Effect of the 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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Abstract

Foot-and-mouth disease (FMD) is one of the highest-risk factors affecting the animal industry worldwide. 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 extended periods after a single 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 indirectly evaluate their effects through several reproducible laboratory experiments. The present study aimed to evaluate the effect of Faradarmani CF, 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's and Alum) were prepared, and 30 male Wistar rats were immunized with the vaccines. Rats were divided into six groups (n=5 per group). Four groups of rats were studied using different treatment combinations, 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 rat serum samples after each immunization. The results showed that Faradarmani CF induced viral replication *in vitro*. In addition, antibody levels in treated groups under both Freund's adjuvant and Alum adjuvant vaccines increased significantly compared to groups without Faradarmani CF treatment. In conclusion, our data suggest that Faradarmani CF may offer a practical approach to improving immunization and vaccination success against FMDV serotype O. It is recommended that the effects of TCFs across different vaccine types be investigated.

Keywords: Foot-and-mouth disease, 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 significant financial losses in the livestock industry due to its severe reduction in animal productivity, rapid transmission, and high mortality in newborn animals caused by myocarditis (Grubman et al., 2004). This disease is characterized by high fever and causes the formation of vesicles on the mouth, tongue, nose, snout, hooves, teats, and other hairless areas of the animal's 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 exhibit high antigenic and genetic variation, so antibody responses induced by one serotype are not effective against other 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 used to protect against each serotype and topotype. Furthermore, vaccines should be administered continuously to maintain antibody titers. Nevertheless, these approaches are time-consuming, and the efficacy of these vaccines remains unclear.

While vaccination policy has been applied to prevent and treat FMD, there are numerous limitations to the vaccines available on the market (Lyons et al., 2019; de Los Santos et al., 2018; Mahapatra et al., 2018). Some limitations include the need for repeated vaccination, the need for a long time to achieve a protective level of vaccine-mediated antibodies, low and short-lived antibody titers, and inadequate host protection due to a humoral immune response alone. Therefore, innovative methods for FMD vaccines are needed to overcome current limitations and improve their efficacy (Lee et al., 2020).

According to Taheri's theory, there are different T-Consciousness Fields with various functions that are a subset of the Cosmic Consciousness Network. These fields do not have a physical or frequency nature and cannot be measured directly using quantitative tools. More details about this theory are provided in the Common Considerations section of this issue. In short, the possibility of practical investigation in these fields allows researchers to study them through a range of experiments (Taheri, 2013).

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

Material and Methods

Faradarmani CF application

The samples studied were influenced by the Faradarmani Consciousness Field, according to the guidelines set up at the CosmoIntel Research Center (www.COSMOintel.com).

In the present study, Faradarmani CF was announced simultaneously with the vaccine injections in treatment groups. Similarly, for *in vitro* examinations, at the same time the virus was inoculated into the cell culture flasks, the treatment groups were exposed to Faradarmani CF.

FMD virus replication

FMD serotype O virus (FMDV serotype O) was initially prepared at the Pirbright Institute and used in this project. BHK (Baby hamster kidney cell line was cultured in Dulbecco's Minimum Essential Medium (DMEM) high glucose, 5% fetal bovine serum (FBS), 1% antibiotics (penicillin and streptomycin), and incubated at 37 °C and 5% CO2. Following 80% confluency of the monolayer of BHK Cell cultures in T-25 flasks, 0.5 mL of FMDV serotype O was inoculated with 107 TCID50 into each Faradarmani CF-treated and control flask. Each day, the cytopathic effect (CPE) was recorded under an inverted microscope for 48 hours. After

48h, samples were sent for real-time PCR testing. All procedures were conducted in a biosafety level-3 (BSL-3) laboratory

Viral titration

96-well cell culture microplates were employed to determine viral titers (10^{-5} to 10^{-9} dilutions) using the Reed & Muench method (Reed et al., 1938). Then, the plates were incubated for 72 h at 37 °C and 5% CO2. The flasks were examined daily to assess the appearance of cytopathic effects.

Viral RNA extraction and real-time RT-PCR reaction

Total RNA from the samples was extracted using the High Pure Viral RNA Kit (Roche) according to the manufacturer's instructions. The Real-time reaction was carried out using the Superscript III/ Platinum Taq one-step kit (Invitrogen). A set of primers was used to detect an FMDV fragment of the 3D (polymerase RNA) gene. The total volume of 20 µL reaction mix contained 0.4 µL MgSO4, 10 µL 2x-reactions buffer, 2.6 µL diethylpyrocarbonate (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 cells (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 cells and the virus were then frozen at -70 °C and thawed twice to lyse the cells and release 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 in 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

Vaccine containing aluminum hydroxide gel

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

Vaccine containing Adjuvant Freund

To prepare the vaccine containing Freund's adjuvant, two types of complete and incomplete adjuvants (Sigma Co.) were used. In this vaccine, 50% antigen was mixed with 50% Freund's adjuvant. Homogenization of the vaccine was performed using a 10 mL syringe, with the tube filled and emptied repeatedly. In the first injection, the vaccine containing complete Freund's adjuvant was used, and in subsequent injections, the vaccine containing incomplete Freund's adjuvant was used.

