Preparation of Oil Adjuvant Inactivated Avian Reovirus Vaccines

Madbouly, H.M.*; Hussein, A.S.; Zaki, T.K.**
and Ensaf, M.H.**
**Dept. Virol., Fac. Vet. Med., Beni-Suef Univ.; ** Vet. Serum and Vaccine Res. Institute*

Abstract

Three types of reovirus inactivated vaccines adjuvated with mineral oil, vitamin E- selenium oil and *Nigella sativa* oil were prepared and checked for their sterility, potency, purity, and safety. The obtained results revealed that these vaccines are sterile, pure, safe and potent when used for young chicks. The efficacies of these vaccines were evaluated by determining the antibody titer elicited by them using ELISA and VNT. The cell mediated immune response was evaluated by lymphocytes blastogenesis, macrophage activity % and macrophage index. The most effective one of them is *Nigella sativa* adjuvated reovaccine followed by vitamin E- selenium adjuvated reovaccine and finally mineral oil adjuvated reovaccine.

Introduction

Reovirus (Orthoreovirus) is one of the six genera of the family Reoviridae, characterized by a genome comprising 10 segments of double-stranded RNA enclosed within a double protein capsid shell 80 nm in diameter (**17; 18 and 25**). These agents have been classified into two groups according to their natural hosts: mammalian reoviruses and avian reoviruses (**13 and 17**). Reovirus infections are prevalent worldwide in chickens, Turkeys and other avian species (**30**). Economic losses caused by Reovirus infections are frequently the result of lameness and poor performance, including diminished weight gains, high feed conversion, and reduced marketability of the affected birds (**10; 8**). Avian Reovirus may cause immunosuppression in chickens (**32; 25 and 26**).

Many different types of compounds are known to improve vaccine efficacy but most of the commercially available products are still supplemented with classical adjuvants including mineral oil emulsions (**15**).

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Fac. Vet. Med. (Moshtohor), Benha Univ

Tissue reactions in chickens injected with mineral oil emulsion vaccine remain a source of poultry condemnations and are of financial concern to the poultry industry. The mineral oil phase alone, which persists for months, may cause undesirable tissue reactions and is considered to have carcinogenic potential for consumers (**39**). Another concern is that accidental injection of operators with mineral oil emulsion is a potential source of liability claims for personal injury (**33**).

For the above-cited reasons, the development of suitable replacements for the mineral oil portion of the vaccines is desirable. The replacement must have high potency, low viscosity, long shelf life, and minimal tissue reactivity. Also, they must be compatible with mass production techniques, homogenous in appearance, cost effective and has the ability to activate cellular immunity to compensate the effect induced by live attenuated vaccines. Some studies were planned to avoid the undesirable effect of the mineral oils by replacing them with suitable animal, vegetable or synthetic oil as has been described by (**35**) and (**20, 21** and **22**). *Nigella Sativa* oil was one of these adjuvants that could be used as a replacement for the mineral oil due to its non-specific immunostimulant effect (**6 and 14**) besides other different desirable effects such as anti-microbial effect (**13**) and anthelmintic effect (**1**).

Accordingly, this investigation was planned to study the possible effect of non-specific immunostimulants (*Nigella sativa* oil and vitamin E - Selenium) and compare with mineral oil and used as control with prepared inactivated Reovirus *Nigella sativa* adjuvant vaccine, inactivated Reovirus vitamin E-Selenium adjuvant vaccine and inactivated Reovirus mineral oil adjuvant vaccine not only to rise the resistance of birds but also to improve their immune response to the vaccine.

Materials and Methods

1-Chicks: Seven hundreds and twenty of one day old ISA chicks were obtained from Poultry Parents Egypt Company. The chicks were floor reared and feed on balanced commercial poultry ration consisting of 21% protein, 3.2% fat, 3.1% fibers and free from mycotoxins and antimicrobial agents, the ration were obtained from Cairo Poultry Company. The chicks were kept under standard hygienic conditions. They divided into 8 groups (90 chicks in each group).

2-Embryonated chicken eggs: 9-11 SPF embryonated chicken eggs were used for propagation and preparation of a batch of Reovirus vaccine.

3- Vaccinal strains of Reovirus: Modified live egg adapted Reovirus lyophilized vaccine kindly supplied by Intervet Company, strain S1133 with a titer of $10.^{8.1}$ EID_{50}/ml. The vaccine was preserved at 4°C till date of propagation in ECE for vaccine preparation and were also used for the vaccination of some chicks of the tested chicken (7th groups).

