Nigerian J. Anim. Sci. 2022, 24 (1): 75-89

Computational Sequence Analysis of Foot and Mouth disease Virus in Cattle

[1*]Ahmed, A. B., [1]Thomas, S. E. and [2]Christopher, K. S.

[1]Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, Lafia, Nigeria
[2]Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State.

***Corresponding Author:** ahmedhabdullahi@gmail.com, **Phone Number:** 08064716949*

***Target Audience:** livestock breeders, cattle farmer, researches, drug developers.*

Abstract

It has been proven that foot and mouth disease virus (FMDV) is a highly contagious, severe and economically devastating viral disease worldwide, which affects animals with cloven hoof such as cattle, deer, pigs, sheep and goats. Thus, this study aimed at investigating the molecular genetic variation of FMD virus of cattle. A total of twenty FMD virus amino acid sequences of cattle were retrieved from the GenBank. Various serotypes of FMD virus were identified. The obtained phylogeny based on amino acid sequences of the FMD virus revealed differential clustering among the sequences. The predicted 3D FMD protein structures of cattle aligned well with the templates. The present information on FMDV biodiversity and evolution could be exploited in tracing FMDV sources and transmission events, as well as to ensure vaccine coverage of corresponding field of FMDV extractions mostly in a developing country such as Nigeria.

***Keywords:** Foot-and-mouth disease, phylogeny, serotypes, protein, cattle*

Description of the Problem

The economic devastation caused by FMD outbreak in bovine (cattle) in some countries has resulted to losses of billions of dollars to their economy; therefore FMD is a threat to animal production.

Foot and mouth disease virus (FMDV) is a virus of the genus Aphthovirus and belongs to the family of Picornaviridae. FMDV has seven distinct serotypes namely, SAT 1, SAT 3, SAT 2, Asia 1, O, A, and C, that infect cloven hoofed animals including cattle, goats, swine, and sheep. Infection with one serotype doesn't confer immunity against another (1). FMDV is amongst the most economically devastating and contagious viral disease in animals. The etiologic agent of the disease contains a single-stranded positive-sense RNA genome of c. 8500 nucleotides surrounded by an icosahedral capsid composed of 60 copies each of 4 structural proteins VP1-4 (also termed 1D, 1B, 1C, and 1A) (2) (3). Hosts susceptible include several domesticated (e.g. cattle, sheep, swine, goats, and Asian buffalo) and wild (e.g. wild boar and African buffalo) cloven-hoofed animals (4). FMDV trigger infection through detection of one of at least four cell-surface integrin molecules αvβ1, αvβ3, αvβ6, or αvβ8 by well

conserved Arg-Gly-Asp (RGD) amino acid sequence motif located in the G-H loop of VP1. Within the host animal, the αvβ6 interaction is observed to be the most relevant (5). The high mutation rate of FMDV due to the error-prone RNA polymerase lacking proofreading ability, leads to the genetic diversity of the FMDV (6). Common cases of FMD are usually characterized by excessive salivation, vesicles on epithelia of the tongue, in the mouth, fever, vesicular condition of the feet, nasal secretion and, in cows, vesicle on the epithelia of the teats. The disease causes severe production losses and while the bulk of affected animals recover, the disease often leaves them weakened and debilitated. In young animals, clinical symptoms varied from mild to severe, and mortality may also occur. Morbidity approaches 100% in susceptible population. Comparatively, animals reared under intensively system are more prone/susceptible to the disease than breeds raised traditionally. In adult animals, mortality is rare but often high mortality is seen in young animals as a result of myocarditis or by lack of milk when the lactating dam is infected by the disease (7). The FMD virus may occur occasionally in some of the FMD free areas, as it can be found in all excretions and secretions of the infected animal, the virus may be present in semen and milk for about four days before showing clinical symptoms in animal. However, animal can still be the carriers of the virus after been recovered from infection. This virus can be spread when animals that have been infected breathe out a large chunk of aerosolized virus, which can be transmitted to other animals through the oral or respiratory routes (8).

The World Organization for Animal Health has identified FMD as one of the most important constraint to international trade of animals and animal products (9). Therefore, countries that are free of FMD prohibit importation of FMD prone/ susceptible animals or their products from infected countries. Thus, the economic consequences of FMD outbreak are often devastating as demonstrated by the 1997 FMD outbreak in Taiwan, and therefore the 2001 outbreak within the UK. In both countries, millions of animals were slaughtered and there was a cost of billions of dollars to their economies (10) (11) (12).

These prompted the need for this study, which aimed at investigating the molecular genetic variation, various physico-chemical properties, and the tertiary structure (3D) prediction of FMDV in bovine (Cattle); to provide pertinent genetic information for the control and eradication of the FMD virus in bovine (cattle). This may lead to better livestock production and more financial benefits to the cattle farmer.

Materials and methods

Sequences of Species: A total of twenty (20) FMDV protein sequences of the bovine (cattle) species were obtained from the databank of the National Center for Biotechnology Information (NCBI).

The protein sequences accession numbers were: AAM640032.1, AAM63996.1, AAM69474.1, AAM69479.1,

AHX03083.1, AHX03082.1, AHX03089.1, AHX03087.1, AHY00740.1, AHY00726.1, AFU55192.1, AFZ75249.1, AFU55202.1, AFU55198.1, AFU55178.1, AFU55199.1, AFU55176.1, ACN81735.1, ACN81729.1, and ACN81750.1.

The Protein Sequence alignment and translation: translation, sequences alignment, and comparisons was carried out using ClustalW as Larkin described it (13).

