

## Original Article

# Assessment of the Immunogenicity of Foot and Mouth Disease Vaccine Produced by Razi Institute against Types of A13, A15 and O2010 of FMD Virus

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## Abstract

**Background and Aims:** Foot-and-Mouth Disease (FMD) is a highly contagious infectious disease of livestock which has made a barrier to hygiene causing severe loss in livestock and their products. The aim of this study was the assessment of antibody response against foot and mouth disease virus types A13, A15, O2010, after injection of FMD vaccine candidate produced by Razi Institute.

**Materials and Methods:** twenty non-vaccinated healthy calves were purchased and their health was evaluated. In order to ensure the absence of antibodies against FMD virus of all types, the blood of animal was sampled and subjected to serum neutralization test (SNT).

The SNT method was performed by the micro-neutralization test. Serum samples were tested before and after vaccination. Six wells of dilutions, 1/8 to 1/256 of serum were prepared and after adding the FMD virus they were incubated and then were added to the cell culture. After 48 hours the CPE was checked.

**Results:** The mean serum titers of antibodies against all three viral type Average A13, A15 and O2010 prior to vaccination was equal to 0.6. One week after the injection, the antibody titer increased especially against A15 in a significant difference (p value=0.017) compared to two other types. The serum antibody titers increase in the three virus types were continued one month after injection. Since then the A13 and A15 type antibody titer underwent increasing but declined against O2010 type. In the second month after the injection, the titer against A13 and A15 remained in stationary state and declined against O2010 type. The statistical analysis showed that the antibody level against the viral types was significantly different in 7 days, 1, 2, 4, 6 and 7 months after the injection.

**Conclusions:** The FMD vaccine produced by Razi institute showed the ability to protect animals become dependent on test conditions, the type O2010 for 6 months and for the type A15 and A13 for 7 months after vaccination.

**Keywords:** foot and mouth disease virus, vaccine, antibody response

## Introduction

Foot and mouth disease (FMD) was firstly discovered in Italy in 1514. The causative agent can spread through the air and

wind (to far away) and livestock contact. The causative agent is classified in the family of picornaviridae and genus Aphthovirus. The disease is highly contagious and severely impacts the livestock production regarding economic loss. The virus infects all ruminants and more than 70 wild animal species with several clinical symptoms including fever (firstly 40-41°C following viremia), decrease in the milk production, fatigue, increase the level of saliva and vesicular ulcers on the linguae,

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mouth, mammary and feet. The rate of morbidity is very high (100%), but the mortality rate is low and mostly the young livestock are affected (6,7).

Seven serotypes have been identified in the genus Aphthovirus including A – O- Asia 1 – C-SAT 3 – SAT 2 and SAT 1. The strains A, O and less extent ASIA1 are predominant subtypes causing FMD in Iran.

The RNA containing virus lacks envelope and undergoes severe antigenic variations which occurs mainly in endemic regions and leads to the appearance of novel variants (minor antigenic variations) and even new subtypes (major antigenic variations up to 15%) in each strain (4,5).

Inactivated FMD vaccine used (manufactured by Razi institute) contains serotypes: type A2013 (A13), Type A2015 (A15) and O2010. The FMD is produced in suspension cell culture of BHK cells (BHK21). the adjuvants are aluminium hydroxide [AL(OH)3] gel and saponine. Five milliliter of vaccine were administered subcutaneously in the neck. The vaccinated animal was monitored for adverse reaction of injection. severity and clinical signs of disease and if the vaccination plan would be fulfilled following a regular and concentrate pattern, it will be more effective and thus culminates in the control of the disease disorders.

Nowadays, by the time and progresses in vaccination designs, the process has been altered and the monitoring and quantification of immune responses will be conducted, and likewise the former pattern is substituted with the new pattern known as discover- validate-characterize- apply.

## Methods

**Vaccine preparation.** Inactivated FMD vaccine used (manufactured by Razi institute) contains serotypes: type A2013 (A13) ,Type A2015 (A15) and O2010. The FMD is produced in suspension cell culture of BHK cells (BHK21). The adjuvants are aluminium hydroxide [AL(OH)3] gel and saponine. Five milliliter of vaccine were administered subcutaneously in the neck two time (Second

vaccination 21 day after first vaccination ). The vaccinated animal was monitored for adverse reaction of injection .

