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EVALUATION OF IMMUNE RESPONSE IN EGYPTIAN BALADY SHEEP VACCINATED WITH ATTENUATED RVF AND PPR VACCINES

(With 6 Tables)

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**تقييم الاستجابة المناعية في الأغنام البلدية المصرية المحصنة بلقاحي
حمى الوادى المتصدع وطاعون المجترات الصغيرة المستضعفين**

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أجرى هذا البحث باستخدام عشرين من الأغنام المصرية قسمت إلى أربع مجموعات بخمسة رؤوس لكل مجموعة - حصنت المجموعة الأولى بلقاح حمى الوادى المتصدع النسيجي الحى المستضعف، وحصنت المجموعة الثانية بلقاح طاعون المجترات الصغيرة النسيجي الحى المستضعف، وحصنت المجموعة الثالثة باللقاحين ممزوجين معا واحتفظ بالمجموعة الرابعة كضوابط غير محصنة. وقد أظهرت النتائج ما يلي: لم تحدث أية أعراض مرضية أو تغيرات إكلينيكية على الحيوانات قيد التجربة طوال فترة الدراسة. كذلك حدث انخفاض فى عدد كرات الدم البيضاء الكلية خلال الأسبوع الأول عقب التحصين ثم ارتفعت هذه الأعداد ثانية لتعود إلى معدلاتها الطبيعية ابتداء من الأسبوع الثانى عقب التحصين وذلك فى المجموعات المحصنة مقارنة بمجموعة الضوابط. ارتفعت نسبة البروتين الكلى فى أمصال الحيوانات المحصنة كما ارتفعت نسبة الألبومين إلى الجلوبيولين. هذا ولم يحدث تأثير على الوظائف الطبيعية للكبد والكلى فى مجموعات الحيوانات المحصنة مقارنة بالضوابط. أمكن تسجيل حدوث زيادة فى المناعة الخلوية خلال الأسابيع القليلة الأولى عقب التحصين. كذلك أستدل من نتائج الفحوص السيرولوجية على اكتساب الأجسام المناعية المعادلة لفيروس حمى الوادى المتصدع بأعلى معيار لها خلال الأسبوع الرابع عقب التحصين فى المجموعة الأولى وكذلك اكتساب الأجسام المناعية المعادلة لفيروس طاعون المجترات الصغيرة بأعلى معيار لها خلال الأسبوع الرابع عقب التحصين فى المجموعة الثانية - كما ثبت إكتساب الأجسام المناعية المعادلة لكل من الفيروسين فى المجموعة الثالثة.

SUMMARY

Twenty Egyptian Balady sheep were divided into 4 groups each of 5 animals. The first group was vaccinated with Rift Valley fever cell culture live attenuated vaccine. The second group received Peste Des Petits Ruminants cell culture live attenuated vaccine. The third group was vaccinated with the two vaccines mixed together. The fourth group was kept as an un-vaccinated control group. All animals remained clinically normal throughout the experimental period. A decrease in the total leukocytic count could be detected only through the first week post vaccination. There was an increase in the total serum protein and in the albumin-globulin ratio. Kidney and liver functions were not affected. Cell-mediated immune response could be detected in the first few weeks post vaccination. Neutralizing antibodies to RVF and to PPR could be detected in the first and in the second group by the first and second week respectively. Peak titres could be detected by the 4th week in both groups. The third group acquired neutralizing antibodies to both viruses.

Key words: Sheep - Immune Response - Vaccination RVF - PPR

INTRODUCTION

Rift Valley Fever (RVF) is an acute infectious viral disease of sheep, cattle, other animals and man. characterized by high abortion rate among pregnant ewes and cows. It produces heavy mortalities in young lambs and calves (Easterday *et al.*, 1962). Several outbreaks of RVF were reported in Egypt (Iman Gabery *et al.*, 1994).

Peste des petits ruminants (PPR) was reported to occur in Egypt for the first time by Ikram *et al.*, in 1988 and secondly by Mouaz *et al.*, in 1995. Although the occurrence of PPR in Egypt was considered as to be in the form of limited outbreaks; a specific homologous live PPR vaccine was developed by Khodeir and Mouaz (1997), which might be in need of, at emergency. Quality control standard measures proved the vaccine to be quite safe and potent (Abeer, 1997). Since a live RVF vaccine is currently used now in Egypt (Ahmed, 1997); it was the idea to experiment a controlled vaccination trial in sheep using both RVF and PPR live vaccines mixed together; aiming at studying the results of such a trial. A special interest was given to humoral and cell mediated immunity responses as well as assessment

of the normal nephro-hepatic functions as a safety criterion for using two viral live vaccines, mixed together.

