

## Comparative immunological studies between tissue culture and egg adapted duck plague vaccines.

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**Abstract:** This study was designed to compare the immune antibody levels either with tissue culture adapted vaccine or egg adapted one response of duck vaccinated against duck plague (DP) used in duck farms in Egypt. Five day old two hundred and ten ducklings were divided into equal seven groups. Groups (1 & 2) were vaccinated with two commercial imported egg adapted (DP) living vaccine; groups (3 & 4) with two commercial locally prepared egg adapted one. While ducks in group (5) vaccinated with commercial imported tissue culture adapted (DP) living vaccine. Groups (6&7) not receive vaccine and served as (+ ve & – ve) control respectively. Blood samples collected weekly post vaccination for monitoring antibody levels by using three serological tests (SNT; PHA and AGPT) and every two weeks duckling in groups (1-6) were individually challenged with  $10^{5.5}$  EID<sub>50</sub>/dose DP virulent strains. The protection evaluated for ten days post each inoculation. The results of this study showed that under experimental condition the commercial egg adapted vaccine either locally prepared or imported one gave higher protective antibodies lasting for long time than tissue culture (DP) vaccine and can be used for controlling duck plague problems in Egypt.

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### 1. Introduction:

Duck plague virus (DPV) also known as duck virus enteritis (DVE) by *Bos (1942)*. This was proposed as the official name by *Jansen & Kunst (1949)*. It is an acute contagious herpes virus infection of duck, geese swans and other water fowl within the order Anseriformes (*Davison et al., 1993*). DPV characterized by vascular damage, tissue haemorrhages, and digestive mucosal eruptions lesions of lymphoid organs and degenerative changes in parenchymatous organs (*Gough & Alexander, 1990; Sandhu & Leibovitz, 1997; Converse & Kidd, 2001 & Kaleta et al., 2007*). DP has produced significant economic losses due to high mortality rate and decrease egg production (*Marlier et al., 2001 & Malmarugan & Sulochana 2002*). It was classified as a member of family herpes viridae, subfamily alpha herpes virinae genus anatis herpes 1 duck plague virus (An HV-1). (*Murphy et al., 1999*). On the basis of differences in the cellular tropism, genome organization, and gene content, herpes viruses have been classified into three subfamilies: Alpha-Beta and gamma herpes-virinae (*Alba et al., 2007*). Avian herpes viruses were grouped into the subfamily. Alpha herpes virinae except for DPV, which was assigned as an unclassified virus within the family Herpes viridae (*Fauquet et al., 2005*). Lack of genome sequences and genomic organization information are factors that limit DPV taxonomy and currently, DPV has not yet grouped into any genus according to the Eighth

International Committee Taxonomy of viruses (*Fauquet et al., 2005 & Ming-Sheng et al., 2010*).

In Egypt the DPV disease was reported for the first time in large flock of white pekin ducks in Bahtim Province, where the disease caused high morbidity and mortality (*Sabry et al., 1986*). DP caused great economic losses with mortality rate ranging 1-16% in breeders and 1-40% in broilers and drop in egg production ranged from 0.5-99.5% (*Sultan, 1990 & Kheir El Dine et al., 1992*). At 2012 *Susan et al.*, found in studying of sequence analysis of the glycoprotein envelopes gene of DP; a great similarity was found between the UL 35 gene amplified from either local or imported vaccinal strain but the antigenicity profile alone with the dot blot matrix revealed that the UL 35 protein antigen (antigen VP 26) from local isolate is more antigenic and thus the genome of the local strain would be a suitable templates for amplification of the UL 35 gene.

So that we design our study for detect the immunological effect of some commercial (DP) vaccines either locally prepared or imported on duck flocks in Egypt

### 2. Material and Methods:

#### Living duck plague vaccine:

Five DP commercial live attenuated vaccines were used; four egg adapted DP vaccines: two imported from Merial, IFT. Batch No. (L 378936 & L388650); and another two from VSVRI. Batch No. (8 & 9)

which locally prepared vaccines. And one tissue culture adapted (DP) vaccine from Merial, IFT. Batch No. (L353644).

The vaccines under test titrated according to *OIE (2012)* and the end point calculated according to *Reed & Muench (1938)*.