**Calves and vaccine injection.** Twenty healthy and naive calves were purchased and their health was assessed exactly. For the insurance of no antibody response against the FMD disease; especially studied serotypes (O2010, A13, and A15), blood samples were collected before vaccine injection and in 7, 14, 21, 30, 60, 90, 120, 150, 180, 210 days after first vaccination. Separate the serum from collected blood ant treated in 56C for 30 minute and store -20°C.

**Serum neutralization test.** The constant virus and varying serum method was use. types A13 , A15 and O2010 viruse was diluted to contain approx . 1000 TCID50% / ml (100 TCID50% / well) and two fold adilution series of serum (1/8 to 1/256) was prepared. Reaction mixtures were prepared containing 50 ul of each dilution of serum with 50 ul of virus suspen-tion in 96 well cell culture microplate (6 well for each dilution of serum). the mixture were incubate at 37C and 3% CO2 for 1 hour at shaking mode in CO2 incubator. Then add 106 IBRS2 cell / ml or 50,000 per well and incubate at 37C and 3 % CO2 in stationary mode after 72 hours observe the plates under reverse microscope to detect the appearance of cytopathic effect. Serum neutralization titer were expere-sed as the reciprocal dilution of serum wich reduce CPE in number of wells by 50%. Canculte the titer of antibody against the viruses in serums by reed and meunch method. Serum titer less than 1.2 log10 (1/16 Dilution), has not enogh antibody to protect 50% of animals from FMD virus and more than 1.2 indicate the serum has enogh antibody to protect 50% of animals from FMD virus.

**ELISA assay.** For the serum antibody titer detection, the ELISA test was performed with the Elisa Kit for FMDV serology (O, A, Asia1), prepared from Perbrghite, England. In this test, firstly 50ul of the rabbit anti FMD virus antibody (1/1000 dilution with bicarbonate buffer) was coated in the 96 wells Maxisorb ELISA microplate and incubated for 1hour at 37°C with low shaking. The wells were washed four times with the phosphate

buffer saline , 0.002M and pH:7.4 (PBS). Next, the antibody-antigen interaction was conducted with various dilutions of serum and virus in u bottom 96 wells microplate and incubate in 37C, 1 hours for reaction. After incubation time 50 ul of mixture added to the wells of coated microplate , and incubated at 37°C with shaking. The wells were washed and then 50ul, 1/100 dilution of Rabbit Anti Guinea Pig HRPO Conjugated antibody with peroxidase enzyme was added to the wells.

After 1h incubation at 37°C with shaking, the plates were washed as explained before. Next, 50ul of chromogenic TMB was added to the wells and placed at dark media for 10 minutes and then 50ul of 1.25 normal sulfuric acid was added to the wells and read with ELISA reader in 450 nm wavelength. The dilution less than 1/40 was considered negative result means the serum has not enough neutralizing antibody to protect 50% of animal from FMD virus, the dilution=1/40-1/90 the test should be repeat and more than 1/90 was considered a positive result and serum has enough neutralizing antibody to protect animal from FMD virus.

The present ELISA for measuring antibody level, just can detect antibody agaist FMD serotype like A,O but cannot differentiate level of antibody against A15 with A13.

## Results

Antibody titre before and after vaccine after administration wich produced by Razi Institue. As exhibited in Figures 1-3, the antibody levels against A13, A15 and O2010 sub-types with the SNT technique before vaccine injection is 0.6 for all types. Seven days after injection of vaccine After the injection of vaccine after day7, the antibody titer increased gradually, however this enhancement was significantly higher level for A15 than other two sub-types and the increasing rate followed for three sub-types for one month and thereafter the increasing trend continued for A13 and A15 subtypes and decreased regarding O2010. The statistical analysis showed that antibody titer against three sub-types was significantly different in day 7 and months 1, 2, 4, 6 and 7. In time spans of 2 to 6 months, the antibody level was

in steady state against A15, but decreased against A13 at first and increased again, and decreased for the both sub-types A after month 6. Likewise, the titer was decreased for the all three sub-types after month 7 (Figure 4).

**ELISA results.** comparison of average ELISA and SN result against type O , and type A , has been shown in figures 5, 6.

