Vet.Med.J.,Giza. Vol.53, No.4. (2005):901-909.

# PREPARATION AND EVALUATION OF COMBINED INACTIVATED ALUMINIUM HYDROXIDE GEL VACCINE AGAINST DUCK VIRUS HEPATITIS AND DUCK PLAGUE DISEASES PRIVATE

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**Received:** 9. 8. 2005

**Accepted:** 24. 8. 2005

## SUMMARY

A combined bivalent alhydragel vaccine against both duck plague virus (DPV) and duck virus hepatitis (DVH) was prepared. The efficacy of the new vaccine was evaluated in different groups of ducklings compared with a single vaccine against each virus alone. Evaluation depended upon estimation of both humoral and cellular immune response. The prepared combined vaccine offered a high titre against each virus. Obtained results have shown that the antibody titre obtained with the new vaccine are never inferior to the titres obtained with the separate single vaccine.

## INTRODUCTION

Vaccination is the basic tool for prevention and control of duck viral hepatitis (DVH) and duck plague (DP) (Crighton and Woolcock, 1978). The live attenuated vaccine is the most common use for one day old ducklings, but more recently the possibility for using inactivated vaccines become of a wide range specially of producing maternally immune duckling (Gough and Spackman, 1981).

Young ducklings hatched from eggs derived either from vaccinated or unvaccinated dams, so their sera may or not contain maternal antibody, however the main advantage of adjuvanted vaccine is of their ability to overcome the inhibitory effect of the maternal antibody to neutralize the circulating antibody. So, whenever the immunological status of duckling is ignored, it is preferable to use inactivated adjuvanted vaccine, aluminium compounds are repository adjuvants as their main adjuvant action is attributed to their "depot" effect, in addition they produce local granulomas that contain antibody producing plasma cells as well as they have shown to activate the alternative complement pathway which provokes

chronic inflammation at the site of inoculation. Demakov et al. (1979) improved the effectiveness of attenuated DVH by aluminium hydroxide adsorption. The inactivated DVH or DPV alone or combined were adjuvanted with aluminium hydroxide (Fan et al., 1993, Abd El-Khaleck et al., 1999 and Mervat et al., 2000).

The purpose of the present study is to give preliminary data on using a combined inactivated aluminium hydroxide gel vaccine for vaccination of young ducklings especially which come from unvaccinated dams against both duck viral hepatitis and duck plague virus.

## MATERIAL AND METHOD

### 1. Seed Viruses:

**a. Duck viral hepatitis vaccinal strain:**

Freeze dried living attenuated vaccinal strain E52 Rispens of DVH propagated in SPF chicken embryos was obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo with a titre of 108 EID50/ml.

**b. Duck plague virus strain:**

Freeze dried attenuated live DPV vaccinal Jansen strain was obtained from VSVRI with a titre of $10^{7}.57$ $EID_{50}$/ml.

### 2. Embryonated chicken eggs:

Specific pathogen free (SPF) embryonated chicken eggs (9-11) days were obtained from Nile SPF eggs farm, Koum Osheim, Fayoum, Egypt were used for propagation, titration and assurance of complete inactivation of viruses.

### 3. Alhydragel:

Sterile aluminium hydroxide 2% gel was obtained from Honil Limited Company, and used as adjuvant.

### 4. Ducklings:

One day old ducklings were obtained from commercial hatchery.

### 5. Viruses propagation and titration:

Each of DVH and DPV was propagated separately in 10 days old embryonated SPF chicken eggs according to the methods of Toth (1969) and Jansen (1964). Obtained viruses were titrated in SPF chicken eggs according to Anon (1971) and the titre was calculated according to Reed and Muench (1938). Titres were 108.6 and 108.2 EID50/ml for both viruses in order.

