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PREPARATION OF A SPECIFIC INACTIVATED PESTE DES PETITS RUMINANTS VACCINE

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SUMMARY

Cell culture adapted local strain of peste des petits ruminants virus "PPRV" (Egypt-87), was inactivated by treatment with binary ethyleneimine (BEI) at the concentrations of 1%, 2% and 3%. A concentration of 3% of 0.1 M BEI was found to be the faster one to obtain complete inactivation of 106.5 TCID50 PPRV within 2 hours. 20% alhydragel was added as an adjuvant and different doses were tested to immunize sheep. There was no significant differences in the immune response of animals vaccinated with either 1, 2 or 3ml of the inactivated vaccine. Boostering dose after 1 month of the 1st vaccination resulted in a better duration of immunity extending to 8 months while it was 5 months on using a single dose of the vaccine.

INTRODUCTION

Peste des petits ruminants (PPR) is a contagious viral disease of small ruminants caused by a paramyxovirus. It is characterized by pyrexia, catarrhal nasal and ocular discharge, necrotic stomatitis and an intestinal mucosal lymphoid tissue reaction syndrome (Appiah, 1982).

In Egypt, the disease was recorded among sheep and goats firstly by Ikram et al. (1988) and lastly by Mouaz et al. (1995).

As it is well known, vaccination is the best mean and corner stone for animal protection against infectious diseases. Among PPR, live attenuated virus vaccines were developed to protect sheep and goats against the disease (Couacy et al., 1995, Khodeir and Mouaz, 1998 and Afaf, 1998). Such live vaccines were found to be safe and

immunogenic protecting animals for not less than one year post vaccination. Some workers, especially in countries where the disease is not endemic, do not prefer the use of live vaccines and like to use inactivated vaccines. Recently, there are serious objections to the use of live vaccine in countries free from rinderpest and there is fear of importing rinderpest virus in meat from cattle immunized with live vaccine (Mirchamsy et al., 1974).

Binary ethyleneimine (BEI) was described by Bahemann (1975) as a viral inactivator and found that it is more specifically acts on the nucleic acid and did not alter the antigenic components of the virus (Bahnemann, 1997).

Adjuvants can increase the immune response induced by a given quantity of an antigen, reduce such a needed quantity of antigen to generate a protective immune response and, prolong the duration of immune response (Bonford, 1997). Alhydragel is an alternative ready prepared Al(OH)3 gel used as adjuvant for many inactivated viral vaccines (Bonford, 1997).

Since Egyptian sheep and goats are threatened by the spread of PPR among the sheep and goat population (Eissa, 1991) and according to the restriction measures adopted by the General Organization of Veterinary Services, on the use of live rinderpest and PPR viruses, the present study is a trial planned to prepare a specific inactivated potent and safe PPR vaccine.

MATERIALS AND METHODS

1. Viruses:

1.1. Peste des petits ruminants virus (PPRV):

A VERO cell adapted live attenuated PPR virus at its 20th passage (Khodeir and Mouaz, 1998) was used in the present work to prepare an inactivated vaccine and for serum neutralization test.

1.2. Rinderpest virus (RPV):

A cell culture adapted RPV vaccinal strain (Singh et al., 1967) was used for SNT to screen test animals before vaccination to ensure that they are free from RP antibodies.

1.3. VERO cells:

African Green Monkey Kidney cells established by Yasumura and Kawatika (1963) were used for PPRV propagation and for SNT.

1.4. Binary ethyleneimine (BEI):

A working solution of 0.1 M BEI, was prepared according to Girard et al. (1977).

1.5. Sodium thiosulphate solution:

A 20% sodium thiosulphate solution was used as a neutralizing agent for excess non reacting BEI.

1.6. Alhydralgel:

A ready prepared Al(OH)3 gel (Alhydragel) was supplied by a United Kingdom Company and used as an adjuvant at 20% ratio to the inactivated PPR vaccine.

1.7. Inactivation of PPRV:

The harvest of PPRV infected VERO cells was titrated using the microtitre technique according to Rossiter et al. (1985) and virus titre was calculated according to Reed and muench (1938). It had a titre of 106.5 TCID50/ml. Virus inactivation was done according to Hassanin (1983) using concentrations of 1%, 2% and 3% of BEI. Samples of virus-BEI mixture were taken every 30 minutes after incubation at 37°C on a magnetic stirrer. BEI was neutralized by 20% sodium thiosulphate solution. The virus infectivity was determined till complete inactivation.

