

## Molecular Characterization of Foot-and-Mouth Disease Virus Collected from Al-Fayoum and Beni- Suf Governorates in Egypt

<sup>1</sup>M.K. EL-Bayoumy, <sup>1</sup>K.A. Abdelrahman, <sup>1</sup>A.M. Allam,  
<sup>1</sup>T.K. Farag, <sup>1</sup>Hala A.A. Abou-Zeina and <sup>2</sup>M.A. Kutkat

<sup>1</sup>Department of Parasitology and Animal Diseases, National Research Center, Giza, Egypt

<sup>2</sup>Department of Poultry Diseases, National Research Center, Giza, Egypt

**Abstract:** Foot-and-mouth Disease (FMD) is a serious contagious viral disease principally affecting cloven hoofed animals. Although serotypes A and O of Foot-and-mouth disease Virus (FMDV) has been continuously circulating in Egypt, during 2012, cases of FMD-SAT 2 have been reported in domesticated livestock. This study was conducted on cattle and sheep suspected of being infected with FMD raised in AL-Fayoum and Beni-Suef Governorates respectively. Samples from sheep were collected during FMD outbreak in 2012, while a spot infection of cattle occurred nine months later. Results of (IDEXX FMD 3ABC Bo-Ov) ELISA revealed infection with FMDV in 100% of tested animals. The objective of this study is to determine genetic characterization of isolated FMD serotypes. Epithelial specimens were collected and analyzed using universal primers to amplify the 5' untranslated region (UTR) by reverse-transcription polymerase chain reaction (RT-PCR) and then O, A, C and SAT 2 primers for serotyping. RT-PCR and sequencing proved that cattle were infected with mixed infection of both types A and SAT 2, while sheep was infected with type SAT 2 only. Phylogenetic analysis of VP3 and VP1 to characterize biodiversity revealed close relation to previously recorded sequences in Egypt.

**Key words:** Cattle • 3ABC Elisa • RT-PCR • Foot-and-Mouth Disease Virus & Sheep

### INTRODUCTION

Foot-and-mouth disease (FMD) is a contagious trans-boundary disease infecting cloven hoofed animals and leads to huge economic losses. It is caused by 7 immunologically distinct serotypes, O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2 and SAT 3. Several of these serotypes circulate currently or periodically in the Middle East and North Africa [1]. It is characterized by fever, lameness and vesicular lesions of the feet, tongue, snout and teats. These debilitating effects, rather than high mortality rates, are responsible for severe productivity losses [2, 3]. FMD is also enzootic in many developing regions of Asia, Africa and South America that lack the adequate veterinary services and resources necessary to undertake control and eradication efforts [4, 5]. Animal movements across international borders have been known to be an important factor in spreading of the virus across countries [6], trade restrictions on animals

and animal products have failed to prevent incursions of FMDV from enzootic hot spots into FMD free areas and hemispheric eradication efforts have met with limited success [7-9]. FMD is enzootic in Egypt and outbreaks have been reported since 1950 [10]. The FMD outbreaks in Egypt were officially reported by the OIE on March 2012. Many outbreaks were recorded in different governorates mainly in the Delta area and few along the Nile in the southern parts of the country. The affected species include cattle and buffalo, where young buffaloes appeared to be the category of animals more severely affected [11].

Sheep and goats have generally been neglected with regard to their epidemiological role in the spread of FMD [12], Sheep played a major role in the 2001 UK FMD epidemic and it was important to obtain virological and aerobiological data for that species infected with the UK strain of virus [13, 14]. Clinical signs of FMD in sheep are frequently mild or not apparent [15]. But while sheep may

not manifest clear clinical signs of FMD, they can secrete and excrete considerable amounts of FMDV and therefore may play a significant role in FMDV transmission [16-18]. Clinical disease in sheep is characterized by lesions on the feet and mouth, fever and viremia. It has been reported, however, that up to 25% of infected sheep may fail to develop lesions and an additional 20% may form only one lesion [19, 20].

