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Use of Rabbits as a Laboratory Model for Evaluation of the Combined Inactivated Respiratory Virus Vaccine (Pneumo-3) Adjuvanted by Montanide Oil (ISA 206)

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Abstract: In this study, fifteen rabbits were used to assess the efficacy of the combined inactivated respiratory viruses vaccine Pneumo-3 adjuvanted by Montanide oil. The possibility to use rabbits as an alternative laboratory animal model in evaluation of the vaccines instead of the original host was assessed. Laboratory evaluation tests proved safety and potency of the oil vaccine. No clinical signs were observed in the safety group that received 2 vaccinal doses. Montanide oil stimulated the immune response of vaccinated rabbits to Pneumo-3 oil vaccine and remained in protective level till the end of the experiment against each fraction of the vaccine. The results indicated that rabbits can be used successfully instead of cattle to evaluate the immunogenicity of the vaccine.

Key words: Oil vaccines · Montanide oil · Rabbits · Respiratory viruses

INTRODUCTION

Bovine respiratory disease complex (BRD) is a major health problem of cattle world wide. It Inflicts considerable financial losses in beef herds [1,2] is the most common cause of mortality in dairy cattle. The causality is multi factorial and the disease appears to be a result of the interaction of infectious micro-organisms and such predisposing factors as host defense, environment and stress [3].

Bovine herpes virus type 1 (BHV-1) [4], bovine virus diarrhea (BVD) [5,6] and Parainfluenza type3 (PI-3) viruses have all been incriminated in the etiology of acute respiratory diseases in cattle and calves feedlot all over the world and Egypt [7-9]. These viruses are causing serious losses each year due to the controlling of BRD which is a major focus of veterinary health programs [10].

Many authors succeeded to use the rabbits as model for evaluation of different types of vaccines [11,12]. Others proposed the rabbits as an experimental host for studies of pathogenesis and infectivity of many animal pathogens [13,14]. Donofrio *et al* [15,16] have used rabbits to evaluate a recombinant bovine herpes virus 4 (BoHV-4), expressing BVD virus NADL strain proteins. Immunization of rabbits resulted in the induction of neutralizing antibodies against both viruses in the absence of an adjuvant and after a single injection dose. Also, Frölich and Streich [17] found that 40% of sera were positive for antibodies of the BVDV-NADL strain in the free ranging rabbits from northern Germany but they did not identify the virus. Valera *et al* [18] induced the BHV-1 infection in the rabbits and confirms the infection through isolation of the virus by classical and modern techniques. They suggested their method to be suitable for experimental infection with other respiratory viruses in this animal model.

Therefore, the purpose of the study is to assess the possibility of using the rabbit as an experimental animal as an alternative cheap and easy model instead of calves to detect the immunogenic response of the combined inactivated respiratory viruses vaccine Pneumo-3 vaccine adjuvanted by Montanide oil against BVDV, BHV-1 and PI3 viruses.

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MATERIALS AND METHODS

Viruses:

- BVD virus, Iman strain ($10^{6.5}$ $TCID_{50}$/ml).
- BHV-1 virus, Abou Hammad strain ($10^{7.5}$ $TCID_{50}$/ml).
- PI3 virus, strain 45 (10^{8} $TCID_{50}$/ml).

The viruses were kindly obtained from the department of the Rinderpest like diseases, Veterinary Serum and Vaccines Research Institute (VSVRI) Abbassia, Cairo.

Cell Culture: Monolayer Maiden Darby Bovine Kidney (MDBK) cell culture was tested to be free from the non cytopathic BVDV and used for propagation and titration of viruses.

Oil Adjuvant: Montanide™ISA 206 (Immunosol) supplied by SEPPIC France.

Preservative: Thiomersal, B.P. 58 powder was used after inactivation as preservative.

Inactivant: Two-bromoethyleneimine hydrobromide (BEI).

