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Molecular evaluation of prepared Rift Valley Fever (RVF) vaccine using Montanide Gel 02 and its effect on the genetic material

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Abstract

Rift Valley fever (RVF) is a zoonotic, arthropod-borne viral disease that affects domestic animals. The disease is caused by the Rift Valley fever virus (Phlebovirus) that belongs to the Phenuiviridae family. Rift Valley fever (RVF) is an animal origin viral bleeding disease that affects the health of both humans and animals because it's a Phlebovirus that makes it very dangerous. Conventional technologies were used to develop the first vaccines, such as inactivated vaccines and the live-attenuated Smithburn vaccine, using virulent RVF virus isolates. Various vaccines against the RVF virus have been developed, the modern trends in vaccine production are going to choose. The adjuvant used in the vaccine production process is a significant consideration. Therefore, in this study we explored the use of Maintained gel 0.2 as adjuvant. The proper adjuvant that can appear high and long-standing immunity. Maintained gel 0.2 is generic of maintaining as water-based adjuvant designed recently to improve efficacy of aqueous type vaccine. Therefore, we investigated the effect of inactivated RVF vaccine with three different concentrations of Montanide Gel 02 (10%, 15% and 20%). The immune response was evaluated and evaluated of the immune response in vaccinated rabbits using real time PCR (RT-PCR). Also, and in other side, starting coding target (SCOT) technique was were used to study the genetic effect of the vaccine on the rabbit's genetic material. RT-PCR The real time PCR results for interferon γ showed that there was dramatically upregulation in transcripts levels of interferon γ with the increases of Montanide gel doses throughout the whole entire period of the experiment. A total of 10 primers were tested for selective amplification of DNA fragments. The ten SCoT primers produced reliable PCR products. However, no SCoT primers showed discernible polymorphism between all tested samples. However, no Scot primers showed discernible polymorphism between all tested samples. The study revealed that with increasing Montanide gel 02 concentration in the rabbit's vaccination, the immune response and its duration increased, with no influence on the genetic material, indicating that Montanide gel 02 was safe and effective. The immune response and its duration were increased with increasing Montanide gel 02 concentration in the rabbit's vaccination, and there was no effect on the genetic material, which gives is give an indication that Montanide gel 02 was safe and effective.

Key words: Rift Valley fever (RVF), Montanide gel 0.2, RT-PCR and Start Codon Targeted (SCoT).

1. Introduction

Rift Valley fever (RVF) is a viral bleeding illness that affects both humans and animals. It is caused by a phlebovirus, which is a member of the Phenuiviridae viral family and is exceedingly hazardous [1]. The virus was first isolated and defined as a result of the abrupt deaths of 4,700 lambs and ewes on a single farm in Kenya in 1931, notably around the shores of Lake Naivasha [2].

Since then, the RVF virus has triggered a slew of economically devastating epizootics marked by sweeping abortion storms and fatality rates of around 100 % among neonates and 10 % to 20 % among adult ruminant cattle (especially sheep and cattle) [3]. Several mosquito species are capable vectors of RVFV, and they play a key role in viral transmission in both the enzootic and epizootic/epidemic cycles between domestic animals and humans [2].

Rift Valley fever virus (Family Bunyaviridae; Genus Phlebovirus) is an enclosed spherical particle with a tripartite single-stranded negative-sense RNA genome (80 to100 nm in diameter). (approx. 11.9 kilobases) [4]. The RVF virus genome is made up of three single-stranded RNA segments with negative or ambient polarity: S segment (1690 nt), M segment (3885 nt), and L segment (1690 nt) (6404 nt) [4]. RVF virions have a spherical form with a diameter of 80–120 nm. It is made up of a ribonucleocapsid (RNP), an icosahedral capsid with 122 capsomers (110 hexamers and 12 pentamers), and an envelope comprising Gn and Gc glycoprotein heterodimers [5].