Phylogenetic trees analysis: Two (2) Neighbor Joining (NJ) trees was constructed; the twenty (20) protein sequences of the FMDV was used for the construction of the first NJ tree, using Poisson model and complete deletion gap/missing data treatment. The construction done was on the basis of genetic distances, showing phylogenetic relationships among the FMDV amino acid sequences. Bootstrap confidence values with 1000 bootstrap iterations using MEGA 6 software was used to calculate the reliability of the trees (14). Similarly, eight (8) protein sequences of the FMDV out of the twenty were used to construct the second NJ tree using Maximum Parsimony tree with 1000 bootstrap iteration. Subtree-Pruning-Regrafting (SPR) algorithm was used to obtain the Maximum Parsimony tree (15).

Percentage Similarities of the Different FMDV Protein Sequences: BLAST (Basic Local Alignment search Tool) tool from the NCBI server was used for the percentage similarities of the different FMDV amino acid sequence, to blast the amino acid sequences of the FMDV to get their percentage similarities.

Physico-chemical properties analysis of FMDV: ProtParam Tool was used for the computation of the FMDV amino acid sequences, for their physical and chemical parameters. The parameters computed for were; amino acid composition, theoretical pI, extinction coefficient, molecular weight, estimated half –life, grand average of hydropathicity, aliphatic index, instability index, and atomic composition (GRAVY) (16).

The tertiary structure (3D) prediction of the FMDV: the eight (8) distinctive FMDV protein sequences used for the 2nd NJ tree was used in this stage of the study. The 3D structure was obtained using the Phyre2 servers for prediction of the structures. The server uses homology modeling techniques and principles in predicting the 3D structure of the amino acid sequence (17).

Results and Discussion

Various FMDV protein sequences retrieved from the GenBank revealed the different serotypes of the virus (Table 1). Length variation in the amino acid sequences of the various serotypes was observed.

Table 1: Various FMDV protein sequences obtained from the NCBI data bank.

Serotype	Species	Protein ID	Accession No.	No. of AA	Country
A	Cattle	1D protein, partial	AAM64003.1	206	India
A	Cattle	1D protein, partial	AAM63996.1	209	India
O	Cattle	1D protein, partial	AAM69474.1	96	India
O	Cattle	1D protein, partial	AAM69479.1	92	India
A	Cattle	polyprotein, partial	AHX03083.1	234	Egypt
A	Cattle	polyprotein, partial	AHX03082.1	230	Egypt
O	Cattle	polyprotein, partial	AHX03089.1	397	Egypt
O	Cattle	polyprotein, partial	AHX03087.1	248	Egypt
A	Cattle	VP1, partial	AHY00740.1	213	Kenya
A	Cattle	VP1, partial	AHY00726.1	212	Kenya
SAT2	Cattle	VP1, partial	AFU55192.1	216	Kenya
O	Cattle	VP1, partial	AFZ75249.1	213	United Kingdom
O	Cattle	polyprotein, partial	AFU55202.1	213	South Korea
SAT2	Cattle	VP1, partial	AFU55198.1	216	Nigeria
SAT2	Cattle	VP1, partial	AFU55178.1	216	Cameroon
SAT2	Cattle	VP1, partial	AFU55199.1	216	Gaza Strip
SAT2	Cattle	VP1, partial	AFU55176.1	216	Bahrain
Asia1	Cattle	VP1, partial	ACN81735.1	210	Malaysia
Asia1	Cattle	VP1, partial	ACN81729.1	211	Iran
Asia1	Cattle	VP1, partial	ACN81750.1	211	Pakistan

Table 2: Percentage similarity of the FMDV protein Sequences obtained using NCBI BLAST Tool

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	–	93%	55%	61%	25%	87%	72%	25%	83%	81%	48%	70%	71%	46%	47%	46%	48%	66%	67%	67%
2		–	55%	62%	56%	85%	71%	56%	80%	78%	46%	70%	70%	46%	48%	47%	48%	65%	66%	66%
3			–	77%	24%	55%	76%	24%	55%	53%	42%	77%	77%	43%	435	42%	44%	59%	57%	57%
4				–	24%	64%	88%	24%	60%	625	46%	87%	87%	46%	475	45%	51%	65%	64%	63%
5					–	42%	25%	99%	29%	36%	24%	100%	18%	36%	18%	36%	NS	NS	NS	NS
6						–	74%	42%	84%	82%	47%	70%	73%	47%	49%	47%	49%	66%	66%	66%
7							–	NS	71%	69%	50%	88%	92%	52%	50%	51%	50%	70%	71%	70%
8								–	NS	29%	32%	24%	99%	18%	36%	18%	36%	NS	NS	NS
9									–	83%	49%	70%	69%	43%	44%	47%	48%	67%	67%	67%
10										–	47%	69%	69%	49%	49%	48%	49%	65%	66%	67%
11											–	49%	52%	81%	81%	84%	95%	47%	48%	48%
12												–	99%	48%	49%	48%	50%	71%	71%	70%
13													–	48%	49%	48%	52%	70%	70%	69%
14														–	94%	92%	81%	48%	47%	48%
15															–	92%	82%	47%	47%	48 %
16																–	83%	47%	47%	47%
17																	–	48%	49%	47%
18																		–	89%	89%
19																			–	91%
20																				–

NS = Not Significant

The above figures on the horizontal and vertical area of the above table (1 to 20) represents following Accession Numbers;
1=AAM64003, 2=AAM63996, 3=AAM69474, 4=AAM69479, 5=AHX03083, 6=AHX03082, 7=AHX03089, 8=AHX03087, 9=AHY00740, 10=AHY00726, 11=AFU55192, 12=AFZ75249, 13=AFU55202, 14=AFU55198, 15=AFU55178, 16=AFU55199, 17=AFU55176, 18=ACN81735, 19=ACN81729, 20=ACN81750.

