Antibody responses by calves after vaccination with commercial and enhanced-potency inactivated pneumo-3 vaccines.

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A B S T R A C T

A highly potent pneumo-3 vaccine of combined inactivated Bovine viral diarrhea virus types 1 (BVDV-I), bovine herpesvirus type 1 (BHV-I) and parainfluenza 3 virus (PI3V) could play a valuable role in respiratory disease control program in calves. We determined the influence of virus concentration by polyethylene glycol (PEG) precipitation on the immune response of calves to the inactivated pneumo-3 vaccine. Both the concentrated and commercial pneumo-3 vaccine was administered twice to six month old seronegative calves through intramuscular (IM) route. Different doses, 2ml, 2.5ml and 3ml of the concentrated vaccine were tested compared with 5ml for the commercial pneumo-3 vaccine. The induced BVDV-I, BHV-I, PI3Vantibodies were followed up in the sera of vaccinated calves up to 24 week post vaccination (wpv) using serum neutralization test (SNT) and indirect enzyme linked immune sorbent assay (ELISA). Virus concentration had a significant effect on serum antibody levels by the 2nd wpv till 24th wpv as well as the economy of the vaccine, where the same immunological effect for the commercial vaccine produced by half dose (2.5ml) of our concentrated pneumo-3 vaccine.

Keywords: inactivated pneumo-3 vaccine, polyethylene glycol, SNT, ELISA

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1. INTRODUCTION

Bovine respiratory disease (BRD) is the most prevalent and costly disease afflicting the cattle industry not only due to abortion, reducing growth rate gain, and food conversion rate, congenital abnormalities but also due to treatment cost (Callan and Garry, 2002).

Bovine viral diarrhea virus types 1 (BVDV-I), bovine herpesvirus type 1 (BHV-I) and parainfluenza 3 virus (PI3V) represent the most important pathogen associated with BRD leading to drastic upset among calves (Juarez-Barrance et al, 2003) and widely spread not only worldwide but also in Egypt (El-Sabbagh et al, 2001). Therefore, multivalent vaccines are widely used to control the BRD. Live and inactivated vaccines with different combinations of antigens are available. The efficacy of these products is usually assessed by the measurement of the humoral and/or cellular immune response against one or more infectious agents under laboratory (Ellis et al., 1995; Fulton et al., 1995; Odendaal et al., 1997; Kerkhofs et al., 2004) or field conditions (West and Ellis, 1997).

The use of inactivated vaccines against these respiratory diseases produces good results for protection of calves from pneumo-enteritis and death (Knezevic et al, 1990). Inactivated virus vaccines have an advantage in that vaccine virus dose not replicate in the host tissues. So, there has been interest in replacing modified live vaccine (MLV) with

inactivated ones, largely because of safety issues. Also, concentration and purification of antigen in vaccine production has many advantages to produce large quantities of product that can be stored in smaller units, also improve immunological activity of viruses and decrease the dose given to the animal (Zeinab and Nehal, 2010)

This work was carried out to investigate the serological responses for a potent PEG concentrated inactivated combined vaccine containing BVDVI, BHV-1 and PI3V (Pneumo-3) compared with the commercial (unconcentrated) vaccine in six month old calves and estimate the best dose introduce the same immunogenic response obtained by the commercial vaccine dose.

2. MATERIAL AND METHODS

2.1. Virus strains

BVDV genotype -1(Egyptian BVDV cytopathic Iman strain of a titer 106.5 TCID50/ml), BHV-I (A local Abou Hammad strain of a titer 107.5 TCID50/ml) and PI3V (Reference Egyptian strain "strain 45" of a titer 108 TCID50/ml) were adapted on MDBK cell line and kindly obtained from the department of the Rinderpest like diseases, Veterinary serum and vaccines research institute (VSVRI), Abbasia, Cairo. These strains were the seed in the used inactivated pneumo-3 vaccine in this study and as reference viruses for SNT and ELISA.

