

Sero Survey on current status of Foot and Mouth Disease in some Egyptian Governorates 2013 - 2015

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting both domesticated and wild cloven-hoofed animals worldwide and it is a disease with high economic importance. In Egypt, FMD has taken as enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV serotype (O) was the most prevalent until serotype (A) appeared in 2006 then during April and May 2012, six outbreaks of FMD serotype (SAT 2) were reported in Egyptian governorates. This study based on evaluation of vaccinated animals by detection of antibodies against serotypes of FMDV (A),(O) and (SAT-2) by (SNT) as well as by analysis of negative Non Structural Protein sera by solid phase competitive ELISA for differentiation between natural infected and vaccinated animals by detection of Non-Structural protein of FMDV by pricocheck test. Five hundred sera were collected from vaccinated cattle and buffalo from five Egyptian governorates (Two governorates represent Delta region: El-Gharbia – Kafr El Sheikh , Two governorates represent upper Egypt: El-Fayoum – El-Menya , and one governorate represents central : El-Giza). The sera collected from diseased and apparent healthy animals. Results of Serum Neutralization Test were 260 of cattle sera out of 280 and 167 of buffalo sera out of 220 were +ve by SNT. Pricocheck test was applied on two hundred cattle and buffalo sera from the positive sera of SNT its results indicated that 48 sera were positive(29 for cattle and 19 for buffalo) , serotyping for positive sera were applied with statistical results showed no significance difference between serotypes in governorate , species and percent of inhibition . Solid Phase Competitive ELISA was applied for the negative 152 sera samples to measure the titer of antibody against strains A,O, SAT-2 with high titer of antibody against serotype (A) was 87 , serotype (O) was 72 and serotype (SAT-2) was 53 . Matching between SNT and ELISA was applied for negative sera samples.

Key Words: Cattle, buffalo, Foot and Mouth Disease and sero survey

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting both domesticated and wild cloven-hoofed animals worldwide. FMDV infects diverse hosts, affecting over 70 species of wild and domestic cloven-hoofed species such as cattle, sheep and swine . The variety of hosts and diversity of serotypes synergistically complicates disease prevention (Smith *et al.*,2014). Foot and mouth disease (FMD) is the most important disease of the international organization of epizootics (OIE) . List A, and one of the most contagious disease among domestic

animals (Carroll *et al.*, 1984; OIE/FAO/WHO,1996 ; Saiz *et al.*, 2002 and Michael *et al.*, 2007). In Egypt, FMD has taken an enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV type O was the most prevalent until serotype A appeared in 2006 (Moussa *et al.*, 1984; Daoud *et al.*, 1988 and Farag *et al.*, 2005) then during April and May 2012, six outbreaks of FMD type SAT 2 were reported in Egyptian governorates (El-Moety *et al.*, 2013). The disease is characterized by the formation of vesicles in the mucosa of the mouth ,external nares and in coronary band of

claws , other areas including udder and teats . Lameness is seen, reduced lactation mastitis and abortion are common clinical signs range from a mild or in apparent infection to one that is sever. Death may result in some cases , mortality from a myocarditis is the most common seen in young animals myositis may also occur in other sites (FAO, 1984).

Sero Diagnosis of FMD as non-structural protein identification, an indirect ELISA was established to specifically identify antibodies induced by FMDV infection but not those induced by vaccination (He *et al.*, 2010). The serum neutralization test is the “gold standard” method for serology EU FMD (2007) .The virus neutralization test (VNT) and the liquid-phase blocking ELISA (LPBE) or solid-phase competition ELISA (SPCE) are the recommended tests for determining the FMD antibody status of livestock OIE (2004). The solid-phase competition ELISA has sufficient sensitivities and specificities for use as serological diagnostic tests for the qualitative and quantitative detection of antibodies against FMDV Li *et al.*, (2012).

MATERIAL&METHODS

1. Serum samples

500 Serum samples were collected from apparently healthy cattle and buffalo of varying ages and sex from different five Egyptian governorates (Gharbia, Kafr El Sheikh, Giza, El Fayoum and El Menya).

2.Serum Neutralization Test applied according to OIE (2009) for FMDV Ab detection.