MATERIALS and METHODS

1. Vaccines :

1.1. RVF vaccine :

The Smithburn RVF live vaccine (Smithburn, 1949) was used in the present study. It is currently produced on Vero cells at the RVF Res. Dep., Vet. Serum and Vaccine Res. Inst., Abbasia, Cairo.

1.2. PPR vaccine :

A vero-cell adapted PPR virus vaccine (Khodeir and Mouaz, 1997), produced at the Rinderpest Res. Dep., Vet. Serum and Vaccine Res. Inst., was also used in the present work.

2. Animals :

Twenty local breed healthy sheep, 9-12 months old, were housed in a BL-3-isolation facility. They were randomly grouped into five's and were samely managed throughout the whole experimental period.

Animals were proven to be seronegative to both RVF and PPR antibodies, just prior to the vaccination trial. One group was vaccinated with the Smithburn RVF live vaccine in a S/C dose of 10³ TCID₅₀ per head. The second group received PPR live vaccine in a S/C dose of 10³ TCID₅₀ per head. The third group was given both vaccines mixed together in a S/C dose of 10³ TCID₅₀ of each, per head. The fourth group was held as an unvaccinated control one. All animals were bled at predetermined intervals and their blood sera samples were monitored for specific antibodies.

3. Cell cultures :

Certified Vero cells were used in cultures for vaccine manufacture of either RVF or PPR as well as for virus titrations and serum-virus neutralization tests. The methods of cell propagation, cell storage and recovery were essentially those mentioned by Dea et al. (1980).

4. Virus titration :

The infectivity titres of either RVF virus or PPR virus were estimated in microtitre plates according to Rossiter and Jessette (1982).

5. Serum-virus neutralization test (SNT) :

Both quantitative and qualitative SNT were done using the microtitre technique as described by Rossiter and Jessette (1982) in microtitre plates. The neutralizing antibody titres were expressed as the reciprocal of the

highest serum dilution that inhibited the appearance of Cytopathogenic effect (CPE) produced by 100-200 TCID₅₀ per 0.1 ml of either RVF virus or PPR virus. The calculation was done according to Cottral (1978).

6. Measurements for cell mediated immune response :

These measurements were applied according to the following:

- a. Separation of lymphocytes according to Boyum (1968).
- b. Total lymphocyte count Per mm³ blood according to Hudson and Hay (1980).
- c. Standardization of lymphocyte concentration for blastogenesis according to cell viability at a final concentration of 2 X 10⁶ / ml.
- d. Preparation of mitogenes :
 - d.1. Phytohaemagglutinin (PHA) according to Hiroshi and Toshikauzui (1986).
 - d.2. Concanavalin-A (Con.A) according to Hiroshi and Toshikauzui (1986).
- e. Lymphocytic transformation assay according to Tadar et al. (1986).

7. Total and differential leukocytic counts :

These were done according to Archer and Jeffcott (1977).

8. Blood Serum total protein and serum albumin :

These were determined according to Weichselbaum (1946) and Ness (1965), respectively.

9. Blood serum alanine aminotransferase (SALT) and aspartic transferase (S AST) and alkaline phosphatase were determined according to Reitman and Frankel (1957).

10. Serum urea, uric acid and creatinine :

These parameters were assessed according to the methods mentioned by Coulombe (1963), and Wendell (1955), respectively.

RESULTS

Serological as well as cell mediated immune responses studied in this work are described in Tables (1 and 2), respectively. Leucocytic status as well as serum protein levels evaluated in this study are depicted in Tables (3 and 4), respectively. Hepato-nephric parameters measured throughout the work are found in Table (5 and 6), respectively.

DISCUSSION

Live virus vaccines are cornerstones for controlling epidemic animal diseases caused by viruses. RVF live vaccine proved to be of value in combating the disease in Egypt (Gabery et al., 1994). The work presented aimed to explore the influences on sheep vaccinated with the RVF vaccine mixed with the recently developed specific PPR live virus vaccine.

