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Full Length Research Article

Generic Variance Indicating Mutations in The *Vp2* Gene of Infectious Bursal Disease Virus (Ibdv) Variants

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ABSTRACT

Infectious bursal disease (IBD) is a disease caused by infectious bursa virus (IBDV). The disease is highly affecting young chickens, representing a serious threat to the poultry industry worldwide. This research aims to determine the pattern of mutation and the trend of evolutionary relationship among different variants of infectious bursal disease virus (IBDV). A total of 26 sequences of *VP2* gene of different IBDV variants, was retrieved from the National Centre of Biotechnology Information (NCBI BLAST) Gen Bank. Multiple sequence alignment was carried out with AF248612.1 as reference sequence. The nucleotide sequences were also translated to amino acids and checked the mutation based on the amino acids' substitutions. From the result, mutation was observed at position S-254-G, E-270-A and S-335-V. However, phylogenetic study categorized the IBDV variants into different geographical continents which comprises Malaysia, China, Finland, Iran, Brazil, Korean, Algeria, Japan, Israel, Russia Germany, and UK Isolates. Understanding the mutation particularly in the VP2 gene may provide insight on the pattern of amino acid substitution which may ease the selection of variant for best vaccine candidate.

Keywords: - Amino acids, Mutation, Phylogenetic, VP2 Gene

INTRODUCTION

Infectious bursal disease (IBD) is caused by infectious bursal disease virus (IBDV).The disease is highly contagious affecting young chickens, representing a serious threat to the poultry industry globally (Castón *et al*., 2008) infection via oral route cause, IBDV replication in several lymphoid organs; the Bursa of fabricius (BF) (a bird-specific primary lymphoid organ where the maturation of the avian B cells occurs) is IBDV main target. Once in the BF, IBDV replicates

actively in immature IgM+ B cells, causing bursal atrophy, immunosuppression and sometimes death (Castón *et al*., 2008).

Based on the classification IBDV belongs to the family *Birnaviridae*, genus Avibirnavirus, and is a naked and bisegmented double-stranded RNA virus. Segment A (3.2 kbp) encodes two open reading frames (ORFs), which are partially overlapping. The first ORF encodes 21 kDa non-structural viral protein 5 (VP5), while the second one codes for a 110 kDa polyprotein which is further cleaved by autoproteolysis to yield mature VP2 (capsid protein, 42 kDa), VP4 (protease, 28 kDa) and VP3 (scaffold protein, 32 kDa). The smaller segment B (2.8 kbp) encodes VP1 (97 kDa), the RNA-dependent RNA polymerase, RdRp (Eterradossi and Saif, 2020). VP2 contains a hypervariable region (hVP2) which carries the immunogenic determinants and spans from amino acid position 206 to 350 (Bayliss *et al*., 1990; Coulibaly *et al*., 2005). Mutations in hVP2 positions 253, 279, 284 and 330 have been related with both adaptation to avian and mammalian cell culture and viral attenuation in chickens of pathogenic field strains. Correspondingly questions about unique genetic mutations involved in the evolution and expansion of vvIBDV remain open. Attenuated vaccines currently used to protect from IBD elicit a more robust immune response when compared to inactivated vaccines, but they convey the risk of reversion toward higher virulence (Eterradossi and Saif, 2020). Thus, a better knowledge of the evolution of IBDV as well as new strategies in the development of safer and more stable vaccines are required to improve the control of emerging IBDV strains (Eterradossi and Saif, 2020).

Previous study revealed that, viral stocks produced before and after serial passages were deep-sequenced to explore the composition of their viral populations and provide insights in the evolutionary trajectories, genetic variability and host adaptability of IBDV. VP2 is the major host-protective capsid protein of the virus that carries the immunogenic determinants and is able to elicit neutralizing antibodies. Recently, IBDV has been classified into seven genogroups based on the marked changes in the amino acids in the hypervariable region of the capsid protein VP2 (hVP2) among the different groups, wherein genogroup 1 is distributed globally. Currently, the disease is controlled by live attenuated or inactivated IBDV vaccine, but the live vaccines can revert to virulence and the traditional vaccines may not give full protection against vvIBDV strain (Nguyen, 2014). The inactivated or killed vaccines are usually given to birds in pre-laying stage to induce higher levels of antibody production for at least 2 weeks. However, with the emergence of antigenic variants and very virulent IBDV (vvIBDV), chickens are not fully protected by conventional IBD vaccines.

