

Matching of 2012 Foot-and-Mouth disease virus SAT2 isolates and vaccine strain in Egypt

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DURING February and March 2012, foot and mouth disease virus (FMDV) had caused wide-spread field outbreaks in Egypt. The disease was reported mainly in Delta area and along the Nile. The affected species included cattle, buffaloes. Calves were more severely affected. Monitoring circulating virus serotypes to ensure that vaccine strain matched field virus was performed in the current work. In addition, tongue epithelial tissue samples from cattle were collected from Monufia, Qalyubia and Sharkia Governorates to perform genetic and antigenic characterization to monitor the circulating virus strain. FMD virus was determined using virus isolation, RT-PCR and real-time RT-PCR using primer pair derived from 3D polymerase and serotype specific primers. The antigenic matching of FMD virus isolates and vaccine strain (r-value) was performed by virus neutralization test and ELISA. These tests used 21 days antiserum raised against vaccine strain. The results showed that the causative agent of the outbreak was FMDV serotypes SAT2, which was detected in all collected samples. Vaccine matching tests (r-value) revealed that the fifteen FMDV isolates were antigenically closely related to vaccine strain. Finally, monitoring the emergence of new FMDV strains in Egypt is important to enable appropriate vaccine to be selected and control measures to be implemented as rapidly as possible.

Keywords: FMD SAT2, PCR, BHK cells, Vaccine matching.

Foot-and-Mouth disease virus belongs to family Picornaviridae, genus Aphthovirus and causes highly contagious vesicular disease that affects cloven hoofed animals. FMD virus exists as seven antigenically and distinct serotypes O, A, C, Asia1, Southern African Territories 1, 2, 3 SAT1, SAT2, SAT3 that can be divided into a number of temporally and spatially distributed topotypes. The probability risk of penetration of new viral strains to Egypt associated with illegal animals and by-products movement through the common borders with neighboring countries and through tunnels of Gaza. Between 1964 and 2005 serotype O was reported in Egypt. Wide spread outbreak due to serotype A occurred by importation of infected cattle in 2006 (Knowles *et al.*, 2007). Serotype O and A were lastly isolated in 2012 (Aidaros, 2002 and Laila El-Shehawy *et al.*, 2011& 2012).

During February and March 2012, foot and mouth disease virus (FMDV) caused wide-spread field outbreaks in Egypt. The affected species included cattle, buffaloes. Calves were more severely affected with mortality rate up to 50%. Mortalities in young stock might be high as a result of lack of maternal immunity. The outbreaks occurred in Delta area and along the Nile. The causative agent was identified as FMD virus serotype SAT2 (Ahmed *et al.*, 2012). Rapid and accurate detection of FMDV is very important. Definitive diagnosis of FMD requires the detection of the virus antigen or genome in clinical materials. Ideally, the sample of choice should be vesicular epithelium from clinically infected animals during the acute stage of the disease. Virus isolation using cell culture, in addition to, complement fixation test (CFT) and ELISA are important assays to prove the presence of virus in samples of suspected materials (Ferris, 2004, King *et al.*, 2006 and OIE, 2008). Polymerase chain reaction is highly specific, rapid and the most procedure carried out for laboratory diagnosis of FMDV (Stram, 1993). The development of real-time RT-PCR procedure has provided an additional tool for diagnosis (Alexandersen *et al.*, 2006).

A variety of serological methods could be used to quantify antigenic differences between FMDV strains and there by estimate cross-protection between a vaccine strain and a field isolate. Genetic characterization and antigenic profile could reveal the emergence of new strains. Appropriate vaccine strain selection is essential for control of FMD and application of vaccination programs. Effective vaccination against FMD requires monitoring of circulating virus serotypes and their evolution to ensure that vaccine strain match field virus displaying antigenic diversity. The high rate of mutation during replication of RNA is conducive for development of escape mutant. In vaccinated cattle, mutation has been found in the major antigenic site of FMD that involved in receptor recognition (Schat *et al.*, 2005 and Mumford *et al.*, 2007).

The aim of this study was the diagnosis and detection of FMD virus in field samples from Monufia, Qalyubia and Sharkia governorates. Virus isolation on cell culture, real-time RT-PCR and RT-PCR using serotype specific primers were performed. Besides, the serological relationship (r-value) was determined by ELISA and virus neutralization test.

