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RESEARCH

Evaluation of vaccination with local and imported vaccine against foot and mouth disease virus in Kalubeya governorate

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ABSTRACT

Background: FMD virus poses a serious threat to the inter-national animal trade. The preventive vaccinations followed by sero-survey have been implemented for the control of FMD.
Objectives: 3ABC ELISA is highly effective for early identification of infected and vaccinated animals. Our study was designed for assessment of vaccination by local and imported FMD vaccines in cattle sera in Kalubeya Governorate.
Methods: 220 serum samples were tested for FMD NSP by 3ABC Trapping ELISA.20 serum samples were used as control (no vaccination),100 serum samples collected from animals received local vaccine and 100 serum samples from animals received imported vaccine.
Results: 34/220(15.5%) were +ve and 186/220(84.5%)were –ve for FMD NSP test preinoculation with vaccines.Serotyping of FMD antibodies were adopted on examined sera for imported and local vaccines using solid phase competitive ELISA 2,4,8,and 10 weeks post vaccination(wpv). Results were 45(24.2%), 65(34.9%)2 WPVand 55(29.5%) ,68(36.5%) 4WPV- 58(31.2%),71 (38.2%) 8WPV and 58(31.2%), 69(37.1%) 10 WPV for serotype A.For serotype (O):55(29.5%) ,58(31.2%)2WPVand 60(32.3%), 62(33.3%) 4WPV and 62(33.3.5%) , 62(33.3%) 8 WPV 60 (32.3%),63(33.9%)10 WPV.For serotype (SAT2):30(.16.1%), 22(11.8%) 2WPV and 39(20.9%), 41(22%)4WPV and 37(19.9%),41(22%) 8/ WPV/ and 27(14.5%), 35(18.8%) 10 weeks post vaccination.
Conclusion: vaccines are a fundamental strategies aimed at global control and eradication of FMD. Control of FMD requires vaccines that will provide differentiating infected from vaccinated animals (DIVA).

Key words: Cattle ; DIVA; ELISA; FMD; NSP; WPV.

BACKGROUND

FMD virus is a highly contagious viral disease of cloven-hoofed animals, specialy high-yielding dairy animals, and is one of the most significant economic diseases of domesticated animals **(Reid *et al.,* 2010 ; Xu *et al.,* 2012)**.FMD is endemic in Africa, Asia, and South America and crosses international boundaries to cause epidemics in areas, which were virus-free previously **(Grubman and Baxt, 2004).**FMD is endemic in the Middle East, Central and South Asia, Africa, and some countries in South America **(Thompson et al., 2001)** and causes an acute disease characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats.**(Depa et al.,2012).**FMD has a high morbidity, low mortality, and contagiousness that can lead to severe economic losses **(Khamees, 2013).**

Foot and mouth disease virus is a small, non-enveloped, single-stranded positive-sense RNA virus, genus Aphthovirus in the family Picornaviridae **(Lewis-Rogers *et al.,* 2008)**. The FMD virus exists in the form of seven serologically and genetically types(O, A, C, Asia1, SAT1, SAT2, and SAT3, but a large number of subtypes have evolved within each serotype **(Neeta et al., 2011 and Depa et al., 2012).**The replication of the virus is rapid after entry through the upper respiratory tract

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or lung, viremia seeding infection into the epithelium where secondary virus multiplication results in vesicles and shedding from the udder in milk **(Larska *et al.*, 2009).**Spread of foot and mouth disease virus is by contact between an infected and a susceptible animal. All secretions and excretions from an infected animal will contain virus, and infection may occur either across damaged epithelium or orally**.In Egypt**, FMD serotype O was endemic since 1950s and up till now, while an epidemic of FMD serotype A stroke Egypt in 2006 and several foci of infection with serotype A is still existing. During 2012, there has been a dramatic spread in FMD serotype SAT2**, (Suzan, 2010).**Although the regular program to control and eradicate FMD, it still endemic in Egypt **(El-Sheikh and Azab, 2005 and Lewis-Rogers *et al.,* 2008)**.All foot and mouth disease virus vaccines are based on cell culture-derived preparations of inactivated whole virus. FMD vaccines may be monovalent or polyvalent. The monovalent vaccines using a field derived or outbreak strain. Foot and mouth disease virus vaccines may provide protection within 4-5 days after vaccination. it depends on the vaccine and on the severity of challenge. Vaccination initiates a short period of immunity, requiring revaccination at intervals of every six to twelve months and on occasion more often for protection against heavy challenge **(Grubman and Baxt 2004; Doel 2003).**

