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Molecular Epidemiological study on SAT2-FMD virus in the Nile basin countries

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Abstract: *Foot and mouth disease virus is a highly contagious disease affecting wide range of animals characterized by high morbidity and low mortality. This virus has 7 serotypes, A, O, C, Asian, SAT1, SAT2 and SAT3. During 2012, Egypt was attacked by an outbreak of SAT2 serotype infection which implements more difficulties to control and overcome the FMD virus infection. Due to the dynamic status of the FMD virus and its reaction to the surrounding environment and the open accessibility of huge number of nucleotide sequences in FMD virus serotypes, the investigation of these serotypes based on the molecular characterizations that give more clear picture on the different alleles of SAT2 serotype circulating in certain geographical areas. The Nile basin countries are so closely related to each other due to sharing the same river and many economic ties. This study was concerned with mining and retrieving the different alleles of VP1 gene (SAT2-FMDV) from GeneBank by their accession numbers belonging to the Nile basin countries, 135 sequences was retrieved, aligned and analyzed using different bioinformatic tools. The results showed high degree of diversity between the different alleles of VP1 gene (SAT2-FMD virus).*

Key words: *SAT2 serotype, FMDV, Phylogenetic analysis, molecular epidemiology*

I. Introduction

Foot and mouth disease virus (FMDV; family Picornaviridae, genus: Aphthovirus) causes a highly contagious disease of ruminants and swine, it is exists as seven immunologically distinct serotypes, O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3 (Rweyemamu et al., 2000; Vosloo et al., 2002). FMD is endemic in most of the African countries where serotypes O, A, SAT 1 and SAT 2 predominate and infection or vaccination with one serotype does not confer immunity against the others (Biswal et al., 2014). FMD is endemic in Egypt as the country is dependent on importation of live animals and meat from many countries all over the world (Knowles et al., 2007; FAO, 2012 and Salem et al., 2012). Three serotypes of FMDV have been detected in Egypt: O, A and SAT-2. Serotype O is the most endemic since 1970 (Samuel et al., 1990; Kitching, 1998;Samuel and Knowles, 2001and Hamza and Beillard, 2013), while serotype A was isolated and identified in 2006 after importation of live animals from Ethiopia (Knowlesand Samuel, 2003; Abed El-Rahman et al., 2006; El-Kholy et al., 2007and Knowles et al., 2007). The newest serotype is SAT-2, which was detected in 2012 (EL-Shehawy et al., 2012 and Valdazo-González et al., 2012). It has been proposed that the different SAT virus types may have differential abilities in crossing species (Bastos, 2001 and Sangare et al., 2004). A common and major epitope of FMDV is located within the surface protein VP1, containing the immuno-dominant GH-loop and the RGD-integrin binding motif, essential for cell attachment (Fox et al., 1989).

Changes in this protein may cause vaccine failure and changes in host specificity (Hernandez et al., 1996). In this study, we describe the genetic diversity and phylogenetic analysis of the nucleotide sequence which encodes for the VP1 protein of SAT2-FMD virus circulating in the Nile basin countries, all sequences were retrieved from the NCBI GeneBank (http://www.ncbi.nlm.nih.gov/) and FAO World Reference Laboratory for Foot-and-Mouth Disease (http://www.wrlfmd.org/) . The data was analyzed with regard to potential epidemiological information and to establish possibly whether FMD outbreaks were caused by viruses persistently circulating and evolving or introduced in Egypt and other countries in the Nile basin valley.

II. Materials and methods

Investigated Area

This study was concerned to investigate the SAT2-FMD virusserotype infection with the Nile basin countries include Egypt, Sudan, Eretria, Ethiopia, Uganda, Kenya, Rwanda, Burundi, Tanzania and Congo. These countries are so closely related to each other as they belong to the Nile basin valley and there are socio-economical relationships especially in animal trades of both living, slaughtered animals and their bio-products.

