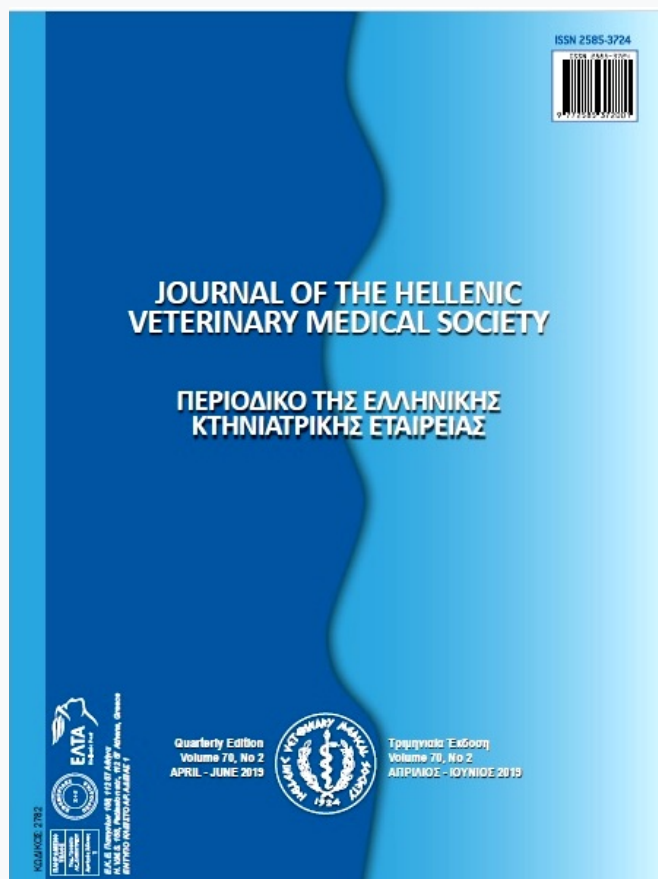


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Assessment of the efficacy of routine vaccination on the magnitude of Foot and Mouth Disease outbreak in Kafrelsheikh governorate, Delta Region, Egypt

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ABSTRACT. Foot and mouth disease (FMD) is a highly contagious viral disease causes a serious economic impact on livestock production and trading. FMD is an endemic disease in Egypt and a national control program that depends on routine obligatory vaccination of all ruminant species is being followed for disease control. A nation-wide epidemic of FMD was commenced in early 2015 and typical clinical signs of the disease were observed even in vaccinated animals. The morbidity and case fatality rates were high enough to be investigated. In the current study, non-vaccinated and vaccinated animals of different sex and ages were examined to evaluate the efficacy of FMD different vaccines used in Egypt. Clinical, post-mortem and serological examinations were used to confirm the infection, while the molecular investigation was applied to identify the serotype responsible for this epidemic. The incidence rate and the attributable proportion (fraction) of FMD cases which could be avoided by vaccination and vaccine efficacy were calculated. The obtained results confirmed the infection with FMD virus (FMDV) serotype O in both non-vaccinated and vaccinated animals. The incidence of FMD was 86.67% among non-vaccinated animals, while it was ranged from 15% to 31.8% among vaccinated animals according to the type of vaccine used. The attributable fraction was 73.9% and the efficacy of the three used vaccines was 63.3%, 76.92% and 82.25% for Tri-Aphthovac, VSVRI and Meriel vaccines, respectively. In conclusion, vaccination in Egypt is able to minimize the magnitude of outbreaks caused by the same serotype found in the vaccine but was not able to prevent the infection and eliminate the disease. The highest vaccination efficacy was found in Mid-aged animals and male cattle.