Nucleotide sequencing was first used for the study of the epidemiology of FMD by Beck and Strohmaier [21] who investigated the origin of outbreaks of types O and A in Europe over a 20 years period. Since then a number of similar studies have been published. Understanding the epidemiology of a disease is essential for the formulation of the most effective control strategies. Determining the source of outbreaks is an important element of epidemiological investigations and for FMD this can be done by nucleotide sequencing [22].

The aim of this study is to determine molecular identification and genetic analysis of the causative strains of FMDV in infected cattle and sheep at AL-Fayoum and Beni-Suef Governorates during and after FMD outbreak of 2012 in Egypt.

## MATERIALS AND METHODS

**Study Design:** Oral epithelium tissue suspension & swabs were collected from clinically infected cattle and sheep for detection and molecular identification of FMDV serotypes. Seventeen (Friesian-Holstein) cattle at AL-Fayoum Governorate, Egypt and twenty local breed (Osseimi) sheep at Beni-Suef Governorate, Egypt suffering from clinical signs of FMD Virus were used. Samples from sheep were collected during the FMD outbreak from March to May 2012, while samples from cattle were collected from severely infected farm at January 2013.

**Detection of Nonstructural Protein 3ABC by ELISA:** Blood samples from each animal were collected and stored at -80°C according to Edwards *et al.* [23]. Serum samples from bovine and ovine origin were used to monitor antibody against nonstructural polyprotein (NSP) 3ABC of FMD antigen using ELISA (IDEXX FMD 3ABC Bo-Ov), it was performed in accordance to the manufacturers' instructions. According to the ELISA test kit manual, samples with percentage values >30% were considered positive, <20% as negative and samples between 20% and 30% were considered suspicious.

**Molecular Serotyping of FMD Virus:** According to Kitching and Donaldson [24] oral swabs and epithelial samples were maintained in transport medium containing equal volumes of glycerol and phosphate-buffered saline (pH 7.2-7.6) containing 2 % antibiotic-antimycotic (BioWhittaker, Walkersville, MD). Using a QIAamp Viral RNA Extraction Kit (QIAGEN, Germany), viral RNA was extracted from 140 µL of each of the collected samples; in accordance to the manufacturers' instructions. The extracted RNA was subjected to RT-PCR using universal primers 1F/1R (10 pM for each) [25]. RT-PCR was performed using a (Thermo Scientific Verso 1-Step RT-PCR Reddy Mix Kit) with an initial 30-min incubation at 50°C; followed by 95°C for 15 min; 35 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 90 s; and a final 10-min incubation at 72°C. PCR results were then analyzed by gel electrophoresis according to the method described by Ireland and Binopal [26]. These validated 1F/1R primers "Table 1" generate a 328-bp product regardless of serotype. RT-PCR was performed on FMD positive samples using the conditions described above for detection of serotypes A, C, O and SAT using the serotype-specific diagnosis primers in "Table 1", [27].

**Sequencing of the PCR Products:** PCR products were separated from agarose gel, excess primers and unincorporated nucleotides using the MinElute gel extraction kit (QIAGEN, Hilden, Germany) as per manufacturer's instructions and then sequenced in a commercial sequencing laboratory (Macrogen - Korea).

**Phylogenetic Analysis:** Obtained sequences were subjected to Basic Local Alignment Search Tool (BLAST) analysis to search the GenBank for homologous nucleotide sequences and to determine whether the sequences were of host or parasite origin [28]. Sequences were downloaded from GenBank for the phylogenetic analysis, multiple sequence alignments were generated using the default settings of Clustal W [29]. A phylogenetic tree was performed using the Maximum likelihood (ML) method, in the MEGA6 software [30].