#### **Virus:**

Virulent strain of duck plague virus was supplied from (*Central. Lab. for Evaluation of Vet. Bio (CLEVB)*) with  $10^{8.5}$  EID<sub>50</sub>/ ml .Virus was propagated in duck ling according to *Lin et al., (1984)* and titrated in SPF egg according to *OIE (2012)* and calculated according to *Reed & Muench (1938)*.

#### **Experimental Hosts:**

**Specific pathogen free (SPF) embryonating chicken eggs (ECE):** SPF eggs were obtained from the SPF production farm, Koum Osheim, El-Fayoum, Egypt. Eggs were kept in the egg incubator at 37°C with humidity 40-60% used for titration of egg adapted vaccine at (10-12) days old on chorioallantoicmembrane (CAM) according to *Jansen, (1961)*.

**Tissue Cultures and cell culture media: (*Lennette, 1964*).**

Primary chicken embryo fibroblast cell (CEF) as obtained from (CLEVB); which prepared as described by *Schat & Purchase (1989)*. Trypsin-inversion solution prepared according to *Lennette (1964)*; Hank's balanced salt solution (HBSS) prepared according to *Hank &Wallance (1949)*; Minimum Essential Medium (MEM) was prepared according to the manufacturer's instructions; and Bovin serum was mycoplasma free and virus screened "Gibco Limited, Scotland and UK". The method used for inoculation in the microtitre plates was done according to *Rossiter & Jessett (1982)*. CEF used for titration of tissue culture adapted vaccine and for SNT test.

#### **Duckling:**

One day old Muscovy ducklings were obtained from El-Wafa Farm. They were reared under strict hygienic measures and tested the maternal antibodies by using SNT. They were used for evaluation of vaccine under test.

#### **Serological tests:**

##### **Serum neutralization test (SNT):**

A beta micro-neutralization procedure was carried out according to *Beard (1989)*. It was used for monitoring of DPV antibodies in duck sera.

##### **Passive haemagglutination test (PHA):**

This test was carried out with the micro titer technique according to the method described by *Tripathy et al., (1970) & Ming et al., (1983)* The (DP) virus antigen prepared according to *Naqi (1990)*.

##### **Agar gel precipitation test (AGPT):**

The microtechnique of agar gel precipitation (AGP) test was used according to *Woernle (1959)*.

#### **Polymerase Chain reaction (PCR): Council of Europe(1999):**

PCR used for detection of Identity of Commercial vaccines under test: Genomic DNA extraction kit using purification kit with Batch No. (00086242) and PCR React mix PCR kit with Batch No (KK5101)

#### **Statistical analysis:**

It was applied using Epi-Info-Computer programmer designed by *Dean et al., (1994)* and produced by World Health Organization (WHO). The calculation was according to *Knapp & Miller (1991)*.

#### **Experimental Design:**

Seven groups of 30 Muscovy ducklings each were used in this study. Duckling from groups (1 to 5) were vaccinated subcutaneously (S/C) with 0.5 mL of different examined commercial vaccines at five day old as shown in table (1). While ducklings in groups (6&7) were kept as control (+ve & - ve); respectively.

Every two weeks after vaccinations five ducklings from groups (1-6) were challenged for ten weeks post vaccination with 5.5 log 10 EID<sub>50</sub> per duck of (DP) challenge virus; administered via(S/C) route. Ducks in group (7) were left as an unchallenged control. Blood samples were collected weekly for ten weeks from vaccinated and unvaccinated duckling to determine the (SNT; passive HA and AGPT) antibody titer for different type of vaccines under test. Following challenge, all duck were observed daily for clinical signs attributable to DPV infection for ten days post each challenge.

