Foot and Mouth Disease (FMD) in Egypt 2010

By


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ABSTRACT

Eight Egyptian governorates were involved in this study representing Lower, Med and Upper Egypt (Ismailia, Dakahlia, Monofia, Sharkia, Helwan, Giza, Beni Suef and Menya). Eight hundred random serum samples were collected from cattle (hundred sample (from each governorate). Eighty virological samples were collected (for virus isolation) on the base of clinical signs FMD (salivation (or epithelial tongue eruption). The collected materials (samples) were submitted for diagnosis and epidemiological monitoring of circulating FMD virus strains in cattle in Egypt.

The serum samples were examined for the circulating antibodies against FMDV serotypes (O1 & A) by using ELISA. Prio CHECK FMDV Non-structural protein blocking ELISA was adopted to differentiate between vaccinated and antibodies produced from previously infected cattle. The virological samples for virus isolation were inoculated in unweaned baby mice and primary calf kidney cell culture for isolation of FMDV. The positive samples were checked by ELISA test to differentiate between O1 & A FMDV serotypes. The results showed that, the circulating antibodies (in cattle) are present moderately in the 8 governorates; the Prio-check blocking ELISA indicated that, about 20% of these antibodies were due to previous infection, while 68% due to vaccination by FMD bivalent locally produced inactivated (FMD) vaccine. The virological investigation indicated that, FMDV serotypes O1&A still existing and circulating in Egyptian cattle.

Keywords: FMD, ELISA, Prio-check, virus isolation, FMDV serotypes O1&A, Egypt.

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INTRODUCTION

Foot and Mouth Disease (FMD) is caused by 7 immunological distinct serotypes O,A,C,Asia1,SAT1,SAT2 and SAT3 which belong to the species FMD virus (genus Aphthovirus, family Picornaviridae). Several of these serotypes (are) currently or periodically in the Middle East and North Africa (Knowles and Samuel, 2003). Since the 1950s, attention has been drawn to the economic importance of FMD in Egypt, after several outbreaks of the disease affected cattle, buffaloes, sheep, goats and camels, with the predominant isolation of FMD virus (FMDV) serotype O1 (Moussa et al. 1974, Daoud et al. 1988 and El-Nakashly et al. 1996). The last outbreak due to serotype (O) was in 2006 Abd El-Rahman et al.(2006). Other serotypes have not been reported since 1972 (Aidaros, 2002). In 2006 clinical cases of FMD were recognized on a cattle farm in Ismailia, Northeastern Egypt. Samples were submitted for laboratory investigation and serotypes determination by using ELISA test, and RT-PCR and FMDV type (A) was confirmed (Knowles et al. 2007). The FMDV type (A) was designated as A/Egy/2006.

Since 2006 A/Egy/2006 inactivated virus strain began to add to the locally produced FMD vaccine, so, the locally produced bivalent (FMD) vaccine is containing both O1 and A/Egy/2006. The FMD outbreaks still reported since July 2006 till now, both serotypes (O1 and A) were isolated (GOVS, 2009 and Ghoneim et al., 2010).

In this study serum and virological materials (OP & Tongue epithelium) were collected from 8 different Egyptian governorates (Ismailia, Dakahlia, Monofia, Sharkia, Helwan, Giza, Beni-Suef and Menya), represent lower, Med and upper Egypt. The serum samples were submitted for determination of circulating antibodies against both types of FMDV O1 & A and to differentiate between antibodies produced from vaccinated and previously infected cattle using (Priocheck blocking ELISA (test)). The virological samples were investigated for isolation and identification by antigen detecting ELISA, the objective of this investigation is to evaluate the current status of FMD in Egypt 2010.
MATERIAL and METHODS

1) Unweaned baby mice:
unweaned Swiss baby mice, 2-4 days old (Charles River Strain, USA) were supplemented from animal house (VSVRI), Abassia, Cairo. They were used for isolation of FMDV by intraperitoneal route (I/P) as 0.1 ml of treated sample (OP or tongue epithelium).

2) Tissue culture:
Primary calf kidney cell culture was prepared at FMD department, VSVRI, Cairo, Egypt. The tissue culture was used to isolate FMDV from virological collected samples, each treated sample was passaged three successive passage for detection of specific CPE of FMDV.

3) Foot and Mouth Disease virus (FMDV)
Serotypes O1/EGY/93 and A / EGY/2006 were typed and subtyped at the FMD department, VSVRI, Abassia, Cairo, Egypt and confirmed by FMD world reference laboratory, Pirbright, UK.

4) FMDV antisera
Hyperimmune serum against FMDV type O1/EGY/93 and A / EGY/2006 were prepared in calves according to Traub, Mansa (1944). They were prepared at FMD department, Abassia, Cairo, Egypt. The antisera used in ELISA to differentiate between serotypes O, A.

