Detection of FMD virus infection in non-vaccinated and vaccinated sheep and goats in Egypt

Gabr F. El-Bagoury [1], Ehab M. El Nahas [1], Dina A. Abd-Elwahed [2], Sayed A.H Salem [2]

[1]*Department of Virology, Faculty of Veterinary Medicine, Benha University, Moshtoher, Benha, Egypt,*[2] *Department of Virology, Animal Health Research Institute, Dokki, Giza, Egypt.*

A B S T R A C T

In this study, 2024 serum samples representing 1341 sheep (625 non vaccinated and 716 vaccinated) and 683 goats (301 non vaccinated and 382 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 (IZSLER) based on 3ABC non-structural proteins that differentiate infected from vaccinated animal. Also direct FMD virus serotype detection from 30 vesicular fluid samples, twenty from suspected sheep and ten from suspected goat was carried out using Sandwich typing ELISA. Results showed that, 3ABC ELISA kit were positive 18.72 %(117/625) of unvaccinated sheep, 20.93 %(63/301) of unvaccinated goats, 22.20% (159/716) of vaccinated sheep and 19.10% (73/382) of vaccinated goat. From a total of 30 samples submitted for direct detection and serotyping of FMDV by Sandwich typing ELISA, 4 were typed as serotype O, two A and one as serotype SAT2. Three of serotype O was detected in sheep and the other one in goats while the positive sample for serotype A and SAT2 were detected in sheep. In conclusion, FMD virus is still endemic in Egypt despite regular control program to eradicate. Serotype O was the most prevalent serotype in sheep and goats beside presence of A and SAT2 in sheep.

Key words: FMD virus; sheep and goats; 3ABC ELISA kit; Egypt.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 502-507, 2018)**

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 (OIE, 2009). It affects cattle, sheep, goats, deer and pigs (Ferguson et al., 2001). 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). Control of FMD virus depend on early diagnosis that confirmed by objective diagnostic tests. So, diagnostic test procedures should be rapid, sensitive and specific (Ferris et al. 2004). ELISA was applied for diagnosis of FMD with possible serotyping of FMD virus at high sensitivity and specificity (Knowles et al. 2007). So, the present study was conducted to detect FMD virus infection in non-vaccinated and

vaccinated sheep and goats' population in Egypt using different ELISA format.

2. MATERIALS AND METHODS

2.1. Serum samples

A total of 2024 sera representing 1341 sheep (625 non-vaccinated and 716 vaccinated) and 683 goats (301 non-vaccinated and 382 vaccinated) were collected from 24 different Egyptian governorates during the year 2016 (table 1). The vaccinated sheep and goats 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 20 sheep and 10 goats 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.

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

No.	Governorate	sheep			goatss		
		Non vaccinated	vaccinated	Total	Non vaccinated	vaccinated	Total
1	Giza	95	61	140	20	5	39
2	Fayoum	13	20	78	34	32	33
3	Cairo	5	8	17	10	7	13
4	Beheraa	117	70	243	41	53	47
5	Kafer El Sheikh	13	31	49	23	9	25
6	Monofya	24	56	82	30	25	53
7	Garbia	15	28	75	9	39	14
8	Kalubia	15	12	47	18	30	43
9	Sharkia	28	52	124	26	55	45
10	Domiat	0	9	15	0	6	0
11	Qina	55	20	83	11	3	8
12	Asuot	13	43	75	11	17	21
13	Elmnya	47	18	73	10	6	13
14	Bani-Sweif	10	35	68	11	30	26
15	Sohag	0	0	112	0	0	38
16	Dakhalia	10	117	75	5	14	79
17	El Wadi El Gedid	84	0	90	6	0	0
18	Luxur	0	15	15	0	0	0
19	Port Said	22	8	37	8	7	8
20	Alexandria	31	28	65	19	12	25
21	Ismailia	4	36	79	3	16	12
22	Suez	9	11	18	6	4	12
23	Aswan	15	27	74	0	4	0
24	South of Sinia	0	11	11	0	8	0
	Total	625	716	1341	301	382	683