Material and Methods

Vaccine strain

FMD virus SAT2/2012 vaccine strain was prepared by propagation of the virus on BHK-21 cells. Antisera against the virus was collected 21 days post vaccination from cattle vaccinated by monovalent SAT2 vaccine. FMD virus SAT2/2012 vaccine strain was also used in virus neutralization test (VNT), ELISA and as positive controls in real-time RT-PCR and RT-PCR assays.

Tongue epithelial samples

Numbers of samples collected from cattle were 6, 6 and 4 from Sharkia, Monufia and Qalyubia, respectively. Epithelial tissues were minced, suspended in veronal buffer, centrifuged and the supernatants were filtered. Some of these supernatants were inoculated onto monolayer BHK cells for isolation of the causative agent. Other part of the epithelial supernatants was used for RNA extraction, virus neutralization test (VNT), ELISA.

One step reverse transcription polymerase chain reaction (RT-PCR)

RNA was extracted from tongue epithelium suspension using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacturer's protocol. RT-PCR was carried out as previously described to amplify target genome sequence of FMDV ((Knowles & Samuel, 1994 and Laila El-Shehawy et al., 2011). For rapid screening of all extracted RNAs, one-step real-time RT-PCR (rRT-PCR) was performed to amplify 3D coding region as described previously (Azab et al. 2012). Primer pair PoR/PoF for FMDV RNA detection was used. PoF (5'- CCT ATG AGA ACA AGC GCA TC -3') and PoR (5'- CAA CTT CTC CTG TAT GGT CC -3') were derived from the virus 3D polymerase to amplify 422 bp expected target sequence (Shin et al., 2003). For specific amplification of SAT 2, the previously reported oligos were used (Knowles and Samuel, 1994). The oligonucleotide set used for the RT-PCR amplification of the South African Territories (SAT) serotypes of FMDV is SAT-1D209F/ FMD-2B208R. The former primers works for all three SAT serotypes and results in a PCR products of approximately 730 bp, 715 bp and 718 bp for SAT 1, SAT 2 and SAT 3, respectively.

Serological matching between vaccine strains and field virus isolates

Serological relationship between field virus isolates and vaccine virus, "r" value was determined by virus neutralization test and indirect ELISA (Rwenyemamu, 1984, Pereira 1978 and OIE, 2008). "r" value, is the relation between field and vaccine strains as follows,

$$\text{"r1" value} = \frac{\text{reciprocal titer of reference serum against field virus}}{\text{reciprocal titer of reference serum against vaccine virus}}$$

Interpretation of the results

- 0.4-1.0: close relationship between field isolates and vaccine strain. A patent vaccine contains vaccine strain conferee protection.
- 0.2-0.39: The field isolate is antigenically related to the vaccine strain. The potent vaccine with vaccine strain is used and immunization must be more than one.
- 0.2: The field isolate is only distantly related to the vaccine strain and the vaccine strain is unlikely to protect against challenge with field isolate.

Results and discussion

Representative results of rRT-PCR with suspected samples of FMD are illustrated (Fig. 1). All fifteen cattle samples were positive by virus isolation and RT-PCR assays. Isolation of FMD on BHK cells revealed that the collected samples had FMDV characteristics cytopathic effect (CPE). Fig. 2 and 3 showed FMD virus genome detected in clinical samples by RT-PCR. The table illustrated that r-value was ranged between 0.66-0.93 by VNT and 0.74-0.92 by ELISA (Table 1). This indicates close relationship between field isolate and vaccine strain. In consequence of that, FMD vaccine containing the investigated SAT2 vaccine strain will confer protection versus this serotype.

