Egypt. J. Vet. Sci. **Vol. 39**, pp.21-35 (2005)

Preparation of Cell-Culture Inactivated Infectious Laryngotracheitis Virus Vaccine Adjuvanted with *Nigella sativa* Oil

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ISOLATED and identified infectious laryngotracheitis virus was inactivated by (BEI) and tested for its sterility, safety and potency then the inactivated ILT virus was mixed by addition of oil adjuvant, which consists of Nigella sativa and paraffin oil (PANISA oil) in the ratio of 1: 1. Two vaccines were made, the first where the aqueous phase to the oil phase was 1: 4 while the second vaccine where the ratio was 1: 2. Quality control tests were made such as drop test, safety and sterility test. The emulsion was oil in water, safe and free from bacterial, fungal and Mycoplasma contamination. Three experiments were made; the first where the vaccines were giving to chicken and the antibody pattern during 16 weeks were demonstrated using ELISA. The second experiment where comparison between the inactivated vaccines and the live attenuated vaccine was done. The third experiment included three successive challenges with four weeks intervals. Vaccines prepared adjuvanted with Nigella sativa oil produced higher humeral response than live attenuated vaccine.

Infectious Laryngotracheitis is one of the most serious respiratory diseases affecting poultry industry in Egypt. The survived cases failed to gain the presumed body weight in case of meat production and drastic decrease in egg production during the disease course in case of flocks for egg production as the disease infect poultry at any age. The signs of the disease are usually acute with high flock morbidity. Gasping follows nasal discharge, lacrimation and moist rales; in the later stages dyspnea occurs with extension of the head and neck. In acute cases and depending on the virulence of the virus, expectoration of bloody mucus may occur. In some affected flocks, this sign was so prevalent that the walls and equipment were spattered with dried blood (Bagust, 1986 and Bagust, *et al.*, 2000). Prophylactic vaccination has been adopted by the use of imported live attenuated ILTV vaccine. Problems may occur due to vaccination with a live virus vaccine, such as the spread of virus and perhaps disease production as it may fail to provide immunity to most birds in the flock and spread of the vaccine virus to non-vaccinated flock (James, *et al.*, 1990). Moreover, increased virulence of the virus may occur due to bird-to-bird passage (Guy, *et al.*, 1991) as well as creation of carrier birds (Williams, *et al.*, 1992).

Nigella sativa was recommended in preparation of oil adjuvant as a very powerful immuno-stimulant used to induce cellular immune response (Madbouly and Tamam 2000; Madbouly, *et al.*, 2001; and 2002). *Nigella sativa* oil extract stimulate T-cell specially T_h and enhancing interleukin production which in turn stimulate macrophages. In addition, Nigella sativa oil act directly on stimulation of macrophage and initiating immunity mediated by NK cells and IFN-gamma (Salem and Hussain 2000).

A trail has been succeeded using inactivated ILT virus vaccine adjuvanted with *Nigella sativa* oil as a non-specific immunostimulant adjuvant that have the ability to activate the immune system of the vaccinated birds (Madbouly and Tamam, 2000). However this vaccine is expensive due to the use of specific pathogen free embryonated chicken egg (SPF-ECE) and the expensive Nigella sativa oil alone.

We aimed to produce a low cost inactivated vaccine through producing a tissue-culture–adapted vaccine to yield large seed of the virus and by using mineral oil with *Nigella sativa* in the ratio of 1: 1.

Material and Methods

Virulent virus

Hot strain of ILTV was isolated from tracheal tissue showing hemorrhagic inflammation taken from broilers, located at Cairo and El-Qaluobia governorates, suffered from bloody coughing and various respiratory disorders. The virus was characterized and identified by Electron microscopy, Dot-ELISA and serum neutralization test (SNT) (Zaher, 2004).

Virus vaccine

Tissue culture attenuated infectious laryngeotrachietis virus (ILT Ivax, American Scientific Laboratories Schering Corp., Cream Ridge,N.J.,Batch no 32935). It was used for vaccination of chicken.

Chicken

Total of 425 Baladi layered- 4 weeks old were used as follow: Fifteen chickens for studying the potency, ten for safety, about 400 chickens (100 injected I/M with 0.3ml of vaccine1, 100 for vaccine 2, 100 for live attenuated vaccine as eye drops, 50 non-vaccinated non-challenged, 50 non-vaccinated challenged). The sera of these chickens before vaccination were proved free from ILTV antibodies by SNT and ELISA according to Madbouly, (1989). Challenge tests were performed after four weeks post vaccination for three times with four weeks intervals.

