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Study On The Immunostimulant Effect Of Different Additives In Inactivated Newcastle Vaccines

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ABSTRACT

The goal of this study was to determine the immunostimulant effect of bee venom on the chicken vaccinated with inactivated Newcastle (ND) vaccines adjuvanted either with paraffin oil or Montanide oil. The bee venom was used at concentrations of 25, 50, 75 µg/ml. The immune response to the inactivated ND vaccines was evaluated using haemagglutination inhibition (HI) test and protection percent. Our data showed an increase in the HI titre and the protective level of immunity which began earlier and persisted for a longtime when the bee venom was added to the different types of ND vaccines. The increase amount of bee venom exhibits a significant increase in the HI titre. Also, the HI titre and protection percent showed a higher level in the case of inactivated ND vaccine adjuvanted with Montanide oil than that adjuvanted with the paraffin oil. Generally, the oil emulsion adsorbed bee venom preparation causing higher immunity than the aqueous preparation. Finally, our results show the immunomodulatory effect of bee venom, allowing it to be used in the immunotherapy or to improve the quality of different vaccines.

INTRODUCTION

Bee products have been used since ancient times by human, not only as a good quality food but also as a natural remedy against various diseases.

Bee venom is considered as one of the main components of bee products which are used in the therapeutic field. It exhibits many properties as antitumorogenic and antibacterial properties ***(1,2)***. Also, bee venom therapy can be used in many fields as immunological, cardio-vascular, pulmonary, neurological, dermatological, fertilization, ... etc ***(3)***.

Bee venom, as an immunotherapy, is considered as an efficient treatment against many diseases. The mechanism of venom immunotherapy is based on the stimulation of cellular and humoral immune response ***(4,5)***.

So, bee venom can be used as an adsorbent or additive to the various inactivated vaccines to enhance and prolong the immune response against them.

Newcastle disease (ND) is still one of the most devastating poultry diseases. The disease is caused by a paramyxovirus strains showing great variation in pathogenicity and classified as a velogenic, mesogenic and lentogenic. They are also classified into viscerotropic and neurotropic depending on the pathological findings in the host ***(6)***. The clinical signs widely may varied from sudden death with 100% mortality to only a mild respiratory or subclinical infection. They depend on many factors such as the virus strains, host species, age of the host, the presence of other organisms, environmental stress and immune status of the host ***(7)***.

There are many types of vaccines used to control the disease in the field. The inactivated vaccines induce a satisfactory immunity in comparable to live ones ***(8)***. Different adjuvants and adsorbents were utilized to enhance and prolong the immune response against inactivated Newcastle disease vaccines.

So, the aim of this work was directed for:

1. Studying the effect of bee venom used with different concentrations on the immune response of chicken vaccinated with inactivated Newcastle disease vaccines.
2. Studying the effect of different adjuvants used in manufacture of Newcastle disease vaccines on the immune response.
3. Studying the effect of using of the bee venom in water or entrapped in adjuvant on the immune response.

MATERIAL AND METHODS

Materials

1. Vaccinal Newcastle disease (ND) strain

Live attenuated ND virus, LaSota strain, was obtained from Intervet Company, Boxmeer, Holland, and was used for vaccine preparation. Its titre was $10^{9.5}$ EID_{50}/ml.

2. Virulent ND virus

It is a field local isolate, Velogenic Viscerotropic Newcastle Disease Virus (VVNDV) ***(9)*** and was obtained from Newcastle Disease Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. Its titre was $10^{9.0}$ EID_{50} /dose and used in challenge test.

3. Embryonated Chicken Eggs (ECE)

Ninety, nine-day-old, specific pathogen free (SPF) embryonated chicken eggs were obtained from SPF Egg Production Farm, Koum Oshiem, Fayoum, Egypt and used for propagation, titration and testing the completion of inactivation of the virus.