Study Approach

The world GeneBanks contain a wide array of nucleotide sequences of SAT2-FMD virus (VP1 gene) which were submitted from different countries during different outbreaks. Hence, the main idea of this study is

to look on the diversity of nucleotide sequences of SAT2-FMD virus (VP1 gene) in a trial to understand the interrelationships between different isolates belonging to the Nile basin countries at the molecular level.

Study design

135 nucleotide sequences of submitted SAT2-FMD virus (VP1 gene) to the NCBI GeneBank (http://www.ncbi.nlm.nih.gov/) and FAO World Reference Laboratory for Foot and Mouth Disease (http://www.wrlfmd.org/) were retrieved and subjected to different bioinformatics tools to analyze those sequences at the molecular level.

SAT2-FMD virus (VP1 gene) GeneBank accessions numbers

All nucleotide sequences of VP1 gene of the SAT2-FMD virus belonging to the Nile basin countries were retrieved from the NCBI GeneBank. 28 accession numbers isolated from Egypt (JX570637, JX570635, JX570633, JX570631, JX570629, JX570627, JX570625, JX570623, JX570621, JX570617, JX570619, JX570615, JX570613, JX570611, JX570636, JX570634, JX570632, JX570630, JX570628, JX570626, JX570624, JX570622, JX570620, JX570618, JX570616, JX570614, JX570612 & JX570610), 5 accession numbers isolated from Sudan (GU566071, GU566072, GU566073, AY343939 & AY442014), 4 accession numbers isolated from Eretria (AY343933, GU194494, AF367126 & AY343934), 8 accession numbers isolated from Ethiopia (AY343935, FJ798158, AY343937, FJ798161, AY343936, AY343938, FJ798159&FJ798160), 18 accession numbers isolated from Uganda (HM623682, GU323171, GU323172, GU323173, GU323174, GU323175, GU323176, GU323177, GU323178, GU323179, AY343969, DQ009731, AY343968, AY343964, AY343966, AY343963, AY343965 & AY343967), 64 accession numbers isolated from Kenya (AY344505, AF335008, AF453256, AJ251473, AF367131, AF367132, AF367133, AY343940, AY343941, AY343942, AY343943, AY343944, AY343945, AY343946, AY343947, AY343948, AY343949, AY343950, AY343951, AY343952, AY343953, AY343954, AY343955, AY343956, AY343957, AY343958, AY343959, AY343960, AY343961, AY343962, DQ009729, GQ294636, GQ294637, HM623678, HM623679, HM623680, HM623681, HM623683, HM623684, HM623685, HM623686, HM623687, HM623688, HM623689, HM623690, HM623691, HM623692, HM623693, HM623694, HM623695, HM623696, HM623697, HM623698, HM623699, HM623700, HM623701, HM623702, HM623703, HM623704, HM623705, HM623706, HM623707, HM623708 & HM623709), 2 accession numbers isolated from Rwanda (AF367134 & DQ009730), 1 accession numbers isolated fromBurundi (AF367111), 4 accession numbers isolated from Tanzania(AB490330, AB490329, AY343970 & AY343971), and 2 accession numbers isolated from Congo (DQ009737 & AF367100).

Bioinformatic analysis

Complete VP1 nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall, 1999) and Clustal W 1.83 (Thompson et al., 1994). These alignments were used to construct distance matrices using the Kimura-2-parameter nucleotide substitution model (Kimura, 1980) as implemented in the program MEGA 5.2 (Tamura et al., 2011). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 135 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 113 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).Substitution pattern and rates were estimated under the Jones-Taylor-Thornton (1992) model.

III. Results

MULTIPLE SEQUENCE ALIGNMENT

All amino acid sequences of VP1 gene belonging to Egypt (28 sequence) were aligned. There were high degree of diversity between these sequences, the most variable regions in the multiple sequence alignment were region 1 (AA position, 23-58), region 2 (AA position, 135-140), region 3 (AA position, 156-160), and region 4 (AA position, 196-201). 3 conserved regions found, region 1: position 1 to 22 (TTSAGEGADVVTTDPSTHGGNV), region 2: position 66 to 82(LRASTYYFCDLEIACVG) and the 3rd region: position175 to 194 (PVDVYYRMKRAELYCPRPLL) (figure 1 and 2).