Keywords: FMD, Vaccine efficacy, Egypt

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INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. Foot and Mouth Disease virus (FMDV) virus belongs to genus *Aphthovirus* of family *Picornaviridae* and has 7 distinct serotypes under which many subtypes exist. In Egypt, outbreaks are being reported since 1950 and FMDV serotype O is the most prevalent circulating serotype in most of these outbreaks (Aidaros, 2002; Knowles, et al., 2007). FMDV serotype A was reported in 1953, 1956, 1958, 1967 and in a major outbreak during 2006 (Mackay et al., 1998; Farag et al., 2006; Knowles et al., 2007). In addition, FMDV serotype SAT2 was reported in 1950 and a major outbreak in 2012 (FAO, 2012; Kandeil et al., 2013; Shawky et al., 2013).

The disease has serious economic effects on bovine production and trading because of its transboundary nature of transmission (OIE, 2009). FMD is characterized by fever, lameness, oral lesions and marked salivation. Carrier state usually develops when immunized animals are subjected to infection as well as after the clinical recovery of the diseased animals. Control of FMD depends on the prevention of virus transmission from infected to susceptible animals (in free countries) or by reducing the number of susceptible animals through vaccination (in endemic countries) (Aidaros, 2002). FMD vaccines are usually multivalent because of the limited cross protection between FMDV serotypes (Kandeil et al., 2013). In Egypt, three different FMD vaccines are available; the governmental vaccine prepared by Veterinary Serum and Vaccine Research Institute (VSVRI, Abbasia, Egypt), Tri apthovac vaccine produced by ME VAC company (Cairo-Egypt) and polyvalent Merial vaccine (Merial, France). The two former vaccines contain inactivated A, O and SAT2 serotypes of FMDV. The last vaccine is a commercial vaccine being purchased privately on a wide scale of the farmers, especially large farms to control FMD. This vaccine contains 6 serotypes of FMDV and this is the reason of preference to it by some farmers who believe that this vaccine is more protective and could prevent the deleterious effect of FMD. The first two vaccines are being used in the national control program carried out by the general organization of Veterinary Services (GOVS). This program relies on the vaccination of all ruminant species except camels in Egypt with FMD vaccine twice a year. The vaccination coverage every year does not reach the required figure of target animal population targeted by vaccination due to lack

of funding to produce sufficient doses of vaccine every year. This study aimed to assess the vaccination efficacy of the three mentioned vaccines under the field conditions at Kafrelsheikh Governorate (Egypt), describe the main clinical signs in clinically infected animals and identify the circulating FMDV serotype in both non-vaccinated and vaccinated animals in this locality.

MATERIAL AND METHODS

Ethical statement This study was conducted following the ethical protocols and guidelines of the Medical Ethics Committee, Faculty of Medicine, Assiut University, Egypt.

Study area

The current study was conducted in Kafrelsheikh Governorate; an area of 3,437 km² with a very high density of livestock in heart of the Nile Delta. The Governorate consists of 10 districts and 206 villages. Kafrelsheikh is located in the northern part of Egypt, along with the western branch of the Nile and its capital is Kafrelsheikh City (Fig. 1).

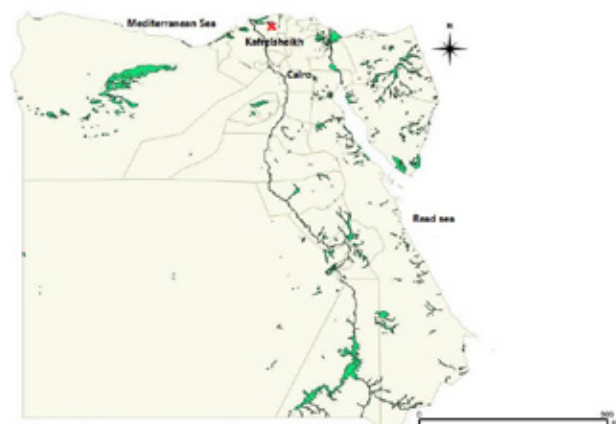


Figure 1. Map shows the area of the study.

