

## TRIALS FOR PREPARATION OF INACTIVATED BIVALENT AVIAN ENCEPHALOMYELITIS AND REO VIRUS VACCINE

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### **Abstract**

Inactivated monovalent and bivalent oil adjuvanted vaccines against Avian Encephalomyelitis (AE) and Reo viruses were prepared. Immunological responses to such experimental vaccines were evaluated by injecting them into groups of susceptible chickens. Blood samples were collected at weekly intervals. Serological and cellular immune response were evaluated by SNT, lymphocyte blastogenesis and macrophage activity test, respectively. The vaccinated chicks were subjected to challenge tests against both viruses. The results indicated that prepared vaccine was efficient, safe, immunogenic and produced satisfactory dual protection against AE and Reo viruses infection.

### **INTRODUCTION**

The modern industry is the most highly developed segment of world food animal production. Diseases are the major problem and the biggest depressive factor on profit margin. This problem can be considered as a constant struggle between the host and the invading organism.

Avian Encephalomyelitis (AE) is an infectious and communicable disease of young chickens. In Egypt, AE was firstly isolated by El-Khashaab (1986) from 7-10 days old broiler flocks. AE virus caused considerable economic loss to the poultry industry, breeders and hatchery men because loss from it is largely confined to the first two weeks after hatching, vertical transmission of the disease and marked drop in egg production.

Avian Reo virus has been concerned with the role in avian arthritis or tenosynovitis. Kheir El-Din and El-Sanousi (1986) isolated and characterized avian Reo virus. The local isolates of avian Reo viruses in Egypt were serologically identified with the reference anti-serum of S1133 strain of avian Reo virus (Nadia, 1986). The use of killed vaccines in oil emulsion is necessary to maintain high and uniform levels of protective maternal antibody in hatching chicks that can be protected for the first 4-5 weeks of age (Baxendale and Luticken, 1981). The high levels of humoral antibody

and the consistency of these levels throughout a flock vaccinated with inactivated oil emulsion provide effective protection against field challenge by virulent virus (Thayer *et al.*, 1983). Combined vaccines have the advantages of providing protection against more than one disease, reducing vaccination cost and number of vaccination per farm, as well as, saving time and labour and reducing the stress reactions.

Each of AE and Reo diseases bearing effects on the movement of birds and leads to paralysis resulting in decrease rate of growth and egg production. So, the object of this study was to prepare and evaluate the immune response of bivalent vaccine of AE and Reo viruses in single dose to avoid these economic losses.

## MATERIALS AND METHODS

### Viruses

#### 1- Field virulent local isolate of AEV

The virus was isolated and identified in Egypt by El-Makaky (2000). It was used for vaccine preparation as well as challenge test. Its titre was  $10^{5.9}$  EID<sub>50</sub>/ml.

#### 2- Avian Reo viruses

##### 2.1. vaccinal strain of avian Reo virus

Modified live egg adapted Reo virus vaccine (strain S1133) was kindly supplied by Intervet Company. It was used for preparation of monovalent and bivalent oil inactivated vaccines. Its titre was  $10^{8.5}$  EID<sub>50</sub>/ml.

##### 2.2. Virulent Reo virus

Virulent Reo virus strain S1133 was used in challenge test and obtained from Newcastle Vaccine Research Dept., Vet. Serum and Vaccine Research Institute, Abbasia, Cairo.

### Chicken eggs

Six and nine days old embryonated chicken eggs (ECE) SPF (Ministry of Agriculture, specific pathogen free egg production farm, Koum Osheim, Fayoum, Egypt) were used for propagation, titration of the preparation batches of AE and Reo viruses, respectively, and testing of complete inactivation to the inactivated batches.

### Experimental chicks

One hundred, one-day-old Hubbard chicks (United Company for poultry production) were reared under complete hygienic measures in isolated and disinfected metal cages, commercial broiler ration was used throughout the experimental period.

