F S	930 TTTCTGAGTAGT F L S S	940 TTTGTTTATAAG F V Y K	950 960 GAGTCTAATTT E S N F
H120-P1 Frame 1	910 GGTTATTATAAT G Y Y N	920 TTTAATTTTTCC F N F S	930 TTTCTGAGTAGT F L S S	940 TTTGTTTATAAG F V Y K	950 960 GAGTCTAATTTT E S N F
Consensus Frame 1	970 ATGTATGGATCT MYGS	980 TATCACCCAAGT Y H P S	990 T <mark>GTAATTTTAGA</mark> CNFR	1,000 CTAGAAACTATT L E T I	1,010 1,020 AATAATGGTTTG N N G L
H52-P1 Frame 1	970 ATGTATGGATCT M Y G S	980 TATCACCCAAGT Y H P S	990 TGTAATTTTAGA C N F R	1,000 CTAGAAACTATT L E T I	1,010 1,020 AATAATGGTTTG N N G L
H120-P1 Frame 1	970 ATGTATGGATCT M Y G S	980 TATCACCCAAGT Y H P S	990 TGTAATTTTAGA C N F R	1,000 CTAGAAACTATT L E T I	1,010 1,020 AATAATGGTTTG N N G L
Consensus Frame 1	1,030 TGGTTTAATTCA W F N S	1,040 CTTTCAGTTTCA L S V S	1,050 ATTGCTTACGGT I A Y G	1,060 CCTCTTCAAGGT PLQG	1,070 1,080 GGTTGCAAGCAA GCKQ
H52-P1 Frame 1	1,030 TGGTTTAATTCA W F N S	1,040 CTTTCAGTTTCA L S V S	1,050 ATTGCTTACGGT I A Y G	1,060 CCTCTTCAAGGT P L Q G	1,070 1,080 GGTTGCAAGCAA G C K Q
H120-P1 Frame 1	1,030 TGGTTTAATTCA W F N S	1,040 CTTTCAGTTTCA L S V S	1,050 ATTGCTTACGGT I A Y G	1,060 CCTCTTCAAGGT P L Q G	1,070 1,080 GGTTGCAAGCAA G C K Q