4- Virulant strain of Reovirus: Virulant strain of reovirus S1133 was obtained from the Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. The virus was found to have a titer of 10^3 EID_{50}/ml.

5- Reference chicken anti-Reovirus S1133 serum: A product of SPAFAS Comp., USA was obtained from Immunology Department, Animal Health Research Institute (AHRI), Dokki, Egypt. It was used for virus identification by VNT.

6-Monoclonal anti-avian Reovirus S1133 serum: It was obtained from Department of Poultry Science, Auburn University, Alabama, USA. It was used for virus identification and purity test.

7- *Candida albicans*: It was kindly supplied by Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt. Twenty four hours old subculture of *Candida albicans* was used as an antigen for evaluating macrophages phagocytic activities.

8- Oil adjuvants:

A). *Nigella sativa* oil: *Nigella sativa* seeds obtained from commercial sources and were pressed. The obtained oil was filtered to remove fine seeds' particles, then sterilized by filtration through 0.22 µm filter (Sartorious – Germany), and kept at 4°C till used for preparing the vaccine.

B) Mineral oil: Mineral oil was kindly supplied by Newcastle Department, Veterinary Serum and vaccine Research Institute, Abbassia, Cairo, Egypt. It was used for preparing another type of vaccine.

C) Vitamin E - Selenium: Vitamin E – Selenium was supplied by the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt, Batch No. 110680.It was used for preparing the vaccine as an immunostimulant adjuvant.

9- Emulsifiers:

A) Sorbitan Monooleate (Span): Sorbitan Monooleate Span was supplied by Ubichem Ltd Code 5241H, Lot 882. It was used for preparing oil phase o f the vaccine.

B) Tween 80 (polyoxyethylene sorbitan): Tween 80 (polyoxyethylene sorbitan) was supplied by Sigma Co., Lot 8340550.and was used for preparing the aqueous form of the vaccine as surfactant.

10-Propagation of reovirus in SPF-ECE: The SPF-ECE was inoculated with vaccinal strain of Reovirus via CAM in embryonated chicken eggs (9-11) days (0.2 ml)/egg. Titration of the obtained virus was carried out and the EID_{50} was calculated according to Reed and Muench formula (**27**).

11- Inactivation of the propagated Reovirus fluid: Inactivation of the virus was made by the addition of ethyleneimine – binary ethyleneimine (BEI) – as described by (**3**).

12-Safety test of inactivated virus in embryonated chicken eggs: The safety of inactivated virus was measured by inoculation of 0.2 ml of inactivated virus fluid via the CAM of each of ten, 9 days old, embryonated chicken eggs. The inoculated eggs incubated at 37°C and candled twice daily for 7 days. The inoculated eggs were individually examined for the specific lesions of Reovirus infection. Fluid harvested from the dead and survived embryoes were pooled and passaged blindly for 2 successive further passages before the batch of the prepared inactivated virus fluid was considered safe.

13-Preparation of inactivated oil adjuvant vaccine: Three types of inactivated oil adjuvant reovirus vaccines were prepared according to (**36**). The adjuvant used for preparation of water-in-oil emulsion vaccines consisted of 88 parts of (*Nigella sativa* oil or vitamin E-selenium oil and/or

mineral oil), 10 parts of span 80 and 2 parts tween 80 added to antigen liquid part.

14-Evaluation of the prepared vaccines:

A- The physical properties (Emulsion type, Relative viscosity, and Emulsion stability) of emulsions were investigated according to (**34**).

B- Quality control of the prepared vaccine:

I-Sterility test:

It was applied to confirm that the prepared vaccines were free from any microbial contaminants before inactivation by inoculation into thioglycolate broth, nutrient agar, MacConky and PPLO media. The inculated media were incubated at 37°C for 72 hours, but Saboraoud maltose agar plate and incubated at 25°C for 14 days.

ii- Safety test in young chicks:

After preparation of the inactivated vaccine, twenty chicks for each vaccine at 21 days old were inoculated with 1.0 ml/bird (2 doses) intramuscularly and 20 chicks were kept as control. The chicks were observed for 2 weeks for any signs of local reactions as abscess formation, irritation or systemic reaction. After one week of vaccination, five birds from each group were subjected to PM examination to detect any pathological lesions, the prepared vaccines were considered safe when there are no clinical signs or pathological lesions.

iii- Potency test:

