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SEROPREVALANCE AND SEROTYPE DETECTION OF FOOT AND MOUTH DISEASE VIRUS IN THREE EGYPTIAN GOVERNORATES DURING 2012 AMONG CATTLE AND BUFFALOES

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A B S T R A C T

Foot and mouth disease (FMD) is still endemic in Egypt despite regular control program to eradicate. In this study, 240 serum samples were collected from Mounofya, Gharbia and Kalubia governorates during 2012 for seroprevalence using commercial ELISA kit (SVANOVIR) based on 3ABC non-structural proteins that differentiate infected from vaccinated animal. Also direct serotype detection from field samples in the herd was carried out using indirect sandwich ELISA. Results showed that, commercial ELISA kit (SVANOVIR) were positive for 35.8% of cattle and 42.0% of buffalo. From a total of 53 samples submitted for direct detection and serotyping of FMDV by indirect sandwich ELISA, 5 were typed as serotype A , 6 as serotype O and 14 as SAT2. All indirect ELISA negative samples also tested negative for FMDV isolation on BHK-21 cell line. In conclusion, serotype SAT2 was circulated and most prevalent serotype in cattle and buffaloes, and different ELISA formats were sensitive and rapid techniques for seroprevalance and serotype detection of FMD virus

Key Words: SVANOVIR ELISA, FMDV, BHK-21, Indirect sandwich ELISA

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1. INTRODUCTION

FMD is a highly contagious, vesicular disease affecting primarily cloven-hoofed animals with severe economic consequences worldwide [**1**]. The causative agent, FMD virus (FMDV), is the prototype species of the Aphthovirus genus within Picornaviridae and seven distinct serotypes A,O, C, South African Territories (SAT) types 1–3 and Asia-1, are distinguished. Till now only Serotypes O and A have been isolated on the Egyptian governorates [**2]**. The RNA genome of approximately 8.5 kb comprises four structural protein genes 1A, 1B, 1C and 1D, which make up the viral capsid and eight non-structural protein (NSP) genes L, 2A, 2B, 2C, 3A, 3B, 3C and 3D responsible for proteolytic cleavage and viral replication [**3**].

Detection of virus-specific antibody can be used for diagnosis, virus neutralization tests (VNTs) and ELISAs for antibodies to structural proteins are used as serotype-specific serological tests **[4]**. In recent years, the non- structural proteins (NSP) 2C and 3ABC has been well documented for differentiation of infected from vaccinated animals with FMDV (DIVA) [**5**]. Perhaps the most reliable single NSP indicator is the polyprotein 3ABC antibodies, which appear to provide conclusive evidence of previous infection [**6**]. The antibodies against 3ABC have been detected up to 395 days post infection in both cattle and buffaloes [**7**]. In Egypt, FMD has taken an enzootic form since over 60 years, where the disease had appeared from time to time

and attacked susceptible animals causing heavy losses in milk, meat and sometimes causing death of young animals. **[8].**

The present study was conducted to investigate the seroprevalence of FMDV antibodies differentiating infected from vaccinated animals among cattle and buffalo herds in Mounofya, Gharbia and Kalubia governorates during 2012 by commercial SVANOVIR, FMD NS ELISA, and rapid serotype detection of FMD virus in suspected field samples obtained from this herd.

2. MATERIALS AND METHODS

2.1. *Serum samples:*

A total of 240 serum samples were randomly collected from cattle and buffaloes in three Egyptian governorates (Mounofya, Gharbia and Kalubia) during 2012. Age of animals ranged from less than one year up more than to 3 years. The samples data is showing in Table (1).

2.2. *Field Samples:*

Fifty three samples include 47 epithelial tissues and 6 osphageo-pharyngeal fluids (OPF) were collected from FMD-suspected cattle and buffalo in Mounofya, Gharbia and Kalubia governorates during 2012. The samples were prepared and tested by indirect sandwich typing ELISA.