2.2. *Commercial inactivated pnumo-3 vaccine*

Tissue culture binary-ethyleneimine (BEI) inactivated pnumo-3 vaccine was produced from reference virus strains without antigen concentration and provided by department of the Rinderpest like diseases, Veterinary serum and vaccines research institute (VSVRI).

2.3. *The prepared Concentrated inactivated pnumo-3 vaccine*

The vaccine was prepared as previously described (Samira et al, 2001) with modification through viral antigens concentration using polyethylene glycol 6000 (PEG-6000). Briefly all reference viruses (BVDV-1, IBRV and PI3V) were initially propagated separately in MDBK. The viruses were harvested and titrated (Mohanty and Lillie, 1965). Inactivation process was initiated with a final concentration of 0.01M BEI at 37˚C and stopped by stopped by addition of cold sodium thiosulphate with a final concentration of 2%. The inactivated viruses were concentrated by PEG-6000 (Killington et al, 1996). The total protein content of the prepared vaccine was estimated as described by Bradford (1976) and the aluminum hydroxide gel adjuvant was added as 20% to the prepared inactivated pnumo-3 vaccine. The prepared vaccine was sterile, pure, and safe and subjected to evaluation using different vaccine dose.

2.4. *Reference hyper-immune sera*

Reference hyper-immune sera against BVDV-1, BHV-1 and PI3V were obtained from Department of the Rinderpest like diseases, VSVRI, Abbassia, Cairo. It was used in SNT and ELISA

2.5. *Calves and experimental design*

Fifteen Friesian apparently healthy unvaccinated calves aged 6-9 month and of about 150-200Kg body weight. These calves were sero-negative for BVDV-1, BHV-1 and PI3V as screened by SNT. They were allotted into G1A, G1B, G1C, G2 and G3 groups (3 calves for each group) and kept in separate breeding rooms. The divided groups were as follow:

G1A: each of three calves was vaccinated intramuscularly with 2 ml of the concentrated inactivated pnumo-3 vaccine as two doses with 2 weeks apart.

G1B: each of three calves was vaccinated intramuscularly with 2.5 ml of the concentrated inactivated pnumo-3 vaccine as two doses with 2 weeks apart.

G1C: each of three calves was vaccinated intramuscularly with 3 ml of the concentrated inactivated pnumo-3 vaccine as two doses with 2 weeks apart.

G2: each of three calves was vaccinated intramuscularly with 5 ml of the commercial inactivated pnumo-3 vaccine as two doses with 2 weeks apart.

G3: the three calves left as non vaccinated group

The sera from calves were collected for follow up the humoral immune response against both the concentrated and commercial inactivated pnumo-3 vaccine.

2.6. *Serum samples*

Serum samples were collected from vaccinated and unvaccinated calves each 2 weeks post vaccination (wpv) till the first month, and then collected every 4weeks (monthly) till 24 wpv. The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C till used in the serological tests.

2.7. *Serum neutralization test (SNT)*

It was performed on MDBK cell line using the micro technique as described (Rossi and Kiessel, 1971).

2.8. *Enzyme Linked Immunosorbent Assay (ELISA)*

It was carried out according to Voller et al, (1976) to determine antibodies against BVDV-1, IBRV and PI3V.

3. RESULTS

3.1. Evaluation of humoral immune response in sera of calves following vaccination with concentrated inactivated pneumo-3 vaccine

It was observed that, neutralizing antibodies in sera persist at their higher level from the 2nd wpv (time of 2nd dose of vaccination) till the 24th wpv for all reference viruses contained in the vaccine as measured by SNT. The groups of calves vaccinated with 2.5ml (G1B) or 3ml (G1C) mostly gave the same immune response but higher than that given 2ml (G1A), table (1). The results of mean ELISA titers (table 2) were confirmative and correlated to that of SNT. The control non-vaccinated group showed no neutralizing antibody response or valuable ELISA titer (Data not shown).