Eighty chicks of 3 weeks old (20/group of 3 different prepared inactivated reovirus vaccines were inoculated with a full dose (0.5 ml) via I/M route for each prepared vaccine and twenty chicks were kept as non vaccinated controls. Three weeks post vaccination, all vaccinated chicken and non vaccinated control chicken were challenged by injection 10^3 EID_{50} /ml virulent Reovirus into foot pad and observed through 14 days. The vaccine proved to be potent when chicks vaccinated with different types of prepared inactivated reovirus vaccine and control chicks were not protected.

iv- Purity test:

The purity test were done to be sure that the prepared inactivated Reovirus vaccine is free from any other virus as like (Newcastle, Leucosis, Gumboro, Avian Encephalomylitis "AE") by using HA test against Newcastle, agar gel

precipitation test to Leucosis, Gumboro, AE Also, the purity test is repeated after the preparation of adjuvated vaccines.

Experimental design for studying the efficacies and potency of the prepared vaccines: Vaccination of young chicks with prepared inactivated Reovirus Nigella sativa, vitamin E selenium and /or mineral oil adjuvated vaccines and live attenuated Reovirus vaccine:

The chicks were vaccinated at first week of age by injection (I/M) 0.5ml of the locally prepared vaccines and 0.2 ml S/C live attenuated Reovirus vaccine at the site of neck according to the following table:

Table (1): grouping of vaccinated chicks:

Group	Number of chicks	Treatment
1	90	Chicken vaccinated with inactivated Reovirus adjuvated with mineral oil
2	90	Chicken injected by mineral oil alone as control for group 1.
3	90	Chicken vaccinated with inactivated Reovirus vaccine adjuvated with vitamin E-selenium oil
4	90	Chicken injected by vitamin E-selenium oil alone as control for group 3
5	90	Chicken vaccinated with inactivated Reovirus vaccine adjuvated with *Nigella sativa* oil
6	90	Chicken injected by *Nigella sative* oil alone as control for group 5.
7	90	Chicken vaccinated with live attenuated Reovirus vaccine
8	90	Chicken not vaccinated as control

I-Evaluation of humoral immune response of chicken: All chicks were bled at one week intervals post vaccination over six weeks for the following parameters:

A-Enzyme linked immunosorbent assay (ELISA): It was carried out according to the manufacture of IDEXX kit for determining (Reo) antibody.

B-Virus Neutralization test (VNT): It was carried out according to (Thayer et al., 1983).

C-Challenge test for Reovirus: All birds (vaccinated and control) were challenged via foot pad with 0.1 ml of a virulent Reovirus strain S1133 10^3 EID_{50} at 7, 10, 12, 15, 18, and 21 days post vaccination and were observed for 15 days after challenge.

II-Evaluation of cell mediated immune response of chickens:

A-Evaluation of lymphocyte blastogenesis by using tetrazolium dye (MTT): according to (**37**)

B-Phagocytic activity of chicken macrophages by using *Candida albicans*: The test was carried out according to (**28**), (**5**) as modified by (**10**).

Results

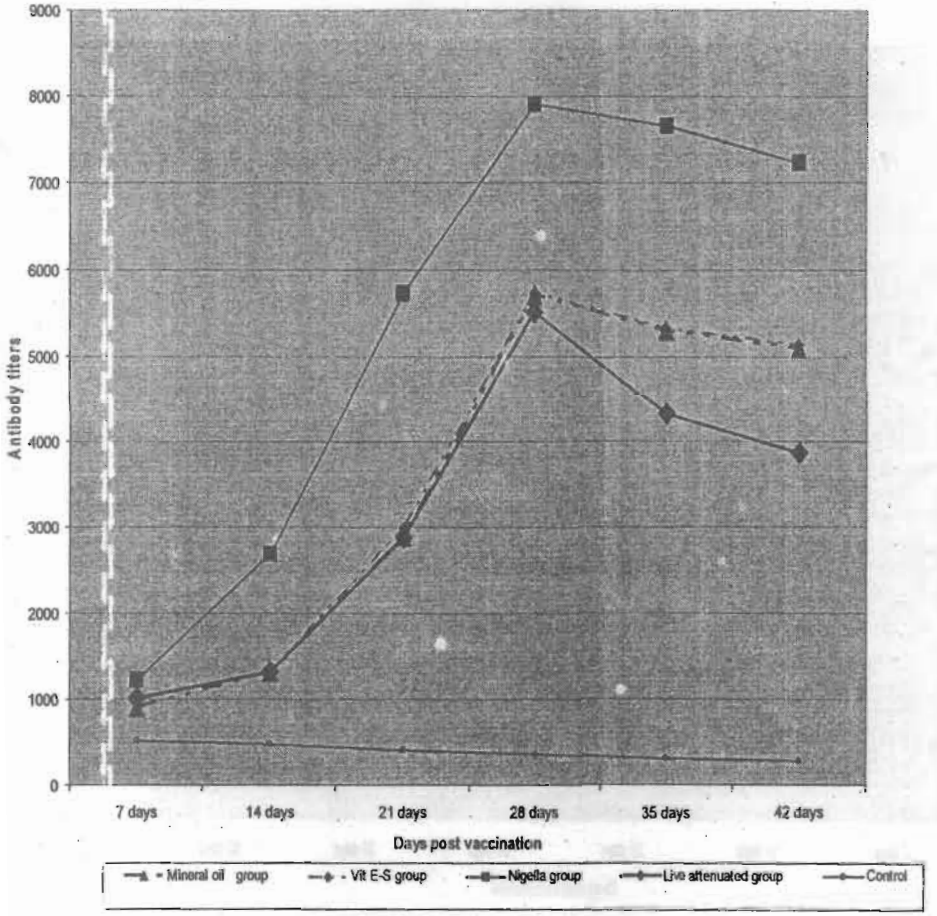
Table (2): Potency of inactivated Reovirus in young chicks 21 days post vaccination

