Effect of Faradarmani Consciousness Field on immune response induced by an inactivated vaccine against Foot and Mouth disease virus (FMDV) in rats and replication of FMDV *in vitro*

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***Dr. Laleh Amani was an outstand-ing, compassionate, and enthusiastic researcher in the Cosmointel. Inc studies who passed away in 2021. We extend our sincere condolences and appreciation for her extraordi-nary efforts in this research and pray for her peace.*

ABSTRACT

Foot-and-mouth disease (FMD) is one of the highest risk factors affecting the animal industry throughout the world. Currently, available commercial FMD vaccines have numerous limitations, such as slow induction and short-term maintenance of antibody titers. Therefore, a novel approach is needed that can induce high neutralizing antibody titers to protect the host in the early stages of FMD virus (FMDV) infection and maintain high antibody titers for long periods after one vaccination dose. There are several T-Consciousness Fields (TCFs), introduced by Mohammad Ali Taheri. TCFs are not matter or energy so they cannot be measured directly. However, we can evaluate their effects indirectly through several reproducible experiments in the laboratory. The present study aimed to evaluate the effect of Faradarmani CF as a type of the TCFs on FMDV replication, titer, and RNA copy number as well as the humoral immune response against two types of inactivated FMDV vaccines with different adjuvants in rats. Two types of FMD vaccines with different adjuvants (Freund and Alum) were prepared, and then 30 male Wistar rats were immunized with vaccines. Rats were divided into 6 groups (n=5 per group). Four groups of rats were studied using the different combinations of treatments and two groups served as positive and negative controls. Vaccination intervals were every 14 days three times. Serum neutralization test (SN) and Enzyme-linked immunosorbent assay (ELISA) were used to assess changes in antibody levels in the serum samples of rats after each immunization. The results showed that Faradarmani CF induced the replication of the vi-rus in vitro. In addition, antibody levels in treated groups under both Freund adjuvant and Alum adjuvant vaccines increased significantly compared to groups without Faradarmani CF treatment. In conclusion, our data suggest that Faradarmani CF may provide an effective approach to increase the success of immunization and vaccination against FMDV serotype O. It is recommended that the effects of TCFs on different types of vaccines be investigated.

Keywords: FMDV, Faradarmani Consciousness Field, Taheri Consciousness Fields, Vaccine

INTRODUCTION

One of the highly contagious viral diseases is Foot-and-mouth disease (FMD), which can affect cloven-hoofed livestock and cause vast financial losses in the industry of livestock due to its severe decrease in productivity of animals, rapid transmission, and high mortality in newborn animals caused by myocarditis (Grubman et al., 2004). This disease is associated with a high fever and causes the formation of vesicles on the mouth, tongue, nose, snout, hooves, teats, and other hairless parts of the animal skin (Arzt et al., 2011).

Foot-and-mouth disease virus (FMDV) is the etiological agent of FMD, which is a single-stranded, positive-sense RNA virus belonging to the Aphthovirus genus of the Picornaviridae family. There are seven distinct serotypes (A, O, C, Asia1, SAT1, SAT2, and SAT3) of this virus (Robinson et al., 2016). The most prevalent serotype of FMDV is serotype O, which circulates in several parts of the world (Klein, 2009).

FMDV serotypes have a high degree of antigenic and genetic variations, so antibody production stimulated by one serotype cannot be effective against different serotypes, and vaccination does not provide cross-protection (Yang et al., 2008, Knowles et al., 2003). Therefore, vaccine strains for each type should be prepared and utilized for protection against each serotype and topotype. Furthermore, vaccines should be continuously injected to maintain antibody titer potency. Nevertheless, these approaches are time-consuming, and the efficacy of these vaccines remains unclear.

While vaccination policy has been applied for the prevention and treatment of FMD, there are numerous limitations of FMD vaccines available in the market (Lyons et al., 2019, de Los Santos et al., 2018, Mahapatra et al., 2018). Some of the limitations are included; the need for repeated and regular vaccination, the requirement for a long time for the establishment of a protective level of vaccine-mediated antibodies, low and short-lived antibody titers, and inadequate host protection with only humoral immune response.

Therefore, innovative methods for use in FMD vaccines are needed to overcome the current limitations of FMD vaccines and increase their efficacy (Lee et al., 2020).

The nature of consciousness and its place in science have received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the1980s, Mohammad Ali Taheri introduced novel fields with a non-material/non-energetic nature named Taheri Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. According to this theory, there are various TCFs with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN). The major difference between the theory of TCFs and other theoretical concepts about consciousness is related to the practical application of the TCFs. These fields can be applied to all living and non-living creatures, including plants, animals, microorganisms, materials, etc.