The frequencies of each amino acid within VP1 gene were calculated for the 28 amino acid sequences in Egypt (table 2).

Maximum Likelihood Estimate of Substitution Matrix

Each entry is the probability of substitution (r) from one amino acid (row) to another (column). Substitution pattern and rates were estimated under the Jones-Taylor-Thornton (1992) model. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to

100, The amino acid frequencies are 7.69% (A), 5.11% (R), 4.25% (N), 5.13% (D), 2.03% (C), 4.11% (Q), 6.18% (E), 7.47% (G), 2.30% (H), 5.26% (I), 9.11% (L), 5.95% (K), 2.34% (M), 4.05% (F), 5.05% (P), 6.82% (S), 5.85% (T), 1.43% (W), 3.23% (Y), and 6.64% (V). For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -1220.441. The analysis involved 28 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 215 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013) (table 3).

Neutrality test

The analysis involved 28 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 215 positions in the final dataset (table 1).

Table 1. Results from Tajima's Neutrality Test (Tajima, 1989).

m	S	p_s	Θ	π	D
28	55	0.255814	0.065737	0.104750	2.248118

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic.

Phylogenetic analysis and evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 4.93128599 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), and are in the units of the number of base substitutions per site. The analysis involved 135 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 113 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013)(figure 1).

The phylogenetic analysis showed that all sequences were divided into two main clusters and the Egyptian isolate sequences were isolated also into two groups, each group belongs to a separate cluster. The first group contains the following isolates(JX570629, JX570628, JX570630, JX570611, JX570613, JX570610, JX570614 & JX570612) and the second group contains the following isolates (JX570637, JX570635, JX570633, JX570631, JX570627, JX570625, JX570623, JX570621, JX570617, JX570619, JX570615, JX570636, JX570634, JX570632, JX570626, JX570624, JX570622, JX570620, JX570618&JX570616) (figure 1).

The most closely related sequences to the first Egyptian group were the following isolates from Kenya (HM623702, HM623703, HM623704, HM623705, HM623706 & HM623708), while the most closely related sequences to the second Egyptian group were the following sequences (AY343933, GU194494, AF367126 & AY343934) and (GU566071) which belongs to Eretria and Sudan respectively (figure 1).

IV. Discussion

This study describes the molecular analysis of VP1 gene of SAT2-FMDvirus isolates which exist and circulate in the Nile basin region, 135 nucleotide sequences had been retrieved from the NCBI GeneBank (http://www.ncbi.nlm.nih.gov/) and FAO World Reference Laboratory for Foot-and-Mouth Disease (http://www.wrlfmd.org/), and subjected to different bioinformatics tools to be analyzed at the molecular level.

This study used the multiple sequence alignment and phylogenetic analysis to define the genetic relationships between different sequences of SAT2-FMDV isolated from Egypt and those that have been collected from neighboring countries in the Nile basin valley. 28 amino acid sequences belongs to SAT2-FMDV isolates of Egypt showed high degree of diversityand this feature was recorded in other studies (Sahle et al., 2007; Kasanga, et al., 2010, Sangula et al., 2010).

This study used phylogenetic analysis to define the genetic relationships between SAT2-FMD sequences recorded in the Nile basin countries(Figure1). From the phylogenetic trees constructed, it is possible to infer the genetic relationship of isolates, and how FMD viruses might be dispersed between countries. The phylogenetic analysis showed all sequences were divided into two main clusters and the Egyptian isolate sequences were isolated also into two subgroups, The most closely related sequences to the first Egyptian group were certain isolates from Kenya while the most closely related sequences to the second Egyptian group were belongs to Eretria and Sudan(Figure1). This indicates that the SAT2-FMDV is constantly evolving with time and geographic location and gives rise to variant viruses that are genetically diverse. This is in agreement with other studies (Sangare et al., 2004, Valdazo-González et al., 2012; Sobhy et al., 2014).

