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STUDIES ON THE IMMUNE-RESPONSE POST VACCINATION WITH INACTIVATED TISSUE CULTURE RABIES VACCINE

By

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SUMMARY

The present work was planed to study the immune response of Egyptian dogs to the recently prepared inactivated tissue culture rabies vaccine.

Ten dogs of different ages (puppies and adults) were vaccinated with the prepared vaccine at the field dose. All animals showed a detectable titer of rabies antibodies in their sera from the first week post vaccination, reached its peak by the 4th week in adults and 6th week in puppies. All vaccinated dogs remained healthy without showing any abnormal clinical signs all over the experimental period. Also, these animals had a protective level of antibodies up to about 1 year post vaccination. Control contact dogs did not show any signs of illness and remained seronegative.

The results of serum neutralization test (SNT) and enzyme linked immunosorbant assay (ELISA) were found to be parallel to each other.

So, the inactivated tissue culture rabies vaccine can be considered a safe and potent vaccine to protect dogs against rabies virus infection, simple in its preparation and economic.

INTRODUCTION

Rabies disease is one of the most important dangerous viral diseases for both animals and human. Therefore, the ideal method for preventing rabies both in man and animal, is to induce, active immunity by means of vaccination.

Since several years in Egypt and many other developing countries, the LEP vaccine (Flury Low Egg Passage) which is living attenuated vaccine was produced for dog vaccination against rabies virus infection.

WHO and Californial congress (1979) recommended the production of inactivated viral

vaccines specially rabies vaccines.

Many workers deal with the subject of rabies vaccines produced in tissue culture since 1960, including living attenuated and inactivated vaccines. Finally they suggested that BHK21 cell line was the best cell type yielding the highest rabies virus titer (Wiktor, 1971) and the Binary Ethylenumine (BEI) was the best inactivator (Larghi and Nebel, 1980). Therefore, studies were conducted to produce a locally inactivated tissue culture rabies vaccine (ERA -strain), on BHK21 cell line and inactivated by BEI (Edries, 1995).

The present study aimed to spot more light on the immunogenicity and duration of immunity which could be induced in vaccinated dogs and puppies with such vaccine.

MATERIAL AND METHODS

1. Vaccine :

A locally inactivated tissue culture rabies vaccine was prepared in the Dept. of Pet. Animal Vaccines Researches, Vet. Serum and Vaccine Research Institute, Abbassia, Cairo.

This vaccine was prepared in BHK21 cell line using ERA strain of rabies virus and inactivated by BEI, finally rehydragel was added as an adjuvant.

2. ERA Vaccinal Strain :

ERA strain was kindly supplied from Pasteure Institute, it was a tissue culture adapted strain on BHK21 cell line, then it was propagated to obtain a high titer (108.5 TCID50/ml). The same strain was used in application of SNT, and preparation of virus antigen.

3. Virus Antigen :

The cell culture adapted ERA strain was inoculated in a monolayer of BHK cell culture, when a full CPE was obtained, the infected cells were subjected to 2 cycles of freezing and thawing. Such infected fluid was centrifuged at 3000 rpm for 15 minutes. Then the supernatant fluid was concentrated to its one tenth using cellulose bag and polyethylene glycol. The obtained antigen was titrated before its use in ELISA technique (Habashi et al., 1987).

4. Laboratory Animals :

Dogs : 7 native breed adult dogs (over 1 year old) and 7 young puppies (less than 6 months old) were used. These animals were found to be seronegative to rabies virus, free from internal and external parasites and clinically normal. 5 adult dogs and 5 puppies were vaccinated (each with the field dose given I/M) while 2 dogs and 2 puppies were kept as unvaccinated contact control animals.

5. Seroconversion :

Blood samples were taken form all vaccinated dogs post vaccination as well as control ones, on the 7th, 14th and 21st days, then monthly up to 1 year.

Rabies antibodies were monitored in the sera of the vaccinated animals using SNT and ELISA.

a) Serum-Neutralization Test (SNT) :

It was carried out according to Atanasiu (1973)

and the antibody titer was calculated as the reciprocal of serum dilution which neutralize 100-200 TCID50 of the virus according to Snigh et al. (1967).

b) Enzyme Linked Immuno-sorbant Assay (ELISA):

This technique was applied as described by Voller and Bidwell (1970).

RESULTS

The obtained results of dogs and puppies vaccination revealed that there was no clinical abnormal post vaccinal reaction in all animals. All animals showed normal levels of rectal temperature and healthy behaviour allover the period of observation.

Results of the serological assay of rabies neutralizing antibodies as tabulated in Table (1) clarifies that adult dogs respond immunologically in a good manner to the inactivated T.C rabies vaccine, Rabies antibodies start to appear in the sera of adults from the 1st weak, reach the peak by the 4th week post vaccination and remained unchanged up to about 10 months.

Among puppies, it was found that rabies antibodies could be detected in their sera by the 2nd week with a peak titre on the 8th week post vaccination and also remained constant till 7 months later.