Mohammad Ali Taheri, the founder of Erfan Keyhani Halqeh, a school of thought, introduced a new science in 2020 as a branch of this school. He coined the term Sciencefact for this new science because it utilizes scientific investigations to prove the existence of T-Consciousness as an irrefutable phenomenon and a fact. Although science focuses solely on the study of matter and energy

and Sciencefact, by contrast, explores the effects of the [non-material/non-energetic] TCFs, Sciencefact has provided a common ground between the two by conducting reproducible laboratory experiments in various scientific fields, and it has used the scientific approach in proving TCFs.

The influence of the TCFs begins with the Connection between CCN as the Whole Taheri Consciousness of the universe and the subjects of study as a part. This Connection called "Ettesal" is established by a Faradarmangar's mind (a certified and trained individual who has been entrusted with the TCFs). The human mind has an intermediary role (Announcer) which plays a part by fleeting attention to the subject of study and then the main achievement obtained as a result of the effects of the TCFs. These fields cannot be directly measured by science, but it is possible to investigate their effects on various subjects through reproducible laboratory experiments (Taheri, 2013).

The research methodology in the study of T-Consciousness has been founded on the process of *Assumption, Argument, and Proof*, in which the basic Assumption is: The Cosmos was formed by a third element called T-Consciousness that is different from matter and energy.

The Argument: The existence of TCFs can be demonstrated by their effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, etc.)

The Proof is the scientific verification of the effects of TCFs on matter and energy (according to the Argument) through various reproducible scientific experiments.

Accordingly, to investigate and verify the existence, effects, and mechanisms of TCFs, the following five research phases (Phases 0 through 4), and the aims of each phase are outlined below.

Phase-0 studies aim to prove the existence of TCFs by observing their effects. The nature of T-Consciousness and what it is will not be addressed in this phase. Phase-1 explores the varied effects of different TCFs. Phase-2 examines the reason behind the varied effects of these fields. Phase-3 investigates the mechanism of TCFs effects on matter and energy. Finally, Phase-4 draws significant conclusions, particularly with regard to the *mind and memory of matter* and their relation to the T-Consciousness, etc.

This study aimed to evaluate the effect of Faradarmani CF on FMD virus replication, titer, and RNA copy number as well as a humoral immune response against the two types of FMD vaccines with different adjuvants in rats.

METHODS AND MATERIALS

Faradarmani CF application

TCFs were applied to the samples according to the protocols regulated by COSMOintel research center (www.COSMOintel.com). A request for Connection to the CCN to utilize TCFs can be placed through the COSMOintel website in the "Assign Announcement" section. This access is available for everyone at no cost. In order to study and experience this Connection, the researchers can register on the website at any time and in order to report the experiment to the COSMOintel research center. Certain details of the experiment must be provided to the center; for example, the characteristics or number and name of samples and controls must be specified. This entire experiment was carried out as a double-blind method where lab technicians were completely unaware of TCFs theory, and the Faradarmangar at the COSMOintel research center who established the Connection was unaware

of the details of the study. Double-blind is a gold standard that is common in science experiments in the field of medicine and psychology, involving theoretical and practical testing.

In the present study, Faradarmani CF was announced simultaneously with the vaccines injections in treatment groups. In a similar way, for *in vitro* examinations, at the same time as the virus was inoculated in the cell culture flasks, treatment groups were under influence of Faradarmani CF.

FMD virus replication

FMD serotype O virus (FMDV serotype O) was originally prepared at Pirbright Institute and was used in this project. BHK (Baby hamster kidney) cell line was cultured in Dulbecco's Minimum essential medium (DMEM) high glucose, 5% FBS, 100 mg/ml penicillin, and streptomycin and incubated at 37 °C and 5% CO_2. Following 80% confluency of the monolayer of BHK Cell cultures in T-25 flasks, 0.5 ml of FMDV serotype O were inoculated with 10^7 TCID50 to each Faradarmani CF treated and control flasks. Each day cytopathic effect (CPE) was recorded under an inverted microscope for 48 hours. After 48h, samples were directed for Real-time-PCR tests. All the procedures were conducted in a biosafety level-3 laboratory (BSL-3).

2.3 Viral titration

96-well cell culture micro-plates were employed to determine viral titration (10^{-5} to 10^{-9} dilutions) using Reed & Muench method (Reed et al. 1938). Then, the plates were incubated for 72 h at 37 °C and 5% of CO_2. The flasks were daily examined to check the appearance of cytopathic effects.

