Detection of FMD virus infection in non-vaccinated and vaccinated cattle and buffaloes in Egyptian governorates during 2016

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A B S T R A C T

In this study, 2299 serum samples representing 1745 cattle (453 non-vaccinated and 1292 vaccinated) and 554 buffaloes (131 non vaccinated and 423 vaccinated) were collected from 24 different Egyptian governorates during the year 2016. These samples were adopted for detection of FMD virus infection using commercial ELISA kit based on 3ABC non-structural proteins that differentiate infected from vaccinated animal. Also direct FMD virus serotype detection from 30 vesicular samples, twenty eight from suspected cattle and two from suspected buffalo was carried out using Sandwich typing ELISA. Results showed that, 3ABC ELISA kit were positive 26.04 %(118/453) of unvaccinated cattle, 39.69 % (52/131) of unvaccinated buffaloes, 23.68% (306/1292) of vaccinated cattle and 34.75% (147/423) of vaccinated buffalo. From a total of 30 samples submitted for direct detection and serotyping of FMDV by Sandwich typing ELISA, 4 were typed as serotype O and one as serotype A. three of serotype O were detected in cattle and the other one in buffalo while the positive sample for serotype A were detected in cattle. In conclusion, FMD virus is still endemic in Egypt despite control program to eradicate and serotype O was the most prevalent serotype in cattle and buffaloes.

Key words: FMD virus; cattle and buffalo; 3ABC ELISA kit; Egypt.

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1. INTRODUCTION

FMD is a highly contagious, vesicular disease affecting primarily cloven-hoofed animals with severe economic consequences worldwide (Sutmoller, 2003). 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, A and SAT2 have been isolated on the Egyptian governorates (Salem et al., 2009 and Shawky et al., 2013). 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 (Clavijo et al., 2004). 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 (OIE, 2009). 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) (Lu et al., 2009). Perhaps the most reliable single NSP indicator is the polyprotein 3ABC antibodies which appear to provide conclusive evidence of previous infection (Mackay et al., 1998). The antibodies against 3ABC have been detected up to 395 days post infection in both cattle and buffaloes (Sorensen et al., 1997). The present study was conducted to detect FMD virus infection in non-vaccinated and vaccinated cattle and buffalo population in Egypt using different ELISA format.

2. MATERIALS AND METHODS

2.1. Serum samples

A total of 2299 sera representing 1745 cattle (453 non-vaccinated and 1292 vaccinated)

and 554 buffaloes (131 non vaccinated and 423 vaccinated) were collected from 24 different Egyptian governorates during the year 2016 (table 1). The vaccinated cattle and buffaloes had a previous history to be vaccinated with locally produced inactivated FMDV vaccine containing serotypes O, A and SAT2. These sera were frozen at -20°C until they were used in ELISA for screening sera to the presence of FMDV non-structural proteins antibodies.

2.2. Vesicular fluid samples

A total of 30 vesicular samples were collected from 28 cattle and two buffaloes during the year 2016. These animals were clinically suspected to be infected by FMD virus. These vesicular fluids were frozen at -20°C until they were used in Sandwich typing ELISA for screening sera to the presence of FMDV serotypes O, A, SAT1 and SAT2.

2.3. FMDV 3ABC ELISA

The commercial FMD 3ABC ELISA kit (IZSLER: Brescia, Italy) was used for detection FMDV infection based on serum samples. The test detects antibodies against non-structural proteins of FMDV. The kit was used according to manufacture instructions. The cut off value is 10%, thus samples which gave a value of less than 10% were considered negative while that give values of 10% or higher were considered positive.

2.4. Sandwich typing ELISA

The FMD viral antigen ELISA kit (IZSLER: Brescia, Italy) was used for detection FMDV serotype O, A, SAT1 and SAT2 in vesicular samples. The kit was used according to manufacture instructions. Samples with optical density (OD) < 0.1 were considered negative for FMDV while samples OD ≥ 0.1 were considered positive for FMDV O, A, SAT1 and SAT2 based on the catching type specific monoclonal antibodies (MAbs). The Positive untyped FMDV samples will give OD ≥ 0.1 with the pan-FMDV MAbs and < 0.1 with type specific MAbs.