## Viruses propagations

### 1- AEV propagation

Propagation and titration of the virus was carried out in SPF embryonated chicken eggs, and the obtained titre was expressed as embryo infective dose as determined by Reed and Muench (1938). The titre was  $10^{5.9}$  EID<sub>50</sub>/ml.

### 2- Reo virus propagation

It was done according to Abou El-Khair (1996). The titre was  $10^{8.5}$  EID<sub>50</sub>/ml.

### Inactivation of the viruses

Inactivation of each virus suspension of AE and Reo viruses was carried out using formalin in a final concentration of 0.2%. The fluids were blended using magnetic stirrer for about 24 hours at room temperature. Samples from the inactivated viruses were tested for safety in 9-11 days old embryonated chicken egg (0.2ml/egg). Three successive blind passages were carried out in ECE.

### Preparation of oil inactivated vaccines

The monovalent and bivalent oil adjuvanted vaccines were prepared according to the method of Thayer *et al.* (1983) with aqueous to the oil ratio (A:O) 1:3 to facilitate preparation of low viscosity emulsion. The prepared vaccines were dispensed into 50ml bottles and stored at 4°C till used.

### Quality control of the experimentally prepared vaccines

#### Purity and sterility tests

The prepared vaccines were tested to be free from bacterial and fungal contaminants.

#### Experimental design

One hundred, one-day-old Hubbard chicks were used. Chicks were reared in isolated conditions. These birds were as far as could be free from clinical signs suggestive of bacterial, viral and parasitic diseases. These chickens were checked for their susceptibility against AE and Reo viruses before inoculation. They were divided into four groups, 25 chicks each as follows:

- Group (1)      → vaccinated with locally prepared bivalent AE and Reo viruses inactivated oil emulsion vaccine.
- Group (2)      → vaccinated with locally prepared AE monovalent inactivated oil emulsion vaccine.
- Group (3)      → vaccinated with locally prepared Reo monovalent inactivated oil emulsion vaccine.
- Group (4)      → non-vaccinated negative control.

On the 21<sup>st</sup> day of age, each chick of vaccinated groups received 0.5ml of its appropriate vaccine by I/M route. Ten random blood samples were collected weekly

from each group for 12 weeks post-vaccination. The separated serum samples were tested for both humoral and cellular immune response against AEV and Reo virus using the following tests:

**1- Serum neutralization test (SNT)**

It was carried out on serum samples for estimating the neutralizing antibodies against AEV and Reo viruses according to the method of Villagas (1990).

**2- Assay of Lymphocyte blastogenesis**

Two random blood samples were collected weekly from each group up to four weeks post vaccination. The test was applied according to Charles *et al.* (1978). Evaluation of the test using MTT [3-(4-5-dimethyl-thiazole-2-yl) 2-5 diphenyl-tetrazolium bromide] according to Mosmann (1983), results of the test were expressed as delta optical density ( $\Delta OD$ ).

$$\Delta OD = (OD \text{ of PHA}) - (OD \text{ of media}) - (OD \text{ of cell} - OD \text{ of media}).$$

PHA = phytohaemagglutinin .

**3- Macrophage activity test**

The test was carried out according to El-Enabawy (1990). The percentage of phagocytosis and the phagocytic index were calculated as follows:

$$\text{Phagocytic percentage} = \frac{\text{No. of phagocytes which ingest candida}}{\text{Total number of phagocytes}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{Total No. of phagocytes which ingest more than two candida}}{\text{Total No. of phagocytes which ingest candida}}$$

**4- Challenge test**

Ten birds from each group were challenged 4 weeks post-vaccination with their corresponding virus. Chickens challenged with AE virus were inoculated intracerebrally with 0.3ml of local field isolate of AEV at titre  $10^5$  EID<sub>50</sub>/ml and were observed for 15 days after challenge. Those challenged with Reo virus were inoculated in the footpad with 0.1ml containing  $10^3$  EID<sub>50</sub>/ml of virulent Reo strain S1133, and were observed for 15 days after challenge. Dead birds and those showing symptoms during the period of observation were kept for post-mortem (P.M.) examination.