Viral RNA extraction and Real-time RT-PCR reaction

Total RNA of samples was extracted using High Pure Viral RNA Kit (Roche) according to manufactures instructions. The Real-time reaction was carried out by Superscript III/Platinum Taq one-step kit (Invitrogen). A set of primers was used for detecting FMDV fragment 3D (polymerase RNA gene). The total volume of 20µl reaction mix contained 0.4 µl $MgSO_4$, 10µl 2x-reactions buffer, 2.6 µl Diethyl Pyrocarbonate (DEPC) water, 0.4 µl Enzyme Superscript III/Platinum, 0.25 µl of each primer, 0.1 µl probe, 6µl extracted RNA. Then, the tubes were placed into a Corbett Rotor-Gene device. The steps of amplification were done in the following temperature cycles: Reverse transcription (one cycle), 50 ºC for 30 minutes, the initial denaturing (one cycle), 95 ºC for 2 minutes, 95 ºC for 30 seconds, and 60 ºC for 30 seconds (40 cycles).

Preparation of complete FMD virus antigen for vaccines

Each flask of BHK cell (80% confluency) was inoculated with 2 ml of virus ($10^{6.8}$ TCID50/ml). After 18 hours, all flasks showed full cytopathic effect (CPE). The flasks containing the cell and the virus were then frozen at -70 °C and thawed twice to destroy the cells and extract the intracellular viruses. In the next step, the resulting suspension was filtered through a 0.2 µm filter. The viral suspension (500 ml) was centrifuged at 3000 rpm for 10 min at 4 °C. Deactivation of the virus was performed by binary ethylenimine at a concentration of 5 mM for 24 hours at 25 °C. Unused ethyleneamine in the viral suspension was neutralized by sodium thiosulfate (5 mM). Polyethylene glycol (8% w/v, 6000 kDa) was used to concentrate the inactivated virus. Finally, the prepared antigen was collected at a volume of 10 ml. The concentration of the prepared antigen was 2.5 mg/ml determined by the Lowry method (Lowry et al., 1951).

VACCINE PREPARATIONS

The vaccine containing aluminum hydroxide gel.

In the preparation of the aqueous vaccine, 33% aluminum hydroxide gel with a pH of 7.2 and saponin (6 mg/dose) were used.

The vaccine containing Adjuvant Freund

To prepare the vaccine containing Freund adjuvant, two types of complete and incomplete adjuvants (Sigma Co.) were used. In the composition of this vaccine, 50% antigen was mixed with 50% adjuvant Freund. Homogenization of the vaccine was performed with a 10-ml syringe with successive filling and emptying of the tube. In the first injection, the vaccine containing complete Freund, and in the subsequent injections, the vaccine containing incomplete adjuvant Freund was used.

Grouping of rats

In this study, 30 male Wistar rats weighing about 300 gr on average were used. Rats were divided into 6 groups (n=5 per group). Subcutaneously, 0.5 ml of the vaccine was injected into each rat in the area between the two shoulders. The 5 rats were considered as the negative control group. Grouping was done as follows:

- Group 1: FMDV (Type O) + Alum adjuvant
- Group 2: FMDV (Type O) + Alum adjuvant + Faradarmani CF
- Group 3: FMDV (Type O) + Freund adjuvant
- Group 4: FMDV (Type O) + Freund adjuvant + Faradarmani CF
- Group 5: Alum + recombinant VP1 (Positive Control)
- Group 6: Negative Control

Vaccination intervals held were every 14 days. Blood samples were taken on day zero and before each immunization dose. Blood samples were centrifuged at low speed (1500 ×g for 10 min at 4 oC) and their serum was isolated. The sera were incubated at 56 ° C for 30 minutes to inactivate their complement. Then, they were stored at -20 ° C until serological tests.

SEROLOGICAL TESTS

Serum neutralization test (SN) and ELISA (Enzyme-linked immunosorbent assay) were used to assess changes in antibody levels in the serum samples of rats after vaccination.

SN test

At first, the sera were serially diluted with Roswell Park Memorial Institute (RPMI) medium and 50 µl of each dilution was poured into each well in duplicate. Second, 50 µl of serotype O virus with a titer of 10^3 TCID50/ml was added to each well. Then, plates were placed in an incubator at 37 °C for 60 minutes. Next, 50 µl of IBR-S2 cell line (0.5×10^5 / well) was added. Finally, the plates were incubated at 37 °C. After 48 hours, the plates were examined for CPE effects. Test results were determined based on CPE observation. The highest dilution of serum, which was able to prevent CPE in 50% of wells, was considered a neutralizing antibody titer. Finally, serum titers were calculated.

