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SIMULTANEOUS VACCINATION OF COMBINED INACTIVATED RESPIRATORY VIRUS VACCINE (PNEUMO-3) WITH RIFT VALLEY FEVER IN SHEEP

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SUMMARY

A total of twelve seronegative sheep against Infectious Bovine Rhinotracheities (IBR), Bovine Viral Diarrhoea (BVD), Parainfluinza-3[a] (PI-3) and Rift Valley Fever (RVF) was randomly divided into four groups. One group received (pneumo-3) vaccine alone, another one simulatenously received pneumo-3 and Rift Valley (RVF) vaccine. The third group received RVF alone and the fourth group served as a non-vaccinated control. No side effects or clinical reaction or disease syndrome could be detected in any of the vaccinated groups. All tested animals remained clinically normal throughout an observed period of 6 weeks post vaccination. Very satisfactory serological responses were detected in animals simultaneously inoculated with both vaccines as well as in animals soldy vaccinated with either vaccine. No significant differences could be found in IBR, BVD and PI-3 serum neutralizing antibody titres of all groups of vaccinated animals also there is no significant difference could be found in RVF serum neutralizing antibody titre. The results clearly indicated the safety and efficacy of simultaneous inoculation of sheep with pneumo-3 with RVF vaccines without impairing the response to individual vaccines and no antagonism between the vaccination penumo-3 and RVF also this good medication for preparing combined vaccine of pneumo-3 and RVF.

INTRODUCTION

Infection with respiratory vaccines produces great economical losses to livestock industry due to poor weight gains and stunted growth, which lead to loss in meat production in addition to

prolongation of the feeding period. Since PI-3 virus acts as a devitalizing agent. It renders the respiratory tissues more susceptible to microbial invasion and multiplication resulting in pneumonia (Hamdy et al., 1965).

BVD virus is multisystemic viral disease of cattle with widely disparaged clinical manifestation (Paton et al., 1989) . BVD, IBR and PI-3 viruses were proved to be incriminated in pneumoenteritis problem (Baz, 1975).

Rift, Valley Fever (RVF) is an infectious, vector transmitted, viral disease of sheep and cattle. It is an abortifacient and causes high mortality in young lambs and calves (Easterday et al., 1962 and Eissa et al., 1977). The Egyptian veterinary researchers succeeded in preparing a safe and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle the disease (El-Nimr, 1980).

The increasing number of vaccine makes it necessary to search for national schems for their administration. The potential for simultaneous vaccination is being greater because of the higher quality of vaccines produced. Efforts , funds and time would be saved at launching vaccination campaign for more than one disease, provided the protective response to each vaccine is not impaired.

The aim of this study was to evaluate the efficiency of synchronous vaccination of sheep with pneumo-3 and Rift Valley Fever vaccines through monitoring of specific antibody titres against 2 vaccines by serum neutralizing test (SNT) for 4 months post vaccination (PV).

MATERIAL AND METHODS

Animal:

A total of twelve healthy sheep of local breed of approximately 3-5 months old were used in this study. These sheep were kept at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt, Rinderpest like Disease Dept. and tested to be free from antibodies against RVF, IBR, BVD and PI-3 before vaccination with RVF and pneumo-3 vaccines.

Vaccines:

A. Local combined inactivated vaccine (pneumo-3):

Containing PI-3 (strain 45) 8 $\log_{10}$ $TCID_{50}$/ml (Samira, 1992) IBR (Abou, Hammad strain) 7 $\log_{10}$ $TCID_{50}$/ml (El-Sabbagh, 1993) and BVD-MD (Iman strain) 6 $\log_{10}$ $TCID_{50}$/ml (Ghaly, 1993). The antigens were inactivated by binary ethyleneimine and adsorbed by 30% alhydrogel in 50ml bottle and registrated in General Organization of Veterinary Services. The dose was 3ml of combined inactivated respiratory

vaccine (pneumo-3) injected I/m2 times 2 weeks apart. The validity of vaccine was 6 months post manufacturing. The vaccine is produced in the Department of Rinderpest Like Diseases and Blue Tongue, in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

B. Rift Valley Fever Vaccine:

Baby Hamster Kidney (BHK) tissue culture BEI inactivated RVF virus vaccine, the final titre of RVF $10^{7.5}$ $TCID_{50}$ before inactivation with 1% of 0.1M binary ethyleneimine (BEI) in 0.2 N NaOH according to Eman (1990) was used. It was obtained from Vet. Serum and Vaccine research Institute, Abbasia, Cairo, RVF Dept.