Animals

A total of 180 cattle were selected randomly under the field conditions during 2015 FMD outbreak in Kafrelsheikh Governorate. Animals included in the study were divided into two groups, 105 animals were non-vaccinated and 75 animals were vaccinated according to the vaccination program in Egypt (two doses with 6 month interval). These vaccinated animals were vaccinated with one of the three different vaccines commonly used in Egypt; 40 and 22 animals were vaccinated with the vaccine produced by Veterinary Serum and Vaccine Research Institute (VSVRI)

and Tri-Aphthovac vaccine (commercial vaccine produced by the Middle East for Vaccines (ME VAC) company), respectively. These vaccines are being used in the national compulsory vaccination campaigns of the GOVS. Also, 13 animals were vaccinated with the commercial vaccine produced by Merial.

Clinical examination

The animals were routinely examined for the clinical symptoms of FMD with special attention for the body temperature, mouth cavity, inter-digital space and heart sounds (Jackson and Cockcroft, 2002)

Serological Diagnosis

During the virus replication in the infected animals there are different types of the non-structural proteins (NSPs) are generated and they are a potential target for the immune system. Therefore, those infected animals produce a considerable titer of antibodies (Abs) against NSPs. These Abs are the target of 3ABC ELISA test to differentiate between clinically infected or carrier animals from immunized animals after vaccine application (Brocchi et al., 2006; Clavijo et al., 2004). Serum samples were collected from animals included in the study and tested by SVANOVIR® FMDV 3ABC-Ab ruminant (Boehringer Ingelheim Svanova, Sweden). This ELISA kit differentiates between the antibodies of the active infection and vaccination and positive samples have percent positive positivity (PP) ≥ 48

Histopathology

Tissue samples from the oral and lingual mucosa were collected from clinically infected animals. Oral, lingual mucosa heart and lung were collected from the dead animals; all these samples were subjected to histopathology (Alexandersen, 2003; Alexandersen

and Mowat, 2005).

Molecular Diagnosis

RNA-Extraction

Tissue samples and vesicular fluid were collected from clinically infected animals. Samples transported to the laboratory on ice in viral transport media containing equal volumes of glycerol and phosphate buffered saline (pH 7.2- 7.6). Viral RNA was extracted using the GeneJET RNA Purification Kit (Thermo Fisher SCIENTIFIC - K0731).

Reverse Transcription PCR (RT-PCR)

All samples underwent testing using a two-step RT-PCR. During the first step, Applied Biosystem Kit Cat. No. 4374966 was used for cDNA synthesis. All cDNAs were tested using the P1/P2 universal primers, as well as the serotype-specific primers, respectively (Table 1). Thermal cycling conditions for the universal primers were as follows: 95 °C for 5 min, followed by 45 cycles of 94 °C for 30 seconds, 48°C for 30 seconds, and 72 °C for 1 min. A final extension was performed at 72 °C for 10 minutes. The annealing temperature (48 °C) was modified when serotype-specific primers were used. It was 46 °C for serotype O primers, 60 °C and 56 °C for general SAT and serotype SAT2 primers and 55 °C for serotype A primers (EL-Kholy et al., 2007; EL-Shehawy et al., 2011; EL-Khabaz and AL-Hosary, 2017).

Gel Electrophoresis

The PCR products were subjected to 1% agarose gel electrophoresis using ethidium bromide staining and gel documentation system to detect positive bands 216, 402, >700, 880, 863-866 bp which are specific of FMD universal primer, serotype O, SAT, SAT2 and Serotype A, respectively.