3.PrioCHECK FMDV NS : ELISA for in vitro detection of antibodies against Foot and Mouth Disease Virus .

Supplied by : Prionics Lelystad B.V. (Netherlands)

Interpretation

The mean percentage inhibition of the weak Positive Control must be > 50% ,The mean percentage inhibition of the Positive Control must be > 70% , PI = < 50% (negative)Antibodies against the NS protein of FMDV are absent in the test sample , PI = \geq 50% (positive)Antibodies against the NS protein of FMDV are present in the test sample .

4. SOLID – PHASE COMPETITIVE ELISA (SPCE)FOR ANTIBODIES SPECIFIC TO FMDV SEROTYPE A,O,SAT 2: Supplied by IZSLER Biotechnology Laboratory (Italy)

Calculation of results

Percentage inhibition produced by the positive control and test sera are calculated as follows: % inhibition = $100 - (\text{serum OD} / \text{reference OD} *) \times 100$

*Reference OD = mean OD of four wells processed with the negative control = 100% reaction = 0%inhibition

Interpretation

For screening purpose, test sera are considered :

Positive when producing an inhibition \geq 70% at the 1/10 dilution ;

Negative when producing an inhibition < 70 % at the 1/10 dilution ;

For titration : serum end-point titre corresponds to the highest dilution producing 50 % inhibition .

RESULTS

Serum Neutralization Test was applied on 500 sera of vaccinated and apparent healthy animals of different sex, age and season (280 cattle and 220 buffalo) which collected from five Egyptian

governorates (2 governorates represent Delta region, 1 governorate represents middle and 2 governorates represent upper region) . On 200 sera solid phase competitive ELISA was applied for differentiation between natural infected and vaccinated animals by detection of Non-Structural protein of FMDV by

priocheck test, then serotyping was done on +ve NSP , Solid Phase Competitive ELISA was applied for the negative sera to measure the titer of antibody against strains A,O, SAT-2 and matching between SNT and ELISA was applied for negative sera samples .

Table 1. Screening by Serum Neutralization Test (SNT) in Cattle sera for detection FMDV Antibodies Serotypes A, O and SAT-2

Governorate	Total Samples	Results		+VE sera at Ab.Titer (32)			+VE Sera Sex		+VE Sera Age			+VE Sera Season	
		+VE	-VE	(A)	(O)	(SAT-2)	Male	Female	6M-1Y	1Y-3Y	3Y-5Y	Winter	Spring
Gharbia	60	57	3	28	43	28	9	48	7	29	21	32	25
Kafr El-Sheikh	60	52	8	39	31	31	2	50	7	21	24	31	21
Giza	70	68	2	39	45	40	37	31	27	37	4	39	29
El-Fayoum	54	51	3	30	35	29	12	39	12	32	7	28	23
El-Menya	36	32	4	22	21	10	2	30	6	13	13	15	17
ToTal	280	260	20	158	157	138	62	198	59	132	69	145	115

Table 1 showed +VE results of screening were 260 out of 280 of cattle sera, the highest titer of antibodies at level (32) were 158, 157 and 138 cattle sera for serotypes (A),(O) and (SAT-2) respectively. In relation of positive sera to sex, age and season results showed highest positivity for female, age (1Y-3Y) and in winter season.

Table 2. Screening by Serum Neutralization Test (SNT) in Bufflo sera for detection FMDV Antibodies Serotypes A, O and SAT-2

Governorate	Total Samples	Results		+VE sera at Ab.Titer (32)			+VE Sera Sex		+VE Sera Age			+VE Sera Season	
		+VE	-VE	(A)	(O)	(SAT-2)	Male	Female	6M-1Y	1Y-3Y	3Y-5Y	Winter	Spring
Gharbia	40	29	11	24	26	24	5	24	5	17	7	21	8
Kafr El-Sheikh	40	27	13	14	17	10	7	20	1	11	15	19	8
Giza	30	22	8	20	19	15	14	8	2	18	2	15	7
El-Fayoum	46	44	2	31	25	29	16	28	5	25	14	25	19
El-Menya	64	45	19	39	38	21	5	40	11	26	8	16	29
ToTal	220	167	53	128	125	99	47	120	24	97	46	96	71

Table 2 showed +VE results of screening were 167 out of 220 of bufflo sera, the highest titer of antibodies at level (32) were 128, 125 and 99 bufflo sera for serotypes (A),(O) and (SAT-2) respectively. In relation of positive sera to sex, age and season results showed highest positivity for female, age (1Y-3Y) and in winter season.