2.3. *SVANOVIR FMDV NS ELISA:*

The commercial SVANOVIR, FMD NS ELISA kit provided by IDEXX Laboratories, Netherlands and manufactured by IDEXX Lieberfeld-bern Switzerland. The test detects antibodies against non-structural proteins of FMD. The kit is used according to **[9]**. Samples give percent of inhibition IP =<50% considered negative and that give IP ≥50% considered positive.

2.4. *Indirect sandwich typing ELISA:*

An ELISA kit provided by FAO/IAEA Division, Vienna, Austria was used. The kit was based on a standard indirect sandwich ELISA technique to determine the presence of FMD virus antigens in tissue samples as described by **[10]**.

2.5. *Isolation of suspected FMDVsamples in BHK-21 cell line:*

Samples tested negative by indirect sandwich ELISA were isolated on BHK-21 cell according to **[11]**.

Table 1. Number of serum samples of cattle and buffaloes in relation to the age

Governorates	Age class-1*		Total Age class-1	Age class-2**		Total Age class-2	Age class-3***		Total Age class-3	Total
	B	C		B	C		B	C		
Mounofya	13	17	30	7	13	20	12	16	28	78
Gharbia	19	23	42	10	15	25	13	17	30	97
Kalubia	10	13	23	6	14	20	10	12	22	65
total	42	53	95	23	42	65	35	45	80	240

*Age class-1(age less than 1.5 year), **Age class-2(age less than 3year),***Age class-3(age above 3 year), B: buffaloes, C: cattle.

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3. RESULTS

*3.1. Results of serum samples examined by SVANOVIR ELISA test***:**

As shown in Table (2) the number of total positive samples were 92 samples out of 240 samples (38.3%). The higher percent of positive were found in Mounofya Governorate (31.7%) and the lower is Kalubia Governorate (36.9%).

As shown in Table (3) the numbers of positive samples of cattle were 50 samples out of 140 samples (35.7%) while numbers of positive samples of buffaloes were 42 samples out of 100 samples (42%).

As shown in Table (4) the numbers of positive samples of young stock below 1.5 year were 34 samples out of 95 samples (35.9%) and for age between 1.5-3 year were 28 samples out of 65 samples (43.1%) while numbers of positive samples of adult

were 30 samples out of 80 samples (37.5%).

Table (2): Results of SVANOVIR FMD NS ELISA at Mounofya, Gharbia and Kalubia governorates during 2012.

Governorates	Total tested sera	No .of positive	(%)
Mounofya	78	31	39.7
Gharbia	97	37	38.1
Kalubia	65	24	36.9
total	240	92	38.3

Table (3): The relation between positive serum and animal species.

species	Total tested sera	No .of positive	(%)
cattle	140	50	35.7
buffaloes	100	42	42
total	240	92	38.3

Table (4): The relation between positive serum and animal age.

Age	Total tested sera	No .of positive	(%)
<1.5	95	34	35.9
1.5-3	65	28	43.1
>3	80	30	37.5
total	240	92	38.3

3.2. Direct detection and serotyping of FMDV in field samples using indirect sandwich ELISA: From a total of 53 samples submitted, 5 were typed as FMDV serotype A, 6 as serotype O and 14 as SAT2. The other 28 samples tested negative by ELISA and virus isolation as revealed in table (5).

Table (5): FMDV Serotypes O, A and SAT2 detected in field samples Using Indirect Sandwich ELISA

Type of samples	No .of tested sample	No. of samples positive by ELISA			No. of samples negative by ELISA and virus isolation
		O	A	SAT2	
Epithelial tissue	47	5	4	13	25
OPF*	6	1	1	1	3
total	53	6	5	14	28

* OPF : osphageo-pharyngeal fluid.

3.3. Virus isolation: All indirect ELISA negative samples also tested negative for FMDV isolation on BHK-21 cell line as revealed in table (5).