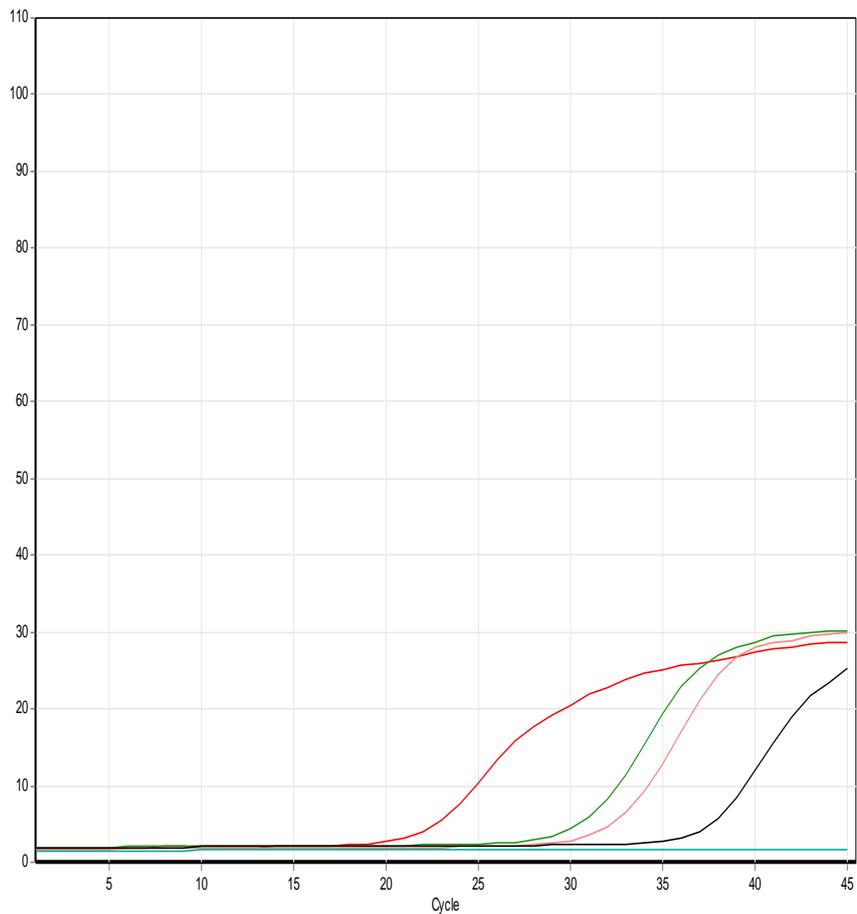


Fig. 1. Real Time RT-PCR result of FMDV isolates. Black is positive control, aqua color baseline is negative control, other curves are positive viral samples.

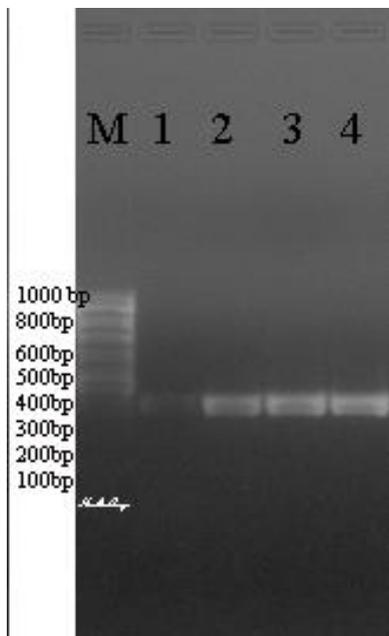


Fig. 2. FMDV detection by RT-PCR. Primers PoF/PoR was used for targeting 3D coding region of the virus. M: 100 bp DNA ladder. Lanes 1 2, 3 &4: positive FMDV isolates (422bp).

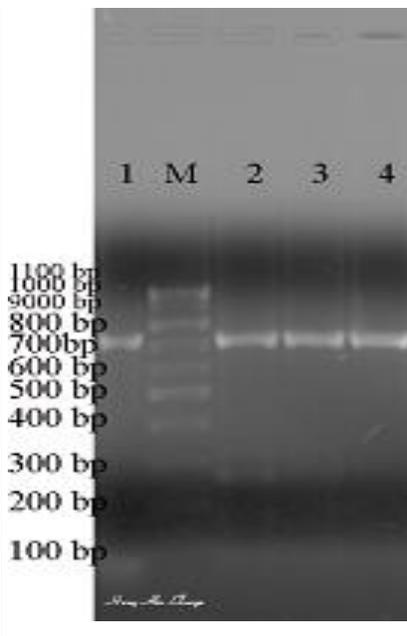


Fig. 3. RT-PCR identification of FMDV SAT2. M: 100 bp ladder. Lanes 1-4 (715-730 bp).

