THE PERFORMANCE OF BOTH PEST - DES PETITS RUMINANTS (PPR) AND BRUCELLA MELITENSIS (REV 1) VACCINES CONCURRENTLY ADMINISTERED TO SHEEP

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ABSTRACT

In a trial to evaluate the safety, reactogenicity and immunogenicity of PPR and Brucella vaccines when given simultaneously to susceptible sheep, these sheep were divided into 4 groups : group one vaccinated against PPR, group 2 vaccinated against PPR and Brucella (using Brucella melitensis Rev1 vaccine), group 3 vaccinated against Brucella using Rev1 vaccine and group 4 was left unvaccinated as control. The results obtained were identical serologically for PPR and Brucella vaccines when given solely but when given simultaneously, Brucella live vaccine act as adjuvant and lead to increase the immune response against PPR vaccine but PPR living attenuated vaccine suppress the immune response against Brucella vaccine, results were discussed.

INTRODUCTION

In countries, which use vaccination programs against many diseases may suffer from the use of different vaccines at the same time in the same animal. It is well known that in living viral vaccines, an interference phenomenon may produce deficiency of immune response of some strains of the same virus or other virus in combined vaccines in the same animal. Bacterial vaccine when inoculated at the same time with viral vaccine should be evaluated because some living viral vaccine may induce leucopenia for a time before developing the immune response. The phenomena may induce retardation of immune response of bacterial vaccine or may produce latent infection to appear and this called post vaccinal reaction. In the present study simultaneous vaccination of sheep with PPR and Brucella melitensis Rev1 vaccines, were used to evaluate this method on both response to PPR and Brucella Rev 1 vaccines.

MATERIAL AND METHODS

1. Vaccines used :

A. For vaccination:

** PPR Living attenuated vaccine **(Khodeir and Mouaz, 1998).**

** Brucella melitensis Rev1 Vaccines CZ veterinaris, S. L. Espana dose [2 x 10^9 Colony forming unit (CFU)].

B. For diagnosis :

* Rinder pest (RP) vaccine **(Singh et. al., 1967).**

2 Antigens:

** For PPR diagnosis:

For serum neutralization test (SNT), PPR living attenuated vaccine used (as antigen) for PPR ELISA prepared according to **Schmidt and Emmons (1989).**

** For Brucella :

Rose bengal kindly supplied by Vet. Serum and Vaccines Res. Institute and the test was done as **Alton et al, (1988)** and Brucella LPS antigen used for ELISA prepared as **Plakett et al, (1976)** and the test was done according to **Alton et al, (1988).**

3. Experimental animals :

** Sheep . Twelve apparently healthy balady sheep, about one year old age, Sero-negative to PPR, Rinder pest (RP) and Brucella.

** Animal vaccinations : Animals were divided into 4 groups :

1st group : were vaccinated against PPR subcutaneously (S/C) with 1ml/ head (3 $\log_{10}$ $TCID_{50}$.).

2nd group : were vaccinated simultaneously against PPR (S/C) with 1ml/ head (3 $\log_{10}$ $TCID_{50}$ and Brucella melitensis Rev1 2ml/ head containing 2 x 10^9 CFU.

3rd group : were vaccinated against Brucella with Brucella melitensis Rev1 2ml/ head containing 2 x 10^9 CFU/4th group : were left as control unvaccinated animals.

Animals from all groups were bled weekly for 4 weeks (one month) and monthly until the end of the experiment and serum were kept freeze (-20°C) until the end of the experiments.

Tests for PPR and Brucella :

**** Serum neutralization test for PPR :**

Both qualitative and quantitative serum neutralization tests were carried out according to the methods described by **Rossiter and Jessette (1982).** The neutralizing antibody titres were calculated as the reciprocal of the final serum dilution inhibiting the cytopathogenic effect (CPE) produced by 100 (average of 100 - 200) $TCID_{50}$ / 0.1 ml of PPR and RP virus on VERO cells.