ILT- hyperimmune serum

It was kindly supplied by Zaher (2004). This serum was prepared by injecting Egg adapted live attenuated vaccine (TAD ILT vaccine 1000 ds, Lohman animal

GMH& coikg, Heinz- Lohman str.4-2-7471 Couxhiven Germany, Batch no 1071121) S/C in 4 weeks old chicken.

Inactivation

The locally isolated strain of ILT virus, was propagated on Vero cells and when the virus titer reached 7.56 Log10/ml, it was seeded for virus inactivation by Binary Ethyleneimine solution (BEI) according to (Barhoom, *et al.*, 1986)

Preparation of vaccine batch

The inactivated virus vaccine consisted of aqueous phase and oil phase; the aqueous phase was containing 95% inactivated ILT suspension mixed with 5% tween 80. While the oil phase (PANISA oil) was consisted of 1 part Nigella sativa oil and 1 part mineral oil where mineral oil part consisted of 90% mineral on 10% of span 80. The stable emulsion was prepared according to (Stone, *et al.*, 1978). The vaccines were prepared as follow: Vaccine no 1 where the aqueous phase to the oil phase in a percentage 1:4. While vaccine no 2 the percentage was 1:2.

Quality control tests

The prepared vaccine was subjected to a serial of tests according to The USDA code of Federal regulation 9CER (1987) testing 113026, 113.27, 113.30 to be free from any bacteria, mycoplasma, and fungi (El sayed 1999). These quality control testes include: Drop test: where drops of the prepared vaccines were dropped on the surface of water to determine the emulsion type. Other testes Sterility test, safety test, efficacy tests as well as potency tests. For studying the safety of the inactivated ILT virus vaccine, it was inoculated in 10 SPF-ECE of 11-12 day old via CAM rout; intratracheally into 5 chickens of one month old, 0.2 ml per chicken and another 5 chicken were kept untreated as control; and on Vero cell culture. The inoculated host systems were remained under observation for 5 days (ECE), 10 days for observing clinical signs and P.M, lesion for chicken, 10 days for CPE on Vero cells. For studying the potency of the inactivated virus suspension: 15-chickens were divided into two groups. One group of 10 chickens were injected by 0.3 ml for each chicken I/M by the inactivated virus suspension and the other 5 chicken kept as control group unvaccinated. The two groups were inoculated intratracheally by 1000 $TCID_{50}$ of hot strain of ILTV, 4 weeks later and kept under observation for 10 days.

Evaluation of the prepared virus vaccine

Serum samples of all used chicken for vaccine evaluation were taken weekly and kept at –20°C till tested by ELISA which was performed according to (Madbouly, 1989).

Challenge tests

Every month after vaccination 10 chickens were taken from each group of the first three groups and challenged with 1000 $TCID_{50}$/1ml by intratracheal route. The fourth group unvaccinated and challenged, was treated as the other three groups, while the fifth group was kept unvaccinated unchallenged as control group.

Result

The infectivity titer of ILT virus was determined before and after treatment with BEI. The obtained results are presented in Fig. 1. The infectivity titers of ILT virus were decreased gradually after treatment of the virus with BEI. It reached 5.33 log_{10} after 20 hours of treatment and (0 log_{10}) after 24 hours of treatment.

The result of drop test and safety test are summed in Table 1 and result of sterility tests was included in Table 2 while results of protection test are mentioned in details in Table 3. The prepared vaccines were safe and have good emulsification. It offered good protection for vaccinated birds against challenge with virulent ILT virus.