Table 1. Different primer used during this study according to EL-Kholy et al., 2007 and EL-Shehawy et al., 2011.

| Primer | | Sequence (5' to 3') | Length (nt) | Specific band |
|----------------------------|------------|-----------------------------------|-------------|---------------|
| Universal primer | P1 | 5'- CCTACCTCCTTCAACTACGG-3' | 20 | 216 bp |
| | P2 | 5'-GAAGGGCCCCAGGGTTGGACTC -3' | 21 | |
| Serotype O 1D/2B region | PH1 | 5'-AGC TTG TAC CAG GGT TTG GC-3' | 20 | 402 bp |
| | PH2 | 5'-GCT GCC TAC CTC CTT CAA-3' | 18 | |
| Serotype SAT primers | SAT-ID209F | 5'CCACATACTACTTTTGTGACCTGGA -3' | 25 | ≥ 700 bp |
| | FMD-2B208R | 5'-ACAGCGGCCATGCACGACAG -3' | 20 | |
| Serotype A primers | PH9 | 5'-TAC CAA ATT ACA CAC GGG AA-3' | 22 | 863-866 bp |
| | PH10 | 5'-GAC ATG TCC TCC TGC ATC TG -3' | 20 | |

Epidemiological examination

Risk “Cumulative Incidence” was calculated in both non-vaccinated and vaccinated groups according to the following equation

Number of disease onsets/ the number of animals exposed to risk x 100

The attributable fraction which means the expected percentage of reduction in number cases following vaccination and was calculated according to the following equation:

(Number of disease onsets/ the number of animals exposed to risk) x 100

The attributable fraction which means the expected percentage of reduction in number cases following vaccination and was calculated according to the following equation:

((The Risk for exposed group - risk for the unexposed group)/ Risk for exposed group) x100

Vaccine efficacy or Vaccine effectiveness for each vaccine separately was calculated according to the following equation

((Risk among non-vaccinated group – risk among the vaccinated group)/ Risk among the non-vaccinated group)) X 100 (Thrusfield, 2005).

Risk estimates and vaccine efficacy were also estimated for different age groups and sex of the animals used in this study.

RESULTS

Results of clinical, post-mortem and histopathological examination

The entire seropositive animal showed the clinical symptoms of the disease which include fever ($\geq 40^{\circ}\text{C}$), excessive salivation, vesicles and erosions on the dorsum of the tongue and hard palate and interdigital space associated with interdigital dermatitis and lameness. Some cases suffered from additional complications like inappetence, detached claws, erosions and ulceration on teats and udder and incurable chronic mastitis. Some cases died suddenly without developing any clinical signs.

Fifty-three animals died due to infection with FMD (12 animals from the vaccinated group and 41 from the non-vaccinated group). All of them showed the clinical signs of the disease. All of these animals were subjected to post-mortem examinations showed congestion of the heart and lung. Histopathological

examination revealed the presence of the hydropic degeneration in spinosum cells at covering oral mucosa, this degeneration leads to the appearance of vesicular lesions and subsequently followed by erosive stomatitis. Also, histopathological examination revealed the occurrence of congestion and hemorrhage of the myocardium, lymphocytic interstitial myocarditis, myocardiolysis and extensive myocardiolysis with lymphocytic cell infiltration which is one of the pathognomonic lesions of FMD affections (Fig. 2, 3).