**** ELISA for PPR and Brucella :** The test was done according to **Alton et.al, (1988)** Briefly ELISA plates were coated with PPR antigen or Brucella lipolysaceharide antigen left overnight at 4°C washed, serum under test was added, incubated at 37°C for 2 hours washed, antiantibodies conjugated with horse raddish peroxidase was added incubated at 37°C for 2 hours, washed, then the orthophenylene diamine substrate (OPD) was added left till the colour changed about 15 to 30 minutes and read at wavelength 492 nanometer cutoff for PPR was 0.1957 (optical density) or more considered positive according to **Lee et. al., (1989),** on the other hand, cutoff for Brucella was 20 ELISA Units or more considered positive according to **Alton et. al., (1988).**

**** Rose Bengal for Brucella :** 30 ml of Rose Bengal antigen Mixed with 30 ml of serum under test, any agglutination within 4 minutes consider positive according to **Alton et. Al, (1988).**

**** Microagglutination test :** Test was performed according to **Brown et al., (1981)** Briefly, sera were geometrically diluted with phenolized saline dispensed 50 ml in each well of V shape microtitration plate. Antigen was diluted in phenolized saline containing 0.005% O - safranin and 50 ml was added to diluted sera, mixture was mixed by hand tapping and incubated at 37°C for 24 hours. Agglutination was indicated by carpet of red agglutinated stained cells with antibodies of the sera covering the bottom of the well, while absence of agglutination indicated by a button of stained cells in the center of the well, dilution of 1 / 40 which equal 80 International Units (I. U.) and more 1 / 80 and so on consider positive.

RESULTS AND DISCUSSION

Bovine or ovine brucellosis is particularly difficult to control due to the nature of the organism and its pathogenesis. In the intracellular environment, the organism is able to invade the immune system of the host and is only partially susceptible to chemotherapeutic agents **(Holman et.al., 1985)** and its control is of particular interest due to its economic losses in the cattle in-

dustry result from abortion and reduction in fertility and milk production **(O.I.E. 1996)**.

Pest des petits ruminants (PPR) is a highly contagious disease fatal for small ruminants sheep and goats characterized by high mortality and morbidity caused by of a virus of morbillivirus subfamily group **(Scott, 1990; OIE, 1996)** and its control is by vaccination only **(O.I.E. 1996)**.

Combined or simultaneous vaccination with bacteria and virus is not a recent ideas to control diseases **(Macadam, 1964)** virus combined to other virus or to bacteria or two or more bacteria from the same family or different families together are used **(Golding et al, 2002)** This simultaneous vaccination methods of PPR and Brucella may be for female breeding stock or sheep reared for breeding because Brucella vaccines were given to females only, due to that the brucella vaccines cause orchitis to males and males were bred for beef industry.

The successful trials of simultaneous vaccination of animals with more than one vaccine were reported before for RP, Black quarter vaccines and Anthrax spore vaccine **(Macadam, 1964)** for RP and Contagious bovine pleuropneumonia **(Brown and Taylor, 1966)** for anaerobe, Anthrax and Foot and mouth disease (FMD) **Darie et. al, 1979)** for FMD and RP **(Hedger et.al., 1986)** for Rift Valley Fever (RVF) and sheep pox **(Taha et.al., 1990 and 1991)** for PPR and RVF **(Mouaz et.al., 1998)** for PPR and sheep pox **(Samira et.al., 1999)** for PPR and Clostridia **(Nahed et.al., 2003)** and PPR, RVF and BCG **(Afaf et. al., 2003)**

Results of group one shown in tables (1 & 2) vaccinated with PPR only revealed that antibody titers were slightly low in 1st week that morbillivirus may cause slight leucopenia from the 1st day to 6th day followed by leukocytosis **(Peter, et. al., 1967)** and the immune response evaluated by ELISA in table (1) were comparable to the results obtained through the neutralization tests, this observation was found in animal groups, either vaccinated solely or simultaneously with both vaccines.

Results of group 3 shown in table (1 & 3) with Brucella melitensis Rev 1 vaccine show normal immune response last about 24 weeks as reported in **(Abd El- Ghany, 2002; El - Bayoumi, 1993)**

Combined vaccination (Brucella with PPR vaccines) induce immune response for both diseases with a protective titre higher than vaccination with PPR alone while in case of Brucella titres last for short term about one month, gave immunity lesser than the vaccination with Brucella vaccine alone which remain more than 6 months from day of vaccination.