[6] and [7] reported that RVFV first appeared in Egypt in 1977-1978, with symptoms including rigors, myalgia, headache, conjunctivitis, nausea, and conjunctivitis, as well as ocular problems. The best means of protecting animal populations, and indirectly humans, is vector control and vaccination with the use of safe and effective inactivated vaccines. Therefore, other studies have been conducted to improve the inactivated vaccine by using binary ethyleneimine instead of formalin for its safe effect on viral antigens and the completeness of the inactivated process [8].

Various vaccines against the RVF virus have been established, such as formalin inactivated virus

[9] and [10], live attenuated virus [11] and [12], viral subunits [13], recombinant virus vectors [14], viral cDNA [4], and by use of recombinant live-attenuated RVF virus containing complete deletions of known virulence genes [15]. During epizootics of RVF, the use of live attenuated Smith burn vaccine is recommended [16] but limitation to be used in pregnant animals due to fear from teratogenic or abortogenic effect and ability to be converted into virulent state [17].

Aluminum salts and oil-based vaccines are the most common adjuvant technology used in veterinary vaccinations [18] and [19]. Oil based vaccines used to obtain field strong immune [20]. Aluminum salts are utilized as a safety reference and are employed with sensitive species due to their low toxicity.

Montanide gel 0.2 is a water-based adjuvant that was developed lately to boost the effectiveness of aqueous type vaccines. It was discovered that employing Montanide gel as an adjuvant resulted in an adequate early immune response in vaccinated calves, as well as a faultless safety profile, regardless of the antigen type [21].). It was also observed that the antibodies produced were greater than those produced by aluminum-based vaccinations [22] and [23].

Correlation between molecular markers and phenotypes becomes very important in significant developments which show genetic similarity, variation, and detection of genomic regions responsible for the different trait. SCoT (Starting Codon Target) is one of these markers which characterized as dominant markers and generally reproducible, so its successfully to use in assess genetic diversity and structure identify genotypes which is the bias of quantitative trait loci (QTL) mapping and DNA fingerprinting in different rabbits' genotypes [24].

In this study, we investigated the effect of inactivated RVF vaccine with three different concentrations of Montanide Gel 02 (10%, 15% and 20%) and evaluated the immune response in vaccinated rabbits using RT-PCR. Also, the genetic effect of vaccination on the rabbit's genetic material was studied using SCOT approach.

2. Materials and Methods

The RVF ZH501 virus strain utilized in this investigation was provided by the Veterinary Serum and Vaccine Institute's viral collection (VSVRI). This strain was widely utilized for vaccine manufacturing at the RVF vaccine research department, VSVRI, Abbasia, Egypt, by Dr. Kareem Elden Zaky. The strain was isolated from the viral stock and kept at –80°C with a titer of 108 TCID50/0.1ml.

2.1. Adult rabbits

30 days old free Sinai Gabali rabbits supplied by the rabbits' farm, Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. The rabbits were used for toxicity test of different concentrations of Montanide Gel 02TM, titration of the virus, evaluation of the immune response of the prepared vaccines and other studies. Baby hamster kidney cells (BHK21) were cultured and maintained according to [25] for RVF virus propagation and vaccine production.

The Montanide Gel 02 TM (Seppic, Paris, France) was prepared in three different concentrations (5%, 10% and 15%) and tested for its toxicity on three groups of rabbits, each containing fifteen rabbits (0.5 ml/rabbit). RVF virus was inactivated according to [26]. The safety evaluation of the provided inactivated virus was performed on five rabbits two weeks old according to [27].

The vaccine was formulated according to the technical bulletin of Montanide 02 oil prescribed by the manufacturer. A total weight of 50-gm inactivated virus suspension was diluted in 50 gm of adjuvant (weight/ weight). Montanide IMS 1313 VG NPR oil-based vaccine was assessed for sterility using thioglycolate and soybean casein digest medium (free from aerobic and anaerobic).