Table 3. Screening Cattle sera for FMDV NSP by Prio check Kit “ELISA Test” and analysis of positive samples FMDV NSP in depend on % of inhibition

Governorate	Total Samples	Results		% of inhibition of +ve FMDV NSP		
		+VE	-VE	50 – 65 %	65 – 80 %	80 – 100 %
Gharbia	20	5	15	1	2	2
Kafr El-Sheikh	26	6	20	1	1	4
Giza	30	10	20	1	4	5
El-Fayoum	23	2	21	1	1	0
El-Menya	23	6	17	2	2	2
ToTal	122	29	93	6	10	13

Table 3 showed +VE results of screening of 122 cattle sera for FMDV NSP by Prio check test were (29) naturally infected and (93) negative FMDV NSP, the highest number in % of inhibition of +ve FMD NSP was in range between 80 – 100% .

Table 4. Screening Buffalo sera for FMDV NSP by Prio check Kit “ELISA Test” and analysis of positive samples FMDV NSP in depend on % of inhibition

Governorate	Total Samples	Results		% of inhibition of +ve FMDV NSP		
		+VE	-VE	50 – 65 %	65 – 80 %	80 – 100 %
Gharbia	20	5	15	1	2	2
Kafr El-Sheikh	14	4	10	0	4	0
Giza	10	2	8	0	1	1
El-Fayoum	17	6	11	1	4	1
El-Menya	17	2	15	2	0	0
ToTal	78	19	59	4	11	4

Table 4 showed +VE results of screening of 78 buffalo sera for FMDV NSP by Prio check test were (19) naturally infected and (59) negative FMDV NSP, the highest number in % of inhibition of +ve FMD NSP was in range between 65 – 80% .

Table 5. Analysis for positive FMDV NSP samples of cattle and buffalo sera for detection of serotypes

Governorate	Cattle					Buffalo				
	+ve No.	A	O	SAT-2	A&O	+ve No.	A	O	SAT-2	A&O
El-Gharbia	5	1	2	1	1	5	1	1	0	3
Kafr El-Sheikh	6	1	1	1	3	4	1	1	0	2
El-Giza	10	2	4	1	3	2	0	1	0	1
El-Fayoum	2	2	0	0	0	6	1	3	0	2
El-Menya	6	3	2	0	1	2	1	1	0	0
Total	29	9	9	3	8	19	4	7	0	8

Table 5 showed results of analysis for positive FMDV NSP samples for detection of serotypes , in cattle sera number of infected cattle with : serotype(A) was 9, serotype (O) was 9, mixed infection serotype(A&O) was 8 and with serotypes SAT-2 was 3. In buffalo sera number of infected buffalo with : mixed infection serotype(A&O) was 8, serotype (O) was 7, serotype(A) was 4 and no infection with serotypes SAT-2 .

Table 6. Matching between SNT& ELISA for FMDV serotype A,O and SAT-2 in cattle sera and analysis of PI for Negative FMDV NSP samples by “ ELISA KIT”

Governorate	Total Samples	SNT			ELISA			PI of Negative FMDV NSP samples by “ ELISA KIT”											
		(A)	(O)	(SAT -2)	(A)	(O)	(SAT -2)	(A)				(O)				(SAT-2)			
								< 70% (-ve)	70 – 80%	80 – 90%	90 – 100 %	< 70% (-ve)	70 – 80%	80 – 90%	90 – 100 %	< 70% (-ve)	70 – 80%	80 – 90%	90 – 100%
Gharbia	15	14	14	12	15	15	12	0	4	7	4	0	5	6	4	3	4	4	4
Kafr ElSheikh	20	17	11	10	18	12	10	2	4	8	6	8	0	2	10	10	4	4	2
Giza	20	16	13	11	17	14	12	3	5	4	8	6	3	3	8	8	4	4	4
El-Fayoum	21	13	15	10	15	15	11	6	4	5	6	6	4	5	6	10	0	1	10
El-Menya	17	7	10	10	9	10	13	8	2	4	3	7	2	3	5	4	6	1	6
ToTal	93	67	63	53	74	66	58	19	19	28	27	27	14	19	33	35	18	14	26

