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Improvement of Inactivated Rabies Vaccine using Montanide Pet Gel-A

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Abstract

The present work was designed to investigate the ability to use montanide pet gel-A (polymeric adjuvant manufactured under Good Manufacturing Practice, GMP rules) in the production of inactivated cell culture rabies vaccine for pet animals. The test of the National Institute of Health (NIH) indicated that antigenic values were 2.5, 2.0 and 3.0 for the reference, aluminum hydroxide gel and montainde pet gel-A vaccines, respectively. The prepared montanide pet gel-A vaccine was found to be safe and immunogenic for experimental cats and dogs. Smaller dose of the prepared vaccine (1ml/cat or dog) was found to be immunogenic for such animals instead of a dose of 2ml of the aluminum based vaccine. Serum Neutralization Test (SNT) and antigen specific Enzyme Linked Immunosorbent Assay (ELISA) were used to assess the immune response induced in vaccinated cats and dogs showing titers of 64, 64 and 128 by SNT and 5, 5 and 6 $\log_2$ by ELISA in dogs and 64, 64 and 128 by SNT and 5, 5 and 5.5 $\log_2$ by ELISA in cats vaccinated with the reference, aluminum hydroxide gel vaccine and pet gel A vaccine respectively. Montanide pet gel-A could be recommended to be used as an adjuvant to rabies vaccine for pet animals using smaller dose and inducing higher antibody levels. With smaller doses high production rate could be suggested.

Key words: Rabies vaccines, Pet gel-A, Aluminum hydroxide gel, Serum neutralization test, Enzyme linked immunosorbent assay.

1. Introduction

Rabies is a zoonotic disease that affects the central nervous system (CNS), causing acute and fatal encephalitis in its affected hosts. The disease etiologic agent is the rabies virus which is a neurotropic, RNA virus belonging to the order Mononegavirales, family Rhabdoviridae genus Lyssavirus. Transmission of rabies infection mainly occurs when infected saliva reaches nervous tissues through a bite wound or skin injuries, or breached mucous membranes [1] .

For anti-rabies vaccine production adjuvants technologies specialized to veterinary sensitive species are mainly aluminum salts [2] and [3]. The other main technologies used in the production of other veterinary vaccines depend on emulsified oil based formulations. Those types of formulations are poorly compatible with the safety demands of pet's models. So, added to the animal sensitivity to vaccine injections [4] the emotional links between the animals and their owners lead to perfect safety profile expectations, no local or general reactions would be tolerated in companion animals. Aluminum salts are sometimes considered as a reference regarding safety and are used in the more sensitive species because of their safety profile [5].

Pet animals are sensitive species where they can react strongly to vaccine. Compared to farm animals, owner's sensitivity to vaccine safety is exacerbated due to emotional links between animal and owner. Adjuvant selection during vaccine development is a key parameter driving vaccine safety and efficacy profile.

Adjuvants performances were highlighted by general safety parameters but also through vaccine efficacy to trigger a protective immune response against the pathogen, safety was followed through monitoring behavior and body temperature of vaccinated animals. Furthermore, histology studies were performed to assess the local reaction in the injection site. Safety performance of montanide pet gel-A was superior to aluminum based vaccines and antibodies production induced by montanide pet gel-A based vaccines was higher than aluminum based vaccines.

New adjuvant generation is therefore needed, presenting an equivalent safety profile, compared to aluminum salts adjuvant but inducing better immune stimulation. To answer this question a new adjuvant based on a dispersion of a high molecular weight polyacrylic polymer was created. Produced under GMP like conditions, this sterile adjuvant is a dispersion of highly stable calibrated spherical micronic gel particles of sodium polyacrylate in water [6]. This polymeric technology, montanide gel, has already been used in several vaccine models, including pet animals vaccines, with a promising safety and efficacy profile[7], [8] and [9] These findings indicate the safety and efficacy profile of this polymer based adjuvants specialized to species where vaccine safety is of higher importance.

This work aims to improve the quality and immunogenicity of the locally produced cell culture inactivated rabies vaccine by using montanide pet gel-A.

2. Material and methods

2.1 Baby hamster kidney cell culture (BHK21)

BHK_{21} was supplied by the Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI) Abbasia Cairo Egypt, and used for preparation of virus suspension and application of SNT to estimate rabies neutralizing antibody titers in vaccinated cats and dogs.

2.2 Viruses

2.2.1Cell culture adapted rabies virus

Evelyn Rokitnicki Abelseth (ERA) strain of rabies virus adapted to BHK-21 cell line with a titer of 7.5 - 8 $\log_{10}$ $TCID_{50}$ /ml was supplied by (DPAVR), (VSVRI), Abbasia Cairo Egypt. It was used for vaccine preparation and in serum neutralization test to estimate the induced antibody titer in immunized animals.