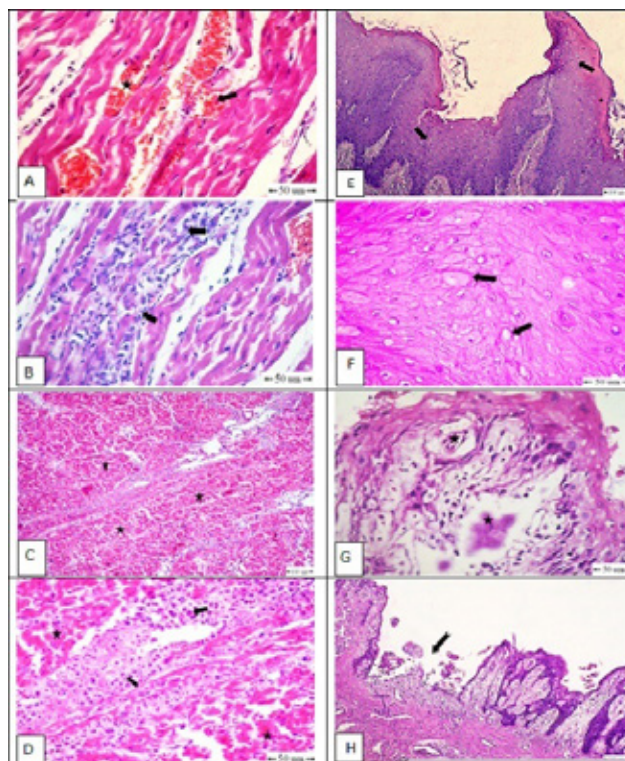


Figure 2. Histopathological finding (I) of heart (A, B, C&D) and oral mucosa (E, F, G &H) from calves infected with FMD. A, Sever congestion (star) and hemorrhage (arrow) in the myocardium (bar=50). B, Lymphocytic interstitial myocarditis (arrow) (bar=50) C, Myocardiolysis (star) (bar=100). D, Extensive myocardiolysis (star) with lymphocytic cell infiltration (arrow) (bar=50). E&F, Hydropic degeneration in spinosum cells at the covering oral mucosa (arrow) (bar=100 & bar=50 respectively). G, Vesicular stomatitis (star) (bar=50). H, Erosive stomatitis (notched arrow) (bar=100).

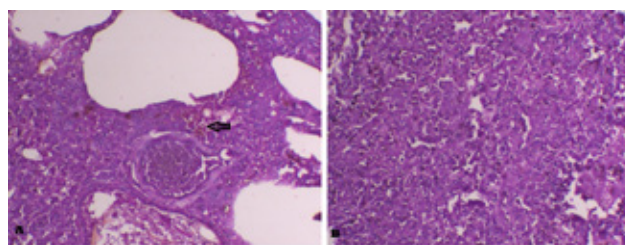


Figure 3. (A) Sever congestion of myocardium and (B) Sever congestion of the lung.

Table 2. Incidence rates in both unvaccinated and vaccinated animals and vaccines efficacy rates.

| Non-Vaccinated | Seropositive | Seronegative | Incidence | Vaccinated | | Seropositive | Seronegative | Incidence | Efficacy |
|----------------|--------------|--------------|-----------|-----------------------|------|--------------|--------------|-----------|----------|
| | | | | Tri-Aphthovac-vaccine | (22) | | | | |
| | | | | (VSVRI) Vaccine | (40) | 7 | 15 | 31.8 % | 63.3% |
| (105) | 91 | 14 | 86.67 | Meriel vaccine | (13) | Seropositive | Seronegative | Incidence | Efficacy |
| | | | | | | 8 | 32 | 20 % | 76.92 % |
| | | | | | | Seropositive | Seronegative | Incidence | Efficacy |
| | | | | | | 2 | 11 | 15.38 % | 82.25 % |

Table 3. Incidence rates in both unvaccinated and vaccinated animals and vaccines efficacy rates according to animal's sex and age.

| Unvaccinated | | | | Vaccinated | | | |
|------------------|--------------|--------------|-----------|--------------|--------------|-----------|------------------|
| sex | seropositive | seronegative | incidence | seropositive | seronegative | incidence | Vaccine efficacy |
| Females | 52 | 8 | 86.7% | 12 | 28 | 30% | 70% |
| Male | 39 | 6 | 86.7% | 5 | 30 | 14.28% | 85.71% |
| Unvaccinated | | | | Vaccinated | | | |
| Age | seropositive | seronegative | incidence | seropositive | seronegative | incidence | Vaccine efficacy |
| Blow one year | 9 | 0 | 100% | 12 | 8 | 60% | 40% |
| ↑1 year:↓5 years | 62 | 14 | 81.58% | 4 | 50 | 7.4% | 92.6% |
| ↑5 year | 20 | 0 | 100% | 1 | 0 | 100% | 0% |

Results of the serological and epidemiological examination

Within the non-vaccinated group 91 out of 105 animals were seropositive (86.7%), while within the vaccinated group only 17 out of 75 animals were seropositive (22.7%); (7, 8 and 2 seropositive out of 22, 40 and 13 selected animals vaccinated with Tri-Aphthovac, VSVRI and Meriel vaccines, respectively) . All these animals were seropositive against the 3ABC NSPs of the virus (Table 2).