TABLE 1. Vaccine matching" r" value of FMDV serotype SAT2 isolates and vaccine strain .

No. of samples	Origin of sample	VNT	ELISA	r ₁ value	
				VNT	ELISA
1	Vaccine strain	2.25	2.42	1.0	1.0
2	Sharkia governorate	2.15	2.24	0.93	0.92
3		1.8	2.15	0.8	0.88
4		1.81	2.15	0.8	0.88
5		1.5	1.81	0.66	0.74
6		1.5	1.81	0.66	0.74
7		1.5	1.8	0.66	0.74
8		Monofia governorate	1.8	2.15	0.8
9	2.15		2.24	0.93	0.92
10	1.5		1.81	0.66	0.74
11	1.81		2.15	0.8	0.88
12	1.5		1.8	0.66	0.74
13	Qalyubia governorate	1.5	1.8	0.66	0.74
14		1.5	1.8	0.66	0.74
15		2.19	2.24	0.93	0.92
16		2.15	2.24	0.93	0.92
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Real-time RT-PCR provided an extremely sensitive and rapid procedure that contributed to improve laboratory diagnosis of FMD (Ferris *et al.*, 2004, OIE, 2008 and Laila *et al.*, 2011). FMDV SAT2 specific primers used in RT-PCR assay for detection of the virus in field samples of epithelium tissue determined that all fifteen collected samples were FMDV SAT2. Gel electrophoresis gave specific band of 715 bp. Previous authors, Reid *et al.*, 1999 and 2000, used serotype specific primers designed from 1D and 2B regions of FMD viral genome for detection and identification of FMDV serotypes. Real-time RT-PCR assay had shown superior diagnostic sensitivity on the other assays.

During the current study, FMD virus serotype SAT2 was recorded in high rate. It was isolated from all collected clinical samples from cattle. Therefore, further studies are essential, where serotype SAT2 is prevalent in neighboring countries. Serious attention should be given during importation of animals and at livestock movement across the border areas. Furthermore, the epidemiological situation of foot and mouth disease in Egypt needs more investigation for improved vaccine based control.

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مطابقة عزلات فيروس مرض الحمى القلاعية النوع سات ٢ وسلالة اللقاح في مصر

ليلي إسماعيل الشهاوي ، هاني إبراهيم أبو النجا ، حسام جمال الدين فوزي ، مجدي عبد العاطي ، حليلة وطني ، فاطمة عبد الحميد موسى و عيبر عزت منصور
قسم بحوث الحمى القلاعية - معهد بحوث الأمصال واللقاحات البيطرية - العباسية - القاهرة - ص.ب ١٣١ - مصر .

خلال شهري فبراير ومارس ٢٠١٢، تفشي فيروس مرض الحمى القلاعية في مصر. وذكرت تقارير أن المرض أساسا في منطقة الدلتا وعلى طول نهر النيل. وشملت الأنواع المتضررة الماشية والجاموس. وكانت العجول أكثر تضررا في العمل الحالي، تم رصد ومسح وبائي للعترات الفيروس لضمان تطابق سلالة اللقاح مع فيروس الحقل. وبالإضافة إلى ذلك، تم جمع عينات الأنسجة الطلائية للسان من الماشية من محافظات المنوفية، القليوبية والشرقية لتنفيذ التوصيف الوراثي والمولدات المضادة (Antisera) لرصد السلالة الفيروسية المنتشرة. تم تحديد فيروس الحمى القلاعية باستخدام عزل الفيروس، اختبارات اختبار البلمرة المتسلسل و النسخ العكسي. و قد تم مقارنة سلالة اللقاح ضد الفيروسات المنتشرة بتحليل مطابقة اللقاح (r-value) مستخدما اختباري ELISA and VNT . وقد كشف تحليل مطابقة لقاح (r-value)، أن خمسة عشر عزلة كانت ترتبط ارتباطا وثيقا بسلالة اللقاح. وأخيرا ، فإنه من الأهمية استمرار رصد أي سلالات جديدة من أجل معرفة قدرة اللقاح على مجابهة المرض.