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Nanoparticles as Immune Modulatory Reagents for Production of Rift Valley Fever Hyperimmune Serum in Sheep

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Abstract

Rift Valley Fever (RVF) since its discovery has been recognized as a source of numerous outbreaks in Africa and Arab peninsulas. It is a zoonotic highly pathogenic mosquito born virus disease. The first line of defense against such disease is the prevention of its the occurrence by periodic vaccination. The use of adjuvant is of fundamental importance in vaccines formulations and hyperimmune serum production. This study aimed to prepare hyperimmune serum against RVF virus to be used in diagnostic purposes in addition to its possible use for treatment in emergence cases. To establish this goal, we use Montanide IMS 3015 investigating its immune modulatory effect in immunized sheep. The experimental sheep received 3 injections of inactivated RVF virus adjuvant with Montanide IMS 3015 one week intervals, the fourth injection was virulent virus. The obtained serum 7 days after the last injection was discovered to be safe and free of foreign pollutants. For mice; specific for RVF virus with mean serum neutralizing antibody titer 20480 and ELISA optical density 1.402. It was found that this serum was able to protect mice against experimental infection with RVF virus in the ratios 100%; 100%; 90% and 60% of it was administrated to mice 24 hours pre-virus infection; simultaneously with the virus; 24 hours post-infection and 48 hours post infection respectively while later serum administration was unable to protect mice against viral infection.

KEYWORDS
Rift Valley Fever virus, Hyperimmune serum, Nano adjuvant.

INTRODUCTION

Rift Valley Fever (RVF) is an acute viral hemorrhagic disease that primarily affects domestic animals (including cattle, buffaloes, sheep, goats, and camels), though it can sometimes affect people. RVF outbreaks can have a considerable negative influence on society, resulting in substantial financial losses and decreased trade. The disease mostly affects livestock, resulting in severe illness and uncompleted birth, the latter is a major source of income for many. Epizootic RVF outbreaks also raise the possibility of human contact with ill animals, which may result in RVF outbreaks in humans (CDC, 2020). In 2017, the World Health Organization (WHO) ranked RVFV among the ten most dangerous pathogens most likely to cause wide epidemics in the near future, requiring urgent attention (WHO, 2017).

A single-stranded RNA virus known as Rift Valley fever virus belongs to the genus Phlebovirus and family Bunyaviridae. The M (medium) and L (large) regions of its genome are negatively oriented, while the S (small) segment possesses ambisense polarity. The nucleocapsid protein (N-protein) and the non-structural protein Nss are both encoded by the S-segment. Whereas the L-segment codes for the RNA-dependent RNA-polymerase, the M-segment encodes the two glycoproteins Gn and Gc as well as the genetic code for a non-structural protein Nsm (Pèpine *et al.*, 2010).

Because RVFV can spread across international and national borders and because there aren't any effective preventative or therapeutic treatments, it's crucial to get a quick and precise diagnosis. When combined with clinical observations, epidemiological data, and/or when seroconversion is shown, serological tests can offer a very accurate method for diagnosing of RVF (Pèpine *et al.*, 2010).

Montanide IMS 3015 is a nano emulsion with droplet size range from 50-60 nm in diameter. The antigen can be occurred in the internal of the emulsion core (Shah *et al.*, 2010). Adsorption of antigen on the surface of the nanoparticle depends on charge interaction (Wendorf *et al.*, 2006).

The main goal of this study was to prepare RVF hyperimmune serum in sheep using Montanide oil IMS 3015 for rapid detection of RVF virus to reach accurate rapid diagnosis, help in the disease control and to be used in emergence cases aiding to minimize economic losses and saving animal wealth.

MATERIALS AND METHODS

Ethical Approval

Institutional Animal Care and use committee at Veterinary Serum and Vaccine Research Institute, acknowledge the research

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manuscript and it has been reviewed under our research authority and deemed compliance to bioethical standards in good faith.

Animals

Mice

Swiss albino mice, 21-28 days old were used for virus titration (80 mice), toxicity (20 mice), safety (70 mice), and for prophylactic test (60 mice).

Sheep

Five Baladi sheep, less than 1 year old, were used through the experimental design for preparation of the hyperimmune serum.

RVF virus strain

ZH501 strain of RVF virus with titer of $10^{7.5}$ $TCID_{50}$/ml was supplied by RVF Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. It was propagated on VERO cell line. The virus was titrated according to El-Nimr (1980) and the titer was calculated according to Reed and Muench (1938).

