Isolation and Characterization of FMD viruses types A and O in years 2009 and 2010 in Egypt

Thesis

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Abstract

Eighty seven samples [8 Buffy coat, 15 saliva, 31 nasal swabs, 4 Tongue epithelium, 16 buccal swabs, 3 oropharyngeal fluid (O.P.F) and 10 swabs] were collected from suspected cows and buffaloes from different Egyptian governorates [Alexandria – Dokahlia, Gharbia, Mounofya, Kalubia, Cairo, Suez, Ismailia, Al Fayoum and El Menia]. Isolation in BHK21 cell revealed that 16 samples showed CPE. The 16 isolates were characterized by antigen detection ELISA. The 16 isolates were pooled as five groups and Identified by real Time RT PCR.

Multiplex Reverse transcription polymerase chain Reaction (RT- PCR) was done using universal primer (1F, 1R) and serotype specific primers for serotype O (OMEL2, KH61) and serotype A (EG 154F, NK61). Five samples out of eleven were positive for serotype O, six samples out of eleven were positive for serotype A, eleven samples out of Eleven were positive for universal primer the RT-PCR products were subjected to direct nucleotide sequencing, Blast searches, multiple alignment and phylogenetic analysis of VP1 nucleotide sequence.
The phylogenetic tree for serotype O revealed that local FMDV detected in examined samples were related to O/Iran/2010, O/Israel -07-6387, D/Pak-40-2006, O1-Sharquia EGY-72 and EG-101-2009 and for serotype A revealed that rebated to A/Iran/2005/A/Jor/4/2006 and A/Eth/1/94.

Serum neutralization Test and Liquid phase bloking Elisa were done for detection of FMDV serotypes O and A antibodies on 471 serum samples from four governovates [Behaira, El-Sharquia, Kafr El-Sheikh and El-Menia]. PrioCHEKIT -FMD-ELISA was used for detection of non-structural protein against FMDV in the same 4 Egyptian governorates.

**Key words:** Foot and mouth disease –virus-Egypt-governorates-2009-2010.
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Dedicated to my family.

My mother, My father, My dear husband, My lovely sons and daughter.
Conclusion

From our study of the current situation of FMD in Egypt it was concluded that:

1. Real Time PCR was used for identification of isolates.
   It has proven to be highly sensitive and specific
2. RT PCR was done and proved that it is accurate rapid sensitive, applied with large scale.
3. Gene sequencing identified 3 samples for serotype O and 3 samples for serotype A.
4. Phylogenetic analysis was done and proved that variants of FMDV are circulating in Egypt.

The circulating variants of FMDV is a major problem of FMDV in Egypt so, must follow up the continuous mutation in the virus from time to time in the field and according to the variant results the vaccine must be updated according to the recent results.

That means cooperation between research diagnostic sector and field sector must be continuous to control or gradually eradicate the disease.
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V
1. Introduction

Foot and mouth disease (FMD) is the most important disease of the international organization of epizooties (OIE). List A, and one of the most contagious disease among domestic animals (Carroll et al., 1984; OIE/FAO/WHO, 1996; Saiz et al., 2002 and Michael et al., 2007).

Foot and mouth disease (FMD) known as aphthous fever is an economically important and highly contagious viral disease that affects cloven-hoofed domestic and wild animals and is considered the most economically important disease world wide (Paixoe et al., 2008).

FMD is caused by Family picornaviridae of genus aphtho virus; it contains a single stranded RNA molecule. The virus has seven major serotypes: A, O, C, SAT\(_1\), SAT\(_2\), SAT\(_3\) and Asia 1, infection with one serotype doesn’t confer immunity against another. The virus is easily spread by several means, the most important being recovered animals or products from such animals (Anthony and Werner, 1992).

FMD virus has 4 structural proteins (SP) (VP\(_1\), VP\(_2\), VP\(_3\) and V\(_4\)) forming the capsid and when it replicates during infection, results in the production of numbers of non structural proteins (NSP) of which some are immunogenic (Tesar et al., 1989).

The most important non-structural protein are 2C 3A and 3C there are three proteins are responsible for cell membrane vesicle proliferation, the pathogenesis and inhibition of host cell protein transcription, respectively (Mason et al., 2003 and Pariente et al., 2005).
The disease is characterized by the formation of vesicles in the mucosa of the mouth, external nares and in coronary band of claws, other areas including udder and teats. Lameness is seen, reduced lactation mastitis and abortion are common clinical signs range from a mild or in apparent infection to one that is sever. Death may result in some cases, mortality from a myocarditis is most common seen in young animals myositis may also occur in other sites (FAO, 1984).

The severity of clinical signs varies according to the strain of virus, the exposure dose, the age and breed of the animals, the host species and its degree of immunity. The signs can range from a mild or in apparent infection to one that is sever. Death may result in some cases (Bastos, 1998).

