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EVALUATION OF LOCALLY PREPARED EGG AND TISSUE CULTURE ADAPTED FOWL POX VACCINES FROM WP_1 STRAIN

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SUMMARY

Fowl pox virus WPI strain was passaged to 6 passage on SPE embryonated chicken eggs (ECE) and chicken embryo fibroblasts (CEF) for vaccine preparation. The maximum titre of the inoculated cultures were around the 5th and on the 4th day after ECE and CEF inoculation respectively. The prepared vaccines were safe, sterile and the best temperature for virus preservation was -20℃ . To compare the efficacy of the locally prepared vaccines with imported commercial vaccines; challenge of vaccinated birds by virulent virus was done which revealed protection 96% for ECE vaccine and 92% for CFE adapted vaccine. Moreover; seroconversion using ELISA and SNT proved better immunogenicity of the local ECE vaccine over the imported or CEF locally vaccines.

INTRODUCTION

Fowl pox has a world wide distribution and is caused by a DNA virus of the genus Avipox of Family Poxvirdae . Its incidence is variable in different areas because of differences in management and hygiene or the regularlity of vaccination.

The use of fowl pox virus (FPV) vaccines is indicated commerically in areas where the disease is endemic or on premises where infection has been diagnosed . Fowl pox vaccines of high potency and quality are now produced in different parts of the world by propagation of the virus in embryonated chicken (ECE).

However, the ECE can be contaminated by a variety of microbial egg born agents including viral, bacterial and mycoplasma species. The vaccine production must turn to use specific

pathogen free (SPF) eggs. This vaccine should contain the virus at a minimum concentration of 10^4 EID_{50}/ml (Winterfield and Hitchner, 1965).

Cell cultures have been proved to be a suitable system for the multiplication of many viruses and be able to offer a suitable replacer for the ECE.

Chicken embryofibroblast (CEF) is the most suitable cell culture for the multiplication of fowl pox virus and that the virus titre in CEF was one log10 lower than that in ECE (Soad, 1986). Vaccines prepared from fowl pox virus propagated in chicken embryo tissue culture were suitable for practical use and protection against field exposure (Bengelsdorff and Schneider, 1963).

Immunity could be established only when the virus is a live and that the cutaneous route was the most effective method for inoculation and usualy using a bifurcated needle (wing web route) (Mayr and Danner, 1976). This skin puncture technique gave a solid immunity within 2 weeks post vaccination (Jurado, 1947).

In Egypt, Sabban (1954) prepared fowl pox virus vaccine from whole CAM of infected ECE with the Beaudette strain of fowl pox. This vaccine was used for vaccination of chicken by stick wing web method. A modified poduction technique for fowl pox virus vaccine from CAM fraction was described (Crowther, 1963). Different researches proved that 4-6 weeks of age is a more suitable age for vaccination against fowl pox (Jordan, 1990; Tripathy and Reed, 1997) to avoid maternal immunity and to give the maximum benefit vacciantion response. So, in Egypt, prophylactic immunization has been adopted to use fowl pox virus vaccine at 6 weeks of age. The aim of the present work was to study:

The suitability of WP_1 strain of fowl pox virus for production of fowl pox virus vaccine by using SPF embryonated chicken eggs and chicken embryo fibroblast cell culture. The potency of the prepared egg ropagated and tissue culture adapted vaccines was studied by determination of the immune response of chickens vaccinated with the two produced vaccines in comparison with two commercial fowl pox vaccines using challenge, Enzyme Linked Immuno Sorbent Assay (ELISA), and Serum Neutralization Test (SNT) as criteria.

MATERIAL AND METHODS

1. Strains Used:

1.1. Vaccinal strains:

a. Egg adapted freeze dried WP_1 strain of fowl pox virus was obtained from Intervet International B.V. Lab. Boxmeer, Holland. The titre was $10^{7.3}$ EID_{50} /ml. This strain was used for production of SPF embryonated eggs and chicken embryo fibroblast adapted vaccines.

b. Diflose CT live freeze dried fowl pox vaccine Lot. No. 53N25 with glycerin solvent, was used in a dose of 10^3 $CCID_{50}$ / bird as imported tissue culture origin vaccine for field application by wing web method.

c. TAD freeze dried fowl pox vaccine, lot No. 951001 was used as imported egg adapted vaccine for field application by wing web method.

1.2. Challenge strain:

Virulent strain (locally isolated field strain) of fowl pox virus was used as challenge virus. It was isolated and identified by Sabban (1954). Its titre was $10^{5.7}$ EID_{50}/ml when determined in embryonated chicken eggs. It was used in a dose of 10^3 EID_{50}/bird.