### 6. Inactivation of both DHV and DPV:

Formalin (BDH limited Poole, England) was used as inactivator for both viruses at a final concentration of 0.2% and added to each virus dropwise during stirring, inactivation continued for 24 hours at room temperature. Two successive blind passages from each virus were carried out in embryonated SPF chicken eggs before the batches of the inactivated viruses were considered safe.

## 7. Vaccines preparation:

**Monovalent and bivalent DVH and DPV alhydragel vaccines:**

200 ml of each of monovalent or bivalent vaccines were prepared. For preparation of the monovalent vaccine 50ml of inactivated duck hepatitis or duck plague viruses were diluted with 50ml physiological saline, the mixture was added to 100ml alhydragel as adjuvants, while being stirred, the prepared vaccines were dispensed into sterile bottles and stored at +4°C till used.

The bivalent vaccine was prepared by mixing 50 ml of the inactivated duck viral hepatitis with 50 ml of the inactivated duck plague virus and the mixture was added to 100 ml alhydragel as adjuvant and prepared in the same way as the monovalent vaccines.

**Purity and safety tests:**

The prepared vaccines were tested for both purity and safety according to the Code of American Federal Regulations (1985).

**Experimental Design:**

One hundred of three day old balady ducklings were obtained and divided into 5 equal groups each of 20 duckling raised in separated pens and vaccinated with the prepared vaccines as follows:

**Group (1):**

Vaccinated with monovalent alhydragel inactivated duck plague vaccine.

**Group (2):**

Vaccinated with monovalent alhydragel inactivated duck viral hepatitis vaccine.

**Group (3):**

Vaccinated with the combined bivalent alhydragel duck plague and duck viral hepatitis vaccine.

**Group (4):**

Boostered with the combined bivalent alhydragel inactivated duck plague-duck viral hepatitis vaccine 6 weeks after primary vaccination with the same vaccine.

**Group (5):**

Non-vaccinated control.

Each bird of the vaccinated groups received a dose of 0.5 ml/IM whether with monovalent or bivalent vaccine containing approximately $10^{7.7}$ and $10^{7.2}$ for DVH and DPV respectively. Birds of (group 4) received the same dose 6 weeks post primary vaccination.

Five random blood samples were collected weekly, starting one week post vaccination till the 8th week and then every two weeks till the end of the experiment (16 weeks post vaccination). Serum was separated and subjected for estimation of humoral immune response against both DVH and

DPV using the following serological tests:

**i. Serum neutralization test (SNT):**

It was carried out according to the method described by Kaleta (1988).

**ii. Enzyme linked immunosorbent assay (ELISA):**

This test was conducted after the method of Zhao et al. (1991).

**Assay of lymphocyte blastogenesis:**

This test was carried out specifically according to the method described elsewhere by Lucy (1977) and evaluation of the test using (MTT) according to Mosmann (1983). The test was carried out for the first week post vaccination and the results were expressed as Delta optical density.

## RESULTS

The prepared combined vaccine was completely sterile from any bacterial, fungal and mycoplasma contaminants and safe for inoculated ducklings.

Tables (1) and (2) summarize the neutralizing antibodies against duck plague virus (DPV) and duck viral hepatitis (DVH). It was noticed that the level of neutralizing antibody for DPV was increasing gradually in groups 1 and 3 till reached its maximum on the 8th week, while the 4th group (boostered group) the level of antibody was still high (128) from the 6th to 12th week then declined to 64 on the 16th week.

In table (2), it was noticed that neutralizing antibody titre of DVH in group 2 and 3 gradually increased till reached the peak (173.6) on the 7th week and (192) on the 10th week while in the 4th group (boostered) the antibody titre was still high from 7th week till the end of experiment with its maximum (512) on the 10th week.

Tables (3) and (4) summarize the humoral immune response against both viruses using ELISA, similar pattern of immunological response was recorded, a maximum ELISA reading for group (3) were (1.91) and (1.033) that recorded at 3rd and 7th weeks post vaccination for both DVH and DPV in order.