3.2. Comparative evaluation of concentrated and commercial (non-concentrated) inactivated pneumo-3

The mean neutralizing antibodies in sera of calves vaccinated with concentrated inactivated pneumo-3 were gradually increased from 2 weeks post vaccination and reached to the highest level at 8 weeks and still in a protective level till the end of the experiment for all reference viruses contained in the vaccine as measured by SNT. For calves vaccinated with commercial inactivated pneumo-3 (G2), valuable neutralizing antibody titers were detected by the 4th WPV with lower antibody titer compared with different doses of concentrated vaccine till the end of experiment as shown in table (3).The results of mean ELISA titers (table 4) were confirmative and correlated to that of SNT. The control non-vaccinated group showed no neutralizing antibody response or valuable ELISA titer (Data not shown).

Table (1): Mean neutralizing antibody titer of BVD (genotype 1), IBR, and PI-3 viruses in sera of calves vaccinated with different doses of concentrated inactivated pneumo-3 vaccine.

Weeks post vaccination	Mean serum neutralizing antibody titers expressed in log10								
	G1A			G1B			G1C		
	BVDV-1	IBRV	PI-3V	BVDV-1	IBRV	PI-3V	BVDV-1	IBRV	PI-3V
Zero day*	0.45	0.45	0.2	0.51	0.35	0.2	0.4	0.3	0.2
2 wpv**	1.2	0.9	1.25	1.6	1.2	1.2	1.65	1.2	1.2
4 wpv	1.5	1.5	1.8	1.85	1.8	2.1	1.92	1.8	2.1
8wpv	1.8	1.8	2.1	2.1	2.1	2.4	1.95	2.2	2.4
12 wpv	1.65	1.8	2.1	1.95	2.1	2.2	1.85	2.1	2.4
16 wpv	1.5	1.5	1.8	1.8	1.95	1.95	1.8	1.65	2.1
20 wpv	1.35	1.5	1.5	1.5	1.65	1.8	1.5	1.5	1.8
24 wpv	1.0	1.2	1.2	1.2	1.5	1.65	1.2	1.5	1.65

*time of 1st dose of vaccination, **time of 2nd dose of vaccination, the groups of cattle were vaccinated with 2ml (G1A) or 2.5ml (G1B) or 5ml (G1C) of concentrated inactivated pneumo-3. The expected protective titers were 0.9 for BVDV-1 and 0.6 for IBRV, and PI-3V (Fulton et al, 1995).

Table (2): Mean ELISA antibody titer of BVD (genotype 1), IBR, and PI-3 viruses in sera of calves vaccinated with different doses of concentrated inactivated pneumo-3 vaccine

Weeks post vaccination	Mean ELISA antibody titers								
	G1A			G1B			G1C		
	BVDV-1	IBRV	PI-3V	BVDV-1	IBRV	PI-3V	BVDV-1	IBRV	PI-3V
Zero day*	0.45	0.60	0.61	0.51	0.60	0.35	0.40	0.35	0.45
2 wpv**	1.55	1.30	1.62	1.92	1.60	1.41	1.94	1.65	1.50
4 wpv	1.92	2.51	2.10	2.10	2.75	2.32	2.01	2.84	2.5
8wpv	2.40	2.83	2.32	2.41	3.56	2.71	2.53	3.78	2.80
12 wpv	2.10	2.83	2.26	2.26	3.51	2.40	2.30	3.75	2.70
16 wpv	1.82	2.45	1.93	2.10	3.25	2.10	2.14	3.65	2.30
20 wpv	1.56	2.53	1.82	1.91	2.61	1.96	1.84	2.89	1.98
24 wpv	1.32	1.56	1.5	1.34	2.55	1.80	1.42	2.47	1.91

*time of 1st dose of vaccination, **time of 2nd dose of vaccination, the groups of cattle were vaccinated with 2ml (G1A) or 2.5ml (G1B) or 5ml (G1C) of concentrated inactivated pneumo-3.

Table (3): patterns of neutralizing antibody titer of BVD (genotype 1), IBR, and PI-3 viruses in sera of calves vaccinated with either concentrated or commercial inactivated pneumo-3 vaccine.