2. Laboratory animals:

2.1. Embryonated chicken egg:

a. Nine days old, embryonated specific pathogen free (SPF) chicken eggs were used for the preparation of chicken embryo fibroblast (CEF) and embryonated chicken egg (ECE) adapted vaccines.

b. Commercial embryonated eggs 9-11 days old, were used for titration of different produced fowl pox vaccine and for serum neutralization test.

2.2. Experimental chickens:

Three hundred Leghorn broiler chickens obtained from United Company for Poultry Production as one day-old, were reared for 6 weeks old, then they were divided into the five respective groups and placed in isolated units to the end of the experiment. The birds were fed on a balanced commercial ration. The chicken groups were vaccinated with the various vaccines by wing web method.

3. Sera:

a. Hyperimmune serum:

Locally prepared anti-fowl pox serum in 6 unvaccinated chickens 4 months old was used.

b. Normal rabbit sera:

Serum samples were collected from healthy unvaccinated rabbits and prepared for usage as negative control sera.

4. Chemicals, and Buffers:

Antibiotics, Earle's minimum essential medium (MEM), bovine serum, and Trypan blue, phosphate buffer saline (PBS), and Trypsin solution 0.25% were used.

5. Fowl pox antigen:

According to Tripathy et al. (1970), fowl pox virus suspension (WP_1 strain) was prepared in ECE. The clarified virus suspension was used as antigen in ELISA (Mockett et al., 1987).

6. ELISA Kite:

IDXX laboratories, Inc., Maine, USA , was used.

7. Titration of the original virus strain on the chorioallanotic membrane of ECE: Titration was undertaken according to Dhillon et al. (1968).

8. Determination of fowl pox strain (WP_1) strain growth rate:

a. In chicken embryo fibroblast cell culture:

Chicken embryo fibroblast monolayer culture tubes were each infected with 0.1ml of tissue culture adapted virus (6th passage) with titre of 10 $TCID_{50}$/ml and a titre of 10 $TCID_{50}$/ml. After one hour incubation at 37℃, all tubes were fed with 2ml of maintenance media and incubated at 37℃. Two hours thereafter, 4 culture tubes were pooled to assay cell free, cell associated and whole culture virus infectivity, the remaining culture were further incubated at 37℃ and harvested at certain time intervals. The virus titration was carried out on the CAM. According to Mishra et al. (1995).

b. On embryonated chicken eggs (ECE):

The egg propagated fowl pox virus in a titre of 7.6 EID_{50}/ml was inoculated on the CAM of 11-12 days old ECE. The inoculated eggs were incubated at 37℃ for 6 days with daily candling and collection of CAM from 5 live inoculated eggs every 24 hours. The collected CAM were prepared to give clear virus suspension which assayed for virus titration in embryonated chicken eggs. According to Gunenkov et al. (1991).

9. Adaptation of fowl pox virus egg adapted strain to chicken embryo fibroblast cell culture: This was carried out according to El-Zein et al. (1974).

Sterility test:

The prepared vaccine was tested before and after addition of stabilizer and lyophilization for presence of either bacterial or mycotic contaminants.

10. Propagation of fowl pox virus in embryonated chicken eggs for vaccine production: This was carried out after Crowther (1963).

11. Titration of the vaccines:

In embryonated chicken eggs and On tissue culture cells: were adopted after Dhillon et al. (1968) and Villegas and Graham Purchase (1989); respectively.

12. Evaluation of the produced vaccines:

According to Seeliger and Price (1956).

a. Purity test: The vaccines were subjected to serological examination against non haemaggluting viruses (HA viruses) using specific antisera by the agar gel precipitation test (AGP) according to the technique of Ouchterlony (1962), and against HA viruses by rapid plate haemagglutination test

according to Anon (1971).

b. **Sterility test:** Sterility tests were done for detection of extraneous bacteria, salmonella, fungi and mycoplasma.

c. **Determination of the keeping quality;** It was designed to choose the best and available preservation temperature of the produced fowl pox vaccines.

d. **Biological safety test: Evaluation of the safety of the locally prepared fowl pox vaccines in susceptible birds:**

Fifty Leghorn chickens, 6 weeks old were used for evaluation of safety. Birds were divided into 5 groups; 10 birds each. The first and second groups were inoculated with the field dose from the tissue culture and egg propagated locally produced vaccines (by wing web route) contain not less than 10^3 EID_{50} dose (tissue culture vaccine $10^{3.9}$ EID_{50}/ml and egg propagated vaccine $10^{4.5}$ EID_{50}/ ml), respectively. The third and fourth groups were inoculated with 10 times the field dose from both locally produced vaccines. The fifth group of birds were kept as uninoculated control group. All birds were kept under observation for 21 days post inoculation for evidence of takes and for the absence of adverse effects attributable to the vaccine.