Therefore, the main objectives of this study were to detect mutations and their antigenic consequences, particularly on the VP2 gene among different global IBDV variants and to select base vaccine candidates.

MATERIAL AND METHODS

To determine the different mutational changes that may occur at different strains of IBDV. Bioinformatics software's which comprises BioEdit (USA version.7.0.5.3; Therapeutics, Ibis) and MEGA 6.0 (Molecular Evolutionary Genetics Analysis version 6.0) was used to determine the mutations and pattern of evolutionary relationship among different IBDV variants.

Nucleotide Sequencing and Analysis of different IBDV variants

A total of 26 sequences of VP2 gene of different IBDV variants, was retrieved from the National centre of biotechnology

information (NCBI BLAST) Gen Bank and saved in the fasta format. Multiple sequence alignment was carried out using AF248612.1 as reference observed based on the amino acid substitutions (Salisu *et al*., 2021).

Construction of Phylogenetic Tree

Phylogenetic tree was constructed from the 26 nucleotide sequences retrieved from .

NCBI. All aligned sequences were exported to BioEdit (USA version.7.0.5.3; Therapeutics, Ibis) and the phylogenetic tree was constructed using MEGA 6.0 Distance- based neighbour joining with 1,000 Bootrap replicates used to evaluate the evolutionary distances (Salisu *et al*., 2021).