4. Binary Ethyleneimine (BEI)

The NDV was inactivated as previously described ***(10).*** 0.1 M binary ethyleneimine was formed through the cyclization of 2-bromo-ethylamine hydrobromide in previously warmed 0.15 N-sodium hydroxide (NaOH) at 37°C. The contents were incubated in water bath at 37°C for one hour, the solution was used as virus inactivator by different concentrations.

5. Sodium hydroxide

It was dissolved in double distilled water as 4% solution and sterilized by autoclaving. The solution was stored at 4°C till used in binary ethyleneimine cyclization.

6. Sodium thiosulphate solution

It was prepared as 40% solution in distilled water and sterilized by autoclaving. It was used for stopping the action of binary ethyleneimine.

7. Normal physiological saline (pH 7.2)

It was prepared of 8.5 % of sodium chloride in distilled water and used as a general diluent.

8. Antibiotic solution

Crystalline penicillin and dihydro-streptomycin were used as: 100 International Units of crystalline penicillin and 50ug of dihydro-streptomycin/ml.

9. Thiomersal

It was supplied by Fluka Company and used as a preservative with concentration of 1:10,000/100 ml.

10. Bee Venom

It was supplied by VacSera Company, Egypt. Its concentration was 100 µg/ml and used in concentrations of 25, 50 and 75 µg/ml.

11. Adjuvants

a. Paraffin oil

It consisted of liquid paraffin, sorpiten monooleate and tween 80. They were supplied by Sigma Company, USA and used for preparation of oil emulsion vaccine.

b. Montanide ISA-206

It was supplied by Sigma Company, USA.

12. Chicken erythrocytes suspension

Red blood cells from adult susceptible chickens were collected by wing vein in 4% sodium citrate as anticoagulant (1ml of anticoagulant + 9ml blood). An equal amount of physiological saline (0.85%) was added to the suspension and the cells were sedimented by centrifugation (3000 rpm at 4°C for 10 minutes). The cell sediments were subjected to 3 cycles of washing in physiological saline and centrifuged as before. The packed red cells were then diluted in saline as 10% solution for the rapid slide haemagglutination (HA) test, 1% for the micro techniques of the haemagglutination inhibition (HI) test, respectively.

Methods

1. Propagation and titration of the ND virus in ECE

Propagation and titration of NDV was done in specific pathogen free (SPF) 9-day-old embryonated chicken eggs ***(11).***

2. Inactivation of ND virus by BEI

Inactivation and testing of complete inactivation was done according to ***(12).*** Virus fluid-BEI inactivator mixture was incubated with continuous stirring at 37°C for 24 hours. From the inactivated virus suspension 3-6 ml were collected every hour in sterile tubes

containing 0.4 ml of 20% sodium thiosulphate solution to stop the action of BEI at different intervals. All samples were assayed for virus infectivity by titration in ECE to determine the rate of virus inactivation.

3. Completion of inactivation

According to **British Pharmacopoeias *(13)*,** in five ECE 9-day old, each sample was inoculated in allantoic cavity and incubated at 37°C for six days. The inoculated eggs candled twice daily, embryos dying 24 hours post inoculation were discarded as non-specific deaths. On primary isolation, 3 blind passages in 9-day-old ECE were carried out. The complete inactivation of virus detected by HA test on the allantoic fluids harvested from the inoculated eggs.

4. Preparation of inactivated ND vaccines

a. Using Paraffin oil

It was prepared by emulsifying the previously prepared ND inactivated fluids with Tween 80 (4 %) to form aqueous phase then added slowly to oil phase which consisted of mineral oil and sorbitan monoleate in a ratio of 1:1*(14)*.

b. Using Montanide ISA-206 adjuvant

It was prepared by emulsifying the ND virus suspension into Montanide ISA_{206} adjuvant (volume / volume) *(15)*.

c. Addition of Bee Venom

Bee venom was prepared in three concentrations; 25, 50 and 70μg/ml and added to inactivated virus suspension before using the adjuvants.

