MOLECULAR SIMILARLY BETWEEN INFECTIOUS BRONCHITIS VIRUSES AND COMMON VACCINES

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Abstract

Infectious bronchitis is an acute extremely infectious respiratory illness caused by the avian gamma-corona virus. Infection with infectious bronchitis virus predisposes the bird to subsequent bacterial infection, worsening the situation. Infection causes severe morbidity and variable mortality in broilers, as well as a significant decrease in layer production of eggs. Samples were collected from clinical cases submitted for necropsy at local veterinary clinics.This study was conducted to detect the molecular similarity in S1 gene sequence between field viruses and commonly used vaccines. In order to compare the sequences of field viruses with vaccinal viruses, two vaccines are chosen based on their popularity in veterinary clinics. These are MA5 strain and H120 strain. Molecular identification was done by using polymerase chain reaction (PCR) which was employed using primers target the S1 gene. Four positive field cases and two vaccine samples were sent to sequencing. The results of sequence alignment showed that vaccine viruses differ by more than 30% when compared to sequences of all the field viruses. The difference between genetic sequence leads to vaccine failure due to difference in the antigenic molecules on the spike protein of IBV.

Keywords: Sport psychology. Sport exercise. Infectious bronchitis virus. Chickens. Sequence identity. vaccines. S1 gene

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Introduction

Infectious bronchitis is an acute extremely infectious respiratory illness caused by the avian gamma-corona virus. Chickens and other avian species can be infected with IBV (OIE, 2018). Infection with infectious bronchitis virus predisposes the bird to subsequent bacterial infection, worsening the situation. Infection causes severe morbidity and variable mortality in broilers, as well as a significant decrease in layer production of eggs (Ibrahiem, 2016). The virus may be found all around the world and is spread by respiration or direct bird-to-bird contact or exposure to contaminated equipment, litter, tools, or more premises. Although in-ovo spread of the pathogen did not recorded yet, it may contaminate the eggshells by shedding from the reproductive or alimentary system (Jackwood & de Wit, 2020; Mohammed et al., 2013). The virus is an enveloped virus that varies in morphology from round to pleomorphic. The virions are roughly 120 nm in size and have club-shaped outer appendages called spikes and these are approximately 20 nm long, giving the virus a look of a crown. Corona is a latin word means crown (Khataby et al., 2020).

The symptoms of IB in affected young birds include general respiratory signs such as nasal secretion, respiratory rales, coughing, sneezing, and gasping. Watery eyes and even dilated sinuses have been seen in chicks. Other concurrent disease could be contributing to the severity of some cases (Ellakany et al., 2019; Hassan et al., 2017; Khamas, 2008). The chicks could be spotted curled up next to a heat source and seem depressed. Feed intake and growth might both be decreased. If the flock is not properly investigated, the sickness may even go undetected (Jackwood & de Wit, 2020). Even in situations with evident production reductions and the laying of bleached eggs, respiratory abnormalities in laying chickens might be absent or extremely slight. The degree of the production reduction can range from minor to severe, depending on parameters such as the viral serotype and birds immunity, the lay phase during which the infection occurred, and concurrent infections (Najimudeen et al., 2020). The trachea, nasal cavity, and sinuses of affected hens contain exudate. During the acute infection, the air sacs may be frothy, then turbid and have a yellow caseous discharge. Inflammation of lung tissues that surround big bronchi. Infections with strains of renal tropism can cause enlarged, faint kidneys due to the occupation of tubules with urate (Benyeda et al., 2009; Ziegler et al., 2002).