Detection of FMDV in clinical samples is still performed using conventional techniques as Enzyme Linked Immuno-Sorbent Assay (ELISA)**(OIE, 2005).** 3ABC Enzyme Linked Immune Sorbent Assay (ELISA) tests were based on the fact that viral replications result in the production of NSPs in the host and will therefore induce the production of anti-NSP antibodies **(Clavijo et al.;2004).**Detection of FMDV in epithelial tissue suspensions carried out using ELISA is usually accompanied by the application of ELISA and cell culture isolation and to any samples showing a cytopathogenic effect (CPE)**(Marquardt et al., 2000; Hanaa et al., 2012)).**

Our study aims to Assessment of vaccination with local and imported vaccine against foot and mouth disease virus in Kalubeya Governorate.

MATERIALS AND METHODS

Serum samples

220 serum samples were collected from cattle aged 6-8 months from different localities of Qalubeyia Governorate and used for detection of FMD NSP antibodies using 3ABC ELISA test.20 non vaccinated serum samples were used as control ,100 serum samples were collected from animals received local vaccine and 100 from cattle received imported vaccine.

Local FMD vaccine

Polyvalent inactivated FMD vaccine:

Inactivated FMD virus serotype A/EGY/1+2012(A Iran05) 10^6 $TCID_{50}$ per dose
Inactivated FMD virus serotype O/EGY-4-2012(O Panasia2) 10^6 $TCID_{50}$ per dose
Inactivated FMD virus serotype SAT2 EGY-A-2012) (Sat 2)10^6 $TCID_{50}$ per dose
The vaccine is water in oil emulsion and is given 3ml /dose subcutaneous.Supplied by VSVRI – Cairo.Egypt.

Imported FMD vaccine

Polyvalent Killed FMD vaccine in saponin and aluminium hydroxide Adjuvant used in aqueous vaccines for cattle: The vaccine contains (SAT2 Ery - SAT2 Zim - A Iran 05 - A4165 – O manisa) strains and tested against the local FMD strains. The dose of vaccine was 4ml s/c to cattle. Supplied by commercial company.

ELISA Kits

3ABC Trapping FMDV NSP ELISA kit:

It was supplied by IZSLER Biotechnology Laboratory, Italy. The 3ABC ® FMDV NS ELISA was used for detection of antibodies directed against the nonstructural protein of FMDV in serum of cattle and buffaloes. It detects FMDV infected animals independent of the serotype that causes the infection according to **(Sørensen et al., 1998).**

Principle of the test

Trapping –indirect ELISA for the détection of antibodies to the non-structural polypeptide (NSP) 3ABCof FMD virus in serum or plasma samples of large and small ruminant.The test can be applied to detect infected animals and vaccinated and FMD serotype that cause the infection.The use of anti-3ABC specific monoclonal antibody (Mab) coated to the solid phase to trap the recombinant 3ABC polypeptide expressed in E coli microtiter plates are supplied pre-coated with the 3ABC antigen captured by Mab. Approprciately diluted test sera are incubatd with the trapped antigen, enabeling the specific antibodies eventually present in sample to bined to the 3ABC. After washing to remove unbound material, an anti-ruminant IgG, peroxidase- conjugated Mab is dispensed: the anti-ruminant IgG bind to the FMD virus antibodies of the positive samples immune-complex with 3ABC.After incubation the unbound conjugate is removed by washing, and the TMB-chromogen substrate is delivered into wells. Acolorimetric reaction developes if conjugate has bound to sample antibody.