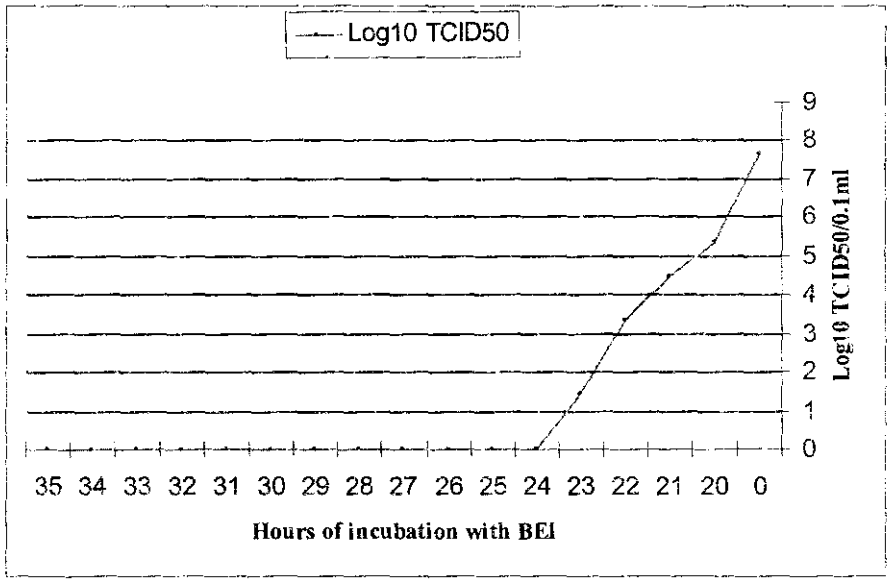

Fig.1. Inactivation curve .

TABLE 1. Results of drop test and safety tests of prepared inactivated ILT virus vaccine .

Test	Result
1-Drop test	Oil on water (the drops remain discrete over the surface of water).
2-safety test: a-on Vero cells b-on ECE c-on chicken	 No CPE. No pock lesion and no stunting of inoculated chicken embryos. Neither symptoms nor P.M. lesion.

TABLE 2. Result of sterility test for inactivated ILT virus .

Types of used medium	Type of microorganism	Result
Nutrient agar	Aerobic bacteria	-ve for bacterial growth
Mac-Conkey agar	Aerobic gram –ve bacteria (*Enterobacteriaccae* and *Enterococcus)*	-ve for bacterial growth
Meat extract agar	Anaerobic bacteria	-ve for bacterial growth
Sabaroud dextrose agar	Fungi	-ve for fungal growth
PPLO's agar	Mycoplasma	No colonies

TABLE 3. Results of protection (potency) test.

Group	No of birds	After infection		
		No of birds show no signs	No of birds show signs	No of died birds
Vaccinated	10	10	0	0
Control	5	0	5	5

Results of Vaccination Using ELISA

Data presented in Table 4 showed clearly that the live attenuated vaccine elicited higher antibody titers at the 4^{th} week P.V. (17256). Then felled down at the 5^{th} week P.V. (5617) and gradually decreased to 2370 at the 7^{th} week P.V., then increased abruptly to 2306 at the 9^{th} week P.V., then decreased gradually till the 16^{th} week P.V. While group II that received N.S. vaccine (1:2) induced, the highest titer that increased gradually from 1^{st} week post vaccination till the 4^{th} week P.V. (24988) then decreased gradually in a sloping manner till the 16^{th} week post vaccination. Group I showed antibody titer parallel to that induced by group II but the antibody titers were lower than that of group II.

Results of the Challenge Tests

a- Results of the first challenge

The level of antibody titers during the first challenge in-groups I and III are parallel to group II but the antibody titers is lower than group II. The antibody titer in-group III become decreased to (348) in the 2^{nd} week post challenge then

elevated gradually and reached to maximum level at 5^{th} week post challenge then decreased also gradually and become not detectable at 3^{rd} month post challenge. Group V (non-vaccinated not challenged) did not show any detected antibody titer during the period of the experiment Table 5.

b- Results of the second challenge

In Table 6 showed gradual decrease on antibody titer from the 1^{st} Wk.P.C.till the 2^{nd} week post challenge (Wk.P.C.) and then gradually increase and reached the maximum level at the 4^{th} week post challenge in all 3 groups (I, II, III) then gradually decreased to the 8^{th} week post challenge. Group II showed the highest titer other than the groups I and III.

c- Result of the third challenge

Data presented in Table 7 showed that the antibody titers in the 1^{st} week post challenge showed gradual decrease in its titer till the 4^{th} week post challenge in all first three groups. The second group showed higher antibody titer than groups I and III.

Data presented in Table 8 showed that all birds in the first 3 groups were 100% protected after 1 month of vaccination when challenged by virulent ILT virus. While the protection % decrease to 80% and 50% in the third group at the 2^{nd} and 3^{rd} challenge whereas it remained constant with 100% protection for group I and II in during the same challenges.