Vaccinated or recently infected poultry are resistant to infection with the same virus strain, while immunity against infection with different IBV strains is variable. The challenge of vaccinated birds with a homologous virus (same strain) leads to much less viral shedding and for a limited duration than in unprotected birds (Vagnozzi et al., 2010). For IBV vaccination, attenuated and killed vaccines are employed. For priming of breeders and layers, live vaccinations are employed and they are also used for broilers. Attenuation is done by repetitive passage in chicken embryos, occasionally in conjunction with thermal processing (Jackwood et al., 2010). There are different vaccine strains used for the imunization of birds against IBV. These include: the Massachusetts strains (Mass41 and H120) (Jackwood & de Wit, 2020), Arkansas (Ark), Connecticut (Conn), Delaware (Del), Georgia98 (GA98), Georgia 08 (GA08), and Georgia 13 (GA13) in the United states, and 793/B, QX, and Q1 in Europe, Asia, and South America (Jordan, 2017). In Iraq, multiple vaccines strains are used including: H 120, MA5, 4/91, QX, Variant2, D274 and M48 (Abdulmaged, 2017; Ali Ameen & Hussein Raoof, 2013; Al-Khafaji, 2013; AL-Zuhariy, 2017; Hammadi & Zahid, 2015; Kadhym & Zahid, 2017; Saood & Al-Mayah, 2017; Zahid et al., 2011).

Our study focused on S1 gene sequence because a few changes in the amino acid sequence could lead to the emergence of a new virus strain (Cavanagh, 2007). When there is new strains, meanwhile, vaccine producers and farm vaccination programs are still relaying on old vaccinal strains will lead to lack of immunological protection of the bird. Hence, this will result in IB outbreaks even in vaccinated birds (Y. Ennaji et al., 2020).

Materials and methods

Sampling

Samples were collected from clinical cases submitted for necropsy at local veterinary clinics. Tissues for molecular detection included tracheas, lungs and kidneys. Those organs were placed on petri dishes and small pieces were cut and put in 1.5 ml microcentrifuge tubes covered with TRIzol™ Reagent and kept in the freezer then sent to PCR laboratories.

Vaccines

In order to compare the sequences of field viruses with vaccinal viruses, two vaccines are chosen based on their popularity in veterinary clinics. These are MA5 strain and H120 strain. Those vaccines were sent for molecular detection along with tissue samples.

Molecular Detection

Polymerase chain reaction (PCR) was employed using primers designed by Raoof et al., (2021). These primers include the forward primer f-IBV-S1 5`- GTT TAC TAC TAC CAA AGT GCC TT -3` and the reverse primer 5`- GTG TAA ACA AGG TCA CCA TTT A -3`. Those oligonucleotides target the S1 gene and produce a 448bp PCR product.

Sequencing and Sequence Analysis

for sequencing step, PCR products were sent to Macrogen Co., Seoul, Republic of Korea. Four positive field cases and two vaccine samples were sent to sequencing. Once the sequence was ready the company emailed the sequence in FASTA format.

To analyse the sequences, two programs were used. The program Geneious Prime was used to generate the sequence similarity percentage and table between the sequences of our study between each other. BLAST® was used to compare the study sequences with sequences of other similar viruses on the NCBI GenBank. The later software was also used to create phylogenetics trees.

Results and Discussion

Sequence alignment of IB 120 vaccine virus sequence and the four cases sequences revealed that the percent identity of the IB 120 vaccine sequence and case 1 sequence was 69.85%. The exact differences in the sequences is

detailed in Figure 3 (Figures 1-3). There was 108 substitution mutations and 12 addition mutations. On the other hand, case 2 sequence was 69.58% identical to IB 120 sequence. There was 110 substitution mutations and 13 addition mutations (Figure 4). Likewise, The percent identity of the IB 120 vaccine sequence and case 3 sequence was 69.52%. There was 110 substitution mutations, 16 addition mutations and 2 deletion mutations (Figure 5). Moreover, case 4 sequence was 68.77% identical to IB 120 sequence. There was 111 substitution mutations, 12 addition mutations and one deletion mutations (Figure 6).