Group No	Total no of Examined Birds	Challenge test at 21 day old		
		No of Infected birds	Survival no	Protection %
1	20	4	16	80
3	20	3	17	85
5	20	1	19	95
8	20	20	0	0

Table (3): Pathological changes at the sites of injection in chicken injected with prepared vaccines and its control

Time post Inoculation	Pathological changes	Severity of changes induced in different chicken groups					
		G1	G2	G3	G4	G5	G6
72 hours	Acute inflammation	+	+	+	+	+	+
	Cyst formation	+	+	+	+	+	+
	Abscess	-	-	-	-	-	-
1 week	Acute inflammation	-	-	-	-	-	-
	Cyst formation	+	+	+	+	+	+
	Abscess	-	-	-	-	-	-
2 week	Cyst formation	+	+	+	+	+	+
	Abscess	-	-	-	-	-	-

Figure: (1): Mean antibody titers of chickens vaccinated with different types of inactivated reovirus vaccine beside live attenuated Reo virus vaccine *at p<0.05 using*



ELISA.

Figure (2): Mean neutralizing antibody titers in different groups of chickens vaccinated with differents types of inactivated reovirus vaccine and live attenuated one by using VNT.

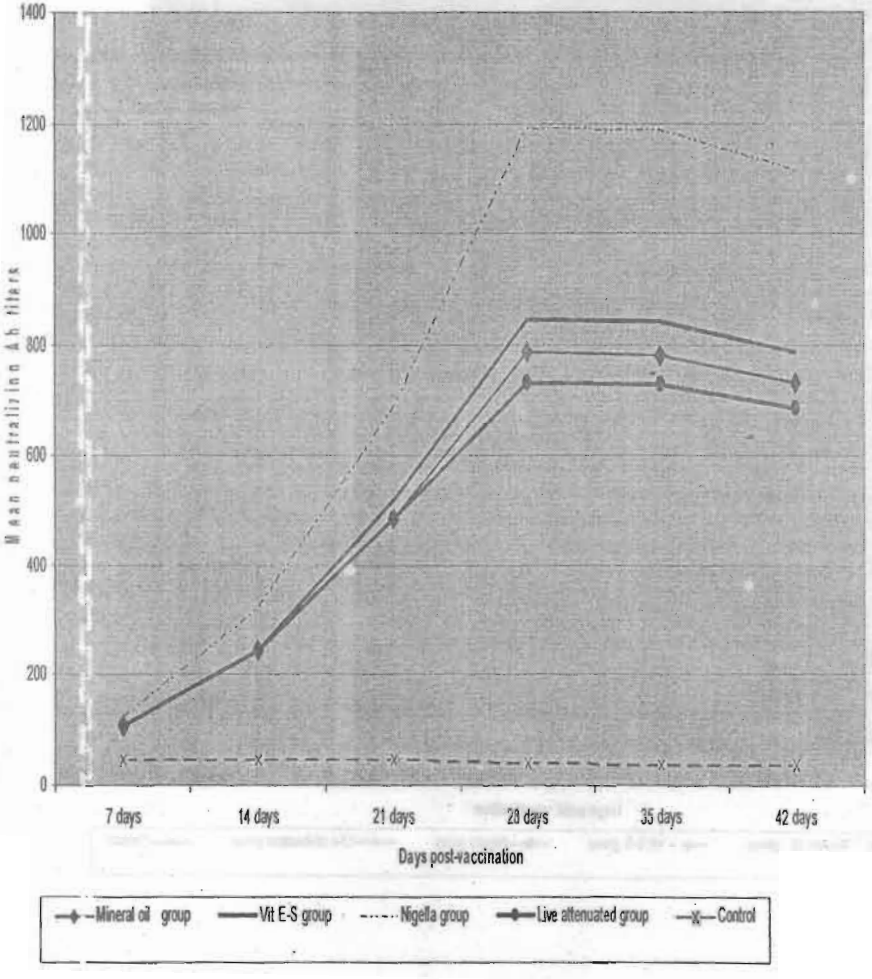


Table (4): Lymphocyte blastogenesis of PBL of chickens vaccinated with different types of inactivated reovirus and live attenuated one by using MTT

Group	1	2	3	4	5	6	7	8
