STUDIES FOR PREPARATION OF A TRIVALENT INACTIVATED VACCINE AGAINST NEWCASTLE, GUMBORO AND EGG DROP SYNDROME

By

Abo-Zaid A. Abo-Zaid; Salwa A. El-Assily; Hoda I. Tawfik and Ensaf Khashabah

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

ABSTRACT

Monovalent inactivated oil vaccines against Newcastle disease (ND), infectious bursal disease (IBD) and egg drop syndrome (EDS) in addition to a trivalent one were prepared and subjected to the quality control tests. Each vaccine was inoculated in one of four chicken groups while a fifth chicken group was kept as non-vaccinated control. The humoral immunity was weekly detected and evaluated up to 24 weeks post vaccination in all groups. The results of haemagglutination inhibition (HI) test and serum neutralization test (SNT) revealed that all the used vaccines induced good levels of humoral immunity. Comparing results of monovalent and trivalent use of vaccine indicated that there was no interference between the used viruses in chicken immune response to each virus. So, we recommended use of trivalent vaccine to save time, cost and effort of vaccination as well as avoid repeated vaccination as a stress factor.

INTRODUCTION

Infectious bursal disease (IBD), Newcastle disease (ND) and egg drop syndrome (EDS) are the most important viral diseases which affected economically poultry industry in many countries including Egypt, where NDV causes high mortality rates, reduction of meat and egg production (**Biswal and Morril, 1954**). IBD is an important viral disease of poultry because it ended with prolonged immuno-suppression in infected chickens, which resulted in increased chicken susceptibility to other infections and interfered with the effective vaccination against other diseases (**Okoye, 1984**). EDS causes dropping in egg production in the laying fowl, usually associated with poor egg-shell quality and colour (**McFerran *et al.*, 1978**).

The main aim in poultry industry is the protection of chickens against infectious diseases. Such protection is depending mainly on vaccination using

specific potent vaccines. A large number of poultry vaccines had been developed either in an attenuated or inactivated form, singly or combined (**Allan *et al.*, 1973; Mouaz, 1986; Afaf, 1990; Madkour, 1995; Hala, 1996; Afaf *et al.*, 1999; Khodeir and Amina, 1999; Susan *et al.*, 1999 and Afaf *et al.*, 2000**).

The combined vaccines have the advantages of providing protection against more than one disease at the same time reduce both vaccination expensive and number of vaccination per farm as well as saving time and labour costs. Besides that, combined vaccines reduces the stress reactions.

So, the aim of this work is the preparation of a trivalent inactivated vaccine to protect chicken against the 3 diseases at one shot.

MATERIAL AND METHODS

1. Virus strains:

1.1. Newcastle disease virus (NDV):

NDV LaSota strain was supplied by the Central Veterinary Laboratory, Weybridge, England.

1.2. Infectious bursal disease virus (IBDV):

Bursa Vac M strain of IBD virus as an attenuated strain was kindly supplied by the College of Agricultural Science. Delwar University, USA,

1.3. Egg drop syndrome (EDS) virus:

EDS-76 virus strain, Code PA0081 Weybridge, England.

2. Embryonated eggs:

A commercial embryonated duck eggs and chicken eggs were obtained from United Company for Poultry Production. The embryonated duck eggs were used for propagation, testing of complete inactivation and preparation of batch of EDS inactivated vaccine. The embryonated chicken eggs were used for preparation of NDV and IBD viruses.

3. Cell cultures:

African green monkey kidney cells (Vero) established by **Yasumura and Kawatika, (1963)** were used in SNT to estimate IBD neutralizing antibodies in the sera of vaccinated chickens.

Chicken embryo rough cells (CER) established by **Smith *et al.*, (1977)** were used for detection of EDS neutralizing antibody titres in sera of vaccinated chickens using SNT.

4. Experimental chicks:

One hundred and fifty, one day old mixed sex commercial Hubbard chicks were supplied by the United Company for Poultry Production. The

chicks were reared under complete hygienic measures in isolated and disinfected wire floored cages and fed commercial broiler ration.

5. Virus propagation:

5.1. NDV was propagated in embryonated chicken eggs according to **Allan *et al.*, (1973)**. The obtained virus was titrated according to the standard methods described in **FAO Publication (1978)**. It had a titre of 10^{11} EID_{50}/ml.