Table 6 showed matching of cattle sera between protective results (at antibody titer 32) by SNT for serotypes A, O and sat-2 were 67, 63 and 53 respectively and negative results of NSP for serotypes A, O and sat-2 which were 74, 66 and 58 respectively. The highest percent of inhibition by ELISA KIT for serotype(A) ranged from 80-90% , for serotype(O) ranged from 90-100% and for serotype(SAT-2) ranged from 90-100%

Table 7. Matching between SNT& ELISA for FMDV serotype A,O and SAT-2 in buffalo sera and analysis of PI for Negative FMDV NSP samples by “ ELISA KIT”

Governorate	Total Samples	SNT			Protective by ELISA			PI of Negative FMDV NSP samples by “ ELISA KIT”											
		(A)	(O)	(SAT -2)	(A)	(O)	(SAT -2)	(A)				(O)				(SAT-2)			
								< 70% (-ve)	70 – 80%	80 – 90%	90 – 100 %	< 70% (-ve)	70 – 80%	80 – 90%	90 – 100 %	< 70% (-ve)	70 – 80%	80 – 90%	90 – 100%
Gharbia	15	13	14	10	15	15	12	0	4	5	6	0	5	8	2	3	4	5	3
Kafr ElSheikh	10	6	2	1	6	2	1	4	0	6	0	8	0	0	2	9	0	0	1
Giza	8	5	3	1	7	3	2	1	3	0	4	5	0	1	2	6	0	1	1
El-Fayoum	11	5	2	0	6	3	1	5	1	0	5	8	3	0	0	10	0	0	1
El-Menya	15	6	5	1	7	6	1	8	1	3	3	9	1	3	2	14	0	0	1
ToTal	59	35	26	13	41	29	17	18	9	14	18	30	9	12	8	42	4	6	7

Table 7 showed matching of buffalo sera between protective results (at antibody titer 32) by SNT for serotypes A, O and sat-2 were 35, 26 and 13 respectively and negative results of NSP for serotypes A, O and sat-2 which were 41, 29 and 17 respectively. The highest percent of inhibition by ELISA KIT for serotype(A) ranged from 90-100% , for serotype(O) ranged from 80-90% and for serotype(SAT-2) ranged from 90-100%

DISCUSSION

Foot and Mouth Disease (FMD) represents one of the most devastating disease affecting cloven-hoofed livestock including cattle, buffaloes, sheep and goats (**summerfield et al., 2009**). It is characterized by the vesicles on the tongue, nose, muzzle and

coronary bands of infected animals. The virus causes one of the most economically devastating diseases in the world. It may be transmitted easily by contact and aerosolic mode (**Abdel-Rahman et al., (2006)** It was first detected in Egypt in 1950 with an

outbreak caused by SAT2 strain, and in 1952, 1956 and 1958 with outbreaks caused by strain A several foci were detected from 1961 up to 2006 with strain O and O1 till the appearance of strain A in the FMD epidemic during January - June 2006 (**Salem *et al.*, 2009**). In this study, sero-surveillance in 5 Egyptian governorates in order to determine the immune status. Also evaluation of FMD vaccine under field condition. 500 sera were collected from vaccinated cattle and buffalo for investigation by SNT, 200 sera were examined by prio check test to differentiate between naturally infected and vaccinated cattle and buffalo and matching between ELISA and SNT. **Table (1&2)** showed Screening by Serum Neutralization Test (SNT) in Cattle and buffalo sera for detection FMDV antibodies Serotypes A, O and SAT-2 the +ve results of in the 5 Egyptian governorates in cattle sera were 260 out of 280 with total percent (**92.9%**) & in buffalo sera were 167 out of 220 with percent (**75.9%**). The antibody titer at level 32 for serotypes A, O and SAT-2 was : 158 (60.7%), 157 (60.3%) and 138 (53.1%) respectively & in buffalo sera 128 (76.6%), 125 (74.8%) and 99 (59.2%) respectively. The results also showed the relation between antibody titer & Sex, Age and Season. The highest positive sera was in Female cattle (198), in Age from 1Y-3Y (198) and in winter season (145) & The highest positive sera was in Female buffalo with total number (120), in Age from 1Y-3Y (97) and in winter season (96). Our results agreed with **Condy and Hedger (1978)** found that the FMD virus is transmitted horizontally and most young buffalo acquire infection soon after maternally derived passive immunity wanes sufficiently, between 3