## RESULTS AND DISCUSSION

New strategies are urgently required for development of new and more efficient poultry vaccine. Oil adjuvants are known to be one of the best immune stimulants that are incorporated into inactivated poultry vaccines. They were even

more efficient than aluminum gel as they produce long-lasting immunity (Adu *et al.*, 1989). The possibility of producing effective local combined vaccine against AE and Reo viruses was the main aim of this study to induce a simultaneous protective immunity against both of them.

In Table 1, the neutralizing antibody titre in group (1) and group (2) showed that the serum neutralizing antibody titre increased from the first week post-vaccination and gradually increased reaching maximal titre at the 4<sup>th</sup> week, then, the bivalent vaccine of AE and Reo produced high titre than the monovalent AE vaccine. Similar findings were reported by Nedelciu and Sofei (1990) who mentioned that groups of chickens inoculated with oil inactivated vaccine, either bivalent (ND and IB) or trivalent (ND, EDS and IB) showed higher immunogenicity than the monovalent vaccines.

Regarding the result of serum neutralization test of inactivated Reo vaccine (Table 2) revealed slight increase in the monovalent vaccinated group till 7<sup>th</sup> week post-vaccination, then, the titre became similar in both groups till the end of experiment. These results agreed with those of Abou El-Khair (1996) who obtained similar immunological trends using inactivated oil Reo virus vaccine.

Table 1. Value of AEV neutralizing antibody titre in chickens vaccinated with monovalent AEV and bivalent AE and Reo viruses inactivated oil emulsion vaccines.

Chicken groups	Titre of neutralizing antibodies *											
	Weeks post vaccination											
	1	2	3	4	5	6	7	8	9	10	11	12
Group (1)	2	32	32	64	64	32	32	64	64	64	32	32
Group (2)	8	32	64	64	32	32	16	32	32	32	64	16
Group (4)	0	0	0	0	0	0	0	0	0	0	0	0

\* Antibody titre = The reciprocal of serum dilution which neutralized and inhibited, the CPE of 100-200 TCID<sub>50</sub> of the virus.

Group (1) = vaccinated with locally prepared bivalent AE and Reo virus inactivated oil emulsion vaccine.

Group (2) = vaccinated with locally prepared AE monovalent inactivated oil emulsion vaccine.

Group (4) = control non-vaccinated.

Table 2. Value of Reo neutralizing antibody titre in chickens vaccinated with monovalent Reo and bivalent AE and Reo viruses inactivated oil emulsion vaccines.

Chicken groups	Titre of neutralizing antibodies *											
	Weeks post vaccination											
	1	2	3	4	5	6	7	8	9	10	11	12
Group (1)	4	16	16	32	ND**	32	32	64	64	64	64	32
Group (3)	4	16	32	64	64	64	64	64	64	64	64	32
Group (4)	0	0	0	0	0	0	0	0	0	0	0	0

\* Antibody titre = The reciprocal of serum dilution which neutralized and inhibited, the CPE of 100-200 TCID<sub>50</sub> of the virus.

\*\* Not done

Group (1) = vaccinated with locally prepared bivalent inactivated oil emulsion vaccine.

Group (3) = vaccinated with locally prepared monovalent Reo inactivated oil emulsion vaccine.

Group (4) = control non-vaccinated.

Fig. 1. Value of AEV neutralizing antibody titre in chickens vaccinated with monovalent AEV and bivalent AE and Reo viruses inactivated oil emulsion vaccines.