1.8. Quality control tests:

Freedom of the prepared vaccine from contaminants, as well as safety and potency tests were done according to Anon (1994).

1.9. Sheep:

Thirty Barkiy sheep of 1-1.5 years old were used in the present work. They were proved to be free from rinderpest and PPR neutralizing antibodies through the screening of their sera by SNT. They were divided into 6 groups, including 5 animals in each as follow:

- Each of groups 1, 2 and 3 received 1, 2 and 3 ml of the prepared vaccine, respectively as one dose only given S/C.
- Group (4) was vaccinated twice with a month in between using a vaccinal dose of 2ml.
- Each animal in group (5) was inoculated S/C with 10ml of the vaccine for the safety test, while animals in group (6) were kept as a non vaccinated control group.

All animal groups were kept under hygienic measures and serum samples were obtained from them weekly for 4 weeks and then monthly for 9 months post vaccination.

1.10. Serum neutralization Test (SNT):

Both screening and quantitative SNT were done using the microtitre neutralization technique according to Rossiter et al. (1985) and the antibody titre was calculated as the reciprocal of serum dilution which neutralize 100-200 $TCID_{50}$ of PPR virus according to Singh et al. (1967).

RESULTS

Table (1): Losses in PPR virus titre due to the inactivation by BEI.

Post inactivation periods in minutes	Virus titre*		
	1% BEI	2% BEI	3% BEI
0	$10^{6.5}$	$10^{6.5}$	$10^{6.5}$
30	10^{6}	$10^{5.5}$	$10^{5.0}$
60	$10^{5.5}$	$10^{4.5}$	$10^{3.5}$
90	$10^{4.5}$	$10^{3.5}$	10^{2}
120	10^{4}	$10^{2.5}$	$10^{0.5}$
150	10^{3}	$10^{1.5}$	0
180	$10^{2.5}$	$10^{0.5}$	0
210	10^{2}	0	
240	$10^{1.5}$	0	
270	10^{1}		
300	$10^{0.5}$		
330	0		
360	0		

* Virus titre expressed as $\log_{10}$ $TCID_{50}$/ml.

Fig. (1): Losses in PPR virus titre due to the inactivation by BEI

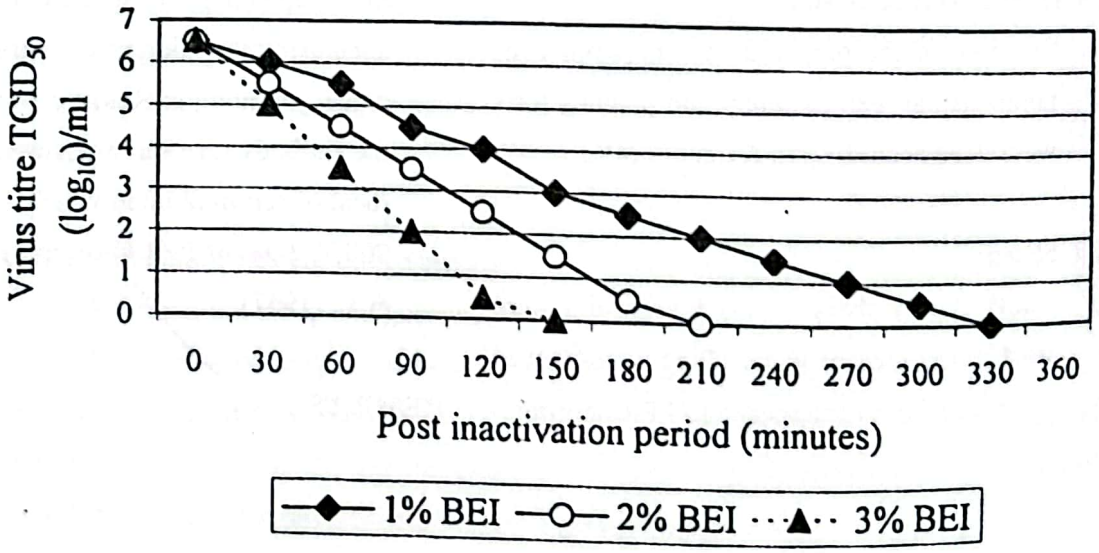

Table (2) : PPR neutralizing antibody titre in sheep sera after vaccination with inactivated PPR vaccine.