Weeks post vaccination	Mean serum neutralizing antibody titers											
	G1A			G1B			G1C			G2		
	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V
Zeroday*	0.45	0.45	0.2	0.51	0.35	0.2	0.4	0.3	0.2	0.35	0.3	0.2
2 wpv**	1.2	0.9	1.25	1.6	1.2	1.2	1.65	1.2	1.2	0.9	0.9	0.9
4 wpv	1.5	1.5	1.8	1.85	1.8	2.1	1.92	1.8	2.1	1.5	1.3	1.3
8wpv	1.8	1.8	2.1	2.1	2.1	2.4	1.95	2.2	2.4	1.8	1.95	2.1
12 wpv	1.65	1.8	2.1	1.95	2.1	2.2	1.85	2.1	2.4	1.7	1.8	1.8
16 wpv	1.5	1.5	1.8	1.8	1.95	1.95	1.8	1.65	2.1	1.5	1.65	1.6
20 wpv	1.35	1.5	1.5	1.5	1.65	1.8	1.5	1.5	1.8	1.2	1.5	1.3
24 wpv	1.0	1.2	1.2	1.2	1.5	1.65	1.2	1.5	1.65	0.9	1.1	0.75

*time of 1st dose of vaccination, **time of 2nd dose of vaccination, the groups of cattle were vaccinated with 2ml (G1A) or 2.5ml (G1B) or 5ml (G1C) of concentrated inactivated pneumo-3. G2 were vaccinated with 5 ml of commercial inactivated pneumo-3. The expected protective titers were 0.9 for BVDV-1 and 0.6 for IBRV, and PI-3V (Fulton et al, 1995).

Table (4): patterns of ELISA antibody titer of BVD (genotype 1), IBR, and PI-3 viruses in sera of calves vaccinated with either concentrated or commercial inactivated pneumo-3 vaccine

Weeks post vaccination	Mean ELISA antibody titers											
	G1A			G1B			G1C			G2		
	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V	BVDV1	IBRV	PI3V
Zeroday*	0.45	0.60	0.61	0.51	0.60	0.35	0.40	0.35	0.45	0.35	0.45	0.4
2 wpv**	1.55	1.30	1.62	1.92	1.60	1.41	1.94	1.65	1.50	1.22	1.21	1.21
4 wpv	1.92	2.51	2.10	2.10	2.75	2.32	2.01	2.84	2.5	1.84	1.58	1.64
8wpv	2.40	2.83	2.32	2.41	3.56	2.71	2.53	3.78	2.80	2.14	3.11	2.4
12 wpv	2.10	2.83	2.26	2.26	3.51	2.40	2.30	3.75	2.70	2.03	2.81	2.11
16 wpv	1.82	2.45	1.93	2.10	3.25	2.10	2.14	3.65	2.30	1.79	2.52	1.93
20 wpv	1.56	2.53	1.82	1.91	2.61	1.96	1.84	2.89	1.98	1.45	2.52	1.64
24 wpv	1.32	1.56	1.5	1.34	2.55	1.80	1.42	2.47	1.91	1.03	1.54	1.21

*time of 1st dose of vaccination, **time of 2nd dose of vaccination, the groups of cattle were vaccinated with 2ml (G1A) or 2.5ml (G1B) or 5ml (G1C) of concentrated inactivated pneumo-3. G2 were vaccinated with 5 ml of commercial inactivated pneumo-3

4. DISCUSSION

The use of inactivated polyvalent vaccines produces good results for protection of calves against BRD with reduction of mortality and decrease incidence of the respiratory disease. The efficacy of these vaccines are variable depending on many factors including animal's age and immune status, virus pathogenicity and its dose and the presence of multiple viral and bacterial infections (Engelken, 1997).

Vaccines can be effective for reducing not only susceptibility but also for reducing shedding of infectious BRD agents to other calves (Frank et al., 2002, 2003)

In Egypt, since fifty and sixty years of the last century, a much attention was drawn to the pneumo-enteritis disease complex syndrome. The control and preventive measures of these infections are based on mainly hygienic management and effective prophylactic vaccination (Durham and Hassard, 1990).