On conclusion, there were 135 different alleles of SAT2-FMDV that circulating in the Nile basin countries including Egypt, the Pairwise alignment showed high degree of polymorphism between different

sequences which indicating that this virus is continuously evolving. The phylogenetic analysis showed that there were two main clusters and the same is applied to the Egyptian SAT2-FMDV sequences. Future investigation is needed to collect different samples from the cloven footed animals in Egypt and to look for more different and evolving isolates of SAT2-FMDV.

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Table 2. The difference in amino acid frequencies of VP1 gene of SAT2-FMDV in Egypt

	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total
JX570637	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570635	9.3	1.9	6.9	3.2	5.1	7.9	3.7	2.3	4.6	6.5	1.4	3.7	6.0	1.9	7.4	3.2	11.6	8.8	0.5	4.2	216
JX570633	9.3	1.9	6.9	3.2	5.1	7.9	3.7	2.3	4.6	6.5	1.4	3.7	6.0	1.9	7.4	3.2	11.6	8.8	0.5	4.2	216
JX570631	8.8	1.9	6.5	3.7	4.6	7.9	3.7	2.3	4.6	6.9	1.4	3.7	5.6	1.9	7.4	3.7	11.6	8.8	0.5	4.6	216
JX570629	8.8	1.9	7.0	3.3	4.7	6.0	4.2	1.9	4.2	6.5	1.9	4.2	6.0	2.3	8.4	2.8	11.2	9.3	0.5	5.1	215
JX570627	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570625	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570623	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570621	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570617	11.1	1.9	6.5	3.7	4.6	7.4	3.7	2.3	4.6	6.9	1.4	2.8	5.6	1.9	7.9	3.2	11.6	8.3	0.5	4.2	216
JX570619	10.6	1.9	7.4	3.2	4.2	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	5.1	216
JX570615	10.6	1.9	6.5	3.7	5.1	7.4	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	4.2	11.1	7.4	0.5	4.2	216
JX570613	8.8	1.9	6.5	3.3	4.7	6.5	4.7	1.9	3.7	7.0	2.3	4.2	5.6	2.3	8.4	2.8	11.2	9.3	0.5	4.7	215
JX570611	8.8	1.9	6.5	3.3	4.7	6.5	4.7	1.9	3.7	7.0	2.3	4.2	5.6	2.3	8.4	2.8	11.2	9.3	0.5	4.7	215
JX570636	9.7	1.9	6.0	3.7	5.1	7.9	3.7	2.3	4.2	6.9	1.4	4.2	5.6	1.9	7.4	3.7	11.6	8.3	0.5	4.2	216
JX570634	9.3	1.9	6.9	3.2	5.1	7.9	3.7	2.3	4.6	6.5	1.4	3.7	6.0	1.9	7.4	3.2	11.6	8.8	0.5	4.2	216
JX570632	8.8	1.9	6.5	3.7	4.6	7.9	3.7	2.3	4.6	6.9	1.4	3.7	5.6	1.9	7.4	3.7	11.6	8.8	0.5	4.6	216
JX570630	8.8	1.9	7.4	3.3	4.7	6.5	4.2	1.9	4.2	6.5	1.9	3.7	6.0	2.3	8.4	2.3	10.7	9.8	0.5	5.1	215
JX570628	9.3	1.9	7.0	3.3	4.7	6.5	4.2	1.9	3.7	6.5	2.3	4.2	6.0	2.3	8.4	2.3	10.7	9.3	0.5	5.1	215
JX570626	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570624	10.2	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.6	8.8	0.5	4.6	216
JX570622	10.2	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.6	8.8	0.5	4.6	216
JX570620	10.6	1.9	7.4	3.2	4.2	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	5.1	216
JX570618	10.6	1.9	7.4	3.2	4.6	6.9	3.7	2.3	4.2	6.9	1.4	3.2	6.0	1.9	7.9	2.8	11.1	8.8	0.5	4.6	216
JX570616	9.7	1.9	6.5	3.7	5.1	7.4	3.7	2.8	4.2	7.4	1.4	3.2	5.6	1.9	7.9	4.2	11.1	7.9	0.5	4.2	216
JX570614	8.8	1.9	6.5	3.3	4.7	6.5	4.7	1.9	3.7	7.0	2.3	4.2	5.6	2.3	8.4	2.8	11.2	9.3	0.5	4.7	215
JX570612	8.8	1.9	6.5	3.3	4.7	6.5	4.7	1.9	3.7	7.0	2.3	4.2	5.6	2.3	8.4	2.8	11.2	9.3	0.5	4.7	215
JX570610	8.8	1.9	6.5	3.3	4.7	6.5	4.7	1.9	3.7	7.0	2.3	4.2	5.6	2.3	8.4	2.8	11.2	9.3	0.5	4.7	215
Avg.	9.8	1.9	7.0	3.3	4.7	7.1	3.9	2.2	4.2	6.9	1.6	3.6	5.9	2.0	7.9	3.0	11.2	8.8	0.5	4.6	215.7