The above table shows the maximal regions of high similarity between the database sequences and the query sequence, which revealed high similarities among most of the protein serotypes of the FMDV sequences obtained from the NCBI server, using the BLAST tool (Table 2). However, accession numbers; AHX03083 (5) against AFU55176 (17), ACN81735 (18), ACN81729 (19) and ACN81750 (20), AHX03089 (7) against AHX03087 (8) and AHX03087 (8) against AHY00740 (9), ACN81735 (18), ACN81729 (19) and ACN81750 (20) were not significant (NS). Some sequences also showed low scores of similarities.

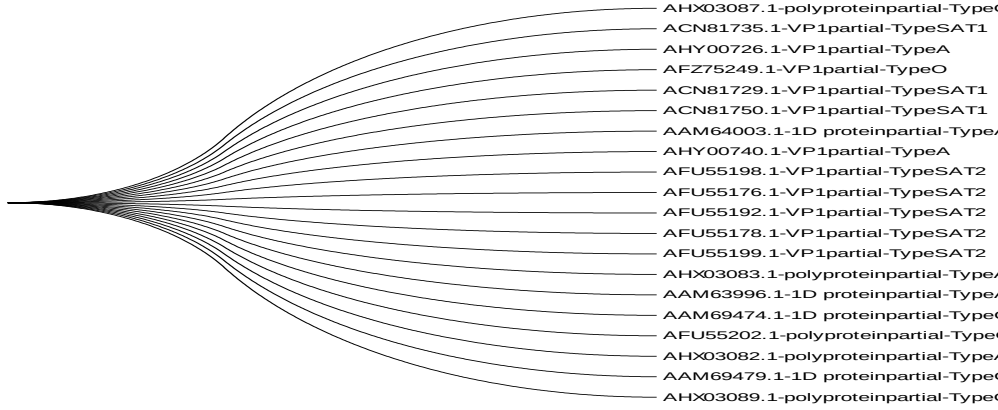


Figure 1: Neighbour-joining tree of the twenty (20) FMDV protein sequences obtained using MEGA 6, and derived using the Neighbor-Joining method. Modeled with a gamma distribution (shape parameter = 0.4) is the rate variation among sites. The VP1partial-TypeSAT2 sequences seemed to cluster better than others.

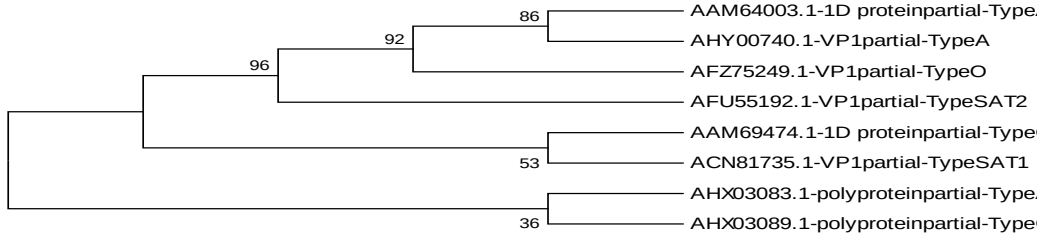


Figure 2: Maximum Parsimony tree of the eight (8) distinctive amino acid sequences of the FMDV, showing the evolutionary history. The retention index is (0.704545) the consistency index is (0.919255), and the polyprotein, partial-Types A and O clustered together.

Table 3: Amino Acid Composition

Amino Acid (%)	AAM64003.1-1D protein, partial Type A	AAM69474.1-1D protein, partial Type O	AHX03083.1-polyprotein, partial Type A	AHX03089.1-polyprotein, partial Type O	AHY00740.1-VP1, partial-Type A	AFU55192.1-VP1, partial-Type SAT2	AFZ75249.1-VP1,partial-Type O	ACN81735.1-VP1,partial-Type SAT1
Ala	11.1	7.9	6.0	12.6	11.7	8.8	9.9	10.5
Arg	6.8	6.2	5.1	4.3	7.5	8.3	7.0	8.1
Asn	4.3	4.2	5.1	4.3	3.3	3.7	4.7	3.8
Asp	3.9	5.3	7.7	5.8	2.3	7.4	3.8	4.3
Cys	0.5	1.4	1.7	1.0	0.5	2.3	0.9	0.5
Gln	4.3	2.5	2.1	4.3	3.3	2.8	4.7	4.8
Glu	4.3	5.3	5.6	3.5	6.1	3.2	4.7	4.3
Gly	7.2	5.6	7.3	5.8	5.6	6.5	4.2	4.8
His	4.3	4.2	4.7	2.8	4.2	4.2	3.3	2.4
Lle	4.8	5.6	6.8	2.5	1.9	1.9	3.8	3.8
Leu	8.7	7.6	5.6	9.6	11.3	6.9	9.9	11.0
Lys	3.9	4.5	4.3	4.5	2.8	4.2	4.7	3.8
Met	1.4	2.8	3.8	1.5	1.4	1.9	1.4	0.5
Phe	2.9	3.9	5.6	4.0	2.3	4.6	1.9	1.9
Pro	5.3	5.6	3.4	6.3	5.6	6.0	7.0	6.7
Ser	3.9	4.5	5.1	3.8	6.1	2.3	3.8	2.9
Thr	10.6	8.4	6.8	10.6	10.8	10.2	11.3	12.9
Trp	0.5	1.4	1.3	0.8	0.5	0.5	0.5	0.5
Tyr	3.9	4.8	4.7	4.8	4.7	5.1	4.7	4.3
Val	7.2	7.3	7.3	7.3	8.0	9.3	8.0	7.1
Pyl	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Sec	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The above abbreviations represent the following:
Sec= Selenocysteine, Glu= Glutamic acid, Arg= Arginine, Cys= Cysteine, Asp= Aspartic acid, Asn= Asparagine, Gln= Glutamine, Ala= Alanine, Gly= Glycine, Pro= Proline, Ile= soleucine, Leu= Leucine, Lys= Lysine, Met= Methionine, Phe= Phenylalanine, His= Histidine, Ser= Serine, Trp= Tryphotphan, Thr= Threonine, Try= Tyrosine, Val= Valine, Pyl= Pyrrolysine.
Amino acid composition varied among the eight specific FMDV proteins in Table 3 above.

