INVESTIGATION OF FOOT AND MOUTH DISEASE VIRUS AT DIFFERENT GOVERNORATES IN EGYPT
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ABSTRACT

Foot and Mouth Disease Virus (FMDv.) was diagnosed in oesophageal, pharyngeal fluid and tongue epithelial samples from clinically suspected cattle in some Egyptian governorates (Monofia, Kalubia, Beni-suef and Sharkia) between 2009–2010. The virus was successfully isolated on unweaned mice and BHK-21 cell line. The virus serotypes were identified using ELISA as FMD serotypes A and O. 43 out of 260 collected samples (17.2%) were positive to serotype (O) in comparison to 13 out of 260 (5.0%) were positive to serotype (A). The identified FMDv. serotypes were confirmed by molecular identification of VP1 coding region on the viral genome with Reverse Transcription–Polymerase Chain Reaction (RT-PCR) using specific primers that gave 800 bp and 402 bp amplification product for FMDv. serotypes A and O, respectively.

KEY WORDS: Foot and Mouth Disease Virus, RT-PCR, Serotypes A and O

1. INTRODUCTION

Foot and Mouth Disease (FMD) is a contagious disease of cloven-hoofed animals caused by FMD virus and characterized by vesicular erosion of the feet, buccal mucosa and mammary glands[1]. It affects cattle, sheep, goats, deer and pigs [2]. FMDv. belongs to genus Aphthovirus of the family Picornaviridae. It is a single stranded positive sense RNA virus [3]. It exists in seven serotypes (A, C, O, Asia 1, and SAT 1, 2 and 3) with no cross-protection conferred among the seven serotypes [4]. In Egypt, FMDv. attacked susceptible animals each year causing drastic losses in milk and meat production and death of young animals [5-8]. Control of FMDv. depend on early diagnosis that confirmed by objective diagnostic tests. So, diagnostic test procedures should be rapid, sensitive and specific [9]. ELISA was applied for diagnosis of FMD with possible serotyping of FMDv. at high sensitivity and specificity [10]. Molecular techniques, such as real-time PCR, are replacing conventional techniques such as virus isolation and antigen detection ELISA (Ag-ELISA) for FMDv. diagnosis for several reasons among which are the ease of automation and rapidity of the results [11]. In the present study diagnosis and typing of FMDv. was attempted using isolation of the virus from suspected samples on unweaned mice and BHK-21 cell culture then identification using antigen ELISA and RT-PCR.

2. MATERIALS AND METHODS

2.1. Viral Samples:

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Two hundred and sixty samples were collected from FMD suspected cattle during 2009-2010 from different governorates (Monofia, Kalubia, Beni-suef and Sharkia) in Egypt. Samples included 250 oesophageal-pharyngeal (OP) fluid and 10 tongue epithelium (TE). They were collected and prepared according to [9].

2.2. Susceptible host systems:

2.2.1. Unweaned mice:
Un-weaned baby Swiss Albino suckling mice of 2-3 days old were used to isolate the virus. Each sample was inoculated in 4 baby mice by intraperitoneal route in a dose of 0.1 ml. Positive samples exhibit paralysis of hind limb and death of Baby mice 48 hours post inoculation. They were supplied by Laboratory animal house at Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo.

2.2.2. Cell Line:
Baby Hamster Kidney cells line (BHK-21 clone 13) were used to isolate the virus. They were supplied by Laboratory animal house at Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo. Positive samples exhibit the pathognomic cytopathic effect (CPE) of FMDv. within 48 hours post inoculation.

2.3. Reference antisera:
Table 1 FMDv. specific primer sequences:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Orientation</th>
<th>Sequence (5’-3’)</th>
<th>Serotype specificity</th>
<th>Genomic location</th>
<th>Bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Forward</td>
<td>TACCAAATTAACACGGGAA</td>
<td>A</td>
<td>1D</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>Reverse</td>
<td>GACATGTCCTCCTGCATCTG</td>
<td>A</td>
<td>1D</td>
<td>800</td>
</tr>
<tr>
<td>3</td>
<td>Forward</td>
<td>AGCTTGTCACAGGGTTTGGC</td>
<td>O</td>
<td>1D</td>
<td>402</td>
</tr>
<tr>
<td>4</td>
<td>Reverse</td>
<td>GCTGCTACCTCCTCCCAA</td>
<td>O</td>
<td>1D</td>
<td>402</td>
</tr>
</tbody>
</table>

2.4. Reference FMDv:
Reference FMDv as locally isolated FMDv. strains type A/EGY/2006 and O1/EGY/93 was collected from cattle used in this study were obtained from FMD department, Veterinary serum and vaccine research institute, Abassia, Cairo, Egypt.