4. DISCUSSION

The results of SVANOVIR ELISA test were proved the presence of antibodies against non-structural protein (NSP) of FMDV in cattle and buffaloe population in Mounofya, Gharbia and Kalubia Governorates which may be attributed to natural infection of FMDV. As shown in Table (2) the higher percent of positive were found in Mounofya (39.7%), Gharbia (38.1%) and the lower was Kalubia (36.9%).As well as circulation of infection among cattle and buffaloes populations (table 3). This indicates recent outbreaks were reported among cattle and buffaloes in these governorates [**12**].

The highest percent of positive found at samples collected from animals above 3 years. While the lowest found at samples collected from animals less than one year (Table 4). This indicates that the immunity afforded by vaccines does not last long [**13**]. These results indicate that the ELISA-3ABC method could be used as a complementary method for sero epidemiological studies as an indirect indicator of viral activity, as long as the age and vaccination status of the animals being sampled are taken into consideration [**14**].

Indirect sandwich Enzyme Linked Immunosorbent Assay (ELISA) was used for detection and serotyping of FMDV in field samples from cattle and buffaloes in Mounofya Gharbia and Kalubia Governorates governorate (table 5) reported that epithelial tissues contain an abundance of virus, making the detection of the virus easily attainable the OPF and Epithelial tissue were better than other samples as mentioned by **[15]**. Also results confirmed that FMDV serotypes SAT2, A and O circulated in Mounofya Governorate and this come in agreement with **[16]**. The dominant serotype detected were FMDV serotype SAT2 and this clarifies the introduction of new serotype beside

endemic serotypes A and O among animal population.

BHK-21 cell cultures have been shown to be the useful for FMDV isolation [**17**] The failure to isolate FMDV in BHK-21 cells from ELISA-negative samples, suggested that the ELISA was almost as sensitive as virus isolation in BHK-21 cells, at least for the detection of serotype O , A and SAT2 [**18**].

In conclusion, SVANOVIR FMD-NS ELISA, and Indirect sandwich ELISA proved to be simple to perform, rapid and has high sensitivity, it will be very useful for FMD control and diagnosis.

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الانتشار السيرولوجى والكشف عن العترة السيرولوجية لفيروس مرض الحمى القلاعية بين الابقار والجاموس فى ثلاث محافظات مصرية اثناء عام 2012

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الملخص العربى

ما زال مرض الحمى القلاعية مستوطنا مصر على الرغم من الاجراءات المبذولة للسيطرة عليه. لذللك ففى تلك الدراسة تم تجميع 240 عينة سيرم من محافظات المنوفية والغربية والقليوبية خلال عام 2012 لتحديد انتشار الاجسام المناعية لفيروس الحمى القلاعية باستخدام الاليزا التجارية للبروتينات الغير تركيبية 3ABC للفيروس للتفرقة بين الحيوانات المصابة والمحصنة . ايضا تم الكشف المباشر عن عترة الفيروس الموجودة بالعينات الحقلية الماخوذة من تلك الحيوانات باستخدام اختبار الساندوتش اليزا الغير مباشر. وقد اسفرت النتائج واعتمادا على الاليزا التجارية للبروتينات الغير تركيبية 3ABC للفيروس للتفرقة بين الحيوانات المصابة والمحصنة كانت النتائج ايجابية لحوالى 35.8% من الابقار و 42% من الجاموس. وبالكشف المباشر عن عترات فيروس الحمى القلاعية فى 53 عينة حقلية بواسطة اختبار الساندوتش اليزا الغير مباشر تبين ان 5 عينة كانت ايجابية للعترة A و 6 للعترة O و14 عينة SAT2 . محاولة عزل الفيروس على خلايا كلى اليربوع للعينات السالبة للاليزا كانت سالبة. وختاما فان العترة SAT2 لفيروس الحمى القلاعية متواجدة و الاكثر انتسارا فى الابقار والجاموس وان الاشكال المختلفة للاليزا كانت سريعة و ذو حساسية عالية لدراسة الانتشار السيرولوجى والكشف عن العترات السيرولوجية لفيروس الحمى القلاعية.

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