e. **The efficacy of the locally prepared fowl pox vaccines in susceptible birds:**

Two hundred and forty, 6 weeks old, susceptible broiler chickens were divided into 5 groups each of 50 chickens wree used. The first 4 groups were vaccinated with one of the tested vaccines (prepared tissue culture, prepared egg propagated vaccines, imported egg progated and imported tissue culture vaccines) using wing web staping method in one wing; respectively according to Seeliger and Price (1956), as 0.02ml of Fowl pox vaccine containing $2X10^6$ EID_{50}/ bird. The fifth group were left as unvaccinated control. Vaccinated birds were checked for takes at the 7th and 10th day post vaccination, the percentage of takes was calculated.

Four weeks post vaccination, half of each vaccinated and control chickens groups were challenged with standard challenge dose of local isolate virulent fowl pox virus containing $10^{3.7}$ EID_{50} per bird by wing web method in the other wing. The challanged birds were checked for lesions 10 days post challenge. The other half of each birds group were challenged 10 weeks post vaccination by the same method. The challenged birds were examined for takes at 5,7 and 10 days. Protection rate was calculated.

1. **Evaluation of Pathological changes:** This was adopted by take count.
2. **Protection percentage by challenge test:** This was carried out 4 & 10 weeks post vaccination, with avirulent strain of fowl pox virus by wing web method. Challenged birds were observed for 7-10 days. Any challenge reactions as pox lesions or takes were recorded.
3. **Evaluation of humoral immunity:**

A. Serum Neutralization Test (SNT): Fifteen serum samples were collected from each vaccinated group (pooled into 5 samples) before vaccination and weekly after vaccination (for 8 weeks), and post challenge (for 4 weeks). The method was that described by Hitchner et al. (1958). Serum samples mixed with different fowl pox virus dilution (serial ten fold dilution), in equal volume, then incubated at 37℃ for one hour, then inoculated on the CAM of 12 days old embryonated chicken eggs. Negative control eggs were inoculated by serum dilutions only. The inoculated eggs were examined 5 days after inoculation and the neutralizing index (NI) was calculated

B. Enzyme Linked Immuno-Sorbent Assay (ELISA): This carried out according to Buscaglia et al. (1985). It was performed on the pooled serum samples collected pre and post vaccination (for 8 weeks) and post challenge (for 4 weeks) of each group. The antibody titre was calculated by S/P ratio according to William (1987).

13. **Statistical Analysis:** In serological tests, the significance of differences between the mean neutralizing index and between mean S/P ratio in vaccinated birds groups was done according to Cochran and Cox (1960).

RESULTS

Obtained results are shown in tables 1-6.

1. The titre of the used WP_1 strain was $10^{7.3}$ EID_{50}/ml.
2. Results of multiplicity of input (MOI) for egg inoculation: showed that the optimal virus titre was obtained by MOI dose of $3X10^5$ EID_{50}/ml inoculum.
3. A. The virus reached maximum titre around the 120 hours post inoculation CEF which indicated that the best time for harvesting of the inoculated culture is the 5th day post inoculation. There was gradual increase in virus titre between 48 hours and 96 hours while a clear decrease began after 132 hours post inoculation. The result of titration of fluids and cells on the CAM indicated that the cell associated (intracellular) virus was approximately one log higher than the cell free (extracellular) virus. The titre of the virus in whole culture was nearly the mean value of both cell free and cell associated virus titre.

B. The highest virus titre appeared on the 4th day post inoculation on ECE which indicate the best

time post inoculation for harvesting of the inoculated eggs.

4. The titre of egg propagated vaccine in susceptible fowls was $10^{4.8}$ CID_{50}/ml while the tissue culture adapted vaccine was $10^{3.9}$ CID_{50}/ml.

5. **The results of keeping quality of the prepared fowl pox vaccines:**

a. At -20℃, there was no change in titre of both tissue culture adapted and egg propagated FPV vaccines either with or without stabilizer, during the period of the experiment (9 months).

b. At -4℃ there was no change in titre of both vaccines with sucrose/lactalbumin stabilizer, while there was slight decrease in titre started at the 4th month on tissue culture vaccine and from the 6th month on the egg propagated vaccine without stabilizer.

c. AT +4℃; in case of vaccines with stabilizer, there was slight decrease in titre, while in case of vaccines without stabilizer the drop of titre started as early as 4 months but of low degree in the egg propagated vaccine, and stareted from the 2nd month in the tissue culture adapted vaccine, the titre decrease gradually till the end of the 9th month.