RESULT

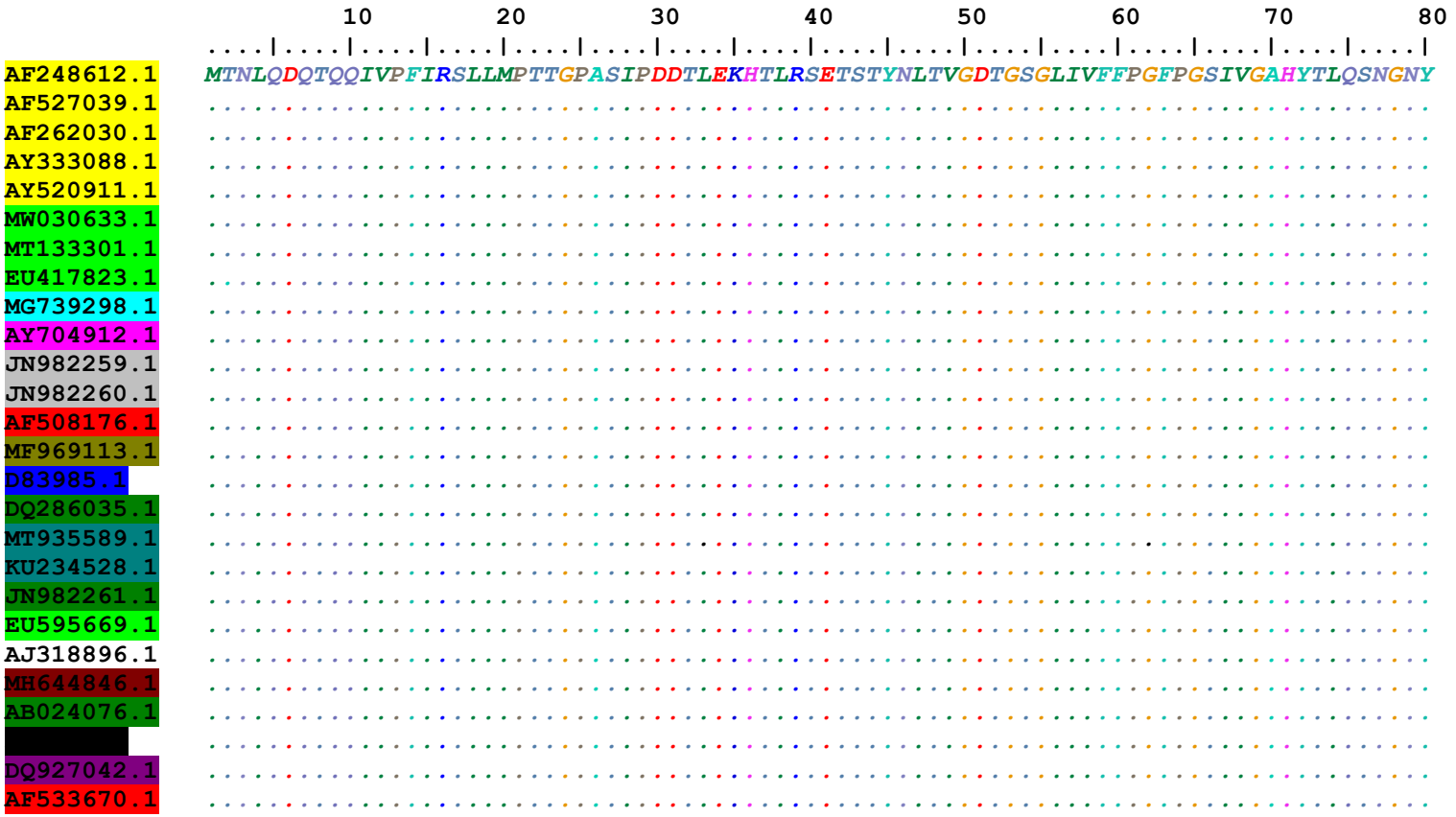


Figure1a: Multiple Sequence Alignment of IBDV VP2 Gene Sequences of the World IBDV Variants to Determine the Pattern of Mutation in the VP2 Gene. A dot (.) indicate position where the sequences a identical to other variants, to other strain the colour indicate IBDV variants, Malaysian variants (yellow), China variants (green), Korean variants (orange), UK variants (dark blue), Iran variants (purple), Brazil variants (grey) and Finland (green)

