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Preparation and evaluation of inactivated oil emulsion vaccine against swollen head syndrome in chickens

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In the present study, inactivated single swollen head syndrome oil emulsion vaccine (SHSV) was prepared and evaluated. SHSV was successfully propagated on the yolk sac of 5-7 days of SPF embryonated chicken eggs. The virus was harvested 48-72 hours post inoculation. The titre of the virus reached $10^{6.2}$ EID_{50}/ml. One hundred and fifty chickens were divided into 3 equal groups. The first group was vaccinated with the prepared SHS vaccine, 2nd group two was vaccinated with imported SHS vaccine, while the third group was kept as non-vaccinated control. Evaluation of the prepared vaccine was done by measuring both cell-mediated and humoral immunity. The results of both T-lymphocyte transformation and ELISA tests revealed that the prepared vaccine induced good protection and has good potentiality for use in chicken in the Egyptian poultry industry.

Key words:

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INTRODUCTION

Respiratory diseases have always been a feature of intensive poultry productions and a wide variety of syndromes of known etiology have been associated with such disease in chickens. A distinct novel upper respiratory disease of chicken: Swollen Head Syndrome (SHS) has been reported for the first time in South Africa **(Buys and DuPreeze, 1980)**. Pneumovirus infection of poultry is associated with serious economic and animal welfare problems even in countries where vaccination against avian pneumovirus has become a routine practice. 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 opisthotonos **(Buys et al., 1989)**. Egg production and quality of eggs are also affected **(Jones et al., 1988)**.

The more severe form of associated disease probably results from dual or secondary infection. The characteristic "Swollen Head" appears as a result of infection with secondary adventitious bacteria, usually *Escherichia coli*. The pneumovirus infection was reported in Egypt by **Ahmed (1991)** and therefore the current study was designed for preparation and evaluation of an inactivated oil emulsion vaccine to swollen head syndrome.

MATERIALS AND METHODS

Seed Virus:

Freeze-dried live vaccine against Swollen Head Syndrome prepared from a modified live virus (PL21 strain) multiplied in VERO cells (Nemovac: Merial, Lyon, France) was used in the study.

Laboratory host:

- SPF embryonated chicken eggs:

Specific pathogen free embryonated chicken eggs were obtained from Koum Osheim SPF Farm, Fayoum, Agriculture Research Center. The eggs were used for propagation and titration of the prepared vaccine.

- Chickens:

One hundred and fifty one-day-old, Hubbard chicks (United Company for Poultry Production) were reared under complete hygienic measures in isolated and disinfected wire floored cages, commercial broiler ration were used for evaluation of

experimentally prepared vaccine and divided as follow:

* Group I: 50 birds vaccinated with the prepared inactivated oil emulsion SHS vaccine; 0.5 ml/bird intramuscularly.
* Group II: 50 birds vaccinated with imported inactivated oil emulsion SHS vaccine; 0.5 ml/bird intramuscularly.
* Group III: 50 birds kept without vaccination as control.

- Virus propagation:

It was performed as reported by **Allan et al. (1973).** The virus was diluted 10^{-2} in sterile physiological saline to which 200 units penicillin and 200 mg streptomycin were added. 0.1 to 0.2 ml of virus suspension was inoculated in the yolk sac of 7 days-old SPF embryonated chicken eggs. Three serial successive passage of virus were carried out in ECE. The harvesting fluid and embryos of third passage were used for preparation of vaccine.

- Titration of virus suspension:

It was carried out according to **Anon (1971)** in SPF ECEs for each passage.

- Inactivation of the virus:

The inactivation process was carried out according to **Stone et al. (1980).** Different concentrations of formalin (0.1 %, 0.5 % and 0.25 %) were used for inactivation of SHS virus suspension. Samples from treated suspensions were collected every 2 hours for 18 hours after addition of 20% sodium bisulphate as a neutralizing agent. The inactivated fluid was kept at 4°C for infectivity test.

- Testing of complete inactivation by infectivity test:

To assure that the prepared inactivated virus fluid was completely inactivated, inactivated virus suspension was inoculated in SPF ECE via yolk sac. Eggs were examined daily for any mortalities and the survival were examined for any lesion. Three successive blind passages were done. The complete inactivation was achieved when there are no any mortalities or lesions in survival eggs.

Preparation of inactivated oil emulsion vaccine against swollen head syndrome:

The adjuvants used for preparation of water in oil emulsion consist of 88 parts paraffin oil, 10 parts span 80 (Sorbitan monooleate) and 2 parts Tween-80 (poly-oxyethylene sorbitan monooleate). All the components were sterilized by

autoclaving at 121°C for 10 minutes (15 lb pressure). The vaccine was prepared as described by **Thayer et al. (1983)**. 10 ml from the aqueous phase (inactivated virus plus tween) were added to 30 ml of oil phase while it was stirred and the mixture was emulsified for 10 minutes.

