Trials for preparation and evaluation of oil inactivated vaccine against Newcastle disease, infectious bronchitis, egg drop syndrome and swollen head syndrome

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Abstract

Inactivated viruses of Newcastle disease (ND), infectious bronchitis (IB), egg drop syndrome (EDS) and swollen head syndrome (SHS) were incorporated into water in oil emulsion vaccine (monovalent) or as in combined (Tetravalent vaccine). Immunological response of the new tetravalent vaccine was evaluated by injecting groups of susceptible chickens with either tetravalent or monovalent vaccines. Humoral immune response was evaluated by direct haemagglutination inhibition test for NDV and EDS virus and enzyme linked immunosorbent assay (ELISA) for SHS and IBV. Challenge test was carried out against NDV using velogenic viscerotropic Newcastle disease virus (VVNDV). The results proved that the humoral immunity of the new tetravalent vaccine were never inferior to those obtained with the monovalent ones and also there is no practical differences in the percentage of the protective immunity induced with NDV. The prepared tetravalent (NDV, IBV, EDS and SHS) inactivated vaccine was immunogenic safe and produced satisfactory protection.

Introduction

Viral disease of poultry constitute one of the most major problems facing the rapidly expanding poultry industry in Egypt causing considerable economic losses due to serious mortality associated with different infectious viruses. Newcastle and infectious bronchitis diseases are among the highly contagious diseases of the respiratory tract of chickens (**11**). Mass vaccination against both diseases has become necessary especially in high density of the poultry population to minimize economic losses.

EDS-76 is a disease of laying hens characterized by sudden and frequently large drop in egg production, with the laying of soft-shelled eggs (**12**).

On the other hand, distinct novel upper respiratory tract disease firstly reported in the late 1970, in South Africa (**5**). It has implicated as factor in swollen head syndrome of chicken SHS (**16**).

Swollen head syndrome (SHS) is caused by avian pneumovirus (APV) infection, which is characterized by swelling of the periorbital and infraorbital sinuses, torticollis, cerebral disorientation and spisthotonos egg production and quality of eggs are affected.

The more severe form of associated disease probably results from dual or secondary infection. The characteristic "Swollen head" appears as result of infection with secondary adventitious bacteria usually Escherichia coli. In Egypt, the existence of infection with pneumovirus was reported by (**1**).

The effective prevention of the disease is still based upon live and inactivated vaccines. The use of oil emulsion inactivated vaccine induced a satisfactory immunity as the vaccination resulted in high level of antibodies in addition to the freedom of vaccinated birds from disease and drop in egg production (**4**). So, the objective of this study was to prepare and evaluate the immune response of tetravalent vaccine of NDV, IBV, EDS and SHS in single and combined form for protection against diseases caused by these agents.

Material and Methods

Seed Viruses:

1. Newcastle disease seed virus:

LaSota strain (supplied by the Central VeterinaryLaboratory, Weybridge, England).

2. Infectious bronchitis disease seed virus:

Strain H120 was obtained as allantoic fluid from Department of Animal Science and Agricultural Biochemistry, University of Delmare, New York, USA.

3. Egg drop syndrome disease seed virus:

EDS-76 live virus product code PA0081 handled by Prof. Dr. Nadia Mohamed Hassan, from Weybridge, England.

4. Swollen head syndrome (SHS) seed virus:

Freeze-dried live vaccine against swollen head syndrome in chickens was supplied by Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo.

Virulent strain:

A velogenic viscerotropic Newcastle disease virus local strain identified by (2) was used for challenge (10^6 EID_{50}/ml).

Embryonated chicken eggs:

Embryonated duck eggs were obtained from United Company for Poultry Production. It was used for propagation of the virus, testing of complete inactivation in the prepared batch of EDS virus vaccine.

Six to nine days old embryonated SPF eggs (SPAFAS) were obtained from Koum Osheim SPF garm, Fayoum, Agriculture Research Center, were used for propagation and testing of complete inactivation in the prepared batches of ND, IBV, (9 days old ECE) and swollen head syndrome (6 days old ECE) vaccines.

Virus Propagation:

1. NDV propagation:

The method of (2) was conducted. Obtained virus was titrated according to the standard methods described in (10). The titre was 10^{11} EID_{50}/ml.