Both vaccines were inoculated in one mixed shot representing a subcutaneous dose of 103 TCID₅₀ of each. Such a procedure was compared to the conventional vaccination of each vaccine solely. An exciting result of great interest was that the third group of sheep behaved immunologically, approximately as those in the first group for RVF vaccine and in the second group for PPR vaccine. The axiomatic interpretation of such a result is that neither of the vaccines suppressed the response to the other. The tabulated results envisage the appreciable response to both vaccines either given solely or mixed together. Such a response was essentially approximate in targeted animals whether humoral or cell mediated.

Additionally, all inoculated animals were found to be clinically normal throughout the whole period of study and their nephro-hepatic parameters as well as the studied blood parameters remained normal as found in control group of animals. Such interesting results might pave the way to encourage the direction of utilization RVF and PPR vaccines mixed together; thus saving time, effort and money needed for the separate procedures. Although Egypt is not practicing now, vaccination of sheep to PPR, however, it is of value to consider such a procedure at emergency.

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Table (1) : Mean serum neutralizing antibody titres in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

No. of animals	Mean Antibody titres to	Weeks post inoculation							
		1	2	3	4	5	6	7	8
5, vaccinated solely with RVF vaccine	RVF	4*	8	32	128	128	128	128	128
	PPR	0	0	0	0	0	0	0	0
5, vaccinated solely with PPR vaccine	RVF	0	0	0	0	0	0	0	0
	PPR	0	4	32	64	64	64	64	64
5, vaccinated with both RVF & PPR vaccines mixed together	RVF	2	16	32	128	128	128	128	128
	PPR	0	8	32	64	64	64	64	64
5, unvaccinated control animals	RVF	0	0	0	0	0	0	0	0
	PPR	0	0	0	0	0	0	0	0

ere expressed as the reciprocal of the last serum dilution that inhibited the appearance of CPE produced TCID₅₀ per 0.1 ml of either of the two viruses.

Table (2) : Cell-mediated immune response in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

Group No.	No. of animals	Mean value of:	Weeks post inoculation						
			1	2	3	4	5	6	7
1	5, vaccinated solely with RVF vaccine	C	1.871*	1.571	1.396	1.197	0.835	0.492	0.455
		P	1.987	1.657	1.757	1.557	0.953	0.691	0.657
2	5, vaccinated solely with PPR vaccine	C	1.411	1.211	1.385	1.210	0.786	0.409	0.194
		P	1.571	1.378	1.518	1.323	0.872	0.591	0.220
3	5, vaccinated with both RVF & PPR vaccines mixed together	C	2.157	1.679	1.796	1.422	1.396	0.539	0.455
		P	2.393	1.879	1.989	1.540	1.512	0.651	0.657
4	5, unvaccinated control animals	C	0.210	0.221	0.283	0.172	0.166	0.189	0.214
		P	0.220	0.188	0.221	0.297	0.250	0.189	0.203

C : Concanavaline.

P : Phytohaemagglutinin.

* Parameter was lymphocyte blastogenesis expressed by stimulation index.

Table (3) : Mean leucocytic values in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

Group No.	No. of animals	Item	Weeks post inoculation		
			1	2	3
1	5, vaccinated solely with RVF vaccine	T	9250	9125	10335
		N	27	33	28
		E	4	5	2
		B	0	1	0
		L	65	58	68
		M	5	4	3
2	5, vaccinated solely with PPR	T	11350	11175	11575
		N	24	38	29
		E	3	3	1
		B	0	0	0
		L	71	57	66
		M	5	5	4
3	5, vaccinated with both RVF & PPR vaccines mixed together	T	10350	9000	11620
		N	34	33	20
		E	2	1	2
		B	1	0	1
		L	65	60	73
		M	6	5	5
4	5, unvaccinated control animals	T	11900	11980	11900
		N	40	42	42
		E	2	2	3
		B	0	0	1
		L	55	57	57
		M	2	2	2
T	Total leucocytic count /mm ³ .		L	Lymphocyte %.	
N	Neutrophil %.		M	Monocyte %.	
E	Eosinophil %.				
B	Basophil %.				