The FMD virus is transmitted via inhalation, ingestion and direct contact with infected animals (APHIS, 2001 and Sakamoto et al., 2002).

Movement of animals between premises is considered to be the main risk factor for the introduction of FMD to farms (Sanderson et al., 2000, Bates et al., 2003; and Ortiz-Pelaez et al., 2006).

In Egypt, foot and mouth disease (FMD) was first detected in 1950 when strain SAT2 caused an outbreak, then in 1952, 1956 and 1958, when outbreak caused by strain A, several foci were detected during years 1961-1970 where no other strains of FMD than O have been detected since 1970. Regularly several foci have been recorded from 1970 to May 2001 (OIE, 2005). Till appearance of strain A during 2006 (Salem., 2009).
Regarding the Middle East, over two hundred outbreaks of FMD were reported in 1989 in: Turkey, Syria, Israel, Jordan, Saudi Arabia and other (GCC), Egypt, Tunisia and Libya (Kitching, 1990).

Methods for the diagnosis of foot and mouth disease consistent with Office International des Epizooties (OIE) standards for FMD diagnosis and include: antigen-capture ELISA for viral antigen typing, liquid-phase blocking ELISA (LPBE) for detection of antibodies against FMDV, and an indirect ELISA for detection of antibodies against the non structural protein (NSP) 3 A B C several molecular diagnostic methods have also been developed for detection of fragments of FMD genome within viral samples such as multiplex RT-PCR, typing RT-PCR and real time RT-PCR (Lu et al., 2008).

Differentiation of infection from vaccination based on antibody to the NSP (Rodriguez et al., 1994).

Vaccination constitutes an important control policy for FMD in affected areas with advanced eradication programs, as well as in free regions that decide to use immunization as a control measure after a recent introduction of the disease. However, considering that vaccinated animals exposed to FMD virus can establish subclinical infection and eventually remain persistently infected (Bergmann et al., 2003A).

- The plan of the present study is to deal with the following items:

1. Epizootiological study on FMD in 4 Egyptian governorates as a pilot governorate to detect the early diagnosis of infection foci by determination of antibodies against “NSP” of FMD virus which
indicate the natural infection as well as to differentiate between the vaccinated and non vaccinated infected animals (Priocheckit.)

2. Trials of isolation of the FMD virus by inoculation on TC “BHK cell line” and viral antigen identification by antigen capture ELISA and real time PCR and multiplex PCR. Nucleotide and amino acid sequence were done.

3. Serological investigation for detection of antibodies against FMD virus by serological techniques as serum neutralization test “SNT” and ELISA (LPBE) for serotyping.
2. Review of Literature

2.1. History

The earliest description of FMD was recorded in 1514 by Hieronymus Fracatovirus in Northern Italy. There was confusion about the specific nature of the disease and other contagious disease, such as rinder pest and anthrax. He also mentioned that FMD was recorded in animals in Germany in 1751. Its first appearance in Britain was in July 1839, in diaries in Stratford and London, this outbreak reached its peak in 1840 and 1842. As a result FMD was considered notifiable in 1971. The author suggested that the ignorance of the disease’s infectiousness resulted in outbreaks from visit of foreign farmers (Anon, 1978).

FMD virus is the etiologic agent of FMD attack-cattle, swine and other cloven-footed animals. Great loss from the disease results from reduced production of meat, milk, and other animal products. FMD characterized by the formation of vesicles on the tongue, nose, muzzle and coronary bands of infected animals. The case with which it may be transmitted by contact and aerosol (Shahan, 1962; Bachrach, 1968; Mayer, 2001).

FMD is perhaps the most infectious disease to veterinary medicine. Put in consideration early recognition epidermiology occurrence around the world and sampling and diagnostic method are the most important points to control the disease. The practitioner must be acute in his or her herd inspection of animals in which vesicular disease is suspected and knowledgeable as to differential diagnosis (Lubroth, 2002).
FMD virus has 7 immunological distinct serotypes. Susceptible species are mainly cattle, sheep, goats, bison and deer. All body fluids of infected animals can contain the virus and are considered infective (Musser, 2004).

2.2.1. Geographical distribution of FMD

The United State has been free from FMD since the 1920, when several outbreaks occurred in California. Outbreak began in pigs and spread to cattle and deer across the central part of state. It took 2 years to eradicate FMD from the local deer population in one national park and 22,000 deer were slaughtered (McVicar et al., 1974).

FMD has not been reported in Japan since 1908 except for one outbreaks that took place at a quarantine station in 1933 (Konigshoffer, 1975).

Serotype “O” continued to be isolated from outbreaks Middle East during the period of 1981 – 1988 (Samuel et al., 1990).