6. **Results of safety test:** revealed that there was no symptoms in the birds vaccinated with 10 fold field dose more than that in birds vaccinated with the field dose in case of both tissue culture and SPF eggs locally prepared vaccines.

Birds of the control group showed severe reaction (takes) at site of inoculation on the 7th on the 10th day post inoculation.

7. **The results of efficiency of egg propagated and tissue culture adapted fowl pox virus (WP_1 strain) prepared vaccines compared with the imported field vaccines:** are shown in tables 1 & 2.

A. Pathological Evaluation:

Results of table (1) revealed that:

1. Most of vaccinated birds showed takes at the site of vaccination, the percent of takes at 10 days post vaccination were 96% at birds vaccinated by the fowl pox egg propagated vaccine and imported tissue culture adapted vaccine (RM) while the tissue culture local adapted vaccine gave 90% takes and the imported egg propagated (TAD) vaccine gave 86% takes, which mean less response and efficacy than the other groups.
2. In vaccinated birds challenged 4 weeks post vaccination there was one bird from 25 birds had lesion at the site of inoculation in E and RM groups, with 96% protection. While, there were lesions on two birds from 25 birds vaccinated by tissue culture vaccine (92.0% protection), and on three birds from 25 birds vaccinated by TAD vaccine (88.0% protection).

Table (1): Take counts after vaccination of the various experimental groups.

Bird group	No.of chicks/ group	Number of birds showing takes				Percent of reaction
		7 days post vaccination		10 days post vaccination		
		+	-	+	-	
E	50	46	4	48	2	96%
TC	50	42	8	45	5	90%
TAD	50	38	12	43	7	86%
RM	50	47	3	48	2	96%
Control	40	0	40	0	40	0%

E : Local egg propagated vaccine.
TC : Local TC adapted vaccine. Ø
TAD: Imported egg propagated vaccine.
RM : Imported TC adapted vaccine.

B- Protection evaluation:

In table (2) the results protection evaluation revealed that:

1. Birds challenged 10 weeks post vaccination showed slight decrease in percent of protection in different degrees but still in permissible limit of protection 85% in imported egg propagated and local tissue culture adapted vaccines, and 90% in imported tissue culture adapted and local egg propagated vaccines.
2. All birds of the control unvaccinated group showed severe pox lesion and takes at site of inoculation with generalization in some birds in form of nodular lesions on the eyelids and around the peak.

Table (2): Results of challenge test in birds vaccinated with different pox vaccines.

Challenge time	Bird group	No.of challenged birds/group	No.of birds showing lesion post challenge			Protection percent (%)
			5dpc	7dpc	10dpc	
4 WPV	E	25	-	1	1	96%
	TC	25	-	1	2	92%
	TAD	25	-	2	3	88%
	RM	25	-	-	1	96%
	Control	20	15	20	20	0%
10 WPV	E	20	-	1	2	90%
	TC	20	-	1	3	85%
	TAD	20	1	3	3	85%
	RM	20	-	2	2	90%
	Control	15	12	15	15	0%

dpc :days post challenge.
WPV :Weeks Post Vaccination.
E : Local egg propagated vaccine.
TC : Local TC adapted vaccine.
TAD: Imported egg propagated vaccine.
RM : Imported TC adapted vaccine.

C. Humoral imunity Evaluation;
1. Serum Neutralization Test (SNT):
1. Pre-and Post-Vaccination:

Table (3): Results of SNT on sera of birds vaccinated by the locally prepared and imported egg propagated FPV vaccine.

Weeks post vaccination	Mean of neutralizing index				
	EV	TAD	Control	TCV	RM
0 day	0.0	0.0	0.0	0.0	0.0
1 week	1.3±0.26	1.1±0.20	0.0	1.2±0.43	1.3±0.26
2 week	1.6±0.36	1.5±0.43	0.0	1.7±0.43	1.8A±0.50
3 week	2.4±0.20*	1.9±0.44	0.0	2.0±0.26	2.2±0.30*
4 week	2.2±0.30*	1.9±0.36	0.0	1.9±0.26	2.0±0.17
5 weeks	1.9±0.26	1.7±0.42	0.0	1.8±0.20	2.0±0.26
6 weeks	1.9±0.17	1.5±0.35	0.0	1.8±0.43	1.9±0.46
7 weeks	1.8±0.21	1.4±0.44	0.0	1.6±0.30	1.9±0.36*
8 weeks	1.8±0.26	1.4±0.46	0.0	1.6±0.40	1.8±0.46

* Significant difference at $P<0.05$.

