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

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\textbf{A BST RACT}

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 un-weaned 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.

\textbf{KEY WORDS}: Foot and Mouth Disease Virus, RT-PCR, Serotypes A and O


\textbf{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.

\textbf{2. MATERIALS AND METHODS}

\textit{2.1. Viral Samples:}
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:
Reference antisera against FMDv. type “O” and FMDv. type “A” used in this study was obtained from FMD department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt (VSVRI).

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, Abbasia, 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.

<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>TACCAATTTACACACGGGAA</td>
<td>A</td>
<td>1D</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>Reverse</td>
<td>GACATGTCCTCCTGATCTG</td>
<td>A</td>
<td>1D</td>
<td>800</td>
</tr>
<tr>
<td>3</td>
<td>Forward</td>
<td>AGCTTGTTACACGGGTTTGC</td>
<td>O</td>
<td>1D</td>
<td>402</td>
</tr>
<tr>
<td>4</td>
<td>Reverse</td>
<td>GCTGCTACCTCCTTCAA</td>
<td>O</td>
<td>1D</td>
<td>402</td>
</tr>
</tbody>
</table>
Investigation of FMD virus in Egypt.

The 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-seuf 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 Oseophageal 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>%</th>
<th>Tissue culture No.</th>
<th>%</th>
<th>FMDV-type O1 No.</th>
<th>%</th>
<th>FMDV-type A No.</th>
<th>%</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>9</td>
<td>18.0</td>
<td>8</td>
<td>16.00</td>
<td>1</td>
<td>2.00</td>
</tr>
<tr>
<td>Beni-seuf</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-Seuf 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. 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-Seuf 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-Seuf 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. Table 3 and Fig. 2 showed the isolation of FMDv. from 10/10 (100%) Tongue Epithelium (TE) samples collected from Monofia, Kalubia, Beni-Seuf and Sharkia showed positive results as 2/2 (100%), 3/3 (100%), 2/2 (100%) and 3/3 (100%).
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(100%) respectively, on both baby mice and tissue culture inoculation while 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>No. of positive</th>
<th>%</th>
<th>No. of positive</th>
<th>%</th>
<th>FMDV-type O1 No.</th>
<th>%</th>
<th>FMDV-type A No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monofia</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kalubia</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beniseuf</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sharkia</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>66.7</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>10</td>
<td>100</td>
<td>9</td>
<td>90.0</td>
<td>1</td>
<td>10.0</td>
</tr>
</tbody>
</table>

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 agreement 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 are 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).

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 402bp, and 3 and 4: Positive FMDV type (A) at 800bp.

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

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استبيان فيروس مرض الحمى القلاعية بمختلف محافظات مصر

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الملخص العربي

إجراء هذه الدراسة بإشراف برل مدي انتشار فيروس مرض الحمى القلاعية والمناطقية في عدد من محافظات مصر.

تم تجميع عينات سائل البلعوم والخلايا الطبيعية للمنائ من أبقار شُخصت إصابتها بالمرض من محافظات المنوفية، القليوبية، بني سويف، الشرقية بين عامي 2009 و 2010. تم عزل الفيروس عن طريق الحقن في رضيع الفئران والشرقية. ثم تم التعرف على العترات (O و A) للفيروس باستخدام اختبار الايلازا.

أوضح نتائج اختبار الايلازا أن 34 من إجمالي 260 عينة بنسبة 17.2% كانت إيجابية للعترة O للفيروس بينما كانت 13 من إجمالي 260 عينة بنسبة 5.0% كانت إيجابية للعترة A للفيروس. تم التأكيد على الفيروس المعزول بواسطة التعرف الجزيئي على الجين المسؤول عن البروتين الفيروس-1 على جينوم الفيروس باستخدام النسخ العكسي لتفاعل RT-PCR، و باستخدام بادئ التفاعل المتخصص الذي أعطي ناتج تضاعفي يساوى 800 قاعدة مزوجة و 402 قاعدة مزوجة لعترات A و O لفirus مرض الحمى القلاعية على التوالي.