Extremely high titre against both viruses were noticed for group (4) specially after the birds had been boostered with the combined vaccine (table 1, 2, 3, 4).

Table (5) summarize the cellular immune response estimated by lymphocyte blastogenesis, approximately similar values (0.376) (0.387) were obtained using the monovalent inactivated vaccines (group 1 and group 2), whereas a maximum value (0.422) was obtained when using the combined vaccine that recorded 3rd week post vaccination.

Table (1): Duck plague serum neutralizing antibody titres in ducklings vaccinated by monovalent duck plague vaccine and combined duck plague-duck hepatitis vaccine

| WPV / Group | 1 | 2 | 3 | 4 | 5 | 6** | 7 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3* | 8 | 16 | 21.33 | 42.6 | 53.3 | 90.6 | 128 | 85.3 | 53 | 16 | 18.6 |
| 3 | 2 | 4 | 21.3 | 3 26.7 | 53.3 | 106.7 | 117.35 | 128 | 96 | 53 | 53.15 | 53.3 |
| 4 | - | - | - | - | - | - | 128 | 128 | 128 | 128 | 96 | 64 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Group (1): Ducklings were vaccinated with monovalent inactivated DP vaccine
Group (3): Ducklings were vaccinated with combined inactivated DP+DVH vaccine
Group (4): Ducklings were vaccinated with booster combined inactivated DP+DVH vaccine
Group (5): Unvaccinated control group
* Mean antibody titre = Mean of the reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 TCID50 of DPV.
** Booster time.

Table (2): Duck virus hepatitis serum neutralizing antibody titres in ducklings vaccinated by monovalent duck virus hepatitis vaccine and combined duck plague-duck hepatitis vaccine

| WPV / Group | 1 | 2 | 3 | 4 | 5 | 6** | 7 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 6.66 | 12 | 18.6 | 48 | 106.6 | 117.3 | 173.6 | 64 | 64 | 32 | 8 | 4 |
| 3 | 10.6 | 21.3 | 26.7 | 48 | 53.3 | 106.6 | 149.3 | 149.3 | 192 | 138.6 | 85.3 | ND |
| 4 | - | - | - | - | - | - | 170.6 | 341.3 | 512 | 426.6 | 426.6 | ND |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Group (2): Ducklings were vaccinated with monovalent inactivated DVH vaccine
Group (3): Ducklings were vaccinated with combined inactivated DP+DVH vaccine
Group (4): Ducklings were vaccinated with booster combined inactivated DP+DVH vaccine
Group (5): Unvaccinated control group
* Mean antibody titre = Mean of the reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of DVH.
** Booster Time
ND: Not Done

Table (3): Mean absorbance values of ELISA test for serum of vaccinated groups by DPV vaccine

| WPV / Group | 1 | 2 | 3 | 4 | 5 | 6** | 7 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.603 | 0.898 | 0.879 | 0.897 | 0.741 | 0.719 | 0.776 | 0.817 | 0.804 | 0.792 | 0.745 | 0.755 |
| 3 | 0.734 | 0.880 | 0.875 | 0.906 | 0.952 | 0.889 | 1.033 | 0.927 | 0.877 | 0.827 | 0.823 | 0.803 |
| 4 | - | - | - | - | - | - | 1.116 | 1.131 | 1.034 | 0.937 | 0.931 | 0.921 |

Group (1): Ducklings were vaccinated with monovalent inactivated DP vaccine
Group (3): Ducklings were vaccinated with combined inactivated DP+DVH vaccine
Group (4): Ducklings were vaccinated with booster combined inactivated DP+DVH vaccine
Group (5): Unvaccinated control group
Absorbance values of negative control serum = 0.261
Absorbance values of positive control serum = 0.897
WPV: Weeks Post Vaccination.

