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Research Article

Use of Guinea Pigs as an Alternative Lab Animal for Quality Control of FMD Vaccines

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ABSTRACT

Foot and mouth disease (FMD) is a primarily communicable disease of cloven footed animal (buffalo, cattle). It causes high economic losses. Vaccination is the potential and mandatory step for prevention and control of FMD virus in field. The Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) and is the only organization in EGYPT that authorized for the quality control of FMD vaccines either local or imported, CLEVB uses calves for evaluation process, but it faced many problems as; they often carry antibodies against FMD serotypes as EGYPT is an endemic country, lack of some Biosafety measures in animal isolators and its price was doubled at last three years, that's why we looked for an alternative model to the original host. In this current study Guinea Pigs (G. pigs) were used as an alternative model for evaluation of FMD vaccines. As it's cheap, easily handled, well secured and free from FMD antibodies. The last five released local FMD vaccine batches were inoculated with 0.5 ml S/C in G. pigs, Sera samples were collected after 28 days according to evaluation protocol, SNT and ELISA were carried out. Comparisons between results of both animals were done. It was found that the antibody titer for G. pigs were protective and less than those of cattle by one log. So it is recommended to use G. pigs instead of cattle for evaluation of FMD vaccine in CLEVB.

Key words: Foot and mouth disease (FMD), CLEVB, G. pigs

INTRODUCTION

Foot and mouth disease is a contagious disease that affects cloven-hoofed animals (Satya, 2009). FMDV is a member of picornaviridea family, its caused by 7 immunologically serotype; A, O, C, Asia1, south Africa territories (SAT1), SAT2 and SAT3 (Paton et al., (2005). Several of these serotypes circulate currently or periodically in North Africa. In Egypt serotype A, O and SAT2 is responsible for recent outbreaks which causes huge economic losses (Aidaros: (2002) & (Shawkyetal., (2012). Although FMDV does not cause high mortalities in adult but it causes high morbidity up to 100%(Knowles et al., (2003). In order to achieve better control of the disease in endemic countries, it's essential to monitor the current variants of the prevalent serotypes of FMDV in the field to ensure most appropriate vaccinal strain to combat the circulating virus (Bulletin, (2014). There were some difficulties to find experimental animals (calves) completely free from antibodies against FMDV to be used in potency test beside using these experimental animals is very expensive. G. pig is cheap and free from any antibodies against FMD serotypes (Zeb, (2015), Thus in current work we used G. pig parallel to cattle for evaluation of FMD vaccine as an alternative experimental model.

MATERIALS AND METHODS

Evaluation in calves: NT and ELISA results of last five released FMD vaccine batches were evaluated in Central Laboratory for evaluation of veterinary biologics. Eighty five male calves (local breed) of six to eight months old of about 200 – 300 kg body weight were used in 5 FMD vaccine batches evaluation. The sera from these calves were previously screened by SNT for the presence of specific antibodies against FMD viruses type O, A and SAT2 and did not reveal any specific antibodies (sero-negative). Eighty five were allotted into 5 groups as the following:

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Group A (17): injected with 1X field dose of 1st vaccine batch via deep S/C route for 15 calves (potency) and 2 calves kept as control Negative.
Group B(17): injected with 1X field dose of 2nd vaccine batch via deep S/C route for 15 calves (potency) and 2 calves kept as control Negative .
Group C(17): injected1X field dose of 3rd vaccine batch via deep S/C route for 15 calves (potency) and 2 calves kept as control Negative .
Group D(17): injected1X field dose of 4th vaccine batch via deep S/C route for 15 calves (potency) and 2 calves kept as control Negative .
Group E(17) injected1X field dose of 5th vaccine batch via deep S/C route for 15 calves (potency) and 2 calves kept as control Negative.

Evaluation in Guinea Pig: Sixty healthy G. pig of both sexes 3-5 months of age weighting 0.4 to 0.5 Kg (free from antibodies against FMDV) were used in the present study. Fifty of these were used in the potency test for evaluation of the same previous local five batches (0.5ml S/C/G, pig) and Ten animals were used as negative control. Sixty G. pig were allotted into 6 groups: Each group contain 10 G. pig as following:
Group A: injected with 0. 5 ml of 1st vaccine batch via S/C route.
Group B: injected with 0. 5ml of 2nd vaccine batch via S/C route.
Group C: injected with 0. 5ml of 3rd vaccine batch via S/C route.
Group D: injected with 0. 5ml of 4th vaccine batch via S/C route
Group E: injected with 0. 5ml of 5th vaccine batch via S/C route
Group F: 10 G. pig were kept as control Negative.