5. Serological Tests

a. Rapid slide haemagglutination test

It was carried out for quick detection of haemagglutinin in the amnioallantoic fluid of virus inoculated eggs *(16)*.

b. Standard quantitative haemagglutination (HA) test

This test was done to determine the haemagglutination titre in amnioallantoic fluid of virus inoculated eggs ***(16)*.**

c. Haemagglutination Inhibition (HI) test

It was done using the beta-procedure (constant virus plus diluted serum) as described by *(17)*. This test was used for measuring the antibody response of vaccinated chickens.

6. Challenge test with virulent ND virus

It was carried out to measure the protection percent in vaccinated and non-vaccinated control chickens by using velogenic viscrotropic ND virus (VVNDV) at 2 and 3 weeks post vaccination using 0.5 ml /bird intramuscularly containing $10^{6.0}$ EID_{50}.

7. Experimental Design

A total of 400, four weeks old chickens were used for evaluation of the immune response of the prepared inactivated ND vaccines. The chickens were divided equally into 8 groups (each contain 50 chickens). The chickens were examined for maternal antibodies against ND before vaccination. The number of chicken in each group and treatment are shown in Table 1.

Table 1. Experimental design for evaluation of the immune response against different types of inactivated ND vaccines.

Experiment No.	Group No.	Treatment	No. of birds	Administration route	Aim of study
(I)	1	Chicken vaccinated with inactivated ND vaccine containing 50 µg bee venom and adjuvanted with paraffin oil	50	I/M	* Study the immunostimulant effect of bee venom on the immune response of vaccinated chickens * Study the effect of different types of adjuvant in presence or absence of bee venom on the immune response
	2	Chicken vaccinated with inactivated ND vaccine containing 50 µg bee venom and adjuvanted with Montanide oil	50	I/M	
	3	Chicken vaccinated with inactivated ND vaccine adjuvanted with paraffin oil	50	I/M	
	4	Chicken vaccinated with inactivated ND vaccine adjuvanted with Montanide oil	50	I/M	
(II)	1	Chicken vaccinated with inactivated ND vaccine containing 50 µg bee venom and adjuvanted with paraffin oil	50	I/M	* Study the effect of different concentrations of bee venom on the immune response
	5	Chicken vaccinated with inactivated ND vaccine containing 25 µg bee venom and adjuvanted with paraffin oil	50	I/M	
	6	Chicken vaccinated with inactivated ND vaccine containing 75 µg bee venom and adjuvanted with paraffin oil	50	I/M	
(III)	1	Chicken vaccinated with inactivated ND vaccine containing 50 µg bee venom and adjuvanted with paraffin oil	50	I/M	* Study the effect of bee venom when used either as an extract in water or with adjuvant on the immune response
	7	Chicken injected with 50 µg bee venom in water then vaccinated with inactivated ND vaccine adjuvanted with paraffin oil	50	I/M	
Negative control	8	Unvaccinated control chickens	50		* Served as negative control
(IV)	1, 2, 3, 4, 5, 6, 7, 8	All the above groups were challenged with VVNDV at 2 and 3 weeks post vaccination	15 per each	I/M	* Determination of the protection %

* Blood samples were collected pre and post vaccination weekly till 4th weeks post vaccination (WPV) then every 2 weeks till 8th WPV and after that monthly till 8th month post vaccination (MPV). The serum was separated from blood by centrifugation and kept at -20°C till used in the serological tests.

* Each vaccinated group was divided into 3 subgroups. The first and second subgroups (15 chickens per each) were challenged with virulent local NDV at 2nd week and 3rd week post vaccination. The third subgroup (20 chickens) was used to follow serologically the immune response against each type of inactivated ND vaccines.