Sequence alignment of MA 5 vaccine virus sequence and the four cases sequences revealed that the percent identity of the MA 5 vaccine sequence and case 1 sequence was 69.42%. The exact differences in the sequences are detailed in Figure 1. There was 110 substitution mutations and 12 addition mutations. On the other hand, case 2 sequence was 69.01% identical to MA 5 vaccine sequence. There was 114 substitution mutations, 13 addition mutations and one deletion mutation (Figures 7, 8). Furthermore, The percent identity of the MA 5 vaccine sequence and case 3 sequence was 68.98%. There was 108 substitution mutations, 15 addition mutations and 2 deletion mutations (Figure 9). Moreover, case 4 sequence was 69.55% identical to MA 5 sequence. There was 110 substitution mutations, 11 addition mutations and one deletion mutations (Figure 10).

Sequence alignment of the four cases sequences revealed that case 1 sequence was identical to case 2 sequence by 98.99% identity. The exact differences in the sequences is detailed in Figure 11. There was 4 substitution mutations in case 1 when compared to case 2. In addition, There was 97.84% identity between case 1 and case 3. There was 8 substitution mutations and one addition mutation (Figure 12). Moreover, There was 97.08% identity between case 1 and case 4. There was 9 substitution mutations and 3 deletion mutation (Figure 13). On the other hand, case 2 and case 3 was 99.75% identical with one addition mutation in case 2 sequence when aligned to case 3 sequence (Figure 14). Furthermore, case 2 and case 4 was 98.25% identical. There was

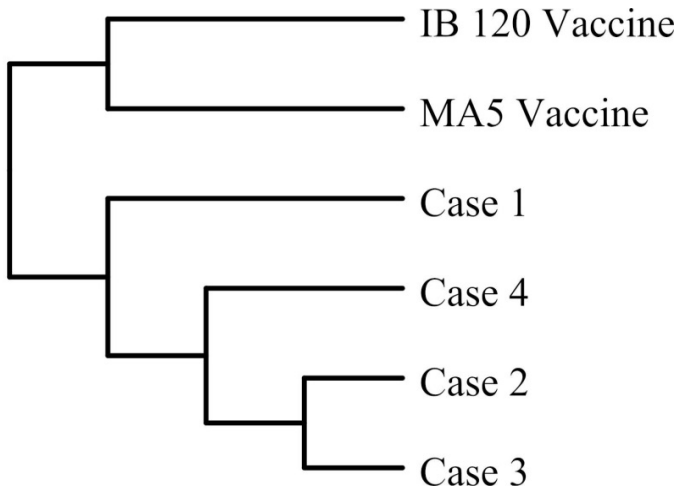

Figure 1: Molecular phylogenetic tree of the 6 sequences in our study.

Note: No field virus from the cases is closely related to the two vaccines. Although, case 1 was the most similar to the vaccines followed by case 4. Case 2 and 3 are the most closely related.

```
Query  7    ATGGGTGGCATTTACATGGGGGTGCGTATGCGGTTGTTAATATTTCTAGTGAATCTAATA  66
Sbjct  7    ........-..............-......G.................................  64

Query  67   ATGCAGGCTCTTCATCTGGGTGTACTGTTGGTATTATTCATGGTGGTCGTGTTGTTAATG  126
Sbjct  65   ............................................................  124

Query  127  CTTCTTCTATAGCTATGACGGCACCGTCATCAGGTATGGCTTGGTCTAGCAGTCAGTTTT  186
Sbjct  125  ............................................................  184

Query  187  GTACTGCATACTGTAACTTTTCAGATACTACAGTGTTTGTTACACATTGTTATAAACATG  246
Sbjct  185  ............................................................  244

Query  247  TTGGGTGTCCTATAACTGGCATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGAAAA  306
Sbjct  245  G...........................................................  304

Query  307  ATGGCCAGCTTTTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTTTTAAATCAT  366
Sbjct  305  ............C...............................................  364

Query  367  TTCAGTGTGTTAATAATTTAACATCCGTATATTTAAATGGTGCAttttttt  417
Sbjct  365  .........................................AC..G....  415
```

Figure 2: Comparison between IB 120 and MA5 vaccine sequences.