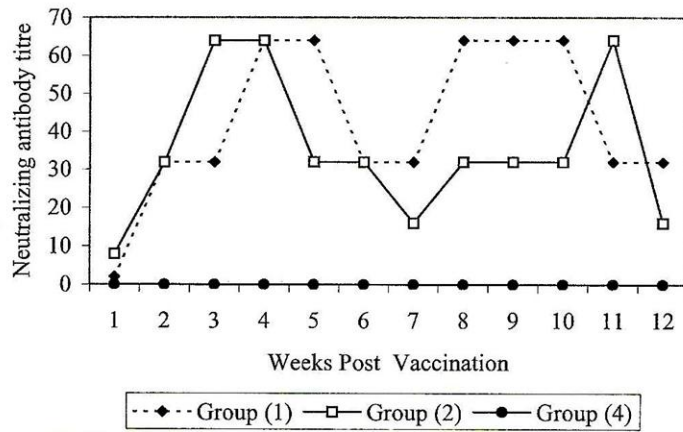
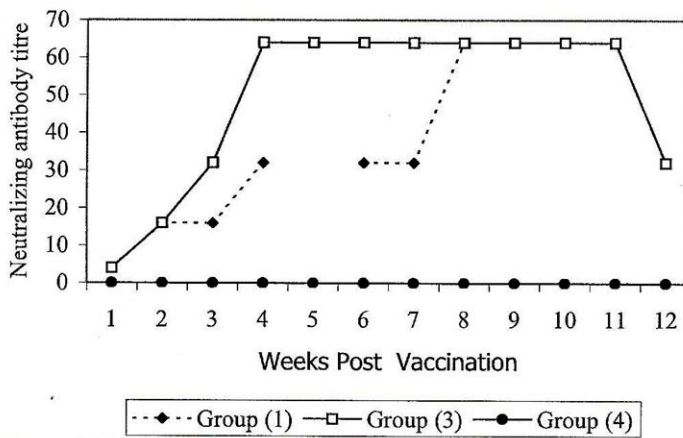


Fig. 2. Value of Reo neutralizing antibody titre in chickens vaccinated with monovalent Reo and bivalent AE and Reo viruses inactivated oil emulsion vaccine.



Concerning the cellular immune response, results of lymphocytes blastogenesis as represented in Table 3 revealed that a maximum response of T cells expressed as delta optical density (OD) was recorded for different groups two weeks post-vaccination, then, the values declined till the end of the test (4 weeks post-vaccination). However, birds of groups (2) and (3) showed higher stimulation 0.092 and 0.094, respectively, followed by group one 0.074. More confirmation of cellular immune response was achieved using macrophage activity test, results represented in Table 4 as expressed by phagocytic percentage and Table 5 that expressed by phagocytic index where running parallel to the former test. The results of cellular immune responses agreed with Timms and Bracemell (1983) as they stated that once the humoral immune response became established, there was a corresponding decrease in the cellular immune response.

Regarding the protection percentage against either virulent AE or Reo virus (Table 6), it was 100% for group (1) and 90% for group (2). On the other hand, protection percent against virulent Reo virus was 100% for both group (1) and (3). Obtained results after challenge test against both viruses were in contact with those obtained by Giambone and Hathcock (1991) where they found that Reo virus vaccine did not interfere with Newcastle disease infectious bronchitis and infectious bursal disease vaccines in one day old chicks when measured by challenge studies.

So, the present investigation indicated that the prepared bivalent AE and Reo inactivated oil emulsion vaccine could elicit the production of protective antibody titres against two used viruses. No mutual enhancement or competition was detected. Similar observations were recorded by Thayer *et al.* (1983) where they did not find any practical difference in amplitude of antibody response when ND antigen used alone or combined with IB antigen. El-Mahdy *et al.* (1999) found that a satisfactory immune response to IB antigen could be obtained when evaluated under laboratory condition in a combined inactivated vaccine with ND virus without any antagonistic action from each other.

In conclusion, the locally prepared bivalent inactivated AE and Reo viruses oil emulsion vaccine had the advantage of providing protection against more than one disease at the same time in one dose, thus, reducing vaccination cost and the number of vaccination per farm, as well as, saving time and labour costs, and more potent and efficient.



Table 3. Cell mediated immune response of chickens vaccinated with bivalent AE and Reo, monovalent AE and monovalent Reo by lymphocytes transformation expressed by delta optical density.