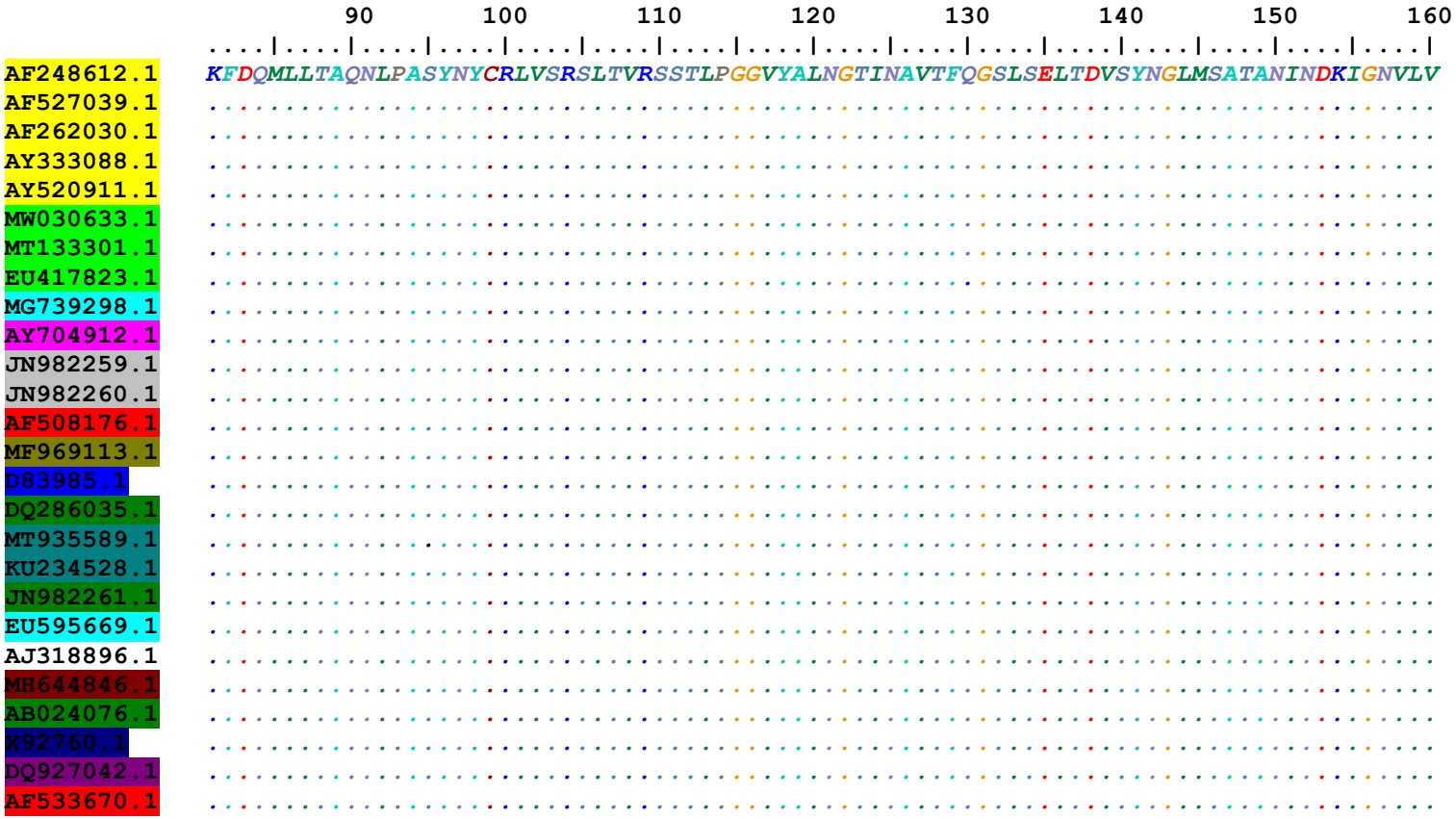


Figure 1b: Multiple Sequence Alignment of IBDV VP2 Gene Sequences of the World IBDV Variants to Determine the Pattern of Mutation in the VP2 Gene. A dot (.) indicate position where the sequences a identical to other variants, to other strain the colour indicate IBDV variants, Malaysian variants (yellow), China variants (green), Korean variants (orange), UK variants (dark blue), Iran variants (purple), Brazil variants (grey) and Finland (green)

