Phylogenetic Placement of Foot and Mouth Disease Virus during 2014 in Buffaloes, Egypt

Hanan A. Fahmy¹, Nashwa O. Khalifa² and Mervat E.I.Radwan³

¹Biotechnology Department, Animal Health Research Institute (AHRI), Giza, 11331, Egypt. ²Zoonoses Department, Faculty of Veterinary Medicine, Benha University, Toukh, 13736, Egypt ³Department of infectious Diseases, Faculty of Veterinary Medicine, Benha University, Toukh, 13736, Egypt

Abstract:

Foot-and-mouth disease virus (FMDV) SAT2 serotype is endemic in Egypt since 2012. The objectives of the present study were to investigate strain identification of FMDV infecting water buffaloes (Bubalus bubalis) in April, 2014, Qalyubia, Egypt. Therefore partial sequences were generated after detection by real time RT-PCR and subsequent gel purification of RT-PCR amplified products of VP1 gene of FMDV- SAT2. Partial sequencing of purified virus revealed that SAT2 serotype of FMD was circulating in this region. Sequences were further examined by sequence analysis and subsequent phylogeny to compare these sequences from known strains of FMDV-SAT2 circulating globally and retrieved from GenBank. Nucleotide substitution generates polymorphism at position 13 nucleotide, where a Cytosine replaced a Thymine and at the levels of 22 nucleotide where Guanine substituted Adenosine. A partial sequence of SAT2 showed the highest level of homology 99.4% similarity with sequences from Egypt 2012 with diversion 0.6 but it is variable from its neighbor countries isolates. Phylogenetic analysis showed a robust tree clustering all samples with sequences belonging to the FMDV-SAT2 variant with strong bootstrap values at relevant nodes and the evolutionary distance between groups is very short. There is a substitution in the sequences of amino acids at the position of 8, where an Alanine is changed to a Threonine. These findings demonstrate the recent picture of FMDV-SAT2 which incriminated for buffalo infectivity and responsible for its persistence in the endemic areas. Such epidemiological data could guide the application of efficient control strategies of FMDV in Egypt.

Keywords: FMDV, buffalo, sequences, nucleotide, amino acid, mutation. **Introduction:**

Foot-and-mouth disease (FMD) results from infection with FMD virus (FMDV), the prototypic *Aphthovirus* within the *Picornaviridae* family (Jamal and Belsham, 2013)[1]. Seven serotypes of FMDV are known; serotypes O and A are widely distributed, while the Southern African Territories (SAT) serotypes (1, 2, and 3) usually are restricted to Africa. Serotype Asia 1 has never circulated within Africa; serotype C has not been identified anywhere since 2005 (Sangula et al., 2011)[2]. Recently FMDV serotype SAT3 detected in long-horned ankole calf, Uganda (**Dhikusooka** et al., 2015)[3].

SAT 2 is the serotype most often associated with outbreaks of footand-mouth disease (FMD) in livestock in southern and western Africa and is the only SAT type to have been recorded outside the African continent in the last decade. Its epidemiology is complicated by the presence of African buffalo (*Syncerus caffer*), which play an important role in virus maintenance and transmission (**Bastos et al., 2003**)[**4**]. This region is also threatened by sporadic incursions of different topotypes and other FMD serotypes that are normally restricted to Sub-Saharan Africa (**Knowles et al., 2007**)[5]. During 2012, severe FMD outbreaks due to introduction of SAT2 serotype for the first time in Egypt causing mortality rates of up to 50% due to multifocal myocarditis, especially in young animals (**Ahmed et al.[6], 2012 and Valdazo Gonzalez et al.,[7] 2012**), with evidence for clinical infection with FMD-SAT2 in Egyptian buffalo by **Fahmy et al., (2014)**[8]. The applications of the molecular biological techniques of PCR amplification and nucleotide sequencing have been significant advances in the understanding of FMDV epidemiology (Knowles and Samuel, 2003[9] and Aggour et al., 2014)[10].

Phylogenetic analyses will help to understand the molecular nature of virus circulating for the selection of vaccination and strategies for the control of FMD in the country. The emergence of SAT2 has required a regular development FMD control programme to select appropriate vaccines to prevent future outbreaks.

The aim of the present study is to throw light on genotyping to characterize the FMDV recovered from Egyptian buffalo and phylogenetic analyses to define antigenic determinants of the virus, and to compare our findings to those related to known strains of FMDV circulating globally.

Materials and Methods:

For continuation of our previous work, under publication, blood samples, tongue epithelium and vesicular fluid recovered from water buffalo (*Bubalus bubalis*) suffering from characteristic clinical signs of FMD virus in April, 2014 in Qalyubia, Egypt were used as follows; samples were used for genetic characterization and stored at -20°C until used. The viral RNA from clinical samples was extracted and subjected to genotype the topotypes of FMDV by one step real time RT-qPCR using oligoprimers and probes for universal (Callahan 3D) gene for common FMDV (Callahan et al., 2002)[11] and (VP1) gene for serotypes A, Iran O, Asia and SAT2 (Ferris et al., 2009)[12]. Positive sample was subsequently amplified by conventional RT- PCR using two pairs of oligonucleotide primers of VP1 gene SAT2 primers and the expected fragments 716bp were identified.

Sequencing of Egyptian FMDV- SAT2 in buffalo:

The PCR products were gel purified by using QIAquick gel extraction kit (Qiagen, Valencia, Calif.) following the manufacture's instruction The purified PCR product was sequenced by using BigDye Terminator v3.1 Cycle Sequencing Kit on an automatic sequencer (ABI 3100 Genetic Analyzer; Applied Biosystems, Foster City, CA). The nucleotide sequences were then aligned with existing sequences of known genotypes from other countries in the GenBank databases using BLAST programs and databases of the NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) (www.blast.ncbi.nlm.nih.gov/Blast.cgi). **Phylogenetic Analysis:**

Partial VP1 nucleotide sequences were aligned using BioEdit 7 software (Hall, 1999)[13] and Clustal W 1.83 program (Thompson *et al.*, 1994)[14]. These alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model (Kimura, 1980)[15] as implemented in the program MEGA software v5.0 (Tamura *et al.*, 2011)[16]. Phylogenetic tree were constructed using the neighbourjoining of MegAlign program from LaserGene Biocomputing Software Package (DNASTAR, Madison, WI).

Result:

Partial sequencing of the VP1 gene produces a sequence for approximately 362bp for each sample and submitted to the GeneBank database with the accession number (KP686058). Sequence alignment was compared with previously reported references of genotypes of the most similar sequences retrieved from GenBank to identify the genotype of the isolate (**Figure 1**). Nucleotide sequencing revealed the occurrence of nucleotide substitution generating a single nucleotide polymorphism at position of 13 nucleotide, where C instead of T and also substitution of G instead of A at position 22 (**Figure1**). The analysis of genetic diversity based on partial sequencing represented the percent of diversion and identity between the new Egyptian isolate and nineteen selected sequences circulating globally and retrieved from GeneBank displayed in (**Table 1**), it revealed that our sequence showed typical identity (99.4%) with Egyptian SAT2, Buffalo, 2012 with accession number KF112931.1, JX570619.1, JX5013960.1 and JX013978.1 with diversion 0.6% but it is variable from its neighbor countries isolates and identity reached to its lowest similarity 85.3 % with AY343934.1 Eritrea.