2. IBV propagation:

Propagation and titration was carried out after the method described by (8). The titre of the virus was 10^7 EID_{50}/ml.

3. EDS propagation:

Virus propagation in embryonated duck eggs was applied according to (2). The titre of the virus was 10^{10} EID_{50}/ml.

4. Swollen head syndrome (SHS) virus propagation:

It was performed as reported by (2). The titre was $10^{7.83}$ EID_{50}/ml.

Inactivation of the viruses:

Inactivation of virus suspension of ND, IBV was carried out using formalin in a final concentration of 0.1 % for 18 hours. While, EDS virus

was inactivated by 0.1 % formalin for 48 hours according to (19). SHS virus was inactivated by 0.5 % formalin concentration for 5 hours according to (20).

Three successive blind passages were done. The complete inactivation was achieved when there are no any mortalities or lesions in inoculated eggs.

Preparation of oil emulsion vaccines:

a. Monovalent vaccine:

The vaccine was prepared as described by (23).

b. Tetravalent vaccine:

The tetravalent vaccine was prepared by mixing equal volume from each viruses ND, IB, EDS and SHS (aqueous phase) to oil phase (paraffin oil and span) by ratio 1:3. The doses of the antigens were adjusted to be contained at least 10^{10} EID_{50} from NDV, 10^{6} EID_{50} from IBV, 10^{10} EID_{50} from EDS and 10^{7} EID_{50} from SHS.

Quality control of the prepared vaccines:

Purity and sterility tests:

The prepared vaccines were tested to be free from contaminants according to (24).

Physical characterization:

The prepared vaccines were subjected to drop test (18), emulsion viscosity (3) and emulsion stability (6).

Experimental design:

Three hundred, one day old, Hubbard chicks were reared in isolated conditions. Their sera were free from antibodies against NDV, IBV, EDS and SHS viruses.

The chicks were divided into six equal groups (50 bird of each) as follows:

Group (I): It was vaccinated with monovalent oil emulsion NDV vaccine.

Group (II): It was vaccinated with monovalent oil emulsion IBV vaccine.

Group (III): It was vaccinated with monovalent oil emulsion EDS vaccine.

Group (IV): It was vaccinated with monovalent oil emulsion SHS vaccine.

Group (V): It was vaccinated with tetravalent oil emulsion vaccine (NDV+IBV+EDS+SHS).

Group (VI): It was kept as unvaccinated negative control.

Each chick received 0.5 ml of its appropriate vaccine I/M at 21 days of age. Ten random blood samples were collected weekly from each group and stored at -20°C until used for detection of corresponding antibodies against ND, IB, EDS and SHS viruses.

Serological tests for viruses:

a. Haemagglutination Inhibition (HI) test:

It was used for estimating the haemagglutinating antibodies against ND virus and EDS virus. It was done according to **(15)**. The beta-procedure of HI test was employed for antibody assay in case of adenovirus strain 127 using micro-method of **(22)** using virus suspension containing 4 HA units.

b. Enzyme linked immunosorbent assay (ELISA):

The IDEXX ELISA kits (flock cheek system) were used to determine the level of serum antibodies against IBV and SHS virus.

c. Bio-assay test (Challenge test):

Challenge test was applied for group vaccinated with NDV. Challenge dose 0.5 ml I/M from virus with 10^6 EID_{50}/ml.

Results and Discussion

Recently, great attention is directed toward the poultry industry in Egypt to meet the increasing demand of animal protein. The poultry industry has expanded and integrated in the last years. Thus, poultry are considered without any doubt, the most appropriate source of protein supply of high nutritive value for human beings. This is due to efficient cost of production and its short life cycle.

Testing the prepared emulsion by drop, viscosity and emulsion stability test for vaccine characterization agreed with those obtained by **(7) and (18)** in which the prepared vaccine had the criteria of ideal oil emulsion vaccine with low viscosity and stable emulsion.

Purity and sterility for the prepared vaccine were also carried out which revealed that no microbial contaminants (fungi, bacteria and mycoplasma).