Table (4): Mean absorbance values of ELISA test for serum of vaccinated groups by DHV vaccine

| WPV / Group | 1 | 2 | 3 | 4 | 5 | 6** | 7 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.627 | 0.752 | 0.75 | 0.839 | 0.806 | 0.773 | 0.963 | 0.977 | 0.871 | 0.830 | 0.731 | 0.624 |
| 3 | 0.835 | 0.968 | 1.91 | 0.936 | 0.907 | 0.879 | 0.916 | 1.070 | 0.952 | 0.834 | 0.832 | 0.830 |
| 4 | - | - | - | - | - | - | 0.906 | 1.186 | 1.179 | 1.172 | 1.129 | 1.086 |

Group (2): Ducklings were vaccinated with monovalent inactivated DVH vaccine
Group (3): Ducklings were vaccinated with combined inactivated DP+DVH vaccine
Group (4): Ducklings were vaccinated with booster combined inactivated DP+DVH vaccine
Group (5): Unvaccinated control group
Absorbance values of negative control serum = 0.371
Absorbance values of positive control serum = 0.830
WPV: Weeks Post Vaccination.

Table (5): Cellular immune response of ducklings vaccinated by different prepared vaccines using lymphocyte blastogenesis assay

| Group | Weeks Post Vaccination | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1 | 0.077 * | 0.249 | 0.376 | 0.036 |
| 2 | 0.080 | 0.294 | 0.387 | 0.082 |
| 3 | 0.070 | 0.326 | 0.422 | 0.141 |
| 5 | 0.002 | 0.051 | 0.082 | 0.0064 |

Group (1): Ducklings were vaccinated with monovalent inactivated DP vaccine
Group (2): Ducklings were vaccinated with monovalent inactivated DVH vaccine
Group (3): Ducklings were vaccinated with combined inactivated DP+DVH vaccine
Group (5): Unvaccinated control group
* ( OD: Delta Optical Density values.

## DISCUSSION

Duck viral hepatitis (DVH) and duck plague virus (DPV) are the highly destructive contagious viral diseases threatening duck industry. Control of both diseases presented a very serious problem. Occasionally, the biosecurity and hygienic measures while are being important but are insufficient for prevention of the both diseases and vaccination against them is essential to secure satisfactory level of protection of young ducklings. Combined vaccines offered this goal where it could provide protection against more than one disease at the same time.

The prepared combined vaccine composed of inactivated DVH and DPV adjuvanted with alhydragel induced the production of antibodies to DVH and DPV in susceptible ducklings. Obtained serum neutralizing titres against both virus considered highly protective at a titre of 1/32 and 1/8 for both viruses respectively according to Golubnichi and Malinovskaya (1984) and Abd El-Khalick (1997). On the other hand, the pattern of humoral antibodies obtained by ELISA test (Tables 3 and 4) were similar as those obtained by SNT (Tables 1 and 2).

This finding is fully agreed with those obtained by Sun et al. (1997 and Mervat et al. (2000) where they found a direct correlation between the titres obtained by ELISA and those obtained by SNT.

Furthermore, the extremely high titre against both virus that was obtained specially after boostering with the new prepared vaccine support the finding of Gough and Spackman (1981) where they reported that the effective level of protection can be secured by administrating three doses of inactivated vaccine.

According to Erickson et al. (1974) and Dardiri (1975), the cell mediated immunity could have a possible role after vaccination with duck plague vaccine. However, a benefit and successful value was obtained with the combined vaccines and these results disagreed with those obtained by Madkour (1995) who found a high response in group of birds vaccinated with the monovalent inactivated Newcastle disease vaccine rather than other group vaccinated with the combined Newcastle and fowl cholera vaccine.

In conclusion, the study has shown that the antibody titres with the new vaccine are never inferior to the titres obtained with the separate single vaccine and the expectation that the bivalent inactivated alhydragel vaccine would provide the best protective titres was consequently justified and this hope however was fulfilled specially on using this vaccine as a boostering.