Consensus Frame 1	1,090 TCTGTCTTTAGT S V F S	1,100 GGTAGAGCAACC GRAT	1,110 TGTTGTTATGCT C C Y A	1,120 TACTCATATGGA Y S Y G	1,130 1,140 GGTCCTTTGCTG G P L L
H52-P1 Frame 1	1,090 TCTGTCTTTAGT S V F S	1,100 GGTAGAGCAACC G R A T	1,110 TGTTGTTATGCT C Y A	1,120 TACTCATATGGA Y S Y G	1,130 1,140 GGTCCTTTGCTG G P L L
H120-P1 Frame 1	1,090 TCTGTCTTTAGT S V F S	1,100 GGTAGAGCAACC G R A T	1,110 TGTTGTTATGCT C Y A	1,120 TACTCATATGGA Y S Y G	1,130 1,140 GGTCCTTTGCTG G P L L
Consensus Frame 1	1,150 TGTAAAGGTGTT CKGV	1,160 TATTCAGGTGAG YSGE	1,170 TTAGATCATAAT L D H N	1,180 TTTGAATGTGGA F E C G	1,190 1,200 CTGTTAGTTTAT L L V Y
H52-P1 Frame 1	1,150 TGTAAAGGTGTT C K G V	1,160 TATTCAGGTGAG Y S G E	1,170 TTAGATCATAAT L D H N	1,180 TTTGAATGTGGA F E C G	1,190 1,200 CTGTTAGTTTAT L L V Y
H120-P1 Frame 1	1,150 TGTAAAGGTGTT C K G V	1,160 TATTCAGGTGAG Y S G E	1,170 TTAGATCATAAT L D H N	1,180 TTTGAATGTGGA F E C G	1,190 1,200 CTGTTAGTTTAT L L V Y
Consensus Frame 1	1,210 GTTACTAAGAGC VTKS	1,220 GGTGGCTCTCGT G G S R	1,230 ATACAAACAGCC I O T A	1,240 ACTGAACCGCCA TEPP	1,250 1,260 GTTATAACTCAA VITO
H52-P1 Frame 1	1,210 GTTACTAAGAGC V T K S	1,220 GGTGGCTCTCGT G G S R	1,230 ATACAAACAGCC I Q T A	1,240 ACTGAACCGCCA T E P P	1,250 1,260 GTTATAACTCAA V I T Q
H120-P1 Frame 1	1,210 GTTACTAAGAGC V T K S	1,220 GGTGGCTCTCGT G G S R	1,230 ATACAAACAGCC I Q T A	1,240 ACTGAACCGCCA T E P P	1,250 1,260 GTTATAACTCAA V I T Q
Consensus Frame 1	1,270 CACAATTATAAT H N Y N	1,280 AATATTACTTTA N I T L	1,290 AATACTTGTGTT N T C V	1,300 GATTATAATATA DYNI	1,310 1,320 TATGGCAGAACT YGRT
H52-P1 Frame 1	1,270 CACAATTATAAT H N Y N	1,280 AATATTACTTTA N I T L	1,290 AATACTTGTGTT N T C V	1,300 GATTATAATATA D Y N I	1,310 1,320 TATGGCAGAACT Y G R T
H120-P1 Frame 1	1,270 CACAATTATAAT H N Y N	1,280 AATATTACTTTA N I T L	1,290 AATACTTGTGTT N T C V	1,300 GATTATAATATA D Y N I	1,310 1,320 TATGGCAGAACT Y G R T
Consensus Frame 1	1,330 GGCCAAGGTTTT G Q G F	1,340 ATTACTAATGTA I T N V	1,350 ACCGACTCAGCT TDSA	1,360 GTTAGTTATAAT VSYN	1,370 1,380 TATCTAGCAGAC Y L A D
H52-P1 Frame 1	1,330 GGCCAAGGTTTT G Q G F	1,340 ATTACTAATGTA I T N V	1,350 ACCGACTCAGCT T D S A	1,360 GTTAGTTATAAT V S Y N	1,370 1,380 TATCTAGCAGAC Y L A D
H120-P1 Frame 1	1,330 GGCCAAGGTTTT G Q G F	1,340 ATTACTAATGTA I T N V	1,350 ACCGACTCAGCT T D S A	1,360 GTTAGTTATAAT V S Y N	1,370 1,380 TATCTAGCAGAC Y L A D
Consensus Frame 1	1,390 GCAGGTTTGGCT A G L A	1,400 ATTTTAGATACA I L D T	1,410 TCTGGTTCCATA S G S I	1,420 GACATCTTTGTC DIFV	1,430 1,440 GTACAAAGTGAA VQSE
H52-P1 Frame 1	1,390 GCAGGTTTGGCT A G L A	1,400 ATTTTAGATACA I L D T	1,410 TCTGGTTCCATA S G S I	1,420 GACATCTTTGTC D I F V	1,430 1,440 GTACAAAGTGAA V Q S E
H120-P1 Frame 1	1,390 GCAGGTTTGGCT A G L A	1,400 ATTTTAGATACA I L D T	1,410 TCTGGTTCCATA S G S I	1,420 GACATCTTTGTC D I F V	1,430 1,440 GTACAAAGTGAA V Q S E
Consensus Frame 1	1,450 TATGGTCTTAAT Y G L N	1,460 TATTATAAGGTT YYKV	1,470 AACCCTTGCGAA N P C E	1,480 GATGTCAACCAG D V N Q	1,490 1,500 CAGTTTGTAGTT Q F V V
H52-P1 Frame 1	1,450 TATGGTCTTAAT Y G L N	1,460 TATTATAAGGTT Y Y K V	1,470 AACCCTTGCGAA N P C E	1,480 GATGTCAACCAG D V N Q	1,490 1,500 CAGTTTGTAGTT Q F V V
H120-P1 Frame 1	1,450 TATGGTCTTAAT Y G L N	1,460 TATTATAAGGTT Y Y K V	1,470 AACCCTTGCGAA N P C E	1,480 GATGTCAACCAG D V N Q	1,490 1,500 CAGTTTGTAGTT Q F V V
Consensus Frame 1	1,510 TCTGGTGAAA SGGGK	1,520 TTAGTAGGTATT L V G I	1,530 CTTACTTCACGT L T S R	1,540 AATGAGACIGGT NETG	1,550 1,560 TCCCAGCTTCTT S 0 L L
H52-P1 Frame 1	1,510 TCTGGTGGTAAA S G G K	1,520 TTAGTAGGTATT L V G I	1,530 CTTACTTCACGT L T S R	1,540 AATGAGACTGGT N E T G	1,550 1,560 TCCCAGCTTCTT S Q L L
H120-P1 Frame 1	1,510 TCTGGTGGTAAA S G G K	1,520 TTAGTAGGTATT L V G I	1,530 CTTACTTCACGT L T S R	1,540 AATGAGACTGGT N E T G	1,550 1,560 TCCCAGCTTCTT SQLL
Consensus Frame 1	1,570 GAGAATCAGTTT E N Q F	1,580 TACATCAAAATC Y I K I	1,590 1,590 ACTAATGGAACA T N G T	6	
H52-P1 Frame 1	GAGAATCAGTTT E N Q F	TACATCAAAATC Y I K I	1,590 ACTAATGGAACA T N G T	6	
H120-P1 Frame 1	1,570 GAGAATCAGTTT E N Q F	1,580 TACATCAAAATC Y I K I	1,596 ACTAATGGAACA T N G T	ö	