Table 4: Physico-chemical characteristics of the eight (8) distinctive FMDV Proteins sequences

Protein	No. of (AA)	Molwt (kDa)	pI	Q	EC	Half life (hr)	II (%)	AI	GRAVY
AAM64003.1-1D protein, partial Type A	207	22733.8	9.41	+ve	17420	7.2	29.18	84.88	-0.287
AAM69474.1-1D protein, partial Type O	356	40641.6	7.37	Neutral	53080	5.5	31.47	80.53	-0.292
AHX03083.1-polyprotein, partial Type A	234	26674.1	5.61	-ve	33140	1.3	28.11	75.38	-0.308
AHX03089.1-polyprotein, partial Type O	397	43428.1	6.63	-ve	45060	30	19.71	80.93	-0.204
AHY00740.1-VP1, partial-Type A	213	23458.6	9.24	+ve	20400	7.2	29.41	86.15	-0.248
AFU55192.1-VP1, partial-Type SAT2	216	24341.5	8.78	+ve	22140	7.2	25.08	69.95	-0.436
AFZ75249.1-VP1,partial-Type O	213	23796.2	9.46	+ve	20525	7.2	32.18	86.10	-0.374
ACN81735.1-VP1,partial-Type SAT1	210	23329.2	9.63	+ve	18910	7.2	31.96	88.76	-0.337

AA= amino acid, Q= Net Charge, pI= Isoelectric point, GRAVY=Grand average of hydropathicity, II= Instability index, molwt =Molecular weight, AI=Aliphatic index, Half-life=Estimated half-life. The above table revealed variation among the studied FMDV protein sequences.