Note: Dots indicate identical nitrogenous base while mutations are colored with red. Keys: Query= MA 5 vaccine, Subject= IB 120 vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

```
Query  26   GGGTGCGTATGCGGTTGTTAATATTTCTAGTGAATCTAATAATGCAGGCTCTTCATCTGG  85
Sbjct  28   ......T.....A..A.C....G.....TTA....A.GC...C........A..TG.ACA  87

Query  86   GTGTACTGTTGGTATTATTCATGGTGGTCGTGTTGTTAATGCTTCTTCTATAGCTATGAC  145
Sbjct  88   C.......CA..GGC....T..T.GA..AAAAA.T...C...A......G....C.....  147

Query  146  GGCACCGTCATCAGGTATGGCTTGGTCTAGCAGTCAGTTTTGTACTGCATACTGTAACTT  205
Sbjct  148  A.....TGGTA........T.......A.CT.A...A........G..GC..........  207

Query  206  TTCAGATACTACAGTGTTTGTTACACATTGTTATAAACATG------TTGGGTGTCCTAT  259
Sbjct  208  C..G...TT.........C..................AG..GTTCAAA..TA.....AC.  267

Query  260  AACTGGCATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGAAAAATGGCCA-GCTT-  317
Sbjct  268  ...A..TC.TA.C.C.AGTGGC.A...T...A.C.....C....CG..A..AA.TA...C  327

Query  318  ----TTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTTTTAAATCATTTCAGTG  373
Sbjct  328  CTTG.........C........CCA..GA....A.......AA.....G..TC.G..A..  387

Query  374  TGTTAATAATTTAACATCCGTATATTTAAATGGTGCAt  411
Sbjct  388  ....G.......T.....T..G.............A..  425
```

Figure 3: Comparison between IB 120 vaccine and case 1 sequence.

Note: Dots indicate identical nitrogenous base, while mutations are colored with red. Keys: Query= Case 1, Subject= IB 120 vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

```
Query  21   CATGGGGGTGCGTATGCGGTTGTTAATATTTCTAGTGAATCTAATAATGCAGGCTCTTCA  80
Sbjct  19   ..........T.....A..A.C....G.....TTA....A.GC...C........A..T  78

Query  81   TCTGGGTGTACTGTTGGTATTATTCATGGTGGTCGTGTTGTTAATGCTTCTTCTATAGCT  140
Sbjct  79   G.ACAC.......CA..GGC....T..T.GA..AAAAA.T...C...A......G....C  138

Query  141  ATGACGGCACCGTCATCAGGTATGGCTTGGTCTAGCAGTCAGTTTTGTACTGCATACTGT  200
Sbjct  139  .....A.....TGGTA........T.......A.CT.A.G.A........G..GC.....  198

Query  201  AACTTTTCAGATACTACAGTGTTTGTTACACATTGTTATAAACATG------TTGGGTGT  254
Sbjct  199  .....C..G...TT.........C..................AG..GTTCAAA..TA...  258

Query  255  CCTATAACTGGCATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGAAAAATGGCCA-  313
Sbjct  259  ..AC....A..TC.TA.C.C.AGTGGC.A...T...A.A.....C....CG..A..AA.T  318

Query  314  GCTT-----TTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTTTTAAATCATTT  368
Sbjct  319  A...CCTTG.....C..CC........CCA..GA....A.......AA.....G..TC.G  378

Query  369  CAGTGTGTTAATAATTTAACATCCGTATATTTAAATGGTGC  409
Sbjct  379  ..A......G.......T.....T..G..............  419
```

Figure 4: Comparison between IB 120 vaccine and case 2 sequences.