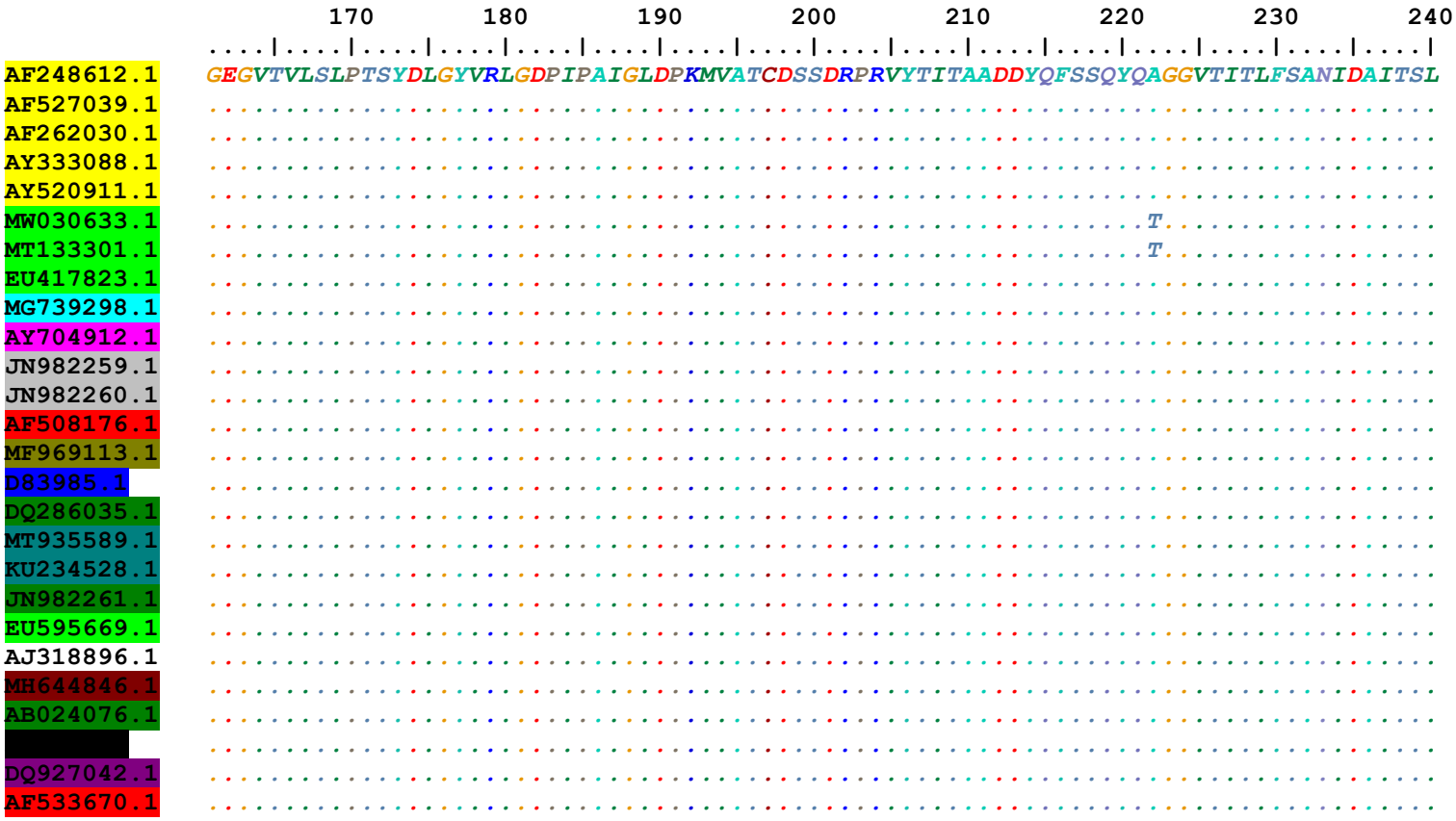


Figure 1c: Multiple sequence alignment of IBDV VP2 gene sequences of the world IBDV variants to determine the pattern of mutation in the VP2 gene. A dot (.) indicate position where the sequences a identical to other variants, to other strain the colour indicate IBDV variants, Malaysian variants (yellow), China variants (green), Korean variants (orange), UK variants (dark blue), Iran variants (purple), Brazil variants (grey) and Finland (green)