2. Virus:

A.Rift Valley Fever Virus:

The original virus was that isolated from a human patient in Zagazig, Sharquia Governorate and supplied by NAMRU-3 after being identified to be RVF virus. It was twicely passaged intracerebrally into suckling mice and has a final titre of 2 X 10^7 $TCID_{50}$/ml. It was designated as ZHMC2, and was considered our seed virus.

B. PI-3:

A reference Egyptian strain of PI-3 virus (strain 45) 8 $\log_{10}$ $TCID_{50}$/ml which was isolated and identified by Singh and Baz (1966).

C. IBR:

A reference Egyptian strain of IBR virus (Abou Hammad strain) which 8 $\log_{10}$ $TCID_{50}$/ml was isolated and identified by Hafez et al. (1976).

D. BVD:

The Egyptian Bovine Diarrhoea-Mucosal disease virus (Iman strain) 7 log10 $TCID_{50}$/ml isolated from a Friezian calf with severe pneumoenteritis at Tahrir Province by Baz (1975).

3. Tissue culture:

A. Madin Darby Bovine Kidney (MDBK):

Cell line culture (Marcus and Moll, 1968) tested to be free from the non cytopathic (NCP) BVD-MD virus was used for growing IBR. BVD and PI-3 viruses.

B- BHK21 and BHK47 cells:

The baby hamster kidney cells growing in monolayers were obtained from Padua Institute, Italy. The cells were used for growing RVF virus.

C. CER cells:

Obtained from Wister Institute, USA. These cells were used for the same purpose as BHK cells.

4. Media and solutions:

The media, as well as the solution were prepared according to the methods described in the "Diagnostic procedures" for viral and Rickettsia infections (1969) as well as to the instruction of

the manufactures.

199 medium:

Difco tissue culture medium 199 (Morgan et al., 1950). It was prepared according to the method described by the manufactures . It could be used as a growth medium for the propagation of BHK and CER cells as well as a maintenance medium.

Minimum Essential Medium Eagle's (MEM):

Modified with Eagle's salt without sodium bicarbonate was obtained from Flow laboratories, UK and used to grow Madin Darby Bovine Kidney (MDBK) cell line.

Serological tests:

Neutralization test:

The test was performed for the measurement of specific BVD, IBR and PI-3 viruses in pneumo-3 vaccine and RVF virus according to Robson et al. (1980) and Walker et al. (1970), respectively.

Vaccination of sheep:

Twelve sheep were divided into 4 groups each of 3 animals as the following:

Group (1): Three sheep each was inoculated intramuscularly with 3 ml of pneumo-3 vaccine twice 2 weeks apart.

Group (2): Three sheep each was vaccinated with one dose 1 ml subcutaneously of RVF vaccine and 3ml of pneumo-3 vaccine intramuscularly simulaneously and injection of pneumo-3 was repeated after 2 weeks.

Group (3): Three sheep each vaccinated with RVF vaccine by injection of sheep 1ml subcutaneously (single dose).

Group (4): Three sheep were kept as non-vaccinated control animals under the same management conditions and were subjected to daily clinical examination. Sheep were weekly monitoried serologically for 6 weeks post inoculation and monthly for 4 months post vaccination.

RESULTS

No clinical reactions or side effects could be detected in any of the vaccinated group. Tested animals remained ciinically normal throughout an observation period 6 weeks post inoculation.

Response to pneumo-3 vaccine:

The means of IBR, BVD and PI-3 serum neutralizing antibody titres in sera of vaccinated sheep were shown in table (1). There was no significant difference in IBR, BVD and PI-3 neutralizing antibody titres in (group 1) receiving pneumo-3 alone and (group 2) simulatenously vaccinated with pneumo-3 and RVF vaccines.

Response to RVF vaccine:

The results shown in table (2) indicates that the response was the similar in animals of group 3

Table (1): Results of IBR, BVD and PI-3 viruses (pneumo-3 vaccine) using serum neutralization test.

