IMMUNE RESPONSE OF SHEEP VACCINATED WITH INACTIVATED COMBINED FOOT AND MOUTH DISEASE, RIFT VALLEY FEVER AND SHEEP POX VACCINE

TALAAT, ABEER A., MERVATE M.ALI AND LILY S.SALAMA

Veterinary Serum and Vaccine Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza-Egypt.

(Manuscript received 9 march 2004)

Abstract

A combined vaccine was constructed to contain FMD O_1, RVF ZH_{501} and sheep pox Egyptian strain inactivated with Binary ethylenemine (BEI) and adsorbed on aluminium hydroxide gel. Comparative studies were conducted to evaluate the prepared vaccines (three monovalent FMD, RVF and Sheep Pox separately and the combined one).

It was found that the serum neutralization antibody titer of sheep vaccinated with the combined vaccine (FMD and RVF) reached its peak earlier than those vaccinated with each as monovalent vaccine, separately, while, there was no significant difference in efficacy of immune response between combined vaccine and sheep pox vaccine. So, it is recommended that animals should be boostered at the 4th week post the first dose.

INTRODUCTION

Combined vaccines are important approach to control the risk of live stock as well as human being diseases. Combined vaccines give the ability to use more than one antigen at the same time to stimulate the immune response, save time, effort and considered more economic. Daoud *et al.* (2001) found that there was no difference in the obtained results of serological tests for monovalent and combined Rift Valley Fever (RVF) and Foot and Mouth Disease (FMD). Marcoss (1992) found that there was a difference in the immune response between combined RVF / Sheep Pox and RVF or Sheep Pox alone. He found that the antibodies started to appear earlier in the animals vaccinated with combined vaccine than those vaccinated with each vaccine alone.

Abeer (1996) mentioned that there was significant increase in the immune response of FMD in animals simultaneously vaccinated with Sheep Pox and FMD vaccine.

So, it is important to produce polyvalent vaccines for sheep and cattle, to enable the veterinary authorities to save time, effort and cost for control such diseases affecting livestock.

Therefore, the aim of this work is a trial to prepare a combined vaccine comprising FMD, RVF and Sheep Pox antigens, and study its effect on immune response of sheep. Also, a comparison will be done with each type of vaccine separately so that one can judge on the validity of this new combined vaccine.

MATERIALS AND METHODS

1. ANIMALS

Twenty-Three adult susceptible sheep free from antibodies against FMD, RVF and Sheep Pox viruses were used.

2. VIRUSES

A- VIRULENT VIRUSES

I. FMD VIRUS

FMD O_1/93 Egypt, with a titer of 10^8 MLD_{50}/ml was kindly obtained from FMD Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and Serum Neutralization Test (SNT).

II. RVF VIRUS

Rift Valley Fever Zagazig, Human strain (ZH_{501}), isolated from a patient in Zagazig, Sharkyia Governorate (1977) had a titer of $10^{7.5}$ $TCID_{50}$/ml. It was kindly supplied by RVF Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and SNT.

III. SHEEP POX VIRUS

The Egyptian strain having a titer of 10^5 SID_{50}/ml. It was kindly obtained from Pox Vaccine Production Department, Serum & Vaccine Research Institute, Abbassia, Cairo. It was used for challenge and SNT.

B- INACTIVATED VIRUSES

Binary Ethylenemine (BEE) inactivated FMD, RVF and Sheep Pox tissue culture vaccine were obtained kindly respectively from FMD, RVF and Sheep Pox Vaccine Production Departments, Serum & Vaccine Research Institute, Abbassia, Cairo.

3. ANTIGENS FOR ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA)

A- FMD antigen

It was prepared according to Wagner *et al.* (1969).

B- RVF antigen

Lyophilized cell lysate RVF antigen was used for detection of RVF IgG antibodies using ELISA. It was prepared according to Elian and Botros, (1997).

C- Sheep Pox antigen

It was prepared according to House, *et al.* (1990).