In table (3) Results showed that antibodies appeared from the first week PV and the neutralizing index (NI) reached the maximum titre (2.4 and 1.9) on the 3rd week for each the locally prepared (E group) and imported TAD vaccine . In the comparison of the local vaccine and TAD vaccine, the NI in E group was higher than in TAD group specially during 3rd and 4th weeks which was statistically in a significant degree.

In the comparison of the local prepared tissue culture vaccine (TC group) and the imported RM vaccine (RM group). The neutralizing antibodies appeared from the first week PV and reach to the maximum titre (2.0 and 2.2) on the 3rd week PV. The NI in RM group was higher than in the tissue culture group specially during the 3 rd and 7th weeks PV, it was of significant degree.

There was significant difference in neutralizing antibody response between groups of vaccinated and control birds from the first week post vaccination.

2. Post Challenge (PC):

Table (4): Results of SNT in challenged birds 4 weeks after vaccination.

Weeks post vaccination	Mean of neutralizing index				
	EV(local)	TAD(import-	Control	TC (local)	RM(imported)
1 week	1.4±0.46	1.2±0.26	1.0±0.17	1.3±0.40	1.4±0.44
2 week	2.2±0.27*	1.9±0.26	1.6±0.26	2.0±0.30	1.9±0.17
3 week	2.9±0.40*	2.4±0.31	2.0±0.18	2.5±0.21	2.6±0.26
4 week	3.2±0.18*	2.6±0.36	2.1±0.10	2.9±0.46	3.1±0.26

* Significant difference at P<0.05.

Results of table (4) revealed that all vaccinated challenged birds showed slight decrease in the NI at the first week PC, then increase gradually till the end of the experiment (4th week).

2. Solid Phase ELISA:

1. Pre-and Post-vaccination (PV):

The results of table (5) showed that the antibodies appeared from the 1st week PV, and increase till reached the maximum ratio (1.932 and 1.462) at 4th week PV, then decreased gradually till the 8th week PV (1.459 and 1.243) in both the EV group and TAD group.

Table (5): Results of ELISA in sera from chickens vaccinated with the locally prepared and imported egg propagated FPV vaccine.

Weeks post vaccination	Mean of S/P ratio				
	EV	TAD	Control	TC (local)	RM (Imported)
0 day	0.301±0.004	0.301±0.004	0.301±0.004	0.301±0.004	0.301±0.004
1 week	0.600±0.026*	0.411±0.077	0.305±0.020	0.479±0.156	0.583±0.117
2 week	1.252±0.067*	1023±0.269	0.311±0.007	1.083±0.162	1.104±0.191*
3 week	1.693±0.188*	1.262±0.232	0.309±0.026	1.208±0.183	1.548±0.279*
4 week	1.932±0.334*	1.462±0142	0.315±0.016	1.797±0.130	1.991±0.091*
5 weeks	1.772±0.305*	1.354±0.127	0.324±0.014	1.715±0.104	1.800±0.092*
6 weeks	1.554±0.161	1.327±0.328	0.308±0.013	1.461±0.233	1.653±0.162*
7 weeks	1.517±0.233	1.243±0.277	0.317±0.011	1.293±0.193	1.596±0.105*
8 weeks	1.459±0.231	1.243±0.343	0.320±0.021	1.268±0.274	1.573±0.323*

* Significant difference at P<0.05.

S/P ratio: samples positive ratio (above one consider protective).

Table (6): Results of ELISA in sera of chickens Challenged 4 weeks post vaccination with the local prepared and imported FPV vaccine.

Time post challenge	Mean ofS/P ratio				
	EV	TAD	Control	TC(local)	RM (imported) (imported)
1 week	0.950±0.104*	0.717±0.058	0.855±0.008	0.739±0.063	0.909±0.027*
2 week	1.216±0.095*	1.065±0.124	1.308±0.045	1.168±0.172	1.221±0.036
3 week	1.980±0.051*	1.659±0.167	1.595±0.023	1.642±0.284	1.715±0.231
4 week	2.121±0.166*	1.917±0.107	1.735±0.022	1.931±0.093	2.014±0.109

* Significant difference at P<0.05.

In the comparison of the local and the imported FPV vaccine. The ELISA titre in the (E) group was higher than the TAD group and it was statistically significant from the first week PV till the 5th week PV.

2. Post challenge (PC):

Resutls of table (6) in comparison of the local vaccine (TC group), and the imported vaccine (RM group), the ELISA titre in RM group was significantly higher than in the tissue culture group from the 2nd week PV till the end of the experiment (8th week). There was significant differences in ELISA values between groups of vaccinated and control birds from the first week post vaccination till the end of the experiment (8th week).

