Global Veterinaria 17 (4): 310-317, 2016
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2016.17.04.10632

Isolation and Characterization of Foot and Mouth Disease Virus Serotype SAT 2 Circulating in Egypt in 2014

[1]*Eman S. Ahmed,* [2]*H.A. Hussein,* [1]*Nahed A. Mahmoud,* [1]*M.H. Ali and* [2]*A.A. El-Sanousi*

[1]Department of Virology, Animal Health Research Institute, Dokki, Giza, Egypt
[2]Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Foot-and-mouth Disease (FMD) is a serious contagious viral disease affecting cloven hoofed animals. During 2012, cases of FMD due to serotype SAT 2 have been reported in domesticated livestock outside sub-Saharan Africa (Libya, Egypt, PAT and Bahrain). Analysis of VP1 (1D) sequences demonstrated that SAT 2 FMDVs from Libya, Egypt and PAT belong to topotype VII. Out of 402 samples collected from clinically infected animals from Egyptian Governorates and tested for SAT 2 antigen detection using (IZSLER, Brescia, Italy) ELISA, 33 samples were positive. Trials for virus isolation on BHK-21 cell line, from the 33 positive samples (by ELISA), revealed two isolates. RT-PCR was employed to confirm the presences of fragments of FMD viral genome in 4 samples (2 isolates and 2 selected original samples based on strong reactivity in ELISA). Nucleotide sequence and phylogenetic analysis of VP1 gene in the 4 samples show that three samples belong to topotype VII linage SAT 2/VII/Alx-12 and one sample belongs to topotype VII linage SAT 2/VII/Ghb-12. Indeed, the study reports the circulation of Ghb-12 and Alx-12 lineages of SAT 2 in cattle and buffalo in Egypt during 2014.

Key words: Epidemiology · Antigenic Identification · Sequencing · Molecular And Genetic Analysis

INTRODUCTION

Foot-and-mouth disease (FMD) is a contagious trans-boundary disease infecting cloven-hoofed animals and leads to huge economic losses [1]. FMD is caused by seven immunologically distinct serotypes, A, C, O, Asia1, South African Territories (SAT)1, SAT2 and SAT3, of FMD virus (FMDV), that belongs to the genus *Aphthovirus* of the family *Picornaviridae* [2].

FMDV is a non-enveloped virus with icosahedral symmetry. The virion is a 140S particle consisting of a single-stranded RNA genome, approximately 8500 nucleotides (nt) and nucleic capsid of 60 copies each of four structural proteins (VP1 [1D], VP2 [1B], VP3 [1C] and VP4 [1A]) [3, 4].

FMD is endemic in Africa, Asia and parts of South America and has the potential to cause sporadic outbreaks in many other parts of the world [5]. Movements of animals across international borders have been considered to be an important factor in spreading of the virus across countries [6].

Outbreaks of FMD have been reported in Egypt since 1950 [7]. Between 1964 and 2005 only serotype O was stated with the exception of 1972 when type A was introduced from sub-Saharan Africa [8]. Also, in early February 2006, another type A virus has struck animals in different localities of Egypt exerting severe economic losses [9].

During 2012, cases of FMD due to SAT 2 have been declared in domesticated livestock outside sub-Saharan Africa, which is the usual geographical range of this serotype. This expansion of outbreaks has affected countries in North Africa (Libya and Egypt) and the Middle East (Palestinian Autonomous Territories [PAT] and Bahrain) [10].

The FMD outbreaks in Egypt were officially reported by the OIE on March 2012 [11]. Initial cases were recognized in the Delta Governorates (Gharbia and Sharkia) and Alexandria and further outbreaks of disease were also suspected in Upper Egypt including Sohag, Qena and Aswan Governorates [12].

Corresponding Author: Eman S. Ahmed, Department of Virology, Animal Health Research Institute, Dokki, Giza, Egypt.
Tel: +201009447147, E-mail: vet_virology@yahoo.com.

Table 1: Sequence, virus specificity, genomic location and size of PCR amplification product of oligonucleotide primers

Primer	Sequence 5'-3'	FMD virus serotype specificity	Location	Expected size of PCR amplification product (bp)
1F	GCCTGGTCTTTCCAGGTCT	All serotypes	5' UTR	328
1R	CCAGTCCCCTTCTCAGATC		5' UTR	
SAT2 Fcl	GTAACCCGCTTTGCCATC	SAT 2	1D	288
SAT2 Rcl	CGCGTCGAATCTGTCTCTG		1D	

Virus diversity is high among SAT serotypes, especially for the SAT 2 serotype, which is composed of at least 14 topotypes [13]. Analysis of VP1 (1D) sequences shows that SAT 2 FMDVs from Libya, Egypt and PAT belong to topotype VII, whereas the FMDVs from Bahrain belong to topotype IV [10].