2.2. Preparation of RVF vaccine collections

Preparation of 5 collections of inactivated RVF vaccine as follow:

Col. 0 negative control (no treatment)

Col. 1 RVF vaccine without adjuvant (inactivated RVFV)

Col. 2 montanide gel 5% concentration as adjuvant + (inactivated RVFV)

Col. 3 montanide gel 10% concentration as adjuvant+ (inactivated RVFV)

Col. 4 montanide gel 15% concentration as adjuvant+ (inactivated RVFV)

All infected and non-infected rabbits were observed and examined every day for clinical symptoms, mortalities, and body weights then were recorded.

2.3. Isolation of total RNA

Rabbits' lymphocytes were used to obtain the total RNA using RNA isolation Mini kit (A&A Biotechnology, Gdynia, Poland). The concentration and purity were measured using Nano-Drop 2000C spectrophotometer (Thermo Scientific, USA). At A260/A280 absorbance ratio, all samples showed an RNA purity of > 1.9. The integrity of the RNA was verified using a gel electrophoresis image on a 2% agarose gel (Gel Doc. BioRad). RNA samples were subjected directly to reverse transcriptase reaction.

2.4. Reverse Transcriptase (RT) Reaction—cDNA Synthesis

Complementary DNA (cDNA) was synthesized from RNA samples by reverse transcriptase reaction .Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) was used to synthesize cDNA according to the manufacturer's instructions.

2.5. Quantitative Real-Time PCR (RT-PCR)

Triplicate qRT-PCR was carried out for all treated groups, non-template control (NTC) and negative cDNA template. The reactions were performed using the primer pairs that designed previously by [28]. namely IFγ gene Primer F (5-CGGCACAGTCATTGAAAGCCTA-3) and IFγ gene Primer R (5-GTTGCTGATGGCCTGATTGTC-3).

PCR master mix contained 600 ng cDNA, 12.5 µl Maxima SYBR Green Master Mix (Maxima SYBR Green qPCR, Thermo Fisher Scientific), 0·3 µmol of forward and reverse primer, 10 nmol /100 Nm ROX solution, nuclease-free water was added to adjusted the final volume to 25 µl.

The PCR program was: 95 °C for 10 min and 40 cycles of 95 °C for 15 s followed by 60°C for 60 s. At the end of the PCR, a melting curve technique was used, which consisted of heating at 95 ºC for 30 s then 65 ºC for 30 s, and 95 ºC for the 30s. Targeted relative gene expression ratios between treated and control collections were evaluated by the formula: $RQ = 2^{-\Delta Ct}$ [29].

2.6. Genomic DNA extraction

Genomic DNA was isolated from blood samples of 75th rabbits using the CTAB method [30], then the extracted DNA for each collection which consists of 15th rabbits mixed together and canceled to be five samples, so the finally number of samples was 25 samples which is representative to the total number of 75th rabbits to be a prerequisite to multiplex PCR. Short conserved regions of genes are surrounded by the ATG translation (SCOT markers) were used to evaluate the genetic stability of the tested samples [31].

2.7. SCOT-PCR Reactions

Prepared Promega Master Mix were used in a total volume of 25 µL (10 µL Master Mix, 10 µL dH2O, 3ul20pmol of primer and 2 µL 10 ng genomic DNA). Perkin-Elmer/GeneAmp®PCR thermo cycler were used with the following condition: denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 50 sec and elongation at 72°C for 1.5 min. The primer extension segment was extended to 10 min at 72°C in the final cycle. PCR products were resolved at 3% agarose gel and 1kb DNA plus ladder was used as a molecular weight standard.

3. Results and Discussion

Forty-five rabbits (three collections each collet consisted of 15 rabbits) were used to tested the toxicity of different concentration of Montanide gel 02 adjuvant (5%, 10% and 15%), which inoculated with 0.5ml/ rabbit and kept under daily observation for two weeks after inoculation. There was no toxic effect or death with different concentrations and also, no mortalities or any signs of illness (no allergic reaction, no inflammation, no granuloma) were appeared on inoculated two weeks rabbits during the observation period (15day post inoculation) after inoculation by inactivated vicinal virus (safety test).