Chicken groups	Weeks post-vaccination			
	1	2	3	4
Group (1)	0.035	0.074	0.059	0.041
Group (2)	0.047	0.092	0.073	0.052
Group (3)	0.043	0.094	0.069	0.055
Group (4)	0.009	0.007	0.010	0.009

Group (1) = vaccinated with locally prepared bivalent AE and Reo viruses inactivated oil emulsion vaccine.

Group (2) = vaccinated with locally prepared AE monovalent inactivated oil emulsion vaccine.

Group (3) = vaccinated with locally prepared Reo monovalent inactivated oil emulsion vaccine.

Group (4) = control non-vaccinated.

Table 4. Phagocytic activity of chickens vaccinated with bivalent AE and Reo, monovalent AE and monovalent Reo by *Candida albicans* expressed by phagocyte percentage.

Chicken groups	Weeks post-vaccination			
	1	2	3	4
Group (1)	40.3	60.2	53.7	49.6
Group (2)	50.2	73.7	66.2	59.4
Group (3)	51.5	72.5	67.1	57.2
Group (4)	14.1	12.2	13.7	19.3

Group (1) = vaccinated with locally prepared bivalent AE and Reo viruses inactivated oil emulsion vaccine.

Group (2) = vaccinated with locally prepared AE monovalent inactivated oil emulsion vaccine.

Group (3) = vaccinated with locally prepared Reo monovalent inactivated oil emulsion vaccine.

Group (4) = control non-vaccinated.

Table 5. Phagocytic activity of chickens vaccinated with bivalent AE and Reo monovalent AE and monovalent Reo viruses vaccines using *Candida albicans* expressed by phagocytic index.

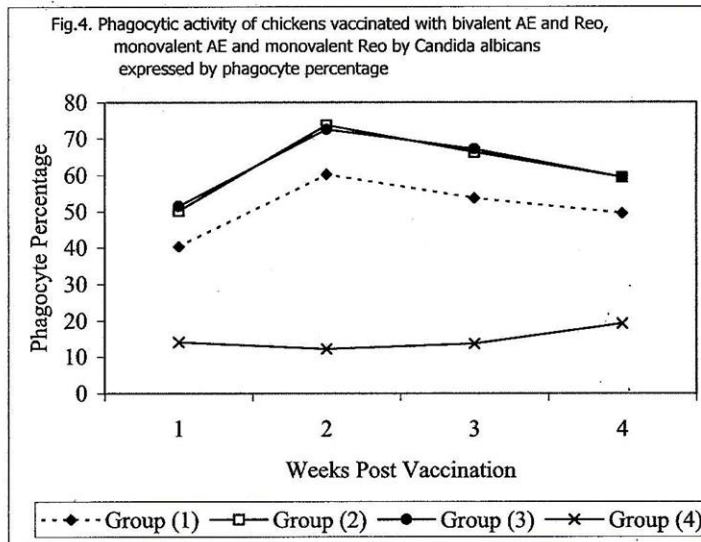
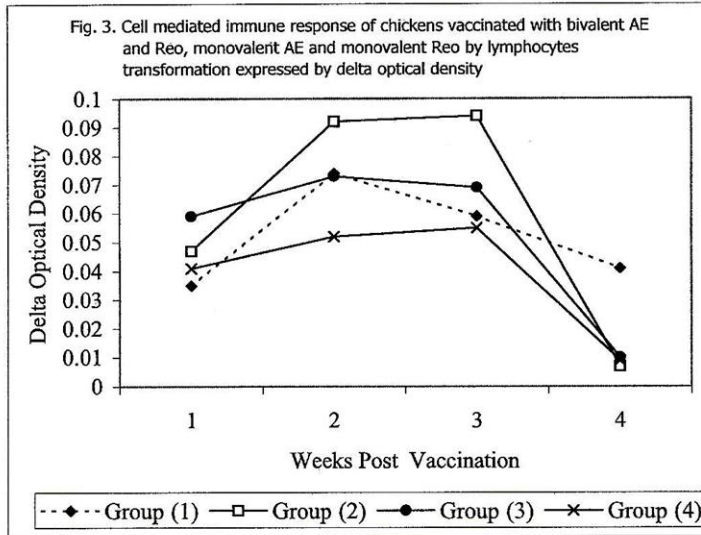
Chicken groups	Weeks post-vaccination			
	1	2	3	4
Group (1)	0.26	0.33	0.29	0.22
Group (2)	0.34	0.42	0.37	0.32
Group (3)	0.33	0.43	0.39	0.31
Group (4)	0.06	0.09	0.07	0.09