Understanding the epidemiology of a disease is necessary for the formulation of the most effective control strategies[14]. The aim of this study is to antigenic and molecular characterization of foot and mouth disease virus serotype Sat 2 circulating strains in the cattle and buffalo population in Egypt during 2014.

MATERIALS AND METHODS

Field Samples: 402 samples (Vesicular fluid, Epithelial tissue, myocardial tissue, tongue tissue and oral swabs) were collected from clinically infected cattle, buffalo, sheep and goat from different governorates in Egypt during 2014 (Table 1). Samples were prepared according to OIE [15].

Antigen Detection by Enzyme-linked Immunosorbent Assay: Initial screening of tissue suspensions prepared from these samples was undertaken using an antigen-detection sandwich ELISA kit (IstitutoZooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna [IZSLER], Brescia, Italy) that detects FMDV serotypes O, A, SAT 1 and SAT 2.

Virus Isolation: Positive FMDV SAT 2 samples in ELISA were subjected to isolation on BHK-21 cell line according to OIE [15].

Antigenic Characterization of Virus Isolates: Clarified viral suspensions were tested using (IZSLER, Brescia, Italy) ELISA for antigenic serotyping of virus isolates.

Molecular Detection of Virus Isolates and Positive SAT2 Antigen Detection Field Samples: RNA was extracted from infected cell culture supernatant and selected original samples by using Pathogene-spin DNA/RNA Extraction Kit (Intron biotechnology). One-step RT-PCR amplification was performed using (Thermo Scientific Verso 1-Step RT-PCR Reddy Mix Kit). Amplification condition was carried according to manufacture instructions. PCR results were analyzed by gel electrophoresis, 1.5% agarose gel containing ethidium bromide, visualized by fluorescence in UV light [16]. The primers used was those reported by Reid *et al.* [17] yielding 328 bp product of FMD viral genome. Additional conventional RT-PCR was applied using serotype-specific primers that targeting VP1 generates a 288-bp product [18] (Table 1).

Sequencing of RT-PCR Products: The target fragment of RT-PCR products was purified from gel, excess primers and unincorporated nucleotides using The Thermo Scientific GeneJET Gel Extraction Kit. Purified RT-PCR products were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit, using SAT 2 Rcl. Comparative analysis of these partial VP1 sequences for the Egyptian FMDV was carried out using available sequences using Basic Local Alignment Sequence Tool (BLAST) of the National Center for Biotechnology Information (NCBI) database.

Phylogenetic Analysis: A phylogenetic tree was performed using the Neighbor-joining (ML) method and the reliability of each tree branch was estimated by performing 1, 000 bootstrap replicates, in the MEGA 7 software [19].

RESULTS

Prevalence of SAT 2 Serotype in Different Governorates: Testing of 402 samples using Antigen-detection sandwich ELISA kit (IZSLER, Brescia, Italy) revealed 33 positive samples with FMDV serotype SAT2. Table 2 shows high occurrence rate of SAT 2 in some Upper Egypt Governorates (Asyut, Qena and Minya) and Suez Governorate.

Table 2: ELISA result of samples collected from clinically infected animals suspecting FMD