3.1. Relative gene expression of interferon γ

IFNγ, or type II interferon, is a cytokine that is critical or innate and adaptive immunity against viral, some bacterial and protozoan infections. IFNγ is an important activator of macrophages and inducer of major histocompatibility complex class II molecule expression. Aberrant IFNγ expression is associated with a number of autoinflammatory and autoimmune diseases [32]. The real time PCR results for interferon γ showed that there was dramatically upregulation in transcripts levels of interferon γ with increases of Montanide gel doses through the whole entire period of the experiment.

The highest level of mRNA was detected for rabbits vaccinated with Montanide gel 15% inactivated RVF vaccine (Col 4) after 8 days from treatment. Despite there were no significant differences among Col 1, Col 2 and Col 3, all collections reveled significant difference compared to control group. In this study the level of interferon γ gene expression in rabbits vaccinated with Montanide gel 02 inactivated RVF vaccine was evaluated by using RT-PCR and compared with positive and negative control as shown in Figure (1).

This in turn was reflected in the 15% inactivated RVF vaccinated rabbits as they showed higher vitality and higher average weight after 30th days from the beginning of the experiment (1.880 kg/ rabbit) more than the rent collections which varied between 1.455kg/ rabbit (Col 0) to 1.667 kg / rabbit (Col 3) as shown in Figure (2).

In accordance with the present findings, the results of [33] revealed that the montanide gel triggered more interferon gene expression with increased doses in rabbits. Similarly, [34] stated the efficiency of montanide (IMS 3015) to improve rift valley fever (RVF) vaccination in sheep causing quick onset and long-lasting immune responses. Early protection against RVFV in goats produced serum IFN-γ, IL-12 and other pro inflammatory cytokines but not IFN-α.innate immunity [35]. Numerous genes were directly or indirectly regulated in their expression which supposedly confer protection and mediate anti-microbial activities by IFN-γ [36].

3.2. Molecular analysis

Why was it necessary to conduct hereditary test to examine the extent of the genetic material of the rabbits in the process of immunization therefore was conducted SCoT marker test. So, a total of 10 primers were tested for selective amplification of DNA fragments.The ten SCoT primers produced reliable PCR products. However, no SCoT primers showed discernible polymorphism between 25 samples.

A total of 160 major SCoT bands (with average 16) were observed (Figure 3), Overall, a high level of genetic similarity ranging from (99.86% to 100%) was revealed among samples using SCOT markers, this means that the genetic material of all treated rabbits compared to the control was not affected by the vaccination process.

These results agree with [24] who founded that only small differences of genetic diversity were observed between 104 rabbits from four Chinese indigenous breeds to study the genetic diversity and population structure and the restriction-site-associated DNA sequencing (RAD-seq) method was effectively used to find genome-wide SNPs.

[37] used Microsatellite Analyzer version 4.05 software, they discovered 81.02 percent of fixed alleles throughout rabbit populations and 79 alleles, 18.98 percent of which were uncommon, during determined New Zealand White, New Zealand Red, Californian White, and Chinchilla rabbit breeds in Nigeria. Moreover, [38] used 10 microsatellite DNA markers to evaluate diversity between 22 rabbit breeds and found that highly polymorphic ranged from 0.4997 to 0.7009 while The lowest genetic distance (0.0087) was observed between American rex rabbit and Germany rex rabbit.

5. Conclusion

Patients with PAH and either having rest Heart rate more than 100 bpm or symptomatic for palpitations may be safely treated with ivabradine. Three-month treatment with ivrabradine coincided with significant improvements in functional capacity of PAH patients. This deserves additional investigation, perhaps via a small randomized study, for confirmation and to understand possible mechanisms of benefit.

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Table (1) Name and Sequence of ten (18-mer) primers used in SCOT- PCR marker.