Table 3. Maximum Likelihood Estimate of Substitution Matrix of VP1 gene of SAT2-FMDV in Egypt.

From\To	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	-	0.14	0.12	0.22	0.06	0.12	0.34	0.67	0.03	0.10	0.15	0.11	0.06	0.03	0.51	1.37	1.39	0.01	0.02	1.01
R	0.21	-	0.10	0.04	0.11	0.64	0.10	0.53	0.38	0.07	0.18	2.01	0.05	0.01	0.19	0.35	0.20	0.09	0.04	0.06
N	0.22	0.12	-	1.48	0.03	0.16	0.19	0.30	0.48	0.13	0.06	0.78	0.04	0.02	0.03	1.79	0.71	0.00	0.12	0.06
D	0.33	0.04	1.22	-	0.01	0.11	2.49	0.49	0.12	0.03	0.03	0.09	0.02	0.01	0.03	0.21	0.13	0.00	0.08	0.11
C	0.23	0.27	0.07	0.03	-	0.02	0.02	0.21	0.09	0.04	0.08	0.02	0.05	0.14	0.03	0.76	0.14	0.08	0.35	0.21
Q	0.22	0.80	0.17	0.14	0.01	-	1.09	0.09	0.68	0.02	0.33	0.91	0.06	0.01	0.42	0.19	0.16	0.01	0.04	0.06
E	0.43	0.08	0.13	2.07	0.01	0.73	-	0.43	0.03	0.03	0.05	0.53	0.02	0.01	0.05	0.11	0.10	0.01	0.01	0.16
G	0.69	0.36	0.17	0.34	0.06	0.05	0.36	-	0.02	0.01	0.03	0.08	0.02	0.01	0.05	0.66	0.10	0.04	0.01	0.16
H	0.09	0.85	0.89	0.27	0.08	1.21	0.08	0.08	-	0.05	0.26	0.16	0.04	0.10	0.30	0.26	0.14	0.01	0.98	0.04
I	0.14	0.06	0.11	0.03	0.02	0.02	0.04	0.02	0.02	-	1.10	0.06	0.59	0.16	0.03	0.14	0.77	0.01	0.05	3.28
L	0.12	0.10	0.03	0.02	0.02	0.15	0.03	0.03	0.06	0.64	-	0.05	0.47	0.53	0.28	0.21	0.08	0.04	0.04	0.61
K	0.15	1.73	0.56	0.08	0.01	0.63	0.56	0.10	0.06	0.06	0.07	-	0.08	0.01	0.06	0.17	0.29	0.01	0.01	0.04
M	0.19	0.11	0.07	0.05	0.04	0.10	0.06	0.05	0.04	1.32	1.82	0.19	-	0.09	0.04	0.10	0.64	0.02	0.03	1.05
F	0.06	0.02	0.02	0.01	0.07	0.01	0.01	0.02	0.05	0.21	1.18	0.01	0.05	-	0.04	0.33	0.04	0.04	0.92	0.20
P	0.78	0.19	0.03	0.03	0.01	0.34	0.06	0.08	0.14	0.03	0.50	0.07	0.02	0.03	-	0.99	0.36	0.01	0.02	0.07
S	1.55	0.27	1.12	0.16	0.23	0.12	0.10	0.73	0.09	0.11	0.28	0.15	0.03	0.20	0.73	-	1.45	0.02	0.11	0.14
T	1.83	0.17	0.52	0.11	0.05	0.11	0.11	0.12	0.06	0.70	0.13	0.30	0.26	0.03	0.31	1.69	-	0.01	0.03	0.39
W	0.03	0.33	0.01	0.02	0.12	0.04	0.04	0.21	0.02	0.04	0.25	0.03	0.02	0.11	0.02	0.11	0.02	-	0.13	0.08
Y	0.06	0.06	0.15	0.12	0.22	0.05	0.02	0.02	0.70	0.08	0.11	0.03	0.02	1.15	0.03	0.22	0.06	0.06	-	0.06
V	1.16	0.05	0.04	0.08	0.07	0.04	0.15	0.18	0.01	2.60	0.83	0.04	0.37	0.12	0.06	0.14	0.35	0.02	0.03	-