	Animal Species					Type of Sample					Results of Antigen detection by ELISA				
Governorate	Cattle	Buffalo	Sheep	Goat	Total	Swab	Epith	Myocardial tissue	Tongue	Vesicular fluid	O	A	SAT 2	-ve	% of positivity
Al Sharqia	4	1	—	—	5	1	4	—	—	—	1	—	—	4	20%
Al-Behera	26	9	5	—	40	8	22	3	5	2	6	1	2	31	22.50%
Alexandria	2	1	5	—	8	—	4	3	—	1	1	—	—	7	12.50%
Asyut	19	11	—	—	30	23	7	—	—	—	—	—	9	21	42.85%
Beni Suef	35	1	—	1	37	4	32	1	—	—	1	2	—	34	8%
Dakahlia	29	25	—	—	54	6	39	3	6	—	11	2	2	39	27.70%
Damietta	8	6	—	—	14	1	13	—	—	—	2	—	—	12	14%
Gharbia	3	5	—	—	8	4	3	1	—	—	2	—	—	6	25%
Giza	5	2	6	—	13	6	7	—	—	—	—	—	4	9	30%
Ismailia	4	7	—	—	11	—	11	—	—	—	2	—	—	9	18%
Kafr El-Sheikh	35	28	4	1	68	33	14	11	9	1	19	—	2	47	29.40%
Luxor	2	—	—	5	7	5	2	—	—	—	—	2	—	5	28%
Mersa Matruh	—	1	2	—	3	—	1	2	—	—	1	—	—	2	33.30%
Minya	19	3	7	—	29	18	10	1	—	—	6	2	3	18	38%
Monufia	7	16	—	—	23	6	14	3	—	—	10	—	—	13	43%
New Valley	—	—	4	—	4	2	2	—	—	—	—	—	—	4	—
Port Said	1	1	2	—	4	2	1	1	—	—	—	1	—	3	25%
Qalyubia	10	3	—	—	13	3	10	—	—	—	4	—	1	8	38%
Qena	7	1	—	—	8	—	8	—	—	—	—	—	6	2	75%
Sohag	7	7	—	—	14	10	—	3	—	1	1	—	1	12	14%
Suez	5	4	—	—	9	6	3	—	—	—	1	—	3	5	44.50%
Total	228	132	35	7	402	138	207	32	20	5	68	10	33	291	
Percentage	56.7%	32.8%	8.7%	1.7%		34.5%	51.5%	7.9%	4.9%	1.2%	21.4%	2.5%	8.2%	72.3%	

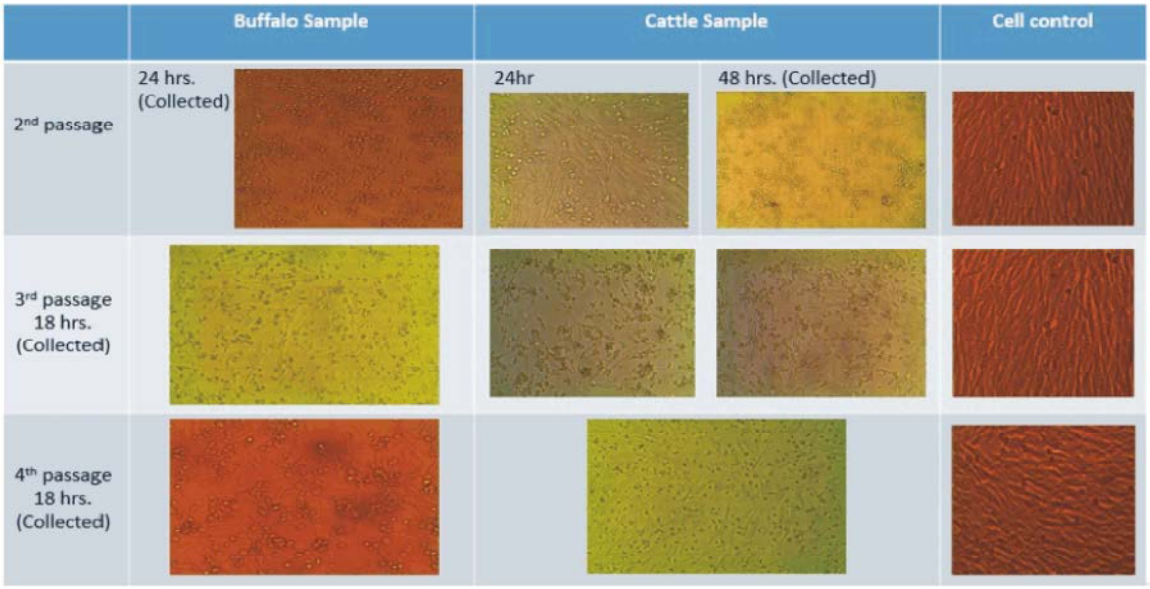

Fig. 1: Characteristic CPE of FMDV isolates post inoculation on BHK-21cells in the form of rounding, granulation and cell detachment

Virus Isolation: By propagation of 33 SAT2 positive samples by Antigen-detection sandwich ELISA on BHK-21 cell line, only two viruses (2 epithelium samples, one of buffalo from Qalyubia Governorate and one of cattle from Suez Governorate) were isolated and adapted on BHK-21 cells and CPE was obvious after 2nd passage 24 hrs. post-inoculation of the cells. Cell rounding, granulation and cell detachment were reported (Fig. 1). Two viruses were isolated.