4. PREPARATION OF THE COMBINED VACCINE

The inactivated FMD virus was mixed together with inactivated RVF virus and inactivated Sheep Pox virus. This combined vaccine was mixed equally with aluminium hydroxide gel. The dose of combined vaccine (3ml) adjusted to contain 10^7 $TCID_{50}$ of RVF, $10^{6.2}$ $TCID_{50}$ Sheep Pox and 10^8 $TCID_{50}$ FMD.

5. EVALUATION OF THE PREPARED VACCINE

a- Sterility test: The vaccine should be free from any fungal, bacterial or micoplasma contamination.

b- Safety and potency tests: were done for FMD, RFV and Sheep Pox vaccines according to Henderson (1970), El Nimr (1980) and OIE Manual (2000), respectively.

6. EXPERIMENTAL DESIGN

Twenty-three adult sheep were classified as follows:

Group A. Three sheep vaccinated subcutaneously (S/C) with 3ml combined vaccine and non-challenged.

Group B. Two sheep vaccinated (S/C) with 3ml-combined vaccine and then, challenged intradermolingual with virulent FMD virus 10^4 $TCID_{50}$/dose.

Group C. Two sheep vaccinated (S/C) with 3ml-combined vaccine and then, challenged **(S/C)** with virulent RVF virus 10^5 $TCID_{50}$/sheep.

Group D. Two sheep **vaccinated (S/C)** with 3ml-combined vaccine and then, challenged intradermal (I/D) with virulent Sheep Pox virus 10^3 SID_{50}/sheep.

Group E. Two sheep were vaccinated (S/C) with 1ml of inactivated FMD vaccine containing 10^8 $TCID_{50}$/sheep.

Group F. Two sheep were vaccinated (S/C) with 1ml of inactivated RVF vaccine containing 10^7 $TCID_{50}$/ sheep.

Group G. Two sheep were vaccinated (S/C) with 1ml of inactivated Sheep Pox vaccine containing $10^{6.2}$ $TCID_{50}$/ sheep.

Group H. Two sheep were used as control non-vaccinated non-challenged.

Group I. Two sheep were challenged intradermolingual with 10^4 $TCID_{50}$/ml FMD virulent virus.

Group J. Two sheep were challenged (S/C) with 10^5 $TCID_{50}$/ml RVF virulent virus.

Group K. Two sheep were challenged (I/D) with 10^3 SID_{50}/ml Sheep Pox virulent virus.

SEROLOGICAL TESTS

1- SERUM NEUTRALIZATION TEST (SNT)

Specific neutralizing antibodies against FMD virus were detected following Ferriera (1976). Antibodies against RVF virus were detected following Pini *et al.*(1973) and specific antibodies against sheep pox virus were detected following OIE Manual (2000).

2- ELISA TEST

This test was used to detect IgG antibodies in serum samples against FMD virus according to Hamblin, *et al.* (1986), against RVF virus according to Meegan *et al.* (1987), and against sheep poxvirus according to Carn (1993).

Table 1. The mean Neutralizing Index (NI) at the following weeks post-vaccination.

Groups	No.	Types of Vaccine	Weeks post-vaccination														
			0	1	2	3	4	6	8	10	12	14	16	18	20	22	24
GA (FMD)	3	Combined FMD, RVF & Sheep pox Vaccine	0.4	1.1	1.35	1.5	1.8	1.95	2.1	2.4	2.4	1.95	1.95	1.8	1.6	1.5	1.5
GA (RVF)			0.5	0.8	1.4	1.8	2.3	2.5	2.6	2.8	2.7	2.5	2.4	2.2	2.0	1.9	1.9
GA (Sheep Pox)			0.35	0.9	1.6	2.3	1.8	1.7	1.7	1.6	1.5	1.4	1.2	1.0	0.9	0.6	0.6
GE	2	FMD	0.3	0.8	1.12	1.35	1.65	1.8	1.8	1.87	1.95	1.8	1.5	1.2	1.2	1.1	0.6
GF	2	RVF	0.5	0.8	1.2	1.5	1.7	2.2	2.4	2.5	2.5	2.2	2.0	1.8	1.5	1.4	1.2
GG	2	Sheep Pox	0.2	0.7	1.5	2.0	1.7	1.6	1.6	1.5	1.5	1.3	0.9	0.7	0.5	0.3	0.3
GH (FMD)	2	Control Non Vaccinated Sheep	0.3	0.6	0.4	0.6	0.4	0.3	0.3	0.6	0.4	0.6	0.5	0.4	0.4	0.3	0.5
GH (RVF)			0.5	0.4	0.7	0.4	0.5	0.5	0.4	0.4	0.5	0.7	0.7	0.4	0.5	0.4	0.4
GH (Sheep Pox)			0.2	0.5	0.4	0.3	0.6	0.6	0.3	0.5	0.3	0.2	0.6	0.5	0.3	0.4	0.4