## RESULTS

Clinical symptoms of FMD suspected animals started with high fever, lameness, anorexia, loss of general health condition, Vesicles and / or ulcers on the dental pad and dorsum of the tongue and foot lesions along the coronary

Table 1: Oligonucleotide primers used for RT-PCR

Primer name	Serotype	Primer sequence (5' to 3')	Region	Expected size (bp)
1F	All 7 serotypes	GCC TGG TCT TTC CAG GTC T	5' UTR	328
1R		CCA GTC CCC TTC TCA GAT C	5' UTR	
ARS4	O	ACC AAC CTC CTT GAT GTG GCT	VP3	1,301
R-NK61		GAC ATG TCC TCC TGC ATC TG	2B	
A-1C562	A	TAC CAA ATT ACA CAC GGG AA	VP3	863-866
R-NK61		GAC ATG TCC TCC TGC ATC TG	2B	
C-1C536	C	TAC AGG GAT GGG TCT GTG TGT ACC	VP3	833-877
R-NK61		GAC ATG TCC TCC TGC ATC TG	2B	
1D209F	SAT	CCA CAT ACT ACT TTT GTG ACC TGG A	VP1	715-730
2B208R		ACA GCG GCC ATG CAC GAC AG	2B	

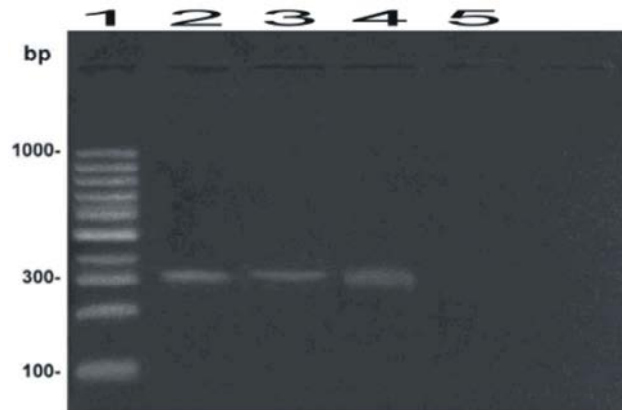


Photo 1: Detection of FMDV genome by RT-PCR using FMD universal primers: lane 1: 100 bp DNA ladder, lanes: 2, 3 & 4: positive FMDV isolates (~328 bp), Lane 5: negative specimen

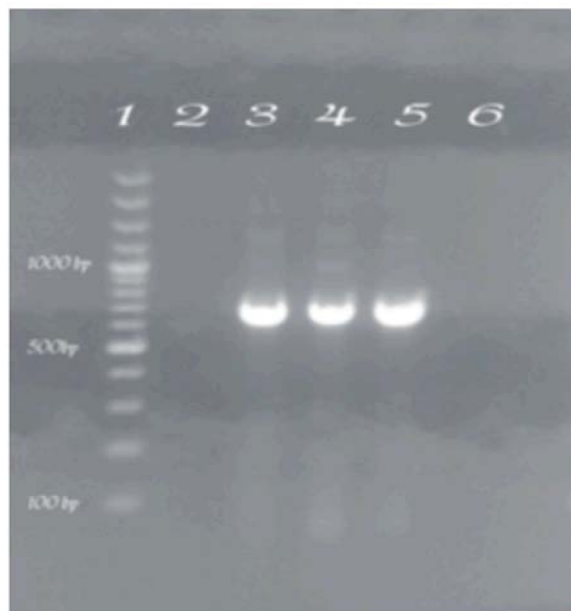


Photo 2: Detection of FMD-SAT genome using RT-PCR. lanes1: 100 bp DNA ladder, lane 2: negative specimen, lanes 3, 4 & 5: positive FMD-SAT isolates (~715-730 bp)

band and inter digital space. Infected animals usually separate itself from the rest of the flock. ELISA (IDEXX FMD 3ABC) results revealed infection with

FMDV with high antibody titers against (NSP) 3ABC of FMD antigen in 100% of the tested animals (cattle and sheep).