## Discussion

FMD virus is an intracellular virus which rapidly multiplies and thus the immune system is not able to response properly in a proper suiFigure time. The humoral part of immune system is the main contributor of combat against the virus by producing antibodies. Therefore, tracing the level of antibodies is indicator of proper status of body immunity against the infection (8,9).

In fact, detection of antibody titer following the vaccination helps to understand vaccine quality for the virus and the titer should be determined against types and sub-types separately because the antibody response against one type does not induce immunity against another type or sub-type.

For instance, presence of antibody against A13 subtype does not confirm the enough titer against A15 sub-type (10). ELISA and SN tests measure the antibody titer. In addition to the vaccine quality, the immunity level is related to several other factors including 1- host properties (species, race, immunity status and maternal antibody, sanitation status, physiologic status such as milking, pregnancy and keeping in special climates (11). 2-vaccine properties such as dose, site and route of consumption, injection method, viral type, volume and purity, adjuvant, booster (like saponin) and salts and vaccine buffers (12).

In this study, the level of response against FMD was measured in calves following the use of a vaccine produced by Razi institute by serum neutralization and liquid phase blocking ELISA (LBP-ELISA) methods.

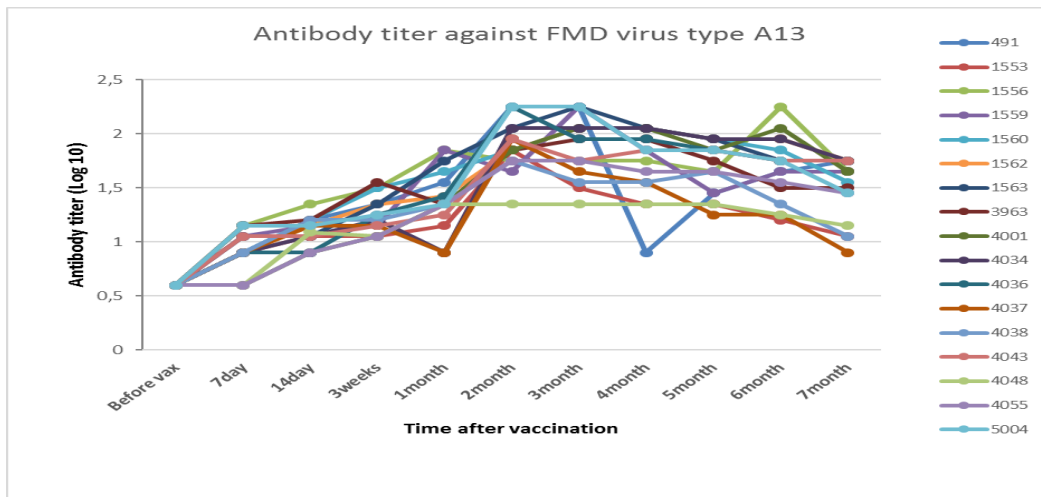


Fig. 1. Antibody titers of serums before and after the injection of vaccine against A13 sub-type in different times based on SNT results.

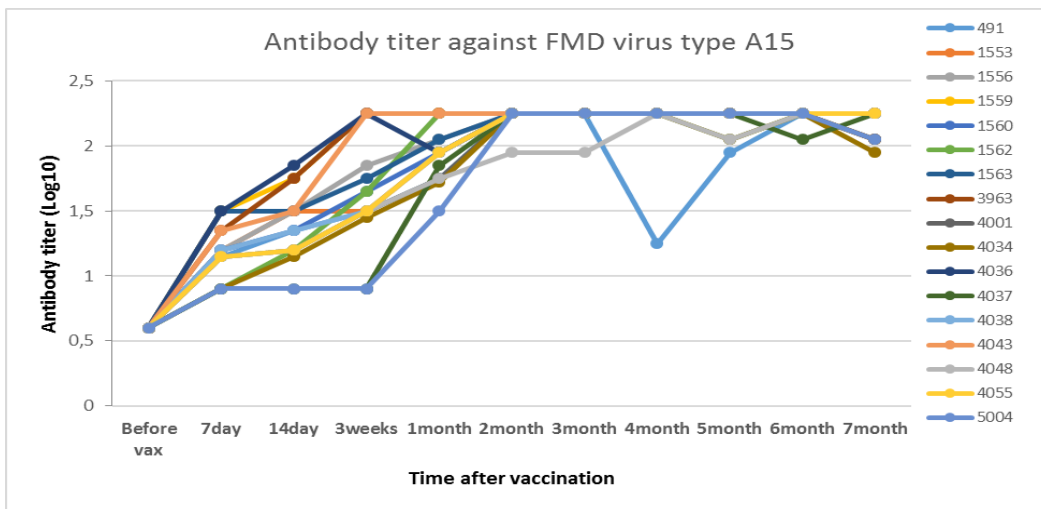


Fig. 2. Antibody titers of serums before and after the injection of vaccine against A15 sub-type in different times based on SNT results.