Note: Dots indicate identical nitrogenous base, while mutations are colored with red. Keys: Query= Case 2, Subject= IB 120 vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

```
Query  14   GCATTTACATGG-GGGTGCGTATGCGGTTGTTAATATTTCTAGTGAATCTAATAATGCAG  72
Sbjct  25   ....G.G....CA......T.....A..A.C....G.....TTA....A.GC...C....  84

Query  73   GCTCTTCATCTGGG---TGTACTGTTGGTATTATTCATGGTGGTCGTGTTGTTAATGCTT  129
Sbjct  85   ..C--.......CACAC.......CA..GGC....T..T.GA..AAAAA.T...C...A.  142

Query  130  CTTCTATAGCTATGACGGCACCGTCATCAGGTATGGCTTGGTCTAGCAGTCAGTTTTGTA  189
Sbjct  143  .....G....C.....A.....TGGTA........T.......A.CT.A.G.A.......  202

Query  190  CTGCATACTGTAACTTTTCAGATACTACAGTGTTTGTTACACATTGTTATAAACATG---  246
Sbjct  203  .G..GC.........C..G...TT.........C..................AG..GTT  262

Query  247  ---TTGGGTGTCCTATAACTGGCATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGA  303
Sbjct  263  CAAA..TA.....AC....A..TC.TA.C.C.AGTGGC.A...T...A.A.....C....  322

Query  304  AAAATGGCCA-GCTT-----TTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTT  357
Sbjct  323  CG..A..AA.TA...CCTTG.....C..CC........CCA..GA....A.......AA.  382

Query  358  TTAAATCATTTCAGTGTGTTAATAATTTAACATCCGTATATTTAAATGGTGCAttttttt  417
Sbjct  383  ....G..TC.G..A......G.......T.....T..G.............A........  442
```

Figure 5: Comparison between IB 120 vaccine and case 3 sequences.

Note: Dots indicate identical nitrogenous base, while mutations are colored with red. Keys: Query= Case 3, Subject= IB 120 vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

```
Query  33   TATGCGGTTGTTAATATTTCTAGTGAATCTAATAATGCAGGCTCTTCATCTGGGTGTACT  92
Sbjct  33   ....-...A.C....G.....TTA...GA.GC...C........A..TG.ACAC......  91

Query  93   GTTGGTATTATTCATGGTGGTCGTGTTGTTAATGCTTCTTCTATAGCTATGACGGCACCG  152
Sbjct  92   .CA..GGC....T..T.GA..AAAAA.T...C...A......G....C.....A.....T  151

Query  153  TCATCAGGTATGGCTTGGTCTAGCAGTCAGTTTTGTACTGCATACTGTAACTTTTCAGAT  212
Sbjct  152  GGTA........T.......A.CT.A...A........G..GC..........C..G...  211

Query  213  ACTACAGTGTTTGTTACACATTGTTATAAACATG------TTGGGTGTCCTATAACTGGC  266
Sbjct  212  TT.........C..................AG..GTCCAAA..TA.....AC....A..T  271

Query  267  ATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGAAAAATGGCCA-GCTT-----TTT  320
Sbjct  272  C.TA.C.C.AGTGGC.A...T...A.A.....C....CG..A..AA.TA...CCTTG...  331

Query  321  TATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTTTTAAATCATTTCAGTGTGTTAAT  380
Sbjct  332  ..C..CC........CCA..GA....A.......AA.....G..TC.G..A......G..  391

Query  381  AATTTAACATCCGTATATTTAAATGGTGCAttttttt  417
Sbjct  392  .....T.....T..G.............AC..G....  428
```

Figure 6: Comparison between IB 120 vaccine and case 4 sequences.

Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 4, Subject= IB 120 vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

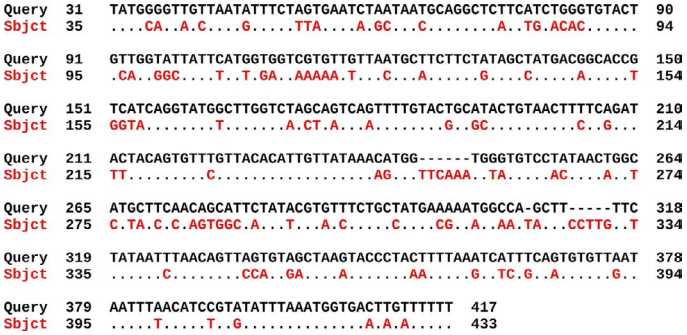

Figure 7: Comparison between MA 5 vaccine and case 1 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 1, Subject= MA 5 Vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

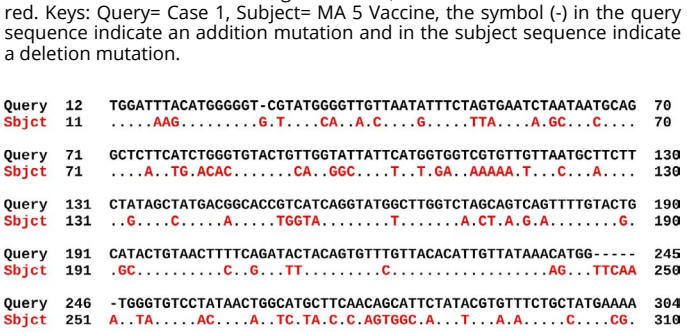

Figure 8: Comparison between MA 5 vaccine and case 2 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 2, Subject= MA 5 Vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Figure 9: Comparison between MA 5 vaccine and case 3 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 3, Subject= MA 5 Vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Figure 10: Comparison between MA 5 vaccine and case 4 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 4, Subject= MA 5 Vaccine, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

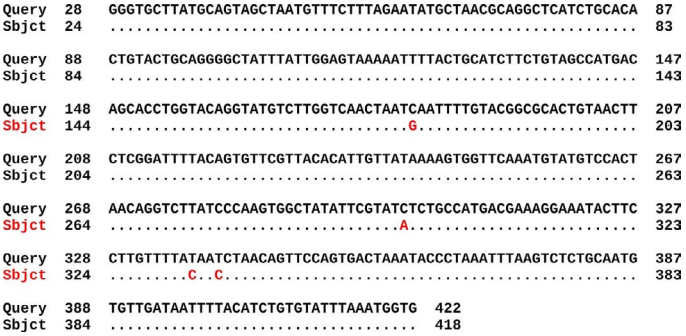

Figure 11: Comparison between case 1 and case 2 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. keys: Query= Case 2, Subject= Case 1, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Figure 12: Comparison between case 1 and case 3 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 3, Subject= Case 1, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Figure 13: Comparison between case 1 and case 4 sequences.
Note: Dots indicate similar nitrogenous base, while mutations are colored with red. Keys: Query= Case 4, Subject= Case 1, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

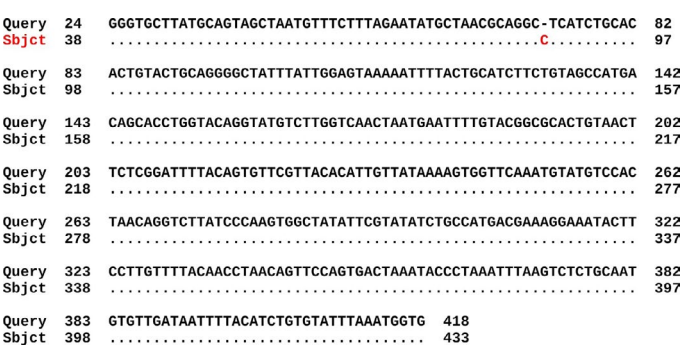

Figure 14: Comparison between case 2 and case 3 sequences.
Note: Dots indicate similar nitrogenous base and mutations are colored with red. Keys: Query= Case 3, Subject= Case 2, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

4 substitution mutations, 2 deletion mutations and one addition mutation in case 2 sequence when aligned to case 4 sequence (Figure 15). Finally, case 3 and case 4 was 96.72% identical. There was 10 substitution mutations and 4 deletion mutations in case 3 sequence when aligned to case 4 sequence (Figure 16).