### **3. Results:**

#### **Detection of viral titration and identity:**

All the five commercial vaccines used in this experiment, titrated in ECE or T.C according to type of vaccines (Table 2) and identified as (DPV) by using PCR: A500 bp amplification band in the lambda control sample indicates the PCR ran successfully (1) 446 bp band in the DP known DNA control indicates the (DPV) primers are working and lan(2) is a tested vaccinal sample at 446 bp indicated that DP viral DNA was present.(Fig -1)

#### **Vaccination and challenge:**

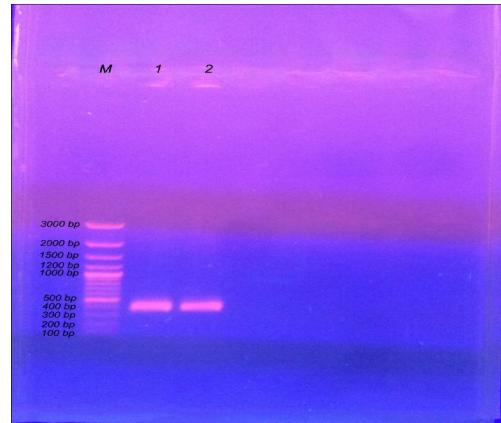
Duckling in groups (1-5) which inoculated with different commercial vaccines remained healthy and no other clinical signs were observed. There was no thermal reaction or loss in body weight in the vaccinated ducks observed. Mild temperature reactions were observed in vaccinated ducks after challenge with virulent (DP) in 2<sup>nd</sup> week post vacc. And all the ducks survived the challenge and remained

healthy. The ducks in group (6) in each time of challenge, exhibited pyrexia and all died between the 4<sup>th</sup> and 6<sup>th</sup> days post challenge showing typical symptoms of (DP), which included profuse greenish diarrhea, loss of voice in some ducks, Lameness, retraction of neck, loss of appetite, loss weight and photophobia.

Necropsy of a few dead ducks was performed and characteristic (DP) lesions were noticed. Principal lesions were hepatomegaly, patchiae in the liver, kidney, spleen and intestinal mucosae.

**Serological tests:**

Three serological tests used for monitoring level of antibodies post vaccination: (SNT; PHA and AGPT). Table (1: A; B & C).



**Fig (1): The PCR amplification of the spike gene of DP polymerase gene vaccines under test**  
The amplification of the bp fragment of the Vp gene of Dp virus of vaccine batches

**Table (1): Humeral immune response of vaccinated duckling by different types of vaccines using (SNT, PHA and AGPT)**

**A. Results of Neutralizing antibody titer (SNT):**

| Groups       | Weeks Post Vacc |    |    |     |     |     |     |     |     |     |
|--------------|-----------------|----|----|-----|-----|-----|-----|-----|-----|-----|
|              | 1               | 2  | 3  | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
| 1)Vaxiduk-1  | 4               | 8  | 32 | 64  | 128 | 256 | 512 | 512 | 512 | 512 |
| 2)Vaxiduk-2  | 4               | 16 | 64 | 256 | 512 | 512 | 512 | 512 | 512 | 512 |
| 3)VSURI-8    | 8               | 16 | 32 | 32  | 64  | 128 | 128 | 256 | 256 | 256 |
| 4)VSURI-9    | 8               | 16 | 32 | 32  | 128 | 128 | 128 | 256 | 256 | 256 |
| 5)Vaxiduk-3  | 4               | 8  | 64 | 128 | 256 | 256 | 256 | 128 | 128 | 128 |
| 6)Cont. + ve | 0               | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 7)Cont. - ve | 0               | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

Vaxiduk (1): DP vaccine with batch no L 378936 egg adapted vacc.

Vaxiduk (2): DP vaccine with batch no L 838650 egg adapted vacc.

Vaxiduk (3): DP vaccine with batch no L 353644 tissue culture vacc.

SNT: Serum neutralization titre; using duck plague virus diluted to contain 100TCID<sub>50</sub> / 50 ul

NB: SNT less than 3.0 log<sub>2</sub> are usually considered to be negative and 8 or greater is considered to be significant positive according to *Docherty & Franson (1992)*.

**Table (1-B): Results of geometric mean titers of different vaccinated duck plague groups using passive haemagglutination test (PHAT).**

| Groups         | Weeks Post Vacc |     |     |     |     |     |     |     |     |     |
|----------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                | 1               | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
| 1)Vaxiduk-1    | 2.8             | 2.2 | 3.2 | 3.4 | 4.8 | 5.3 | 5.5 | 6.4 | 6.6 | 6.6 |
| 2)Vaxiduk-2    | 2.8             | 2.8 | 3.8 | 4.8 | 5.0 | 5.8 | 5.8 | 6.0 | 6.0 | 6.6 |
| 3)VSURI-8      | 2.8             | 2.8 | 4.2 | 5.2 | 6.2 | 5.8 | 5.8 | 6.8 | 6.4 | 6.0 |
| 4)VSURI-9      | 2.8             | 2.8 | 4.4 | 4.2 | 5.2 | 5.0 | 4.8 | 6.4 | 5.8 | 5.8 |
| 5)Vaxiduk-3    | 2.2             | 2.2 | 3.2 | 3.6 | 4.8 | 5.8 | 5.8 | 4.8 | 4.8 | 4.8 |
| 6)Control + ve | 0               | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 7)Control - ve | 0               | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