5.2. IBDV was propagated after the method of **Hitchner, (1970)**. The obtained virus was titrated in embryonated chicken eggs via allantoic sac inoculation and the titre was expressed and calculated according to method of **Reed and Muench, (1938)** and reported to be $10^{8.5}$ EID_{50}/ml.

5.3. EDSV was propagated according to **Allan *et al.*, (1973)** and titrated in embryonated duck eggs. The titre was expressed and calculated according to the method of **Reed and Muench, (1938)** and found to be 10^{7} EID_{50}/ml.

6. Inactivation of viruses:

Inactivation of the used viruses were separately carried out using formalin in a final concentration of 0.1% of the total volume for both NDV (**Mohab, 1992**) and EDS (**Rozhdestvenskii, 1984**) and 0.2% for IBDV (**Li *et al.*, 1986**). The fluids were left on a magnetic stirrer at room temperature for 18, 24 and 48 hours for ND, IBD and EDS, respectively.

Judgement for inactivation was carried out by inoculation of samples from treated viruses in embryonated chicken eggs (0.2ml/egg) for NDV and IBDV; while EDS was tested in embryonated duck eggs to be sure that the required complete inactivation occurred.

7. Vaccine preparations:

The monovalent oil vaccines against each virus as well as the trivalent inactivated oil vaccine were prepared according to **Stone *et al.*, (1978)** with aqueous to oil ratio 1:3. The prepared vaccines were adjusted to contain 10^{9}, $10^{6.5}$ and 10^{7} EID_{50} for ND, IBD and EDS / dose, respectively.

8. Quality Control:

The prepared vaccines were subjected for quality control measures as described by **Stone *et al.*, (1979)**.

9. Serological tests:

9.1. Haemagglutination Inhibition test (HI):

It was used for estimating the haemagglutinating inhibiting antibodies against ND and EDS viruses according to **Majujabe and Hitchner, (1977)**.

9.2. Serum neutralization test (SNT):

It was used for estimating the neutralizing antibodies against both IBDV and EDS. After methods of **Weisman and Hitchner, (1978).**

* IBDV and EDSV neutralizing antibody titre = the reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of the virus.

10. Experimental design:

One hundred and fifty, one-day-old chicks were reared till they became 4 months old. The chicks were divided into 5 groups (30 chicks/each group).

Group (1): Vaccinated with the prepared trivalent inactivated oil vaccine.

Group (2): Vaccinated with the locally prepared inactivated monovalent oil ND vaccine.

Group (3): Vaccinated with the locally prepared inactivated monovalent oil IBD vaccine.

Group (4): Vaccinated with the locally prepared inactivated monovalent oil EDS vaccine.

Group (5): Non-vaccinated controls.

Each chicken in the vaccinated groups was I/M injected by 0.5 ml from the prepared vaccines according to its group.

Ten random blood samples were collected weekly from each group for 24 weeks post vaccination. Sera were separated, collected and stored at 20°C until used for detection of corresponding antibodies against ND, IBD and EDS.

RESULTS AND DISCUSSION

In the present study, an inactivated trivalent vaccine was prepared from standard viral strains and subjected to the standard quality control tests. This vaccine was found to be free from foreign contaminants (bacteria, fungi and mycoplasma), safe and confirming that its quality measures agree with those of the recommended conditions (**Davidson, 1975 and Anon, 1994**).

The results of HI test revealed that there is no great difference in the obtained titre levels against ND and EDS in vaccinated groups either use of monovalent or in trivalent vaccines, where all chickens showed high levels of specific HI ND and EDS antibodies (Tables 1 and 2) (Fig. 1 and 2).

Generally, in comparing results of HI-test obtained from testing chicken sera vaccinated with monovalent and trivalent vaccines; in both ND and EDS; it is clearly observed that log_2 titres in trivalent vaccinated groups started somewhat higher from the 1st week in both ND and EDS groups. Furthermore, these higher titres appear again in the week 24 in ND while the EDS vaccinated group continued higher from the 1st week till the last samples at 24 weeks.