	Mineral oil reo vaccine	Mineral oil	E-Sel reo vaccine	E-se oil	Nig. reo vaccine	Nig	live reo vaccine	control
7	1.14	0.7	1.16	1.0	1.36	1.27	1.15	0.6
14	1.29	0.6	1.38	1.2	1.76	1.44	1.32	0.4
21	1.21	0.54	1.28	1.0	1.72	1.40	1.18	0.3
28	1.14	0.41	1.24	1.0	1.61	1.41	1.09	0.31
35	1.05	0.38	1.14	0.9	1.55	1.23	0.6	0.24
42	0.8	0.34	1.10	0.7	1.38	1.13	0.57	0.21

(Tetrazolium Dye) at $p < 0.05$

PBL= peripheral blood lymphocyte. * = significant points

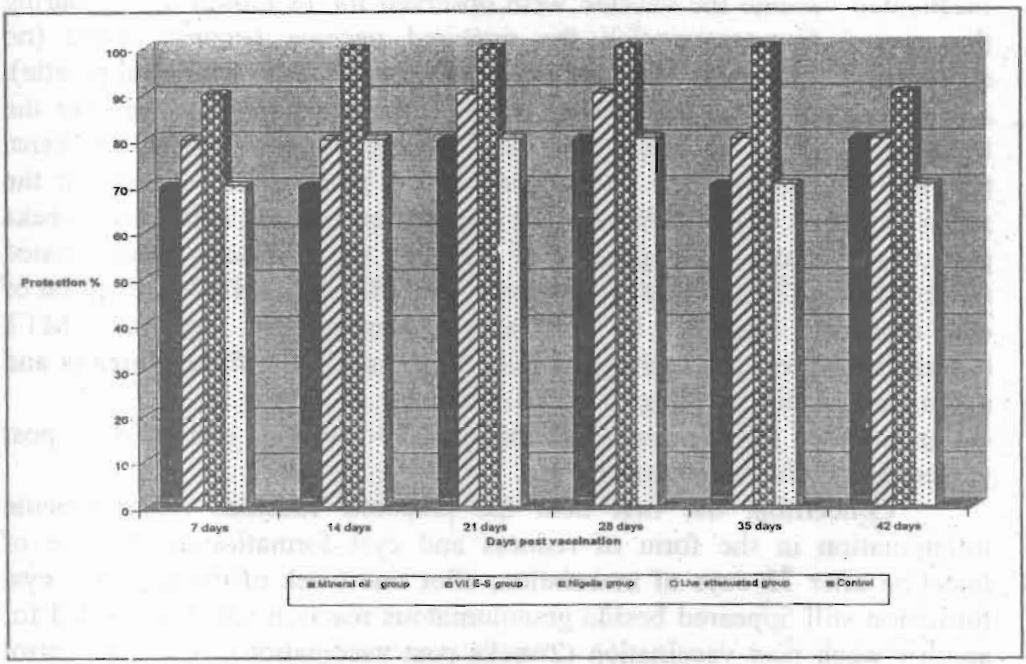
Table (5): Effects of different types of inactivated reovirus vaccine and live attenuated one on macrophages percentage (%) of chickens by using *candida albicans*

Group	1	2	3	4	5	6	7	8
PBL blood collected Post Vacc.	Mineral oil reo-vaccine	Min-eral oil	Vit.E-selen Reo oil vaccine	Vit.E-selen oil	Nigella Sativa Reo oil vaccine	Nigella sativa oil	Live Atten-uated Reo virus vaccine	Control
7	24	13.6	27.2	18.46	45	48.4	28.7	4.4
14	33.7	12.1	34.9	28.9	60.8	54.8	32.6	4.0
21	30.4	8.0	29.8	26.5	58.4	51.4	29.5	3.8
28	28.4	4.4	28.7	23.6	55.3	48.6	26.6	2.4
35	26.5	3.8	26.7	21.5	50.8	42.6	21.5	0.8
42	22.6	3.0	24.6	18.4	46.4	37.3	16.8	0.7

Table (6) Effects of different types of inactivated reovirus vaccine and live attenuated one on macrophages index of chickens by using *candida albicans*.