and 8 months of age, **EU FMD (2007)** reported that serum neutralization test is the "gold standard" method for serology, **OIE (2008)** mentioned that the virus neutralization test is recognized as the standard method for detecting antibodies to FMDV structural protein, **Habiela *et al.*, (2010)** reported that The seasonal incidence of the disease in the cold season has been observed and animal movement seems to play a major role in virus dissemination and FMDV outbreaks were predominantly encountered in the cold season between November and March, but our results disagreed with **Sutmoller and veira (1980)** reported that the cattle having neutralizing antibody titers in excess of 1:64 would seen indicate a high level of protection while titers within the range 1:8 to 1:32 are particularly difficult to interpret in terms of protection on challenge. Statistical Analysis using chi-square in frequency distribution of SNT results in different governorates revealed that significance difference (sig.diff.) between El-Fayoum and El-Menya and no sig. diff. in El-Garbia, Kafr El-Sheikh and El-Giza, in relation to SNT results and species in all governorates showed sig. diff. between cattle and buffalo in El-Garbia, Kafr El-Sheikh and El-Giza, on other hand no sig. diff. in El-Fayoum and El-Menya. In relation between governorates and antibody titer at level (32) the statistical results showed that there is no sig. diff. between various governorates and titer of antibody for serotype (A) but there is sig. diff. for serotype (O) where El-Gharbia governorate had the highest significant percentage while Kafr El-Sheikh had the significantly lowest one also there is sig. diff. for serotype SAT-2 as El-Fayoum had the highest significance difference while El-Menya was significantly the

lowest one, in relation between species and antibody titer at level (32) showed that there was no significant relationship between species and antibody titer for serotype (A) in all governorates except for Kafr El-Sheikh where cattle significantly higher than buffalo with, no significant relationship between species and antibody titer for serotype (O) in all governorates and in serotype (SAT-2) no significant relationship in all governorates except for Kafr El-Sheikh where cattle significantly higher than buffalo. Also our results revealed that there was no sig. diff. in results of SNT in various governorates and in sex (male and female cattle&buffalo). In frequency distribution of SNT test across different age of animals there was no sig. diff. in El-Fayoum and there is sig. diff. in El-Garbia, Kafr El-Sheikh, El-Giza and El-Menya. The age group 1 Y – 3 Y had significantly higher proportion of positive SNT results, while the age group 6M – 1 Y had the significantly lower proportion of positive SNT results. Results revealed no sig. diff. between various governorates in results of SNT and season in all governorates except in El-Gharbia, Kafr El-Sheikh and El-Menya, Buffalo in winter showed highly significant proportion of positive SNT results, while cattle had no sig. diff. between winter and spring. **Table (3&4)** Screening Cattle and buffalo sera for FMDV NSP by Prio check Kit “ELISA Test” and analysis of positive samples FMDV NSP in depend on % of inhibition showed 29 cattle sera naturally infected out of 122 sera with percent (23.7%) and 93 negative sera, the highest level for positivity of the 29 positive cattle sera was 13 sera which ranged from (80 – 100%) and 19 sera buffalo naturally infected out of 78 sera with percent (24.3%) and 59 negative

