Arch. Inst. RAZI (1992) **42/43,** 95-101

Notes of Antibody Response of Calves to Foot-and-Mouth Disease Bivalent Vaccine Containing Strains A-Mardabad and 01

Roustai, M.H., Gholami, M.R., Ahourai, P., Holakoui Naeeni, K., and Bakhtiari, A.

Summary: This investigation was done to assess the neutralizing antibodies and immune respose of Holstein-Friesian calves, vaccinated against strains A-Mardabad and 01 of foot and mouth disease (FMD) virus. Thirty two calves whose sera were free from neutralizing antibodies against the viruses, were divided into four groups of equal numbers. Formalin inactivated saponified FMD vaccine from the viruses propagated in BHK_{21} suspension cells with volumes of 2.5 ml, 5 ml, and 10 ml was injected subcutaneously to calves of first, second, and third groups respectively. The fourth group was kept as control. potency of vaccine was tested by challenge with 10,000 TCID 50/ml of A-Mardabad and 01 strains, currently being used for the vaccine production. Vaccine was also assessed by measuring the neutralizing antibodies in the calves over a period of 4 months. Serum antibody titers from test groups were probably suggestive of having a satisfactory level of protection.

Keywords: *Foot and mouth disease / Vaccine*

Introduction

Foot and mouth disease (FMD) occurs in many Asian countries. it has long been known as an international scourge as it is one of the most important economic animal diseases affecting not only cattle and buffaloes but also sheep and goat species *(1)*. This infection is an important disease of livestock in the Islamic Republic of Iran and assumes a greater importance as the value of

livestock increases. Strains 01 and A-Mardabad are endemic and strain Asia-1 is isolated occasionally. The disease is difficult to control because of the geographical location of the country and insufficient vaccine. However, vaccine control is required for two major reasons: Firstly, the vaccine should be innocuous for the host animal, that is, it should not be capable of inducing any disease symptoms. Secondly, the vaccine should be potent, that is, capable of protecting the animal against the disease in question *(2)*.

Materials and Methods

Vaccine production:

Cattle isolated virulent strains 01 and A-Mardabad of FMD virus were adapted to propagate in BHK_{21} suspension cells for large scale virus prodution. Infectivity of the viruses was assessed by titration in BHK_{21} monolayer cells and complement fixing activity using macro CF test. The vaccine was formulated with chloroform treated virus. Cell debries and other rigid materials were filtered out, and the infectivity titer of each strain was adjusted to $10^{6.72}$ to $10^{7.5}$ TCID 50/ml. Each dose of the vaccine contained 3ml of 01 and 1.5 ml of A-Mardabad strains to which were added 0.5 ml alhydrogel. In order to inactivate the virus, formalin (0.05%) was added at 29-30 C° and kept for 36 hours. Neutral saponin was added as an adjuvant. Final PH of the vaccine was adjusted to7.

Experimental calves:

The calves inculded in this study were Holstein-Friesian breed of 6 month age on which potency test of the vaccine was done. Thirty two sero negative calves for antibodies against strains 01 and A-Mardabad were chosen. These animals were divided into four groups. Half, one, and two doses of the vaccine were injected subcutaneously to the first three groups respectively, and the fourth group was kept in contact with the vaccinees, as control. All the animals were bled 21 days postvaccination before being revaccinated with the same amount and composition of the vaccine. Their sera were then collected 21 and 120 days after second vaccination to determine the titers of neutralizing antibodies.

Potency test in animals:

Potency test in experimental and control calves was done by intradermal inoculation of the tongue with 10000 TCID 50/ml of homologus virulent strain A-Mardabad virus, and nasopharyngeal inoculation with 10000 TCID 50/ml of

homologous virulent strain 01 virus. Blood samples were collected from the calves on 10th and 17 th days after challenge for screening.

Antibody assay method:

Sera from test and control calves collected after vaccination and potency test were assayed for virus specific neutralizing antibodies using BHK_{21} monolayer culture tubes. Each serum sample was incubated at 56°C for 30 minutes before use in the test. Twofold dilutions of sera were made in test medium.A volume of 0.6 ml of each serum dilution was added to 0.1 ml of virus dilution containing 100 TCID 50/0.1 ml and mixed well. After incubation for 1hr at 37°C, each mixture was added to one test tube of the monolayer cell and left in incubator for 72hrs. The serum neutralizing antibody tirer was taken as the reciprocal of the highest intial serum dilution which completely inhibited the appearance of CPE.