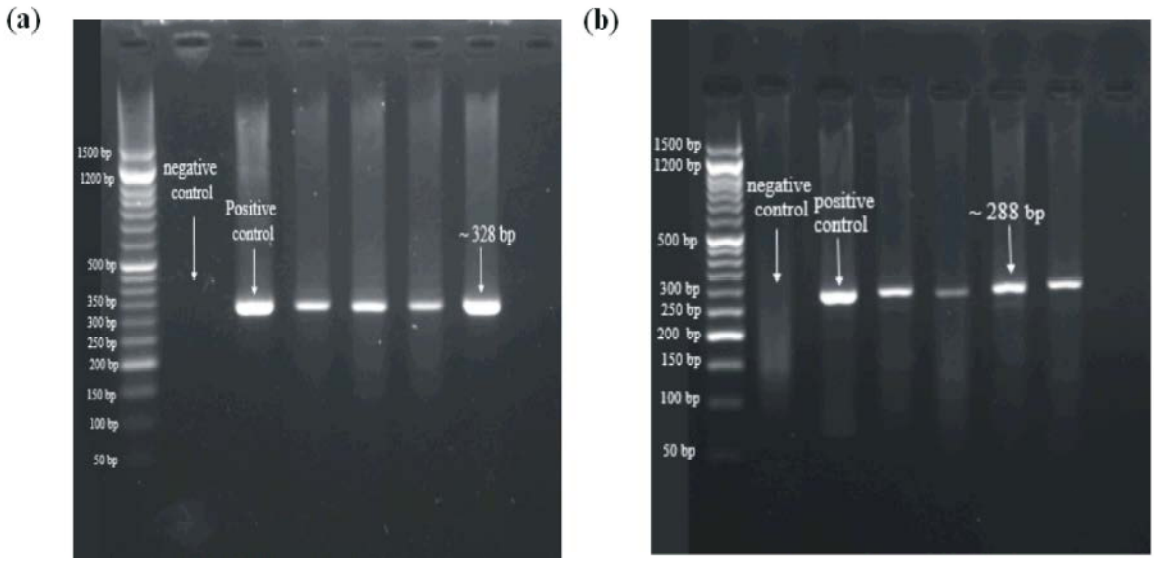

Fig. 2 (a): Ethidium bromide stained agarose gel electrophoresis of amplified products of RT-PCR using FMD universal primers. Lane 1: 50 bp DNA ladder, Lane 2: negative specimen, lane 3: positive control, lanes: 4-5-6-7 positive FMDV (~328 bp). (b) Ethidium bromide staining agarose gel electrophoresis of amplified products of RT-PCR using specific-serotype primers for FMDV-SAT 2 genome. Lane 1: 50 bp DNA ladder, lane 2: negative control, lane 3: positive control, lanes: 4-5-6-7 positive FMD-SAT 2 (~288 bp)