sera, the highest level for positivity of the 19 positive sera was 11 sera which ranged from (65 – 80%) our results agreed with **Abbas et al., (2002)** reported that in serum samples which collected from different areas in KSA, the results of sero-diagnostic tests revealed that 3ABC ELISA is the most reliable test 100% specificity and 100% sensitivity for the detection of FMD viral activity in the vaccinated cattle and sheep with absence of clinical symptoms, **Foord et al., (2007)** said that the current preferred test to differentiate infected from vaccinated animals is a competition ELISA (C-ELISA) designed to detect antibodies to the non-structural protein 3ABC as an indicator of infection, **OIE, (2009)** adopted an ELISA that detects antibodies against FMD NSP and it is the only method used to demonstrate FMD freedom post-vaccination in South America. Our Results disagreed with **Bergmann et al., (1998)** reported that In vaccinated cattle slightly more false positive were found with ELISA. It was suggested that some vaccinated contain residual NSP, and the recent vaccination with these vaccines could result in false positive tests. Therefore, emergency vaccines should be prepared from purified FMD antigens only absence of antibody responses NSP should be demonstrable for such vaccines, **Iman et al., (2005)** reported that the checkit-3-ABC ELISA Kit was recommended to test animals before vaccination if the tested animals were repeatedly vaccinated with improperly inactivated FMD virus vaccine. The statistical analysis revealed that no sig. diff. between various governorates and species (cattle&buffalo) in percent of inhibition of NSP. **Table(5)** explained the analysis for positive FMDV NSP

samples of cattle and buffalo sera for detection of serotypes and that showed in cattle sera infection with Serotype (A), (O) , A&O (mixed infection) and (SAT-2) was 9,9,8 and 3 respectively, in Buffalo sera infection with Serotype (A) , (O), A&O (mixed infection) and Serotype (SAT-2) was 4,7,8,0 respectively . Our results agreed with **Clavijo *et al.*, 2003** mentioned that characterization of the FMDV serotype is essential for tracing source of the virus with proper selection of effective vaccine **El_Tarabili *et al.*, (2009)** examined 54 animals in four different provinces of Egypt (Ismailia, Al-Gharbia, Dakahlia and Kafr El Sheikh) and 35 FMD isolates out of 48 are serotyped as O strain with a percentage of 72.90% while 13 FMD isolates are serotyped as A strain with a percentage of 27.10% from the total number of FMDv isolates. our results disagreed with **(WRLFMD, 2015)** The epidemiological report of FMD in Egypt during the midyear 2014 indicated that FMDV serotypes O and SAT2 are the predominant strains circulating in Egypt, followed by serotype A with 34% , 32% and 15% prevalence, respectively, and the rest (19%) remained unclassified ,The difference in the epidemiology of the FMDv serotypes could be attributed to the diagnostic procedure difference where the WRLFMD used PCR. Statistically results revealed no sig. diff. between various governorates and species in serotype of positive NSP. **Table (6&7)** Matching between SNT& ELISA for FMDV serotype A,O and SAT-2 in cattle & buffalo the analysis % of Negative FMDV NSP samples , in cattle serotype (A), (O) and (SAT-2) were 69, 63 and 55 respectively with SNT while in ELISA were 74 ,66 and 58 respectively regarding to buffalo the

results with SNT for serotype (A), (O) and (SAT-2) were 28, 26 and 12 respectively while in ELISA were 41, 29 and 17 respectively. Our results agreed with **OIE Annual status (2000)** reported that ELISA is serotype specific, sensitive, quantitative, quicker to perform, less variable, not dependant on cell cultures and be performed with inactivated antigens, **Mackay *et al.* (2001)** developed a solid phase competitive ELISA to measure antibodies to FMDV. The limit of detection of solid phase ELISA was similar to that, of liquid phase assay and both tests had lower limit of detection (i.e. were able to detect lower amounts of antibody) than the virus neutralization test. The specificity of the solid phase ELISA was considerably higher than that of the liquid phase blocking ELISA and almost equivalent to the virus neutralization test , **Kris De Clerq (2002)** found that, the virus neutralization test (VNT) is the reference test to detect antibodies against FMDV. Disadvantages of the test include that, it takes 2-3 days to complete, require cell culture facilities and is preferred with live virus. Antibodies can also be detected by ELISA system which is faster and can be performed with inactivated virus, **OIE, (2004)** mentioned that the virus neutralization test can be use for FMD virus diagnosis but requires cell culture facilities and takes 2-3 days to provide results ,ELISA is sensitive, quantitative and has the advantage that it's quicker to perform, less variable and is not dependent on tissue culture systems . Statistically there was no sig. diff. between various governorates and serotype A and serotype SAT-2 in each governorates but there is sig. diff. positive strain O as

well as no sig. diff. between species and serotype A, O and serotype SAT-2 .