The formed antibodies were still at high levels up to 24 weeks post vaccination. These findings came in agreement with those of **Holmes *et al.*,**

(1989) who reported that there was no significant difference in EDS, IBD and ND antibody titres on using of monovalent or trivalent vaccine against the three diseases. Also, **Kozlino *et al.*, (1990)** obtained similar results up to 30 weeks post vaccination with a polyvalent vaccine. Many findings were found to be in agreement with our obtained results (**Madkour, 1995; Hala, 1996; Afaf *et al.*, 1999 and Susan *et al.*, 1999**).

Tables (3 and 4), Fig. (3 and 4) showed that vaccinated chicken with the prepared trivalent vaccine, exhibited good titres of neutralizing antibodies against IBD and EDS extending at high levels up to 24 weeks post vaccination. There was no difference in chicken groups vaccinated with the monovalent and trivalent vaccines showing that there was no antagonizing effect of the 3 antigens on the humoral immune response of vaccinated birds to each one. These results were found to be confirmed by those obtained previously by **Nedelciu and Sofei (1990); Uslangolu (1990); Bidin *et al.*, (1998); Afaf *et al.*, (1999); Khodeir and Amina (1999) and Afaf *et al.*, (2000)**.

So, it could be concluded that the prepared inactivated trivalent vaccine is safe, potent and induce high measured humoral antibody titres for a period of 24 weeks in vaccinated chickens against ND, IBD and EDS at the same time. Also, from the obtained results and from the comparison between titres induced by monovalent and trivalent vaccine we can recommended the use of trivalent one to save time effort, cost and reduce vaccination as stress factor on chickens.

REFERENCES

Afaf, H.A. (1990): Studies on vaccines and vaccination against Gumboro disease. Ph.D. Thesis, Poult. Dis., Fac. Vet. Med., Cairo Univ.

Afaf, H.A.; Khodeir, M.H. and Abou El-Kheir, M. A. (1999): Studies to prepare an inactivated vaccine against Gumboro and Reoviruses. J. Vet. Med. Res, Fac. Vet. Med., Mansoura Univ., 1 (1): 29-43.

Afaf, H.A.; Khodeir, M.H. and Mervat, A. El-Koffy (2000): Trials for preparation of a combined vaccine against Newcastle and Gumboro diseases. Suez Canal Vet. Med. J., Vol. III (2): 453-464.

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, 1115pp.

Anon (1994): Quality control of rinderpest cell culture vaccine. Standard operating procedures. FAO, Report No. (118): 1994.

Bidin, Z.; Cajavac, S.; Sladic, D.; Ergotic, N.; Clzeli, A. and Pokric, B. (1998): Protection of broiler breeders by an inactivated combined water in oil in water viral vaccine. Acta Vet. Ltung, 46 (1): 25-34.

Biswal, G. and Morril, C.C. (1954): Newcastle disease virus. Poult. Sci., 33: 880-897.

Davidson, I. (1975): Testing Veterinary Vaccines. Vet. Rec., 97: 389-392.

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

Hala, M.E. (1996): Trial for preparation of bivalent inactivated Newcastle and infectious bursal disease vaccine. M.V.Sc. Thesis, Diseases of Poultry and Rabbits, Fac. Vet. Med., Cairo Univ.

Hitchner, S. B. (1970): In Diseases of Poultry. Edited by Hofstad, M.S. 7th Edition.

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

Khodeir, M.H. and Amina, A. El-Bayomy (1999): Preparation and evaluation of a combined vaccine against fowl cholera and egg drop syndrome. Egypt. Vet. Med. Assoc., 8 (A), 26 Guli Street, Cairo, Egypt.

Kozlina, B.; Knezevic, N.; Nikoloski, J. and Matovic, V. (1990): Immunoprophylaxis of poultry with inactivated vaccine. I. parallel study of immunogenicity of polyvirol-3 and appropriate inactivated monovalent vaccines. Acta Veterinaria Beograd, 40 (2-3): 129-135.

Li, H.Q.; Zhou, J.; Liu, F.Z. and Chen, L.Y. (1986): Inactivated oil adjuvant vaccine for infectious bursal disease in chickens. Chinese J. Vet. Med., 12 (5): 50-53 (Ch) Inst. Anim. Vet.

Madkour, M. S. (1995): Some trials for preparation of bivalent inactivated fowl cholera and Newcastle disease virus vaccine. Ph.D. Thesis, Microbiology, Fac. Vet. Med., Cairo Univ.