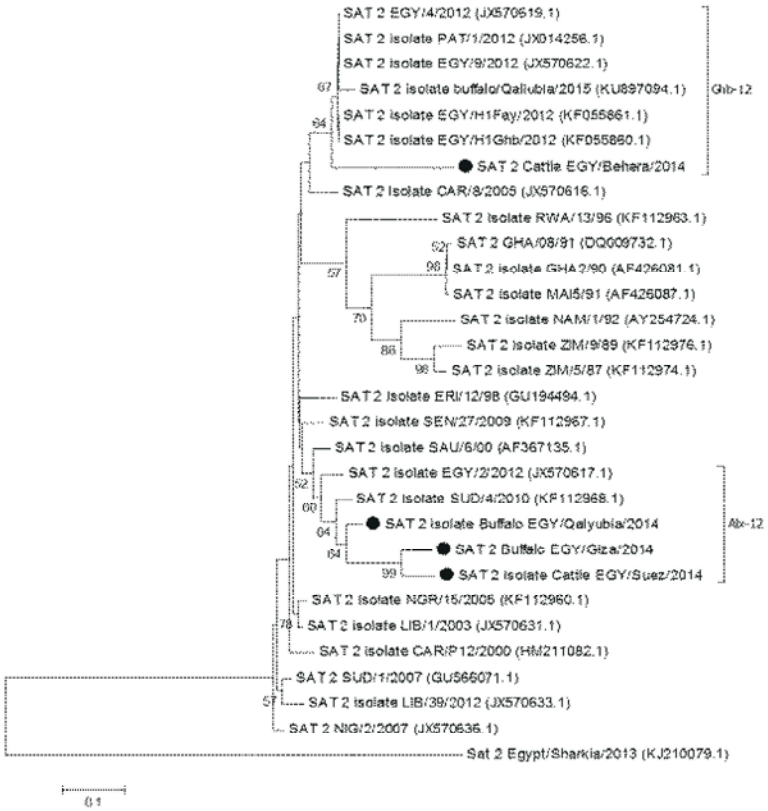

Fig. 3: Neighbor-joining phylogenetic tree based on the virus protein VP1 coding sequence, showing the relationships between the FMDV serotype SAT2 isolate from Egypt and other contemporary viruses. Bootstrap values are shown above the branches

Table 3: Nucleotide homology percent of the obtained sequence of FMD SAT2 isolates and positive field samples in the current study compared with other reference sequences

	Sequence Identity Matrix															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1		94.5	93.3	86.6	86.6	88.4	90	87.8	87.8	86	86.6	96.3	91.5	90	82	1
2	5.5		91.5	89.6	89.6	89	88	88.4	87.8	89	86.6	90.9	86	85	84	2
3	6.7	8.5		87.8	87.8	90.9	92	90.3	88.4	87.2	87.8	90.9	86.6	85	84	3
4	13.4	10.4	12.2		98.7	92.1	90	91.5	85.4	99.3	90.9	86	82.4	82	90	4
5	13.4	10.4	12.2	1.3		92.1	90	91.5	86.6	99.3	90.9	86	82.4	82	92	5
6	11.6	11	9.1	7.9	7.9		96	98.1	87.8	91.5	92.1	88.4	85.4	84	87	6
7	9.7	11.6	8.5	9.7	9.7	4.3		94.5	89	89.6	89	89	86	84	85	7
8	12.2	11.6	9.7	8.5	8.5	1.9	6		88.4	90.9	91.5	87.8	86	84	87	8
9	12.2	12.2	11.6	14.6	13.4	12.2	11	11.6		86	84.2	87.2	83	82	82	9
10	14	11	12.8	0.7	0.7	8.5	10	9.1	14		90.3	85.4	81.8	81	91	10
11	13.4	13.4	12.2	9.1	9.1	7.9	11	8.5	15.8	9.7		87.2	84.2	84	87	11
12	3.7	9.1	9.1	14	14	11.6	11	12.2	12.8	14.6	12.8		93.3	92	83	12
13	8.5	14	13.4	17.6	17.6	14.6	14	14	17	18.2	15.8	6.7		93	81	13
14	9.7	15.2	15.2	18.2	18.2	16.4	16	15.8	18.2	18.8	15.8	7.9	7.3		82	14
15	18.2	15.8	16.4	9.7	8.5	12.8	15	13.4	17.6	9.1	13.4	17	18.8	18		15

1	SAT 2 isolate SUD/4/2010 (KF112968.1)	6	SAT 2 isolate LIB/1/2003 (JX570631.1)	11	SAT 2 isolate CAR/1/2005 (JX570615.1)
2	SAT 2 isolate EGY/2/2012 (JX570617.1)	7	SAT 2 isolate SEN/27/2009 (KF112967.1)	12	SAT2 isolate Buffalo Egy/Qalyubia/2014
3	SAT 2 isolate SAU/6/00 (AY297948.1)	8	SAT 2 isolate NGR/15/2005 (KF112960.1)	13	SAT2 isolate Cattle Egy/Suez/2014
4	SAT 2 isolate EGY/H1Ghb/2012 (KF055860.1)	9	SAT 2 isolate ERI/12/98 (GU194494.1)	14	SAT2 Buffalo Egy/Giza/2014
5	SAT 2 isolate EGY/9/2012 (JX570622.1)	10	SAT 2 isolate PAT/1/2012 (JX014256.1)	15	SAT2 Cattle Egy/Behera/2014

Molecular Characterization of Selected Samples: Amplification assay revealed positive results with the expected correct bands. Figure 2 a and b show the obtained RT-PCR bands confirming the characterization of FMD in the cells as well as the serotyping results of isolated viruses (Fig. 2).