Grouping of rats

In this study, 30 male Wistar rats, weighing about 300 g on average, were used. Rats were divided into six 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 five rats were considered 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's adjuvant
- Group 4: FMDV (Type O) + Freund's adjuvant + Faradarmani CF
- Group 5: Alum + recombinant VP1 (Positive Control)
- Group 6: Negative Control

Vaccination intervals 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 °C), and the 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 rat serum samples after vaccination.

SN test

At first, the sera were serially diluted in Roswell Park Memorial Institute (RPMI) medium, and 50 µL of each dilution was added to 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 the 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 that prevented CPE in 50% of wells was considered the neutralizing antibody titer. Finally, serum titers were calculated.

ELISA

FMDV serotype O was diluted 1/10 with coating buffer (NaHCO3/Na2CO3, 0.05 M, pH 9.5), and 100 µL was added to 96 microplate wells and incubated at 4 °C overnight. All contents of the microplate 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 space between antigen molecules at the bottom of the wells. After blocking, the sample was washed four times with washing buffer.

To determine the appropriate dilution of the sera, two positive and two negative control serum samples were diluted in a checkerboard pattern. Four dilutions (1:25, 1:50, 1:100, and 1:200) of the mentioned sera were prepared and tested in PBS-T and 1% skim milk. Finally, a 1:100 dilution was determined to be the 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 3,3',5,5'-Tetramethylbenzidine (TMB) (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 absorbance (OD) of the samples was read on 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 virus's compatibility with BHK cells and their growth and proliferation were evaluated in cell culture. Results of virus titration (TCID50 calculation) are presented in Table 1. On average, a half-log is added to the virus titer for each passage. The duration of the observed cytopathic effects began at 6 to 7 hours after virus inoculation and reached a peak at 12 hours. In cultures affected by the Faradarmani CF, cells could survive longer in the face of the virus.

The Ct values were used to assess the results of the real-time RT-PCR assay. There were differences in Ct values between the Faradarmani CF treatment and control groups. The lower Ct values in the Faradarmani CF treatment group indicate that the number of viruses was higher in the samples under the influence of Faradarmani than in the controls.

Table 1. TCID50 and Real-time PCR assay results

	NO.	TCID50 (log)	Overall mean (log)	Distance (log)	Ct 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

Serum neutralizing (SN) test and ELISA tests

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

Table 2. Results of the serum neutralization test after injection of different vaccines

Studied groups	First BS (0 day)	Second BS (14 day)	Third BS (28 day)	Fourth BS (42 day)
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's adjuvant	0.3	0.5	0.9	1.2
FMDV (Type O) + Freund's adjuvant+ FCF	0.3	0.8	1	1.9
Alum+ recombinant VP1 (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 immunogenicity.

The antibody concentration was evaluated by ELISA, with whole virus particles used for coating, and the mean OD results are shown in Table 3. The sera of each group were 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 virus serotype O were observed in all vaccinated rat groups. The ELISA results indicate a significant increase in antibody titer in the Faradarmani CF-treated groups compared to the untreated group.

Table 3. Results of the ELISA test after injection of antigens with alum and Freund's 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's 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 (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 with high immunogenicity.

Discussion

In virus culture, Faradarmani CF treatment increased cell survival. 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 the influence of Faradarmani CF, viruses have more time to replicate in host cells.

In the present study, Faradarmani CF increased protective antibodies in rats receiving multiple injections in both the Freund and Alum adjuvant vaccine groups compared to groups without Faradarmani CF. Therefore, the Faradarmani CF can be used to improve immunization and vaccination success against the FMD serotype O virus. The effect of the Faradarmani CF on the immunogenicity of the Freund's adjuvant-containing vaccine was greater than that of 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 may have a greater impact on organic-based adjuvants or those containing organic components. Furthermore, ELISA results indicated a significant increase in antibody titer in groups affected by Faradarmani CF compared to those without Faradarmani CF.

To evaluate the humoral immune response of rats against inactive target antigens in control groups, at least two injections should be administered. However, in groups under Faradarmani CF, this protective effect was complete by the second injection. Furthermore, the immunogenicity of the complete antigen of the killed FMD serotype O virus with Freund's and alum adjuvants was much higher than that of the VP1 protein. Similar to the ELISA results, the serum neutralization test results with the complete adjuvant Freund and the incomplete composition showed higher antibody levels in the Faradarmani CF group than in the control and alum groups. It seems that using Faradarmani CF as a complementary factor in immunization and vaccination can lead to a stronger immune response and improved safety in livestock.