RESULTS

Experiment (I)

It studied the effect of adding 50 μg/ml bee venom to inactivated ND vaccines by 2 types of adjuvants (paraffin oil and Montanide oil) in comparison with the ordinary ND vaccines containing the same types of adjuvants as shown in Table 2. In groups 1 and 2 showed high HI titre began from 2nd WPV (7, 7.3 log_2, respectively). The titre increased to reach the peak 10.7 and 11.1 log_2 for groups 1 and 2 respectively at the 6 MPV then persisted till 7 MPV for group 1 and 8 MPV for group 2. After that, the titre began to decrease at 8 MPV to 10.5 for group 1.

On contrary, group 3 which was vaccinated with inactivated ND vaccine with paraffin oil showed increase in HI titre to reach 8 log_2 at 3 MPV and persisted till 4 MPV then decreased to reach 7.4 log_2 at 8 MPV. But, in group (4), the peak of HI is higher than group 3 (8.7 log_2) at the 4th MPV and persisted till 5 MPV then began to decline to reach 8.3 log_2 at 8 MPV, while group (8) (control non-vaccinated control) birds remain had sera free from any HI antibodies throughout the period of the experiment.

Experiment (II)

Table 3 shows the results obtained by HI test for sera of vaccinated chickens with inactivated ND vaccine containing different concentrations of bee venom. All birds had sera free from any antibody against ND when collected before vaccination denoting their susceptibility.

Chickens of group 5 vaccinated with inactivated ND vaccine containing 25μg/ml bee venom showed increasing in HI titres till reached its peak (9.9 log_2) at 5 MPV and persisted till 6 MPV, then began to decrease till reached 9.4 log_2 at the 8 MPV. While, group (I) chickens vaccinated with inactivated ND vaccine containing 50 μg/ml bee venom showed higher HI titre which reached to the maximum 10.7 log_2 at the 6 MPV and persisted to the 7th MPV then declined at the 8th MPV to reached 10.5 log_2. The highest values of HI antibodies occurred in group (6) which was vaccinated with inactivated ND vaccine containing 75 μg/ml bee venom. The titre reached 7.7 log_2 at 2nd WPV and continue to increase till reached 12 log_2 at 7th MPV and persisted till the 8th MPV.

Table 2. The effect of bee venom on the immune response of chicken vaccinated with different types of inactivated ND vaccines using HI test

Groups	No. of serum sample	Mean HI antibody titre (log_2)												
		Weeks Post Vaccination							Months Post Vaccination					
		Pre	1	2	3	4	6	8	3	4	5	6	7	8
1	15	0	4.4	7.0	8.2	9.0	9.3	9.5	9.7	10	10.3	10.7	10.7	10.5
2	15	0	4.5	7.3	8.5	9.3	9.5	9.8	10.1	10.5	10.8	11.1	11.1	11.1
3	15	0	2.9	4.2	6.5	7.1	7.3	7.5	8	8	7.9	7.7	7.6	7.4
4	15	0	3.7	4.9	6.8	7.7	7.9	8.0	8.5	8.7	8.7	8.6	8.5	8.3
8	15	0	0	0	0	0	0	0	0	0	0	0	0	0

Pre.: Prevaccination

Table 3. Comparison between HI results of vaccinated chickens with inactivated ND vaccine adjuvanted with paraffin oil and containing different bee venom concentrations

Groups	No. of serum	Mean HI antibody titre (log_2)												
		Weeks Post Vaccination							Months Post Vaccination					
		Pre	1	2	3	4	6	8	3	4	5	6	7	8
1	10	0	4.4	7.0	8.2	9.0	9.3	9.5	9.7	10	10.3	10.7	10.7	10.5
5	10	0	4.2	6.5	7.8	8.4	8.6	8.7	9.3	9.7	9.9	9.9	9.6	9.4
6	10	0	4.7	7.7	8.7	9.5	9.8	10	10.4	10.9	11.4	11.7	12	12
8	10	0	0	0	0	0	0	0	0	0	0	0	0	0

Pre.: Prevaccination

Experiment III

It discussed the comparative study of aqueous and oil adsorbed preparations of bee venom. The birds in group 1 showed higher HI antibody titre (7.0 log_2) than those in group 7 (5.7 log_2) at the 2nd WPV. The titre in chickens of group 1 continued to increase till reach the peak (10.7 log_2) at 6 MPV and persisted till 7th MPV then declined to reach 10.5 log_2 at 8 MPV.