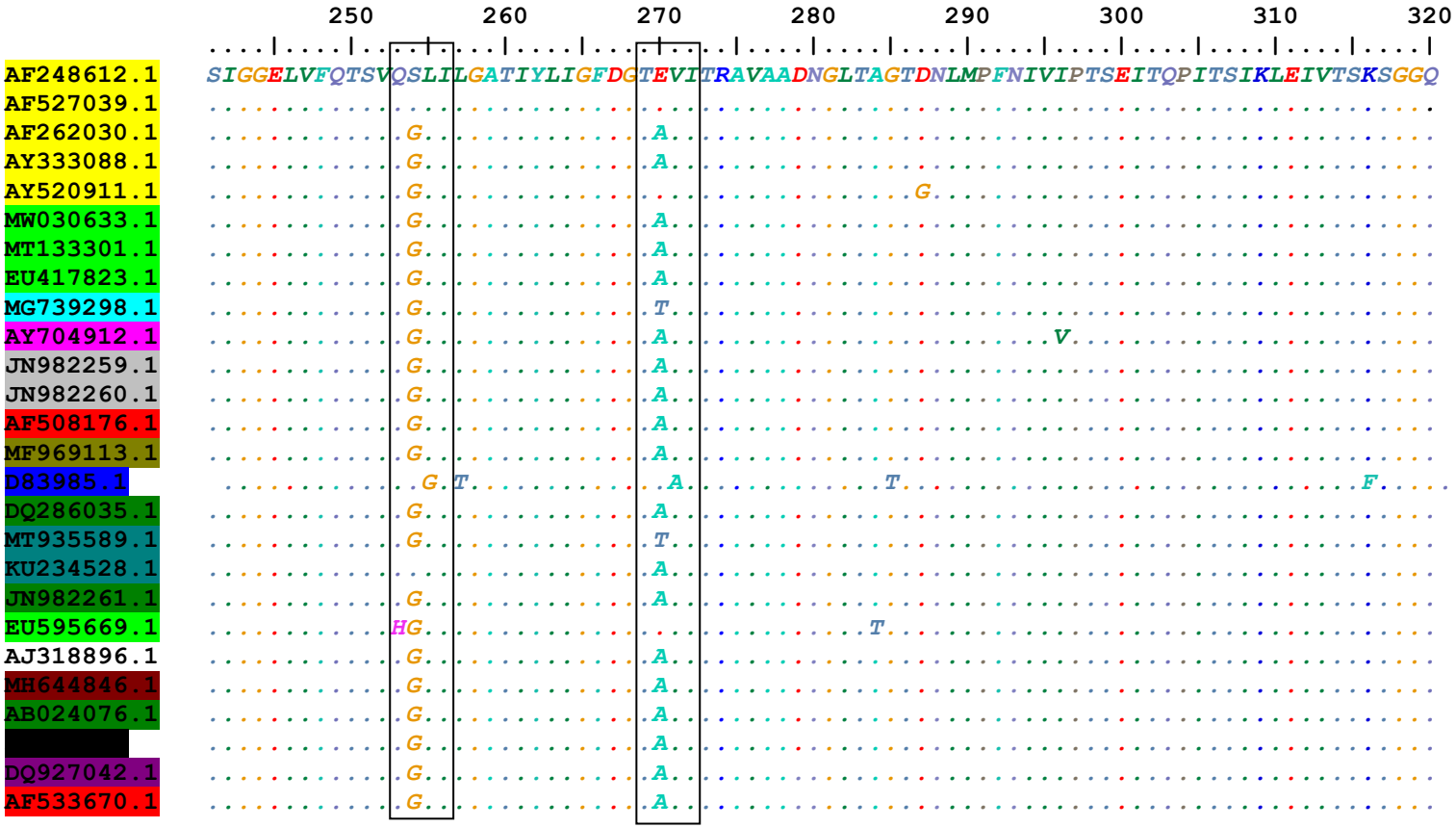


Figure 1d: Multiple Sequence Alignment of IBDV VP2 Gene Sequences of the World IBDV Variants to Determine the Pattern of Mutation in the VP2 Gene. A dot (.) indicate position where the sequences a identical to other variants, to other strain the colour indicate IBDV variants, Malaysian variants (yellow), China variants (green), Korean variants (orange), UK variants (dark blue), Iran variants (purple), Brazil variants (grey) and Finland (green)