In total, sequence alignment showed that vaccine viruses differ by more than 30% when compared to sequences of all the field viruses (Tables 1-2). Hence, the vaccinal viruses are grouped separately from the rest of field viruses in the

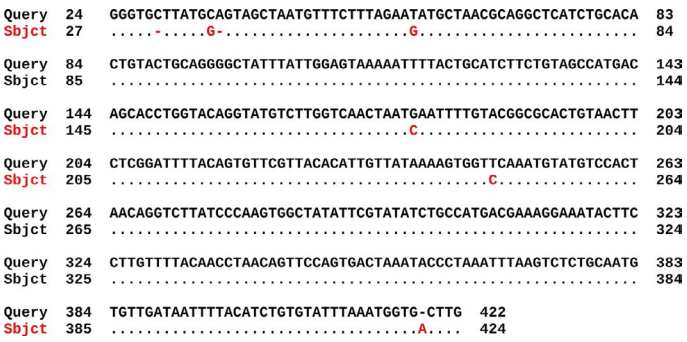

Figure 15: Comparison between case 2 and Case 4 sequences.
Note: Dots indicate similar nitrogenous base and mutations are colored with red. Keys: Query= Case 4, Subject= Case 2, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Figure 16: Comparison between case 3 and case 4 sequences.
Note: Dots indicate similar nitrogenous base and mutations are colored with red. Keys: Query= Case 4, Subject= Case 3, the symbol (-) in the query sequence indicate an addition mutation and in the subject sequence indicate a deletion mutation.

Table 1: The sequenced viruses and their GenBank accession numbers.

Virus	GenBank Accession Number
IB H120 vaccine	OP373738
MA5 vaccine	OP373739
Case 1	OP373740
Case 2	OP373741
Case 3	OP373742
Case 4	OP373743

Table 2: Percent identity of sequences.

	IB 120 Vaccine	MA5 Vaccine	Case 1	Case 2	Case 3	Case 4
IB 120 Vaccine		**98.05%**	69.85%	69.58%	69.52%	68.77%
MA5 Vaccine	**98.05%**		69.42%	69.01%	68.98%	69.55%
Case 1	69.85%	69.42%		**98.99%**	**97.84%**	**97.08%**
Case 2	69.58%	69.01%	**98.99%**		**99.75%**	**98.25%**
Case 3	69.52%	68.98%	**97.84%**	**99.75%**		**96.72%**
Case 4	68.77%	69.55%	**97.08%**	**98.25%**	**96.72%**	

Note: Bold and Empty cells indicate high similarity in the sequences. There was high similarity between the two vaccines but they both differ by more than 30% when compared with all cases.

molecular phylogenetic tree in Figure 1. This difference is a huge difference in molecular terms especially when taking into consideration that the difference between human and mice genome is only about 20% (Mouse Genome Sequencing Consortium, 2002).

Our results emphasize the importance of genetic diversity and confirm the the presence of continuous sequence alterations. This was demonstrated by comparing our sequences to each other and found nucleotide mutations in each pair of isolates compared. The presence of high sequence alterations in IBVs was investigated by Umar et al., (2016). They highlighted the three elements that may cause these alterations. First, There is no RNA polymerase proofreading, this causes errors and hence mutations. Second, the constant use of different live vaccines will lead to recombination and emergence of new strains. Third, continuous circulation of the virus will cause pressure on the birds immune system and increase the chance of errors during replication.

Conclusions

1. There was more than 30% difference in the S1 gene sequence when comparing sequences from vaccines used in Iraq and viruses circulating locally.
2. The difference between genetic sequence leads to vaccine failure due to difference in the antigenic molecules on the spike protein of IBV.
3. Our result showed that gene sequencing provides great benefits in designing and choosing vaccines against local viruses.
4. There was continuous occurrence of mutations in local IBV viruses. This was shown in the comparison between our sequences and previous sequence data in the GenBank.

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