In conclusion FMD is endemic disease in most Egyptian governorates controlled by obligatory vaccination by local inactivated Trivalent vaccine for serotypes A, O and SAT-2

REFERENCES

- Abbas, A.M.; Nagi, G. M. and Mujalli, D.M. (2002):** Application of serodiagnostic tests for differentiation between infected and vaccinated animals with foot and Mouth Disease using bioengineered non-structural proteins in the kingdom of Saudi Arabia.
- Abd El-Rahman, A.O.; Farag, M.A.; Samira El-Kilany, Ali, S. M. and AboYazed, M. (2006):** Isolation and identification of Serotype O of Foot and Mouth Disease virus from imported Bulls and its correlation to the current used vaccine strain O1/3/1993. Proc. 3rd Inter Conf. Vet. Res. Div., NRC, Cairo, Egypt, pp. 91-100 .
- Bergmann, I.E.; Auge de Mello, P; Neitzert, e.; Beck, E. and Gomez, I. (1998) :** Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme linked immunoelectrotransfer blot analysis with bioengineered non-structural viral antigens. Am. J. Vet. Res., 54: 825-831.
- Carroll, A. R.; Rowlands, D. J. and Clark, B. E. (1984) :** Nucleic acids Res-,12; 2461-2472 .
- Clavijo, A., Viera-Pereira, P.J. and Bergmann, I., 2003.** Use of the reverse transcription polymerase chain reaction (RT-PCR) for the rapid diagnosis of foot and mouth disease in South America. Veterinary research communications 27, 63-71.
- Condy, J. B. and Hedger, R. S. (1978):** "Experience in the establishment of a herd of foot and mouth disease free African buffalo (*Syncerus caffer*) ".South African J. of Wildlife Res., 8: 87-89.
- Daoud, A., Omar, A., El Bakry, M., Metwally, N., El Mekkawi, M. and El Kilany, S. (1988) :** Strains of Foot and Mouth Disease virus recovered from 1987 outbreak in Egypt. J. Egypt. Vet. Med. Ass., 48(1): 63-71.
- El-Moety, M.S.A.; El-Aty, M.M.A.; Fakry, H.M.; Daoud, H.M.; Ibrahim, E.E.; El-Din. W.M.G.; Rizk, S.A.; El-Naga, H.A.; Mohamed, A.A.B.; El-Krim, A.S.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.
- El_Tarabili M. M., El_Shahidy M. S., Azab A. M., Abu El_Naga H. I., Abd El_Daiem M. M., Abdelwahab S. A. and M., F., 2009:** serological and molecular diagnosis of fmdv isolates collected from four provinces of egypt. scvmj vix, 33-43.
- EU FMD, (2007):** EU FMD-The European Commission for the Control of Foot-and- Mouth Disease. Report of the 37th Session of EUFMD, Rome 2007. Ref type: internet communication