3. RESULTS

3.1. Results of FMDV 3ABC ELISA

Serological examination of 2024 serum samples obtained from a herd of unvaccinated and vaccinated sheep and goat 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 412 (27.09%) from 2024 total examined sera. FMDV antibodies were detected in 18.72% (117/625) of unvaccinated sheep, 20.93% (63/301) of unvaccinated goat, 22.20% (159/716) of vaccinated sheep and 19.10% (73/382) of vaccinated goat indicate prevalence of infection among both unvaccinated and vaccinated sheep and goat. From the total positive samples (412), vaccinated sheep exposed 38.59% (159/412), followed by unvaccinated sheep 28.39% (117/412), vaccinated goat 17.71% (73/412) and unvaccinated goat 15.29% (63/412) indicate higher FMDV infection in vaccinated and unvaccinated sheep than goat 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, two A and one as serotype SAT2. Three of serotype O was detected in sheep and the other one in goat while the positive sample for serotype A and SAT2 were detected in sheep (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	%
		Sheep			Goat			Sheep			Goat					
		Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative			
1	Giza	95	35	60	20	5	15	61	30	31	5	3	2	181	73	40.3315
2	Fayoum	13	7	6	34	16	18	20	4	16	32	13	19	99	40	40.404
3	Cairo	5	3	2	10	4	6	8	1	7	7	0	7	30	8	26.6667
4	Beheraa	117	20	97	41	3	38	70	7	63	53	7	46	281	37	13.1673
5	Kafer El Sheikh	13	3	10	23	6	17	31	4	27	9	2	7	76	15	19.7368
6	Monofya	24	3	21	30	1	29	56	5	51	25	0	25	135	9	6.66667
7	Garbia	15	6	9	9	0	9	28	5	23	39	16	23	91	27	29.6703
8	Kalubia	15	4	11	18	6	12	12	0	12	30	3	27	75	13	17.3333
9	Sharkia	28	3	25	26	3	23	52	13	39	55	10	45	161	29	18.0124
10	Domiat	0	0	0	0	0	0	9	0	9	6	0	6	15	0	0
11	Qina	55	1	54	11	1	10	20	6	14	3	0	3	89	8	8.98876
12	Asuot	13	4	9	11	3	8	43	8	35	17	3	14	84	18	21.4286
13	Elmnya	47	15	32	10	10	0	18	4	14	6	4	2	81	33	40.7407
14	Bani-Sweif	10	1	9	11	0	11	35	5	30	30	4	26	86	10	11.6279
15	Sohag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Dakhalia	10	2	8	5	1	4	117	31	86	14	1	13	146	35	23.9726
17	El Wadi El Gedi	84	1	83	6	0	6	0	0	0	0	0	0	90	1	1.11111
18	Luxur	0	0	0	0	0	0	15	4	11	0	0	0	15	4	26.6667
19	Port Said	22	1	21	8	0	8	8	0	8	7	0	7	45	1	2.22222
20	Alexandria	31	1	30	19	1	18	28	4	24	12	3	9	90	9	10
21	Ismaillia	4	0	4	3	1	2	36	13	23	16	4	12	59	18	30.5085
22	Suez	9	6	3	6	2	4	11	1	10	4	0	4	30	9	30
23	Aswan	15	1	14	0	0	0	27	14	13	4	0	4	46	15	32.6087
24	South of Sinia	0	0	0	0	0	0	11	0	11	8	0	8	19	0	0
Total		625	117	508	301	63	238	716	159	557	382	73	309	2024	412	20.3557
%*		100	18.72	81.28	100	20.9302	79.0698	100	22.2067	77.7933	100	19.1099	80.8901	100	20.3557	1.00572
%**			28.3981			15.2913			38.5922			17.7184			100	

***** %: indicate positive percent from total tested sera in each species, **%: indicate positive percent from total positive samples (412)

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

Species	Total samples	FMD virus serotype			
		O	A	SAT2	Total
sheep	20	3	1	1	5
goats	10	1	0	0	1

4. DISCUSSION

FMD is economically the most important viral disease of domesticated and mild ruminants such as cattle, buffalo, sheep, goats and dear. 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 by products (Thomson, 1994)

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 sheep and goat 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 18.72 %(117/625) of unvaccinated sheep, 20.93 %(63/301) of unvaccinated goat, 22.20% (159/716) of vaccinated sheep and 19.10% (73/382) of vaccinated goat 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 and 1D) of the P1 region are enclosing the RNA genome of the virus whereas, the eight non-structural proteins (2A, 2B, 2C and 2D, 3A 3B, 3C and 3D) are involved in the live cycle of the virus inside the infected cells. the most important non-structural protein are 2C, 3A and 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 Mabs recognized FMDV strains of wide antigenic and molecular diversity of all seven serotypes (Ferris et al., 2011)

Serotypes O, A and SAT2were 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 sheep and goat beside presence of A and SAT2 in sheep.