Group	1	2	3	4	5	6	7	8
PBL blood collected Post Vacc.	Mineral oil reo vaccine	Mine-ral oil	Vit E-selen Reo oil vaccine	Vit E-selen oil	Nigella Sativa Reo oil vaccine	Nigella sativa oil	Live Attenuated Reo virus vaccine	Control
7	0.14	0.09	0.15	0.12	0.22	0.23	0.16	0.06
14	0.19	0.07	0.17	0.16	0.38	0.29	0.18	0.04
21	0.18	0.06	0.17	0.14	0.32	0.27	0.17	0.03
28	0.16	0.05	0.16	0.13	0.30	0.24	0.15	0.03
35	0.14	0.05	0.15	0.12	0.28	0.21	0.12	0.02
42	0.13	0.04	0.14	0.09	0.23	0.20	0.08	0.02

Figure (3): Protection rate of chickens vaccinated with different types of inactivated reovirus vaccines comparing with live attenuated and control.



DISCUSSION

For preparing the inactivated reovirus vaccine different adjuvants (mineral oil, vit E- selenium oil and *Nigella sativa* oil) were selected for this purpose, the selection of mineral oil for preparing inactivated reovirus vaccine previously selected by (**4**), (**18**), (**7**) and (**2**), while the use of *Nigella sativa* oil for preparing reovirus inactivated vaccine was selected according to (**20**), (**19**), (**21**).

Three types of inactivated vaccine (mineral oil, vitamin E-selenium oil and *Nigella sativa* oil), reovirus inactivated vaccine were tested for potency after 21 days post vaccination by challenge by hot reovirus strain table (2) by foot pad injection. Data present in table (2) revealed that chicks vaccinated with *Nigella sativa* oil adjuvated reovirus vaccine were protected

95% more than other groups that vaccinated either with mineral oil reovirus adjuvated vaccine 80% or with vitamin E-selenium reovirus adjuvated vaccine 85%. For studying the stability and viscosity of prepared reovirus inactivated vaccine the vaccine were observed for 18 month at 4°C during this period for preservation the prepared vaccine become stable (no separation of oil from water) and viscous (easily dropped from 1ml pipette). All three types of the prepared vaccines were sable and viscous over the testes period. For evaluation of prepared vaccine in three days old chickens, the following items were investigated: 1-The pathological changes at the site of injection of prepared vaccines were observed from 3 days to 2 weeks post vaccination.2-Determination of antibody titers in sera of vaccinated chicks after 7-42 days post vaccination by ELISA and VNT.3-Evaluation of cell mediated response by lymphocyte blastogenesis by using MTT (tetrazolium dye), macrophage activity % by using **Candida albicans** and macrophage index by using *Candida albicans* .
4-Challenge of the vaccinated chicks at 7-10-12-15-18 and 21 days post vaccination with hot reovirus strain.

Concerning the first item the prepared vaccines induced acute inflammation in the form of redness and cyst formation at the site of injection after 72 days of inoculation, after one week of injection the cyst formation still appeared beside granulomatous reaction which extended for another week post vaccination (2weeks post vaccination). Another control groups of the same age were inoculated with mineral oil, vitamin E-selenium oil and *Nigella sativa* oil studying their effects alone without reovirus, the obtained results are nearly the same as for those chicks groups vaccinated with the prepared vaccine (Table:3).

