



# Joint use of Ca – Modified Double-Strained RNA as an Immunostimulator with a Vaccine for the Prevention of Food and-Mouth Disease (FMD)

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## Research Article

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## Abstract

The purpose of this work is to study the effect of combined use of the immunostimulant preparation which is double-stranded Ca-modified RNA with inactivated FMD vaccine for cattle against A, O and Asia-1 subtypes of FMD. Preparation have been received from sodium nucleinate or allocated from a killer yeast of *Saccharomices cerevisiae*. The modification of Ca ions assayed by the addition of sterile solution of calcium chloride. Based on received data it can be concluded that the using of immunomodulator in cattle induces more early and high immune response. The FMD vaccine intramuscular administration together with the preparation provides high enough of an immune response and high titer of antibody maintained for a period of 90 days (time of observation).

**Keywords:** FMD; Ca-modified double-stranded RNA; Immunomodulatory

## Introduction

FMD refers to an especially dangerous animal diseases and is one of the most destructive diseases of livestock breeding. Currently there are intensive development of

pharmacological and pharmaceutical industry and despite the creation of a wide variety of vaccines FMD is causing enormous economic damage not only to underdeveloped countries, but such as the United Kingdom and the still to many other countries among the world [1-3]. There are needs

to establishing preventive immunostimulatory preparations. The task of the vaccination is to create an intense and prolonged immunity by maximizing involvement in immunogenesis the protective mechanisms of the organism, which largely connected with the use of immunomodulators. A highly effective strategy for combating infectious diseases is to enhance host defenses using immunomodulators, either preventatively, through vaccination, or therapeutically. The effectiveness of many vaccines currently in use is due in part to adjuvants, molecules that have little immunogenicity by themselves but which help enhance and appropriately skew the immune response to an antigen [4].

The effectiveness of modern vaccines is based on the fact that the vaccine strains have a limited capacity to replicate, but sufficiently stimulate the relevant elements of the immune system. The use of immunomodulators contributes to enhancing the immune status of the organism and strengthens antigenic response to the introduction of vaccines. The vaccine used in the prevention of FMD and a number of other viral diseases, only after some time shows their immune properties, and during this time the virus manages to multiply and infect new cells [5]. Although present conventional FMD vaccines can prevent clinical disease, protection is short lived (~6 months), often requiring frequent revaccination for prophylactic control, and vaccination does not induce rapid protection against challenge or prevent the development of the carrier state [6]. Detection of immunostimulatory activity of RNA and DNA oligonucleotides allowed get new adjuvants and antiviral preparations. Development of technologies for producing members aptamers-structured molecules of DNA and RNA, possessing the ability to selectively interact with specific target molecules, made it possible to design drugs that block the function of the enzyme-receptor activity of regulators of genes [7]. It is known that the exogenous RNA have a stimulating effect on regenerative processes in tissues and organs. Previously we have shown that the active start in preparation of sodium nucleinate (NN) selected from *Saccharomices cerevisiae* yeast is double-stranded RNA (dsRNK) [8]. To enhance the sustainability of specific serum nucleases and for stabilization of the preparation it was received calcium modified form of dsRNK (Ca-dsRNK) (conventional name - Inductor). Testing of this preparation was carried out in conjunction with the Scientific Centre for Risk Assessment and Analysis in Food Safety Area, SNCO of the MOA of RA. The existing mono-, bi- and polyvalent vaccine produced in different countries and protecting animals in producing immunity in approximately 3 weeks after their introduction in an organism. However, in the case of an outbreak of FMD during that period almost all animals can be infected. Applied large economic losses and environmental damage, resulting in significant financial losses. It is extremely important the fact that introduction of

the proposed preparation leads to emergency protection of animals after 6 hours after its introduction with 80-85% of the protection of animals against FMD virus. The preparation has a nonspecific immunostimulant property and creates the urgent protection of animals regardless of strain and type of viruses, and causes no side effects. Previously we have shown that Ca-dsRNK exhibits a high enough immunostimulant action against Newcastle disease of poultry [9].

## Materials and Methods

To obtain Ca-modified RNA preparation (Ca-dsRNK) raw materials served as nucleinate sodium, which received in OJSC "Biosynthesis" (Penza, Russia). It is an easily water soluble yellow-grey powder odourless. Modification of Ca ions assayed the addition of sterile solution 0.1 m calcium chloride approximately 2.5 ml to the appearance of light stable fluid light scattering experiment on 100 ml, containing 100 mg nucleinats and maintained within 40 minutes to form the final complex. From the traditional vaccine proposed product has the following characteristics: has no protein nature; its effect is already with 6 hour after injection; Based on modifications in the cell more than 90% of the preparation penetrates; protected from the action of tissue nuclease; acts regardless of the type and strain of the virus; has expressed adjuvant properties. In solution the preparation active 30 days at a temperature of 4-10° C. Safety and reactogenicity of the preparation tested in 10 guinea pigs and 2 pigs which intramuscular injected 2 and 20 ml, preparation respectively. Animals were observed 10 days during which they had not had any complications, which testify to the safety of the preparation and the absence of reactogenicity [10]. FMD virus of type Asia-1 (2001) was used in experiments. Infection titer of virus was 7.5 lgLD and as working titer 104 dilution has been taken.