Table 1: number of sera collected from non-vaccinated and vaccinated cattle and buffaloes sera in different Egyptian governorates during 2016

No.	Governorate	Cattle			Buffuloes		
		Non vaccinated	vaccinated	Total	Non vaccinated	vaccinated	Total
1	Giza	24	116	140	9	30	39
2	Fayoum	29	49	78	9	24	33
3	Cairo	10	7	17	5	8	13
4	Beheraa	39	204	243	7	40	47
5	Kafer El Sheikh	23	26	49	9	16	25
6	Monofya	8	74	82	7	46	53
7	Garbia	4	71	75	0	14	14
8	Kalubia	0	47	47	0	43	43
9	Sharkia	19	105	124	5	40	45
10	Domiat	0	15	15	0	0	0
11	Qina	43	40	83	3	5	8
12	Asuot	8	67	75	4	17	21
13	Elmnya	25	48	73	3	10	13
14	Bani-Sweif	3	65	68	12	14	26
15	Sohag	54	58	112	28	10	38
16	Dakhalia	0	75	75	0	79	79
17	El Wadi El Gedid	74	16	90	0	0	0
18	Luxur	0	15	15	0	0	0
19	Port Said	37	0	37	8	0	8
20	Alexandria	33	32	65	11	14	25
21	Ismailia	6	73	79	5	7	12
22	Suez	3	15	18	6	6	12
23	Aswan	11	63	74	0	0	0
24	South of Sinia	0	11	11	0	0	0
Total		453	1292	1745	131	423	554

3. RESULTS

3.1. Results of FMDV 3ABC ELISA

Serological examination of 2299 serum samples obtained from a herd of unvaccinated and vaccinated cattle and buffaloes in 24 Egyptian governorates during the year 2016 were screened for FMDV antibodies against non-structural protein using 3ABC IZSLER® Kit ELISA. It was found that the number of positive sera were 623 (27.09%) from 2299 total examined sera. FMDV antibodies were detected in 26.04 %(118/453) of unvaccinated cattle, 39.69 %(52/131) of unvaccinated buffalo, 23.68% (306/1292) of vaccinated cattle and 34.75% (147/423) of vaccinated buffalo indicate prevalence of infection among both unvaccinated and vaccinated cattle and buffaloes. From the total positive samples (623), vaccinated cattle exposed 49.11 %(306/623), followed by vaccinated buffalo 23.59 %(147/623), unvaccinated cattle 18.94 % (118/623) and unvaccinated cattle 8.34% (52/623) indicate higher FMDV infection in vaccinated than unvaccinated cattle and buffalo population (table 2).

3.2. Results of Sandwich typing ELISA

From a total of 30 samples submitted for direct detection and serotyping of FMDV by Sandwich typing ELISA, 4 were typed as serotype O and one as serotype A. three of serotype O were detected in cattle and the other one in buffalo while the positive sample for serotype A were detected in cattle (table 3).

Table (2): prevalence of FMDV non-structural protein antibodies in unvaccinated and vaccinated cattle and buffaloes sera in different Egyptian governorates during 2016