In group 2 tables (1 & 2) vaccinated simultaneously with Brucella and PPR the increase of titer against PPR in comparison with the 1st group vaccinated with PPR only may be due to pres-

ence of lipopolysaccharide of Brucella cell wall which may act here as non-specific immunostimulant as the preliminary studies of **Mastan et. al., (1976)** on the combined vaccines between Brucella abortus vaccine and Foot and Mouth disease virus vaccine and role of cell wall of Brucella. In the same group the decrease of the titer against Brucella at the first few weeks may be due to the presence of leucopenia induced by the virus of PPR of the vaccine, slight leucopenia from the 1st day to 6th day followed by leukocytosis with different response to the vaccines may be due to individual variations taking into consideration that only 3 animals were tried in each group. **(Peter, et. al., 1967 , Phillips, et. al., 1975)** However, antibody titres were increase when PPR vaccine was inoculated with Brucella vaccine as the lipopolysaccharide of the cell wall of Brucella act as immunostimulant, **Berinstein, et. al. (1993)** who made their experiments on the effect of Brucella cell wall indueed on memory cells of mice vaccinated with foot—and-mouth disease virus and **(Goldstein et. al., 1991)** who studied the immunogenicity of lipopolysaccharide derived from Brucella abortus, potential as a carrier in development of vaccines for **AIDS and Asa, et. al., (1999)** who tried to improve the immune response to foot and mouth disease virus vaccine in calves by using Avridine of Brucella cell wall as adjuvant.

The presence of latent infeetion in apparently healthy animals with brucella organisms **(Dolan, 1980).** as in the second group vaccinated with the simultaneous vaccination with one of morbillivirus (PPR) which cause leueopenia and immunosuppression leads to flushing of the disease and appearance of high titres in the 1st few weeks, and this may hinder the control of brucellosis. Immuno-suppression for a brief time may cause wide spread of Brucella infection.

In conclusion, the result of the present work proof the safety of vaccination of sheep using both living attenuated Brucella vaccine and PPR vaccine but its efficiency will be more successful when using dead cells of Brucella as an adjuvant for viral vaccine e.g. PPR but in case of combination with brucella with viral vaccine it was not successful trial, the combination may be used with other bacteria either gram positive or gram negative that will not interfere with its immunological effect and this point needs more investigation and research.

Table (1) Results of ELISA tests for *PPR* and *Brucella* vaccines vaccinated alone or simultaneously.

Weeks	ELISA results							
	Brucella alone		ELISA Units of *Brucella* in (*Brucella* + *PPR*)		ELISA Units of *PPR* in (*Brucella* + *PPR*)		*PPR* alone	
1st week	24.85	+	100.00	+	0.261	+	0.102	-
	20.35	+	85.02	+	0.276	+	0.175	-
	19.22	-	28.46	+	0.236	+	0.127	-
2nd week	50.41	+	81.73	+	0.285	+	0.144	-
	47.02	+	80.20	+	0.310	+	0.280	+
	57.22	+	20.22	+	0.305	+	0.245	+
3rd week	55.84	+	85.52	+	0.315	+	0.199	+
	65.89	+	88.60	+	0.352	+	0.261	+
	66.54	+	22.48	+	0.318	+	0.255	+
4th week	90.61	+	72.66	+	0.336	+	0.216	+
	95.23	+	75.60	+	0.441	+	0.275	+
	98.69	+	25.74	+	0.332	+	0.268	+
8th week	504.51	+	69.04	+	0.343	+	0.218	+
	452.65	+	66.80	+	0.386	+	0.287	+
	550.10	+	16.56	-	0.322	+	0.242	+
12th week	440.20	+	19.34	-	0.357	+	0.223	+
	395.12	+	13.25	-	0.376	+	0.277	+
	520.54	+	12.86	-	0.342	+	0.257	+
16th week	345.66	+	0	-	0.323	+	0.210	+
	324.45	+	0	-	0.431	+	0.282	+
	386.32	+	0	-	0.328	+	0.260	+
20th week	120.36	+	0	-	0.363	+	0.213	+
	112.10	+	0	-	0.381	+	0.268	+
	128.66	+	0	-	0.335	+	0.248	+
24th week	62.14	+	0	-	0.347	+	0.227	+
	58.45	+	0	-	0.364	+	0.276	+
	54.63	+	0	-	0.327	+	0.254	+