Sheep groups	Mean PPR neutralizing antibody titre*/periods post vaccination										
	1 WPV**	2 WPV	3 WPV	4 WPV	1 MPV***	2 MPV	3 MPV	4 MPV	5 MPV	6 MPV	7 MPV	8 MPV
1- Vaccinated with 1ml	0	0	>2	4	8	8	4	2	0	0	0	0
2- Vaccinated with 2 ml	0	0	2	8	16	16	8	4	4	2	0	0
3- Vaccinated with 3 ml	0	0	2	8	16	16	16	8	8	4	0	0
4- Vaccinated twice	0	0	2	8 ↓ Boostering	16	32	32	32	16	16	8	8
5- Unvaccinated control	0	0	0	0	0	0	0	0	0	0	0	0

* Antibody titre = the reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of PPR virus.

** WPV: Weeks Postt Vaccination.

*** MPV: Months Post Vacciantion

DISCUSSION

PPR is a viral infectious disease affecting sheep and goats and cause great economic losses which could not be neglected especially in developing countries which suffer from less income and increased demand for animal protein (Appiah, 1982).

Effective and valuable vaccination is considered the basic tool of controlling such disease. There is a large variety of viral vaccines either live attenuated or inactivated ones. However, inactivated vaccines are more stable and easier to handle under field condition, than attenuated ones (Bahnemann, 1997).

Among PPR control in Egypt, there was a recommendation to produce a specific homologous vaccine where Egyptian sheep and goats are supposed to be threatened by PPR virus (Eissa, 1991). Specific live attenuated PPR vaccines were prepared locally and they were found to be safe and immunogenic protecting sheep and goats for not less than 1 year and may longer (Khodeir and Mouaz, 1998 and Afaf, 1998). But to respect the rules of the General Organization of Veterinary Services for the deprival of the use of the live PPR and RP virus, an inactivated cell culture PPR vaccine was prepared in the present work.

Concerning PPR virus inactivation by different concentrations (1%, 2% and 3%) of 0.1 M BEI, 3% concentration gave the faster virus inactivation (Table 1). Similar results were obtained by Abeer (1999) dealing with RP virus which is a member of the same family (paramyxoviridae). In addition, we suggest that the time of complete inactivation depends on the virus strain and its titre and if it is in organ tissues or in cell culture harvest. So, the present results may agree or differ from other authors.

The obtained inactivated PPR vaccine was found to be free from bacterial, fungal and mycoplasmal contaminants. It was also safe, where the animals which received 10ml dose, did not show any post vaccinal reactions and vaccinated animals showed a protective titre (not less than 8) of formed antibodies (Anon, 1994).

Results presented in table (2) point out that different doses of inactivated PPR vaccine had slight or no difference regading the immune response of vaccinated animals with 2 and 3 ml. All vaccinated animals showed the peak of antibody titre after one month post vaccination showing a disagreement with Khodeir and Mouaz (1998), Afaf (1998) and Hussien (1998) who found that such peak was recorded by the 2nd and 3rd week post vaccination. This difference could be attributed to the type of used vaccine where they used a live vaccine which replicate in the animal body resulting in a much faster and longer immunity.

Boostering of animals with a 2nd dose (2ml) re-

sulted in a longer immunity than in animals that received one dose where it was 8 and 5 months, respectively. In this respect, it was found that higher and longer serological response was similarly elicited with higher doses of inactivated rinderpest vaccine (Mirchamsy et al., 1974 and Abeer, 1999).

Spotting light on the low titre of detected antibodies in vaccinated animals, there is a fact shown that even a serological response as low as only at the level of undiluted serum, will suffice to resist the infection with virulent virus (Plouright and Ferris, 1962b).

So, it could be concluded that the inactivated cell culture PPR vaccine is a safe and immunogenic vaccine and can be used for sheep vaccination without any hazard.

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