Immune response to NDV measured by HI test in vaccinated chicken either the monovalent or tetravalent vaccines are shown in table (1). The HI antibody titres were gradually increased from 3rd week until 10th week post vaccination in both groups I and V and there was no significant differences between them (Table 1). These results come in agreement with **(13)** who mentioned that there were no significant differences between groups received tetravalent or monovalent vaccine.

Regarding the results of EDS in table (4) showed the HI antibody titre of group III and group V run in parallel line. Similar results were obtained by **(25)** and **(14)** who decided that no antigenic interference between the vaccinal antigens contained the polyvalent vaccine.

Dealing with the result of ELISA antibody titre against IBV in table (2) showed that no noticeable difference was detected between groups vaccinated with monovalent IBV vaccine and that vaccinated with polyvalent vaccine, where the titres gradually increased till reached ts maximum value at 7th week post vaccination 3816 and 3890 for group II and V respectively. These results come in agreement with **(17)** who mentioned that groups of chickens were inoculated with oil inactivated vaccines either bivalent (NDV and IBV) or trivalent (NDV, IBV and EDS) showed slight increase in immunogenicity than with single vaccines.

Results of ELISA for group IV vaccinated with SHS and group V in Table (3) revealed that the antibody titres recorded that the titre values increased gradually and reached its peak at the 7th week 6681 and 6016 for group IV and V, respectively. These results were in accordance with **(9)** who stated that ELISA used for measuring SHS antibodies and is considered the most common reliable and practical assay.

Regarding to bioassay test in table (5) revealed that the immunity of chickens vaccinated with monovalent or tetravalent vaccines gave good protection percent ranged from 35-100 % while no protective percent in the non-vaccinated groups.

As regards to the prepared tetravalent inactivated oil emulsion vaccine (NDV+IBV+SHS+EDS), it was found that such vaccine could elicit the production of satisfactory antibody titres against the viruses used and there were no excreted virus where contact birds remain healthy. There is no

interference mutant enhancement or competition between viruses. So, it could be concluded that the prepared tetravalent inactivated oil emulsion NDV+IBV+EDS+SHS vaccine is produced sufficient protective immunity against these diseases and can be used as safe and potent vaccine.

References

1. Ahmed, A.A.S. (1991): Newly emerged diseases of chickens in Egypt. Aerosols, No. 4, Sept. 1991.

2. Allan, W.H.; Lancaster, J.E. and Toth (1973): The production and use of Newcastle disease vaccine. Food and Agriculture Organization, P. 53 Rome, Italy, 1115 pp.

3. Becher, P. (1965): Theory of emulsion stability in: Becher, P. (Ed.) Emulsions. Theory and Practice. 2nd Ed.

4. Box, P.G. and Furminger, I.G.S. (1975): Newcastle disease antibody level in chickens after vaccination with oil emulsion adjuvant killed vaccine. Vet. Rec., 95: 108-111.

5. Buys, S.B. and Dupreeez, J.H. (1980): Swollen head syndrome in chickens. Preliminary report on the isolation of possible aetiological agents. J. South Africa Vet. Assoc., 60: 221-222.

6. Cessi, D. and Nordelli, L. (1973): Requirement for testing oil emulsion inactivated Newcastle disease vaccine. Proc. 42nd Symp. On Requirement for Poultry Virus Vaccines, Lyon, p. 325-328.

7. Cunliffe, H.R. and Graves, J.H. (1963): Formalin treated FMDV. Comparison of two adjuvants in cattle. J. Comp. Med. Vet. Sci., 27: 193-197.

8. Cunningham, C.H. (1973): A laboratory guide in virology. 7th Ed. Burgess Publishing Co. Minneapolis, Minnesota.

9. Eterradossi, N.; Toquin, D.; Guittat, M. and Bennejean, G. (1995): Evaluation of different turkey rhinotracheitis virus used as antigens for serological testing following live vaccination and challenge. J. Vet. Med., B, 42: 175-186.

10. FAO, Animal Production and Health (1978): Newcastle disease vaccine. Their production and use. Series No. 10.

11. Hofstad, M.S. (1984): Diseases of Poultry. 8th Ed. Avian Infectious Bronchitis. Pp. 429-443. Iowa State Univ., USA.

12. Holmes, H.C.; Webb, K.J. and Box, P.G. (1989): Vaccine for the control of egg drop syndrome (1976). Vet. Rec., 124: 309-310.