ELISA

FMDV serotype O was diluted 1/10 with coating buffer ($NaHCO_3$ / Na_2CO_2, 0.05 M, pH = 9.5), 100 µl of which was added into 96 micro-plate wells and incubated at 4 °C overnight. All contents

of the micro-plate were discharged and washed three times with PBS-T (500 µl PBS, 20 µl Tween 20). Skim milk 5% (in PBS-T) was used to block the empty space between antigen molecules at the bottom of the wells. After blocking, washing was done four times with washing buffer.

For determining the appropriate dilution of serums, two positive and negative control serum samples were diluted as a checkerboard. Four dilutions (1:25, 1:50, 1: 100, and 1: 200) of the mentioned sera were prepared and tested in PBS-T and skim milk %1. Finally, a 1: 100 dilution was detected at a proper concentration.

For the addition of unknown sera, 100 µl of rat serum was added to each well and incubated for 75 minutes at 37 °C. The washing steps were repeated four times.

For adding the secondary antibody, 100 µl of Rabbit Anti-Guinea Pig Horseradish Peroxidase Conjugated (Sigma) was added to each well and incubated at 37 °C for 75 minutes. After this step, washing was done 5 times. Substrate (TMB: 3,3',5,5'-Tetramethylbenzidine) (100 µl) was then added to all wells and placed at room temperature and in the dark for 15 minutes. In the end, 50 µl of 1 M hydrochloric acid was added as a stop solution and the optical absorption (OD) of samples was read by an ELISA reader at 450 nm.

RESULTS

Impact of Faradarmani CF on replication, titer, and RNA copy number of FMD virus

In this study, the compatibility of the virus with the BHK cells, their growth, and proliferation were evaluated in the cell culture. Results of virus titration (TCID50 calculation) are presented in Table 1. On average, in each passage, a half-log is added to the virus titer. The duration of the observed cytopathic effects was started from 6 to 7 hours after inoculation of the virus and reached its peak in 12 hours. In cultures affected by the Faradarmani CF, cells could survive longer in the face of the virus.

The results of the real-time RT-PCR assay were assessed by the C_t values. There were differences in C_t values between the Faradarmani CF treat-

Table1. TCID50 and Real-time PCR assay results

Virus	NO.	TCID50 (log)	Overall mean (log)	Distance (log)	C_t values
Faradarmani CF treatment group	1	7.2	6.96	0.53	8
	2	6.7			10
	3	7			8
Control group	4	6.8	6.43		9
	5	6			10
	6	6.5			9
Faradarmani CF treatment group (Second repeat)	1	8.25	8	0.44	6
	2	7.75			7
	3	8			6
Control group (Second repeat)	4	8	7.56		6
	5	7.2			8
	6	7.5			8

ment and control groups. The lower Ct values in the Faradarmani CF treatment group indicate that the number of viruses was greater in the samples under influence of Faradarmani compared to the controls.

Serum neutralization and ELISA tests

In this study, serological evaluation of samples was performed at 14-day intervals after immunizations (Table 2). The sera of each group was mixed (pooled) at each stage of blood sampling. In all groups, a significant change was found in antibody levels in the second blood sampling stage (before the second injection). A significant increase was seen in antibody levels in the titration of neutralizing antibodies in blood samples after the second injection in all groups except the control group. Interestingly, in Faradarmani CF treated groups in Freund adjuvant and Alum adjuvant vaccines, there was a significant increase in protective antibodies in rats who received several injections compared to groups without Faradarmani (Table 2). Therefore, Faradarmani CF can be considered an effective treatment for increasing the success of immunization and vaccination against serotype O FMD virus. Moreover, the rate of immune stimulation rate of the recombinant VP1 protein was lower than in the groups with and without the Faradarmani CF in the third and fourth immunization in the titration of neutralizing antibodies. The effect of the Faradarmani CF on the immunogenicity of the FMD vaccine with Freund adjuvant was more than the alum adjuvant.