- Quality control of the prepared vaccine:

a. Purity and sterility tests:

The prepared vaccine was tested to prove its freedom from any contaminants according to **United State Code of Federal Regulation, USA (1987).**

b. Physical characterization:

The prepared vaccine was subjected to drop test (**Roshdy, 1996**), emulsion viscosity (**Becher, 1965**) and emulsion stability (**Cessi and Nordelli, 1973**).

- Evaluation of immune response:

1. Cellular immune response:

Assay of lymphocyte blastogenesis was applied according to **Lucy (1974)**. Evaluation of the test using MTT was performed according to **Mosmann (1983)**. Results of test were expressed as delta optical density (ΔOD).

2. Humoral immune response: Enzyme linked immunosorbent assay (ELISA):

It was done for estimating antibodies against SHS using commercial kit (Biocheck B.V. Catheth straat, 38c, Holland) according to **Grant et al. (1987).**

RESULTS AND DISCUSSION

Swollen head syndrome (SHS) is the disease caused by the avian pneumovirus. Avian pneumovirus is a member of the subfamily pneumovirinae, belonging to family paramyxoviridae, which is distinguished from other paramyxoviruses by the lack of both haemagglutinating and neuraminidase activities.

The hazard of the disease depends greatly on the extent of infection with other pathogens such as Pasteurella species and E. coli. A drop in egg production as highly as 70 % occurs in laying poultry and takes about three weeks to retain its normal level (**Stuart, 1989**). Studies on the use of oil adjuvant in the preparation of inactivated vaccines show that parentally inoculated antigens adjuvanted with oil emulsion generally stimulate higher and more

persistent antibody titres (**Zanella, 1969**).

Based on the previously reported studies, this work was conducted for the preparation of inactivated oil emulsion vaccine against swollen head syndrome disease.

The results of inactivation of SHS virus suspension by different concentration of formalin indicated that inactivation occurred completely with 0.5 % final concentration of formalin at 5 hours. This result agreed with that obtained by **Abd El-Rahman, (2003)** who stated that turkey rhinotracheitis (TRT) losses its infectivity to SPF ECE after 1-5 and 7 hours at a concentration of 1%, 0.5 % and 0.25 %, respectively.

Results of cellular immune response by lymphocyte blastogenesis assay as represent in table (2) revealed that maximum response of T-cells expressed as ΔOD were 0.162 and 0.153 at 14 days post vaccination then decline to 0.153 and 0.145 at 21 days post vaccination for groups (1 and 2) respectively compared with very low ΔOD of unvaccinated groups. These results agreed with **Timmes and Bracemell (1983)** as they stated that once the humoral immune response becomes established, there was accordance decrease in the cellular immune response.

Results of ELISA (table 3) revealed that the antibody titre reached peak value at 4th week post vaccination where the titre was 12016 and 10547 for group 1 and 2 respectively. At the 7th week post vaccination the antibody titers still high where it reached 2098 and 2079 for group 1 and 2 respectively. At the 8th week post vaccination, the antibody titre start to decline till reached its minimum level 846, 843 at the 10th week these results where accordance with **(Eteradossi et al., 1995)** who stated that ELISA has become the most practical method for evaluating the immunological response of poultry to various vaccination.

Table (1): Inactivation of SHS virus suspension by using different concentration of formalin

Hours of exposure	Final concentration of formalin		
	1 %	0.5 %	0.25 %
0	6.3 *	6.3	6.3
1	3.0	5.5	6.0
3	2	3.0	5.0
5	0	0	4.0
7	0	0	2.4
9	0	0	2.0
12	0	0	1.0
14	0	0	0
18	0	0	0

* Titre was expressed as $\log_{10}$ EID_{50}/ml.

Table (2): Results of lymphocyte blastogenesis assay on chickens vaccinated with the swollen head syndrome inactivated oil emulsion vaccine

Group	Weeks Post Vaccination				
	3 Days	1 Weeks	2 Weeks	3 Weeks	4 Weeks
1	0.094*	0.132	0.162	0.153	0.103
2	0.085	0.131	0.153	0.145	0.102
3	0.016	0.095	0.006	0.006	0.003

* Results expressed as delta optical density (ΔOD).

Group (1): Vaccinated with prepared inactivated oil emulsion SHS vaccine

Group (2): Vaccinated with imported inactivated oil emulsion SHS vaccine

Group (3): Unvaccinated control.

Table (3): ELISA titres in sera of chickens vaccinated with inactivated oil emulsion Swollen Head Syndrome Vaccine (SHSV)

Group	Antibodies titre									
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10
1	318	851	2941	12016	4689	3992	2098	1445	1263	846
2	263	828	2849	10547	4689	3952	2079	1413	1265	843
3	656	656	656	656	656	656	656	656	656	656

- Group (1): Vaccinated with prepared inactivated oil emulsion SHS vaccine

Group (2): Vaccinated with imported inactivated oil emulsion SHS vaccine

Group (3): Unvaccinated control.

Calculation of antibody titre:

$$S/P \text{ ratio} = \frac{\text{Mean of test sample} - \text{Mean of negative control}}{\text{Mean of positive control} - \text{Mean of negative control}}$$

Log 10 titre = 1.0(log10 S/P) + 3.52

Antilog = titre

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