5) Samples
a. Serum samples:
Eight hundred serum samples were collected randomly from cattle in Eight Egyptian governorates (Ismailia, Dakahlia, Monofia, Sharkia, Helwan, Giza, Beni-Suef and Menya), as a hundred samples from each governorates. The serum samples were used to
determine the FMDV circulating antibodies and to detect the non-structure protein by Prio CHECK blocking ELISA as a tool for differentiation between vaccinated and previously infected animals.

**b. Samples for virus isolation**

Eighty samples were collected from the previously mentioned governorates on the base of the presence of clinical signs of FMD, these samples were:

**b.1. Tongue epithelium:**

Two gms of tissue of epithelial tongue was ground with equal amount of sterile sand; veronal buffer was added as 1:2 the mixture was centrifuged at 7000 r.p.m for 10 minutes. The supernatant was collected. The treated fluid was used in virus isolation and strain type identification.

**b.2. Oesophageal-pharyngeal fluid (OP)**

OP fluid was collected by probing sampling cup (*Kitching and Donaldson, 1987*). Each sample was treated with chloroform, centrifuged at 7000 r.p.m for 10 minutes; the supernatants were used in virus isolation and the identification.

**6) ELISA test**

ELISA test was carried out for determination of antibodies against FMDV serotypes O1, A according to *Laila EL-Shahawy et al., (1984)* and *Chenard et al., (2003)*.

**7) Prio CHECK FMDV blocking ELISA test:**

It was used to differentiate between previously vaccinated and infected animals, by detection the 3ABC non-structure protein. It was supplied by Prionics Lelystad B.V., NL-8203 AG. Lelystad, Netherlands.
Fig. (1) Map of Egypt showed governorates under investigation

Table No. (1): Determination of FMDV circulating antibodies in cattle in different Egyptian governorates
<table>
<thead>
<tr>
<th>Governorate</th>
<th>Number of serum samples</th>
<th>ELISA test (O) serotype</th>
<th>Priocheck ELISA test (A) serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ismailia</td>
<td>100</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>Dakahlia</td>
<td>100</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td>Monofia</td>
<td>100</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Sharkia</td>
<td>100</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>Helwan</td>
<td>100</td>
<td>61</td>
<td>19</td>
</tr>
<tr>
<td>Giza</td>
<td>100</td>
<td>44</td>
<td>21</td>
</tr>
<tr>
<td>Beni-Suef</td>
<td>100</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>Menya</td>
<td>100</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>800</td>
<td>309/800</td>
<td>135/800</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td>38.62</td>
<td>16.87</td>
</tr>
</tbody>
</table>

Table No. (2): Isolation of FMDV from some Egyptian governorates in primary kidney cell culture and baby mice.

<table>
<thead>
<tr>
<th>Governorate</th>
<th>*Number and type of collected samples</th>
<th>Baby mice</th>
<th>**CKC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OP</td>
<td>T.E.</td>
<td>OP</td>
</tr>
<tr>
<td>Ismailia</td>
<td>9</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Dakahlia</td>
<td>8</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Monofia</td>
<td>11</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Sharkia</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Helwan</td>
<td>9</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Giza</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Beni-Suef</td>
<td>8</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Menya</td>
<td>9</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>9</td>
<td>50/71</td>
</tr>
<tr>
<td>Percentage</td>
<td>70</td>
<td>66.6</td>
<td>64.7</td>
</tr>
</tbody>
</table>

* OP = oesophageal pharangal fluid. T.E. = tongue epithelum

** CKC = Primary calf kidney cell culture
Table No. (3): Identification of FMDV serotypes O, A by the use of ELISA.

<table>
<thead>
<tr>
<th>Governorate</th>
<th>ELISA test</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OP*</td>
<td>T.E.**</td>
<td>OP*</td>
<td>T.E.**</td>
</tr>
<tr>
<td></td>
<td>(O) serotype</td>
<td>(A) serotype</td>
<td>(O) serotype</td>
<td>(A) serotype</td>
<td></td>
</tr>
<tr>
<td>Ismailia</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dakahlia</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Monofia</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sharkia</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Helwan</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Giza</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Beni-Suef</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Menya</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* Positive 51 OP samples.  
**Positive 6 T.E. samples.

Foot and Mouth Disease (FMD) and its related research aspects represent a wide; interesting and progresable field for veterinary scientists. It was found that Egypt is usually threatened by FMD outbreaks which were; at most cases; caused by FMDV type O & A (Abd El-Rahman et al., 2006). So it is of interest to follow up the circulating antibodies against the virus as well as comparing between the circulating virus serotypes to detect any change in the field isolates rather than the serotypes used in the locally produced vaccine to get a good quality vaccine that could able to protect the Egyptian animals against the old established and a new arisen FMDV strains, because in a country in which control of FMD relies predominately on vaccination, the stability of the currently used vaccine in high potency is the only way to protect susceptible animal against FMD outbreaks (Farag et al., 2005).