Primer	Sequence (5′ - 3′)	TNB	Primer	Sequence (5′ - 3′)	TNB
ST- 1	ACCATGGCTACCAGCTCG	5	ST-16	CAATGGCTACCACTACAG	4
ST- 4	CCCATGGCTACCACCGGC	8	ST-21	ACATGGCTATGACTACAG	6
ST- 9	CGACATGGCGACTCACA	5	ST-22	CAATGGCTACCACTACAG	8
ST- 11	ACCATGGCTATGACCGCA	9	ST-28	GAATGGCTACCACTACAG	8
ST- 15	CCATGGCTACCACTATCG	10	ST-31	CTATGGCTACCATGACAG	5

TNB: Total number of amplified bands.

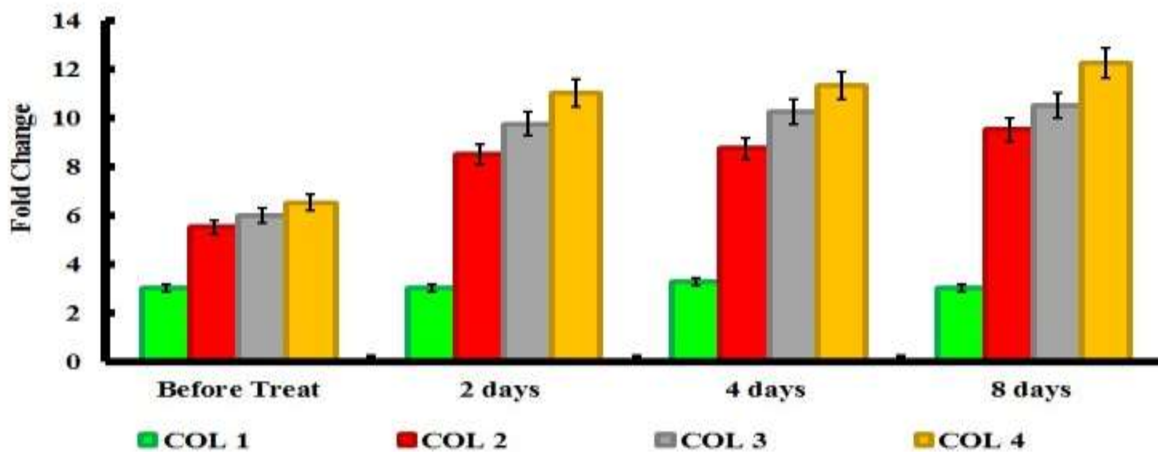

Fig. (1) Mean values of Interferon γ of rabbits vaccinated with different forms of inactivated RVF vaccine and non-treated, Col. 0 negative control (no treatment), Col. 1 RVF vaccine without adjuvant (inactivated RVFV), Col. 2 montanide gel 5% concentration as adjuvant + (inactivated RVFV), Col. 3 montanide gel 10% concentration as adjuvant+ (inactivated RVFV), Col. 4 montanide gel 15% concentration as adjuvant+ (inactivated RVFV).