Discussion

The identified ILTV was subjected for a process of inactivation using binary ethylineimine (BEI) that used for nucleic acid inactivation without any change in the viral protein configuration. Treated samples with BEI were taken every one hour after 20 hours post treatment as previous studies stated that complete inactivation of ILTV occurred 24 hours post treatment (Barhoom, *et al.*, 1986; Madbouly and Tamam, 2000). The inactivation of ILT virus was confirmed by virus titration on tissue culture cells, on ECE and inoculation of chickens, where no CPE, pock lesion or signs were observed on any of the inoculated hosts after 24 hours of inactivation (Table 3). Moreover, the inactivated virus was tested for sterility (free from bacterial, fungal and Mycoplasma contaminants) there was no colonies appeared on any of the used medium (Table 4). The inactivated virus was subjected for vaccine preparation by mixing the aqueous part (inactivated virus) with the oil part. The oil part consisted of equal volumes of *Nigella sativa* oil (which are very immuno-stimulant) with mineral oil (paraffin oil). Although chickens can be successfully vaccinated as early as 1 day old, chickens less than 2 weeks of age do not respond as well to vaccination as do older birds (Hanson and Bagust, 1991). Therefore chickens on this study were vaccinated at the age

TABLE 4. ELISA antibody titers of vaccinated and control chicks

Group*	Result	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week	13th week	14th week	15th week	16th week
I	S/P**	1.757	1.896	2.281	2.292	1.451	1.444	1.201	1.163	1.009	0.911	0.787	0.511	0.484	0.384	0.203	0.130
	AB titer	12048 ±216	13456 ±230	17590 ±628	17717 ±650	9125 ±168	9064 ±172	6937 ±204	6623 ±187	5397 ±171	4646 ±68	3759 ±72	2010 ±26	1865 ±27	1328 ±41	526 ±24	277 ±19
II	S/P	1.656	1.661	1.673	2.906	1.789	1.348	1.080	0.897	0.690	0.511	0.475	0.448	0.435	0.250	0.168	0.133
	AB titer	11057 ±124	11103 ±189	11226 ±462	24988 ±701	12369 ±309	8200 ±90	5945 ±106	4542 ±94	3106 ±84	2010 ±59	1806 ±45	1662 ±24	1589 ±23	710 ±15	399 ±16	284 ±13
III	S/P	0.524	1.246	1.397	2.251	1.038	0.955	0.573	0.583	2.750	1.702	1.481	1.292	1.158	1.107	0.390	0.221
	AB titer	2074 ±52	7316 ±78	8644 ±127	17256 ±618	5617 ±171	4978 ±106	2370 ±75	2432 ±45	23069 ±696	11503 ±287	9401 ±128	7715 ±193	6584 ±167	6167 ±142	1357 ±36	595 ±20
IV	S/P	0.046	0.044	0.056	0.049	0.040	0.039	0.042	0.039	0.040	0.042	0.039	0.039	0.030	0.037	0.031	0.039
	AB titer	58 ±5	57 ±5	80 ±5	67 ±5	58 ±5	48 ±5	53 ±5	48 ±5	58 ±5	58 ±5	48 ±5	48 ±5	33 ±5	44 ±5	34 ±5	48 ±5
F-Value		21.57**	7.64**	3.36*	7.57**	9.59**	5.41**	5.31**	11.30**	18.44**	11.26**	13.51**	16.43**	2.78*	2.78*	1.75*	2.02*

Groups* are:
I: Group that received the inactivated ILT virus vaccine 1:4 (1 aqueous phase: 4 oil phase) II: Group that received the inactivated ILT virus vaccine 1:2 (1 aqueous phase: 2 oil phase) III: Group that received the attenuated ILT virus vaccine IV: Group that received no vaccine and lift as control S/P** represent standard proportion ratio and AB titer represent antibody titers. f-Value *p< 0.05 **P<0.01

TABLE 5. ELISA antibody titers of vacciaated and control chicks before and after the first challenge