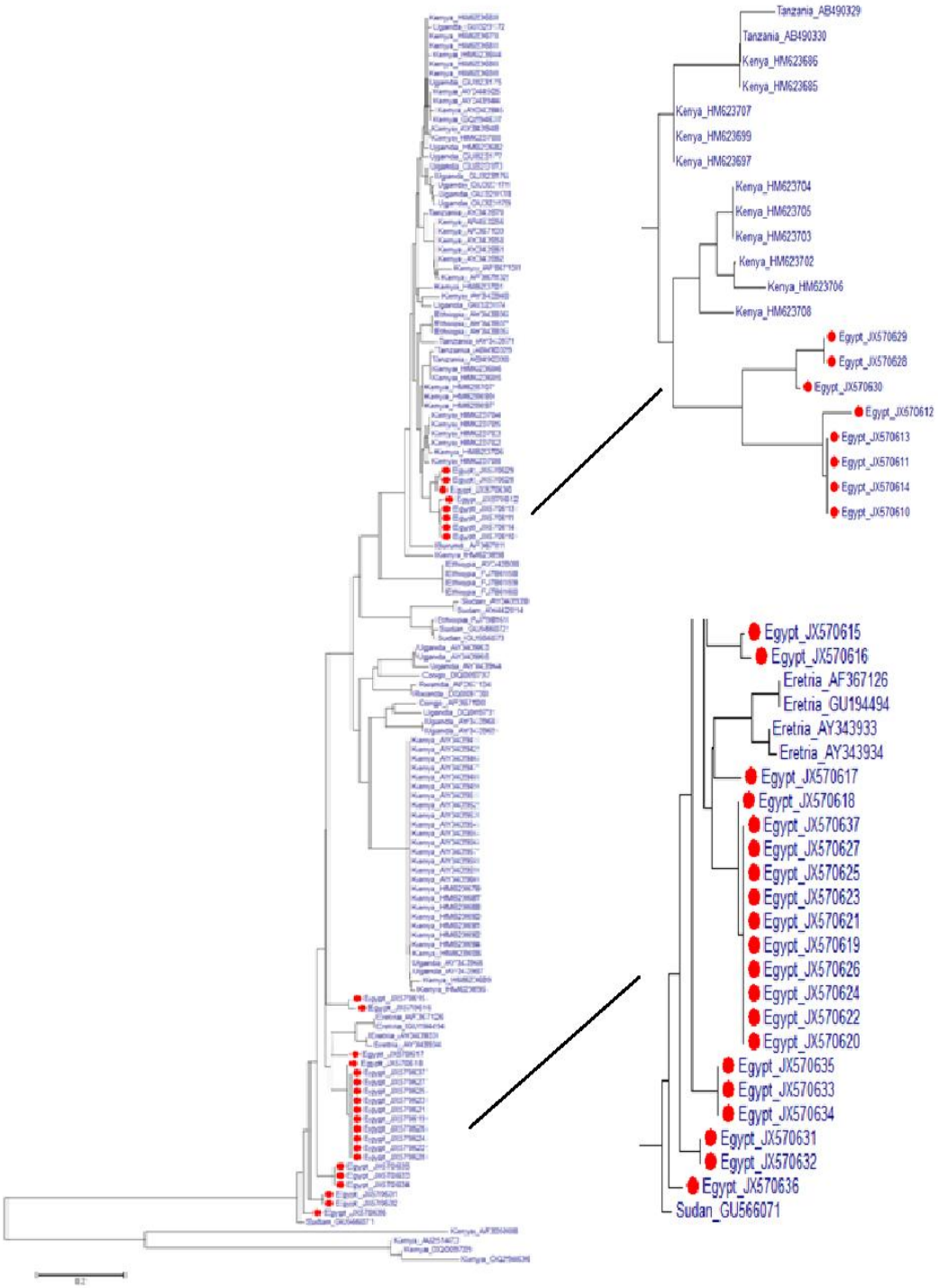

Figure 1. Phylogenetic analysis tree of VP1 gene of SAT2-FMDV in Egypt.