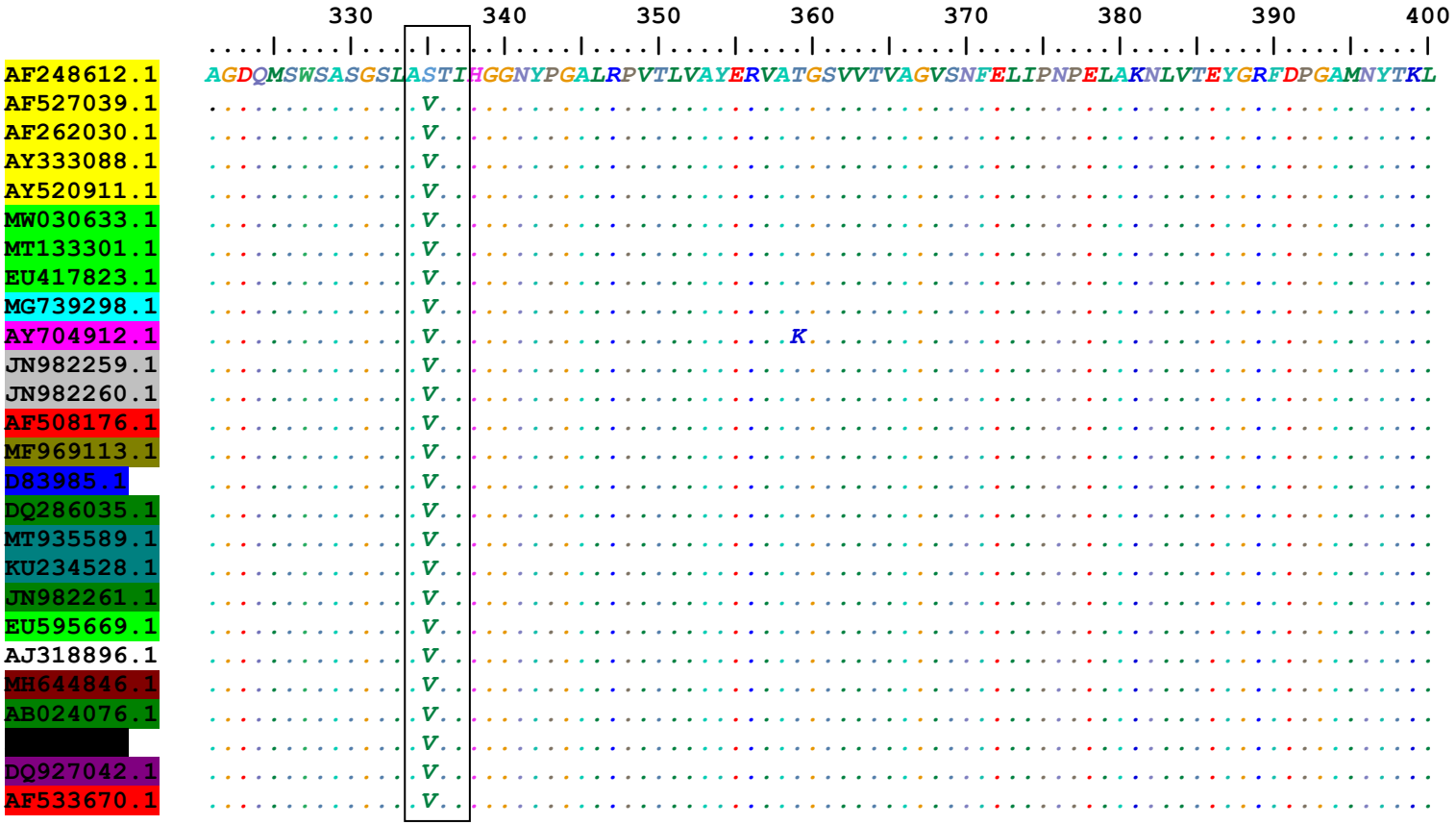


Figure 1e: Multiple Sequence Alignment of IBDV VP2 Gene Sequences of the World IBDV Variants to Determine the Pattern of Mutation in the VP2 Gene. A dot (.) indicate position where the sequences a identical to other variants, to other strain the colour indicate IBDV variants, Malaysian variants (yellow), China variants (green), Korean variants (orange), UK variants (dark blue), Iran variants (purple), Brazil variants (grey) and Finland (green)

Table 1: Molecular marker substitutions in the VP2 gene of the IBDV variants retrieved from the NCBI GenBank

S/N	Accession Number	Country	Amino acid (aa) substitution and position		
			255	270	335
1	AF248612.1*	Malaysia	S	E	S
2	AF527039.1	Malaysia	S	E	V
3	AF262030.1	Malaysia	G	A	V
4	AY333088.1	Malaysia	G	A	V
5	AY520911.1	Malaysia	G	E	V
6	MW030633.1	China	G	A	V
7	MT133301.1	China	G	A	V
8	EU417823.1	China	G	A	V
9	MG739298.1	Finland	G	T	V
10	AY704912.1	Iran	G	A	V
11	JN982259.1	Brazil	G	A	V
12	JN982260.1	Brazil	G	A	V
13	AF508176.1	Korean	G	A	V
14	MF969113.1	Algeria	G	A	V
15	D83985.1	Japan	S	E	V
16	DQ286035.1	Israel	G	A	V
17	MT935589.1	Brazil	G	T	V
18	KU234528.1	France	G	A	V
19	JN982261.1	Brazil	G	A	V
20	EU595669.1	China	G	T	V
21	AJ318896.1	Germany	G	A	V
22	MH644846.1	Russia	G	A	V
23	AB024076.1	Japan	G	A	V
24	X92760.1	UK	S	A	V
25	DQ927042.1	Brazil	G	A	V
26	AF533670.1	Korean	G	A	V

Note. * indicate the reference Variant

Nucleotide Sequencing and Analysis Variants

Based on the VP2 gene nucleotide sequences and the amino acids which correspond to 440 amino acids (Figure 1a-e) segments in the VP2 gene sequences. From the result it has been observed that there is mutation in position S-254-G (Figure 1d), S-270-A (Figure 1d) and S-335-V (Figure 1e).

Phylogenetic Analysis

Phylogenetic analysis based on the twenty-six (26) IBDV VP2 gene sequences. The result categorized the IBDV variants into different continent which includes Malaysia, China, Finland, Iran, Brazil, Korean, Algeria, Japan, Israel, Russia Germany, and UK.