GA = combined FMD, RVF and Sheep Pox vaccinated animals. GE = FMD vaccinated animals. GG= Sheep Pox vaccinated animals.
GF = RVF vaccinated animals. GH = control non vaccinated animals.

Table 2. The mean results of ELISA test of vaccinated sheep with combined or single vaccine as well as control sheep.

Groups	No.	Types of Vaccine	Weeks post - vaccination														
			0	1	2	3	4	6	8	10	12	14	16	18	20	22	24
GA (FMD)	3	Combined FMD, RVF& sheep pox vaccine	0.038	0.106	0.127	0.144	0.186	0.197	0.218	0.243	0.246	0.195	0.193	0.182	0.157	0.142	0.146
GA (RVF)			0.181	0.196	0.225	0.237	0.284	0.296	0.303	0.318	0.306	0.290	0.287	0.276	0.262	0.254	0.249
GA (Sheep Pox)			0.043	0.081	0.132	0.201	0.172	0.150	0.146	0.149	0.135	0.085	0.084	0.082	0.080	0.052	0.049
GE	2	FMD	0.028	0.075	0.106	0.128	0.156	0.187	0.184	0.188	0.196	0.183	0.144	0.117	0.113	0.104	0.059
GF	2	RVF	0.175	0.196	0.221	0.232	0.242	0.273	0.282	0.296	0.294	0.273	0.262	0.251	0.232	0.222	0.212
GG	2	Sheep Pox	0.040	0.061	0.130	0.199	0.160	0.152	0.153	0.128	0.130	0.084	0.079	0.051	0.048	0.040	0.039
GH (FMD)	2	Control Non Vaccinated Sheep	0.027	0.053	0.036	0.055	0.035	0.024	0.028	0.057	0.032	0.056	0.046	0.036	0.034	0.028	0.044
GH (RVF)			0.182	0.174	0.183	0.178	0.182	0.182	0.172	0.177	0.181	0.183	0.182	0.174	0.182	0.178	0.174
GH (Sheep Pox)			0.031	0.044	0.040	0.035	0.060	0.058	0.036	0.046	0.034	0.032	0.054	0.046	0.036	0.042	0.042

ELISA represented as optical density. FMD cut off = 0.082 RVF cut off = 0.185 Sheep Pox cut off = 0.085
Cut off according to *Edward (1985).*

Table 3. The mean results of serological tests of challenged sheep as well as control ones.

