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A TRIAL FOR PREPARATION AND EVALUATION OF LIVE ATTENUATED COMBINED AVIAN ENCEPHALOMYELITIS AND FOWL POX VACCINE IN COMPARISON WITH IMPORTED ONE

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SUMMARY

An experimental trial was carried out to prepare a combined attenuated vaccine against avian encephalomyelitis (AE) and fowl pox diseases. Monovalent live attenuated vaccine was firstly prepared against each disease on specific pathogen free (SPF) eggs. After titration, they were mixed together and lyophilized as a bivalent live vaccine. Chickens were vaccinated with the locally prepared vaccine as well as the imported one. Humoral immune response of the vaccinated chicks was evaluated by using enzyme linked immunosorbent assay (ELISA) for avian encephalomyelitis and neutralization test (NT) for AE and fowl pox viruses. The prepared combined vaccine was found potent, safe and protective against challenge viruses.

INTRODUCTION

Avian encephalomyelitis is an infectious and communicable disease of young chickens, was firstly described by Jones (1932) and designated it as "an encephalomyelitis in the chicken". In 1934, Jones referred to the malady as "epidemic tremor" because it was observed that many affected chickens manifested a tremor or vibration of the head and neck. The disease affects chiefly the central nervous system and ataxia is observed more frequently and earlier in the onset of the disease than the tremor. The term "infectious avian encephalomyelitis" was proposed by Van Roekel et al. (1939). Fowl pox has a world wide distribution and is caused by a DNA virus of the genus Avipox virus of the family Poxviridae. Its incidence is variable in different areas because of differences in management and hygiene or the regularity of vaccination. The use of fowl pox virus (FPV) vaccines is indicated commercially in areas where the

disease is endemic or on premises where infection has been diagnosed. Avian encephalomyelitis (AE) and fowl pox (FP) are among viral diseases that cause considerable economic losses to poultry related to retarded growth in young chickens and drop in egg production in laying birds (Tripathy, 1989).

The advantage of live virus vaccination is that the vaccines are usually sold as freeze dried brain suspension and chorioallantoic membrane CAM from infected embryonated eggs for AE and FP respectively and relatively inexpensive and easy to administer and lend themselves to mass application. Local immunity is stimulated by infection with live viruses and protection occurs very soon after application. Vaccine viruses may spread from birds that have been successfully vaccinated to those that have not (Calnek et al., 1997).

Combined vaccines have the advantages of providing protection against more than one disease, reducing vaccination expense and number of vaccination per farm as well as saving time and labor costs beside reducing the stress reactions. Also, the more manual capture and restraint needed to inject vaccines into poultry specially egg laying hens. So, the objective of this study was to prepare and evaluate the immune response of bivalent attenuated vaccine of AE and fowl pox viruses in single and combined form for protection against diseases caused by these agents.

MATERIAL AND METHODS

1. Viruses:

1.1. Vaccinal strains:

1.1.a. Avian encephalomyelitis (AE) virus:

Avian encephalomyelitis virus Calnek strain 1143, lyophilized with stabilizer (AE Vaccine Nobilis) was obtained from Intervet International B.V., Boxmeer, Holland. Its titre was $10^{6.6}$ EID_{50}/ml.

1.1.b. Fowl pox (FP) virus:

The egg adapted Beaudette strain of fowl pox was obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. Its EID_{50} was determined before starting the experiment and found to be 10^{8}/ml.

1.1.c. Nobilis AE+FP vaccine:

Combined vaccine against AE and FP was obtained from Intervet International B.V., Boxmeer, Holland as freeze dried vaccine. It was packed in two separate units one contain the vaccine vial and the other contain the bottle with sterile dilution (Unisol). The titre of this vaccine was $10^{6.46}$ EID_{50}/ml for fowl pox and $10^{6.6}$ EID_{50}/ml for AE.

1.2. Challenge viruses:

1.2.a. Field virulent local isolate of AE virus:

The virus was isolated and identified in Egypt by (El-Makaky, 2000). It was used for intracerebrally challenge. It has a titre of $10^{5.9}$ EID_{50}/ml and 0.3ml was injected per bird.

1.2.b. Virulent fowl pox:

Egyptian virulent FP virus was used as a challenge virus. It was isolated and identified by Sabban (1954). Its titre was $10^{5.7}$ EID_{50}/ml when titrated in embryonated chicken eggs. It was used in dose of $10^{3.0}$ EID_{50}/bird.

2. Experimental chickens:

Two hundred and fifty broiler Hubbard chickens of six weeks old were obtained from the "United Company for Poultry Production) and reared under complete hygienic measures in isolated floored cages

3. Embryonated chicken eggs (ECEs):

Embryonated SPF (Arab Republic of Egypt, Ministry of Agriculture, Specific Pathogen Free Egg Production Farm, Nile SPF eggs, Koum Osheim, Fayoum, Egypt). Six days old for AE virus inoculation and 11 day old for FP virus inoculation. The eggs were used for vaccines preparation.