While, the HI titre in sera of chickens in group 7 reached to its peak (9.2 log_2) at 4 MPV and persisted till 5 MPV then began to decrease to reach 8.5 log_2 at 8 MPV. The sera of the control group remained seronegative till the end of the experiment. The results are shown in Table 4.

Experiment IV

The experiment showed the protection percent of the chicken groups vaccinated with different types of inactivated ND vaccines and the effect of bee venom extract as an immunostimulant agent. At the 2nd WPV, groups (1, 2 and 6) were protected 100% against local VVNDV (titre 6 log_{10} / ml), while groups (5 and 7) gave lower percent of protection 93.3 % and 80 % respectively.

Also, groups 3 and 4 did not protect against virulent NDV and showed 53.3% and 66.7 % percentage of protection respectively. But, at 3rd WPV, all groups protected from infection with the challenge VVNDV. The protection percent of groups 1, 2, 5, 6 and 7 was 100% while of groups 3, 4 it was 93.6%. Results are shown in Table 5.

Table 4. Results of immune response of chickens vaccinated with inactivated ND vaccine due to the usage of bee venom dissolved in water or with different types of adjuvants

Groups	No. of serum samples	Mean HI antibody titre (log_2)												
		Weeks Post Vaccination							Months Post Vaccination					
		Pre	1	2	3	4	6	8	3	4	5	6	7	8
1	15	0	4.4	7.0	8.2	9.0	9.3	9.5	9.7	10	10.3	10.7	10.7	10.5
2	15	0	4.5	7.3	8.5	9.3	9.5	9.8	10.1	10.5	10.8	11.1	11.1	11.1
7	15	0	4.0	5.7	7.0	8.0	8.2	8.5	9.0	9.2	9.2	9.1	8.7	8.5
8	15	0	0	0	0	0	0	0	0	0	0	0	0	0

Pre.: Prevaccination

Table 5. Results of challenge test (protection %) of chicken vaccinated with different types of inactivated ND vaccines using local virulent NDV (n= 15).

Groups	2nd weeks post vaccination		3rd weeks post vaccination	
	No. of dead birds	Protection %	No. of dead birds	Protection %
1	0	100 %	0	100 %
2	0	100 %	0	100 %
3	8	53.3 %	1	93.3 %
4	5	66.7 %	1	93.3 %
5	1	93.3 %	0	100 %
6	0	100 %	0	100 %
7	3	80 %	0	100 %
8	15	0 %	15	0 %

DISCUSSION

Although, for live Newcastle disease (ND) vaccines, local immunity may contribute to protection, for inactivated vaccines protection is solely based on systemic immunity and more specifically, mainly on the humoral antibody response.

The HI response has found to be a good parameter for protection. Also, in the challenge experiment, protection against lethal challenge was accompanied with high antibody levels. As cited above, the **British Pharmacopoeia** ***(13)*** on inactivated ND vaccines offers an HI antibody induction test as an alternative for challenge test in batch quality control.

Also, the current evidence has defined the immunostimulant properties for bee venom. Thus, these studies direct comparisons of the efficacy and safety of different preparations of bee venom.

The data obtained from Table 2 in experiment I showed that inactivated ND vaccines are able to induce a distinct HI antibody response in the 4 groups of chickens. While, the early induction (at 2 WPV), high values (7, 7.3 $\log_2$) and persistence peak of HI antibody titres occurred from 6 to 7 MPV in group (1) and from 6 to 8 MPV for group 2 of chickens which were vaccinated with inactivated ND vaccine containing the bee venom as an immunostimulant additive is clearly shown in comparison with the response in other 2 groups 3, 4 groups which received ND vaccine adjuvanted either with paraffin oil (induce peak of immunity at 3 MPV and persisted till 4MPV) or montanide oil (induce peak of immunity at 4 MPV and persisted till 5 MPV).