## REFERENCES

Abd El-Khaleck, M.A. (1997): Trials for the production of

duck plague virus vaccine. Ph.D. Thesis Microbiology, Virology, Fac. Vet. Med., Zagazig Univ.

Abd El-Khaleck, M.A.; Samara, A.A. Soliman; Ibrahim, I.S. and Mervat A. El-Koffy (1999): Preparation of combined inactivated vaccine against duck plague and duck cholera diseases. Beni-Suef Vet. Med. J., 9 (3-A): 165-177.

Anon (1971): Studies on an agent causing mortality among ducklings immune to duck hepatitis virus. Avian Dis., 13: 834-846.

Code of American Federal Regulation (1985): Publishing by: the Office of Federal Register National Archives Records Service General Services Administration.

Crighton, G.W. and Woolcock, P.R. (1978): Active immunization of ducklings against duck virus hepatitis. Vet. Rec., 102: 358-361.

Dardiri, A.H. (1975): Duck virus enteritis (duck plague) characteristics and immune response of the host. Ann. J. Vet. Res., 36: 535-538.

Demakov, G.P.; Ogorodnikova, V.N.; Semenovykh, A.P. and Nabatov, F.A. (1979): Improvement of prophylaxis of duck viral hepatitis. Vestn. Skh, Nauki, 10: 85-87.

Erickson, G.A.; Proctors, J.; Pearson, J.E. and Gustafson, G.A. (1974): Diagnosis of duck virus enteritis (duck plague). 17th Annual Processing of American Ass of Vet. Lab. Diagnosticians.

Fan, W.M.; Zhang, J.Y.; Luo, H.L.; Shi, A.H. and Liu, J.P. (1993): Studies on an inactivated duck embryo vaccine against DVH. Acta Veterinaria et Zootechnica Sinica, 24 (4): 354-358.

Golubnichi, V.P. and Malinovskaya, G.V. (1984): Dynamics of post vaccinal antibodies in blood serum against duck hepatitis virus. Vet. Nauk. Proiz (Minsk), 22: 72-75.

Gough, R.E. and Spackman, D. (1981): Studies with inactivated duck virus hepatitis vaccines in breeder ducks. Avian Pathol., 10: 471-479.

Jansen, J. (1964): The interference phenomenon in the development of resistance against duck plague. J. Comp. Pathol. Ther., 74: 3-7.

Kaleta (1988): Duck viral hepatitis type I vaccination: Monitoring of the immune response with a microneutralization test in Pekin duck embryo kidney cell culture. Avian Pathol., 17: 325-332.

Lucy, F.L. (1977): Chicken lymphocyte stimulation by mitogens. A microassay with whole blood cultures. Avian Dis., 22: 296-307.

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

Mervat, A.H. El-Koffy; Hoda, I.T. Mohamed; Susan K. Tolba and Abd El-Khaleck, M.A. (2000): Trials for production of alhydragel inactivated duck hepatitis virus vaccine. Suez Canal Vet. Med. J., III (1): 215-222.

Mosmann, T. (1983): Rapid colorimetric assay for cellular growth and cytotoxicity assays. J. Immunol. Methods, 65: 55.

Reed, L.J. and Muench, H. (1938): A simple method of estimating fifty percent end points. Am. J. Hyg., 27: 493-497.

Sun, Q.Y.; Liu, H.; Li, J.S.; Cai, B.X. and Tao, S.Q. (1997): Study on the detection of antibodies against duck hepatitis virus by an indirect enzyme linked immunosorbent

assay (ELISA). Chinese J. Vet. Sci., 17 (4): 347-349.

Toth, T.E. (1969): Chicken embryo adapted duck hepatitis virus growth curve in embryonated chicken eggs. Avian Dis., 13: 535-539.

Zhao, Xin Liu; Philips, R.M.; Li, G. and Zgong, A. (1991): Studies on the detection of antibody to duck hepatitis virus by enzyme linked immunosorbent assay. Avian Dis., 35: 778-782.