The sera of different groups that vaccinated with different types of prepared inactivated vaccines beside sera of another groups vaccinated with live attenuated vaccine and control non vaccinated group were tested by ELISA and VNT for determining mean antibody titer for a period of six weeks post vaccination with sera collected weekly. The obtained results that presented in figure (1) revealed clearly that chicks vaccinated with *Nigella sativa* oil adjuvated reovirus vaccine elicited higher antibody titer than other groups vaccinated with other types of used (mineral oil, vit.E-selenium oil,

live attenuated reovirus vaccine). The antibody titer increase gradually from the 2nd week post vaccination and reached the peak at the 4th week post vaccination then become to decrease gradually with very low amounts from the 5th and the antiboly titer still elevated even at the 6th week post vaccination of the three types of used vaccines for example the antibody titer by ELISA and VNT in sera of vaccinated chicks by mineral oil ,vitamin E-selenium oil, *Nigella sativa* oil, live attenuated reovirus vaccine) were (1314.3,243), (1321.8 ,243), (2688 ,320) and (1320.2, 243) at the 2nd week post vaccination but this titer increase to (5710.2, 788), (5743.8 ,844), (7911, 1191) and (5512.7 ,733) at the 4th week post vaccination then these titers gradually decreased to reach (5088.8 ,733) , (5143.2 ,788) ,(7222.4 , 1114) and (3806.4 , 686) at the 6th week post vaccination. The sera of control group ranged between 276 and 507 by ELISA and (38-64) by VNT (Figure: 2). From these results it is also clear that the highest antibody titers by VNT corresponds the highest titer by ELISA and lowest titer by VNT correspond to the lowest titer of ELISA but the ELISA is more sensitive than the VNT for detected reovirus antibody. The sensitivity of ELISA than VNT retained to the fact that the ELISA can detect either antibody directed against both surface and internal core antigen while VNT can detect only the antibody directed against the surface antigen, the sensitivity of ELISA was investigated by several authors like (31) .Therefore when checking the profile of antibody titer post vaccination the ELISA is consider the preferred test of choice.

Concerning the evaluation of immune response by lymphocyte blastogenesis data present in table (4) revealed that chicks received Nigella sativa oil adjuvated reovirus vaccine had the highest value of PBL, from the 7th day post vaccination (1.36) and reached the peak at the 2nd week post vaccination (1.76) while the other used vaccine (either inactivated or live attenuated reovirus vaccine) gave lower values than those obtained by Nigella sativa adjuvant vaccine. Data presented in table (4) showed clearly that chicks received *Nigella sativa* oil adjuvated reovirus vaccine had higher values of lymphocytes blastogenesis from the 7th day post vaccination till 35th day post vaccination then the other used vaccines (inactivated and live attenuated) .The highest values were obtained by *Nigella sativa* adjuvated

vaccine (1.76and1.72) followed by vit E- selenium (1.38and1.28) at 2nd and 3rd week post vaccination respectively .The live attenuated and mineral oil adjuvated vaccine gave nearly similar values (1.32and1.18) and (1.29and1.21) at the same weeks respectively .

On the other hand, the non- specific immunostimulant effect of *Nigella sativa*, vit E- selenium was very clear in chicks received it alone. The highest values were obtained with *Nigella sativa* oil (1.44 and 1.40) followed by vit E- selenium oil (1.2and1.0) in the 2nd and 3rd week post injection respectively .The mineral oil gave a very limit effect of stimulation on comparison with those chicks that did not received any oil (control group).

From these results it is very clear also that the highest value of lymphocyte blastogenesis were recorded during the 2nd and 3rd week post vaccination and this agree with those obtained by (**20**), (**21**), (**38**) and by (**11**). The percentage of macrophage activity of vaccinated chickens with inactivated prepared vaccine and live attenuated one by using *Candida albicans* were presented in table (5). These results show clearly that macrophage activity percent was higher in group vaccinated with *Nigella sativa* oil vaccine (60.8 and 58.4%) at the 2nd and 3rd week post vaccination, than other groups that vaccinated with inactivated or live attenuated vaccines. Vit E- selenium vaccine also activated macrophage but to a lesser extent than *Nigella sativa* oil vaccine , it gave (34.9 and 29.8%) at the same time live attenuated and minerl oil vaccines gave nearly similar percentage of activation (32.6 and 29.5%) and (33.7 and 30.4%) respectively. On the other hand, the non specific immunostimulant effect of *Nigella sativa* oil and vit E- selenium was cleared in groups received the oil alone without inactivated reovirus. Concerning the macrophage index of all used vaccine data presented in table (6) showed clearly that the macrophage index percent in group vaccinated with *Nigella sativa* oil vaccine was higher than other groups vaccinated either with inactivated (vit E-selenium oil and mineral oil adjuuvated) or live attenuated vaccines. The highest value was in *Nigella sativa* oil vaccine (0.38and0.32) followed by mineral oil, Vit E-selenium vaccine, these vaccines gave nearly similar or values ranged between (0.17and0.19) in the 2nd nd 3rd weeks post vaccination. On the other side, the injection of *Nigella sativa* oil or Vit E-selenium gave higher

values (0.29and0.27) and (0.16 and 0.14), than mineral oil (0.07and0.06) in the 2nd and 3rd weeks post injection respectively. This obtained results of macrophage activities and macrophage index are agree with those (**20**), (**21**) and (**30**).