Phylogenetic analysis showed a robust tree clustering all isolates with sequences belonging to the FMDV- SAT2 type with strong bootstrap values at relevant nodes. Phylogenetic tree shows the evolutionary relationship of the sequences in which the length of the horizontal line was proportional to the estimated genetic distance between the sequences. Such tree indicated that the evolutionary distance between groups is very short (**Figure 2**). Protein sequence analysis indicated the presence of one substitution in the sequences of amino acids at the position of 8, where an Alanine is substitutes by a Threonine (**Figure 3**).

Discussion:

Egypt is endemic for FMDV-SAT2 since 2012. Foot-and-mouth disease remains a globally important livestock disease affecting cloven-hoofed animals. It remains enzootic in many regions, especially in developing countries where it imposes a trade barrier upon livestock and their products. We selected the specific primers of FMDV-SAT2 based on the highly conserved VP1 gene - coding region (Knowles and Samuel, 2003)[9].

Our data indicated that the purified and partially sequenced PCR products generated 362bp of FMDV-SAT2 genotype. The sequences were aligned by cluster grouping where the clusters aligned the most

similar sequences firstly then progressively more distant groups of sequences until the global alignment was obtained. The NCBI-BLAST search found that our isolates are (100%) homologues to the genotype FMDV SAT2 topotype and its accession number is KP 686058.

In February 2012, a new extensive FMDV SAT2 outbreak struck Upper Egypt (Salem et al., 2012)[17], Delta Governorates (Ahmed et al., 2012[6] and Valdazo Gonzales et al., 2012)[7], Gharbia (Elhaig and Elsheery, 2014)[18] and Alexandria (El-Shehawy et al., 2014)[19] and in African countries serotype SAT2 was mainly responsible for outbreaks (Depa et al., 2012)[20].

A sub clinical or unapparent infection can occur in African buffalo (Jamal and Belsham, 2013)[1]. There are several potential risk factors associated with both introduction and spread of the FMDV infection. The most important of these are biosecurity, movement of live animals and animal products, swill feeding and access to landfill waste (EFSA, 2012)[21].

Sequencing of our samples revealed substitution in two nucleotides generating a change at the level of 13 nucleotide, where a C replaced a T. (**Figure, 1**). In addition, our isolate revealed other substitution at the levels of 22 nucleotides, where G substituted A. These substitution did not express in the previously mentioned Egyptians, 2012 isolates. The causative agent, FMD virus has a rapid mutation rate (**Upadhyaya et al., 2014**)[22].

The nucleotide sequence data indicates that the similarity in nucleotide sequence (99.4%) between SAT2 FMDV, buffalo, Egypt, Qalyubia, 2014 and a virus of buffalo origin obtained during 2012, Egypt KF112931.1, JX570619.1, JX5013960.1 and JX013978.1 with diversity of 0.6. It is worth mention that identity percent 99.2% with the virus in the same locality Banha, Qalyubia, Egypt JX 570625 with 0.8

divergences. The virus diversity is high among SAT serotypes, especially for the SAT 2 serotype that is composed of at least 14 geographically restricted topotypes (**Bastos et al., 2003**)[4]. Phylogenetic tree indicated that the evolutionary distance between groups is very short, suggesting that the genetic divergence is recent (**Figure, 2**). In addition to SAT2 serotype, phylogenetic analysis of VP1 nucleotide sequences demonstrated that viruses from Egyptian field cases fell into other two different serotypes that were belonging to A and O serotypes (**Salem et al., 2012**)[17]

Phylogenetic analysis showed that our isolates clustered with SAT2 FMD virus, revealed that KP686058, Qalyubia , Egypt put in the same category with KJ210079, JX570616, JX570615, JX570617 Egypt, 2012 and closely related to KF112968 Sudan and JX570633 Libya (**Table, 1**). Egypt is a large country with a dense animal population and is bordered by Libya, Sudan and Palestine and FMD is endemic in those three countries (**FAO, 2012[23], Ahmed et al.[6], 2012 and Valdazo-Gonzalez et al., 2012**) [7]respectively. Animals may move between these countries without restriction, leading to the uncontrolled spread of FMDV.

Nucleotide substitution is translated in the protein sequence as our data refer to the presence of an A instead of a T, at the level of 8 (**Figure**, **3**). These substitutions of FMDV SAT2 topotype variation is associated with change in the geographical distribution, infectivity and antigenicity, and can circulate within the buffalo populations at different localities of the Egypt through unrestricted animal movements.

Conclusions:

Our study provides persistence of the circulation of SAT2-type FMD viruses among buffalo population. Therefore monitoring the emergence of SAT2 strains of FMDV in Egypt is important to enable appropriate vaccines selection and control measurement as rapidly as possible.

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Table (1): The percent of identity and diversion for amino acid sequence ofVP1 geneFMD virus topotype SAT 2 from buffalo, Egypt with accessionnumberKP686058 in comparison with nineteen selected sequences globallycirculating from GenBank using DNA star software.