Predicted 3D Protein Structures of FMDV in Cattle

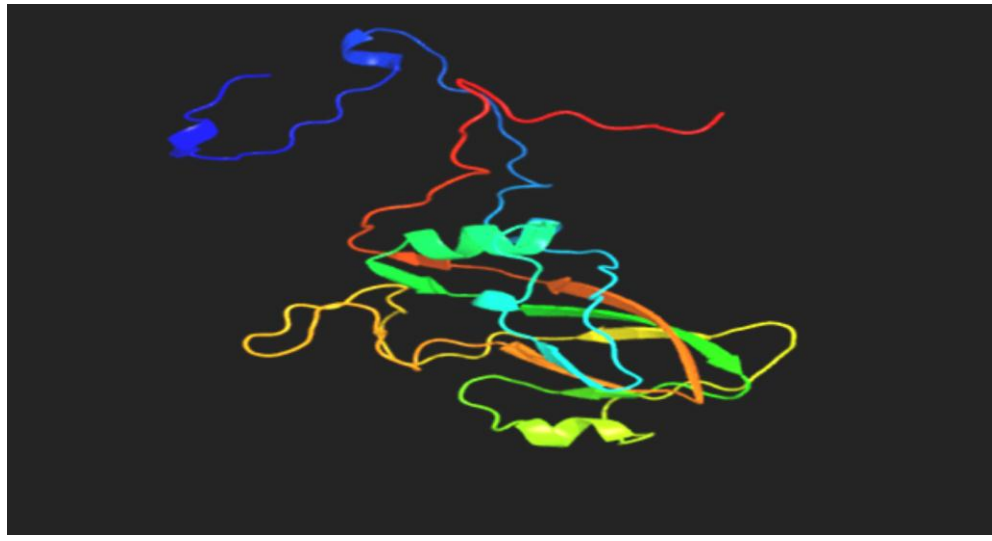


Figure 3: Structure of 1D Protein Partial-Type A

Model dimensions (Å): X: 71.791Y: 54.307 Z: 40.819

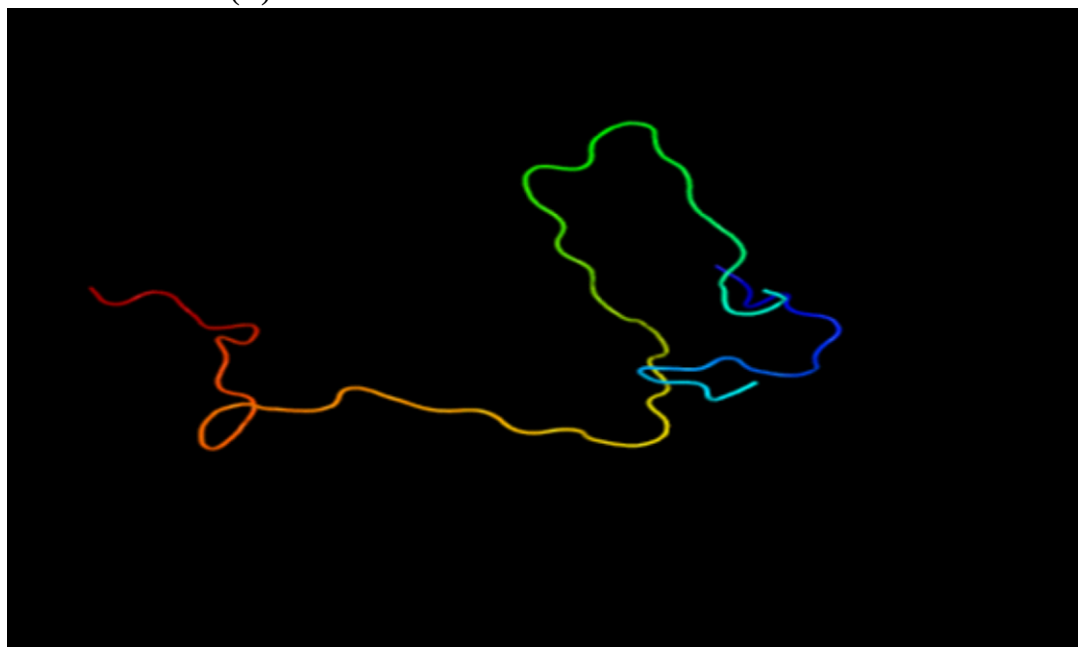


Figure 4: Structure of 1D Protein Partial-Type O

Model dimensions (Å): X: 50.229Y:53.033Z: 26.573