Vaxiduk (1): DP vaccine with batch no L 378936 egg adapted vacc.

Vaxiduk (2): DP vaccine with batch no L 838650 egg adapted vacc.

Vaxiduk (3): DP vaccine with batch no L 353644 tissue culture vacc.

PHA: passive haemagglutination log<sub>2</sub> geometric mean titers.

NB: PHA titer less than 5.0 log<sub>2</sub> is usually considered to be unprotected and 5.0 or greater is considered to be significant protective according to *Ming et al., (1983)*.

Ducks in groups (6&7) are unvaccinated groups.

**Table (1-c): Results of Agar Gel precipitation test (AGPT) of different vaccinated duck plague groups.**

| Groups         | Weeks Post Vacc |   |   |   |   |   |   |   |   |    |
|----------------|-----------------|---|---|---|---|---|---|---|---|----|
|                | 1               | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1)Vaxiduk-1    | -               | - | - | - | - | + | + | + | + | +  |
| 2)Vaxiduk-2    | -               | - | - | - | + | + | + | + | + | +  |
| 3)VSURI-8      | -               | - | - | - | + | + | + | + | + | +  |
| 4)VSURI-9      | -               | - | - | - | + | + | + | + | + | +  |
| 5)Vaxiduk-3    | -               | - | - | - | - | + | + | + | + | +  |
| 6)Control + ve | -               | - | - | - | - | - | - | - | - | -  |
| 7)Control - ve | -               | - | - | - | - | - | - | - | - | -  |

Vaxiduk (1): DP vaccine with batch no L 378936

Vaxiduk (2): DP vaccine with batch no L 838650

Vaxiduk (3): DP vaccine with batch no L 353644 tissue culture vacc.

NB: Ducks in groups (6&7) are unvaccinated groups.

**Table (2): Protection test in ducklings vaccinated with different live commercial attenuated (DP) vaccines:**

| Group | Type of vaccine     | Titration                                      | Protection % |    |     |     |     |
|-------|---------------------|--|--------------|----|-----|-----|-----|
|       |                     |  | 2w           | 4w | 6w  | 8w  | 10w |
| 1     | Vaxiduk-1           | 5.5 Log <sub>10</sub> EID <sub>50</sub> /dose  | 60           | 80 | 100 | 100 | 100 |
| 2     | Vaxiduk-2           | 5.5 Log <sub>10</sub> EID <sub>50</sub> /doses | 60           | 80 | 100 | 100 | 100 |
| 3     | VSURI-8             | 5.2 Log <sub>10</sub> EID <sub>50</sub> /dose  | 80           | 80 | 100 | 100 | 100 |
| 4     | VSURI-9             | 5.2 Log <sub>10</sub> EID <sub>50</sub> /dose  | 80           | 80 | 100 | 100 | 90  |
| 5     | Tissue culture Vacc | 4.6 Log <sub>10</sub> TCID <sub>50</sub> /dose | 80           | 80 | 100 | 100 | 90  |
| 6     | Control + ve        | Challenged + ve                                | 0            | 0  | 0   | 0   | 0   |
| 7     | Control - ve        | Not vacc. Not chall.                           | -            | -  | -   | -   | -   |

Vaxiduk (1): DP vaccine with batch no L 378936

Vaxiduk (2): DP vaccine with batch no L 838650

W: Weeks post vaccination

N.B: The vaccine should contain minimum of 10<sup>3.0</sup> EID<sub>50</sub>/dose at time of use and cause protection% not less than 90% according to OIE 2012

#### 4. Discussion:

Duck plague virus causes substantial economic losses to the world wide duck production areas. Four parameters "including three serological tests (SNT, PHA and AGPT) for detect antibody level and evaluate the protection percentage" were used for monitoring the immunological studies between tissue culture and egg adapted duck plague vaccines to know the effective one for using in duck flocks in Egypt.