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.

McFerran, J.B.; McCracken, R.M.; McKillop, E. R.; McNula, M. S. and Collins, D.S. (1978): Studies on a depressed egg production syndrome in Northern Ireland. Avian Pathol., 7: 35-47.

Mohab, H. A. (1992): Studies of the development of Newcastle disease killed vaccine. M.V.Sc. Thesis, Microbiology, Fac. Vet. Med., Cairo Univ.

Mouaz, M. A. (1986): Studies on the production and use of tissue culture Newcastle virus vaccines. Ph.D. Thesis, Microbiology, Fac. Vet. Med., Cairo Univ.

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

Okoye, J.O.A. (1984): Infectious bursal disease of chickens. Vet. Bull., Wyebridge, 54: 425-436.

Reed, L.T. and Muench, H. (1938): Simple method of estimating 50 percent end point. Amer. J. Hyg., 27: 793-799.

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

Smith, A. L.; Tignor, G.H.; Mifune, K. and Motahasi, T. (1977): Isolation and assay of rabies serogroup viruses in CER cells. Inter. Virol., 8: 92-99.

Stone, D.; Brugh, M.; Hopkins, S. R.; Yoder, H.W. and Bread, C.W. (1978): Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. Avian Dis., 22: 666-674.

Stone, D.; Brugh, M.; Erickson, G. and Beard, C.W. (1979): Evaluation of inactivation of inactivated newcastle disease oil emulsion vaccines. Avian Dis., 24 (1).

Susan, S. A.; Al-Safty, M. M.; Abd El-Baky, M.H. and Elham A. El-Ebiary (1999): Preliminary study on preparation and evaluation of tissue culture combined inactivated vaccine against Newcastle disease, infectious bursal disease and viral arthritis virus. J. Egypt. Vet. Med. Ass., 59 (2/3): 1041-1054.

Uslangolu, B. (1990): Comparison of the antibodies titres obtained by different serological methods (HI, gel precipitation, ELISA) in hens vaccinated against egg drop syndrome 1976 (EDS-76). Doga Turk Veterinerlik Ve Hayvancilik Dergisi, 14 (1): 112-125.

Weisman, I. and Hitchner, S. B. (1978): Virus neutralization, virus agar gel precipitation test for detecting serological response to infectious bursal disease virus. Avian Dis., 22 (4): 598-603.

Yasumara, Y. and Kawatika, Y. (1963): Studies on SV40 virus in tissue cultures. Nihn Rinsho, 21: 1201-1215.

Table (1): Newcastle disease HI antibody titres in vaccinated chicks.

Chicken Groups	ND HI antibody titres (log_2) / Weeks Post Vaccination								
	1	2	3	4	8	12	16	20	24
1	16	64	128	256	1024	256	256	256	256
2	8	16	128	128	1024	256	256	256	128
5	0	0	0	0	0	0	0	0	0

Group (1): Vaccinated with locally prepared trivalent vaccine.
Group (2): Vaccinated with locally prepared monovalent ND vaccine.
Group (5): Control non-vaccinated.

Table (2): Egg drop syndrome HI antibody titres in vaccinated chickens

Chicken Groups	EDS HI antibody titres (log_2) / Weeks Post Vaccination								
	1	2	3	4	8	12	16	20	24
1	8	8	32	128	256	128	128	128	128
4	2	8	64	128	128	64	64	64	32
5	0	0	0	0	0	0	0	0	0

Group (1): Vaccinated with locally prepared trivalent vaccine.
Group (4): Vaccinated with locally prepared monovalent EDS vaccine.
Group (5): Control non-vaccinated.

Table (3): Infectious bursal disease neutralizing antibody titres in vaccinated chickens.

Chicken Groups	IBDV SN antibody titres / Weeks Post Vaccination								
	1	2	3	4	8	12	16	20	24
1	2	8	32	64	128	256	128	64	64
3	2	8	16	128	256	256	128	128	64
5	0	0	0	0	0	0	0	0	0

Group (1): Vaccinated with locally prepared trivalent vaccine.
Group (3): Vaccinated with locally prepared monovalent IBDV vaccine.
Group (5): Control non-vaccinated.