13. Kozalina, B.; Knezevic, N.; Nikolovski, J. and Matovic, V. (1990): Immunoprophylaxis of poultry with inactivated vaccine. 1. Parallel studyof immunogenicity of polyvirol-3 and appropriate inactivated monovalent vaccines. Acta veterinaria Beograd, 40 (2-3): 129-135.

14. Madkour, M.S.; Abdel Wanis, N.A.; Abo-Zaid, A.A. and Abdel Ghany, M.E. (1999): Trials for preparation of trivalent inactivated vaccine against Newcastle, infectious bronchitis and infectious bursal diseases. The 3rd Sci. Conf. Vet. Med. Res., Alexandria University, October, 12-14.

15. Majujabe, K.A. and Hitchner, S.B. (1977): Antibody response to strain combination of Newcastle disease virus as measured by haemagglutination inhibition test. Avian Dis., 21: 576-584.

16. Morley, A.J. and Thomson, D.K. (1984): Swollen head syndrome in broiler chickens. Avian Dis., 28: 238-243.

17. Nedelicu, D. and Sofei, D.M. (1990): Mixed inactivated vaccines in oil for the control of vira. diseases in fowls. Archiva Veterinaria (Bucuresti), 19: 113-120.

18. Roshdy, O.H. (1996): Studies on inactivated oil FMD vaccine. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.

19. Rozhdestvenskii, I.K. (1984): Inactivating the aviadenovirus of EDS (strain EDS-76). Veterinariya, Moscow, USSR, No. 4, 61-62.

20. Salah Shabaan Abdel Rahman (2003): Preparation and evaluation inactivated vaccines against turkey rhinotracheitis virus. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.

21. Sheble, A. and Reda, M.I. (1976): Cited by Khafagy, A.K. (1972). Thesis M.V.Sc., Fac. Vet. Med., Cairo Univ.

22. Takatsy, G.Y. (1956): The use of the spiral loops in serological and virological micro-methods. Acta Microbiol. Hung., 3: 197.

23. Thayer, C.S.; Edison, and Kleven, S.H. (1983): Multivalent inactivated virus oil emulsion vaccines in broiler breeder chickens. Newcastle disease virus and infectious bursal disease virus bivalent vaccines. Poult. Sci., 62: 1978-1983.

24. United States Code of Federal Regulations (1987): Animal and Animal Products, 1987. Published by the Office of the Federal Register National Archives and Records Administrations.

25. Wu Yan Gong; Zheng Ming Qui; Cai Baoxiang and Chen Pu Yan (1994): Evaluation of trivalent oil emulsion vaccine against Newcastle disease, infectious bronchitis and egg drop syndrome (immune response of laying hens vaccinated with trivalent oil emulsion vaccine to ND, IB and EDS-76). Anim. Husb. Vet. Med. China, 26 (5): 206-209.

Tables

Table (1): Mean value of ND HI antibody titre in groups of vaccinated chickens using haemagglutination inhibition test (HI) test

Groups	Weeks Post vaccination									
	1	2	3	4	5	6	7	8	9	10
I	0	2.7	4.0	6.25	8.3	7.8	7.0	6.2	6.3	5.7
V	0	3.1	3.9	7.0	7.8	7.4	7.0	6.33	5.5	5.0
VI	0	0	0	0	0	0	0	0	0	0

Group (I): Vaccinated with monovalent oil emulsion NDV vaccine.
Group (V): Vaccinated with tetravalent oil emulsion vaccine (NDV+IBV+EDS+SHS).
Group (VI): Unvaccinated negative control.

Table (2): Geometeric mean ELISA antibody titre against IBV in groups of vaccinated chickens

Group no.	ELISA antibody titre									
	Weeks Post vaccination									
	1	2	3	4	5	6	7	8	9	10
II	1162	1976	2134	2596	2983	3661	3816	3661	3543	2412
V	1006	1988	2015	2630	2989	3695	3890	3766	3661	2956
VI	0.004	0.002	0.0134	0.004	0.017	0.0134	0.0153	0.004	0.004	0.0134

Group (I): Vaccinated with monovalent oil emulsion NDV vaccine.
Group (V): Vaccinated with tetravalent oil emulsion vaccine (NDV+IBV+EDS+SHS).
Group (VI): Unvaccinated negative control.