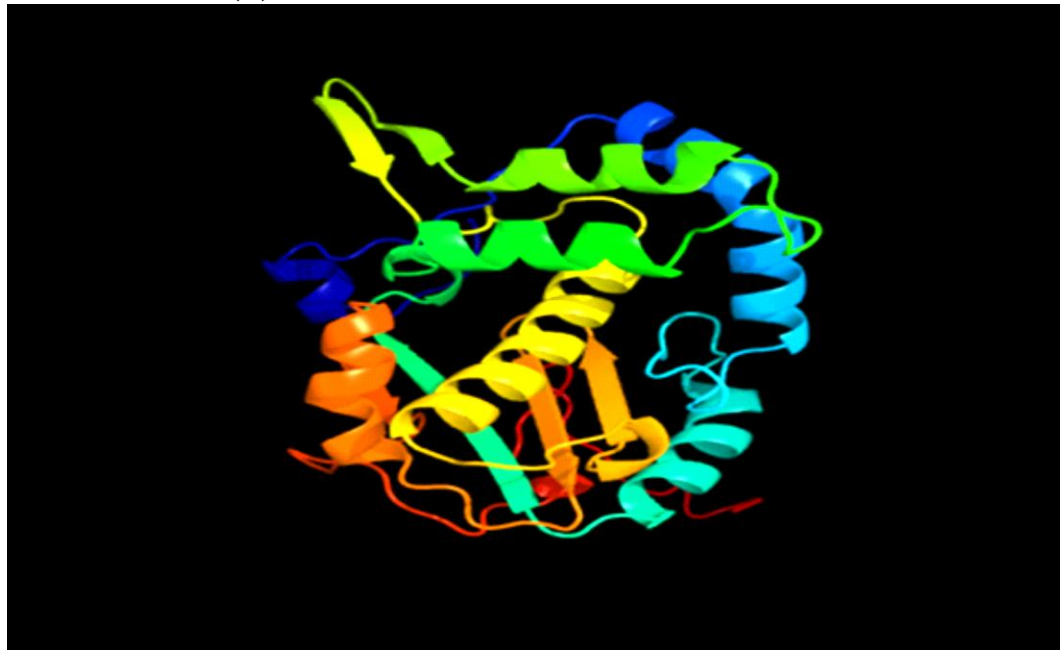


Figure 5: Structure of Polyprotein Partial-Type A

Model dimensions (Å): X: 49.020 Y: 51.410 Z: 51.362

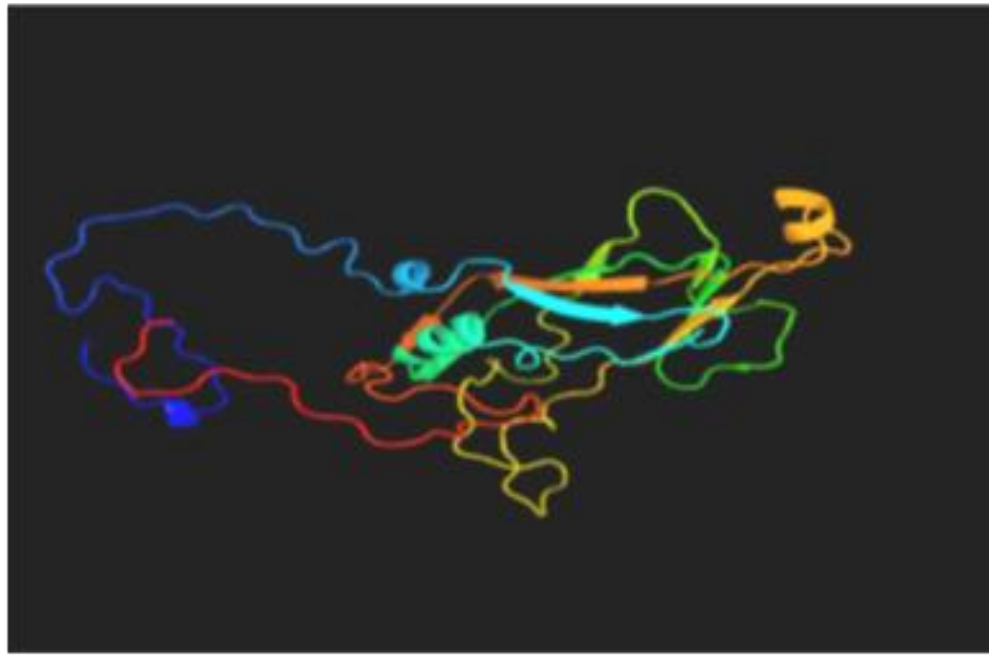


Figure 6: Structure of Polyprotein Partial-Type O

Model dimensions (Å) X: 65.128Y:72.222Z: 49.824

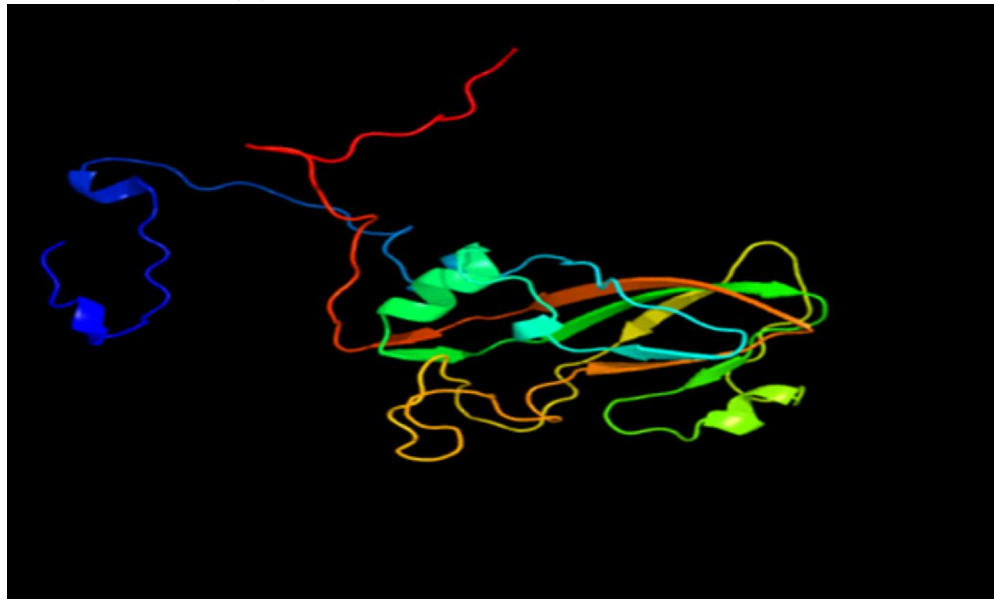
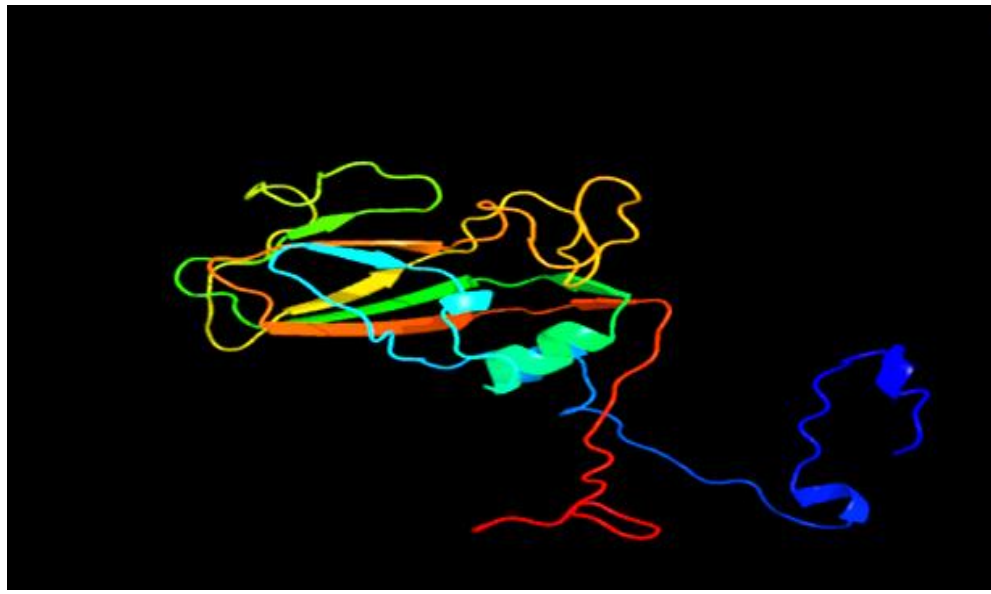