Further more to reach the complete evaluation of the prepared vaccine the vaccinated chicks with the prepared vaccine and live attenuated one were challenged with hot reovirus strain at 7,10,12,15.18 and 21 days post vaccination (Figure:3). Data revealed clearly that the inactivated reovirus .vaccines adjuvated with *Nigella sativa* oil showed higher protection rates (90-100%) even the chicks were challenged at early days post vaccination (7-2 days) or later days (21 days) post vaccination than the other used inactivated vaccines (mineral oil and vit E selenium adjuvant vaccines). Mineral oil adjuvant vaccine gave the lowest protection rate (70-80%).While vit E selenium adjuvated vaccine gave also higher rates of protection (80-90%) but lower than those obtained by *Nigella sativa* oil adjuvant vaccines. On the other hand, the protection rate offered by life attenuated vaccine is also lower than vit E selenium and *Nigella sativa* adjuvant vaccine nearly with the same wat offered by mineral oil adjuvant vaccine.

The obtained results revealed clearly that the prepared vaccine have the ability to elicit high titer of antibody against reovirus but the most effective one of them is the prepared vaccine adjuvated with *Nigella sativa* oil followed by vit E-selenium adjuvated vaccine , mineral oil adjuvated vaccine and finally live attenuated vaccine .The clinical signs that appeared on non protected chicks are typical with field reovirus infection like that feather abnormalities , diarrhea ,swelling at the site of inoculation , poor growth rate and these symptoms appeared after 7th days post inoculation ,the P.M finding lies in tenosynovitis. On the other hand the unvaccinated control challenged chicks showed typical reovirus clinical signs and also P.M lesion. These signs varies in chicks vaccinated and challenged from feather abnormalitis and swelling at the site of inoculation as in all vaccines used except *Nigella sativa* oil adjuvated vaccine which revealed only

ruffled feather and poor growth rate (it occurred on % of infected number only but the survival number show no symptoms).

Finally it is very important to said that this study support the previous study conducted by (**20**), (**21**) in that the *Nigella sativa* oil is highly immunogenic in the choice of vegetable oil in preparing inactivated viral vaccine, in addition the choice of vitamin E-selenium oil as adjuvants in preparing inactivated reovirus vaccine is considered a new step in preparing such vaccine and also considered a new trend in viral vaccine preparation specially those inactivated vaccine. The selection of these both oils depend mainly upon the immunostimulants effects of both oils as described by (**23**), (**20**), (**21**) and (**22**).

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تحضير لقاحات ميتة بقواعد زيتية لفيروس الريو في الطيور

حنفي محمود مدبولي* ، أحمد سعد حسين*، طارق كمال زكي**، انصاف محمد حسبر
*قسم الفيروسات – كلية الطب البيطري – جامعة بني سويف.
**معهد بحوث السيرم واللقاح بالعباسية

الملخص العربي

في هذه الدراسه تم تحضير ثلاثه أنواع من لقاح الريو العتره S1133 وتم استخدام ثلاثة أنواع من الزيوت المختلفة من (زيت حبه البركه ، زيت فيتامين ه – سلينيوم ، زيت معدني) بأضافتها وعمل لقاح الريو المثبط من زيت حبه البركه ولقاح الريو المثبط من زيت فيتامين ه – سلينيوم ولقاح الريو المثبط من زيت معدني . وعمل تقييم علي تأثير اللقاحات الثلاثة علي الأستجابه المناعيه لكتاكيت التسمين من عمر يوم حتي ٤٢ يوم. وتم تيقيم تأثير الأنواع المختلفه من اللقاحات التي تم تحضيرها علي الأستجابه المناعيه لكتاكيت التسمين بأجراء الأختبارات لقياس الأستجابه المناعيه بأختبار التحدي ضد مرض فيروس الريو وتعين المناعه الخلويه و الدمويه بالأختبارات وقد أشتملت الأختبارات المناعيه الخلويه أختبار النشاط المناعي للخلايه المناعيه اللمفاويه ومستوي النشاط للخلايه الأكوله . وبالنسبه للمناعه الدمويه تم قياس الأجسام المناعيه المضاده لفيروس الريو في المصل وأختبار الأليزا وأختبار التعادل الفيروسي .