Table (4): EDSV neutralizing antibody titres in vaccinated chickens

Chicken Groups	EDSV SN antibody titres / Weeks Post Vaccination								
	1	2	3	4	8	12	16	20	24
1	2	8	32	64	128	128	128	64	32
4	3	6.5	32	64	128	128	128	64	32
5	0	0	0	0	0	0	0	0	0

Group (1): Vaccinated with locally prepared trivalent vaccine.
Group (4): Vaccinated with locally prepared monovalent EDS vaccine.
Group (5): Control non-vaccinated.

Fig. (1): Levels of Newcastle disease HI antibody titres in vaccinated chicks

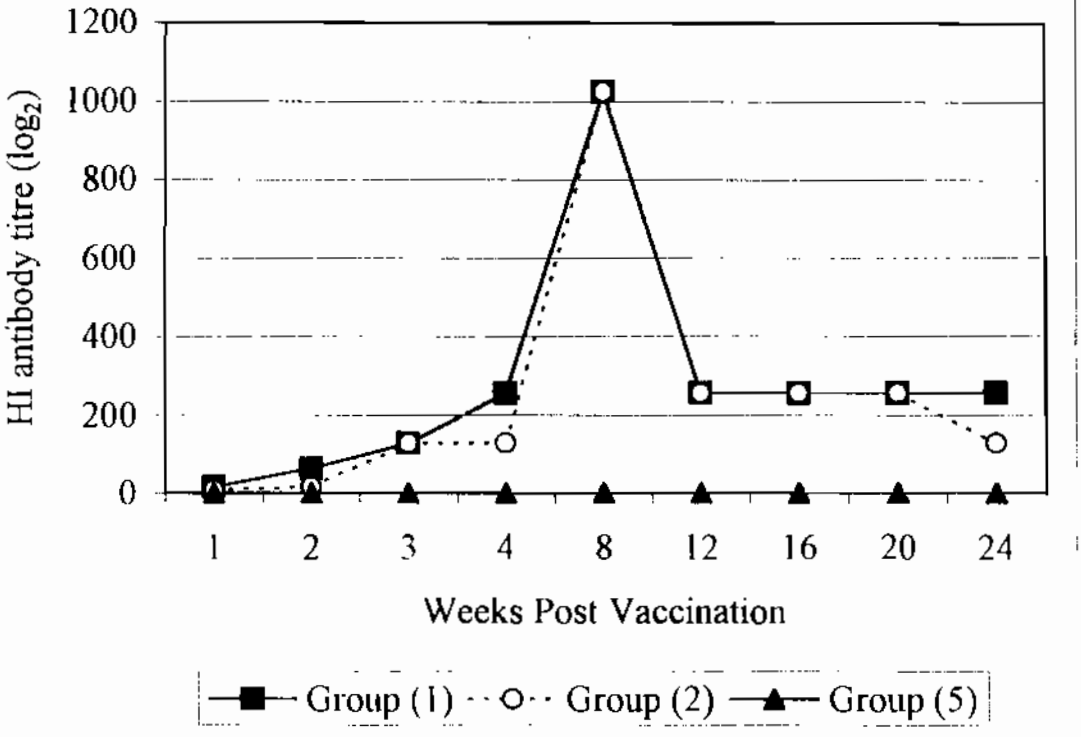


Fig. (2): Levels of egg drop syndrome HI antibody titres in vaccinated chickens

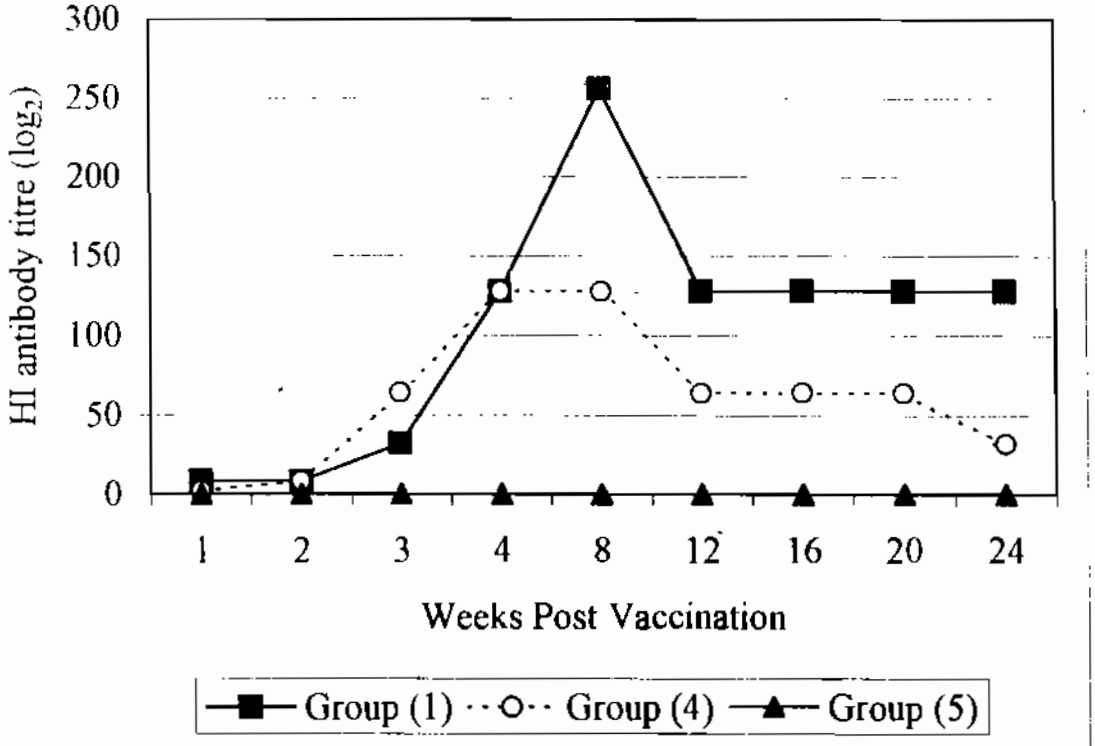


Fig. (3): Levels of infectious bursal disease neutralizing antibody titres in vaccinated chicks