Table (3): ELISA antibody titre against SHS in groups of vaccinated chickens

Groups	ELISA antibody titre									
	Weeks Post vaccination									
	1	2	3	4	5	6	7	8	9	10
IV	1622	1846	2050	3118	3447	5132	6681	6175	5321	4210
V	1251	1648	2150	3416	3415	5121	6016	6017	5616	4612
VI	0.004	0.002	0.0134	0.004	0.017	0.0134	0.0153	0.004	0.004	0.0134

Group (IV): Vaccinated with monovalent oil emulsion SHS vaccine.
Group (V): Vaccinated with tetravalent oil emulsion vaccine (NDV+IBV+EDS+SHS).
Group (VI): Unvaccinated negative control.

Table (4): EDS HI antibody titre in groups of vaccinated chickens using haemagglutination inhibition test (HI) test

Groups	Weeks Post vaccination									
	1	2	3	4	5	6	7	8	9	10
III	2	3.0	4.6	5.5	6.8	8.0	7.5	9.0	10	8.3
V	2	3.0	4.5	6.0	6.5	8.0	7.3	9.0	10	8.4
VI	0	0	0	0	0	0	0	0	0	0

Group (III): Vaccinated with monovalent oil emulsion EDS vaccine.
Group (V): Vaccinated with tetravalent oil emulsion vaccine (NDV+IBV+EDS+SHS).
Group (VI): Unvaccinated negative control.

Table (5): Results of challenge test of chickens against NDV at 3rd and 6th week post vaccination

Groups (Weeks Post Vaccination)	Number of birds/group	Number of deaths in challenged birds	Protection percent (%)
Group (I) 3rd WPV *	20	0/20	100
Group (V) 3rd WPV	20	1/20	95
Group (I) 6th WPV	20	0/20	100
Group (V) 6th WPV **	20	0/20	100
Control Group	20	20/20	Zero

Group (I): Vaccinated with monovalent inactivated oil emulsion NDV vaccine.
Group (V): Vaccinated with tetravalent inactivated oil emulsion (NDV+IBV+EDS+SHS).
Group (VI): Unvaccinated control.
* Challenge 3rd week post vaccination.
** Challenge 6th week post vaccination.
Challenge dose 10^6 EID_{50}/ml/bird

محاولات لتحضير و تقيم لقاح زيتى مثبط ضد امراض النيوكاسل ، الاتهاب الشعبى المعدى ، ظاهرة تدنى البيض فى الدجاج و ظاهرة تورم الراس فى الدجاج .

هاله محمد المكاكى، لمياء محمد عمر، امل احمد سيد، عفاف حمدى امين
معهد بحوث الامصال و اللقاحات البيطريه / عباسية ، المعمل المركزى للرقابة .

تم تخليط فيروسات امراض النيوكاسل ، الالتهاب الشعبى المعدى ، ظاهرة تدنى البيض و ظاهرة تورم الراس فى الدجاج لقاح رباعى زيتى و تحضير لقاح احادى زيتى لكل فيروس على حدا و قد تم تقييم الاستجابة المناعية للقاح الرباعى المحضر و كذا للقاحات الاحادية و ذلك من خلال حقن مجموعات من الطيور القابله للاصابة . تم تقييم المناعة الخلطه باجراء اختيار التلازن الدموى عير المباشر لفيروسى النيوكاسل و ظاهرة تدنى البيض و اجراء اختبار الايزا لكل من الاتهاب الشعبى المعدى و ظاهرة تورم الراس فى الدجاج . تم عمل اختبار التحدى للمجموعات المحقونه بفيروس النيوكاسل . و قد اوضحت النتائج ان اللقاح الرباعى لا يعطى استجابة مناعية اعلى من اللقاحات الاحادية كلا على حدا ، كما اوضحت النتائج ان خلط كلا من هذة الفيروسات فى لقاح واحد لم يؤثر على الاستجابة المناعية و الحماية .
و بناء عليه يوصى باستخدام اللقاح المركب حيث انه امن و ذو كفاءه عاليه .