Sheep Group	No.	Vaccine received	Log10 virus neutralizing antibody titres post vaccination																					
			0 day			1 Week			2 weeks			3 Week			4 Week			8 Week			12 Week			16 Week		
			IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3
1	3	Sheep vaccinated with pneumo-3 vaccine	0	0	0	0	0	0	0.45	0.30	0.75	1.35	1.20	1.50	1.65	1.50	1.95	1.80	1.65	2.10	1.65	1.65	1.95	1.65	1.50	1.80
			0	0	0	0	0	0	0.30	0.30	0.45	1.50	1.20	1.35	1.80	1.80	1.80	2.10	1.80	1.95	1.80	1.80	1.80	1.65	1.65	1.50
			0	0	0	0	0	0	0.30	0.30	0.45	1.50	1.20	1.35	1.80	1.80	1.80	2.10	1.80	1.95	1.80	1.80	1.80	1.65	1.65	1.50
		Means	0	0	0	0	0	0	0.35	0.30	0.55	1.45	1.20	1.40	1.75	1.70	1.85	2.00	1.75	2.00	1.75	1.75	1.89	1.65	1.60	1.60
2	3	Sheep vaccinated with pneumo-3 & PVF simultanously	0	0	0	0	0	0	0.45	0.45	0.60	1.15	1.00	1.50	1.80	1.65	2.10	2.10	1.80	2.35	1.95	1.80	1.95	1.80	1.65	1.95
			0	0	0	0	0	0	0.30	0.30	0.45	1.50	1.15	1.50	1.65	1.80	1.80	1.95	1.80	2.10	1.95	1.80	2.10	1.80	1.65	1.95
			0	0	0	0	0	0	0.45	0.45	0.45	1.15	1.50	1.35	1.80	1.65	1.95	2.10	2.10	2.10	1.80	1.65	1.80	1.65	1.50	1.80
		Means	0	0	0	0	0	0	0.40	0.40	0.50	1.26	1.22	1.45	1.75	1.70	1.95	2.05	1.90		1.90	1.75	1.95	1.75	1.60	1.90
3	3	Sheep vaccinated with RVF vaccine only	There is no SN titre could be detected in this group																							
		Means																								
4	3	Non vaccinated sheep control	No antibody titre could be detected																							
		Means																								

Protective SN titre for PI-3 virus is 0.60 (Mihylovic et al., 1976).
Protective SN titre for IBR virus is 0.60 (Zuffa and Fectova, 1980).
Protective SN titre for PVD virus is 0.90 (Bittle et al., 1968).

Table (2): *Results of serum neutralization test on sera vaccinated sheep against RVF vaccine.*

Group	No. of animal	Vaccine received	Log_{10} serum neutralizing antibody titres post vaccination with RVF vaccine						
			Zero day	1 week	2 week	3 week	4 week	8 week	12 week	16 week
1	3	Sheep vaccinated with pneumo-3 vaccine	0.3	0.4	0.3	0.4	0.2	0.0	2.1	0.0
			0.2	0.3	0.2	0.3	0.2	0.0	2.4	0.0
			0.0	0.3	0.4	0.4	0.3	0.0	2.3	0.0
		Means	0.2	0.33	0.3	0.36	0.23	0.0	2.26	0.0
2	3	Animal vaccinated with pneumo-3 & PVF simultaneously	0.3	0.9	1.2	1.8	2.5	2.6	2.2	1.9
			0.3	1.0	1.35	2.2	2.5	2.8	1.9	2.0
			0.45	1.2	1.5	2.3	2.4	2.6	2.4	2.1
		Means	0.35	1.03	1.35	2.0	2.46	2.70	2.16	2.0
3	3	Animal vaccinated with RVF vaccine only	0.4	1.2	1.6	2.4	2.8	2.8	0.0	2.0
			0.6	1.1	1.3	2.0	2.25	2.0	0.0	2.0
			0.5	1.3	1.5	2.1	2.3	2.5	0.0	1.8
		Means	0.5	1.2	1.46	2.16	2.45	2.40	0.0	1.95
4	3	Non vaccinated Animal control	0.5	0.3	0.5	0.5	0.3	0.0	0.0	0.0
			0.3	0.4	0.2	0.4	0.6	0.0	0.0	0.0
			0.4	0.5	0.3	0.2	0.45	0.0	0.0	0.0
		Means	0.4	0.4	0.35	0.35	0.40	0.0	0.0	0.0

The permissible level for protection against RVF log 2.0 or more (Esterday et al., 1962).
Randal et al. (1964) NI for log 1.7 or greater is adequate to protect susceptible animal for infection.
Walker et al. (1970) NI of log 1.0 or more is positive but 0.7 means a negative one.

vaccinated with RVF alone and in those of group 2 simultaneously inoculated with RVF and pneumo-3 vaccine , control non vaccinated group of animals remained sero negative.

DISCUSSION

Assessment of humoral immune response after vaccination is a mean for evaluation of the protective capacity of vaccines. The commonly used serological test is the neutralization test which has been used extensively as a measure of immunity against combined inactivated respiratory virus vaccine (pneumo-3) and RVF vaccines.