Preparation of Combined Inactivated Respiratory Vaccine (Pneumo-3) Adjuvanted by Montanide Oil: The vaccine was prepared according to the instructions in the manual of *Vaccine mixing process using Montanide™ ISA206 adjuvant* (SEPPIC Company).The vaccine was prepared in the Department of the Rinderpest like diseases, Veterinary Serum and Vaccines Research Institute (VSVRI) Abbassia, Cairo. The binary ethylenimine (BEI) was used as an inactivator for the viruses and sodium thiosulphate was used to stop the action of BEI. The vaccine contains $10^{6.5}$ $TCID_{50}$/ml BVD virus, Iman strain, $10^{7.5}$ $TCID_{50}$/ml BHV-1 virus, Abou Hammad strain and 10^{8} $TCID_{50}$/ml PI3 virus, strain 45. The aqueous inactivated antigen fluids were mixed at ratio of equal volumes of antigens and Montanide oil adjuvant (w/w) to form water-in-oil emulsion vaccine. Low speed mixing for 5 minutes at 250-300 rpm was done. The pH was adjusted to 7.5 and the thiomersal was added as a vaccine preservative at final concentration of 0.001%. The vaccine was distributed in a sterile bottles each had 100 ml then capsulated and labeled.

Rabbits: Fifteen New Zealand White rabbits weighing about 2.0 kg were used to evaluate the combined vaccine. Rabbits were hosted and cared in the Animal House, Veterinary Research Division, National Research Center, Giza Egypt, in accordance with NRC laws for animal experimentation. Rabbits were maintained at 24°C with a controlled light cycle and with food and water ad libitum.

Evaluation of Locally Prepared Combined Inactivated Respiratory Virus Vaccine (Pneumo-3 Oil Vaccine) Purity: It was performed in accordance with USA Code of Federal Regulation [19] testing to be free from bacteria, mycoplasma, fungi and extraneous viruses as non-cytopathic strain of BVDV.

Safety: It was performed in accordance with USA Code of Federal Regulation[19] in laboratory animals (mice and guinea pigs).

Two vaccinal doses of the vaccine formulation was administered to 3 rabbits and observed for local as well as systemic reaction for a period of 7 days. The rectal temperature of these rabbits from 3 days before vaccination until 7 days post inoculation was also recorded.

Immunization of Rabbits: Twelve New Zealand White rabbits about 8 months of age (They were randomly divided into 2 groups, each contains six rabbits). They were kept under observation for 10 days before vaccination.

General clinical examination was carried out as well as serum samples were collected for detection of antibody against BVD, BHV-1 andPI-3 viruses and all proved to be free from any antibodies against mentioned viruses.

Group (1): Each rabbit was intramuscularly immunized with 5ml of locally produced Pneumo-3 vaccine (BVD, PI-3 andBHV-1) by two injections, 3 weeks apart and sera were obtained via the auricular vein at scheduled intervals according to Fulton *et al* [20]. Potency evaluation at one month post booster in this group was divided into two subgroups 3 rabbits each as follows:

Subgroup (A): This group used for studying the duration of immunity. Serum samples were collected from each rabbit at zero, 21 days post second vaccination (DPV) andevery month after the booster dose up to 4 months.

Subgroup (B): The 3 rabbits were challenged at 28 days post injection of the second dose of vaccination with pathogenic virulent strains of the viruses, rabbit for each virus. Rabbits were infected with 5 ml intravenously and 5 ml instilled intranasally of each virus [21].

Group(2): This group divided into 2 subgroups, each has 3 rabbits:

Positive Infected Control Rabbits: Each rabbit was infected with 5 ml of virulent virus.

Negative Control Rabbits: The three rabbits were left as non infected and vaccinated control rabbits.

The effectiveness of the vaccine was evaluated on the basis of clinical observation for 2 weeks post challenge exposure test according to El-Azhary *et al* [22].

Serum Neutralization Test (SNT): Sera were assayed for the presence of antibodies by the micro-neutralization test as described by Rossi and Kiessel, [23] using MDBK cell line and reference viruses.

RESULTS

Results of antibody response to the Pneumo-3 oil vaccine indicated that there was a protective serum neutralizing antibody titer level expressed in $\log_{10}$ (0.6) of antibodies started at 2nd week post vaccination increased to the highest level at 2nd month post vaccination in all antigens as shown in Table 1. The peak of the neutralizing antibody titers against BVD, BHV-1 and PI-3 were reached at approximately 2nd month post vaccination.

DISCUSSION

We aimed to assess the possibility to use rabbits as an alternative laboratory animal model in evaluation of the vaccines instead of the original host. Kelly [13] and Saad *et al.* [25] demonstrated rabbits -as a suitable model- could be available as experimental animal for discussing the immune response of farm animal vaccines instead of cattle. Many authors, Schiller and Lowy [23] and Tsenova *et al.* [12] were succeeded to use rabbits as model for evaluation of different types of human vaccines and its diseases. Valera *et al.* [18] described an alternative technique to inoculate rabbits and to reproduce infection by Bovine herpes virus type 1. They confirmed the infection by observing the clinical signs, viral isolation from nasal swabs, histological lesions found, positive polymerase chain reaction and antibodies production.