Groups		Type Of Vaccine	No.	Weeks post-vaccination					Days post challenge					
				0	1	2	3	4	1	3	5	7	10	15
GB (FMD)		Combined FMD, RVF & Sheep Pox Vaccine	2	0.5	1.2	1.5	1.8	1.95	1.8	1.65	1.95	2.1	2.5	2.5
GB (FMD) ELISA				0.043	0.116	0.145	0.198	0.194	0.182	0.154	0.197	0.219	0.256	0.252
GC (RVF)			2	0.5	0.8	1.5	1.8	2.3	2.2	1.8	2.2	2.5	2.8	2.8
GC (RVF) ELISA				0.182	0.196	0.230	0.252	0.286	0.270	0.252	0.272	0.297	0.318	0.308
GD (Sheep Pox) NI			2	0.4	0.9	1.9	2.2	1.8	1.7	1.5	1.9	2.5	2.6	2.8
GD (Sheep Pox) ELISA				0.045	0.083	0.175	0.200	0.170	0.162	0.134	0.176	0.220	0.231	0.243
GI challenged with FMD		Control Non Vaccinated Challenged Sheep	2	0.2	0.2	0.3	0.4	0.4	0.5	0.9	0.9	1.2	1.2	1.5
	ELISA			0.018	0.015	0.024	0.036	0.034	0.044	0.086	0.083	0.114	0.118	0.145
GJ challenged with RVF	NI		2	0.3	0.5	0.4	0.4	0.5	0.4	0.8	1.2	0.8	2.0	2.4
	ELISA			0.168	0.179	0.174	0.176	0.179	0.176	0.196	0.221	0.231	0.262	0.287
GK challenged with Sheep Pox			2	0.2	0.3	0.2	0.4	0.3	0.5	0.7	1.3	1.5	1.9	2.4
	ELISA			0.041	0.042	0.040	0.043	0.042	0.045	0.046	0.132	0.136	0.174	0.216

ELISA represented as optical density
FMD cut off = 0.082 RVF cut off = 0.185 Sheep Pox cut off = 0.085
No. = Number of animals

RESULTS AND DISCUSSION

The intervention for vaccinating animals by each vaccine singly is troublesome and time consuming. Therefore, since some years, many vaccine producing factories succeeded in combining more than one vaccine to be inoculated in the same dose and at the same time(Taha *et al.*, 1991).

The present study aimed to carry experimental trials for preparing a combined vaccine including foot and mouth antigen, Rift Valley Fever and sheep pox antigens together, and comparing the sero-conversion of sheep vaccinated with this combined vaccine, as well as animals vaccinated with each monovalent.

Regarding serum neutralization test, Table 1 revealed the neutralizing antibodies for FMD vaccine started to appear from 1st week being (0.8) neutralizing index (NI) and reaching its peak at the 12th week (1.95). In those vaccinated with the combined vaccine, the FMD antibodies at the 1st week were (1.1) and reaching their peak at the 10th week (2.4) and still within the protective level up till the end of the experiment {24weeks} (1.5), as recorded by Ferrlera (1976) who found that the level of protection was (1.2). Regarding neutralizing antibodies for RVF vaccine, they started to appear from the 1st week, in animals vaccinated with RVF vaccine alone. The mean of neutralizing index was (0.8) and reached its peak at the 10th week being (2.5). In those vaccinated with the combined vaccine at the 1st week, the neutralizing index was (0.8) and reached its peak at 10th week being (2.8) and still being protective (1.9) up till the end of the experiment {24 weeks} as mentioned by Pini *et al.* (1973) who found that the protective level of antibody against RVF was (1.5) $\log_{10}TCID_{50}$. In animals vaccinated with either FMD or RVF as monovalent vaccine, it was found that the protective level was up till the 18th week in FMD (1.2), and for RVF in the 20th week was (1.5) and then, began to decrease below the protective level.

The antibodies started to appear in the 1st week in sheep vaccinated with sheep pox vaccine. The mean of neutralizing index was (0.7) and reached its peak at the 3rd week (2.0), then, gradually decreased till the 12th week (1.5) as recorded by Cottral (1978) who determined that the NI $\geq$1.5 was considered protective for sheep pox virus. The antibodies in these animals decreased to be (0.3) at the end of the experimental period {24weeks}, while, in those vaccinated with the combined vaccine, the antibodies started to appear in the 1st week being (0.9) and reached their peak at the 3rd week (2.3) and still within the protective level up till the 12th week (1.5), then, gradually decreased up till the end of the experiment being (0.6) at the 24th week.