Figure 2. Full-length characterization and alignment of the S1 gene of H52 and H120 strains from Razi Institute at both nucleotide and amino acid level.

Comparison of these S1 sequences with those available in the GenBank also revealed that they are 100% identical to some sequences isolated in the field which can be resulted from either reisolation of vaccinal viruses circulating in the susceptible hosts (Jackwood et al., 2012) or highly frequent recombination of the IBV genome during co-circulation of the vaccinal and field strains (Capua et al., 1999; Lee and Jackwood, 2001; Jackwood et al., 2010; Jackwood et al., 2012).

The changes of nucleotide and amino acid sequences observed from the 3rd passage of H52 strain made these three passages exactly identical to the H120 strain. As these passages are 52nd and 120th passages of the Massachusetts serotype in embryonated-egg (Bijlenga et al., 2004), it can be assumed that the changes established in the 3rd passage of H52 strain remained constant until the 120th passages with no effects on immunogenicity and pathogencity of these strains. The amino acid G to V substitution, at position 118 within the hypervariable region is reminiscent of G to V substitution at position 110 reported by Cavanagh et al. (1988). It would be of interest to determine whether this specific substitution within the hypervariable region resulted from host adaptation during serially embryonated-egg passages. In this respect, it has been reported that alternate passage of the field strain 793/B in chick and embryonated eggs favored selection of serine and alanine at position 95, respectively,. This change is speculated to be a consequence of hostdriven selection due to internal host factors (Cavanagh et al., 2005).

These data emphasize that the S1 gene of H52 and H120 strains from Razi institute did not show a substantial change during serial passages in embryonated eggs. To determine the genetic change responsible for the distinct virulence of these strains, characterization of the full genome of these strains is recommended. In this point of view, it has been reported that the attenuation of IBV in egg is associated with nonstructural (nsp3) protein (Ammayappan et al., 2009; Phillips et al., 2012).

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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