Immune response to pneumo-3 vaccine, the results obtained in table (1) revealed that the means of serum neutralizing antibody titre of vaccinated sheep. Group 1 and 2 at 21 days post vaccination were sufficient to protect susceptible animals from respiratory viruses (IBR, BVD and PI-3) as reported by Zuffa and Feketleova (1980); Mihylovic et al. (1979) and Bittle et al. (1968) who reported that protective SN titre against IBR, BVD and PI-3 viruses are 0.6,0.6 and 0.90, respectively and there is no significant differences could be detected in mean SN antibody titres against IBR, BVD and PI-3 in group 1 and group 2 post vaccination as recorded by Matsuoka et al. (1966) using PI-3 virus with Pasteurella multocida and Pasteurella haemolytica, Tribe et al. (1968) who applied vaccination of guinea pigs and colostrum deprived calves with bovine adenovirus 3 with parainfluenza-3 virus vaccine. Matsuoka et al. (1972) applied vaccination of calves by inactivated PI-3 and IBR with killed pasteurella multocida and pasteurella haemolytica vaccine. Sampson et al. (1972) applied trials of immunization to calves with an inactivated bovine rhinotracheitis and parainfluenza-3 vaccine with pasteurella bacterin. Burrough et al. (1982) applied intranasal instillation of IBR and PI-3 virus vaccines and Hussien et al. (1996) who studies the effect of sheep pox and combined inactivated respiratory virus vaccine (Triangle-4) in the immune response of calves against IBR, BVD and PI-3 viruses.

Concerning the immune response to RVF vaccine, serological examination showed that serum antibody to the RVF virus were present in some of the sheep before vaccination. These antibody titre might be attributed to the fact that those animals have specific maternal antibodies from colostrum as reported by Nawal (1984) who studied the level and duration of maternal immunity in lambs and tried to determine the age at which these new born lambs become susceptible and have to be vaccinated . The results obtained in table (2) showed that the mean serum neutralizing antibody titre of vaccinated sheep were sufficient to protect susceptible animals from RVF infection (1.7-2.3

$\log_{10}$ $TCID_{50}$/ml) as recorded by Easterday et al. (1962); Randal et al. (1964) and Cottral (1978) who concluded that the protective neutralizing index of vaccinated sheep with RVF vaccine 1.7 $\log_{10}$ $TCID_{50}$/ml but Walker et al. (1970). who reported that NI of $\log_{10}$ 1.0 or more is positive and 0.7 means a negative one. The mean titres remained high for 4 months. These results agreed with Taha et al. (1990) RVF and alum gel Rumanian strain of sheep pox virus. Timour (1992) prepared combined vaccine of RVF and sheep pox vaccine for sheep and Mohamed Abdel Samae et al. (1994) studied the effect of RVF and sheep pox vaccines on the immune response of sheep.

The impact of pneumo-3 and RVF on the livestock industry in Egypt could be greatly minimized through launching regular national wide vaccination campaigns.

The increasing number of vaccines makes it necessary to design schemes for their national use to save time, efforts and fund. Other successful trials of simultaneous vaccination of animals with more than one vaccine were reported before (Macadam, 1964) for rinderpest and black quarter or anthrax spore vaccine (Brown and Taylor, 1966) for rinderpest and contagious bovine pleuropneumonia (Darie et al., 1979) for FMD, clostridia and anthrax (Polydorau et al., 1980) for FMD and anthrax, enterotoxaemia and enzootic abortion . (Joseph and Hedger 1984) for FMD and haemorrhagic septicaemia (Hedger et al., 1986) for rinderpest and FMD (Osman et al., 1987) for rinderpest and BCG as well as (Osman et al., 1990) for rinderpest and haemorrhagic septicaemia and Osman et al. (1991) applied synchronous vaccination of cattle with rinderpest and BCG vaccines.

In this trial, very satisfactory immune response was obtained both to pneumo-3 and RVF (table 1 and 2). It was of interest to find out that the response to both vaccines was the same either given solely (group 1 and 3) or simultaneously group 2. Such results assure that simultaneous vaccination had not effect on the immune response against RVF or pneumo-3.

Our results clearly indicate the safety and efficacy of synchronous vaccination of calves with pneumo-3 and RVF. Such a proposal scheme would be of value to overcome one of the biggest constraints in obtaining adequate vaccination coverage that is the reluctance of livestock owners to muster their fattening calves and sheep for number of time.

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