The vaccine efficacy was 63.3%, 76.9% and 82.3% for Tri-Aphthovac, VSVRI and Meriel vaccines, respectively. Attributable fraction for vaccine effectiveness during this epidemic was 73.9%.

The incidence rate and vaccine efficacy were affected by some risk factors like sex and age. In the non-vaccinated group the incidence rate was 86.7% in both sexes and 100% in the young animals below one year and old animals above five years while it was 81.58% in animals in mid-age (above one year and below five years). In the vaccinated group, the incidence was higher in females than in males. The vaccine was more efficient in males than females; 85.71% and 70%, respectively. The incidence rate was higher in young animals and old animals of the vaccinated group and it possibly reflects the low efficacy of the

vaccine in these age groups (Table 3).

Results of molecular examination

All clinically affected cases were confirmed positive by using both Universal primer (P1/P2) and specific primer for Serotype O 1D/2B region without co-infection with other serotypes (Fig. 4).

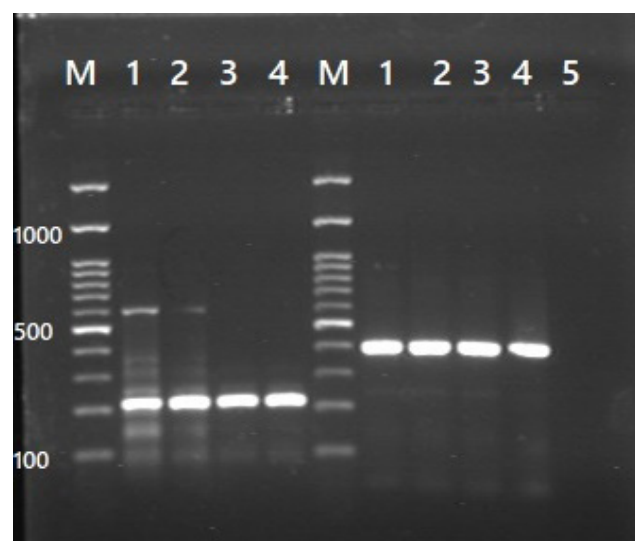


Figure 4. Left, (M) DNA Marker 100bp, Lanes (1:4) positive bands of the universal primer (P1/P2) at 216 bp Right, (M) DNA Marker 100bp Lanes (1:4) positive bands of the specific primer for Serotype O 1D/2B region at 402 bp.

DISCUSSION

Food and mouth disease is an endemic viral disease in Egypt and it remains one of the main obstacles for livestock production. The immunity status of animals is a key factor which influences the clinical outcome of FMD and so, poorly immunized animals are most probably susceptible to this life-threatening disease. On the other hand, animals of intermediate immunity will develop mild symptoms, while those with protective immunity will be asymptomatic. Co-infection with more than one serotype is another important factor influences the clinical severity, the co-infection between serotype O and SAT2 was recorded in previous studies in Assiut Governorate, Upper Egypt (El-Khabaz and Al-Hosary, 2017).

Vaccination is the main preventive measure to protect animals against FMD (Renjun, et al., 2016) particularly in an endemic country of multiple circulating serotypes such as Egypt. On the other hand, all of FMD vaccines provide short-lived immunity and hence it is important to consider the accurate booster vaccinations to prevent the appearance of clinical cases. However, immunization doesn't prevent the development of carrier state and most of the vaccinated animals may have antibody response against the Non-Structural proteins of this virus, particularly against 3ABC, following their exposure to FMDV (Mackay et al., 1998; Parida, 2009). This is maybe possibly the reason for the occurrence of seropositive animals among vaccinated animals observed in the current study.