Table (4) : Serum total protein values in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

Group No.	No. of animals	Mean value of:	Weeks post inoculation		
			1	2	3
1	5, vaccinated solely with RVF vaccine	P	7.64	6.23	8.39
		A	2.89	2.72	3.71
		G	4.74	4.00	4.68
		A/G %	0.60	0.68	0.78
2	5, vaccinated solely with PPR vaccine	P	8.8	7.45	8.52
		A	2.96	2.87	3.27
		G	5.84	5.25	4.83
		A/G %	0.50	0.54	0.67
3	5, vaccinated with both RVF & PPR vaccines mixed together	P	7.28	6.56	8.26
		A	2.65	2.19	3.06
		G	4.57	4.36	5.19
		A/G %	0.57	0.50	0.58
4	5, unvaccinated control animals	P	7.91	7.96	7.90
		A	2.75	2.78	2.52
		G	5.16	5.26	5.13
		A/G %	0.53	0.52	0.49
P	Total protein gm%.				
A	Albumin gm%.				
G	Globulin gm%.				

Table (5) : Hepatic parameters in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

Group No.	No. of animals	Parameter of:	Weeks post inoculation		
			1	2	3
1	5, vaccinated solely with RVF vaccine	SALT (F. unit/ml)	20.1±1.2	20.5±1.2	20.1±0.9
		SAST (F unit/ml)	15.3±1.3	16.1±1.2	12.1±1.2
		Alk. P. (IU/ml)	9.9±1.2	11.5±2.8	8.15±2.1
		Total bilirubin (mg%)	2.9±0.09	2.3±0.5	3.0±0.3
2	5, vaccinated solely with PPR vaccine	SALT (F. unit/ml)	19.2±0.7	19.4±0.9	19.3±1.7
		SAST (F unit/ml)	10.5±0.8	9.2±0.9	9.4±1.6
		Alk. P. (IU/ml)	10.6±0.9	9.1±3.1	10.2±1.6
		Total bilirubin (mg%)	2.8±1.8	2.5±0.61	2.8±0.1
3	5, vaccinated with both RVF & PPR vaccines mixed together	SALT (F. unit/ml)	22.4±2.2	22.2±1.9	22.9±2.1
		SAST (F unit/ml)	11.3±1.0	7.1±0.8	14.4±0.7
		Alk. P. (IU/ml)	11.8±2.4	11.1±2.1	8.4±2.7
		Total bilirubin (mg%)	2.0±0.8	2.8±0.8	2.7±0.7
4	5, unvaccinated control animals	SALT (F. unit/ml)	20.1±1.9	19.4±0.9	19.3±1.7
		SAST (F unit/ml)	8.9±0.7	9.2±0.9	9.4±1.6
		Alk. P. (IU/ml)	6.4±3.1	9.1±3.1	10.2±1.6
		Total bilirubin (mg%)	3.4±0.6	2.5±0.61	2.8±0.1

Table (6) : Nephric parameters in sheep vaccinated solely with either RVF or PPR vaccines and in those vaccinated with the two vaccines mixed together.

Group No.	No. of animals	Parameter of :	Weeks post inoculation		
			1	2	3
1	5, vaccinated solely with RVF vaccine	Urea nitrogen (mg%)	24.8±3.8	24.2±2.9	23.9±1.9
		Uric acid (mg%)	0.38±0.06	0.41±0.08	0.31±0.09
		Creatinine (mg%)	0.71±0.22	0.75±0.35	0.8±0.27
2	5, vaccinated solely with PPR vaccine	Urea nitrogen (mg%)	24.5±4.4	24.3±2.9	24.3±2.37
		Uric acid (mg%)	0.4±0.08	0.40±0.8	0.40±0.08
		Creatinine (mg%)	0.70±0.18	0.75±0.35	0.72±0.26
3	5, vaccinated with both RVF & PPR vaccines mixed together	Urea nitrogen (mg%)	20.4±3.5	20.1±3.5	20.2±3.3
		Uric acid (mg%)	0.38±0.04	0.4±0.08	0.39±0.06
		Creatinine (mg%)	0.79±0.16	0.84±0.28	0.81±0.17
4	5, unvaccinated control animals	Urea nitrogen (mg%)	20.6±4.2	22.1±3.9	20.4±2.8
		Uric acid (mg%)	0.36±0.17	0.32±0.6	0.30±0.1
		Creatinine (mg%)	0.74±0.12	0.71±0.25	0.81±0.26