Group (1) = vaccinated with locally prepared bivalent AE and Reo viruses inactivated oil emulsion vaccine.

Group (2) = vaccinated with locally prepared AE monovalent inactivated oil emulsion vaccine.

Group (3) = vaccinated with locally prepared Reo monovalent inactivated oil emulsion vaccine.

Group (4) = control non-vaccinated.



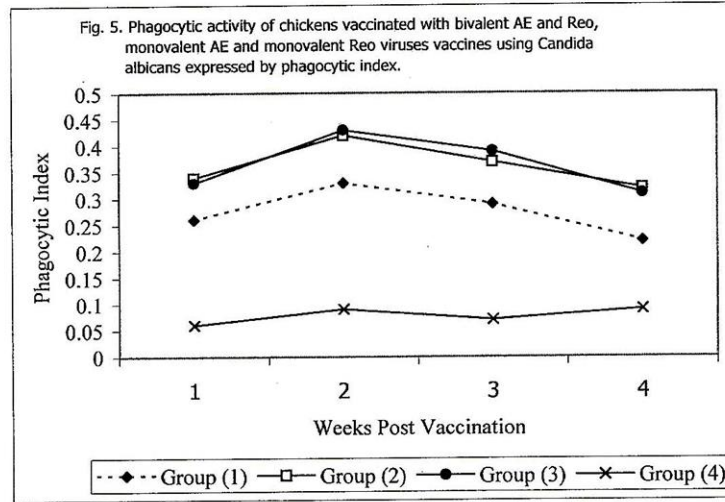


Table 6. Revealed results of challenge test against AE and Reo viruses 4 weeks post vaccination.

Chicken groups	Used vaccine	No. of vaccinated chicken	Challenge virus	No. of survived chicken	Protection %
1	Bivalent AE & Reo viruses vaccine	10	AE virulent	5	100%
			Reo virulent	5	100%
2	Monovalent AEV vaccine	10	Virulent AE	9	90%
3	Monovalent Reo virus vaccine	10	Virulent Reo	10	100%
4	Non-vaccinated control	10	Virulent AE	0	0%
			Virulent Reo	0	0%

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### محاولات لتحضير لقاح مثببط مزدوج ضد مرضى الارتعاش الوبائي والريو

هالة محمد المكاكى، محمد عبد العنى أبو الخير ، مرفت عبد المنعم الكوفى ، محمد سيد مذكور ،  
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معهد بحوث الأمصال واللقاحات البيطرية - مركز البحوث الزراعية - وزارة الزراعة -  
الدقى - جيزة - مصر

تم تحضير لقاح ميت مزدوج ضد كل من مرضى الارتعاش الوبائي والريو ولقاحات ميثة  
فردية ضد كل من المرضين على حدة . وتم تقييم هذه اللقاحات الثلاثة بحقتها فى مجاميع الدواجن  
وقياس المناعة الخلوية باستخدام اختبار التحور الليمفاوى واختبار نشاط خلايا المكروفاج وقياس  
المناعة الخلطية باستخدام اختبار المصل المتعادل. كذلك تم إجراء اختبار التحدى ضد كل من  
الفيروسين وقد أثبت اللقاح المزدوج أنه آمن وذو كفاءة فى التصدى للمرضين معاً.