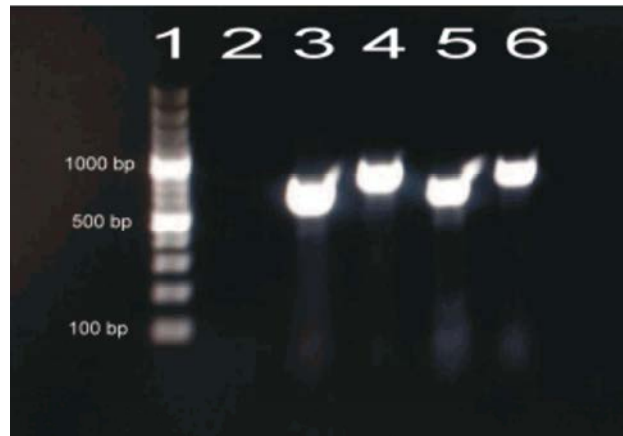


Photo 3: Detection of FMD-SAT & FMDV-A genomes using RT-PCR. lane1: 100 bp DNA ladder, lane 2: negative specimen, lanes 3 & 5: positive FMD-SAT isolates (~715-730 bp), lanes 4 & 6: positive FMDV-A isolates (~863-866bp)

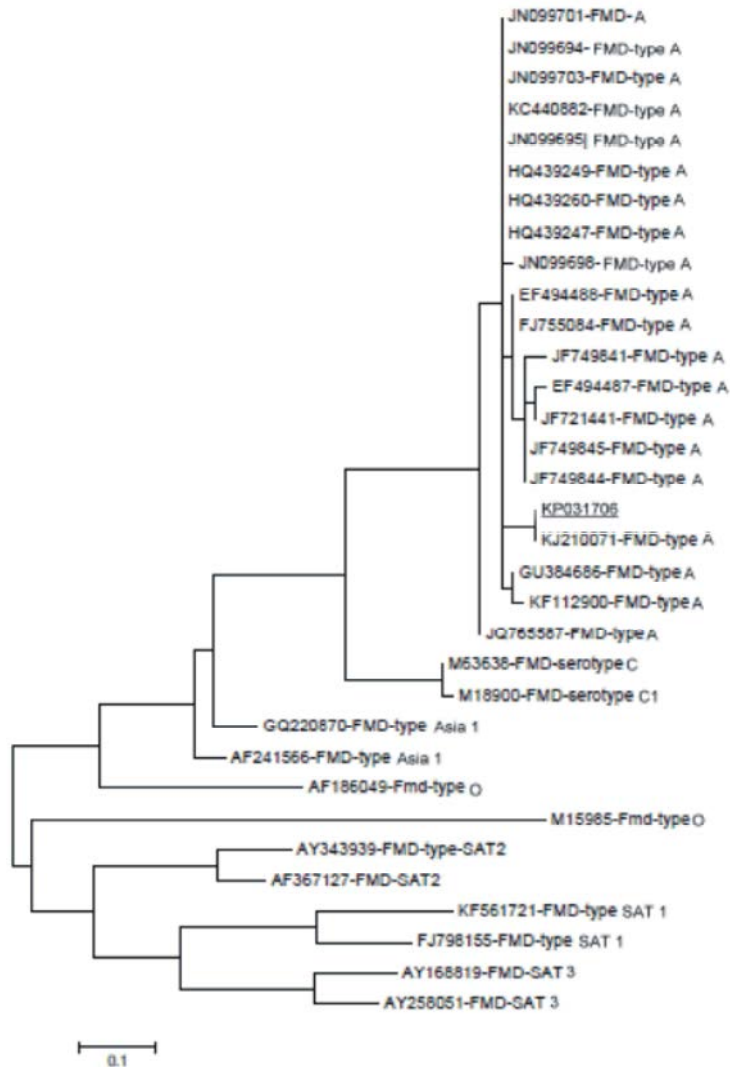


Fig. 1: Molecular Phylogenetic analysis of FM- strain "A" by Maximum Likelihood method