Sequence and Phylogenetic Analysis: A phylogenetic tree was constructed based on the sequence alignment of VP1 gene showing our isolated viruses clustered with the topotype VII strains isolated from Saudi Arabia, Sudan, Libya, Eritrea, Cameroon and also other strains isolated from Egypt during outbreaks occurred in 2012 (Fig. 3).

Identity percent between the selected viruses in this study along with some Egyptian and references SAT 2 viruses were presented in (Table 3). The similarity percent was ranged from 81% to 98%. Three samples found to be similar to Alx-12 lineage of SAT 2 (SAT2 isolate Buffalo Egy/Qalyubia/2014 exhibits 96% identity with SAT2/SUD/4/2010 (KF112968.1) and 91% identity with SAT2/EGY/2/2012 (JX570617.1), SAT2 isolate Cattle Egy/Suez/2014 exhibits 91.5% identity with SAT2/EGY/2/2012 (JX570617.1) and SAT2 Buffalo Egy/Giza/2014exhibits 90.3% with SAT2/SUD/4/2010 (KF112968.1)) whereas SAT2 Cattle Egy/Behera/2014 was similar to Ghb-12 lineage of SAT 2 in percent of identity 91.5% with SAT 2 EGY/9/2012 (JX570622.1) and 91% with SAT 2/PAT/1/2012 (JX014256.1).

DISCUSSION

In this study, representative samples of FMD suspected cases collected from distinct outbreaks during 2014 were tested by antigen ELISA reporting the prevalence of SAT 2 serotype (Table 1). Upper Egypt governorates are most affected which may be due to the long-range airborne transmission nature of FMDV allowing virus traveling over wide-ranging distances causing incursions at previously virus-free premises [20]. Moreover, the importation of live animals from FMDV SAT 2 endemic countries including carrier stage animals can be considered as a contributor in spreading of the virus within quarantine area such as Suez where animals are isolated before introducing into Egyptian market. Having said that, the role of carrier animals in the spread of virus in the field is still argumentative [21].

Around 50% of collected samples were epithelium from unruptured or freshly ruptured vesicles or vesicular fluid (The preferred tissue for diagnosis). Myocardial tissue can be submitted from fatal cases [15]. Oral swabs are sample of choice for virus isolation due to high virus titer.

Trails for isolation on BHK-21 cell line from 33 SAT 2 positive samples (by ELISA) revealed only two viruses. BHK-21 (baby hamster kidney) and others established cell lines such as IB-RS-2 cells (pig kidney cells) are 10 x less sensitive, in detection of low amounts of infectivity, than

primary cultures of bovine thyroid and porcine, calf, or ovine kidney [15, 22]. However, the production of consistent quality, ready for- use primary cells is laborious, time-consuming and expensive [23].

Employing RT-PCR in the present study was based on the amplification of 328 bp of 5'UTR in the positive samples to detect all of seven FMDV serotypes [17].

To confirm the amplification of serotype SAT 2, serotype specific primer set to amplify 288 bp of VP1 gene were utilized [18].

Nucleotide sequence data are commonly used for phylogenetic analysis to pursue transboundary movements of FMDV and can also be used to characterize antigenic determinants of the virus. Additionally, sequence data were also used to develop a sensitive and specific molecular assay to detect FMDV from affected animals [12].

During FMDV SAT 2 outbreaks in 2012, phylogenetic analysis based on the nucleotide sequence of highly variable VP1 describes the genetic relationship between Egyptian isolates and FMDV isolates from neighboring and sub-Saharan African countries showing that Egyptian SAT2 isolates were closely related to each other and previously characterized FMDVs from the Kingdom of Saudi Arabia, Sudan, Libya, Eritrea and Cameroon and all belonged to topotype VII. This signposts that topotype VII has been circulating in the district for at least 14 years and the virus is likely to have been maintained in sheep or goats, species in which clinical disease is less likely to be apparent [24, 25].

Two lineages (designated as SAT 2/VII/Ghb-12, which was found in the Egyptian Governorates of Faiyum, Gharbia, Giza, Kafr el-Sheikh, Minya, Monufia, Qalyubia, Suez and more recently in PAT and SAT 2/VII/Alx-12, which was identified in Alexandria Governorate) were included in this topotype. Across VP1, these two lineages diverge by almost 10% (64–66 substitutions) at the nt level demonstrating that though they share a close evolutionary history, they are not directly related to each other which suggesting that either two independent introductions or a single introduction of two lineages have arisen [12].