Figure 7: Structure of VP1 Partial-Type A

Model dimensions (Å) X: 71.791Y:60.009Z: 38.584



Ahmed et al

Figure 8: Structure of VP1 Partial-Type SAT2

Model dimensions (Å) X: 71.950Y: 50.919Z: 44.520

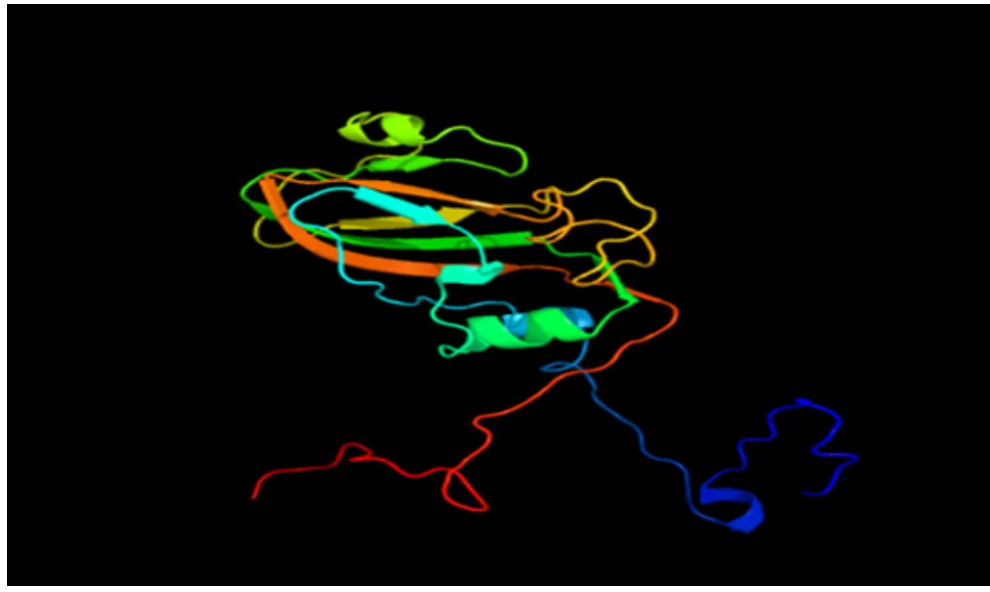


Figure 9: Structure of VP1 Partial-Type O

Model dimensions (Å) X: 57.419Y: 66.816Z: 49.811

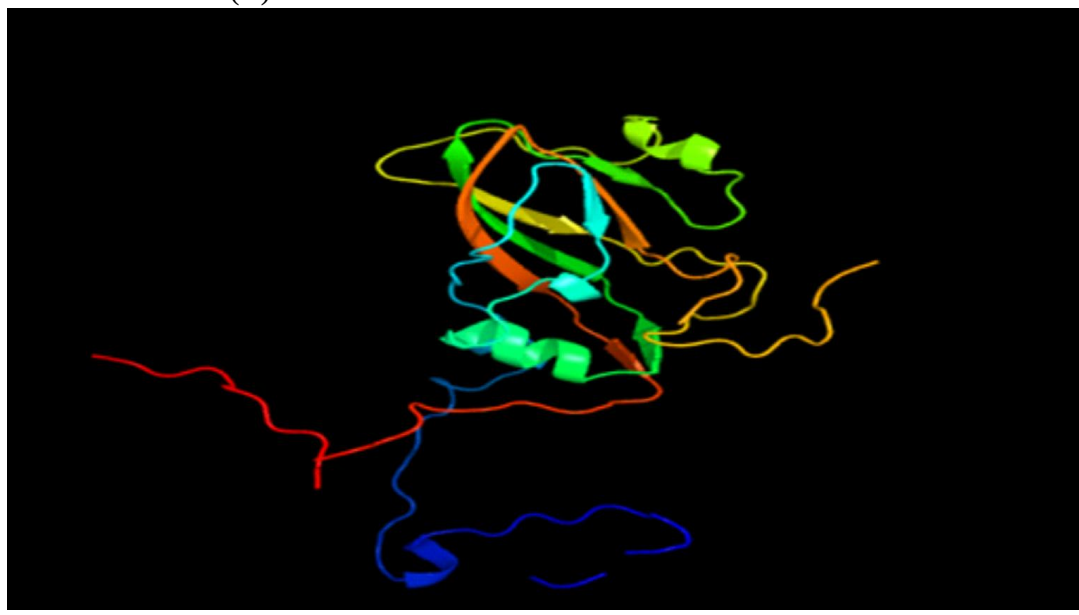


Figure 10: Structure of VP1 Partial-Type SAT1
Model dimensions (Å) X: 73.172Y: 63.456Z: 39.794
The predicted 3D structures of the eight (8) distinctive FMDV Proteins are shown in Figures 3-10 above.

Pertinent information on the epidemiology of FMDV, as well as the roles portrayed by various hosts, is vital for improving prevention and control of the disease (19). In Cattle, during carrier state of the FMDV, the viral persistence at the upper respiratory track is linked to the epithelial cells of the nasopharynx and declined levels of mRNA for numerous immunoregulatory cytokines in the diseased tissues (20). Sequence length varied among the various serotypes of FMDV in the present study. There are instances whereby variability might results from insertions or deletion of sequences, short tandem repeat (STR), DNA rearrangement, DNA duplication. Length variation have been reported to affect the functionality of protein as evident in frame-shift mutations (21).

The neigbour-joining tree revealed clearly that clustering was mostly protein type-wise, and the VP1, partial type-SAT2 sequences clustered more together. The mutation rate of VP1 gene's nucleotide is the most highest in four structural protein in a related study by Knowles (22). Similarly, 1D protein partial type-A clustered with VP1 partial type-A (i.e.less of serotype wise) and among the sequences than the other protein types. The evidence of long-term evolutionary persistence at the locus is as a result of the presence of numerous alleles at

a specific FMDV locus. It has been put forward that the alleles in one serotype are frequently more closely linked to the alleles in serotypes closely related, than to the other alleles in the same serotypes. The serotype-wise clustering might be due to serotype specific residues. High genetic diversity is revealed at the FMDV locus of the studied species.

The findings on the FMDV amino acid composition may be important for molecular biologists, chemist and drug developers. The pH at which a protein carries no net charge is known as the isoelectric point. Thus, the isoelectric point is of importance in purification of protein because it is the pH at which mobility in an electro focusing system is zero (the point at which there will be accumulation of protein) and at which solubility is often minimal. This measure point out what percentage of light is absorbed by a protein at a particular wavelength. Protein half-life is the time it takes before only half of the protein pool for that particular protein is left and these half-life of proteins is highly dependent on making stability of overall protein and the presence of the N-terminal amino acid.

The predicted 3D protein structures of FMDV of the studied species aligned well with the templates. The practical applications of the prediction of protein structure are many and varied, including selecting sites for mutagenesis, rational design of drugs and guiding the development of functional hypotheses about hypothetical proteins (23) (24) (25).

Therefore, the current findings may be exploited into proper understanding of the mechanism of FMD virus in cattle species, especially in the area of detection of new strains of the virus.

Understanding the epidemiology of a disease is important for the formulation of the foremost effective control strategies. Nucleotide sequencing represents a core/main component for tracing outbreak sources and epidemiological investigations. The present information on FMDV biodiversity and evolution could be exploited in tracing FMDV sources and transmission events, as well as to ensure vaccine coverage of corresponding field of FMDV extractions mostly in a developing country such as Nigeria.

Conclusion and Application

1. More molecular approaches which could aid understanding for genetic resistance to FMD: and should be put in place towards curtailing the deadly disease in Nigeria and the whole world.
2. The phylogenetic trees analysis could be used in tracing FMDV sources and transmission events.
3. The current findings throw more light in understanding the mechanism of FMD virus in cattle species, especially in the area of detection of new strains of the virus.
4. The FMDV amino acid composition result obtained in this study is of pertinent value to molecular biologists, chemist and drug developers.

Acknowledgement

The authors would like to show their profound heartfelt gratitude to Prof. A. Yakubu for his invaluable guides and suggestions.