5. REFERENCES

Alexandersen, S., Zhang, Z., Donaldson, A.I., (2002). Aspects of the persistence of foot-and-mouth disease virus in animals-the carrier problem. Microbes Infect. 4, 1099–1110

Alexandersen, S., Mowat, N., (2005). Foot-and-mouth disease: host range and pathogenesis. Curr. Top. Microbiol. Immunol. 288, 9–42

Clavijo, A. and Kitching, P., (2003): The nature and Diagnosis of FMD clinical Microbiology Newsletter; 25 (11), 81-88

Clavijo, A.,Wright, P., Kitching, P.(2004): Developments in diagnostic techniques for differentiating infection fromvaccination in foot-and-mouth disease. Vet. J. 167, 9–22

Ferguson M. N.; Christl A. D. and Roy M.(2001): The Foot-and-Mouth Epidemic in Great Britain: Pattern of Spread and Impact of Interventions. Science, May 11; 292 (5519), 1155-1160

Ferris. N; Grazioli. S; Hutchings. G, and Brocchi. E, (2011): Validation of recombinant integrin avB6 / monoclonal antibody based antigen ELISA for the diagnosis of FMD.Journal of virological methods 175: 253-260

Ferris, N; Scott, R; Donald, K; Ceoff, H and Andrew, S. (2004): Prospects for improved laboratory diagnosis of FMD using real time RT- PCR. Eu Comm. Control of FMD China. Crete. Greece, 11-15 Oct., 2004, Appendix; 42: 261-267

Knowles,N.J., Wadsworth , J., Reid, S. M., Swabey, K. G., El-Kholy, A. A., Abd El-Rahman, A. O., Soliman, H. M., Ebert, K., Ferris, N. P., Hutchings, G. H., Shaw A.E., Reid S.M., Ebert K., Hutchings G.H., Ferris N.P. and King D.P. (2007): Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot- and-mouth disease. Journal of Virological Methods 143(1): 81-5

Lu, Z., Zhang, X., fu, Y., Tian, M., Sun, P., Li, D., Liu, Z. Xie, Q. (2009): Expression of the major epitope regions of 2C integrated with the 3AB non-structural protein of foot- and-mouth disease virus and its potential for differentiating infected from vaccinated animals. J. virol. Methods.170 (1- 2): 128-33

Mackay, D.K.J., Forsyth, M.A, Davis, P.R., Belsham, G.J., Flint, M. and Rayon, M.D. (1998): differentiating infection from vaccination in FMD using a panel of recombinant non- structural proteins in ELISA. Vaccine. 16:446-459.

Maddur MS, Gajendragad MR, Gopalakrishna S, Singh N. (2008). Comparative study of experimental Foot-and-Mouth Disease in cattle (Bos indicus) and buffaloes (Bubalis bubalus). Vet. Res. Commun. 32(6):481-9

OIE (office International des Epizooties) (2000): FMD, chapter 2.1.1 in manual of standards for diagnostic tests and vaccine, 4th Ed. 2000, Paris, 77-92

OIE (office International des Epizooties) (2009): Version adopted by the World Assembly of Delegates of the OIE in May 2009 chapter 2.1.5. Foot and mouth disease

Pereda, A.; Konig, G.; Chimen Ozoth, S.; Borca, M.; Palma, E. and Piccane, M. (2002): Full length nucleotide sequences of FMDV Strain O1 campos / bra 158. Brief. Repot. Arch. Viro., 147 (11); 2225 – 2230

Reid, S.; Pierce, K.; Mistry, R.; Bharya, S.; Dukes, J.; Volpe, C.; Wangh, L. and King, D., (2010): Pan-serotypic detection of FMDV by RT-linear-after-the-exponential PCR.Molecular and Cellular Probes 24; 250–255

Salem, S.A.H.; Eweis M.; Ismail, I.; Said, A.; Nahla Sh Kotb and Fayed, A.A. (2009): Longitudinal study on the detection of different FMDV serotypes using indirect sandwich ELISA. Egypt .J. of Appl. Sci. 24 (4A) 15-28

Shawky M., Abd El-Aty M., Hiam. M. Fakry, Hind M. Daoud, Ehab El-Sayed I., Wael

.

Mossad G., Sonia A. Rizk, Abu-Elnaga H., Mohamed A. A., Abd El-kreem A. and Farouk E. M (2013) Isolation and Molecular Characterization of Foot and Mouth Disease SAT2 Virus during Outbreak 2012 in Egypt. J Vet Adv 2013, 3(2): 60-68

Thomson G. (1994): Foot and Mouth disease in infectious disease of livestock with special reference to South Africa, edited by J.A.W. Cotzer, G.R. Thomson. Cap-Town, London, Newyork, Oxford University press pp. 825-992.