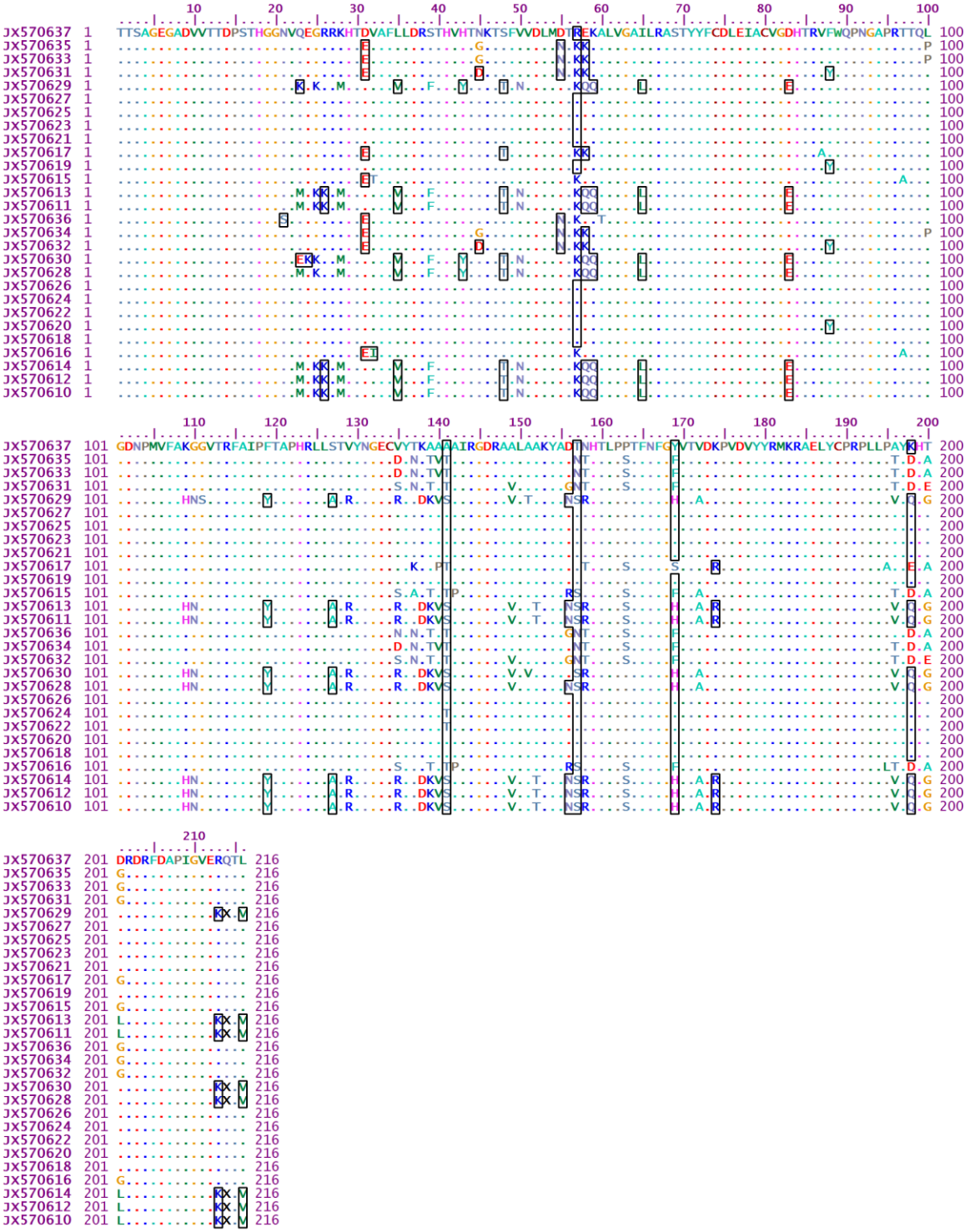

Figure 2. Multiple sequence alignment of amino acid sequences of SAT2-FMDV isolates in Egypt.