BHK (Baby Hamster Kidney) cells: Cells were used for SNT (serum neutralization test) and propagation of FMDV strains (A, O, & SAT2) used in SNT. These cells were obtained from Reference strain bank in Central Laboratory for evaluation of veterinary biologics (CLEVB).

FMDV strains: O/EGY/4/2012, A/EGY/1/2012 and SAT2/EGY/2/2012 strains were used in SNT test for evaluation serum collected from vaccinated animals. These strains were obtained from Reference strain bank in (CLEVB).

Serological tests

Solid phase competitive ELISA (SPCE): The samples were tested for the detection of antibodies against FMDV using solid phase competitive ELISA, validated by IZSLER Brescia Italy. Ready to use kits were used and reagents were prepared according to the instructions given in the manual. Four dilutions were prepared for titration of test sera (1/10, 1/30, 1/90 and 1/270) in antigen coated microplates. The OD values were read at 450 nm using a microplate reader and sera giving PI (percent inhibition) values equal to or greater than 70% were considered as positive.

Serum neutralization assay (SNT): the test was performed by using the micro technique as described by Ferriera (1976).

RESULTS

Estimation of humoral immune response in vaccinated calves (groups A, B, C,D,E) with local commercial vaccines (Batches 1, 2, 3,4,5) against FMDV type O, A and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) obtained at 28th day post vaccination while the humoral immune response in vaccinated Guinea pigs with local commercial vaccines (Batches 1, 2, 3, 4, 5) against FMDV type O, A and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) obtained at 28th day post vaccination in all batches against A, O and SAT2 except SAT2 in batch 1 , O and A in Batch 5 as shown in Table 1.

Estimation of humoral immune response in vaccinated calves and Guinea pigs with local commercial vaccines (Batches 1, 2, 3, 4, 5) against FMDV type O, A and SAT2 using ELISA showed +ve (>70%) in dilution 1/10 in all batches except O and A in Batch 5 for Guinea pigs group E as shown in Table 2.

DISCUSSION

Foot and mouth disease is one of the most important diseases worldwide. It is widely spread in Africa, Asia & South America. This disease causes severe economic destructive losses; like reduce milk &meat production, may cause mortalities in young calves, all of these affects on trading of animals and their yields. Vaccination is the most important and effective choice for controlling eradicating FMDV (Smitsaart *et al*., 1998). Vaccination with FMD vaccines of good quality prevent losses of livestock and reduce the incidence of the disease (Hunter 1998). In this study guinea pigs were used as an experimental animal in parallel to cattle (original host) for the evaluation of local FMD vaccine batches. As these animals were less expensive, easily management &have similar clinical signs as that of the original hosts (Jones *et al*., 1997) and (Fischer *et al*., 2003). According to both OIE and CLEVB manuals all the calves used for evaluation of FMD vaccines must be free from antibodies against the strains (A, O &SAT2) involved and incorporated in the evaluated vaccines.

Table 1: Serum antibody titer for vaccinated cattle and G.Pigs with polyvalent inactivated FMDV vaccine using SNT:

Vaccine batches		SNT Antibody titer	
		Cattle	Guinea pigs
Batch 1	O	1.2	1.2
Group A	A	2.4	2.1
	SAT2	1.2	1.08
Batch 2	O	2.1	1.8
Group B	A	2.65	2.4
	SAT2	1.44	1.38
Batch 3	O	2.1	1.5
Group C	A	2.4	2.05
	SAT2	1.38	1.2
Batch 4	O	1.5	1.2
Group D	A	1.8	1.5
	SAT2	1.5	1.2
Batch 5	O	1.2	0.9
Group E	A	1.2	0.9
	SAT2	1.5	1.2