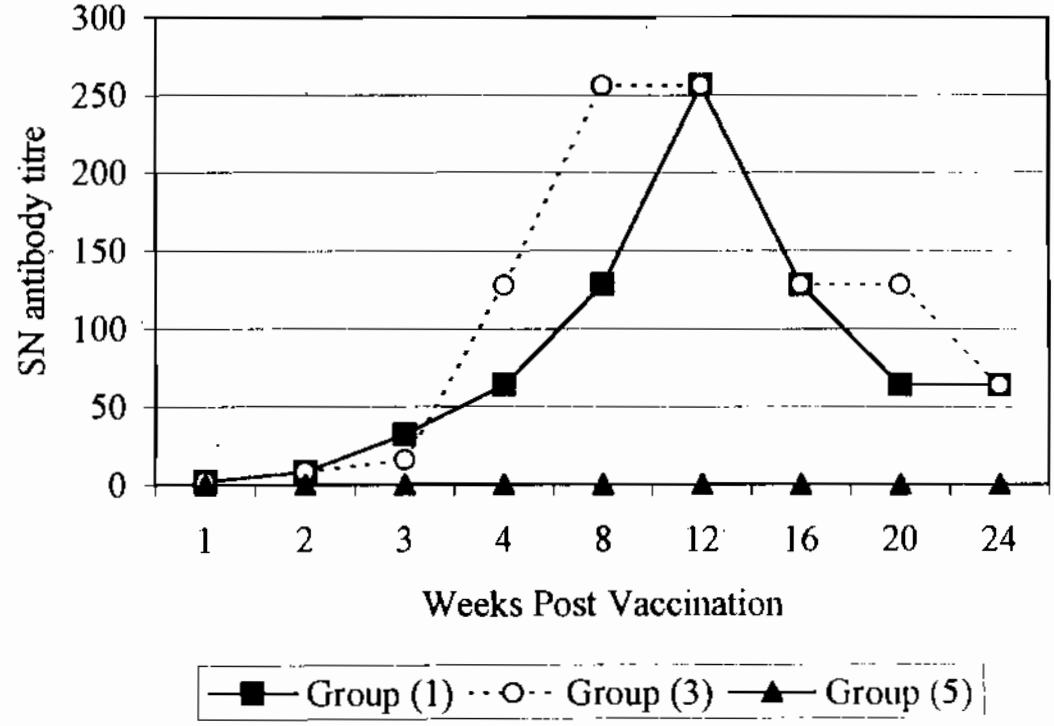
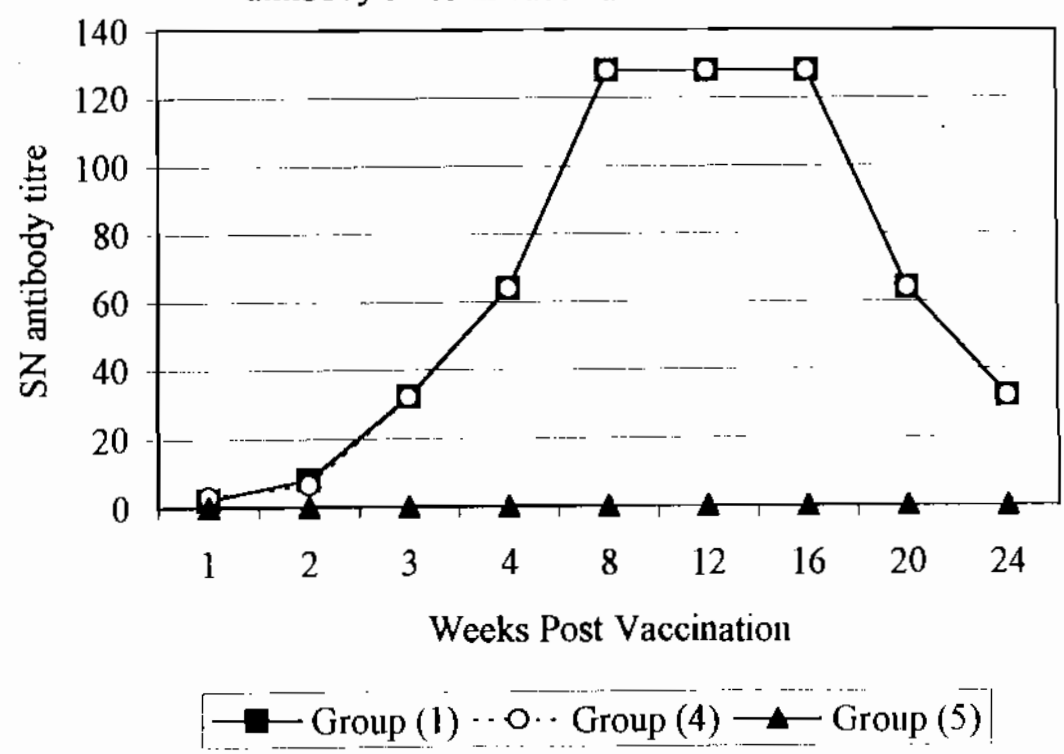


Fig. (4): Levels of egg drop syndrome neutralizing antibody titres in vaccinated chickens



الملخص العربي

محاولات لتحضير لقاح ثلاثي ميت ضد أ مراض النيوكاسل والجمبورو وظاهرة تدنى البيض

أبو زيد أحمد أبو زيد سلوى أنور الأصيلى هدى إبراهيم توفيق . إنصاف خشبة

معهد بحوث الأمصال واللقاحات البيطرية – العباسية – القاهرة

تم تحضير لقاح ثلاثي زيتي ميت ضد أمراض النيوكاسل والجمبورو وظاهرة تدنى البيض مع تحضير لقاح أحادى ضد كل فيروس على حدة. وتم اختبار كفاءة اللقاحات مناعيا بحقن كل لقاح في مجموعة من الدجاج عمر أربع أشهر وتم جمع عينات من أمصال الطيور لمدة ٢٤ أسبوع بعد الحقن لفحصها سيرولوجيا حيث ثبت أن اللقاح الزيتي الثلاثي أعطى كفاءة مناعية عالية ضد الثلاثة أمراض لمدة ٢٤ أسبوع وبمقارنته باللقاحات الفردية تبين انه لم يحدث أي تداخل بين الفيروسات الثلاثة من حيث التأثير على الاستجابة المناعية للطيور ويفضل استخدام اللقاح الثلاثي حيث أنه يوفر الوقت والجهد المبذول والنفقات عند التحصين بلقاحات فردية.