Group*	Result	Before challenge	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week
I	S/P**	2.292	0.265	0.095	0.409	0.936	0.570	0.403	0.376	0.283	0.230	0.088	0.070	0.034
	AB titer	17717 ±650	776 ±29	176 ±13	1457 ±98	4834 ±104	2355 ±47	1423 ±52	1286 ±66	852 ±28	634 ±26	157 ±17	112 ±15	41 ±9
II	S/P	2.906	0.349	0.230	0.395	0.747	0.751	0.561	0.181	0.079	0.025	0.018	0.018	0
	AB titer	24988 ±701	1155 ±59	632 ±43	1385 ±69	3482 ±101	3513 ±46	2301 ±62	446 ±23	134 ±12	25 ±6	15 ±3	15 ±3	0 ±2
III	S/P	2.251	0.174	0.151	0.170	0.421	0.559	0.348	0.296	0.170	0.149	0.145	0.023	0
	AB titer	17256 ±618	422 ±31	343 ±44	406 ±38	1517 ±52	2288 ±35	1153 ±50	912 ±51	406 ±20	337 ±18	322 ±24	22 ±7	0 ±2
IV	S/P	0.049	0.514	0.617	1.156	1.199	0.825	0.825	0.831	0.724	0.667	0.662	0.649	0.625
	AB titer	67 ±5	2026 ±82	2643 ±84	6562 ±103	6921 ±120	4025 ±96	4025 ±96	4065 ±93	3334 ±77	2948 ±37	2923 ±80	2345 ±84	2691 ±29
V	S/P	0.049	0.040	0.025	0.056	0.039	0.031	0.042	0.049	0.038	0.041	0.037	0.031	0.031
	AB titer	67 ±5	58 ±9	25 ±4	80 ±9	48 ±7	33 ±9	53 ±9	67 ±9	46 ±9	51 ±9	44 ±9	34 ±9	34 ±9
F-Value		7.57**	7.29**	4.88**	1.50*	1.61*	7.54**	7.15**	2.01*	2.01*	9.01**	4.92*	9.28**	9.83**

Groups * are:

I : Group that received the inactivated ILT virus vaccine 1:4 (1 aqueous phase: 4 oil phase) II : Group that received the inactivated ILT virus vaccine 1:2 (1 aqueous phase: 2 oil phase) III : Group that received the attenuated ILT virus vaccine IV : Group that was not vaccinated, challenged V : Group that was not vaccinated, not challenged S/P** represent standard proportion ratio and AB titer represent antibody titer. F-Value *p< 0.05 **P<0.01

TABLE 6. ELISA antibody titers of vaccinated and control chicks before and after the second challenge.

Group*	Result	Before challenge	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
I	S/P**	1.163	0.274	0	0.774	1.184	0.691	0.275	0.181	0.135
	AB titer	6623 ±187	816 ±26	0 ±6	3672 ±62	6795 ±95	3113 ±85	816 ±38	445 ±25	291 ±19
II	S/P	0.897	0.119	0.06	1.461	1.901	1.282	0.524	0.142	0.130
	AB titer	4542 ±94	241 ±10	81 ±9	9215 ±293	13505 ±240	7623 ±175	2084 ±57	313 ±24	277 ±18
III	S/P	0.593	0.101	0.123	0.493	0.644	0.565	0.430	0.291	0.108
	AB titer	2432 ±45	191 ±17	255 ±14	1908 ±46	2812 ±69	2326 ±61	1566 ±50	886 ±51	209 ±15
IV	S/P	0.039	0.500	0.590	0.970	1.010	0.980	0.950	0.940	0.900
	AB titer	48 ±5	1947 ±38	2475 ±31	5091 ±73	5398 ±109	5167 ±106	4939 ±97	4864 ±103	4567 ±87
V	S/P	0.039	0.025	0.030	0.028	0.031	0.025	0.027	0.031	0.030
	AB titer	48 ±5	25 ±9	32 ±9	29 ±9	34 ±9	25 ±9	28 ±9	34 ±9	32±9
F-Value		11.30**	9.0**	3.3**	1.5*	5.8**	5.2**	1.6*	1.5*	1.8*

Group* are:
I: Group that received the inactivated ILT virus vaccine 1:4 (1 aqueous phase: 4 oil phase) II: Group that received the inactivated ILT virus vaccine 1:2 (1 aqueous phase: 2 oil phase) III: Group that received the attenuated ILT virus vaccine. IV: Group that was not vaccinated, challenged V: Group that was not vaccinated, not challenged S/P** represent standard proportion ratio and AB titer represent antibody titer's. F-Value *p< 0.05 **P<0.01

TABLE 7. ELISA antibody titers of vaccinated and control chicks before and after the third challenge .