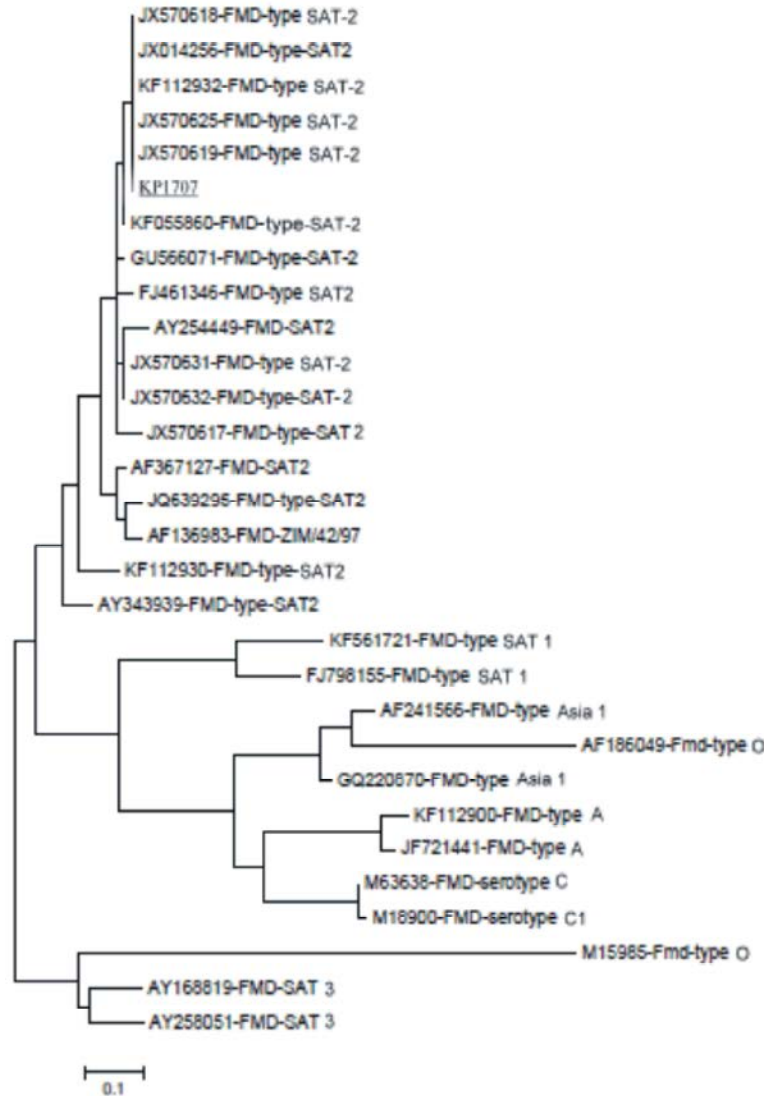


Fig. 2: Molecular Phylogenetic analysis of FM- strain "SAT 2" by Maximum Likelihood method

RT-PCR was run on samples collected from clinically infected animals using the universal primers of FMDV. A total of "37" epithelial samples collected, "20" and "17" samples from sheep and cattle respectively, 35 (89.2 %) of all animals tested positive to universal FMDV, the amplification products were identified at the expected positions of 328 bp in cattle (Photo 1) and as previously recorded in sheep by EL-Bayoumy *et al* [31]. Typing of FMDV-positive samples using serotype specific primers for A, O, C and SAT indicated that all FMDV-positive samples from sheep at Beni-Suef Governorate were of the SAT serotype, the amplification products were identified at the expected positions 715-730 bp for SAT (Photo 2), FMDV-positive samples from cattle at AL-Fayoum

Governorate were mostly of the A serotypes "twelve animals" and some of SAT serotype "three animals" (Photo 3). Sequencing of amplified products demonstrated that the FMDV was of the A and SAT 2 types in samples collected from cattle and of SAT 2 from samples collected from sheep.