In our study, laboratory evaluation tests proved safety of the oil vaccine. There was no death as well as no clinical signs in the vaccinated challenge rabbits. No clinical abnormalities were observed in the safety group that received 2 vaccinal doses. There was neither record of elevation of body temperature nor development of clinical signs of illness in rabbits during 21 days post inoculation.

Table 1: SN titers against the viruses expressed in log10 in calves vaccinated with combined inactivated respiratory viruses vaccines

Virus	Sample Date	Vaccinated Group	Control Negative Group
BVD	Zero Day*	0.3	0.3
	21 DPV**	1.5	0.45
	1 MPV	1.93	0.2
	2 MPV	2.28	0.22
	3 MPV	2.02	0.35
	4 MPV	1.8	0.25
BHV-1	Zero Day*	0.45	0.3
	21 DPV**	1.8	0.6
	1 MPV	2.4	0.42
	2 MPV	2.5	0.35
	3 MPV	2.25	0.3
	4 MPV	2.0	0.25
PI-3	Zero Day*	0.35	0.3
	21 DPV**	1.65	0.4
	1 MPV	2.1	0.25
	2 MPV	2.25	0.42
	3 MPV	2.1	0.3
	4 MPV	1.95	0.22

DPV: Days post vaccination; MPV: Month post vaccination
*: First vaccinal dose; **: Second vaccinal dose (Booster dose)

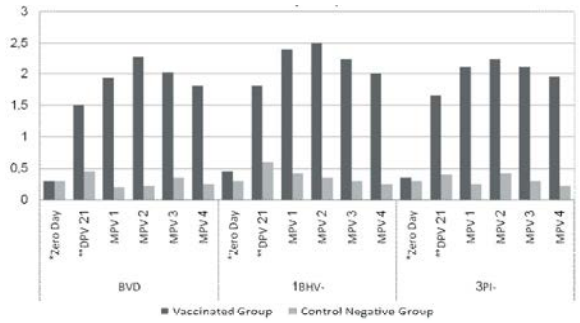

Fig. 1: SN titers against the viruses in log10 in calves vaccinated with combined inactivated respiratory viruses vaccines

Estimation of humoral antibody response to the Pneumo-3 oil vaccine revealed considerable variations both in the rapidity of the development and magnitude of immunity. Results indicated that there was a protective serum neutralizing antibody titer level expressed in $\log_{10}$ (0.6) of antibodies started at 2nd week post vaccination increased to the highest level at 2nd month post vaccination in all antigens as shown in Table 1 and Figure 1. This was related primarily to the immune-stimulation effect of Montanide oil. The duration of immunity elicited by the Pneumo-3 oil vaccine is long-

lived and the antibody titer is remained high after administration. The peak of the neutralizing antibody titers against BVD, BHV-1 and PI-3 were reached at approximately 2nd month post vaccination. These results are agreed with that of Kassem *et al.* [26,27]. They proved that vaccines adjuvanted by Montanide™ ISA oil maintain the antibodies level higher than other adjuvants like the aluminum hydroxide gel which is commonly used in vaccines against the BRD viruses.

The rabbits in this study revealed a rapid and distinct immune response resembling that shown in the cattle. Our results are agreed with that of Saad *et al.* [25]. They succeeded to use the pregnant rabbits as a model instead of pregnant cattle to evaluate the immunogenicity of the live attenuated IBRV vaccine without any adverse reactions. Also, Kelly [13] succeeded to use the rabbits as model to study the pathogenesis of the BHV-1 infection.

Our results are compatible with Stewart-Tull [28] who said that the water in oil emulsion is a good vaccine combination and the enhanced action observed with this vaccine is due to the oil emulsion is responsible for the retention of the antigen at the site of inoculation. This depot provides a slow and prolonged antigenic stimulus to antibody-forming cells.

In conclusion, the combined inactivated respiratory virus vaccine adjuvanted by Montanide oil retained potent for long period and elicited distinct antibody response in rabbits. Moreover, we can use rabbits as a compatible laboratory model for evaluation of the vaccines rather than natural host.

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