DISCUSSION

IBDV is the causative agent of IBD, the disease causes immunosuppression which leads to secondary infections among rearing poultry flocks. Characterization of the virus is important for its control and eradication. The frequent and outbreaks of IBDV, especially in vaccinated poultry flocks have made it mandatory for regular epidemiological studies to be carried out in order to keep track of any genetic changes/variations that may occur in the field. This will enhance the efforts in controlling IBD in Nigeria and also assist in the selection of the right vaccine strain that will be effective in combating IBD outbreaks in the field. In this study molecular markers (amino acids) was determine in the VP2 gene of the different IBDV sequences. From the results mutation was observed critically from three (3) positions from the 26 sequences Serine-255-Glycine, Glutamate-270-Alanine and Serine-335-Valine. Interestingly, these amino acids substitution has further distinguished the IBDV variants into different continents. Equally, based on the molecular markers of the virus, the substitution exist between the reference variant (AF248612.1) indicate that there is amino acid substitution in position 254 from Serine to Glycine in the China, Finland, Iran, Japan, UK and Brazil this might further differentiate them base on the virus virulence nature. Furthermore, this trend of amino acids changes confirmed that the mutation in IBDV VP2 gene is consistent in the majority in the global IBDV sequences.

Mutation in the VP2 gene is critical as its play an important role in antigenic variation by which the virus can escape neutralizing antibodies. Most of the amino acid (aa) changes among the antigenically different IBDVs are clustered in the hypervariable region of VP2 (hVP2). Thus, this hypervariable region of VP2 is the obvious target for IBDV detection.

Moreover, the result also confirmed mutation in position Alanine-270-Glutamate and Serine-335-Valine respectively. Phylogenetic analysis based on the 26 nucleotide sequences of the VP2 gene, has categorized them into different continents (Malaysia, UK, Germany, Brazil, China, Israel, Finland, and Korean). However, the reference sequence were closely related to Israel and Korea variants (79%) and is distantly related to USA variants. Interestingly, the Brazil and Germany variants are closely related.

CONCLUSION

In conclusion, mutation was observed in the position 254, 270 and 330 in the VP2 gene. Mutation in the VP2 gene is very critical and as its play an important role in antigenic variation by which the virus can escape neutralizing antibodies. This study will provide insights on the genomic architecture of the IBDV.

The phylogenetic study has categorized the IBDV variants into different continents. This information might provide the researchers on the evolutionary relationship that exist between world IBDV variants.

Authors' Contributions

SA designed the study, conducted the Bioinformatics analysis and interpreted the data and drafted the manuscript. **MA** and **AA** participated in the study design, analysis and interpretation of data and drafting of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

No competing interests as declare by the authors.

REFERENCES

Bayliss C.D., Spies U., Shaw K. (1990): A Comparison of the Sequences of Segment A of four Infectious Bursal Disease Virus Strains and Identification of a Variable Region in VP2. *Journal of General Virology*.71 (6):1303–1312

Castón, José R., Rodríguez. José, F., Carrascosa, and José L. (2008): "Infectious Bursal Disease Virus (IBDV)". Segmented Double-stranded RNA Viruses: Structure and Molecular Biology. *Caister Academic Press*. ISBN 978-1-904455-21-9.

Coulibaly, F., Chevalier, C., Delmas, B and Rey, F.A. (2010). Crystal Structure of an Aquabirnavirus Particle: Insights nto Antigenic Diversity and Virulence Determinism. *Journal of Virology*.84 (4):1792–1799

Eterradossi, N and Saif, Y.M. (2008): "Infectious bursal disease". In Saif, Y.M. (Ed.). *Diseases of poultry (*12th Ed.). Blackwell P Publishing. p. 185-208.

NCBI Resource Coordinators. (2012): "Database resources of the National Center for Biotechnology Information". Nucleic Acids Research 41 (Database issue): D8–D20.

Nguyen, T. H. (2014): "GUMBORO Disease in Chickens and Methods of Prevention and Treatment" "Infectious Bursal Disease: Introduction". *The Merck Veterinary Manual.* 2006. Retrieved 2007-06-26

Salisu, A., Abdul Razak, M., Mohd H B., Abdul Rahman O., Aini, I and Nurulfiza, M, I.(2021): Molecular Markers and Phylogenetic Analysis of UPMT27, a Field Isolate of the Malaysian Fowl Adenovirus Associated with Inclusion Body Hepatitis. *Pertanika Journal of Science & Technology* 29 (1); 547 – 563