FMD outbreaks continue to occur within the large Saudi dairy herds although many Saudi forms have introduced a program of vaccination against FMD every 3 months (Hafez, 1991). The serotype “O” FMD was endemic in Egypt and Libya and there have been reported in Tunisia during 1990 (Kitching, 1992). The reduction of the risks of introducing exotic FMD virus strains to the Saudi Arabia kingdom through live animals importation was recommended by (Hafez et al., 1993).
In Uruguay: The last outbreak of FMD was in 1990 and still, routinely vaccination of cattle, the country was in 1993 given the unique designation of (free from disease with vaccination) (Anon, 1994).

In Namibia the outbreak of FMD 1994 due to serotype SAT₃ and in South of Africa due to serotype SAT₂ (Thomson, 1995). In the European countries; Bulgaria, Greece, Turkey, Albania and Macedonia outbreaks of FMD type “O” in 1996 (Kitching, 1998).

In Taiwan the outbreak which occurred in March 1997 was caused by a pig-adapted virus strain (O/Taiwan/97) which didn’t infected species of cloven-hoofed animals by natural in Jun 1999. The second strain (O/Taiwan/99), was isolated from infected cattle didn’t develop pathological lesions. During the period of January to March 2000, five outbreaks caused by FMD similar to (O/Taiwan/99) virus and infected species included goats, Chinese yellow cattle and dairy cattle (Huang et al., 2000).

In United kingdoms and main land of Europe was a timely reminder of its devastating effect (Pluimers et al., 2001). The outbreak of FMD type “O” was confirmed in UK this outbreak was caused by an FMD strain. That was responsible for the outbreak in Japan (Blanco et al., 2002).

In Turkey, a total of 29 outbreaks have been reported, 16 due to type “O”, 11 due to type “A” and 2 due to type “Asia 1” (EU FMD Meeting, 2002).

In Niyazaki and Mokkaido Prefectures, Japan, four outbreaks of FMD occurred from March to May 2000. FMD virus isolation was
Review of Literature

achieved by sampling probing materials from Japanese Black Cattle. The FMD was identified as type “O” by ELISA for antigen detection and the nucleotide sequence encoding the VP1 was determined. This FMD was designated as (O/Japan/2000) by World Reference Laboratory in Pirbright Institute, England (Sakamoto et al., 2002).

In Africa six of the seven types of FMD virus occur which is the unique situation in comparison with other regions of the world, of the 6 virus types prevalent in Africa four are widespread (O, A, SAT1 and SAT2) while SAT3 is more or less confined to Southern Africa and type “C” has only been identified in Kenya in the recent past (Thomson et al., 2002). In Africa FMD is endemic and the epidemiology of the disease is more complicated than other parts of the world (Vosloo et al., 2002).

In China the FMD virus type “O” circulated separately in 1958 and 1999; the O/Tibet/CHA/99 strain was the causative virus of FMD in 1999 (Mason et al., 2003b). In outbreaks in Zimbabwe the two serotypes SAT1 and SAT2 were involved (Sammin et al., 2004).

FMD is endemic in Asia, Africa, parts of Europe and most of South America (OIE, 2004).

In Turkey since 1962 serotypes, A, C, O, Asia 1 and SAT1 among the 7 serotypes of FMD circulating globally have been reported from outbreaks of FMD. Asia 1, A and O are currently being reported inspite of considerable efforts to control the disease including vaccination (Parlak et al., 2005).

Russia is constantly threatened by the penetration of FMD from bordering Asian countries; examples, are FMD outbreaks of 1995, 2000,
2004 and 2005 caused by virus penetration from China to Russia (Valarcher et al., 2005).

In Iran the three serotypes of FMD virus including A, Asia 1 and O had been detected (Alamdar et al., 2006).

The countries in which FMD is found reflects in many ways their level of economic development being absent from Europe, North America and Australia, Sporodic in South America and endemic in most of Asia and Africa (Kitching et al., 2007). In many sub-sahara Africa countries FMD serotype SAT-1 seems to be endemic (Sahie et al., 2007).

2.2.2. FMD in Egypt

Many FMD outbreaks in Egypt were recorded in 1953, 1956 and 1958. Type “A” of FMD virus was responsible for these outbreaks, while “SAT 2” strain was responsible for the 1950 outbreak’ (Zahran, M 1960). Different types of FMD virus (O, A and SAT 2) were identified. Type “O” virus was the most prevalent in setting up the disease. Type “A” and “SAT 2” were the main cause of outbreaks during 1953, 1958 and 1960 (Zahran, 1961).

Two strains of type “O” and “A” viruses were isolated from naturally infected cattle. Type “O” virus was in 1966 from cows in Cairo quarantine station and type “A” was isolated from cows contracting FMD in Abis area near Alexandria in August (1967) (Mazhar et al., 1974). Type “O” virus was the most prevalent in the setting the disease among infected cattle and buffaloes in Egypt during the last 25 years (Moussa et al., 1979). In Sharkya a sever outbreak took place in January. This isolate