- (FAO) Food and Agricultural Organization of the United Nations (1984):** Emerging Diseases of Live stock. Vol. 1. the Diseases and their Diagnosis, Geering W. A., ed. FAO, Rome Italy, 43-51.
- Farag, M.A.; Aggour, M.A. and Daoud, A.M. (2005):**ELISA as a rapid method for detecting the correlation between the field isolates of Foot and Mouth Disease and the current used vaccine strain in Egypt. *Vet. Med. J., Giza.* Vol. 53, No. 4: 949-955.
- Foord, A., Muller, J., Yu, M., Wang, L. and Heine, W., (2007):** Production and application of recombinant antibodies to foot-and mouth disease virus non-structural protein 3ABC.
- Habiela, M., Ferris, N. P., Hutchings, G. H., Wadsworth, J., Reid, S. M., Madi, M., Ebert, K., Sumption, K.J., Knowles, N. J., King, D. P. and Paton, D. J., 2010 :** Molecular characterization of foot-and-mouth disease viruses collected from Sudan. *Transboundary and emerging diseases* 57, 305-14.
- He, C., Wang, H., Wei, H., Yan, Y., et al., (2010):** A recombinant truncated FMDV 3AB protein used to better distinguish between Infected and vaccinated cattle. *Vaccine* 28: 3435–3439.
- Iman, M.; Fatehia, M. and Tawfeek, A (2005):** FMD-3ABC as diagnostic in ELISA kit for differentiation between infected and vaccinated cattle. *J. Egypt Med. Assu.*, 65(5); 145-154.
- Kris de Clerq (2002) :** Overview of FMD diagnostic techniques. FMD, Control Strategies International Symposium, 2-5 June, France. Abstract Book, 107.
- Li, Y., Swabey, K., Gibson, D., Keel, P., Hamblin, P., Wilsden, G., Corteyn, M., Ferris, N., (2012):**Evaluation of the solid phase competition ELISA for detecting antibodies against the six foot-and-mouth disease virus non-O serotypes. *Journal of Virological Methods* 183; 125– 131.
- Mackay, D.; Bulut, A.; Rendle, T.; Davidson, E. and Ferris, N. (2001):**A solid phase competition ELISA for measuring antibody to FMDV. *J. Virol. Methods*, 97; 33-48.
- Michael, P . ; Ward, Shawn W.; Laffan and Lina D. High field (2007) :**The potential role of wild and feral animals as reservoirs of foot and mouth disease . *Preventive. Veterinary, medicine.* 80, 9-23.
- Moussa, A.A.M.; Daoud, A.; Hussein, K.; Hassan, N.A.; Fahmy, F.; Azab, A. and El-Shehawy, L. (1984):**Prevalence of FMD in Egypt". *Agric.Res.Rev.*, 62(5B): 55-63.
- OIE/FAO/WHO,(1996) :** Manual of standards for diagnostic tests and vaccines .Saiz, M.; Nunez, J.; Jimenez – Clavero, Metal (2002) : FMD; biology and prospects for disease control . *Microbes Infect.*, 4(11); 1183-1192.
- OIE (Office International des Epizooties) (2000):**Foot and mouth disease, Chapter 2.1.1.In manual of standards for diagnostic tests and vaccine, 4th Ed. 2000, Paris, 77-92.
- OIE (2004):**Manual of diagnostic tests and vaccines for terrestrial animals, 5th edition.Parida, S., Anderson,J., Cox,, S.,Barnett, P. and Paton, D., (2006):Secretory IgA as an indicator of oro-pharyngeal foot-and-mouth disease virus replication and as a tool

- for post vaccination surveillance. *Vaccine* 24 ; 1107–1116.
- OIE, (2008):** Foot-and-mouth disease. In: OIE, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). OIE, Paris, France, pp. 198–199.
- OIE (Office International des Epizootie) (2009) :** Manual of Standards for Diagnostic Tests and Vaccines. Part 2. Foot and Mouth Disease, 5th ed. OIE, Paris (Section 2.1, Chapter 2.1.1).
- Saiz, M.; Nunez, J.; Jimenez – Clavero, Metal (2002) :** FMD; biology and prospects for disease control . *Microbes Infect.*, 4(11); 1183-1192.
- Salem, S.A.H.; M. Eweis; I. Ismail; A. Said; Nahla, S and A.A.Fayed (2009):** Longitudinal study on the detection of different FMDV serotypes using indirect sandwich ELISA. *Egypt. J.of Appl.Sci*24 NO (4A) 5-28.
- Smith, M.T; Bennett, A.M.; Grubman, M.J. and Bundya, B.C. (2014):** Foot-and-mouth disease: Technical and political challenges to eradication *Vaccine* 32 (2014) 3902–3908.
- Summerfield, A.; Guzylack-Piriou, L.; Harwood, L. and McCullough, K.C. (2009):** Innate immune responses against foot-and-mouth disease virus: Current understanding and future directions. *Veterinary Immunology and Immunopathology* 128: 205–210.
- Sutmoller, P. and Veira, A. (1980):** The relation ship of neutralizing antibody titres for FMD and the protection of cattle. *Bull. Del. Centro an Americans de fiebra*, 39/40: 57-62.
- WRLFMD. 2015.** World FMD Reference Laboratory web site <http://www.wrlfmd.org>.