2.5. Enzyme-linked immunosorbent assay (ELISA):
It was carried out according to the method of [12] to identify FMD viral antigens using antigen ELISA.

2.6. Specific oligonucleotide Primers:
Two types of specific primers were used in RT-PCR assay for detection of 1D (VP1) gene of type A and type O FMDv. All primers were synthesized by Metabion, Germany. It was carried according to [13].

2.7. Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR):
It was performed according to [13].

3. RESULTS and DISCUSSION

FMD is economically the most important viral disease of domesticated and mild ruminants such as cattle, buffalo, sheep, goats and deer. It can cause high mortality in young animals and production losses in adults, and is considered the single most important constraint to trade in live animals and animal products and their byproducts [14]. FMDV has a positive sense, single stranded RNA genome of 8400 nucleotides that code for twelve proteins, four of them are structural and make up capsid of the virus and the other eight are non-structural genes and which together allow the virus to replicate in an infected cell. The
In an investigation of FMD virus in Egypt, structural genes are identified as 1A, 1B, 1C and 1D, the non-structural as L, 2A, 2B, 2C, 3A, 3B, 3C and 3D. Samples were collected on the base of clinical FMD signs appeared on infected animals namely salivation and tongue epithelial lesions are the most lesions that make suspicion toward FMD, our findings was similar to that recorded by [15, 16]. The collected samples, were tongue epithelium (T.E) and oesophageal pharyngeal fluid (OP), from Monofia, Kalubia, Beni-Suef and Sharkia governorates along the year 2009-2010 according to the notification of the FMD outbreaks, the sample numbers were 70, 50, 50 and 80, respectively. These samples were submitted to FMDv isolation in baby mice and tissue culture and to identification by indirect ELISA.

### Table 2: FMDv positive Oesophageal Pharyngeal samples using baby mice inoculation, tissue culture inoculation and indirect ELISA.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples</th>
<th>Baby mice No.</th>
<th>Baby mice %</th>
<th>Tissue culture No.</th>
<th>Tissue culture %</th>
<th>FMDV-type O1 No.</th>
<th>FMDV-type O1 %</th>
<th>FMDV-type A No.</th>
<th>FMDV-type A %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monofia</td>
<td>70</td>
<td>15</td>
<td>21.5</td>
<td>15</td>
<td>21.5</td>
<td>13</td>
<td>18.57</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>Kalubia</td>
<td>50</td>
<td>9</td>
<td>18.0</td>
<td>8</td>
<td>16.0</td>
<td>8</td>
<td>16.00</td>
<td>1</td>
<td>2.00</td>
</tr>
<tr>
<td>Beni-Suef</td>
<td>50</td>
<td>8</td>
<td>16.0</td>
<td>8</td>
<td>16.0</td>
<td>4</td>
<td>8.00</td>
<td>4</td>
<td>8.00</td>
</tr>
<tr>
<td>Sharkia</td>
<td>80</td>
<td>14</td>
<td>17.5</td>
<td>14</td>
<td>17.5</td>
<td>9</td>
<td>11.25</td>
<td>5</td>
<td>6.25</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>46</td>
<td>18.4</td>
<td>46</td>
<td>18.4</td>
<td>34</td>
<td>13.60</td>
<td>12</td>
<td>4.80</td>
</tr>
</tbody>
</table>

Results in Table 2 and Fig. 1 showed the isolation of FMDv from 46/250 (18.4%) oesophageal pharyngeal (OP) samples collected from Monofia, Kalubia, Beni-Suef and Sharkia showed positive results as 15/70 (21.5%), 9/50 (18%), 8/50 (16%) and 14/80 (17.5%) respectively, on both baby mice and tissue culture inoculation.

Fig 1. Positive Oesophageal Pharyngeal samples to FMDv tested by using baby mice inoculation, tissue culture inoculation and indirect ELISA.

Results in Table 3 and Fig. 2 showed the isolation of FMDv from 10/10 (100%) Tongue Epithelium (TE) samples collected from Monofia, Kalubia, Beni-Suef and Sharkia showed positive results as 2/2 (100%), 3/3 (100%), 2/2 (100%) and 3/3 (100%) respectively, on both baby mice and tissue culture inoculation while identification of the isolated virus using indirect ELISA showed that FMDv type O was identified in 34/250 (13.6%) oesophageal pharyngeal (OP) samples collected from Monofia, Kalubia, Beni-Suef and Sharkia in 13/70 (18.57%), 8/50 (16%), 4/50 (8%) and 9/80 (11.25%) respectively, after their isolation. FMDv type A identification using indirect ELISA showed that 12/250 (4.8%) oesophageal pharyngeal (OP) samples were positive which were distributed as 2/70 (2.86%), 1/50 (2%), 4/50 (8%) and 5/80 (6.25%) in Monofia, Kalubia, Beni-Suef and Sharkia, respectively. The obtained results was in agree with previous published research [5, 17-19] who stated that, since 2006 FMDv serotypes (O1 and A) are isolated from Egypt and still existing and circulating in Egypt.
El-Bagoury et al., (2011)

Identification of the isolated virus using indirect ELISA showed that FMDV type A was identified in 9/10 (90%) oesophageal pharyngeal (OP) samples collected from Monofia, Kalubia, Beni- Seuf and Sharkia in 2/2 (100%), 3/3 (100%), 2/2 (100%) and 2/3 (66.7%) respectively, after their isolation.