The GenBank accession numbers in bankit for nucleotide sequences are: KP031706 and KP031707 for FMD-A and FMD-SAT 2 respectively. A phylogenetic tree was constructed based on the sequence alignment of VP3 gene of FMDV including sequence "KP031706" is illustrated in (Fig. 1) and phylogenetic tree based on the sequence alignment of VP1 gene including sequence "KP031707" is illustrated in (Fig. 2).

## **DISCUSSION**

Due to low meat production in Egypt, import of live animals especially from Sudan and Ethiopia increased, according to the Egyptian Ministry of Agriculture, from January 1 to March 8, 2012, Egypt imported 15,088 head of cattle from different suppliers (Hungary, Croatia, Ethiopia, Sudan and Brazil). Since Egyptian cattle, buffalo and sheep had not been previously exposed to or vaccinated against FMDV-SAT2, the case fatality rate associated with the newly emerged virus reached 20 % [32]. Between 1964 and 2005, only serotype "O" was reported in Egypt, with the exception of 1972 when type "A" was introduced from Sub-Saharan Africa. In 2006, a novel type "A" strain entered Egypt and rapidly spread throughout the ruminant population, causing severe losses [33]. However, serotype O has been the predominant type where type A has been controlled by vaccination [34]. During 2012, there has been a dramatic upsurge in FMD SAT-2 outbreaks in Egypt. Cattle, water buffalo and small ruminants were affected with severe clinical signs particularly in young animals with a mortality rate of up to 50%, approximately 40,000 cattle and water buffaloes were affected and more than 4,600 animals were killed; only FMDV-SAT 2 was detected, with an overall detection rate of 80.3 % [35, 36]. The clinical disease varies with the species, breed of the animal affected and serotype and strain of FMDV [1].

Comparison of whole genome sequences can provide further discrimination between closely related viruses and help to recreate the transmission pathways between farms within outbreaks [37]. In this study, we reported the genetic characterization of the VP1 gene of FMD-SAT 2 isolates from sheep at Beni-Suef Governorate during extensive outbreak in Egypt 2012 and the VP3 gene of FMD-A from an FMD outbreak in individual farm of Friesian-Holstein cattle at AL-Fayoum Governorate 9 months later. Sequence comparison of isolated strain "A" showed that the nucleotide sequence of those specimens were 100 % identical to FMD-A" isolated from AL-Fayoum Governorate "KJ210071" and exhibited 96 % identity to FMD Outbreak in Iraq 2009 which was a recombinant virus of previously circulating serotypes A/Pak and O/Pak, from Pakistan and Afghanistan during 2002-2009 "HQ439247" and from Egypt between 2011-12 "KC440882". Sequence comparison of isolated strain "SAT 2" showed that the nucleotide sequence of those specimens were 100 % identical to FMD-SAT 2 isolated from Egypt in the outbreak of 2012 "JX013978", "JX570618" and 2013 "KF112932".

The susceptibility of sheep to FMD may vary with the breed of animal and strain of virus [15]; it may play an important role in spreading of FMDV by harboring acute infection without manifesting substantial clinical signs of disease. The disease in sheep is generally mild and can be difficult to distinguish from other common conditions [17]. It is difficult to determine the exact source of the newly emerging virus in Egypt due to the fact that there are no strict measures that prevent animal movements and also due to a lack of comprehensive animal-movement records in sub-Saharan Africa [38].

In conclusion, RT-PCR assays coupled to nucleotide sequencing were validated for identification of the causative agent of the FMD spots in Egypt. We should encourage communication, awareness, more involvement of the private sector and continuous strict control of risk high spots and movement of animals. However, the role of sheep in transmission of FMDV shouldn't be neglected and more epidemiological work with the neighboring Middle East and African countries should be done in order to obstruct crossborder transmission of the disease.

## **ACKNOWLEDGMENT**

Authors acknowledge and thank Dr. Hatem Soliman Toughan, Fish Medicine and Managements-Department of Animal Medicine-Faculty of Veterinary Medicine-University of Assiut, for his excellent suggestions during the writing of the manuscript.