2.2.2 Challenge virus strain (CVS)

Mice brain adapted rabies virus with a titer of 6.5 $\log_{10}$ MLD_{50}/ml was obtained from the DPAVR- VSVRI and used the NIH test to evaluate the antigenic value of the prepared vaccine.

2.3 Vaccines

2.3.1 Local inactivated rabies vaccine

Local inactivated cell culture rabies vaccine with aluminum hydroxide gel adjuvant was supplied by DPAVR and used for vaccination of experimental animals in a comparison with the prepared Montainde pet gel-A vaccine.

2.3.2 Reference rabies vaccine

Defensor-1 rabies vaccine was supplied by Zoetis, USA and used as a reference vaccine in the test of the National Institute of Health (NIH).

2.4 Experimental animals

2.4.1 Mice

Two hundreds weaned Swiss Albino mice (3-4 weeks old) were used for determination of the antigenic value of rabies vaccines using the test of (NIH).

2.4.2 Cats and dogs

Fifteen native breed cats and fifteen native breed dogs of about 3-4 months of age were found to be free from rabies antibodies as screened by serum neutralization test. Each species group was divided into 3 groups (each contains five animals) where the first group was vaccinated with the traditional locally produced inactivated rabies vaccine using a dose of 2ml/animal inoculated subcutaneously (s/c) in the inner aspect of the thigh; the second group was vaccinated with the prepared Montanide Pet Gel-A rabies vaccine using a dose of 1ml/animal through the same route while the third group was kept non-vaccinated as control. Serum samples were obtained from animal groups on week intervals post vaccination up to 6 months.

2.5 Montanide pet gel-A

Montanide pet gel-A used as adjuvant for pets vaccine was supplied by Seppic (SEPPIC SA 22 Terrasse Bellini, Paris, France).

2.6 Preparation of montanide pet gel-A rabies vaccine

ERA virus strain of rabies was propagated in BHK_{21} cell culture and a virus suspension was prepared with a titer of 6.5 $\log_{10}$ $TCID_{50}$/ml then inactivated with binary ethylenemine [10] then Montanide pet gel-A was added at the ratio of 10% as adjuvant according to the manufacturer directions.

2.7 Quality control testing of the prepared rabies vaccine adjuvanted with montanide pet gel-A

Testing of the prepared vaccine for freedom of foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma), safety and potency in mice was carried out [11].

2.8 Serum neutralization test (SNT

SNT was carried out to estimate rabies neutralizing antibodies in serum of test animals[12] and the antibody titer was determined as the reciprocal of the final serum dilution which neutralized and inhibited the appearance of the cytopathic effect (CPE) of 100 $TCID_{50}$ of rabies virus [13].

2.9 Enzyme linked Immunosorbent Assay (ELISA)

Solid phase ELISA was carried out to follow up the induced rabies antibody titers in vaccinated cats and dogs [14].

3. Results and discussion

The present obtained results, tabulated in Table (1) revealed that, these founded antigenic values appear to be higher than recommended values [11] and [15] who concluded that the antigenic value of inactivated rabies vaccine should not be less than 0.3.

Tables (2, 3, 4 and 5) demonstrated that all tested vaccines induced detectable serum neutralizing and ELISA rabies antibodies in vaccinated dogs and cats by the second week post vaccination reaching its peak levels by the 2^{nd} month and nearly remained unchanged up to 6 months later. Such antibodies recorded their mean peak titers post vaccination of dogs and cats with

either vaccine to be 64, 64 and 128 by SNT and 5, 5 and 6 $\log_2$ by ELISA in dogs vaccinated with the reference, aluminum hydroxide gel vaccine and montanide pet gel-A vaccine respectively. In case of cats vaccinated with the same vaccines, SNT and ELISA showed the peak values of 64, 64 and 128 by SNT and 5, 5 and 5.5 $\log_2$ by ELISA respectively. These values indicated that the tested vaccines are safe and effective for dogs and cats when compared to cell culture inactivated rabies vaccine adjuvanted with aluminum hydroxide gel with recommended protective level of rabies serum neutralizing antibodies should not be less than 0.5 IU, (5 $\log_2$) by SNT and ELISA. where similar results were previously recorded by [16], [17], [18], [19], [20] **.**