DISCUSSION

It was recognized during the year 1920 that vaccination was an effective mean of disease controlling and the immunity could be establish only when the virus is a live and that the cutaneous route was the most effective method of vaccination (Tripathy and Reed 1997). However, due to the fact that embryonated eggs can be contaminated by a variety of microbial agents which are vertically transmitted from the mother hen to the egg. This study was designed as a trial to produce FPV vaccine locally by using SPF eggs with new pure FPV strain in order to produce a pure, potent and safe vaccine and measure its efficency to give good immune response.

In the present study, FPV vaccine was prepared in SPF eggs and used in the recommended dose and wing web route comparing it with commercial one, to assure that the imunizing dose will be delivered and consequently give a great stimulation for resistance in vaccinated chickens. Fowl pox vaccines of high potency and quality are now produced in different parts of the world by propagating the virus in embryonated chicken eggs.

Cell cultures have also proved to be a suitable system for the multiplication of many animal viruses, so it offers a suitable replacer for embryonated eggs. So, we tried to adpat the used strain on CEF and to prepare batches of FPV vaccine under the optimal condition as described by Soad (1986).

Fowl pox vaccines must contain at least 10^5 EID_{50}/ml (Winterfield and Hitchner, 1965). Starting with these ideas, the first experiment was designed to test the potency of the used FPV strain (WP1 strain) to produce the pure local vaccine.

The FPV vaccinal strain was titrated before use and it was $10^{7.3}$ EID_{50}/ml and in order to maximize the virus yield from inoculated ECE, it was essential to study the effect of various multiplicity of input (MOI) on the final virus titre.

Orlando et al. (1967) investigated the relation between the virus out put and the MOI and the optimal MOI ratio which gave maximum virus output. In our study, the results revealed that the maximum virus output was resulted when the ECE inoculated by virus suspension containing 3 X 10^5 EID_{50}/ml.

The results of the titre of different prepared vaccine passage in both ECE and susceptible birds showed that the FPV egg propagated vaccine reached its maximum titre in the 5th passage in ECE and it was $10^{7.6}$ EID_{50}/ml. It was higher than in susceptible chicks ($10^{4.8}$ CID_{50}). While, the tissue culture adapted vaccine (6th passage) titre was $10^{6.9}$ CID_{50} and gave higher titre than in susceptible chicks ($10^{3.9}$ CID_{50}). These results showed a difference of 2.8 logs and 3.0 logs respectively. This may be attributed to the relative higher susceptibility of the chicken embryos and cells than the susceptible birds. This findings are similar to those previously recorded by Sokker et al. (1967). However, the tissue culture adapted virus titre rather low ($10^{6.1}$ EID_{50}/ml) than the egg propagated virus vaccine ($10^{7.3}$ EID_{50}/ml) in the first passage, the virus showed gradual adaptation on the cell culture during the different subsequent passage till reached titre of $10^{6.9}$ EID_{50}/ml on the 6th passage. This rise in virus titre with increasing passages gave hope that by more passages a higher titre and more adaptation may be obtained.

In the second step the results determined the growth curve of the fowl pox WP1 strain after being adatped on the CEF measured by using the CAM inoculation. The results indicated that a logarithmic increase in virus titres started from the 2nd day after inoculation with maximum titre on the 5th day. Then a clear decrease occurred from 120 to 144 hours post inoculation which indicated that the best time for vaccine harvesting was the 5th day post inoculation. This agree with

El-Dahaby et al. (1971), Michael (1981) and Soad (1986) who found that the highest titers of FPV propagated in CEF cell culture increased gradually until the 5th day post inoculation when it reached its maximum growth. On the other hand, Gafford et al. (1969) and Rai and Sethi (1972) reported that peak titre of FPV in CEF reached 72 hours post inoculation. The logarithmic difference between the titre of the cell-associated and cell free virus is quite high (about 1-1.8 $\log_{10}$) but it was preferable to include both cell associated and cell-free virus in the harvested material for vaccine preparation. As in primary culture of CEF most of the virus remained cell-associated throughout the multiplication period of 120 hours as reported by Michael (1981).

Maiti et al. (1991) reported that the extra and intracellular viruses have difference in their antigenic make up which help in the development of imunogenicity. Also, Fernands et al. (1981) reported that extracellular virus of FPV was more immunogenic than intracellular viruses which is due to presence of excess antigenic protein.