No.	Governorate	Non vaccinated						Vaccinated						Total	Positive	Negative
		Cattle			Buffuloes			Cattle			Buffuloes					
		Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative			
1	Giza	24	8	16	9	4	5	116	43	73	30	16	14	179	71	108
2	Fayoum	29	17	12	9	5	4	49	15	34	24	13	11	111	50	61
3	Cairo	10	8	2	5	3	2	7	3	4	8	1	7	30	15	15
4	Beheraa	39	8	31	7	2	5	204	37	167	40	11	29	290	58	232
5	Kafer El Sheikh	23	8	15	9	3	6	26	5	21	16	5	11	74	21	53
6	Monofya	8	0	8	7	4	3	74	7	67	46	3	43	135	14	121
7	Garbia	4	2	2	0	0	0	71	23	48	14	7	7	89	32	57
8	Kalubia	0	0	0	0	0	0	47	12	35	43	16	27	90	28	62
9	Sharkia	19	4	15	5	3	2	105	26	79	40	17	23	169	50	119
10	Domiat	0	0	0	0	0	0	15	12	3	0	0	0	15	12	3
11	Qina	43	3	40	3	0	3	40	8	32	5	1	4	91	12	79
12	Asuot	8	2	6	4	2	2	67	7	60	17	2	15	96	13	83
13	Elmnya	25	2	23	3	0	3	48	18	30	10	4	6	86	24	62
14	Bani-Sweif	3	1	2	12	8	4	65	12	53	14	8	6	94	29	65
15	Sohag	54	8	46	28	4	24	58	6	52	10	0	10	150	18	132
16	Dakhalia	0	0	0	0	0	0	75	19	56	79	35	44	154	54	100
17	El Wadi El Gedid	74	5	69	0	0	0	16	1	15	0	0	0	90	6	84
18	Luxur	0	0	0	0	0	0	15	3	12	0	0	0	15	3	12
19	Port Said	37	28	9	8	3	5	0	0	0	0	0	0	45	31	14
20	Alexandria	33	6	27	11	5	6	32	1	31	14	5	9	90	17	73
21	Ismailia	6	2	4	5	2	3	73	35	38	7	3	4	91	42	49
22	Suez	3	2	1	6	4	2	15	0	15	6	0	6	30	6	24
23	Aswan	11	4	7	0	0	0	63	10	53	0	0	0	74	14	60
24	South of Sinia	0	0	0	0	0	0	11	3	8	0	0	0	11	3	8
Total		453	118	335	131	52	79	1292	306	986	423	147	276	2299	623	1676
%*		100	26.0486	73.9514	100	39.6947	60.3053	100	23.6842	76.3158	100	34.7518	65.2482	100	27.0987	72.9013
%**			18.9406			8.34671			49.1172			23.5955			100	

Table 3: prevalence of FMDV infection in cattle and buffaloes by Sandwich typing ELISA in vesicular fluid during the years 2016

Species	Total samples	FMD virus serotype			
		O	A	SAT2	Total
cattle	28	3	1	0	4
buffaloes	2	1	0	0	1

4. DISCUSSION

Diagnosis of FMD begins with clinical investigation of animals suspecting of being infected followed by confirmation of the presence of FMDV antigen or genome using laboratory methods vesicular epithelium is the sample of choice, being in rich in virus during the acute phase of the disease (Reid et al., 2010)

In this study, we firstly report the prevalence FMD virus among non-vaccinated and vaccinated cattle and buffalo population in 24 governorates in Egypt during 2016. The data of 3ABC ELISA test (IZSLER) was proved the presence of antibodies against non-structural protein (NSP) of FMD virus in 26.04 %(118/453) of unvaccinated cattle, 39.69 %(52/131) of unvaccinated buffalo, 23.68% (306/1292) of vaccinated cattle and 34.75% (147/423) of vaccinated buffalo may be attributed to natural infection of FMDV and carrier state in these animals (Alexandersen and Mowat 2005).

The four structural protein (1A, 1B, 1C & 1D) of the P1 region are enclosing the RNA genome of the virus whereas, the eight non-structural proteins (2A, 2B, 2C&2D, 3A 3B, 3C &3D) are involved in the live cycle of the virus inside the infected cells. the most important non-structural protein are 2C, 3A& 3C there are three parties are responsible for cell membrane vesicle proliferation, the pathogenesis and inhibition of host cell protein transcription respectively (Pereda et al., 2002).

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 (Ferris et al., 2011)

The epithelium or vesicular fluid collected from an unruptured or recently ruptured vesicle was considered the sample of choice for FMD virus detection (OIE 2000) and contain high titer of virus during the acute phase (3 to 4 day) of disease (Clavijo and Kitching 2003).

The sandwich ELISA was used recombinant integrin avβ6 as a capture ligand and serotype specific monoclonal antibodies (Mabs) as detecting reagents. The integrin MabElits recognized FMDV strains of wide antigenic and molecular diversity of all seven serotypes (Ferris et al., 2011)

Serotypes O, and A were detected in field samples from non-vaccinated cattle and buffalo by sandwich typing ELISA that represents a potential hazard in many countries resulting in the disease remaining endemic for long periods despite vaccination (Maddur et al., 2008). Also this activates the appearance of new subtypes of FMD virus as a result of presence of infected population beside vaccinated one (Alexandersen et al 2002).

Conclusion: FMD virus is still endemic in Egypt despite regular control program to eradicate and serotype O was the most prevalent serotype in cattle and buffalo.

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