Control animals remained seronegative without any abnormalities inspite of their contact with vaccinated ones.

Also the ELISA results (Table 2) agreed and confirmed the previous results of SNT, indicating that rabies serum antibodies could be detected in the examined sera 2-3 weeks reaching its maximum within 1 month and persists at the high level for about 7 and 10 months post vaccination in puppies and adult dogs respectively.

DISCUSSION

An important factor in the preparation of rabies viral vaccines is that the virus immunogenicity be retained at the highest possible level, while ensuring the complete virus inactivation (Wiktor et al., 1972). In the current work a preliminary study on duration of immunity in response to a newly produced inactivated tissue culture rabies vaccine was performed. Results showed that all the vaccinated dogs seroconverted and neutralizing antibodies started to appear 1-2 weeks post vaccination. All animals remained healthy without any abnormal clinical signs.

Concerning the seroconversion, all the vaccinated dogs (adult and young) showed that the used vaccine succeeded to provoke a protective level of immunity (not less than 1:5) within 1-2 weeks after vaccination. The obtained results agreed with those obtained by Sikes et al. (1971) who

Table (1) : Mean rabies-neutralizing antibody titer in vaccinated dogs.

Periods post vaccinated	Rabies antibodies titer in vaccinated dogs*		
	Puppies	Dogs	Cont.
Prevaccination	0	0	0
1 WPV**	<5	>5	0
2 WPV	5	25	0
3 WPV	25	625	0
1 MPV***	125	625	0
2 MPV	625	625	0
3 MPV	625	625	0
4MPV	625	625	0
5MPV	625	625	0
6MPV	625	625	0
7MPV	625	625	0
8MPV	125	625	0
9MPV	125	625	0
10MPV	25	625	0
11MPV	25	125	0
12MPV	25	125	0

* Reciprocal of serum dilution which neutralized 100-200 TCID50 of the virus.

** WPV : week post vaccination.

*** MPV : month post vaccination.

Table (2) : Results of ELISA applied on dog sera.

Periods post vaccinated	Rabies antibodies titer in vaccinated dogs		
	Puppies	Dogs	Cont.
Prevaccination	-ve	-ve	-ve
1 WPV*	-ve	0.5	-ve
2 WPV	0.5	1.2	-ve
3 WPV	1.1	1.8	-ve
1 MPV**	1.4	1.8	-ve
2 MPV	2.0	2.1	-ve
3 MPV	2.0	2.3	-ve
4MPV	2.0	2.3	-ve
5MPV	2.1	2.4	-ve
6MPV	2.3	2.5	-ve
7MPV	2.2	2.4	-ve
8MPV	2.0	2.2	-ve
9MPV	1.8	2.2	-ve
10MPV	1.4	2.0	-ve
11MPV	1.2	1.9	-ve
12MPV	1.2	1.8	-ve

* WPV : week post vaccination.

** MPV : month post vaccination.

*** Degree of +ve reaction.

**** Results of ELISA reading less than one considered negative.

suggested that the serum neutralization level of 1:5 or greater is a protective level and less than that was considered as negative. These serum neutralizing antibodies reached the peak about 3-4 weeks post vaccination. It was clear that the adult dogs had a better level of neutralizing antibodies than the young dogs (less than 6 m), 10-11 months post vaccination there was a slight decline in the level of neutralizing antibodies but remained at a protective level, therefore these results suggests the necessary of revaccination of dogs yearly.

All these results come in agreement with those obtained by Sikes et al. (1971) who found that inactivated ERA tissue culture rabies vaccine protect 100% of the vaccinated dogs when challenged 1 year post vaccination and protect about 80% of them 3 years post vaccination and Larghi et al. (1976) who found that vaccination of dogs under 1 year old with the inactivated tissue culture rabies vaccine will protect them against street virus challenge 60 days post vaccination.

At the same time these results agree with what obtained by Chomel et al. (1988) who used the inactivated tissue culture vaccine in a mass campaign of canine and feline vaccination under field conditions, indicating that this vaccine would completely protect 97% of the vaccinated dogs 1 year post vaccination.

The obtained results in the SNT-were supported by the results of ELISA which indicated that the presence of rabies antibodies in the sera of the vaccinated dogs could be detected just 1-2 week post vaccination, reached its highest level 3-4 weeks post vaccination and lasts about 10 months, later then slight decline was noticed by the end of the first year. These results came in the same direction with those obtained by Voller and Bidwell (1970 and 1975); Kulonen et al. (1991) and Steimer et al. (1993) who used ELISA for estimation of antibodies in the examined sera of infected or vaccinated hosts.

So, it could be concluded that the newly produced inactivated tissue culture rabies vaccine (ERA strain) is a potent, safe and economic vaccine for dogs vaccination.

Further studies for the use of the same vaccine for all animal species, and trials to elongate the duration of immunity are recommended.

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