The growth curve of the FPV-WP1 strain after propagation on the embryonated chicken eggs, indicated that there was a gradual increase in virus titers till reach its peak on the 4th day post egg inoculation which is the best time to obtain the highest virus titre in CAM collected for vaccine preparation. These results differ with Haig (1951) who described the technique of FPV growing in embryonated eggs and the inoculated eggs were harvested for vaccinal material preparation at 5 days post inoculation.

The prepared vaccine was free from any pathogens after virological, bacterial, and mycological examination. The results cleared that sucrose-lactalbumin was a good preservative stabilizer where it causes no significant loss in the titre at the (+4℃) which was ranged between (0.1-0.4 log), respectively. While, in case of (-20℃ and-4℃) there was no loss in titre till the end of the experiment at 9 months.

The results of preservation at -4℃ were almost similar for FPV with and without stabilizer. The dropping in the titre at end of the experiment at 9 months ranged between (0.1-0.4) logs for FPV with stabilizer at +4℃, respectively. Whle, it ranged between (0.3-1.1) logs for that without stabilizer at - 4℃ and 4℃.

There results agree with Mayr (1962) who recorded that the addition of 5% saccharose and 1% fat-free milk powder prolonged keeping quality of poultry pox viruses up to 57 weeks. From these findings, it was clear that the stabilizer played an important role in the long life of the vaccines.

There was post vaccinal reaction in form of takes at site of inocuation from 7-10 days post vaccination in case of field dose and 10 times the

field dose for both local and imported vaccines. This post vaccinal reaction disappeared on the 15th day i.e the prepared vaccine was safe and not have severe post-vaccinal reactions.

Since takes reflect the ability of the virus to multiply at the site of inoculation leading to the production of skin lesions. They may be taken as a measure for virus pathogenicity for chickens.

Our results in table (1) showed that chicken vaccinated with the locally prepared egg propagated vaccine gave 96% takes within 7-10 days after vaccination, while the tissue culture adapted vaccine gave 90% takes which indicated the higher immunogenicity of egg propagated vaccine than the tissue culture adapted one. In the same manner, the tissue culture adapted imported commercial vaccine gave 96% takes in vaccinated birds which showed the same percentage resulted from locally prepared egg propagated one. The egg propagated imported vaccine gave 86% takes which was lower than the locally prepared one. This showed that the locally prepared egg propagated vaccine is more potent than the imported one and the locally prepared tissue culture adapted vaccine. Beaudette (1949) reported that the development of takes reactions was important in the production of immunity against fowl pox. Seeliger and Price (1956), Bengelsodroff and Schneider (1963), and Saini et al. (1990) observed a close correlation between the occurrence of take at the site of inoculation and the immunity gained on vaccinated birds. Regarding our aforementioned results it is quite clear that the prepared vaccines accord with the Code of Federal Regulations (Animals and Animal Products), prepared vaccine.

Table (2) showed the results of challenging the vaccinated and control chickens with the local virulent fowl pox virus, 4 weeks after vaccination gave 92% and 96% protection (no local take reaction) in chickens vaccinated with the locally prepared tissue culture adapted and egg propagated vaccines, respectively. The imported vaccines gave 96% and 88% protection with the tissue culture adapted and egg propagated vaccines, respectively. This indicated that the locally prepared egg propagated fowl pox vaccine provided superior protection compared to the locally prepared tissue culture adapted one and the imported egg propagated one. The control birds developed positive take lesions at the site of inoculation and systemic skin lesions in some of the control challenged birds. This results agree with those reported by El-Dahaby et al. (1971), Rai and Sethi (1972), El-Zein et al. (1974), Mockett et al. (1990) and Tripathy and Reed (1997) who reported that at least 80% of the vaccinates should be protected from challenge infection to consider the vaccine potent. While, control birds show lesions.

Birds challenged 10 weeks after vaccination showed a slight decrease in protection percent

which ranged from 85% up 90%. It indicated slight decrease in immunity of chickens against fowl pox virus. Seeliger and Price (1956) reported a significant decrease in immunity between the 40 and 80 days post vaccination challenge. While, all birds that had takes at vaccination were immune at 40 days and 92% at 80 days. Sarma and Sharma (1988) recorded 50-80% protection after vaccination with different FPV vaccinal strains for 8 weeks old birds using different routes of vaccination. The higher protection rate in the present study might be due to difference in the used vaccinal and challenge strain of FPV and in the age of vaccination.