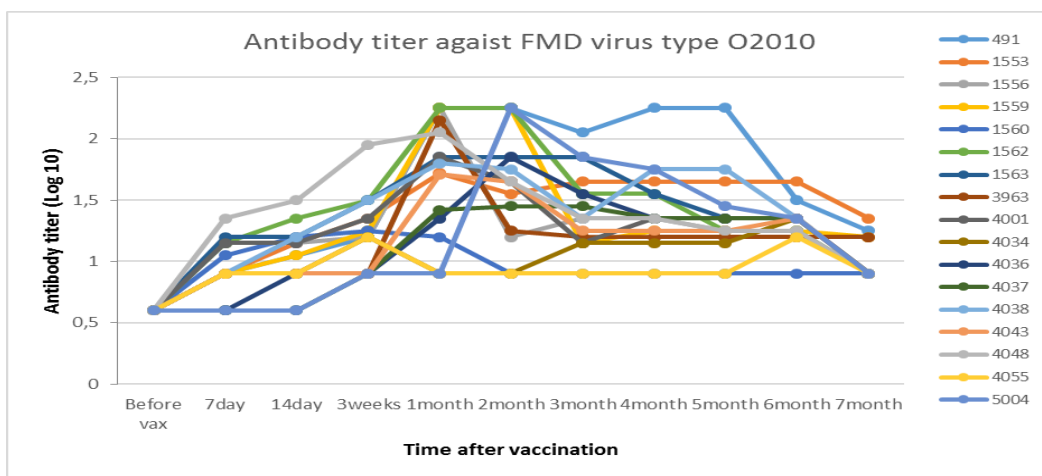


Fig. 3. Antibody titers of serums before and after the injection of vaccine against O2010 sub-type in different times

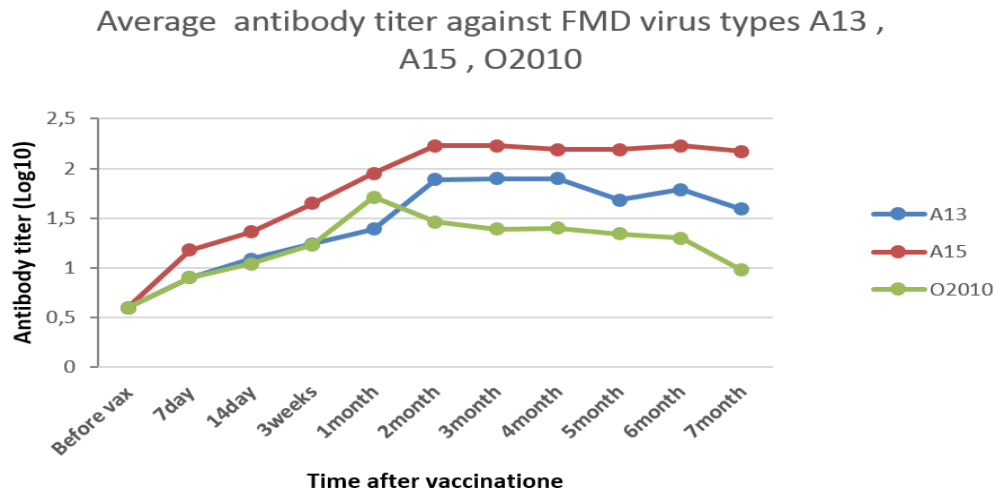


Fig. 4. The comparison of antibody titer levels in calves serum against A13, A15 and O2010 subtypes, based on SNT.