	Percent Identity																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1		99.4	99.4	99.4	99.4	99.2	99.2	99.2	99.2	99.2	99.2	89.8	89.2	89.2	88.7	88.1	88.1	87.5	87.3	87.0	1
2	0.6		100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.7	99.7	90.4	89.8	89.8	89.2	88.7	88.7	88.1	87.8	87.5	2
3	0.6	0.0		100.0	100.0	99.7	99.7	99.7	99.7	99.7	99.7	90.4	89.8	89.8	89.2	88.7	88.7	88.1	87.8	87.5	3
4	0.6	0.0	0.0		100.0	99.7	99.7	99.7	99.7	99.7	99.7	90.4	89.8	89.8	89.2	88.7	88.7	88.1	87.8	87.5	4
5	0.6	0.0	0.0	0.0		99.7	99.7	99.7	99.7	99.7	99.7	90.4	89.8	89.8	89.2	88.7	88.7	88.1	87.8	87.5	5
6	0.9	0.3	0.3	0.3	0.3		99.4	99.4	99.4	99.4	99.4	90.7	90.1	90.1	89.5	89.0	89.0	88.4	87.5	87.3	6
7	0.9	0.3	0.3	0.3	0.3	0.6		99.4	99.4	99.4	99.4	90.1	89.5	89.5	89.0	88.4	88.4	87.8	87.5	87.3	7
8	0.9	0.3	0.3	0.3	0.3	0.6	0.6		99.4	99.4	99.4	90.1	90.1	90.1	89.5	88.4	89.0	87.8	88.1	87.8	8
9	0.9	0.3	0.3	0.3	0.3	0.6	0.6	0.6		99.4	99.4	90.7	90.1	90.1	89.5	89.0	89.0	88.4	88.1	87.8	9
10	0.9	0.3	0.3	0.3	0.3	0.6	0.6	0.6	0.6		99.4	90.1	89.5	89.5	89.0	88.4	88.4	87.8	87.5	87.3	10
11	0.9	0.3	0.3	0.3	0.3	0.6	0.6	0.6	0.6	0.6		90.1	90.1	90.1	89.5	88.4	88.4	87.8	87.5	87.3	11
12	11.2	10.5	10.5	10.5	10.5	10.2	10.8	10.8	10.2	10.8	10.8		88.7	88.7	88.7	96.0	87.0	86.4	88.4	88.1	12
13	11.9	11.2	11.2	11.2	11.2	10.8	11.5	10.8	10.8	11.5	10.8	12.6		100.0	97.7	88.1	92.9	91.8	89.5	89.2	13
14	11.9	11.2	11.2	11.2	11.2	10.8	11.5	10.8	10.8	11.5	10.8	12.6	0.0		97.7	88.1	92.9	91.8	89.5	89.2	14
15	12.6	11.9	11.9	11.9	11.9	11.5	12.2	11.5	11.5	12.2	11.5	12.6	2.3	2.3		88.1	92.9	91.8	89.5	89.2	15
16	13.4	12.7	12.7	12.7	12.7	12.3	13.0	13.0	12.3	13.0	13.0	4.1	13.3	13.3	13.3		87.3	86.7	87.8	87.5	16
17	13.3	12.6	12.6	12.6	12.6	12.2	12.9	12.2	12.2	12.9	12.9	14.6	7.5	7.5	7.5	14.3		98.6	88.4	88.1	17
18	14.0	13.3	13.3	13.3	13.3	12.9	13.6	13.6	12.9	13.6	13.6	15.3	8.8	8.8	8.8	15.0	1.4		87.3	87.0	18
19	14.4	13.6	13.6	13.6	13.6	14.0	14.0	13.3	13.3	14.0	14.0	12.9	11.5	11.5	11.5	13.7	12.9	14.3		99.7	19
20	14.7	14.0	14.0	14.0	14.0	14.4	14.4	13.6	13.6	14.3	14.4	13.3	11.9	11.9	11.9	14.0	13.2	14.7	0.3		20
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	

SAT2, Buffalo, EGY, 2014, KP686058 SAT2.Buffalo,EGY/16/12,KF112931.1 SAT2,EGY/4/2012,VP1,JX570619.1 SAT2,EGY/23/2012,JX013980.1 SAT2,buffa,EGY/7/2012,JX013978.1 SAT2,EGY/H1Fay/2012,KF055861.1 SAT2,EGY/28/2012,KF112935.1 SAT2,Banh,EGY/13/12VP1,JX570625.1 SAT2,EGY/11/2012VP1.JX570624.1 SAT2,EGY/3/2012VP1,JX570618.1 SAT2,buffal,EGY/26/12,JX013979.1 SAT2,EGY/2/2012VP1,JX570617.1 SAT2,LIB/1/2003VP1,JX570631.1 SAT2,LIB/7/2003VP1,JX570632.1 SAT2,NGR/15/2005,KF112960.1 SAT2,SUD/4/10capsid,KF112968.1 SAT2,CAR/8/2005VP1,JX570616.1 SAT2,CAR/1/2005VP1,JX570615.1 SAT2,ERI/1/98VP1(1D)AY343933.1 SAT2,ERI/4/98VP1(1D)AY343934.1

Divergence

	10	20		40		50	79
SAT2, Buffalo.EGY, 2014, KP686058	OCACTACOCA	GCCCGGCGAC				ACCCOCTTTO	CONTROCOUTT
SAT2, PAT/1/2012, JX014256.1		OCTODOCGAC					
SAT2, EGY/3/2012, MC440884.1		OCTODOCGAC					
ShT2, Egy/Sharkia/13, KJ210079.1		OCTOOCGAC				ACCCOCTTTG	
SAT2.Buffal,EGY/16/12,KF112931 SAT2.EGY/4/2012,VP1,JX570619.1		GCTCOGCGAC GCTCOGCGAC					
SAT2, EGY/23/2012, JX013980.1		OCTOGOCGAC					
SAT2, buffalo, EGY/7/12, JX013978		OCTOGOCGAC					
SAT2, EGY/H1Pay/2012, KF055861.1		OCTODOCGAC		TTTTTGCCAA	OOOCOOCOTA	ACCCOCTTTG	CEATCOOGTT
SAT2, EGY/28/2012, KF112935.1		GCTCGGCGAC					
SAT2, VP1Ban, EGY/13/12, JX570625		GCTCOGTGAC					
SAT2,EGY/11/2012VP1,JX570624.1 SAT2,EGY/3/2012VP1,JX570618.1		OCTCOOCGAC OCTCOOCGAC					
SAT2, buffal, EGY/26/12, JX013979		OCTOGOCGAC					
SAT2, EGY/2/2012VP1, JX870617.1		OCTTOOCGAC					
SAT2, LIB/1/2003VP1, JX570631.1		ACTTOGTOAC					
SAT2, LIB/7/2003VP1, JX570632.1		ACTTOGTOAC				ACCCOCTTTG	
SAT2, NGR/15/2005, KF112960.1		ACTTOGTOAC OCTTOGCOAT					
SAT2, SUD/4/10capsid, NP112968.1 SAT2, CAR/8/2005VP1, JX570616.1		ACTTOGTONC					
SAT2, CAR/1/2005VP1, JX570615.1		ACTTOOCGAC					
SAT2, ERI/1/98VP1 (1D) AV343933.1	OGACCACCCA	ACTTOGTOAC	AACCCCATOG	TTTACOCCAN	OCOCOTOTA	ACCCOCTTTG	CTATCCCGTT
SAT2, MurchisonFalls, FJ461346.1		ACTTOOCGAT					
SAT2, ERI/4/98VP1(1D) AY343934.1 SAT2, LIB/39/2012VP1, JX570633.1		ACTTOGTGAC OCCCOOCGAT				ACCOUNTING	
SAT2, LIB/39/2012VP1, 3X570633.1 SAT2, SUD/1/2007VP1, GU566071.1		ACTTOOCGAT					
SAT2, SEN/27/2009, KF112967.1		ACTTOGTGAT					
SAT2, Camer/74/4VP1(1D) AY284451		ACTTOGTGAC					
SAT2, Camer, fd1/74/10VP1, AY2544	OGACCOCCCA	ACTTOGTOAC	ANTOCONTOG	TTTTCGCCAA	GOOCOGTOTO	ACCCOCTTTG	CEATCCCGTT
SAT2, Camer, bbo/39/06VP1, AY2544		ACTTOGTGAT					
SAT2, Camer/3/30VP1(1D)AY254452	GGACCOCCCA	ACTTOGTGAT	ANTOCONTOG	TTTTCOCCAA	OCOTOCICIO	ACCCOCTITIC	CCATCCCGTT
		90	104 1	AM		A.84	
BAT2, Buffalo, EGY, 2014, KP686058	TACOOCTOCA	CACAGOCTOC	TGTCTACTGT	TTACAACOOC	GAGTGTGTCT	ACACCAMOOC	COCCOCTOCC
SAT2, PAT/1/2012, JX014256.1		CACAGOCTOC					
SAT2, EGY/3/2012, MC440884.1		CACAGOCTOC					COCCOCTOCC
SAT2, Egy/Sharkia/13, KJ210079.1 SAT2, Buffal, EGY/16/12, KF112931		CACAGOCTOC					
SAT2, EGY/4/2012, VP1, JX570619.1		CACAGOCTOC					
SAT2, EGY/23/2012, JX013980.1						ACACCAAOOC	
SAT2, buffalo, EGY/7/12, JX013978		CACAGOCTOC					
SAT2, EGY/H1Fay/2012, MF055861.1		CACAGOCTOC					
SAT2, EGY/28/2012, KF112935.1		CACAGOCTOC					
SAT2, VP1Ban, EGY/13/12, JX570625 SAT2, EGY/11/2012VP1, JX570624.1							COCCACTOCC
SAT2, EGY/3/2012VP1, JX570618.1		CACAGOCTOC					
SAT2, buffal, EGY/26/12, JX013979	TACOOCTOCA	CACAGOCTOC					
BAT2, EGY/2/2012VP1, JX870617.1						ACAAGAAOOC	
SAT2, LIB/1/2003VP1, JX570631.1		CACAGOCTOC					
SAT2, LIB/7/2003VP1, JX570632.1 SAT2, NGR/15/2005, KF112960.1		CACAGOCTOC					
SAT2, SUD/4/10capsid, KF112968.1		CACAGGTTGC					
SAT2, CAR/8/2005VP1, JX570616.1						ACACCANGAC	
SAT2, CAR/1/2005VP1, JX570615.1		CACAGOCTGT					
SAT2, ERI/1/98VP1 (1D) AV343933.1		CACAGOCTOC					
SAT2, MurchisonFalls, FJ461346.1		CACAGACTAT					
SAT2, ERI/4/98VP1(1D) AY343934.1 SAT2, LIB/39/2012VP1, JX570633.1		CACCOGCTOC					TGTTACTOCC
SAT2, SUD/1/2007VP1, GU566071.1		CACCOGCTGC					
SAT2, SEN/27/2009, KF112967.1		CACAGGTTGC					
SAT2, Camer/74/4VP1 (1D) AY254451						ACAACAAAAC	
SAT2, Camer, fdl/74/100P1, AY2544		CACAGOCTOC					
SAT2, Camer, bbo/39/06VP1, AY2544 SAT2, Camer/3/30VP1 (1D) AY254452		CACAGOCTOC					
	1.0		371	1.00	1.00		210