The specific antibody response to viral vaccination has been considered a major indicator of vaccine efficacy and the benefits of increasing antigen payload with respect to the humoral antibody response was examined in detail in the mid 1980s (Rweyemamu et al 1984)

This work was carried out to prepare and evaluate PEG concentrated viruses (BVDVI, BHV-1 and PI3V) combined inactivated alhydra gel adjuvant vaccine (Pneumo-3) in calves to establish whether a correlation exists between antigen payloads and to examine associated changes in the immune response and estimate the effective dose for controlling of such infections.

The inactivated vaccine provided some protection already after the first dose and was therefore considered to be very efficacious after completion of the two-dose vaccination schedule (Mawhinney and Burrows, 2005)

The predicted time to seronegative for BVD-I and 2, BHV-I, and PI-3V ranged widely among individuals from 46 to 299 days (Fulton et al., 2004). By 6 months, it would be expected that most calves would have levels of maternal antibody that were insufficient to interfere with vaccination (Van Donkersgoed et al. 1991)

It is clear that the concentrated virus by PEG 6000 is highly fourth time more than the inactivated virus (Zeinab et al 2003, Zakaria, 2006)

There is evidence that increasing the antigen payload promoted a more rapid and greater systemic antibody response as well as

impeding local virus replication through antibody mediated protection (Barnett et al 2004).

Recently antigen payload FMD vaccines are capable of eliciting systemically detectable cytokine responses IL-6, IL-8 and IL-12 in pigs (Barnett et al 2002), which can be measured for up to 6 months after vaccination (Cox et al 2003). It is likely that similar and/or other immune responses are stimulated in ruminants and these might be augmented by the use of higher antigen payload vaccines.

Immune stimulation due to the vaccine was measured by the neutralization test since it is the most virus specific serological response and neutralization test is a recognized defense mechanism in many viral infections. After the recommended course of 2 IM vaccinations high neutralization activity was recorded against all vaccine components.

The titer of neutralizing and/or ELISA antibodies which was detected in the sera of vaccinated calves is much increased in concentrated pneumo-3 vaccine by increasing its dose, where calves vaccinated with 2.5ml or 3ml mostly gave the same immune response but higher than that given 2ml (table 1 and 2) as the antigen payload in PEG concentrated pneumo-3 vaccine enhanced by the increased dose. This result indicated high potency of the concentrated vaccine which is adequate to protect susceptible animals from infection

Estimation of humeral immune response to the inactivated combined vaccine showed that the mean antibodies titers were gradually increased from 2 weeks post vaccination and reached to the highest level at 8 weeks and still in a protective level till the end of the experiment for all viruses components of the vaccine compared to the non-vaccinated group as measured by SNT and ELISA (table 3 and 4). This agreed with the studies which reported that the minimum accepted neutralizing antibody titers were 0.9 $\log_{10}$ for BVDV and 0.6 $\log_{10}$ for IBRV and PI-3V (Fulton et al, 1995). Duration of immunity elicited by aluminum hydroxide gel vaccine was short-lived and antibody concentration rapidly falls over periods of 4-6 months after administration (Ellis et al, 2005, El-Bagoury et al, 2012)

The previous results revealed that the immune response against the components of the vaccine prepared from PEG concentrated viruses was greater than that prepared from un-concentrated virus, this result agreed with (Lyer et al 2001) who found that vaccines formulated with virus purified with 8% PEG were more immunogenic than the vaccines formulated with untreated harvest viruses

In conclusion, concentration of viruses by PEG improve the quality of antigen used in the vaccine formulation to produce a good quality combined inactivated vaccine by the using of 2.5 ml dose that was able to induce detectable and protective levels of specific antibodies against BVDV, IBRV and PI-3V by the 2nd wpv and continued till 24 wpv as measured by SNT and ELISA.

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