The source of Ghb-12 lineage of the 2012 Egypt/Palestine outbreak was most likely Cameroon whereas Alx-12 lineage seemed most probable to be descending from a Sudanese isolate. In addition, it was also closely related to the virus from 2000 in Saudi Arabia. This does not exclude the incursions being because of the import of the same flock of infected animals from Sudan, since the Ghb-12 lineage, originating in Cameroon, may have traveled east on its route to Egypt [24].

In the present study, comparison of VP1-based sequence and phylogenetic analysis show that two lineages (SAT 2/VII/Ghb-12 and SAT 2/VII/Alx-12) of FMDV SAT 2 have been circulating in Egypt since 2012 and it may be in order that serotype SAT 2 became endemic. However, counter to the defined viruses in 2012 the occurrence rate (Incidence) of SAT 2/VII/Alx-12 is greater than SAT 2/VII/Ghb-12. This is may be explained by repeated introduction of virus, especially that phylogenetic analysis of SAT 2/VII/Alx-12 characterized in this study was similar to those of 2012 showing that it is closely related to both SUD/4/2010 (KF112968.1) and SAU/6/00 (AY297948.1).

Collectively, from the obtained results we recommend strict quarantine measures for importing of live animals from FMDV endemic countries to prevent introduction of other FMDV serotype or strains besides that, in case of inadequate epidemiological statistics about the existing circulating FMD viruses in the neighboring countries, molecular epidemiological studies must be frequently applied not only in order to trace and classify existing viruses from recent outbreaks, but also to monitor evolutionary rates of the circulating viruses which allow Egyptian authorities to expect the breaking point (Threshold) at which evolving of new strain is expected as according to Klein [26]molecular epidemiology develops speculations about developmental variations considering altered selection pressures, caused by the host's immune system and new environmental challenges.

CONCLUSION

Overall, FMDV SAT 2 was isolated from animal population in Egypt during 2014. RT-PCR, nucleotide sequence and phylogenetic analysis indicating that SAT 2 virus topotype VII, two lineages SAT 2/VII/Ghb-12 and SAT 2/VII/Alx-12, is circulating in Egypt since its incursion in 2012.

REFERENCES

1. Knowles, N.J. and A.R. Samuel, 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res., 91: 65-80.
2. ICTV, 2012. Viral taxonomy: 2011 Release. http://ictvonline.org/virusTaxonomy.asp?version=2011.
3. Rueckert, R.R. and E. Wimmer. 1984. Systematic nomenclature of picornavirus proteins. J. Virol., 50: 957-959.