## **REFERENCES**

1. Knowles N.J. and A.R. Samuel, 2003. Molecular epidemiology of foot-and-mouth disease virus. *Virus Res.*, 91: 65-80.
2. James, A.D. and J. Rushton, 2002. The economics of foot and mouth disease. *Rev Sci Tech Off Int Epiz* 21(3): 637-644.
3. Arzt, J., N. Juleff, Z. Zhang and L.L. Rodriguez 2011. The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. *Transbound Emerg Dis.*, 58: 291-304.
4. Sumption, K., M. Rweyemamu and W. Wint, 2008. Incidence and distribution of foot-and-mouth disease in Asia, Africa and South America; combining expert opinion, official disease information and livestock populations to assist risk assessment. *Transbound Emerg Dis.*, 55: 5-13.

5. Rodriguez, L.L. and M.J. Grubman, 2009. Foot and mouth disease virus vaccines. *Vaccine*, 27(Suppl 4): D90-D94.
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. Rweyemamu, M., P. Roeder, D. MacKay, K. Sumption, J. Brownlie and Y. Leforban, 2008. Planning for the progressive control of foot-and-mouth disease worldwide. *Transbound Emerg Dis.*, 55: 73-87.
8. Valarcher, J.F., Y. Leforban, M. Rweyemamu, P.L. Roeder, G. Gerbier, D.K. Mackay, K.J. Sumption, D.J. Paton and N.J. Knowles, 2008. Incursions of foot-and-mouth disease virus into Europe between 1985 and 2006. *Transbound Emerg Dis.*, 55: 14-34.
9. Loth, L., M.G. Osmani, M.A. Kalam, R.K. Chakraborty, J. Wadsworth, N.J. Knowles, J.M. Hammond and C. Benigno, 2011. Molecular characterization of foot-and-mouth disease virus: implications for disease control in Bangladesh. *Transbound Emerg Dis.*, 58: 240-246.
10. 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 Int Epiz.*, 21(3): 451-458.
11. Shawky, M., M. Abd El-Aty, Hiam. M. Fakry, Hind M. Daoud, I. Ehab El-Sayed, G. Wael Mossad, Sonia 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(2): 60-68.
12. Patil, P.K., J. Bayry, C. Ramakrishna, B. Hugar, L.D. Misra, K. Prabhudas and C. Natarajan, 2002. Immune responses of sheep to quadrivalent double emulsion foot-and-mouth disease vaccines: rate of development of immunity and variations among other ruminants. *J. Clin Microbiol.*, 40(11): 4367-71.
13. Gibbens, J.C., C.E. Sharpe, J.W. Wilesmith, L.M. Mansley, E. Michalopoulou, J.B. Ryan and M. Hudson, 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. *Vet. Rec.*, 149: 729-743.
14. Alexandersen, S., Z. Zhang, S.M. Reid, G.H. Hutchings and A.I. Donaldson, 2002. Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. *Journal of General Virology*, 83: 1915-1923).
15. Kitching, R.P. and G.J. Hughes, 2002. Clinical variation in foot and mouth disease: sheep and goats. *Rev. Sci. Tech*, 21: 505-512.
16. Burrows, R., 1968. The persistence of foot-and-mouth disease virus in sheep. *J. Hyg (Lond)*, 66: 633-640.
17. Donaldson, A.I. and R.F. Sellers, 2000 Foot-and-mouth disease. In *Diseases of Sheep*. 3<sup>rd</sup> edition. Edited by Martin WB, Aitken ID. Oxford: Blackwell Science, pp: 254-258.
18. De Bravo Rueda, C., A. Dekker, P.L. Eblé and M.C.M. De Jong, 2014. Identification of factors associated with increased excretion of foot-and-mouth disease virus. *Prev. Vet. Med.*, 113: 23-33.
19. Gibson, C. F., A.I. Donaldson and N.P. Ferris, 1984. Response of sheep vaccinated with large doses of vaccine to challenge by airborne foot-and-mouth disease virus. *Vaccine*, 2: 157-161.
20. Hughes, G.J., V. Mioulet, R.P. Kitching, M.E. Woolhouse, S. Alexandersen and A.I. Donaldson, 2002. Foot-and-mouth disease virus infection of sheep: implications for diagnosis and control. *Vet. Rec.*, 150: 724-727.
21. Beck, E. and K. Strohmaier, 1987. Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *Journal of Virology*, 61: 1621-1629.
22. 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.
23. Edwards, S., S.B. Woods, D.G. Westcott, M. Emmerson, P.C. Jones and A J. Phillips, 1986. An Evaluation of Five Serological Tests for the Detection of Antibody to Bovine Herpesvirus 1 in Vaccinated and Experimentally Infected Cattle. *Res. Vet. Sci.*, 41(3): 378-382.
24. Kitching, R.P. and A.I. Donaldson, 1987. Collection and transportation of specimens for vesicular virus investigation. *Rev. Sci. Tech. Off. Int. Epiz.*, 1987, 6(1): 263-272.
25. Reid, S.M., N.P. Ferris and G.H. Hutching, 2002. Detection of all seven serotypes of foot-and-mouth disease virus by realtime, fluorogenic reverse transcription polymerase chain reaction assay. *J. Virol. Methods* 105, 67-80FAO(2012)Foot-and-mouth disease caused by serotype SAT2 in Egypt and Libya: A Regional concern for animal health in North Africa and the Middle East. *EMPRES WATCH* 25.
26. Ireland, D.C. and Y.S. Binepal, 1998. Improved detection of Capripoxvirus in biopsy samples by PCR. *J. Virol. Methods*, 74: 1-7.