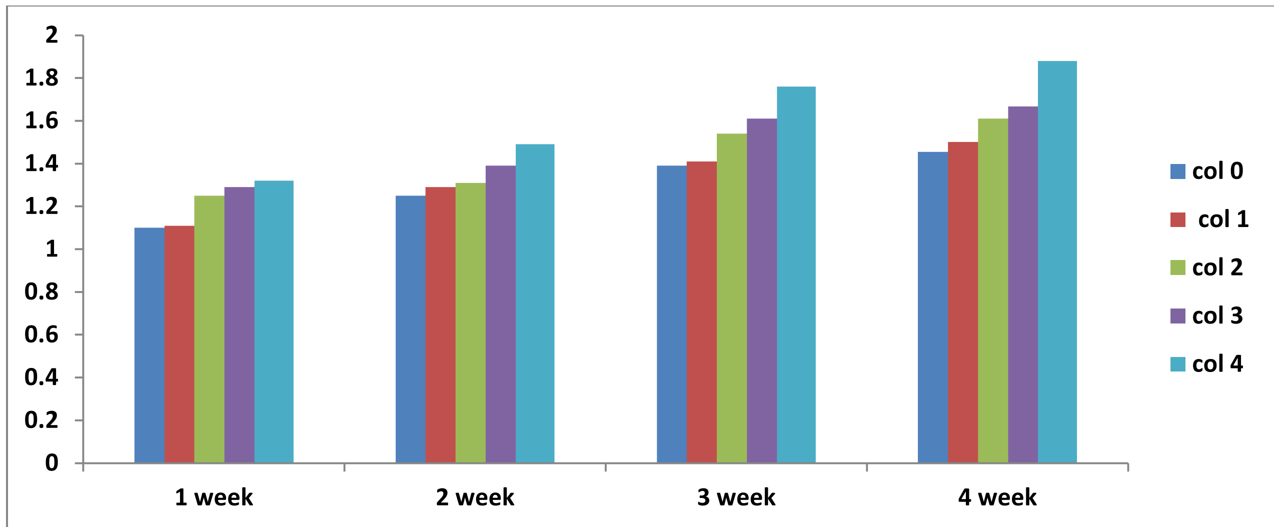

Fig. (2) Mean values of body weights at different ages of rabbits vaccinated with different forms of inactivated RVF vaccine and non-treated, Col. 0 negative control (no treatment), Col. 1 RVF vaccine without adjuvant (inactivated RVFV), Col. 2 montanide gel 5% concentration as adjuvant + (inactivated RVFV), Col. 3 montanide gel 10% concentration as adjuvant+ (inactivated RVFV), Col. 4 montanide gel 15% concentration as adjuvant+ (inactivated RVFV).

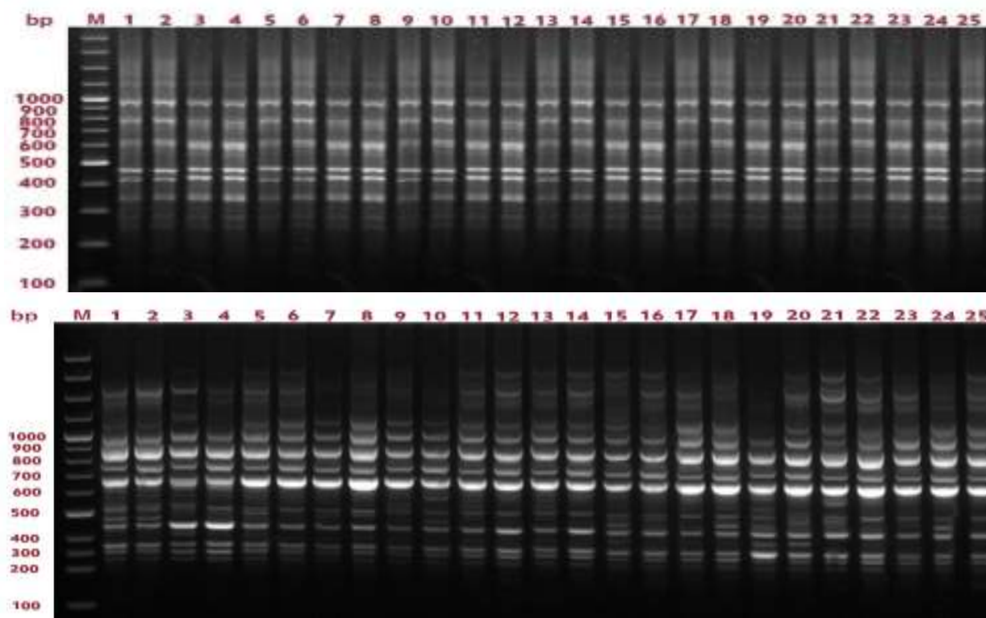

Fig. (3) SCOT profiles of 25th samples (1-25) as detected with primers (A) SCoT- 3 and (B) SCoT- 8. DNA molecular weight standards (M) 1kbp DNA ladder.

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