On the other side, it was clear that the montanide pet gel-A vaccine induced higher rabies antibody titers in both of vaccinated dogs and cats than those induced by the reference and aluminum hydroxide gel vaccine although the used dose of the montanide pet gel-A vaccine was the half (1ml) of that used in aluminum hydroxide gel vaccine (2ml). In this respect, compared to farm animals, owner's sensibility to vaccine safety is exacerbated due to emotional links between animal and owner. Selection of adjuvant during vaccine development is considered key parameter driving vaccine safety and efficacy profile [21]**.** The present study demonstrated the capability to use montanide pet gel-A (polymeric adjuvant manufactured under Good Manufacturing Practice GMP rules) rabies vaccine for pet animals. It was concluded that montanide gel has already been used in several vaccine models, including pet animals vaccines, with a promising safety and efficacy profile[7], [8]and [9]**.** Antigen specific ELISA was used to assess the immune response induced. In cat and dog trials, aluminum based formulation were used as benchmark for montanide formulation. Safety performances of montanide Pet Gel-A were superior to aluminum based vaccines in dogs and cats. The antibodies production induced by this gel based vaccines was higher than aluminum based vaccines. Montanide pet gel-A can be used associated with a wide range of antigenic media and recommended to be used as adjuvant for sensitive animal's vaccines.

4. Conclusion

Depending on the obtained results, it could be concluded that the rabies inactivated vaccines adjuvanted with montanide pet gel-A is preferable than aluminum hydroxide gel vaccine providing high protective levels in vaccinated dogs and cats with a small dose that is preferable by pet animals owners. Also it is recommended using of such adjuvant to the locally produced inactivated rabies vaccine instead of aluminum hydroxide gel.

Table (1) Quality control parameters of rabies vaccines

Tested vaccine	Reference vaccine	Aluminum hydroxide gel vaccine	Montanide pet gel-A vaccine
Antigenic value	2.5	2.0	3.0
Freedom of foreign contaminants	Free from aerobic and anaerobic bacteria; fungi and mycoplasma		
Safety in mice	Safe showing no abnormal clinical signs in inoculated mice with double doses		
Potency in mice	Potent (all vaccinated mice were able to withstand the CVS)		

Table (2) Rabies serum neutralizing antibody titer in vaccinated dogs

Tested vaccine	Mean rabies serum neutralizing antibody titer*									
	0 day **	1WPV ***	2WPV	3WPV	1MPV ****	2MPV	3MPV	4MPV	5MPV	6MPV
Reference	0	2<	8	16	32	64	64	64	64	64
Aluminum hydroxide gel vaccine	0	2	4	16	32	64	64	64	64	64
Montanide pet gel-A vaccine	0	2	8	16	64	128	128	128	128	128
Unvaccinated control	0	0	0	0	0	0	0	0	0	0

*Mean rabies serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of rabies virus

** 0 day= Pre-vaccination ***WPV= Weeks post vaccination

****MPV= Months post vaccination

Table (3) Rabies ELISA antibody titer in vaccinated dogs

Tested vaccine	Mean rabies ELISA antibody titer (log_2)									
	0 day *	1WPV **	2WPV	3WPV	1MPV ***	2MPV	3MPV	4MPV	5MPV	6MPV
Reference	0.0	1	2	3	4	5	5.5	5.0	5.5	5.6
Aluminum hydroxide gel vaccine	0.0	1	2	2.5	3.5	5	5	5	5.2	5.0
Montanide pet gel-A vaccine	0.0	1.5	2.5	4	4.5	6.0	6.0	6.2	6.0	5.9
Unvaccinated control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* 0 day= Pre-vaccination **WPV= Weeks post vaccination
***MPV= Months post vaccination

Table (4) Rabies serum neutralizing antibody titer in vaccinated cats

Tested vaccine	Mean rabies serum neutralizing antibody titer*									
	0 day **	1WPV ***	2WPV	3WPV	1MPV ****	2MPV	3MPV	4MPV	5MPV	6MPV
Reference	0	2<	4	8	32	64	64	64	64	64
Aluminum hydroxide gel vaccine	0	2<	2	8	16	64	64	64	64	64
Montanide pet gel-A vaccine	0	2	8	32	64	128	128	128	128	128
Unvaccinated control	0	0	0	0	0	0	0	0	0	0

*Mean rabies serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of rabies virus
** 0 day= Pre-vaccination ***WPV= Weeks post vaccination
****MPV= Months post vaccination

Table (5) Rabies ELISA antibody titer in vaccinated cats

Tested vaccine	Mean rabies ELISA antibody titer (log_2)									
	0 day *	1WPV **	2WPV	3WPV	1MPV ***	2MPV	3MPV	4MPV	5MPV	6MPV
Reference	0.0	1.0	2.0	2.5	3.0	5.0	5.5	5.0	5.5	5.6
Aluminum hydroxide gel vaccine	0.0	2<	2.0	2.0	2.5	5.0	4.5	5.1	5.0	5.0
Montanide pet gel-A vaccine	0.0	1.5	2.5	3.5	5.0	5.5	6.0	6.2	6.0	6.2
Unvaccinated control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* 0 day= Pre-vaccination **WPV= Weeks post vaccination
***MPV= Months post vaccination

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