Table 2: Serum antibody titre for vaccinated cattle and G. Pigs with polyvalent inactivated FMDV vaccine using ELISA:

Vaccine batches		ELISA Antibody Titre							
		Guinea pigs				Cattle			
		1/10	1/30	1/90	1/270	1/10	1/30	1/90	1/270
Batch 1 Group A	O	80%	65%	30%	10%	82%	68%	27%	11%
	A	95%	88%	78%	45%	98%	94%	88%	68%
	SAT2	77%	62%	22%	8%	81%	65%	25%	10%
Batch 2 Group B	O	88%	81%	64%	30%	94%	89%	79%	47%
	A	97%	94%	87%	67%	98%	95%	90%	81%
	SAT2	84%	68%	32%	11%	86%	70%	35%	15%
Batch 3 Group C	O	85%	73%	45%	21%	94%	89%	83%	55%
	A	92%	86%	75%	46%	98%	93%	89%	68%
	SAT2	79%	67%	35%	12%	83%	67%	34%	9%
Batch 4 Group D	O	84%	75%	53%	25%	76%	63%	45%	21%
	A	97%	95%	83%	58%	86%	77%	55%	26%
	SAT2	83%	72%	34%	9%	77%	67%	33%	14%
Batch 5 Group E	O	82%	67%	30%	12%	65%	53%	35%	7%
	A	85%	68%	33%	14%	67%	56%	35%	8%
	SAT2	83%	67%	34%	9%	80%	65%	38%	18%

In this work 5 local FMD vaccine batches were injected in 5 groups of G. pigs and other 5 groups of cattle (original host). Blood samples were collected regularly each week. The antibody titer against the 3 serotypes (A, O&SAT2) were monitored in serum samples using serum neutralization test &ELISA test.

The results as shown in table (1) reveled that in group A vaccinated with batch 1, the humoral immune response to serotypes O, A&SAT2 are 0.9 $\log_{10}$, 1.8 $\log_{10}$& 0.9 $\log_{10}$ respectively in G. pig while in calves are 1.2 $\log_{10}$, 2.4 $\log_{10}$ &1.2 $\log_{10}$ respectively at 28th day post vaccination. While in group B vaccinated with batch 2, the humoral immune response to serotypes O, A & SAT2 are 1.8 $\log_{10}$, 2.4 $\log_{10}$&1.38 $\log_{10}$ respectively in G. pig while in calves are 2.1 $\log_{10}$, 2.65 $\log_{10}$ & 1.44 $\log_{10}$ respectively at 28th day post vaccination. In group C vaccinated with batch 3 the antibody titer in G. pig were 1.8, 1.75& 0.9 $\log_{10}$ for type O, A& SAT2 respectively, and in calves 2.1, 2.4&1.38 $\log_{10}$ for type O, A& SAT2 respectively at 28th day post vaccination. In group D vaccinated with batch 4 the antibody titer (in G. pigs) is 1.2, 1.5&1.2 $\log_{10}$ for type O, A&SAT2 respectively, in calves group D 1.5, 1.8&1.5 $\log_{10}$for type O, A&SAT2 respectively. In group E vaccinated with batch 5, the antibody titer in G. pigs is 0.9, 0.9& 1.2 $\log_{10}$ while in calves group E 1.2, 1.2 & 1.5 for O.A & SAT2 respectively. The results tabulated in table (2) which shows ELISA results as a confirmatory test that came in parallel manner to the results obtained by SNT. From these results we can conclude that G. pigs can be used in evaluation of FMD vaccines instead of the original hosts. These results agree with Barteling, (1998) who stated that the original host (buffalo & cattle) used in potency test for evaluation of FMD vaccines raises many issues including cost, biosafety & biosecurity measures especially in FMD free countries. Also these results come in compliance with Zeb *et al.*, (2015) who vaccinated G. pigs with FMD vaccines as an alternative laboratory animal to large animal.

From the discussed results we can conclude that G. pigs can be used for evaluation of FMD vaccines considering 0.9 $\log_{10}$ the boarder protective titre using SNT. It could be an alternative laboratory animal model to the original host (cattle, buffalo, etc……) in vaccine evaluation as it has economic advantages as well as other benefits such as time, labour saving, easily handling and biosafety improvement.

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