Concerning the humeral immune response, antibodies were monitored in sera collected from vaccinated and non vaccinated duckling using SNT; PHA and AGP tests, serum as shown in table (1: A,B& C). SNT titers of ducklings vaccinated with five different type of living attenuated (DP) vaccines was ranged from 4 to 8 at 1<sup>st</sup> week and gradually increased to 32 & 64 at 3<sup>rd</sup> week for all groups and at 5<sup>th</sup> week reached its maximum titer 256 and still high till 7<sup>th</sup> week post vaccination (G5) then decline again to 128 till end of experiment while in 1<sup>st</sup> and 2<sup>nd</sup> groups (G1&G2) which vaccinated with imported egg adapted vaccine; the maximum antibody titer 512 from 6<sup>th</sup> & 7<sup>th</sup> to 10<sup>th</sup> week.

Antibody titers in (G3 & G4) reach 128 at 5<sup>th</sup> & 6<sup>th</sup> week post vaccination and increase to 256 at 8<sup>th</sup> week and still high till end of test. The maximum titer (512) for imported and (256) for locally prepared vaccine were still high till end of experiment in ducks

vaccinated with commercial egg adapted (DP) vaccine.

Our results for SNT agree with (Docherty & Franson 1992; Kulkarni et al., 1998 & Mondal, et al., 2010) that reported ducks developed neutralizing antibody against DPV which was measured only in sera collected post vaccination. The neutralizing titer by SNT test of 3 weeks post vaccinated sera were between 1: 8 and 1: 32 and the highest mean SN antibody was 1: 64 and A SN titer of 8 or greater is considered to be significant and is evidence of exposure of DPV. Results of geometric mean titers in vaccinated groups by passive haemagglutination test (PHA) in table (1-B) showed that antibody titer in ducks were significantly increased at 3<sup>rd</sup>, 4<sup>th</sup>, sixth and eight week of vaccination. Those observations are in conformity with Deng et al., (1984) & Islam et al., (2005) who reported that attenuated duck plague vaccine could produce satisfactory levels of humeral immune response and the PHA antibody titer in ducks vaccinated by single dose of DP vaccine showed significantly increase at two, four, six and eight weeks post vaccination. Antibodies can be detected by agar gel precipitation test (AGPT) in table (1-C) in 5<sup>th</sup> week in (G2-4) that ducks vaccinated with locally prepared living attenuated egg adapted (DP) vaccines (G3 & 4) and imported one (G2). While another tested vaccine antibodies can be detected at 6<sup>th</sup> week post

vaccination; our results agree with that reported by (**Kalaimathi & Janakiram 1991**) that using AGPT for screening of duck plague virus antibodies in serum samples

Results in tables (1&2) show correlation were found between the level of antibody titers and the protection percent of vaccinated ducklings. These observations are in conformity with the findings of **Islam et al., (2005)** who reported attenuated duck plague vaccine could produce satisfactory levels of humeral immune response either post vaccination and followed by challenge with virulent virus and could protect agents the virulent challenge. While the SNT results in table (1A) considered the test of choice for the evaluation of the protection as reported by **Abd El-Khaleck (1997)**.

The hepatomegaly, petechial in liver, kidney, spleen and intestinal mucosae which were noticed in few dead ducks as positive control results agree with **Barr, et al., (1992) & Shawky, et al., (2000)**. Finally the relation between humeral antibodies and results of protection percentage agree with **Hossain et al., (2005)** who reported that chicken embryo attenuated live duck plague virus vaccine produced satisfactory level of antibody response and the ducks were resistant to virulent challenge.

Based on the data presented in our study it can be concluded that, under experimental condition the commercial (DP) egg adapted vaccine gave protected level of humeral antibody starting from 3<sup>rd</sup> week post vaccination and still elevated and reach the high level at 5<sup>th</sup> week with protection 90%- 100% against field isolated virulent strain till end of experiment. While in tissue culture one the antibody decline from 8<sup>th</sup> week and gave protection 90%.

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