Also, Experiment IV showed that all inactivated ND vaccines containing bee venom with different concentrations either in aqueous or adjuvanted form could protect chicken earlier at 2nd WPV from infection with local VVNDV. The protection percent ranged from 100 % for groups 1, 2 and 6 and 93.3 % for group 5. But the groups of chickens that vaccinated with inactivated ND vaccines only 3, 4 were not protected at that time (53.3 and 66.7%, respectively). While at 3rd WPV, all groups were protected against challenge virus with different percentages 100% for groups 1, 2, 5, 6 which received vaccine containing bee venom and 93.3 % for groups 3, 4 which did not receive bee venom.

The effect of bee venom which acts as an antigen is based on the stimulation of humoral and local cellular immune responses. This agreed with **Nam et al.,** ***(18)*** who reported that administration of bee venom elicits anti-inflammatory, anti-nociceptive and anti-allergic effects in various animal models. These properties of bee venom may depend on its direct effect on helper T-cell activities and on Th1/Th2 lineage development from CD4(+) T cells by increasing the expression of Th1-specific cytokine, IFN-gamma.

Also, it has been illustrated previously *(5)* that the usage of bee venom as an immunotherapy induce a monocytes activation characterized by a delayed overproduction of IL-12 and INF-alpha. These cytokines could be relevant to the inhibition of Th2 cells during venom immunotherapy. Therefore, venom immunotherapy (VIT) induced tolerance could depend not only on the specific action of venom antigens on T-cells, but also on a secondary non-specific action of monocytes. Also, recent study *(2)* showed the possibility of tumor growth and metastasis inhibiting effects of bee venom in mice and in tumor cell cultures. Intravenous administration of bee venom to mice significantly reduced the number of metastasis in the lung. Thus, the bee venom has an indirect mechanism for tumor growth inhibition which is based on stimulation of the local cellular immune responses in lymph nodes. In addition , to cellular immunity, bee venom elicits high levels of IgG1, IgE and haemagglutinating antibody responses ***(4).***

To investigate the amount of bee venom added to the ND antigen preparations, we compared between the HI antibody titres induced by different concentrations of bee venom as shown in Experiment II and Table 3. It was noticed that the increasing of bee venom concentration from 25, 50 to 75 μg/ml in the prepared vaccine will increase the antibody level 9.9, 10.7 and 12 $\log_2$ at 5, 6 and 7 MPV, respectively and persisting time of the peak of protective level of immunity till 6, 7 and 8 MPV. This results is consistent with that cited previously *(19)* indicated that the increasing amounts of bee venom (between doses of 0.025 – 0.5 g/ml) to human monocytes cell line exhibit a significant increase in proliferative response.

The objective of the Table 4 in Experiment III is to demonstrate that the adjuvant adsorbed bee venom preparation caused higher level of HI antibody titres than the aqueous preparation.

In this study, paraffin oil and Montanide oil adjuvants were used and the results showed increasing HI titres till reached the peak (10.7 $\log_2$) at 6 MPV and (11.1 $\log_2$) at 6 MPV for paraffin oil and Montanide ND vaccines respectively in comparison to 9.2 $\log_2$ at 4MPV for the aqueous preparation of bee venom.

Also, these results indicated that using of Montanide oil was more suitable for its ability to give highly protective level of immunity and persisted for longer time than that obtained by paraffin oil.

The previous data is in coincidence with that of previous study which *(20)* showed that venom immunotherapy (VIT) with aqueous extracts is considered a life-saving treatment in insect, but systemic side effects are quite common.