27. 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 reversetranscription polymerase chain reaction. *J. Virol. Methods*, 89: 167-176.
28. Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSIBLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, 25: 3389-3402.
29. Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins, 1997. The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 25: 4876-4882.
30. Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725-2729.
31. EL-Bayoumy, M.K., K.A. Abdelrahman, T.K. Farag, A.M. Allam and Hala A.A. Abou-Zeina, 2014. Zinc, Vitamin E and Selenium Oral Supplementation Reduces the Severity of Foot-and-Mouth Disease Clinical Signs in Sheep. *Global Veterinaria*, 13(5): 704-710, 2014.
32. OIE (2012) Egypt official report. [http://www.oie.int/wahis/public.php?page=event\\_summary&reportid=9993](http://www.oie.int/wahis/public.php?page=event_summary&reportid=9993)
33. 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: 1593-1596.
34. Ghoneim, N.H., A.K. Abdel-Karim, L. El-Shehawy and K.A. Abdel-Moein, 2010. Foot and mouth disease in animals in Sharkia governorate-Egypt. *Transbound Emerg Dis.*, 57: 19-21.
35. Ahmed, H.A., S.A.H. Salem, A.R. Habashi, A.A. Arafat, M.G.A. 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 Blackwell Verlag GmbH. *Transboundary and Emerging Diseases*, 59: 476-481.
36. 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(3): 619- 627.
37. Cottam, E.M., J. Wadsworth, A.E. Shaw, R.J. Rowlands, L. Goatley, S. Maan, N.S. Maan, P.P.C. Mertens, K. Ebert, Y. Li, E.D. Ryan, N. Juleff, N.P. Ferris, J.W. Wilesmith, D.T. Haydon, D.P. King, D.J. Paton and N.J. Knowles, 2008. Transmission pathways of foot-and-mouth disease virus in the United Kingdom in 2007. *PLoS Pathog.*, 4: 1-8.
38. Habiela, M., N.P. Ferris, G.H. Hutchings, J. Wadsworth, S.M. Reid, M. Madi, K. Ebert, K.J. Sumption, N.J. Knowles, D.P. King and D.J. Paton, 2010. Molecular characterization of foot-and-mouth disease viruses collected from Sudan. *Transbound Emerg Dis.*, 57: 305-314.