Percentage positivity = $\frac{\text{net OD value of test sera}}{\text{net OD value of positive control serum}} \times 100$

Solid-phase competitive ELISA for serotyping of FMD antibodies(A,O,SAT2):

The Solid-phase competitive ELISA using selected neutralizing anti-FMD monoclonal antibody, specific for FMD serotypes (A,O,SAT2) is applied to measure antibodies against these serotypes. Percentage inhibition =100-(serum OD/reference OD*) x100

Reference OD=mean OD of four negative control wells =100%= 0% inhibition

RESULTS

Table (1): Detection of antibodies against FMDV nonstructural protein using 3ABC-Trapping indirect ELISA in cattle sera pre inoculation with local and imported vaccines:

	No. of examined serum samples	+ve serum samples		-ve samples	
		Number	%	Number	%
Control	20	3	1.3	17	7.7
Local vaccine	100	17	7.7	83	37.7
Imported vaccine	100	14	6.3	86	39
Total	220	34	15.5	186	84.5

Table (2): Detection of specific antibodies against FMDVusing solid phase competitive ELISA in cattle sera after (2) weeks post vaccination with local and imported vaccines

	No. of –ve NSP serum samples	Positive serum samples					
		Serotype A		Serotype O		Serotype SAT2	
		Number	%	Number	%	Number	%
Control	**17**	**9**	**4.8**	**9**	**4.8**	**1**	**0.5**
Local vaccine	**83**	**45**	**24.2**	**55**	**29.5**	**30**	**16.1**
Imported vaccine	**86**	**65**	**34.9**	**58**	**31.2**	**22**	**11.8**
Total	**186**	**119**	**63.9**	**122**	**65.6**	**53**	**28.5**

Table (3): Detection of specific antibodies against FMDV using solid phase competitive ELISA in cattle sera after (4) weeks post vaccination with local and Imported vaccines

	No. of –ve NSP serum samples	Positive serum samples					
		Serotype A		Serotype O		Serotype SAT2	
		Number	%	Number	%	Number	%
Control	17	12	6.5	9	4.8	10	5.4
Local vaccine	83	55	29.5	60	32.3	39	20.9
Imported vaccine	86	68	36.5	62	33.3	41	22.1
Total	186	135	72.5	131	70.4	90	48.4

Table (4): Detection of specific antibodies against FMDV/ using /solid phase competitive ELISA in cattle sera after(8) weeks post vaccination with local and imported vaccines

	No. of –ve NSP serum samples	Positive serum samples					
		Serotype A		Serotype O		Serotype SAT2	
		Number	%	Number	%	Number	%
Control	**17**	**12**	**6.5**	**12**	**6.5**	**10**	**5.3**
Local vaccine	**83**	**58**	**31.2**	**62**	**33.3**	**37**	**19.9**
Imported vaccine	**86**	**71**	**38.2**	**62**	**33.3**	**41**	**22.1**
Total	**186**	**141**	**75.8**	**136**	**73.1**	**88**	**47.3**

Table (5): Detection of specific antibodies against FMDVusing solid phase competitive ELISA in cattle sera after (10) weeks post vaccination with local and imported vaccines:

	No. of –ve NSP serum samples	Positive serum samples					
		Serotype A		Serotype O		Serotype SAT2	
		Number	%	Number	%	Number	%
Control	**17**	**9**	**4.8**	**8**	**4.3**	**3**	**1.6**
Local vaccine	**83**	**58**	**31.2**	**60**	**32.3**	**27**	**14.5**
Imported vaccine	**86**	**69**	**73.1**	**63**	**33.9**	**35**	**18.8**
Total	**186**	**136**	**37.1**	**131**	**70.4**	**65**	**34.9**