The humoral immune response in vaccinated birds was measured by SNT, ELISA.The SNT was considered the test of chioce for detection of antibodies for FPV which has been applied by Michael (1981). Results of evaluation of the humoral immune response of chicks by the SNT were shown in tables (3 and 4). It is revealed that the neutralizing antibodies were detected on the different vaccinated groups at the end of the first week post vaccination (PV) which disagree with Pilchard et al. (1962). who found that neutralizing antibodies were detectable beginning 2 weeks after initial vaccination. The neutralizing index (NI) was in average (1.2 and 1.3) in case of local prepared tissue culture and egg propagated vaccines; respectively, and about (1.1 and 1.3) in case of imported egg propagated (TAD) and tissue culture adapted (RM) vaccines; respectively. The NI was increased till reach their maximum (1.9-2.4) at the end of the 3rd week post vaccination in all the vaccinated groups peak at the end of the 3rd week PV.

The NI either remained unchanged (in chicken vaccinated by TAD vaccine) or they started to decrease gradually in very low levels which can be neglected as it could not affect the immune status of the vaccinated birds. These findings are similar to those results previously recorded by Dhanesar and Malik (1983) as neutralizing antibody was ranged between log 1.0 and 2.6 during 2nd, 3rd and 4th weeks of vaccination then decreased to about log 1.0 after 8 and 12 weeks after vaccination.

Higher titre were found in the groups vaccinated with the local egg adapted and imported tissue culture adapted (RM) vaccine, while the lower titers were observed in the groups vaccinated with local tissue culture adapted vaccine and the imported egg propagated (TAD) vaccine. This findings correlated to the percent of the post vaccinal reaction (take percent) observed with these vaccines.

In general, these results were in agreement with the observation of Tripathy et al. (1970) who reported that the production of take was important in the production of immunity and neutralizing antibodies.

The results of post challenge, illustrated in tables (5,6) showed that in the vaccinated challenged chickens, the neutralizing antibodies level was decreased during the first week after challenge, then started to increase gradually from the 2nd week until the end of the experiment (4th week) in all vaccinated group. While, in the control positive chickens (non vaccinated challenged birds), showed gradual increase in the neutralizing antibody.

The ELISA test was used to assay the antibody response to the FPV locally prepared and imported vaccines as shown in tables (7,8,9,10). There was a significant increase in the ELISA antibody titers in all four vaccinated group receiving the local and imported vaccines than those of the control group from the first week post vaccination with low mean titers (0.411-0.600) then began to increase gradually: At the 4th week post vaccination, the ELISA antibody titers reached its maximum levels among all vaccinated groups with mean value (1.767-1.932) in chicken vaccinated with local prepared vaccines and (1.991-1.462) in case of imported tissue culture adapted and egg propagated vaccines respectively. Then, declined gradually but remained in desirable protective range until the end of the test period (8th week post vaccination).

There was a significant increase in ELISA titre in group of chicken received local egg propagated vaccine than the group received the imported one from the first week to the 5th week post vaccination. Also, there was a significant increase in ELISA titre in group received the imported tissue culture adapted vaccine than that received the local one from the 2nd week post vaccination till the end of the test period (8th week post vaccination).

As pointed out by Nagy et al. (1990), the serum ELISA antibody titers correlated with protection against experimental infection of FPV and the degree of the immunological response with vaccination can be judged from the serum mean ELISA antibody titers. Our results revealed that the locally prepared egg propagated vaccine gave superior protection than the imported one and than the locally prepared tissue culture adapted vaccine. This results agree with Dhanesar and Malik (1983) who reported that the infected CAM incorported with allantoic fluid from infected embryonated chicken egg gave better immunity and antibody response than the vaccine of cell culture origin.

The obtained results of serological tests in different vaccinated groups and non vaccinated control chickens suggested that the correlation between ELISA antibody titers and SNT antibody titers were approximately regular. These results agree with Lee et al. (1994) who reported that the correlation rate between SNT and conventional ELISA results was 93.4%.

Whereas, ELISA is more sensitive and producible than the SNT (Buscaglia et al., 1985), and that earlier detection of antibody by the ELISA than the VN test was confirmed by previous finding (Nagy et al., 1990). This disagree with our results as SN antibodies was early detectable from the 1st week post vacination in higher titre than the ELISA antibody, while ELISA gave obvious significant variation between the vaccinated groups.

Consequent of the previous results, FPV adapted in CEF cell culture and embryonated chicken SPF eggs using WP1 strain proved to be potent, safe , and capable to protect birds against challenge with virulent virus using the wing-web route. While, the locally prepared egg propagated vaccine gave better immune response and protection percent compared with the imported one and the locally prepared tissue culture adapted one. The local tissue culture adapted vaccine gave lower protection percent and serological results than the improved one which means that it needs more adaptation on cell cultures.

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