4. Preparation of the vaccines:

Preparation of monovalent vaccine against AE:

It was prepared by intra yolk sac inoculation. Propagation and titration was carried out according to the method described by Berger (1982). The titre of the virus was expressed as embryo lethal dose (ELD_{50}/ml) as determined by Reed and Muench (1938). The obtained maximum titre was $10^{5.4}$ EID_{50}/ml.

Preparation of monovalent vaccine against FP:

The technique for propagation of fowl pox virus in embryonated chicken eggs for vaccine production was run according to Crowther (1963). Titration of the vaccine was carried out according to Dhillon et al. (1968).

Lyophilization of the obtained viruses was performed after addition of 20% skimmed milk as stabilizer.

Preparation of combined bivalent AE and fowl pox vaccine:

Equal amount of both AE and FP vaccines previously prepared was mixed with 20% skimmed milk and dispensed in vials 1ml, lyophilized and stored at -20°C till used.

Purity test:

It was carried out according to Anon (1971).

Safety test:

Ten, six week old apparently healthy and full susceptible chickens, each was inoculated with 100 field dose by wing web method. The birds were kept for 3 weeks under observation for evidence of takes and for the absence of any adverse effects attribute to the vaccine.

Experimental Design:

Five groups of chickens (50 chickens per group), each of six weeks old were used. The chickens were treated as follows:

Group (1): Vaccinated with locally prepared at-

tenuated combined AE and FP vaccines.

Group (2): Vaccinated with the imported attenuated combined AE and FP vaccine.

Group (3): Vaccinated with the single attenuated AE vaccine.

Group (4): Vaccinated with local attenuated FP vaccine.

Group (5): Non-vaccinated control.

Each bird was vaccinated by wing web with a dose containing approximately $10^{5.0}$ EID_{50}/ml, $10^{7.2}$ EID_{50}/ml of AE and fowl pox, respectively.

Ten random blood samples were collected from each group weekly allover the experimental period (8 weeks). The obtained serum samples were tested for evaluation of humoral immune response against AE and fowl pox by the following serological tests:

1. Enzyme linked immunosorbent assay (ELISA):

For AE virus, the test was carried out according to Garrett et al. (1984), Shafren and Tannock (1988) using commercial kit (Kirkegaard and Perry Laboratories Inc. "KPL").

2. Serum neutralization test:

Microtitration test (Beta Procedure) for quantitative estimation of AE antibodies was applied according to Villagas (1990) and Boulter (1957) for fowl pox virus.

3. Challenge test:

For AE virus:

Birds (vaccinated and control) were challenged intracerebrally four weeks post vaccination with 0.1 ml of local virulent AEV ($10^{5.9}$ EID_{50}/ml) and observed 15 days for any signs of AE (Ataxia, paralysis and tremor of head and neck).

For fowl pox virus:

Four weeks post vaccination, half of each vaccinated and control chickens groups were challenged with standard challenge dose of the virulent fowl pox virus containing $10^{3.7}$ EID_{50} per bird by wing web method in the other wing. The challenged birds were checked for takes 15 days post challenge.

RESULTS AND DISCUSSION

New strategies are urgently required for development of new vaccine (Nagaraja et al., 1991). Protection of poultry against more than one disease at the same time is of great importance to reduce labor, costs and stress on vaccinated birds.

Regarding the humoral immune response against AEV, is represented in table (1) which denoted that there are noticeable differences between groups 1, 2 and group 3 where the maximum titre recorded at the 4th week was 4578 and 4499 for groups 1 and 2, respectively, while group 3, the titre reached 1676. The protective antibody titres obtained by serum neutralization test showed that

he results were parallel to that obtained by ELI-5A test. The explanation of these findings may be ttributed to there is no interference or any antag-nistic reaction between two antigens AE and FP when combined as live vaccine but fowl pox act s immune stimulant to AE virus. Moreover, the btained data are encouraging for using a com-ined AE and FP as live vaccine (Abd El-Wanis t al., 1999).

Dealing with the neutralizing antibodies against FPV, results are shown in table (3) revealed that nearly there are no differences between groups 1, and 4 where peak of neutralizing antibody was ecorded at the 4th week post vaccination. Ob-ained results agreed with those of Thayer et al., 1983) who found that no practical differences in alues of antibody response to either antigen used lone or compared to that of bivalent combina-on.

he protection percent of chicks to intracerebral allenge were 95% for groups 1 and 2 and 90% for group 3, which meaning that the vaccine conferred a high degree of protection against challenge (0.1 ml of virulent AEV $10^{5.9}$ ELD_{50}) 4 weeks post vaccination. This result agreed with Macleod (1965) who reported that one dose of vaccine containing $10^{5.9}$ ELD_{50} of virus protect over 90 %.