## The Results of Investigation

Four experimental cattle have been injected by preparation of "Inductor" and after 12 hours they were infected by FMD type Asia-1 virus. Animals of the control group were also infected with the same virus. In control group there was an increase of temperature and was expressed generalization. From 4 experimental animals in one the body temperature climbed to 41°C with the generalization, the other two animals during 7-21 days has not shown any complications and the body temperature was within normal limits. The similar investigations previously was carried out also for pigs and preparation against shows good protective results.

Summarizing the obtained experimental data for the preparation of "Inductor" as a immunomodulator, resulting in emergency protection of animals (pigs, cattle)

from FMD virus, it can be assumed that the effect of this preparation due to its ability to stimulate the synthesis of endogenous interferon with substantial antiviral activity, which noticeably increases the immune response of T- and B- cells. In addition it is known that ds-RNA increases the level of phosphorylation of plasma membrane proteins, activate transmembrane currents extracellular calcium ions, increases the activity of phospholipase A2 which leads to the accumulation and release of lysoform phospholipids and unsaturated fatty acids with immunostimulating properties.

The study the effect of combined use of immunostimulator with vaccines was held on 100 heads of cattle (cows, young cattle 8-12 months, calves 3-4 months) in the premises of

Shirak marz Aregnadem of the Republic of Armenia. Animals in each age group were divided into 2 groups, one of which was only immunized with the vaccine, and another vaccine in conjunction with the immunostimulant. Experiments were conducted using inactivated polyvalent vaccine against FMD from strains of A, O and Asia-1, which injected animals intramuscularly. The vaccine used according to the instructions for use. As an immunomodulator have been used Ca-modified RNA.

Blood serum obtained from cattle before inoculation and through 7, 14, 30, 60 and 90 days after immunization, tested for the presence of specific antibodies for FMD. The results of investigation are presented in Table 1

Group of animals	Quantity of animals	The scheme of immunization	Titre of antibody in reaction of neutralization ( $\log_2$ )					
			Before immunization	After immunization				
				7 day	14 day	30 day	60 day	90 day
Cows	20	Polyvalent vaccine A, O, Asia - 1	3.2±0.3	4.5±0.2	6.8±0.4	7.1±0.4	7.0±0.3	4.8±0.2
	20	Polyvalent vaccine A, O, Asia - 1 + immunostimulator	3.2±0.3	4.6±0.2	6.8±0.4	7.4±0.4	7.0±0.3	5.9±0.1
Young	15	Polyvalent vaccine A, O, Asia - 1	3.0±0.2	4.7±0.2	6.1±0.2	6.5±0.2	4.8±0.1	4.4±0.4
8-12 months.	15	Polyvalent vaccine A, O, Asia - 1 + immunostimulator	3.0±0.2	4.8±0.2	6.4±0.2	6.7±0.3	6.2±0.4	5.4±0.3
Calves	15	Polyvalent vaccine A, O, Asia - 1	2.9±0.1	4.6±0.4	5.8±0.4	5.9±0.2	4.7±0.1	4.2±0.2
3-4 months	15	Polyvalent vaccine A, O, Asia - 1 + immunostimulator	2.9±0.1	4.6±0.4	6.3±0.4	6.8±0.3	5.6±0.1	4.9±0.2

P<0,05

**Table 1:** The influence of immunomodulator in antigenic activity of FMD vaccine.

As can be seen from the table, before vaccination titer of antibodies for FMD in blood was equal to 2.9 -3.2  $\log_2$ . In response to the introduction of vaccines and vaccine along with immunostimulator in the first 2 weeks after vaccination, there was a sudden increase of antibody titers with a certain increase in the next 2 weeks. One month after vaccination in animals immunized with the vaccine only average titer against FMD virus A, O and Asia-1 was 5.9 - 7.1  $\log_2$ , that is high enough. However, in the future, at 60 and 90-s ' days after vaccination in animals immunized with the vaccine only was observed recession of the tension of immunity, unlike animals which received Ca-modified preparation with vaccine. If animals with only vaccination shows titer of antibodies to 90 day amounted to 4.2 -4.8  $\log_2$ , in animals immunized with the vaccine and immunostimulator the titer was markedly higher around 4.9 -5.9  $\log_2$ .

## Conclusion

The proposed Ca-ds-RNK preparation had immunomodulatory properties against FMD virus that it

shows on various experiments both with appropriate FMD vaccine and as a separate preparation. The combined use of Ca-ds-RNK with FMD vaccines had shown that even if after 14-30 days there are similar level of titre of antibodies the vaccines with immunostimulator ensures high-stimulation of the immune response, and additionally supports it at a high enough level for a long time (after 60 days). This fact is important due to fact that FMD vaccination are performs usually with 6 months interval for adults and 3-4 times per year for young animals and when organizing revaccinations of young cattle, the dates for which coincide with the period of animals on pastures where there are not sufficient conditions for their implementation and therefore the calves becoming more susceptible hosts for FMDV introduction.

Based on these data it can be concluded that the using of immunomodulator in cattle induces more early and high immune response and reducing the possibilities for vaccinated cattle (including calves) with being infected with epidemiological strains of FMDV. Intramuscular inoculation of FMD vaccine with immunomodulator provides sufficiently

high immune response and high titer maintained for a period of 90 days (date of observation) (Mkrtchyan O.A. Effectiveness of active prevention of foot-and-mouth disease in the Republic of Armenia // thesis candidate Veterinary Sciences-2016, Yerevan). Based on the data obtained, we proposed for consideration by the new strategy of immunization of cattle by joint application of vaccine with immunomodulator (Ca-modified double-stranded RNA).

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