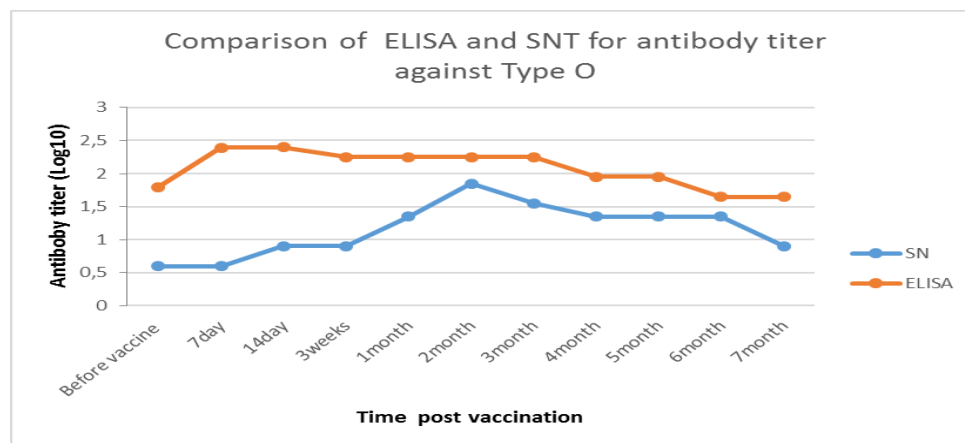


Fig. 5. The comparison of average antibody level against type A based on ELISA and SNT results.

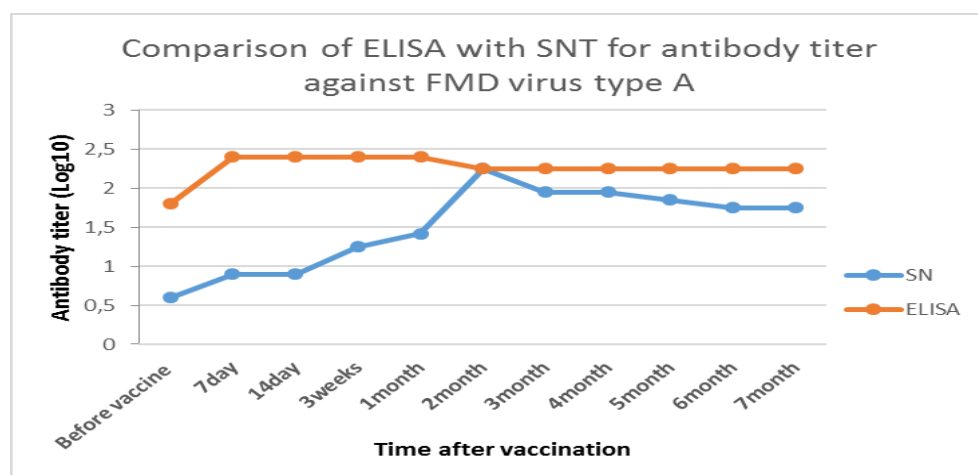


Fig. 6. The comparison of average antibody level against Type A based on ELISA and SNT results.

In this study, the level of response against FMD was measured in calves following the use of a vaccine produced by Razi institute by serum neutralization and liquid phase blocking ELISA (LBP-ELISA) methods.

In the comparison of alterations in the time between SNT and LBP-ELISA, a great level of similarity was observed. The antibody level against O2010 in the ELISA test was protective (1/90) in the month 5, but in SNT was month 6. The antibody level was protective until the month 6 in the ELISA and the month 7 in SNT against type A.

The protective level of antibody against the field virus is 1.2 on the basis of logarithm 2 in SN test according to references (13). According to the McClough et al in 1992 the antibody titer lower than 1.5 (logarithm 0.7 on the basis 2) and between 1.5 and 1.20 is proportional but incomplete immunity and higher than 1.20 is complete immunity. Altogether higher level of antibody shows the higher protection rate against the generalized infection and thus prevents a carrier state of livestock (14). The method of world reference center for FMD in Pirbright, England is based on the relation of SN titer of 90%, protective percentage and PD50 in which the antibody titer shows the level of protective state (15) as indicated followings:

$$\text{Log (SN90)} = 0.923 \log (\text{PD50}) + \text{PA50}$$

$$\text{PA50} = 0.70 \text{ for type O}$$

$$\text{PA50} = 0.54 \text{ for types A and Asia}$$

PD50: Serum titer corresponding to 50% protection

SN90: mean serum titer of calculated antibody response to undiluted vaccine

Note that in this equity each serotype differs and need a separate formula. In this formula, there is a constant number which differs for each type and thus each vaccine producing center should obtain for each purpose. In this study, the results of ELISA and SN test were similar for detection of antibodies. Saadma et al examined four commercial vaccines in aspect of protective effect and antibody titer on calves.