Table 3: FMDV positive epithelial tongue samples using baby mice inoculation, tissue culture inoculation and indirect ELISA.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples</th>
<th>Baby mice</th>
<th>Tissue culture</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of positive</td>
<td>%</td>
<td>No. of positive</td>
</tr>
<tr>
<td>Monofia</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Kalubia</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Beniseuf</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Sharkia</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig 2 Positive epithelial tongue samples tested by baby mice and tissue culture inoculations, and indirect ELISA.

FMDV type A identification using indirect ELISA showed that 1/10 (10%) Tongue Epithelium (TE) samples were positive which was located as 1/3 (33.3%) in Sharkia governorate only. These results were in agree with that of earlier reports [5, 17-19] who stated that, since 2006 FMDV serotypes (O1 and A) are isolated from Egypt and still existing and circulating in Egypt. Also these results match those obtained by previous authors [20-23] who recorded that serotype (O1) FMDV is the most prevalent isolated serotype in Egypt.

It could be concluded that FMDV is still circulating in the governorates under investigation. 43 out of 260 collected samples (OP and T.E.) 17.2% were positive to FMDV serotype (O) in comparison to 13 out of 260 (5 %) were positive to FMDV serotype (A). These results indicated that the prevalence of serotype (O) is higher than serotype (A) in the four governorates. Confirmed identification and typing of FMDV using RT-PCR is based on published sequences for the 1D gene (encoding the VP1 viral protein) for amplification of FMDV. RNA [1]. All serologically ELISA positive serotype (O and A) FMDV. samples under investigation (57 samples) were passaged once in tissue culture cells to be used for viral RNA extraction.

The results of RT-PCR reflected that all serologically ELISA positive samples for FMDV serotypes (O and A) gave positive result with RT-PCR. FMDV. serotype (O) gave positive result with RT-PCR at 402bp, while FMDV. serotype (A) gave positive RT-PCR results at 800bp (photo 1) with variable intensity on ethidium bromide gel. These results are parallel to that reported by [24] who stated that RT-PCR assays were confirmatory to the classical serological and viral isolation methods due to their high sensitivity and speed, also with [25] who used primers PH1/PH2 in a single tube one step RT-PCR, and achieved success when the target FMDV 1D/ 2B sequences (402bp).
Investigation of FMD virus in Egypt.

Photo 1 Agarose gel electrophoresis of RT-PCR products for detection of FMDV type (O) and type (A) using 1D specific primer. L: DNA Ladder (100bp to 10 k bp), 1 and 2: Positive FMDV type (O) at 402 bp and 3 and 4: Positive FMDV type (A) at 800 bp.

Also, the RT-PCR results were in parallel with the results indicated by [26], who used the PH1/PH2 primers and get the band at 402bp for type (O) and 800 for (A). In this field the results of [7] revealed that, the universal primer set P1/ P2 amplified cDNA fragment of 216bp, which was equivalent to the expected amplification product size from any FMDv. genome. Specific cDNA amplified for serotype (A) giving discrete bands at approximately 816bp.

4. CONCLUSIONS

From this study it’s clear that FMDV serotype O1 and A/ Egy/2006 still exist and circulate in Monofia, Kalubia, Beni Suef and Sharkia Governorates.

5. REFERENCES


El-Bagoury et al., (2011)


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Investigation of FMD virus in Egypt.

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الملخص العربي

Execution of this study aimed at determining the distribution of the FMD virus in several Egyptian governorates.

Samples of saliva and epithelial cells from the tongue were collected from cows that suffered from the disease in governorates of Menoufia, Qalyubia, Beni Suef, and Sharqiya between 2002 and 2010. The virus was isolated by inoculating newborn rats and on kidney cells of newborn mice, and then the presence of the units (O and A) of the virus was determined by ELISA test. The results of the ELISA test showed that 43% of the 260 samples were positive for the O unit of the virus, while 31% were positive for the A unit. The isolated virus was confirmed by determining the gene responsible for the virus protein by sequencing the reverse transcriptase-polymerase chain reaction (RT-PCR) and using the specific primer that gave a result of double-stranded units A and O of the FMD virus in turn.

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