DISCUSSION

FMD is an international disease problem and cooperation within and across borders is essential. It is on A list of the infectious diseases of animals and has been considered as the most

important limit to international trade in animals and animal products**,(Grubman and Baxt, 2004, and O'Donnell et al.,2011).**

Early detection of FMD virus is fundamental for effective control of the disease and requires a sensitive and rapid method of diagnosis **(Veerasami et al., 2008)**.No cross protection between the different serotypes.The serotype /of a virus involved in an outbreak cannot be as curtained on the basis of clinical signs. To permit proper control/vaccination programs, the determination of the serotype involved in field outbreaks has to be established within laboratories. Several laboratory techniques have been used for the diagnosis of FMD and to certain the serotype of the virus. ELISA test is alternative test to Virus neutralization (VN). ELISA test/is faster but less variable, quantitative results and is not dependent on cell-culture capabilities. Applications of ELISA are broad. These applications include post-outbreak surveillance, monitoring of vaccination status and international trade. The application of NSP tests after vaccination of animals is dependent on the use of purified, inactivated vaccine that is free (as much as is possible) of NSPs. Differentiation of infected animals from vaccinated animals (DIVA) is essential for proper eradication of FMD by vaccination and the development of carrier animals due to vaccination **(Uttenthal et al., 2010)**. Antibody response against FMD viral non-structural proteins has been widely used for this purpose. As **shown in table (1),**220 were tested for FMD NSP/by 3ABC Trapping ELISA.20 serum samples were used as control(no vaccination),100 serum samples collected from animals received local vaccine/ and 100 serum samples from /animals received imported vaccine.34/220(15.5%)were +ve and 186/220(84.5%)were -ve for NSP. Our results disagreed with those of **(suzan et al.; 2011)** who detected (48%) antibodies against FMD NSP in cattle sera.**Table(2)** showed the serotyping/of FMD antibodies in serum samples by Solid-phase competitive ELISA (A,O, SAT2)2 weeks post vaccination with local inactivated vaccine and imported vaccine. In animals received local vaccine, antibodies were 45(24.2%), 55(29.5%) and 30(16.1%), while animals received imported vaccine, antibodies were 65(34.9%),58(31.2%) and 22(11.8%)for FMD serotype A,O and SAT2 respectively. FMD antibodies 4 weeks post vaccination with local vaccine were 55(29.5%),60(32.3%) and 39(20.9%)and for imported vaccine, 68(36.5%),62(33.3.9%) and 41(22%) as showed in **Table (3).**8 weeks post vaccination with local and imported vaccine, FMD antibodies were 58(31.2%),62(33.3%) and 37(19.9%) and 71(38.2%),62(33.3%) and 41(22.1%)for FMD serotype A,O and SAT2 respectively as showed in **table (4).**

Serotyping of FMD antibodies were 58(31.2%),60(32.3%) and 27(14.5%) for local vaccine and were 69(37.1%),63(33.3%) and 35(18.8%) for FMD serotype A,O and SAT2 respectively as showed in **table (5).**The results agreed with results obtained by **(El-Habbaa et al.:2014).**The highest positive antibody percents against FMDV by ELISA were recorded for serotype A and agreed with those results of **Pattnaik and Vedkataramanan (1989**). Animals may be persistently infected with FMD after challenge with live FMD virus.Vaccines consist of inactivated FMD virus induce antibodies against the structural proteins and not to the non-structural proteins **(Clavijo et al., 2004).**Inactivated FMD vaccines are an important component of control and eradication strategiesboth in enzootic and non-enzootic areas**(Valarcher et al..;2007).**

CONCLUSION

The application of FMD vaccination programsare a fundamental component of strategies/ aimed to global control and eradication of the disease.Eradication of FMD requires vaccines that will allow differentiating/infected animals from vaccinated animals (DIVA). Emergency/response to FMD outbreaks will require fast acting DIVA and reliable/vaccines with

long-term stability of the formulated ready to useproduct. Matching with local and imported vaccine according to our results no significant difference in general except some points refers that imported vaccine rapid arising of FMD antibodies in some serotypes.

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