Basically FMDV circulating antibodies may be due to vaccination or previous infection. So, in this study eight hundred random serum samples were collected from 8 governorates represent lower, Med and Upper Egypt.
The serum samples were investigated by ELISA test and Priocheck blocking ELISA to determine the circulating FMDV antibodies against serotypes (O₁) & (A) and to differentiate between cattle vaccinated with Egyptian bivalent locally produced FMD vaccine and the previously infected one.

Table No. (1), showed that, 544 serum samples out of 800 samples (68%) were contained antibodies against FMDV of both types (O₁) and (A). 38.62% of type (O₁) and 16.87% was due to type (A). Priocheck blocking ELISA indicated that, 92 samples out of 444 samples (positive ELISA these this results indicated that the infection is still occurred (DeDiego et al., 1997; Sorensen et al., 1998; Chung et al., 2002; Clavijo et al., 2004 and Laila et al., 2004). Sorensen et al., 1997 and Chung et al., (2002) found that antibodies to the 3ABC antigen in cattle and sheep could not detected earlier than 10 days post experimental infection, for serotype (O₁) antibodies Helwan was the highest governorate to detect antibodies (61 samples out of 100), while Menya was the lowest (18 samples out of 100). For FMDV serotype (A), the highest governorates to detect antibodies were Ismailia, Giza, Sharkia and Beni-Suef (about 22-20 sample out of 100).

According to serum collected samples, the antibodies against both types FMDV (O₁, A) are moderate (68%), the rest animals are free from antibodies, that is mean that, about 30% of the animals in previously mentioned 8 governorates are susceptible to FMD infection.

On the other hand virological samples were collected on the base of features of FMD (Clinical FMD) Mann and Sellers 1990.

Table No. (2), exhibited, that both types of FMDV (O₁, A) were isolated from OP and tongue epithelium. The collected samples were inoculated in unweaned baby mice and primary calf kidney cell culture (64.7% - 70%) of OP samples were positive and (55.5% - 66.6%) of tongue epithelium were positive too.

Table No. (3) Indicated that, Ismailia, Sharkia, and Beni-Suef were free from serotype (A) according to OP samples collected, while both serotypes were identified in OP
samples collected from Dakahlia, Monofia, Helwan, Giza and Menya. When tongue epithelium used, the results showed that, Ismailia, Monofia, Helwan and Beni-Suef were free from serotype (O), while serotype (A) identified from Ismailia and Helwan. Accordingly, it can be concluded from these results that, both types of FMD O₁ & A still existing and circulating in Egypt with a moderate circulating antibodies (68%), that means that about 30% of Egyptian cattle are under risk of FMD outbreaks.

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ليلى الشهاوي، عادل عزب، حليمة الوطني، مجدى عبد العاطى، هاني أبو النجا، عبير طلعت، وائل مسعد، عمرو إسماعيل، إيهاب السيد
قسم بحوث الحمي القلاعية، معهد بحوث الامصال واللقاحات البيطرية، العباسي – القاهرة
- جمهورية مصر العربية ص. ب. 131

الملخص العربي
تمت هذه الدراسة في ثمانية محافظات تمثل مصر السفلي والوسطى والعليا والمحافظات هي (الاسماعيلية، الدقهلية، المنوفية، الشرقية، حلوان، الجيزة، بني سويف، المنها). تم جمع 800 عينة عشوائية من مصل الابقار بمعدل 100 عينة لكل محافظة كذلك جمعت 80 عينة من الحلق والغشاء الطلائي للسان لحيوانات ظاهر عليها اعراض الحمي القلاعية وخاصة اللعاب والاصابة في طلائي اللسان تم تسخين تلك العينات وتفعيلها. تم اختبار عينات المصل ضد نوعي الفيروس (O1, A) بواسطة استخدام اختبار ELISA. اختبار الليمكوندش ELISA للفيروسات المحصنة بحثل والسباق عدوها والمراض. تم إجراء الاختبارات الفيروسية بواسطة حقن العينات الفيروسية في الفئران السويسرية الضعيفة وكشف النتائج باستخدام اختبار ELISA. اظهرت النتائج ان الأجسام المناعية ضد فيروس حمي القلاعية منتشرة في محافظات الدراسة بنسبة 89٪ بينما ظهرت اختبار الليمكوندش ELISA للعوامل المحصنة باللقاح والسباق عدوها للمرض. هذا الاختبار يفرق بين الحيوان المصاب والمحصن. اظهرت الدراسة ان نوعي العوارض لفيروس حمي القلاعية O1 & A ما زالت موجودة ومنتشرة في الحيوانات المصرية في المحافظات التي تم نشراء الدراسة.

الدراسة جزء من مشروع التوصيف الجئي للعوارض المصرية لفيروس حمي القلاعية بدعم من صندوق العلوم والتنمية التكنولوجية STDF.