The evaluation of antibody concentration was performed by coating the whole particle of the inactivated virus with ELISA and its mean OD results are shown in Table 3. The sera of each group was pooled at each stage of blood sampling. The 0.2 cut-off was determined based on bovine negative serum samples. Increased antibody titers against FMD serotype O virus were seen in all the vaccinated rat groups. The results of ELISA indicate a

Table2. Results of serum neutralization test after injection of different vaccines

Studied groups	First BS (0 day)	Second BS (14 days)	Third BS (28 days)	Fourth BS (42 days)
FMDV (Type O) +Alum adjuvant	0.3	0.5	0.8	1.4
FMDV (Type O) + Alum adjuvant + FCF	0.3	0.7	1.1	1.7
FMDV (Type O) + Freund adjuvant	0.3	0.5	0.9	1.2
FMDV (Type O) + Freund adjuvant + FCF	0.3	0.8	1	1.9
Alum + recombinant VP1 (Positive Control)	0.3	0.5	0.6	0.8
Negative Control	0.3	0.3	0.3	0.3

BS: Blood sampling, FCF: Faradarmani Consciousness Field, VP1 is a structural viral peptide that has high immunogenic characteristics.

Table3. Results of ELISA test after injection of antigens with alum and Freund adjuvants in the studied groups.

Studied groups	First BS (0 day)	Second BS (14 days)	Third BS (28 days)	Fourth BS (42 days)	Fifth BS (56 days)
FMDV (Type O) + Alum adjuvant	-	0.35	0.59	0.92	2.60
FMDV (Type O) + Alum adjuvant + FCF	-	0.5	0.81	1.2	3.1
FMDV (Type O) + Freund adjuvant	-	0.28	1.10	1.57	2.82
FMDV (Type O) + Freund adjuvant + FCF	-	0.41	1.5	2.1	3.5
Alum+ recombinant VP1 (Positive Control)	-	0.30	0.55	0.42	0.65
Negative Control	-	0.10	0.20	0.15	0.12

BS: Blood sampling, FCF: Faradarmani Consciousness Field, VP1 is a structural viral peptide that has high immunogenicity.

significant increase in antibody titer in groups affected by the Faradarmani CF treatment compared to the group without Faradarmani CF treatment.

DISCUSSION

In virus culture, Faradarmani CF treatment increased the survival of the cells. Viruses, as intracellular parasites, rely on their host cells for energy, macromolecular synthesis, and genome replication (Whitaker-Dowling et al., 1999, de Castro et al., 2013). Therefore, in groups under influence of Faradarmani CF, viruses have more time to be replicated in the host cells.

In the present study, Faradarmani CF increased protective antibodies in rats who received several injections in both Freund and Alum adjuvant vaccine groups compared to the groups without Faradarmani CF. Therefore, the Faradarmani CF can be used to increase the success of immunization and vaccination against the FMD serotype O virus. The effect of the Faradarmani CF on the immunogenicity of the Freund adjuvant-containing vaccine was more than the alum adjuvant-containing group. Freund's complete adjuvant is an oily adjuvant containing the Mycobacterium, but alum (aluminum hydroxide) adjuvant is a mineral adjuvant. According to this observation, it seems that Faradarmani CF probably has a greater impact on organic-based adjuvants or those with organic components. Furthermore, the results of ELISA indicated a significant increase in antibody titer in groups affected by Faradarmani CF compared to the groups without Faradarmani CF.

In order to evaluate the humoral immune response of rats against inactive target antigens in control groups, at least two injections should be performed. However, in groups under Faradarmani CF effect, this protective effect was completed in the second injection. Furthermore, the immunogenic power of the complete antigen of the killed FMD serotype O virus with Freund and alum adjuvants was much higher than that of VP1 protein. Similar to the results ELISA, the serum neutralization test results on antigen with complete adjuvant Freund as well as incomplete composition, higher levels of antibodies were found in the serums of the Faradarmani CF group than the control and alum groups. It seems that the use of Faradarmani CF as a complementary factor in immunization and vaccination can cause a greater immune response and better safety in livestock.

In previous studies, the effects of the TCFs on MCF7 cancer cell line (Taheri et al., 2020a), Alzheimer's disease rat models (Taheri et al., 2021b), spatial memory, and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021c), wheat plant (Torabi et al. 2020), bacterial population growth (Taheri et al., 2021d), viral growth (Taheri et al. 2021a), and the electrical activity of the brain during Faradarmani in the Faradarmangars population have been investigated (Taheri et al., 2020b).

As it was mentioned in the introduction section, since TCFs are neither matter nor energy, cannot be measured directly, but it is possible to investigate their effects indirectly through various experiments. We suggest other researchers conduct more experiments to clarify the effects of the Faradarmani CF on different types of vaccines.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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