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SAT2, Buffalo, EGY, 2014, KP686058		ATCOTOCOOC			ACACCAACCA	CACTITOCCO	
SAT2, PAT/1/2012, JX014256.1	ATTOGTOGAG	ATCOTOCOOC	ACTTOCOOCA	ANGTACOCTO.	ACACCAACCA ACACCAACCA	CACTITICCCG	CCAACCTTCA
SAT2, PAT/1/2012, JX014256.1 SAT2, EGY/3/2012, MC440554.1	ATTOGTOGAG		ACTTGCOGCA	ANGTACOCTO	ACACCAACCA ACACCAACCA ACACCAACCA	CACTITIGCOG CACTITIGCOG CACTITIGCOG	OCAACOTTCA OCAACOTTCA
SAT2, PAT/1/2012, JX014256.1 SAT2, EGY/3/2012, NC440884.1 SAT2, Egy/Sharkia/13, KJ210079.1 SAT2, Buffal, EGY/16/12, KJ112931	ATTOGTOGAG ATTOGTOGAG ATTOGTOGAG	ATCOTOCOOC ATCOTOCOOC ATCOTOTOCO ATCOTOCOOC	ACTTGCOGCA ACTTGCOGCA ACTTGCOGCA ACTTGCOGCA	AAGTACOCTG AAGTACOCTG AAGTACOCTG AAGTACOCTG	ACACCAACCA ACACCAACCA ACACCAACCA ACACCAACCA ACACCAACCA	CACTITIGCOG CACTITIGCOG CACTITIGCOG CGATITIGCOG CACTITIGCOG	CEAACETTEA CEAACETTEA CEAACETTEA
ShT2, PhT/1/2012, JK014256.1 ShT2, EGY/3/2012, KC440554.1 ShT2, Egy/Sharkia/13, KJ210079.1 ShT2, Buffal, EGY/16/12, KF112931 ShT2, EGY/4/2012, VP1, JK570619.1	ATTEGTOGAG ATTEGTOGAG ATTEGTOGAG ATTEGTOGAG	ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC	ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA	АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО	ACACCAACCA ACACCAACCA ACACCAACCA ACACCAACCA ACACCAACCA ACACCAACCA	CACTITIGCOG CACTITIGCOG CACTITIGCOG CGATITIGCOG CACTITIGCOG	
ShT2, PhT/1/2012, JK014256.1 ShT2, EGY/3/2012, KC440884.1 ShT2, Egy/Sharkin/13, KC120079.1 ShT2.Buffal, EGY/16/12, KF112931 ShT2, EGY/2012, VP1, JK570619.1 ShT2, EGY/2012, JK013980.1	ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG	ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC	ACTTGOOGCA ACTTGOOGCA ACTTGOOGCA ACTTGOOGCA ACTTGOOGCA	АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС	АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА	CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG	
ShT2, PhT/1/2012, JX014256.1 ShT2, EGY/3/2012, KC440884.1 ShT2, EGY/3harkia/13, KK210079.1 ShT2 Buffal, EGY/16/12, KT112931 ShT2, EGY/32012, JX013976.1 ShT2, EGY/32/2012, JX013976.1	ATTOGTOGAG ATTOGTOGAG ATTOGTOGAG ATTOGTOGAG ATTOGTOGAG ATTOGTOGAG	ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC	ACTTGODGCA ACTTGODGCA ACTTGODGCA ACTTGODGCA ACTTGODGCA ACTTGODGCA	АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС АЛСТАСОСТС	АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА АСАССААССА	CACTITIGOOD CACTITIGOOD CACTITIGOOD CACTITIGOOD CACTITIGOOD CACTITIGOOD CACTITIGOOD CACTITIGOOD	CCAACCITCA CCAACCITCA CCAACCITCA CCAACCITCA CCAACCITCA CCAACCITCA
SAT2, DAT/1/2012, JX014256.1 SAT2, EGY/3/2012, KC440584.1 SAT2, Egy/Sharhia/13, KZ10075.1 SAT2.Buffal, EGY/16/12, KF112931 SAT2, EGY/3/2012, VD1, JX570615.1 SAT2, EGY/23/2012, JX013950.1 SAT2, Duffalo, EGY/7/12, JX013975 SAT2, SGY/IIFay/2012, KY055661.1	ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG ATTCGTGGAG	ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC	ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA	АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО		CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG CACTITIGCOG	
ShT2, PhT/1/2012, JX014256.1 ShT2, EGY/3/2012, KC440884.1 ShT2, EGY/3harkia/13, KK210079.1 ShT2 Buffal, EGY/16/12, KT112931 ShT2, EGY/32012, JX013976.1 ShT2, EGY/32/2012, JX013976.1	ΑΤΤΟΟΤΟΟΙΑ ΑΤΤΟΟΤΟΟΙΑ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ ΑΤΤΟΟΤΟΟΙΑΟ	ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC ATCOTOCOC	ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA ACTTGOGGCA	АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО АЛОТАСОСТО	ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА ХСЛОСАЛОСА	CACTITIGOOG CACTITIGOOG CACTITIGOOG CACTITIGOOG CACTITIGOOG CACTITIGOOG CACTITIGOOG CACTITIGOOG	000A00TTCA 000A00TTCA 000A00TTCA 000A00TTCA 000A00TTCA 000A00TTCA TCAA00TTCA 000A00TTCA
ShT2, PhT/1/2012, JX014256.1 ShT2, Ext/3/2012, KC44084.1 ShT2, Egy/Sharkia/13, KJ210079.1 ShT2, Buffal, EdY/14/12, KT112831 ShT2, EdY/4/2012, VM1, JX670619.1 ShT2, EdY/4/2012, VM13950.1 ShT2, EdY/142012, KT105861.1 ShT2, EdY/18-gy/2012, KT105861.1 ShT2, VM1Ban, EdY/14/12, JX570625 ShT2, Sty/11/2012VM1, JX570625	ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ ΑΤΤΟΟΤΟΘΑΟ	ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC	ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA ΑCTTGOGGCA	ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG ANGTACOCTG	ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА ХСАССАЛССА	CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS CACTTTRECCS	00000000000000000000000000000000000000