Previous studies have explored the influence of TCFs across diverse biological and physiological contexts, including their impact on the MCF7 breast cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior in an Alzheimer's disease rat model (Taheri et al., 2021a), wheat plants (Torabi et al., 2020), bacterial population (Taheri et al., 2021b), and alterations in brain electrical activity observed during Faradarmani Connection among Faradarmangars (Taheri et al., 2020b).

As mentioned in the introduction section, since TCFs are neither matter nor energy, they cannot

be measured directly; however, their effects can be investigated indirectly through various experiments. We suggest that further research be conducted to clarify the effect of Faradarmani CF on various vaccines.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Arzt, J., Baxt, B., Grubman, M., Jackson, T., Juleff, N., Rhyan, J., Rieder, E., Waters, R., & Rodriguez, L. (2011). The pathogenesis of foot-and-mouth disease II: Viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus–host interactions. *Transboundary and Emerging Diseases, 58*(4), 305–326.

de Castro, I. F., Volonté, L., & Risco, C. (2013). Virus factories: Biogenesis and structural design. *Cellular Microbiology, 15*(1), 24–34.

de Los Santos, T., Diaz-San Segundo, F., & Rodriguez, L. L. (2018). The need for improved vaccines against foot-and-mouth disease. *Current Opinion in Virology, 29*, 16–25.

Grubman, M. J., & Baxt, B. (2004). Foot-and-mouth disease. *Clinical Microbiology Reviews, 17*(2), 465–493.

Klein, J. (2009). Understanding the molecular epidemiology of foot-and-mouth disease virus. *Infection, Genetics and Evolution, 9*(2), 153–161.

Knowles, N., & Samuel, A. (2003). Molecular epidemiology of foot-and-mouth disease virus. *Virus Research, 91*(1), 65–80.

Lee, M. J., Jo, H., Park, S. H., Ko, M.-K., Kim, S.-M., Kim, B., & Park, J.-H. (2020). Advanced foot-and-mouth disease vaccine platform for stimulation of simultaneous cellular and humoral immune responses. *Vaccines, 8*(2), 254.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*(1), 265–275.

Lyons, N. A., Knight-Jones, T. J., Bartels, C., Paton, D. J., Ferrari, G., Vermillion, M. S., Brooks, A. W., Motroni, R., Parker, E., & Berquist, M. L. H. (2019). Considerations for design and implementation of vaccine field trials for novel foot-and-mouth disease vaccines. *Vaccine, 37*(7), 1007–1015.

Mahapatra, M., & Parida, S. (2018). Foot-and-mouth disease vaccine strain selection: Current approaches and future perspectives. *Expert Review of Vaccines, 17*(7), 577–591.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology, 27*(3), 493–497.

Robinson, L., Knight-Jones, T. J., Charleston, B., Rodriguez, L., Gay, C., Sumption, K. J., & Vosloo, W. (2016). Global foot-and-mouth disease research update and gap analysis: 5–Biotherapeutics and disinfectants. *Transboundary and Emerging Diseases, 63*(1), 49–55.

Taheri, M. A. (2013). *Human from another outlook* (2nd ed.). Interuniversal Press. ISBN-13: 9781939507006; ISBN-10: 1939507006

Taheri, M. A., Semsarha, F., Mahdavi, M., Afsartala, Z., & Amani, L. (2020a). The influence of the Faradarmani consciousness field on the survival and death of MCF-7 breast cancer cells: An optimization perspective. *SSRN Electronic Journal.*

Taheri, M. A., Semsarha, F., & Modarresi-Asem, F. (2020b). An investigation on the electrical activity of the brain during Fara-Darmani connection in the Fara-Therapist population. *SSRN Electronic Journal.*

Taheri, M. A., Torabi, S., Nabavi, N., & Semsarha, F. (2021a). Influence of Faradarmani consciousness field (FCF) on spatial memory and passive avoidance behavior of scopolamine model of Alzheimer disease in male Wistar rats. *SSRN Electronic Journal.*

Taheri, M. A., Zarrini, G., Torabi, S., Nabavi, N., & Semsarha, F. (2021b). Influence of Faradarmani consciousness field on bacterial population growth. *bioRxiv.*

Torabi, S., Taheri, M. A., & Semsarha, F. (2020). Alleviative effects of Faradarmani consciousness field on *Triticum aestivum* L. under salinity stress. *F1000Research, 9*, 1089.

Whitaker-Dowling, P., & Youngner, J. S. (1999). Virus–host cell interactions. In *Encyclopedia of Virology* (pp. 1957–1965). Academic Press.

Yang, M., Holland, H., & Clavijo, A. (2008). Production of monoclonal antibodies against whole virus particles of foot-and-mouth disease virus serotype O and A and their potential use in quantification of intact virus for vaccine manufacture. *Vaccine, 26*(28), 3377–3382.

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