From the previous results, it is clear that sheep pox vaccine acted as an immuno-potentiating agent, and the use of the combined vaccine is of much benefit for increasing the duration of the immuno-response of the animals. This agrees with Taha *et al.* (1991) who found that the NI in sheep vaccinated with combined vaccine reached its peak earlier than in those vaccinated with each vaccine alone. Also this agrees with Abeer (1996) who mentioned that there was significant increase in the immune response of FMD in animals simultaneously vaccinated with sheep pox and FMD vaccines.

It is also clear that, in animals vaccinated with sheep pox vaccine, the antibody level decreased from the 14^{th} week below the protective level. It is recommended that these animals should be boostered with sheep pox vaccine at the 4^{th} week from the first dose as said by Manal *et al.* (2003) to prolong the period of immunity. On the other side , in animals non-vaccinated (Group H), the NI did not exceed (0.7).

From Table 3, it is clear that the combined vaccine can protect animals against the three viruses when challenged four weeks post-vaccination with virulent FMD virus ($10^4 MLD_{50}$/ml), RVF virus (10^5 $TCID_{50}$/ml) and sheep pox virus (10^3 SID_{50}/ml). These results agreed with the data obtained by Marcoss (1992) who prepared RVF/sheep pox combined vaccine and also protected sheep when challenged with both virulent viruses (RVF and sheep pox virus). Also, Daoud *et al.* (2001) found that combined vaccine (RVF/FMD) could protect animals against both viruses when challenged with virulent RVF and FMD viruses.

In non-vaccinated challenged sheep, there was no rise in the NI before challenge (Table 3). Then, it began to be detectable on the 7^{th} day for FMD, RVF and sheep pox, respectively, and reached its peak on the 15^{th} days post-challenge for FMD, RVF and sheep pox viruses, respectively.

The results of SNT and ELISA tests were in parallel to each other and equally sensitive. This agreed with Niklasson *et al.*, (1984). Finally, from the previous mentioned data, it is clear that FMD/RVF and sheep pox combined vaccine could be used safely for protection of sheep against these diseases.

REFERENCES

1. Abeer, Ezzat Mahomoud. 1996. Effect of using foot and mouth disease vaccine in comination with another vaccine on the immune status in farm animals. Thesis, Ph.D. Fac. Vet. Med., Cairo University.
2. Carn, V.M. 1993. Control of capripox virus infection. Vaccine, 11: 1275 – 1279.
3. Cottral, G.E. 1978 . Pox viruses. In Manual of standardized methods for VeterinaryMicrobiology, ed. G.E.Cottral. Cornell University press (Ithaca and London). P.P 273 – 91.
4. Daoud, A.M., Wafaa El deghaidy, M. Shawky, K. Z. Hassan. and M.M. Taha. 2001.Trial for preparation and evaluation of inactivated alhydra-gel combined vaccine for Foot and Mouth Disease and Rift Valley Fever. Beni Suef Vet. Med. J., 11 (2): 599-607.
5. Edward, K. 1985.Progress in Enzyme immunoassays; Production of reagents, experimental design and interpretation.Bull. W. H. O.,63 (4): 793-811.
6. Elian, K. A. and B. Botros. 1997. Production and evaluation of RVF diagnostic antigens by ELISA technique. 3rd Arab Vet. Med. Cong., March 22-26, 1997 Cairo.
7. El Nimr, M.M. 1980. Studies on the inactivated vaccine against " RVF" . Thesis, Ph.D. Fac. Vet. Med. Assiut University.
8. Ferriera, M. E. V. 1976. Microtiter neutralization test for the study of FMD antibodies. BLTN. Centro Panamericano des Fiebre Aftosa, 21: 17-24.
9. Hamblin, C., I. T. R. Barnett and J. Crowther. 1986. A new enzyme linked immunosorbent assay (ELISA) for detection of antibodies against FMD virus. J. Immunol. Methods, 93: 115-121.
10. Henderson, W. M. 1970. Foot and Mouth disease; a definition of the problem and routes on its isolation. Br. Vet. J., 126: 115-120.
11. House, J. A., M. Terronce, T. M. Wilson, N. El Nakashly, I. Abdel Karim, I. Ismail, N. El Danaf, M. A. Mousa and N. N. Ayoub. 1990. The isolation of lumpy skin disease virus and bovine herpes virus 4 from cattle in Egypt. J. Vet. Diag. Invest., 2: 111-115.
12. Manal, Awad, A. Miclkael, M. Soad Soliman, S. S. Samir and A. M. Daoud. 2003. Trial for preparation of inactivated sheep pox vaccine using Binary ethylenemine. Egyp. J. of Immunol., 10 (2): 2003.