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ShT2, PhT/1/2012, JX014256.1 ShT2, Ext/3/2012, KC44084.1 ShT2, Ext/3/2012, KC44084.1 ShT2, Duffal, EXt/13, KJ210079.1 ShT2, Duffal, EXt/14, NS10495.1 ShT2, Duffal, EXt/14, NS12975 ShT2, Duffal, EXt/14, NS12975 ShT2, Duffal, EXt/14, NS12975 ShT2, CY/12/2012, KF11955.1 ShT2, VIBan, EXt/14/12, JX570625 ShT2, EXt/11/2012VP1, JX570625.1 ShT2, DST/11/2012VP1, JX570615.1 ShT2, DST/11/2012VP1, JX570615.1	Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά	ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC ATCOTOCOGC	Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ Ασττοσόσολ	ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO	ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА ХСКСОЛОССА	CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS CACTITISCOS	ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА ССААССТТСА
ShT2, PhT/1/2012, JX014256.1 ShT2, EGY/3/2012, KC440854.1 ShT2, EGY/3/2012, KC440854.1 ShT2, Buffal, EGY/16/12, KF112931 ShT2, Buffal, EGY/16/12, KF112931 ShT2, Buffalo, EGY/12, VD1, JX570619.1 ShT2, Buffalo, EGY/7/12, JX013975 ShT2, CGY/H1Fay/2012, KF11295.1 ShT2, CGY/20/2012, KF11295.1 ShT2, CGY/11/2012VP1, JX570625.1 ShT2, EGY/11/2012VP1, JX570624.1 ShT2, EGY/1/2012VP1, JX570624.1 ShT2, EGY/1/2012VP1, JX570624.1 ShT2, EGY/1/2012VP1, JX570624.1	Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά Αττοστοσικά	ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE ATCOTOCODE	Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ Ασττοςοσολ	ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO ANGTACOCTO	аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса, аскерлеса,	CACTITISCOS CACTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS CALTITISCOS	
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SAT2, EGY/2/2012, HC440884.1	ACTTOGOGTA	COTGACOGTT	GACAAAACCAG	TOGACOTTTA	CTACCOGATO	AAGAGOOCTG	AGCTOTACTO
SAT2, Egy/Sharkis/13, KJ210079.1	ACTTOOOGTA	TOTGACOGTT	GACANACCAG	TCGACOTTTA	CTACCOGATO	AAGAGOOCTG	AGCTGTACTG
SAT2.Buffal,EGY/16/12,KF112931	ACTTOGOGTA	COTGACCOTT	GACANACCAG	TOGACOTITA	CTACCOGATO	ANGNOGOCTO	AGCTGTACTO
SAT2, EGY/4/2012, VP1, JX570619.1	ACTTOGOGTA	COTGACCOTT	GACARACCAG	TOGACOTITA	CTACCOGATG	ANGAGOGCTG	AGCTGTACTG
SAT2, EGY/23/2012, JH013980.1						AAGAGGGCTG	
SAT2, buffale, EGY/7/12, JX013978						ANGAGOOCTO	
SAT2, EGY/H1Fay/2012, KF055861.1						ANGLOOOCTO	
SAT2, EGY/28/2012, KF112935.1						Алалоооста Алалоооста	
SAT2, UP1Ban, EGY/13/12, JX570625 SAT2, EGY/11/2012UP1, JX570624.1	ACTICOUGTA	COTOACCOTT	Check have been	TOGACOTTTA	CTACCOGATO	AAGAGGGCTG	AGCTOTACTO
SAT2, EGY/3/2012VP1, JX570618.1						AAGAGOOCTG	
SAT2, buffel, EGY/26/12, JX013979						ANGAGOGCTG	
SAT2, EGY/2/2012VP1, JX570617.1	ACTTCOOOTC	COTOACCOTC	GACAGACCAG	TEGACGTITA	CTACCOGATO	ANGAGAGCTO	AGTTGTACTO
BAT2, LIB/1/2003VP1, JX570631.1						ANGAGOGCTG	
SAT2, LIB/7/2003VP1, JX570632.1	ACTTOODOTT	COTGACCOTC	GACANACCAG	TCGATGTTTA	CTACCOGATO	ANGAGOGCTG.	AOCTOTACTO
SAT2, NGR/15/2005, MF112960.1	ACTITOOGIT	COTGACOGTC	GACANACCAG	TCGATGTTTA	CTACCOGATO	ANGAGOGETG	AGCTGTACTG
SAT2, SUD/4/10capsid, KF112968.1	ATTTOGOGITT	COTOACCOTC	GACAGACCAG	TCGATGITTA	CTACCOGATG	ANGAGAGETG	AGTTGTACTG
SAT2, CAR/8/2005VP1, JK570616.1 SAT2, CAR/1/2005VP1, JK570615.1	ACTTOGOGITT	TOTGACCOTC	GACAAACCAG	TEGACOTITA	CTACCOGATG	AAGAGOGCTG AAGAGOGCTG	AGCTGTACTG
SAT2, ERI/1/2005001, JAS70615.1 SAT2, ERI/1/98001(1D) AY343933.1	ACTICOUGHT	OTTOACCOCC	GACANOCCAG	TOGACOTTINA	CTACCOGATO	ANGAGOGETG	AGCTOTACTO
SAT2, MurchisonFalls, FJ461346.1	ACTICOOGTT	COTGACOGTC	GACAAACCAG	TAGACOTITA	CTACCOGATO	ANGAGOGETG	AACTGLACTG
SAT2, ERI/4/98UP1 (1D) AV343934.1	ACTTCOOGTT	COTGACOGTO	GACAAGCCAG	TEGACOTITA	CTACCOGATO	AAGAGGGCTG	AOCTOTACTO
SAT2, LIB/99/2012VP1, JX570693.1	ACTTCOOGTT	COTGACCOTC	GACANACCAG	TOGACOTITA	CTACCGAATG	ANGAGOOCTO	AGCTOTACTO
SAT2, SUD/1/2007VP1, GU566071.1	ACTTCOOGTT	COTGACOGTC	GACANACCAG.	TOGACOTITA	CTACCGAATG	ANGAGOGCTG	AGCTGTACTG