Table (2): Results of serum neutralizing antibody titers in sheep vaccinated with both living attenuated *PPR* and *Brucella* vaccines

Animals group	N°	Vaccine received	Geometric mean of *PPR* serum neutralization test / week																			
			0 week	1st week	Mean	2nd week	Mean	3rd week	Mean	4th week	Mean	8th week	Mean	12th week	Mean	16th week	Mean	20th week	Mean	24th week	Mean
1	1	*PPR*	0	2	3.17	8	12.7	32	80.6	64	161	64	161	64	161	64	161	64	161	64	161
	2		0	4		16		128		256		256		256		256		256		256	
	3		0	4		16		128		256		256		256		256		256		256	
2	1	*PPR* and *Brucella*	0	2	2	8	12.7	128	128	256	256	256	256	256	256	256	256	256	256	256	256
	2		0	2		16		128		256		256		256		256		256		256	
	3		0	2		16		128		256		256		256		256		256		256	

The titre was expressed as the reciprocal of the last serum dilution that inhibited the appearance of CPE produced by 100 – 200 $TCID_{50}$ / 0.1 ml of PPR Virus.

Table (3) Results of *Brucella*, vaccinated alone or simultaneously vaccinated with *PPR* vaccine.

Weeks	*Brucella* results				
	Rose Bengal for *Brucella*	TAT for *Brucella*		TAT for *Brucella* in [(*Brucella* + *PPR*) vaccines]	
1st week	+	40	-	320	+
	-	40	-	160	+
	-	40	+	20	-
2nd week	+	40	-	160	+
	+	80	+	160	+
	+	80	+	20	-
3rd week	+	160	+	160	+
	+	320	+	160	+
	+	320	+	20	-
4th week	+	320	+	80	+
	+	320	+	80	+
	+	640	+	20	-
8th week	+	1280	+	20	-
	+	1440	+	20	-
	+	1440	+	-ve	-
12th week	+	320	+	10	-
	+	320	+	10	-
	+	320	+	-ve	-
16th week	+	80	+	10	-
	+	160	+	10	-
	-	160	+	-ve	-
20th week	+	80	+	10	-
	+	80	+	10	-
	-	80	+	-ve	-
24th week	+	68	-	10	-
	-	84	+	10	-
	-	80	+	-ve	-

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الملخص العربى

فاعلية لقاحى طاعون المجترات الصغيرة والبروسيلا عند حقنهما تزامنيا فى الأغنام

المشتركون فى البحث

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فى محاولة لتقييم سلامة وفاعلية ومناعة لقاحى طاعون المجترات الصغيرة والإجهاض المعدى (البروسيلا) تم حقنهما متزامنين فى أغنام تم التأكد سيرولوچيا من خلوهما من طاعون المجترات الصغيرة والطاعون البقرى والبروسيلا، وقسمت الحيوانات المحقونة إلى ثلاث مجموعات المجموعة الأولى حقنت بلقاح طاعون المجترات الصغيرة المجموعة الثالثة تم حقنها بلقاح البروسيلا أما المجموعة الثانية فقد تم حقنها باللقاحين معاً وتركت مجموعة رابعة بدون حقن كضابط، المجموعتين التم تم حقنها بلقاحات منفردة أعطت نتائج متطابقة لنتائج اللقاحات فى الأغنام أما المجموعة التى تم حقنها باللقاحين متزامنين فقد زاد تأثير لقاح طاعون المجترات الصغيرة للتأثير المحفز للجدار الخارجى للبروسيلا، أما تأثير لقاح طاعون المجترات فقد أدى إلى تهبيط تأثير لقاح البروسيلا وفى البحث تمت مناقشة النتائج.