The obtained results confirmed that the inactivated FMD vaccines provide a protective immunity ranging from 63.3% up to 82.25% according to the type of vaccine against infection with FMDV serotype O which is the only serotype isolated in this study and incriminated in this epidemic. The insufficient vaccine efficacy in this study could result from the short incubation period of the disease which gives the chance for the infection to spread widely before the vaccine protective titer achieved. This theory may explain the appearance of the clinical case and sudden deaths in some vaccinated animals observed in the current study. Because of this scenario, farmers in Egypt think that the vaccine itself is responsible for the development of the clinical cases, particularly in official national vaccination campaign where animals of a village are collected together in one place for vaccination.

The lowest efficacy was observed in case of vac-

cine prepared by ME VAC company - Middle East, Egypt (Tri aphthovac) followed by the vaccine prepared by VSVRI, Abbasia, Cairo, Egypt and the highest efficiency was recorded by Merial commercial vaccine (Merial, France). This finding may be attributed to the way of vaccine handling and injection and the persons who carry out the process of vaccination. Vaccination with the former two vaccines are usually applied by paramedical who usually carries out the vaccination process during the obligatory field vaccination campaign and this causes many problems with the vaccination process. Faults that may have direct effects on the vaccine efficacy include the dose, route or preservation as well as lack of experience, training and understanding of the vaccination protocols before and after vaccine administration. On the other hand, commercial vaccine such as Merial vaccine provides much protection because it is usually applied by a veterinarian rather than paramedical. This agrees with the conclusion of (Fawzy et al., 2017) who declared that vaccination by paramedical and assistant is a weak point in the vaccination process due to faults being followed during the vaccination process.

Some other risk factors have direct effects on the success of vaccination; these factors include animal's age and animal's sex. In the current study, the finding of the animal sex comes in agreement with previous studies that reported that animal sex has no effect on the susceptibility and seropositivity of FMD in non-vaccinated animal (Kibore et al., 2013). On the other hand, some other studies reported a higher incidence rate in female than in males (Hailu et al., 2010). Animal's age plays an important role in the animal susceptibility as in this study the incidence rates were higher in young animals below one year and old animals above five years. This finding may be closely associated with immunity and the ability to produce specific antibodies against this virus (Kibore et al., 2013). On the other hand, the vaccine efficacy was greatly affected by both animal's sex and age. The vaccine was more efficient in males more than females. The incidence rate and efficacy were 14.28% and 85.71% in males and 30% and 70% in females, respectively. This finding may be attributed to some stress factors associated with females like pregnancy and lactation which have direct effects on the ability of immune system to produce the efficient amount of antibodies to protect female animals against this infection. According to the age, the vaccine was more efficient during mid-age (above one year and below five years) where the incidence rate was 7.41% and

the efficiency of vaccination recorded 92.59%. This finding also closely related to the ability of the immune system to produce specific antibodies against this virus. In old and young animals, the immune system is unable to produce enough antibodies (Renjun et al., 2016).

Vaccination against FMD does not provide complete protection against the periodical occurrence of outbreaks in Egypt and the whole vaccination process in the country needs periodical evaluation and updating. On the other hand, active surveillance and molecular epidemiological studies are much required to detect any changes in the virus components and disease epidemiological patterns.

CONCLUSION

This study showed that the vaccination against FMD in Egypt has a protective effect against disease spread and it has a big tendency to minimize the severity of outbreaks caused by the same serotype found

in the vaccine. On the other hand, these vaccines were not able to prevent the infection and eliminate the disease. Vaccination is more possible to protect mid-aged animals and males than other age groups and sex. Finally, we concluded that the vaccine being used has to contain the circulating FMDV serotypes in the study area and this could be achieved through continues epidemiological surveys and molecular identification of circulating virus

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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