SAT2, SEN/27/2009, HF112967.1	ATTTCOODTT	COTGACCOTT	GACANACCAG	TOGATOTTTA	CTACCOGATO	хлалаоаста	AOCTOTACTO
SAT2, Camex/74/4VP1(1D) AY254451	ACTITOOOTT	TOTOLCOOTC	GACAACCAG	TCOATOTTTA	CTACCOGATO	ANDADOOCTO	AACTOTACTO
SAT2, Camer, Ed1/74/10UP1, AY2544	ACTTCOOGTT	таталесате	GACAACCAG	TCOATOTTTA	CTACCOGATO	ANGLOOOCTO	AACTOTACTO
SAT2, Camer, bbo/39/06VP1, AY2544 SAT2, Camer/3/30VP1(1D) AY254452	ACTTEGOGTT	TOTOLCOGTC	GACAJACCAG	TOGATOTTTA	CTACCOGATO	ллаласста	AACTOTACTO
SA12, CAMEE/3/30VP1(1D) A1254452	ACTICODODITI	Turuxcoure	GAERACCEAG	TCOATOTTTA	CTACCOGATO	ANGNOOCTO	AACTOTACTO
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		**********	the state and state and a state				
SAT2, Buffalo, EGY, 2014, KP686058					CANENCALITICS.	ACCALCAT	
SAT2, PAT/1/2012, JX014286.1						ACOCOCCCAT	
BAT2, EGY/2/2012, KC440884.1						ACGCGCCCAT	
SAT2, Egy/Sharkis/19, KJ210079.1						ACGCGCCCAT	
SAT2.Buffal,EGY/16/12,WF112991 SAT2,EGY/4/2012,VP1,JX570619.1						ACOCOCCCAT	
SAT2, EGY/29/2012, JX013980, 1						ACGCGCCCAT	
SAT2, buffalo, EGY/7/12, JX019978						ACOCOCCAT	
SAT2, EGY/H1Fay/2012, KF055861.1	COCACOCCA	CTOCTOCCAG	OCTACAAACA.	CACAGACAGA	GACAGATTOS	ACOCOCCAT	COOCOTCGAA
SAT2, EGY/28/2012, MF112935.1						ACOCOCCAT	
SAT2, VP1Ban, EGY/13/12, JX570625						ACOCOCCAT	
SAT2, EGY/11/2012VP1, JX570624.1						ACOCOCCAT	
SAT2, EGY/3/2012VP1, JX570618.1						ACOCOCCAT	
SAT2, buffal, EGY/26/12, JX013979 SAT2, EGY/2/2012VP1, JX570617, 1						ACOCOCCCAT	
SAT2.LIB/1/2003VP1.JX570631.1						ACOCOCCCAT	
SAT2, LIB/7/2003VP1, JX570632,1						ACOCOCCAT	
SAT2, NGR/15/2005, NF112960.1			CCTACGACCA			ACOCACCCAT	
SAT2, SUD/4/10capsid, KF112968.1	TOCACOCCCA	CTOTTACCAG	COTACOAACA	COCNOOCNON		ACOCOCCAT	
BAT2, CAR/8/2005VP1, JX570616.1	CCCOCOCCCA	CTOTTOCTAN	CCTACGACCA	COCAOGTAGA	GACAGATTCG	ACOCOCCAT	TOOCOTCOAG
SAT2, CAR/1/2005VP1, JX570615.1						ACOCOCCAT	
SAT2, ERI/1/98VP1 (1D) AY343933.1	TOCACOCOCA	CTACTOCCTG	CCTACGACCA	COCANOCAGA	GACAGATTOG	ACOCOCCAT	COOCGTOGAA
SAT2, MurchisonFalls, FJ461346.1						ACCCCCCAT	
SAT2, ERI/4/98VP1(1D)AY249934.1 SAT2, LIB/39/2012VP1, JX570633.1	TOCACOCOCA	CTACTOCCTG	OCTADGADCA	COCAGCAGA	GACAGATTOG	ACGCGCCCAT ACGCGCCCCAT	COCOTCOAA
SAT2, SUD/1/2007VP1, GU566071.1						ACOCOCCAT	
SAT2, SEN/27/2009, MF112967.1	OCCACOCCA	TTOCTOCCAG	CCTATGACCA.	COGAGATAGA	GACAGATTTG	ACOCACCCAT	COOTOTTONO
SAT2, Cames/74/4VP1 (1D) AY254451			CCTACGAACA				
SAT2, Camer, fd1/74/10VP1, AY2544	TOCACOCCCG	CTOTTOCCAG	CCTACGAACA	CACAGACAGA	GACAGATTOS	ACOCO	
SAT2, Camez, bbo/39/06VP1, AY2544	TCCACOCCCG	CTOTTOCCAG	CCTACGAACA	CACAGACAGA	GACAGATTOS	ACOCG	
SAT2, Camer/3/30VP1 (1D) AY254452	теслевессв	статтасска	CCTACGAACA	слеталенан	GACAGATTCO	ACOCG	
AND							
SAT2, Buffalo, EGY, 2014, KP686058	MANCHANCOC	TG					
SAT2, PAT/1/2012, JX014256.1	MANCHANCCC	TO					
SAT2, EGY/3/2012, KC440884.1	AGACAGACCC						
SAT2, Egy/Sharkia/13, KJ210079.1	AGACAGACCC						
SAT2. Buffal, EGY/16/12, KP112931 SAT2, EGY/4/2012, VP1, JX570619.1	AGACAGACCC						
SAT2, EGY/23/2012, JX019980.1	AGACAGACCC						
SAT2, buffalo, EGY/7/12, JX010978	AGACAGACCC						
SAT2, EGY/H1Fay/2012, KF055861.1	AGACAGACCC						
SAT2, EGY/28/2012, MF112925.1	AGACAGACCC						
SAT2, VP1Ban, EGY/13/12, JX570625	AGACAGACCC						
SAT2, EGY/11/2012VP1, JX570624.1	MANCHANCOC						
SAT2, EGY/3/2012VP1, JX570618.1	AGACAGACCC	TG					
SAT2, buffal, EGY/26/12, JX013979	AGACAGACCC						
<pre>SAT2,EGY/2/2012VP1,JX570617.1 SAT2,LIE/1/2003VP1,JX570631.1</pre>	AGACAGACCC						
SAT2, LIB/7/2003VP1, JX570632.1	AGACAGACCC						
SAT2, NGR/15/2005, KF112960.1	AGACAGACCC	TO					
SAT2, SUD/4/10capsid, KF112968.1	AGACAGACCC	TG					
SAT2, CAR/8/2005VP1, JX570616.1	AGOCAGACCC	TA					
SAT2, CAR/1/2005VP1, JX570615.1	AGOCAGACCC	TA					
SAT2, ERI/1/98VP1 (1D) AY343933.1	NOOCHANCCC						
SAT2, MuschisonFalls, FJ461346.1	CONCANNEAC						
SAT2, ERI/4/98VP1(1D)AY343934.1 SAT2, LIB/39/2012VP1, JX670633.1	AGOCAGACCC						
SAT2, LIB/39/2012/P1, JX570633.1 SAT2, SUD/1/2007VP1, GU566071.1	AGACAGADOC	TG					
SAT2, SUD/1/2007/P1, GUS66071.1 SAT2, SEN/27/2009, HF112967.1	AGACAGACCC	TO					
SAT2, Camer/74/4VP1(1D) AY254451							
SAT2, Camer, fd1/74/10VP1, AY2544							
SAT2, Camer, bbo/29/06VP1, AY2544							
SAT2, Cames/3/20VP1 (1D) AY254452							