Group*	Result	Before challenge	Post challenge			
			1st week	2nd week	3rd week	4th week
I	S/P**	0.511	0.380	0.295	0.228	0.071
	AB titer	2010 ±42	1307 ±30	906 ±59	624 ±29	114 ±17
II	S/P	0.448	0.442	0.404	0.348	0.148
	AB titer	1662 ±24	1631 ±38	1429 ±29	1149 ±26	331 ±13
III	S/P	1.292	0.412	0.378	0.295	0.135
	AB titer	7715 ±193	1471 ±56	1297 ±28	406 ±19	292 ±11
IV	S/P	0.039	0.400	0.450	0.611	0.603
	AB titer	48 ±5	1409 ±28	1671 ±31	2604 ±73	2679 ±93
V	S/P	0.039	0.030	0.032	0.031	0.039
	AB titer	48 ±5	32 ±9	36 ±9	32 ±9	48 ±7
F-value		8.5**	3.6**	2.0*	2.2*	9.8**

Groups* are: I : Group that received the inactivated ILT virus vaccine 1:4 (1 aqueous phase: 4 oil phase). II : Group that received the inactivated ILT virus vaccine 1:2 (1 aqueous phase: 2 oil phase). III : Group that received the attenuated ILT virus vaccine. IV : Group that was not vaccinated challenged. V : Group that was not vaccinated, not challenged S/P** represent standard proportion ratio and AB titer represent antibody titer's. F-Value *$p < 0.05$ **$P < 0.01$

TABLE 8. Numbers of dead birds and birds showed clinical symptoms after three trials of challenge .

Group	1st challenge				2nd challenge				3rd challenge			
	Show no signs	Show signs	Dead	Protection %	Show no signs	Show signs	Dead	Protection %	Show no signs	Show signs	Dead	Protection %
I	10/10	0/10	0/10	100%	10/10	0/10	0/10	100%	10/10	0/10	0/10	100%
II	10/10	0/10	0/10	100%	10/10	0/10	0/10	100%	10/10	0/10	0/10	100%
III	10/10	0/10	0/10	100%	8/10	2/10	2/10	80%	5/10	5/10	0/10	50%
IV	0/10	10/10	6/10	40%	0/10	10/10	7/10	30%	0/10	10/10	7/10	30%
V	10	0/10	0/10	No chall enge	10/10	0/10	0/10	No chall enge	10/10	0/10	0/10	No chall enge

Groups* are: I : Group that received the inactivated ILT virus vaccine 1:4 (1 aqueous phase: 4 oil phase). II : Group that received the inactivated ILT virus vaccine 1:2 (1 aqueous phase: 2 oil phase). III : Group that received the attenuated ILT virus vaccine. IV : Group that was not vaccinated challenged. V : Group that was not vaccinated, not challenged

of 4-weeks-old. The first question was attempted to answer with the present study whether broilers could be immuned to ILT virus infection via vaccination with the inactivated vaccine and whether the resulted immunity is better than that induced by the life attenuated vaccine. The second question, closely related to the first, whether protection from challenge by virulent virus was associated with levels of serum antibody measurable by ELISA test. Table (6) shows increase in the antibody titer of the first three groups till it reached its peak at the 4^{th} week post vaccination (P.V.) and these results agrees with those obtained by (Burnet, 1936; Hitchner and White, 1958). After that, the antibody titers decreased sharply and this can be explained by the concurrent infection that happened by coccidiosis in-group I and II that offers an immunosuppression leading to decrease in antibody titers. The sharply increase in antibody titers may be due to re-circulation of the live virus vaccine in-between vaccinated chickens and this confirms the result obtained by (Bagust, 1986 and James *et al.*, 1990). The obtained results from tables (5-6-7-8) revealed that groups that vaccinated with *Nigella sativa* adjuvant, which named PANISA oil, vaccine showed increasing in antibody titer after vaccination and challenge when compared with group III and this mean that *Nigella sativa* oil (PANISA oil) play an important role for increasing antibody titer non specifically, and the best concentration of *Nigella sativa* is 1: 2 (1 aqueous part: 2 oil phase) followed by group I which received the vaccine in a concentration of 1: 4 (1 aqueous part: 4 oil phase). While the live attenuated vaccine induced lower antibody titer than the other two inactivated vaccine supplemented with PANISA oil as adjuvant. This picture was confirmed by (Burnet, 1936; Shirrel Davidson *et al.*, 1988 and Jordan, 1990). From the obtained results in this study, it is very clear that antibody levels to ILTV vaccine were demonstrated to vary significantly among groups of birds, depending on the ratio of aqueous to the oil phase adjuvant administered. Furthermore, the ability of a group to withstand challenge by virulent virus was shown to be not correlated to the group's average levels of ILT serum antibody before challenge. However, a similar correlation has been noticed previously (Hayles, *et al.*, 1976). These humoral immune response, although associated with infection, are not the primary mechanism of protection to ILT virus infection (Fahey and York, 1990; and Jordan, 1990). It suggests that besides humeral immunity, both local immunity in the respiratory tract and cell-mediated immunity may be involved in the protective mechanism. Moreover, the results described here confirm the useful effect of *Nigella sativa* adjuvant vaccines and the difficulty in establishing long-term protection so a booster vaccination is recommended. These trials confirmed the safety and efficacy of *Nigella sativa* adjuvant vaccine as a successful oil adjuvant. The propagation of ILTV on cell culture beside the addition of mineral oil to *Nigella sativa* oil lowered the viscosity and the cost of the vaccine dose, than that prepared by (Madbouly *et al.*, 2000), in which SPF-ECE were used a host system for virus propagation and *Nigella sativa* oil was used as adjuvant without any additives. The result obtained by inactivated vaccine in the ratio of 1:2 (1 aqueous part: 2 oil phase) gave the best results than that of 1:4 (1 aqueous part: 4 oil phase), where inactivated vaccine in the ratio of 1: 4 produced abscess formation and deaths among vaccinated birds occur.