Two vaccines contained oil adjuvants and two other contained aluminum hydroxide. Their

method was similar to that from this study. The results showed that 80% of livestock exhibited resistance against the virus O, two oil vaccines, but against the virus A it was 80% and 100% (16). It is expected that oil vaccines show more protective effect but because of delay in the increase in antibody titer, the level maximizes in the day 28 of injection, while for the aluminum hydroxide the time is 21 days after injection, and in their study there is a bias as they challenged in the day 21 for oil vaccine and the titer has not reached the maximum level (17). Muhan and colleagues assessed the secretory mucosal and humoral antibody titer following the vaccination for type Asia.

Their study showed that when the calf infects with the disease natural or artificial, the secretion of humoral antibody is induced. The antibody titer in the infected and vaccinated calves against the Asia type, increased higher than 2 in the basis of logarithm 2 or dilution of 1/128. In comparison to A15 and A13 subtypes the results are similar in which the antibody titer increased higher 2 after 210 days (18).

Raj Kumar et al. described the protective level for types O, A and C and Asia  $\leq 1.5$ ,  $\leq 1$  and  $\leq 1.4$  respectively (19). Doel, evaluated the titer against virus O following vaccination containing aluminum hydroxide and showed the titer was higher when inactivated with ethylene compared to the formalin (20).

The booster dose was injected three weeks after the first vaccine injection and in fact when the titer initiates to decrease and thus the antibody titer increases after the booster injection. In fact, this note is similar to the first and second immune response to the protein antigens. In the day 7 after the injection, the antibody titer increases and continues to 21 days after the vaccination, but decreases gradually because the IgM increases at first with low life span, and thus the booster similar to the second response of humoral part against the protein antigen causes the increase of secretion of IgG type antibody with more life span and protects the livestock for several months (21).

Muhan et al. injected booster in two times of 19 days and 128 days, and the results demonstrated that for 19 days booster the titer reaches the maximum level after 50 days for type O

and remains at the protective status up to 150 days after the first injection. When the injection is the day 120 after the first injection, the antibody titer against O serotype reaches the protective level after the day 40 and remains up to 90 days at this state and thereafter decreases and until the booster injection lowers the protective level. Following the booster injection in the day 120, the antibody titer increases again and remains at the maximum level up to 180 days after vaccination and then decreases.

Considering the results from the mean antibody titer against subtypes A13, A15 and O2010 in the SN test in different times, it reaches the protective level in the day 21 for A13 and O2010, while the protective level against A15 is the day 14. The miligram weight of viruses O and A types differ for immune response by antibody secretion and 2 MG of type A is equal to 4-5 MG of type O to reach the protective level (22).

With consideration of a protective titer of 1.2 on the basis of logarithm 2 or dilution 1/16, in the day 7, 53% of livestock were protected against A15, but it was 11.7% for O2010 and 0% for A13. This amount in the day 14 provided protective level of 82.35% for A15, 29.41% for O2010 and A13 sub-types. In the week 3, the protective percentage in dilution 1/16 was 70.58%, 88.23% and 58.82% for O2010, A15 and A13, respectively.

Interestingly, subtype O2010 could not induce 100% protective effect in any of dilutions and time spans. These findings suggest that immunity against O serotype needs more amount of virus in the vaccine to give an equal protective level to A serotype mainly because of lower ability of immune stimulation.

About the O2010 virus in the SN experiment, antibody titer to the sixth month after the vaccine is protective. In the SN antibody titer of A serotype after the seventh month headline protect their hosts.

The Figures and graphs in the results section, it is quite evident that the average level of antibodies against the virus O to six months after injection of the vaccine remains at doses more than protective titer, but the titer against the

A15 and A13 remains to 7 months at protective titer.

Therefore, the FMD vaccine produced by Razi institute could protect the farm animals 6 months against type O and seven months against A13 and A15 types by injection of booster in the day 21.

Furthermore, the protective level of vaccines regarding antibody titer against viruses was 1.2 on the basis logarithm 2 and dilution 1/16.

## Acknowledgments

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## Conflict of interest

The authors have no conflict of interest.

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