Tissue culture Binary inactivated RVF virus

Tissue culture Binary inactivated RVF virus was prepared according to Eman (1995) and used for hyperimmunization of sheep using Montanide IMS oil 3015 as adjuvant.

Montanide IMS oil Adjuvant

Montanide IMS 3015 was obtained from Seppic, Paris, France. It was added in equal volume to the inactivated RVF virus then gently mixed on low speed steering (250-300 rpm) for 5 minutes according to Barnett and Williams (1998). Stable preparation was obtained by mixing the aqueous medium into Montanide IMS 3015 at a low sheer rate (Sonia, 2008).

African green monkey kidney (VERO) cell line

African green monkey kidney cell line was supplied by RVF Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. The cells were grown and maintained according to Taha (1982). It was used for virus titration and serum neutralization test (SNT).

Serum neutralization test (SNT)

It was carried out according to Swanepoel *et al*. (1986) and Flourence (1992) to screen the sera of sheep before application of the experimental work and evaluation of the prepared of hyperimmune sera.

ELISA test

ELISA text was carried out according to Synder *et al*. (1984).

Experimental design

Toxicity test

Adult 10 mice were inoculated intrapritonially with Montanide oil IMS 3015 and 10 mice were kept as control for 10 days observation period (Eman and Shalakamy, 2005) to study the toxicity of the oil.

Preparation of RVF hyperimmune serum in sheep

Each of the four sheep was inoculated S/C with 2 ml of the mixture of 1 ml IMS 3015 oil with 1 ml of inactivated RVF virus where 1 ml of this mixture was inoculated in each thigh. Such inoculation was repeated 2 times in week intervals, the fourth dose inoculated virulent RVFV. One week after last dose the sheep were bled daily for 7 days, and the sera were separated for detecting the antibody titer using SNT and ELISA. One sheep was kept as a control (Gihan *et al*., 2001; Tradi, 2003).

Evaluation of the prepared hyperimmune serum

Testing the negativity of the prepared RVF hyperimmune serum from aerobic and anaerobic bacteria; fungi and mycoplasma was carried out according to OIE (2018).

Identity of prepared hyperimmune serum: using agar gel precipitating test was carried out on referenced RVF antigen according to Gihan *et al*. (2001) and Tradi (2003).

Serum Neutralization test was done according to Swanepoel *et al*. (1986) and Eman and Shalakamy (2005) and indirect ELISA was applied according to Synder *et al*. (1984) which were carried out to determine the RVF antibody titer in the obtained serum

Practical evaluation of the prepared RVF hyperimmune serum

Prophylactic effect

Prophylactic effect of the prepared RVF hyperimmune serum was tested in 6 groups of adult mice (10 mice/group) as follow:
The first group was inoculated with 0.2 ml/animal intra peritoneal of the prepared hyperimmune serum followed with 0.1ml from Rift Valley Fever virus ($10^3$$MLPLD_{50}$/animal) 24 h later.
The second group was inoculated simultaneously with the prepared hyperimmune serum and Rift Valley Fever virus.
The third group was inoculated with 0.1ml from Rift Valley Fever virus followed by the hyperimmune serum 24 h later.
The fourth group inoculated with 0.1ml from Rift Valley Fever virus followed by the hyperimmune serum 48 h later.
The fifth group was inoculated with 0.1 ml from Rift Valley Fever virus as a control positive.
The sixth group was kept without any inoculation as control negative.
All mice groups were kept in insect proof animal houses with hygienic procedures, eating a balanced diet, and having access to enough water.

RESULTS AND DISCUSSION

In this study, Montanide oil IMS 3015 was used as an immunomodulatory adjuvant to prepare Rift Valley Fever hyperimmune serum in sheep. It is a nanoparticle adjuvant in an aqueous phase containing immunostimulant compound listed as Generally Recognize as Safe (GRAS) substances (Bahgat, 2017).

Results in Table 1 showed that IMS 3015 has a non-toxic effect in inoculated mice which agree with that reported by Bahgat (2017). Table 2 shows the application of quality control tests, and revealed that the prepared RVF hyperimmune serum was free from aerobic and anaerobic bacteria; fungi and mycoplasma; safe. In addition, application of AGPT on RVF virus sample showed clear precipitating line indicating the specificity and the

identity of the prepared hyperimmune serum to detect the RVF virus (Gihan, *et al*., 2001; Tradi, 2003).

Table 1. Toxicity of Montanide oil IMS 3015 used in adult Swiss albino mice.