Figure (1): Nucleotide sequence alignment of FMDV-SAT2 in Buffalo, Egypt, 2014 (KP686058) and comparative analysis with the available sequences using Basic local alignment sequence tool (BLAST) of the National Center for Biotechnology Information (NCBI) database. Two nucleotides substitution in KP686058 at positions13 and 22.



Figure (2): Phylogenetic tree sequences of FMDV-SAT2, Buffalo, Egypt, 2014 (KP686058) and their relationship with reference sequences of other FMDV genotype retrieved from GenBank. The tree analysis was obtained from partial sequence VP1 gene. All isolates cluster with sequences belonging to the FMDV-SAT2 genotype (Accession No. KP686058). A sequence aligned by Clustal W method and the tree was built by using MEGA5 software. Genetic distance is indicated below the tree.

	10	20	30	40	50
		FLPRAAPALP		*********	· · · · · · · · · · · · · · · · · · ·
SAT2 .Buffalo, EGY/ 2014 / KP 686058	CLPSPATALW	FLPRAAPALP	SRLRLHTGCC	LLETTASVST	PRPPLPIVEI
SAT2.Buffalo,EGY/16/12,KF11293		FLPRAAPALP			
SAT2, EGY/4/2012, VP1, JX570619.1 SAT2, EGY/23/2012, JX013980.1	GLPSSATTLW	FLPRAAPALP	SRURLHTOCC	LLETTASUST	PRPPLPEVET
SAT2, buffa, EGY/7/2012, JX013978	GLPSSATTLW	FLPRAAPALP	SRLRLHTOCC	LETTASVST	PRPPLPIVET
SAT2, EGY/H1Fay/2012, KF055861.1	GLPSSATTLW	FLPRAAPALP	SRLRLHTCCC	LLFTTASVST	PRPPLPIVEI
SAT2, EGY/28/2012, KF112935.1	GLPSSATTLW	FLPRAAPALP	SRLRLHTCCC	LLFTTASVST	PRPPLPEVEI
SAT2, Banh, EGY/13/12VP1, JX57062	GLPSSVTTLW	FLPRAAPALP	SRLRLHTGCC	LLETTASVST	PRPPLPIVEI
SAT2, EGY/11/2012VP1.JX570624.1	GLPSSATTLW				
SAT2, EGY/3/2012VP1, JX570618.1	GLPSSATTLW	FLPRAAPALP	SRLRLHTCCC	LLFTTASVST	PRPPLPFVVI
SAT2, buffal, EGY/26/12, JX013979	GLPSSATTLW	FLPRAAPALP	SRLRLHTCCC	LLFTTASVST	PRPPLPFVEI
SAT2, EGY/2/2012VP1, JX570617.1 SAT2, LIB/1/2003VP1, JX570631.1	GLPNLVTILW				
SAT2, LIB/7/2003VP1, JX570632.1	GLPNLVTILW	FSPRAAPALP	SRSRPHTCCC	LLSTMASAAT	TRPPLPSVET
SAT2, NGR/15/2005, KF112960.1	GLPHLVTTLW	TSPRAAPALP	SRSRPHTCCC	LLSTMASAAT	TRPPLPSVEI
SAT2, SUD/4/10capsid, KF112968.1	ELPSLAITPW				
SAT2, CAR/8/2005VP1, JX570616.1	GLPHLVTTPW	FLPRAAPALP	SRSRPLTCCC	LESTHVSAAT	PRPPLPSCEI
SAT2, CAR/1/2005VP1, JX570615.1		FLPRAAPALP			
SAT2, ERI/1/98VP1 (1D) AY343933.1	GPPNLVTTPW				
SAT2, ERI/4/98VP1 (1D) AY343934.1	EPPSPAITPW	FTPRAVPALL	SRSRPHTCCC	PLSTTASAPT	CRPPPPSVET
SAT2, LIB/39/2012VP1, JX570633.1	ELPNLAITPW				
SAT2, SUD/1/2007VP1, GU566071.1 SAT2, SEN/27/2009, KF112967.1	GLPNLVIILW	FSPRAAPALP	FRLRPHTOCC	PLSTMVSAAT	TEPPPPSVET
SAT2, Camero/74/04VP1, AY254451.	GPPNLVTIPW	FSPRAVPALP	SRSRPHTCCC	LETHASVPT	TEPPPPSVET
SAT2, Camero/74/10VP1, AY254471.	GPPNLVTIPW	FSPRAVPALP	SRSRPHTCCC	LESTMASVPT	TEPPPPSVCT
SAT2, Camero/039/06VP1, AY254448	GPPNLVIIPW	FSPRAVPALP	SRSRPHTGCC	PLSTMASAPT	TKPLPPSVET
SAT2, Camero/003/30VP1, AY254452	GPPNLVIIPW	FSPRVVPALP	SRSRPHTCCC	LLFTHASAPX	TKPPPPPSVET
	60	70	=0	90	100
SAT2.Buffalo,EGY/ 2014 / KP 686058	VRILEROSTLT	PTTLCROPST	SCTPLENOST	ETTGROLSCT	AHAHCCOPTH
SAT2.Buffalo,EGY/16/12,KF11293	VRHLROSTLT	PTTLCROPST	SCTPLINGST	FTTGRGLSCT	AHAHCCOPTH
SAT2, EGY/4/2012, VP1, JX570619.1	VRHLROSTLT	PTTLCROPST	SCTPLINOST	FTTGRGLSCT	AHAHCCOPTN
SAT2, EGY/23/2012, JX013980.1	VRHLROSTLT	PTTLCROPST	SCTPLINGST	FTTGRGLSCT	ABAHCCOPTN