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(*Recived* 14/2/2005;
Accepted 8/8/2005)

تحضير لقاح نسيجي ميت ضد فيروس التهاب الحنجرة و القصبة الهوائية المعدي باستخدام زيت حبة البركة

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تم تثبيط الحامض النووي لفيروس التهاب الحنجرة والقصبة الهوائية المعدى بعد تنقيته بمادة البنزي إثيلامين بعد خلطهم لمدة ٢٤ ساعة وتم التأكد من تثبيط الحامض النووي للفيروس عن طريق تمزيره على خلايا الفيرو ولم يعطي أي تغيرات بها. وقد نقل إلى الأغشية اللقائيقية للبيض المخصب ولم يعطي أي حرشوفات . كما أعطى للفراخ الحية فلم يعطي أي أعراض للمرض مؤكدا بذلك تمام عملية تثبيط الحمض النووي كاملا. تم تحضير لقاح مثبط ضد هذا الفيروس بخلط السحتوى المائي للفيروس المثبط مع المحلول الزيتي المكون من خليط من زيت حبة البركة وزيت البرافين بنسبة ١ : ١ وتم تحضير لقاحين الأول بنسبة ١ : ٢ والثاني بنسبة ١ : ٤ (وهي نسبة المحلول المائي : محلول الزيتي).قد تم عمل سلسلة من اختبارات الجودة للقاح وتبين أن اللقاح آمن وخالي من المسببات المرضية الأخرى مثل البكتريا والفطريات والميكوبلازما. تم تحصين أعداد كبيرة من الدواجن من النوعين المحضرين وأخذ عينات دم لفصل المصل لقياس الأجسام المناعية الخاصة بالفيروس واختبارها بواسطة اختبار الأنزيمي المناعي الأرتباطي الاليزا اسبوعيا ولمدة أربعة أشهر ثم مقارنة اللقاح المحضر باللقاح الحي المضعف المباع بالأسواق وثبت أن اللقاح المحضر أفضل في نتائجه عن اللقاح الحي. تم عمل عدوى معملية بعترة قوية ثلاث مرات متتالية يفصل بينها مدة شهر وقد تم جمع السيرم لقياس الأجسام المناعية ضد الفيروس وتم تحليل العينات بواسطة الاختبار الانزيمي الارتباطي الاليزا وقد وجد أن اللقاح ١ : ٢ أعطى أحسن النتائج. وكانت نسبه الحماية للقاح المحلى المثبط المحضر بالقاعدة الزيتية (زيت حبه البركة و زيت البرافين) بالنسب المستخدمه ١:٢ و ١:٤ هى ١٠٠%و ١٠٠% في اختبار التحدي عند عمر شهر و شهرين و ثلاثة أشهر بعد التحصين على التوالي و يتضح من هذه النتائج أن اللقاحين الذين تم تحضيرهما بنسبة ١:٢و ١:٤ أفضل في إحداث مناعة ممتدة و حماية الكتاكيت من العدوى بالفيروس الضاري وان اللقاح المحضر بنسبة ١:٢ أفضل من الآخر المحضر بنسبة ١:٤.