Results

The serologic findings of the tests are reflected in tables 1 and 2. Presence and increase in antibodies after vaccination were evident in all test groups while control group remained negative. The initial level of neulralizing antibodies after first vaccination in groups one and two of calves was considerably less than that following second vaccination. Level of the antibodies remained fairly stable in calves of group three after first and second vaccination. Eight initially seronegative control calves which were kept in contact with test groups for some 5 months, remained seronegative, indicating that there was no probability of virus shedding. Antibodies in the blood samples of the controls taken 10 days after challenge ranged from $\frac{1}{2}$ to $\frac{1}{16}$ against A-Mardabad strain while there were only 3 sera showing $\frac{1}{2}$ to $\frac{1}{8}$ antibodies to 01 strain and the rest remaind negative.

Geometric meantiter (GMT) of sera from each group taken 21 days post-second vaccination was compared with those of the control group taken 17 days after challenge. No significant difference in serum neutralizing antibody titers was observed in calves inoculated for second times with strain A-Mardabad vaccine comparing with the titers of control calves obtained 17 days after challenge, indicating thereby that the level of antibody titers after vaccination was satisfactory. However the level of antibody to 01 strain was significantly higer in test calves.

The titers reached an appropriate level in calves of group 3, after first vaccination, indicating thereby that excess of antigen had a better effect on

antibody response (Table 1)

Discussion

The methods available for potency testing of FMD vaccine by various laboratory techniques have been discussed from time 2, to time and more recently at the International Symposium on Foot and Mouth Disease *(2)* held at Lyon in 1976, where merits and demerits of these methods have been discussed *(3,4,5,6)*. Some investigators belive that potency tests can only be performed in the traget host species of animal, since by definition, they are tests the capacity of the vaccine to protect animals against infection by the pathogen concerned. In the case of FMD, this is measured by the capacity of vaccinated animals to resist a challenge infection by a dose of live virus (homologous strains to the vaccine)known to evoke symptoms of FMD and generalisation of these symptoms in non-immune animals *(2)*.

In the present study, the standardised method of potency control as used in Europe was modified. Though only one batch of vaccine was tested, the data collected from the test indicated that the vaccine was potent and showed a satisfactory immune and antibody response in calves, as judged by challenge with homologus vaccine strains of A-Mardabad and 01 and by measuring serum neutralizing antibody titers. The vaccinated animals responded well and a satisfactory level of antibody titer was obtained on the 21 st day post second vaccination in sera collected from the calves given 2.5 and 5 ml doses, whereas the calves vaccinated with 10 ml dose showed satisfactory level of antibodies on the 21 st day post - first vaccination. Although the antibody titers measured 120 days after second vaccination had lower level, but reached the satisfactory level after challenge.

Based on the results obtained from this study it could be concluded that:

1 - Calves should be vaccinated against FMD with normal dose recommended by the Razi Institute.
2 - Primary immunization of calves begins after maternal immunity is waned. The second dose should be given 21 days later and further doses at 6 - month intervals there after.
3 - The vaccine is safe and there is no risk of virus shedding.
4 - Attention should be given to use of two doses of vaccine at one time, in the case of remote villages and areas, without good access roads.

Ackenowledgments

Authours are grateful to Dr.M.Amighi, Dr.K.Khedmati, T., Ephtekhari and R.Sarmast, for providing necessary facilities.

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Table 1. Serum neutralizing antibody titers following vaccination of calves with FMD bivalent vaccine containing strains A - Mardabad and o1

No. of group	Geometric Mean Titers of post vaccination antibodies					
	21 days post first Vaccination		21 days post second Vaccination		120 days post second Vaccination	
	A-Mard.	01	A-Mard.	01	A-Mard.	01
1	3.6	2.4	12.3	13.5	4.4	4.5
2	5.0	5.2	10.4	8.7	6.7	6.7
3	11.3	8.0	11.3	12.7	6.3	6.3
Control	0	0	0	0	0	0

Table 2. Serum neutralizing antiboldy titers following challenge of calves with 10000 TCID 50/ml of A- Mardabad and 01 vaccine strains

No. of group	Geometic Meantiters of post challenge antibodies			
	10 days after challenge		17 days after challenge	
	A - Mard	01	A-Mard	01
1	4.8	5.7	14.7	8.8
2	5.7	5.2	14.7	8.7
3	4.0	3.5	12.7	9.0
Control	5.2	2.8	13.5	4.4