SAT2, buffa, EGY/7/2012, JX013978	VRHLRQSTLT	PTTLCROPST	SCTPLINQST	FTTGRGLSCT	AHAHCCOPTN
SAT2, EGY/H1Fay/2012, KF055861.1	VRHLROSTLT	PTTLCROPST	SCTPLINOST	FTTGRGLSCT	AHAHCCOPTN
SAT2, EGY/28/2012, KF112935.1		PTTLCROPST PTTLCROPST			
SAT2, Banh, EGY/13/12VP1, JX57062 SAT2, EGY/11/2012VP1.JX570624.1		PTTLCROPST			
SAT2, EGY/3/2012VP1, JX570618.1		PTTLCROPST			
SAT2, buffal, EGY/26/12, JX013979	VRHLROSTLT	PTTLCROPST	SCTPLINOST	FTTGRGLSCT	AHAHCCOPTN
SAT2, EGY/2/2012VP1, JX570617.1	VORSROSTLT	PRTLCROPST	SCPPSTDQST	ITTGRELSCT.	VHANC*OPTN
SAT2, LIB/1/2003VP1, JX570631.1	VRYSROSHLA	TRTLCROPST	SCSPSTNQSM	FTTGRGLSCT	AHAHCCOPTT
SAT2, LIB/7/2003VP1, JX570632.1	VRYSROSMLA	TRTLCROPST	SCSPSTNQSM	TTTGRGLSCT	AHAHCCOPTT
SAT2, NGR/15/2005, KF112960.1		TRTLCROPST			
SAT2, SUD/4/10capsid, KF112968.1		PPTLCROPSI ATTLCROPST			
SAT2, CAR/8/2005VP1, JX570616.1 SAT2, CAR/1/2005VP1, JX570615.1	VERSEOSTLA	ATTLCROPST	SCLEPTNOST	PERCROLSOF	ARAHCCOPTT
SAT2, ERI/1/98VP1(1D)AY343933.1	ACHISRRSTLT	TETLERKPET	SCSPSTSOST	FTTGRGLSCT	VHAILYCLPTT
SAT2, ERI/4/98VP1 (1D) AY343934.1	ACHSRRSTLT	TSTLCRKPST	SCSPSTSOST	FTTGRGLSCT	VHAHYCLPTT
SAT2, LIB/39/2012VP1, JX570633.1	COHSROSMLT	TRTPCROPST	SCSPSTNQST	FTTERGLSCT	AHARCCOPTT
SAT2, SUD/1/2007VP1, GUS66071.1		TRTLCROPST			
SAT2, SEN/27/2009, KF112967.1	VRYSRLSTLA	TRTLEROPSI	SCSPLINGSM	FTTGRGLSCT	AHAHCCOPMT
SAT2, Camero/74/04UP1, AY254451.	ARHSRUSTLA	TRTLCROPST	LGLPSTTOSM	PTTGRGLNCT	VHARCCOPTH
SAT2, Camero/74/10VP1, AY254471. SAT2, Camero/039/06VP1, AY254448	ARUSROSTLA	TRTLEROPST	SCLPSTNOSM	FTTGRGLNCT	VIARCCOPTN
SAT2, Camero/003/30VP1, AY254452	ARYSROSTLA	THTLCROPST	SGLPSTTOSM	FTTGRGLNCT	VHARCCOPTN
					0.000
	110				
SAT2 . Buffalo, EGY/ 2014 / KP 686058	TOTETOSTRP				
	TOTETOSTRP	SASK RP			
SAT2,EGY/4/2012,VP1,JX570619.1	TOTETDSTRP				
SAT2, EGY/23/2012, JX013980.1	TOTETOSTRP	SASKORP			
SAT2, buffa, EGY/7/2012, JX013978	TOTETOSTRP				
SAT2, EGY/H1Fay/2012, KF055861.1	TOTETOSTRP	SASKORP			
SAT2, EGY/28/2012, KF112935.1	TOTETOSTRP				
SAT2, Banh, EGY/13/12VP1, JX57062	TOTETDSTRP	SASKORP			
SAT2, EGY/11/2012VP1.JX570624.1	TOTETOSTRP	SASKORP			
SAT2, EGY/3/2012VP1, JX570618.1 SAT2, buffal, EGY/26/12, JX013979	TOTETUSTRP				
SAT2, EGY/2/2012VP1, JX570617.1	TOAETDSTRP				
SAT2, LIB/1/2003VP1, JX570631.1	TKAETOSTRP	LVSRORP			
SAT2, LIB/7/2003VP1, JX570632.1	TKARTUSTRP	LVSRORP			
SAT2, NGR/15/2005, KF112960.1	TOAETUSTHP	LVSRORP			
	TOARTDSTRP				
SAT2, CAR/8/2005VP1, JX570616.1	TOVETDSTRP				
SAT2, CAR/1/2005VP1, JX570615.1	TOVETOSTRP	LASRCRP			
SAT2, ERI/1/98VP1(1D)AY343933.1 SAT2, ERI/4/98VP1(1D)AY343934.1	TOAETDSTRP				
SAT2, ERI/4/98VP1(1D)AT343934.1 SAT2, LIB/39/2012VP1, JX570633.1	TOAETDSTRP	SASRUPP			
SAT2, SUD/1/2007VP1, GU566071.1	TOVETOSTRP	SASRORP			
SAT2, SEN/27/2009, KF112967.1	TEIETOLTHP				
SAT2, Camero/74/04VP1, AY254451.	TOTETOSTX-				
SAT2, Camero/74/10VP1, AY254471.	TOTETOSTX-				
SAT2, Camero/039/06VP1, AY254448	TOTETDSTX-				
SAT2, Camero/003/30VP1, AY254452	TLTETOSTX-				

Figure (3): Protein sequence alignment of deduced amino acids of VP1 gene of FMDV SAT2 of buffalo, aligned by MEGA5 with known strains references sequences in GeneBank. One substitution in amino acid of FMDV-Sat2, Buffaloes, Egypt, 2014, KP686058 at positions 8.