13. Marcoss, T. N. 1992. Attempts of preparation a combined RVF vaccine with sheep pox vaccine for sheep in Egypt. Thesis, M. Vet. Sci., Fac. Vet. Med., Alexandria University.
14. Meegan, J. M., R. J. Yedloutschnig, B. A. Peleg, J. Shy, C. J. Peters , J. S. Walker and R. E. Shope. 1987. Enzyme-Linked Immunosorbent Assay for detection of antibodies to Rift Valley Fever virus in ovine and bovine sera. Am. J. Vet. Res., 48 (7): 1138-41.
15. Niklasson, B., C. J. Peters, M. Grandie and O. Wood. 1984. Detection of human IgG and IgM antibodies to Rift Valley Fever Virus by ELISA. J. Clin. Micro., 19 (2): 225- 229.
16. Office International Des Epizooties. "OIE". 2000. Manual of standards for diagnostic tests and vaccine 4th Edition.
17. Pini, A., L. J. Lund and S. J. Davis 1973. Fluorescent and neutralizing antibody response to infection by Rift Valley Fever Virus. J. S. Afr. Z. Med. Assoc., 44 (11): 161- 165.
18. Taha, M. M., M. Soad, A. Soliman, A. Mickael, A. El Debegy, A.Y.A. Mohsen and Monira Nassar. 1991. Studies on the immune response of sheep vaccinated with Sheep Pox, RVF combined vaccine in comparison with each Monovalent vaccine. Beni Suef Vet. Med. Res.,1: 183-194.
19. Wagner, G. G., J. L. Card and K. M. Cowan. 1969. Plum Island Animal Disease Laboratory Animal disease and parasitic Research Division Agricultural Research Service, U.S. Department of Agricultural Grean Port, New York, USA.

الأستجابة المناعية للأغنام المحصنة بلقاح مركب ثلاثى لفيروسات الحمى القلاعية وحمى الوادى المتصدع و جدرى الأغنام

عبير أحمد طلعت ، مرفت محمد على ، للى صبحى سلامة

معهد بحوث الأمصال واللقاحات – مركز البحوث الزراعية – وزارة الزراعة – النقي – جيزة – مصر

تم تحضير و تقييم لقاح مركب يحتوى على ثلاث عترات من فيروس الحمى القلاعية العترة (O_1) و فيروس حمى الوادى المتصدع العترة (ZH_{501}) و فيروس جدرى الأغنام العترة المصرية و الذى تم تثبيطها بواسطة مادة البينارى إيثلين أمين و خلطها مع مادة الألومنيوم هيدروكسيد جيل ، و قد أجريت عدة تجارب لتقييم الإستجابةالمناعية للحيوانات المحصنة باللقاحات المنفردة ومقارنة نتائجها مع اللقاح المركب. و وجد أن الأجسام المناعية المتعادلة فى أمصال الحيوانات المحصنة باللقاح المركب وصلت إلى مستوى أعلى فى فترة أقل من الحيوانات المحصنة باللقاحات المنفردة (الحمى القلاعية وحمى الوادى المتصدع) بينما لايوجد فرق معنوى واضح بين الحيوانات المحصنة باللقاح المركب و لقاح جدرى الأغنام المنفرد ، لذا ينصح بإعطاء جرعة منشطة منة فى الأسبوع الرابع من بداية التحصين.