Inoculum	Number of I/P inoculated mice	Number of Affected mice	Toxicity %
IMS 3015	10	0	0
None	10	0	0

The level of the antibody titer was determined using SNT and ELISA (20480 by SNT and1.402 by ELISA) as presented in Tables 3 and 4, which showed that the highest level of the antibody titers was obtained at the 8th to10th day post inoculation recording its peak by the ninth day with neutralizing antibody titer reach to 20480 and ELISA ΔOD 1.402.

Also, hyperimmune serum was able to protect mice against experimental infection with RVF virus when inoculated 24 hours before or after infection or simultaneously with the virus. The prophylactic efficacy of the obtained RVF hyperimmune serum with its highest titer was tested in experimentally RVF infected adult mice (Table 5). The results of this test revealed that the first group of mice (inoculated with hyperimmune serum 24 hours before Rift Valley Fever virus) and the second group (inoculated simultaneously with the prepared hyperimmune serum and Rift Valley Fever virus) were able to withstand the virus infection with a percentage of 100%. the third group (inoculated with Rift Valley Fever virus then it treated 24 h later with the hyperimmune serum) withstood the virus infection with protection percentage of 90% and the fourth group (inoculated with Rift Valley Fever virus

Table 2. Quality Control of prepared RVF hyper immune serum in sheep.

Test	Days post last inoculation						
	8	9	10	11	12	13	14
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
Identity (AGPT)	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Safety	0/10*	0/10	0/10	0/10	0/10	0/10	0/10

* Number of dead mice/ number of tested

Table 3. Serum neutralizing antibody titre of the prepared RVF hyper immune serum in sheep.

Sheep No.	Pre inoculation	Days post last inoculation						
		8	9	10	11	12	13	14
1	0	10240	20480	10240	10240	5120	5120	2560
2	0	5120	10240	5120	5120	2560	2560	2560
3	0	10240	20480	10240	10240	5120	5120	2560
4	0	5120	10240	5120	5120	2560	2560	1280
Control	0	0	0	0	0	0	0	0

Table 4. ELISA optical density of the prepared RVF hyper immune serum in sheep.

Sheep No.	Pre inoculation	Days post last inoculation						
		8	9	10	11	12	13	14
1	0.02	1.21	1.40	1.21	1.19	0.94	0.89	0.83
2	0.01	1.05	1.21	1.01	1.01	0.89	0.82	0.79
3	0.02	1.25	1.35	1.20	1.10	0.92	0.88	0.85
4	0.02	0.97	1.08	0.95	0.93	0.81	0.79	0.72
Control	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Cut off: 0.268

Table 5. Prophylactic effect of prepared RVF hyper immune serum on RVF infected adult mice.

Mice Groups	No. of mice	Survived mice number per day										Survival %	Mortality %
		1	2	3	4	5	6	7	8	9	10		
1st group (Received hyper immune serum 24 Before RVFV)	10	10	10	10	10	10	10	10	10	10	10	100	0
2nd group: (Simultaneously received hyper immune serum and RVFV)	10	10	10	10	10	10	10	10	10	10	10	100	0
3rd group: (Received hyper immune serum 24 after RVFV)	10	10	10	10	10	10	10	10	10	10	10	90	10
4th group: (Received hyper immune serum 48 after RVFV)	10	10	10	10	9	9	8	8	7	7	6	60	40
5th group: (Inoculated with RVF virus alone)	10	10	10	8	5	3	1	0	0	0	0	0	100
6th group: (Control without any inoculation)	10	10	10	10	10	10	10	10	10	10	10	100	0

then it treated 48 h later with the hyperimmune serum) showed protection in 60% of mice. On the other side, the fifth group (inoculated with Rift Valley Fever virus only) showed typical signs of RVF virus infection presented by ruffled fur, hunched posture, poorly responsive and paralysis then death , and the sixth group (non-inoculated control) remained healthy for up to 10 days of clinical observation. These findings indicated that the prepared RVF hyperimmune serum could be used for treatment of infected animals when administrated on the proper time post exposure to the virus infection.

Similar results were found by Khodeir and Daoud (2008), who discovered that passive immunization of experimentally rabies-infected mice were able to withstand the infection when treated with the antiserum on the first, second- and third-day post infection but cannot survive after that. Moreover, Albehwar (2009) demonstrated that post-exposure vaccination of animals against rabies virus infection should be carried out as soon as is practical and within the same time frames as advised following exposure.

CONCLUSION

It could be concluded that nanoparticle adjuvant Montanide oil IMS 3015 could be used as a safe immunomodulatory agent for production of Rift Valley Fever hyperimmune serum.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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