4. Consuelo Carrillo, 2012. Foot and Mouth Disease Virus Genome. In Viral Genomes - Molecular Structure, Diversity, Gene Expression Mechanisms and Host-Virus Interactions, Prof. Maria Garcia (Ed.), ISBN: 978-953-51-0098-0, InTech publisher, pp: 53-68, Available from: http://www.intechopen.com/ books/viral-genomes-molecular-structurediversity-gene-expression-mechanisms-and-host-virus-interactions/meaningful-genomics-of-fmdv.
5. Thompson, J.D., P. Muriel, D. Russell, P. Osborne, A. Bromley, M. Rowland, S. Creigh-Tyte and C. Brown, 2002. Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. Rev. Sci. Tech., 21: 675-687.
6. Abdul-Hamid, N.F., N.M. Hussein, J. Wadsworth, A.D. Radford, N.J. Knowles and D.P. King, 2011. Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. Infect Genet Evol., 11: 320-328.
7. Aidaros, H.A., 2002. Regional status and approaches to control and eradication of foot and mouth disease in the Middle East and North Africa. Rev. Sci. Tech. off IntEpiz., 21: 451-458.
8. Knowles, N.J., J. Wadsworth, S.M. Reid, K.G. Swabey, A.A. El-Kholy, A.O. Abd El-Rahman, H.M. Soliman, K. Ebert, N.P. Ferris, G.H. Hutchings, R.J. Statham, D.P. King and D.J. Paton, 2007. Foot-and-mouth disease virus serotype A in Egypt. Emerg Infect Dis; 13: 1593-6 [available at http://www.Ncbi.nlm.nih.Gov/ pmc/ rticles/PMC2851527].
9. El-Kholy, A.A., H.M.T. Soliman, N.A. Helmy and A.O. Abdel Rahman, 2007. Genetic identification of the foot-and-mouth disease virus caused 2006 outbreak in Egypt. Arab J. Biotech., 10: 193-206.
10. Valdazo- González, B., N. J. Knowles, J. Hammond and D.P. King, 2012. Genome Sequences of SAT 2 Foot-and-Mouth Disease Viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip) Journal of Virology, 86: 8901-8902.
11. Shawky, M., M. Abd El-Aty, H.M. Fakry, H.M. Daoud, I.E. El-Sayed, G.W. Mossad, S.A. Rizk, H. Abu-Elnaga, A. A. Mohamed, A. Abd El-.kreem and E.M. Farouk, 2013. Isolation and Molecular Characterization of Foot and Mouth Disease SAT2 Virus during Outbreak 2012 in Egypt J. Vet. Adv., 3: 60-68
12. Ahmed, H.A., S.A. Salem, A.R. Habashi, A.A. Arafa, M.G. Aggour, G.H. Salem, A.S. Gaber, O. Selem, S.H. Abdelkader, N.J. Knowles, M. Madi, B. Valdazo-González, J. Wadsworth, G.H. Hutchings, V. Mioulet, J.M. Hammond and D.P. King, 2012. Emergence of foot-and-mouth disease virus SAT 2 in Egypt during 2012. Transbound. Emerg. Dis., 59: 476-481.
13. Bastos, A.D., D.T. Haydon, O. Sangaré, C.I. Boshoff, J.L. Edrich and G.R. Thomson, 2003. The implications of virus diversity within the SAT2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. J. Gen. Virol., 84: 1595-1606.
14. Samuel, A.R. and N.J. Knowles, 2000. Foot-and mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). Journal of General Virology (2001), 82: 609-621.
15. OIE (Office International des Epizooties/World Organization for Animal Health). 2012. Chapter 2.1.5. - Footand mouth disease.
16. Ireland, D.C. and Y.S. Binepal, 1998. Improved detection of Capripoxvirus in biopsy samples by PCR. J. Virol. Methods, 74: 1-7.
17. Reid, S.M., N.P. Ferris, G.H. Hutchings, A.R. Samuel and N.J. Knowles, 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J. Virol. Methods, 89: 167-176.
18. Recherche du virus de la fièvreaphteuse par détection de l'ARNgénomique par RT-PCR classique. Atelier De Formation Sur Le Diagnosticde La Fievre Aphteuse, 21-25 mai 2012.ANSES – Laboratoire de Santé Animale de Maisons-Alfort Laboratoire National de RéférenceFièvreAphteuse.
19. Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol., 33: 1870-1874.
20. Alexandersen, S., Z. Zhang, A.I. Donaldson and A.J. Garland, 2003. The pathogenesis and diagnosis of foot-and-mouth disease. J. Comp. Patho, l. 129: 1-36.
21. Dawe, P.S., F.O. Flanagan, R.L. Madekurozwa, K.J. Sorensen, E.C. Anderson, C.M. Foggin, N.P. Ferris and N.J. Knowles, 1994. Natural transmission of foot-and-mouth disease virus from African buffalo (Syncerus caffer) to cattle in a wildlife area of Zimbabwe. Vet. Rec., 134: 230-232.

22. MacLachlan, N.J. and E.J. Dubovi, 2011. Fenner's Veterinary Virology, Fourth Edition Chapter, 26: 434.
23. Jamal, S.M. and G.J. Belsham, 2013. Foot-and-mouth disease: past, present and future. Veterinary Research, 44: 116.
24. Hall, M.D., N.J. Knowles, J. Wadsworth, A. Rambaut and M.E.J. Woolhouse, 2013. Reconstructing Geographical Movements and Host Species Transitions of Foot-and-Mouth Disease Virus Serotype SAT 2.mBio, 4: e00591-1.
25. Kandeil, A., R. El-Shesheny, G. Kayali, M. Moatasim, O. Bagato, M. Darwish, A. Gaffar, A. Younes, T. Farag, M.A. Kutkat and M.A. Ali, 2013. Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch.Virol., 158: 619- 627.
26. Klein, J., 2009. Understanding the molecular epidemiology of foot-and-mouth-disease virus. Infection, Genetics and Evolution, 9: 153-161.