References

1. **World Organization for Animal Health OIE. (2009).** Terrestrial Manual: Foot and Mouth Disease, OIE (Ed.). Vol. 1, Chapter 2.1.5, Office International des Epizooties, Paris, France, Pp. 1-29. Available at: https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/2.01.05_FMD.pdf. Accessed April 2018.
2. **Rueckert, R. R. and Wimmer, E. (1984).** Systematic nomenclature of picornavirus proteins. *Journal of Virology*. 50:957–959.
3. **Grubman, M. J. and Baxt, B. (2004).** Foot-and-Mouth Disease. *Clinical Microbiology Reviews* 17 (2): 465-493.
4. **James, J., Zhu, J. A., Michael, C. Puckette, G. R., Smoliga, J., Pacheco, M. and Luis, L. R. (2013).** Mechanisms of Foot-and-Mouth Disease Virus Tropism Inferred from Differential Tissue Gene Expression. Plos one, 8(5), e64119. https://doi.org/10.1371/*journal.pone*.0064119.
5. **Lawrence, P., LaRocco, M., Baxt, B. and Rieder, E. (2013).** Examination of soluble integrin resistant mutants of foot-and-mouth disease virus. *Virology Journal*, 10: 2. https://doi.org/10.1186/1743-422X-10-2.
6. **Domingo, E., Escarmís, C., Baranowski, E., Ruiz-Jarabo, C. M., Carrillo, E., Núñez, J. I. and Sobrino, F. (2003).** Evolution of foot-and-mouth disease virus. *Virus Research*, 91: 47-63.
7. **World Organization for Animal Health (OIE). (2014).** Foot and Mouth Disease. In: OIE General Disease Information Sheet. Available at: https://www.oie.int/en/disease/foot-and-mouth-disease/#ui-id-1. Accessed January 2019.
8. **World Organization for Animal Health (OIE). (2012).** Foot and mouth disease. In: OIE Terrestrial Manual. Ch.2.1.5. Available at: https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_Cards/FOOT_AND_MOUTH_DISEASE.pdf. Accessed July 2018.
9. **The Center for Food Security and Public Health (2014).** Fact Sheet-Iowa State University: Foot and Mouth Disease (FMD). Available at: https://www.cfsph.iastate.edu/Factsheets/pdfs/foot-and-mouth-diseas.pdf. Accessed May 2018.
10. **Scudamore, J. M. and Harris, D. M. (2002).** Control of foot and mouth disease: lessons from the experience of the outbreak in Great Britain in 2001. *Review Scientific Technical Office International Epizootics*, 21: 699-710.
11. **Thompson, D., Muriel, P., Russell, D., Osborne, P, Bromley, A., Rowland, M., Creigh-Tyte, S. and Brown, C. (2002).** Economic costs of the foot-and-mouth disease outbreak in the United Kingdom in 2001. *Review Scientific Technical Office International Epizootics*, 21:675–687.
12. **Yang, P. C., Chu, R. M., Chung, W. B. and Sung, H. T. (1999**). Epidemiological characteristics and financial costs of the 1997 foot-and-

mouth disease epidemic in Taiwan. *The Veterinary Record*, 145:731–734.

13. **Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R. and McGettigan, P. A. (2007**). ClustalW and Clustal X version 2.0.*Bioinformatics*, 23: 2947–2948. doi:10.1093/bioinformatics/btm 404 View Article.
14. **Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013).** MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725-2729.
15. **Nei, M. and Kumar, S. (2000).** *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
16. **Gasteiger. E., Hoogland. C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., Bairoch, A. and John, M. W. (2005).** *The Proteomics Protocols Handbook.* Humana Press. Pp.571-607.
17. **Kelley, L. A. and Sternberg, M. J. (2009).** Protein structure prediction on the web: a case study using the Phyre server. *Nature Protoc*ols, 4:363-371.
19. **Wekesa, S. N., Sangula, A. K., Belsham, G. J., Tjornehoj, K., Muwanika, V. B., Gakuya, F., Mijele, D. and Siegismund, H. R. (2015).** Characterisation of recent foot-and-mouth disease viruses from African buffalo (Synceruscaffer) and cattle in Kenya is consistent with independent virus populations. *BioMed Central Veterinary Research*, 11:17.
20. **Pachero, J. M.,Smoliga, G. R.,O' Donnell, V.,Brito, B. P.,Stenfeldt, C., Rodriguez, L. L.and Arzt, J. (2015).** Persistent Foot-and-Mouth Disease Virus Infection in the Nasopharynxof Cattle; Tissue-Specific Distribution and Local Cytokine Expression. *PLoS ONE*, 10(5):e0125698.doi: 10.1371/*journal.pone*.0125698**.**
21. **Ebhardt, A. H., Fednak, A. and Fahlman, R. (2010).** Naturally occurring variation in sequence length creates miRNA isoforms that differ in argonaute effector complex specificity. *Silence*, 1:12.
22. **Knowles, N. J. and Samuel, A. R. (2003).** Molecular epidemiology of foot-and-mouth disease virus. *Virus Research*, 91: 65–802.
23. **Watson, J. D., Laskowski, R. A. and Thornton, J. M. (2005).** Predicting protein function from sequence and structural data. *Current Opinion Structural Biology*, 15: 275–284.
24. **Rava, P. and Hussain, M. M. (2007).** Acquisition of triacylglycerol transfer activity by microsomal triglyceride transfer protein during evolution. *Biochemistry*, 46: 12263–12274.
25. **Park, H., Hwang, K. Y., Oh, K. H., Kim, Y. H., Lee, J. Y. and Kim, K. (2008).** Discovery of novel alpha-glucosidase inhibitors based on the virtual screening with the homology-modeled protein structure. *Bioorganic and Medicinal Chem*istry, 16: 284–292.