These results agree with those reported by Brandly (1941), El-Dahaby et al. (1971), Rai and Sethi (1972), Singh et al. (1973), El-Zein et al. (1974), Rao et al. (1978), Mockett et al. (1990) and Tripathy and Reed (1997) who detected that the potent vaccine should protect at least 80% of the vaccinated birds from infection with the virulent virus, while 80% or more of the control non vaccinated ones shows the typical symptoms.

The results of challenging the immunity of the vaccinated and control chickens with the virulent fowl pox virus gave 95% and 90% protection in groups 1 and 2 while group 4 gave 90% protection (no local take reaction) (Table 5).

Table (1): Geometric mean ELISA antibody titre against AE in groups of vaccinated chickens

Chicken Groups	Weeks Post Vaccination							
	1	2	3	4	5	6	7	8
1	1010	2976	4479	4578	4501	3165	2996	2982
2	1003	2610	4231	4499	4030	3037	2891	2811
3	919	1328	1676	1526	1510	1414	1479	1317
5	601	631	812	425	671	432	604	603

Group (1): Chickens vaccinated with locally prepared combined live AE and FP.

Group (2): Chickens vaccinated with imported combined live AE and FP.

Group (3): Chickens vaccinated with locally prepared AE vaccine.

Group (5): Unvaccinated control chickens.

N.B. Correct positive control (CPC) =

Mean positive control (MP) - Mean negative (MN)

$$S/P \text{ ratio} = \frac{OD \text{ of sample} - MN}{CPC}$$

Log_{10} titre = Log10 S/P ratio X 0.717 + 3.906

Titre = Antilog 10^X

OD of positive serum sample was > 0.3

Table (2): The average mean titre of neutralizing antibodies against AEV in groups of vaccinated chickens

Chicken Groups	Weeks Post Vaccination							
	1	2	3	4	5	6	7	8
1	16*	32	128	512	256	256	128	64
2	16	32	64	256	256	256	128	64
3	8	16	32	64	64	32	32	16
5	0	0	0	0	0	0	0	0

Group (1): Chickens vaccinated with locally prepared combined live AE and FP.

Group (2): Chickens vaccinated with imported combined live AE and FP.

Group (3): Chickens vaccinated with locally prepared AE vaccine.

Group (5): Unvaccinated control chickens.

* Titres expressed as the reciprocal of serum dilution.

Table (3): The average mean titre of neutralizing antibodies against FP in groups of vaccinated chickens

Chicken Groups	Weeks Post Vaccination							
	1	2	3	4	5	6	7	8
1	1.2*	3.3	6.9	9.6	8.4	6.0	3.3	2.4
2	2.4	6.0	8.4	11.1	9.6	5.7	4.8	2.4
3	1.2	2.1	6.6	8.4	6.6	6.0	2.4	1.2
5	0	0	0	0	0	0	0	0

Group (1): Chickens vaccinated with locally prepared combined live AE and FP.

Group (2): Chickens vaccinated with imported combined live AE and FP.

Group (4): Chickens vaccinated with locally prepared FP vaccine.

Group (5): Unvaccinated control chickens.

* Serum neutralizing antibody titre.

Table (4): Results of challenge test of chickens against AE at 4th week post vaccination

Vaccinated groups	No. of birds group	No. of birds with symptoms of AE*	Protection percent
1	20	1	95%
2	20	1	95%
3	20	2	90%
5	20	20	0%

Birds shown symptoms of AE (Ataxia, paralysis, walking on hock joint, tremor, incoordination of movement and blindness) or death.

Group (1): Chickens vaccinated with locally prepared combined live AE and FP.

Group (2): Chickens vaccinated with imported combined live AE and FP.

Group (3): Chickens vaccinated with locally prepared AE vaccine.

Group (5): Unvaccinated control chickens.

N.B. The birds were inoculated intracerebrally.

Table (5): Results of challenge test in chickens vaccinated with different fowl pox vaccines

Vaccinated groups	No. of birds group	No. of birds with symptoms of pox*	Protection percent
1	20	1	95%
2	20	1	90%
4	20	2	90%
5	20	20	Zero

* Birds shown symptoms of fowl pox (Takes at sites of inoculation 7-10 days post challenge with generalization in some birds in form of nodular lesions on the eye lids and around the peak "Classical form of fowl pox").

Group (1): Chickens vaccinated with locally prepared combined live AE and FP.

Group (2): Chickens vaccinated with imported combined live AE and FP.

Group (4): Chickens vaccinated with locally prepared fowl pox vaccine.

Group (5): Unvaccinated control chickens.

N.B. The birds were inoculated by wing web.

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