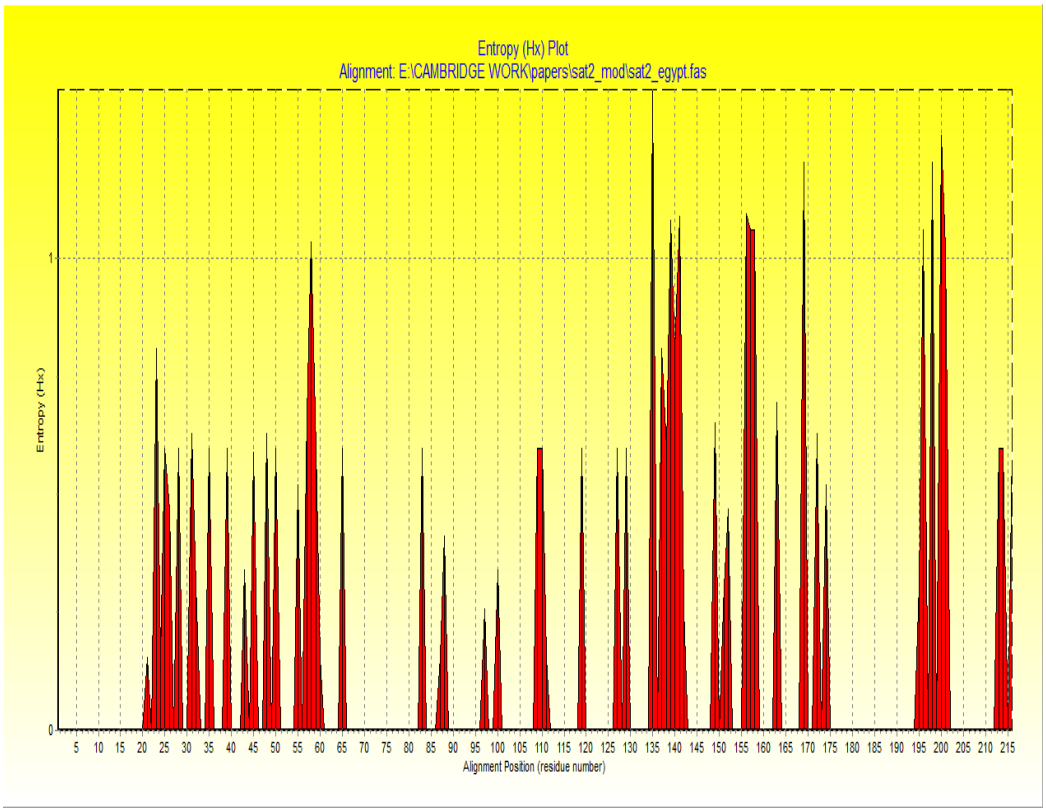

Figure 3. Entropy plot showing the similarity of amino acid sequences of SAT2-FMDV isolates in Egypt.