It has been considered that the immunotherapy with aluminium hydroxide adsorbed bee venom extracts was more safer, immunogenic, effective and the low rate of side-effects may be due to the slow release of the venom in the aluminium hydroxide adsorbed form *(21)*. Thus, antibody responses were raised in mice injected with honey bee venom administered in alum *(22)*. A single injection of 8 μg entrapped bee venom into mice gave the same IgG antibody response as 2 x 80 μg bee venom dissolved in water ***(23).*** By the same method, it was possible to inject 4 times the LD_{50} dose of entrapped bee venom in mice without any significant side reactions.

The previous data support and justify the use of the bee venom component for inactivated ND vaccines, where the study reported that bee venom increases the protective immunity induced by inactivated vaccines and persisted for longer time. So, our findings are of importance for the improvement of immunization and might lead to more effective vaccines. Also the immunostimulant property of bee venom could be effective in future to be use accompanied with some types of vaccines which might cause immunosuppression to the vaccinated individuals.

REFERENCES

1.***Mulfinger, L.S. (1990):*** Evaluation of the Synergistic antibacterial activity of polymyxin-B- and melittin. Toxicon., 41 (7) : 861.

2.***Orsolic, N.; Sver, L.; Verstovsek, S.; Terzic, S. And Basic, I. (2003):*** Inhibition of mammary carcinoma cell proliferation in-vitro and tumor growth in-vivo by bee venom. Toxicon., 41 (7): 861-870.

3.***Almeida, A.B.; Brandeburg, M.A.M.; Satrapa, R.; Jacomin, J.A; Beletti, M.E. and Visintim, J.A. (1999):*** Revista Brasileira de Reproducao Animal, 23 (3): 271.

4.***Fehlner, P.F.; Berg, R.H.; Tam, J.P. and King, T.P. (1991):*** Murine T cell responses to melittin and its analogs. J. Immunol., 146 (3): 799-806.

5.***Magnan, A.; Marin, V.; Mely, L.; Birnbaum, J.; Romanet, S.; Bongrand, P. and Vervloet, D. (2001):*** Venom immunotherapy induces monocytes activation. Clin. Exp. Allergy, 31 (8): 1303-1309.

6.***Alexander, D.J; Wilson, G.W.; Russell, P.H.; Lister, S.A. and Parsons, G. (1985):*** Newcastle disease out of breaks in fowl in Great Britain during 1984. Vet. Rec., 117: 429.

7.***Alexander, D.J. (1997):*** Newcastle disease and other Paramyxoviridae infections. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid and H. W. Yoder, eds Iowa State University Press, Ames, Iowa. pp. 541-570.

8.***Box, P.G. and Furminger, I.G.S. (1985):*** Newcastle disease antibody levels in chickens after vaccination with oil emulsion adjuvant killed vaccines. Vet. Rec., 96: 108.

9.***Reda, I.M. and Sheble, A. (1976):*** Isolation and characterization of local viscrotropic Newcastle disease virus. Unpublished data.

10.***Bahnemann, H.G. (1990):*** Inactivation of viral antigens for vaccine preparation with particular reference to the application of binary ethyleneimine. Vaccines, 8: 290-303.

11.***Allan, W.H.; Lancaster, J.E. and Toth, B. (1973):*** The production and use of Newcastle disease vaccines Food and Agriculture Organization, P. 53, Rome, Italy, 1115 PP.

12.***Madkour, N.S. (1995):*** Some trails for preparation of bivalent inactivated Fowl Cholera and Newcastle disease virus vaccines. Thesis Ph.D., Microbiology, Cairo University.

13.***British Pharmacopoeia (Veterinary) (2002):*** A companion volume providing standards for substances, preparations and immunological products used in veterinary medicine.

14.***Stone, D.; Brugh, M.; Hophins, S.R.; Yoder, H.W. and Peard, C.W. (1978):*** Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. Avian Dis., 22: 666-674.

15.***Barnett, P.; Pullen, L.; Stothon, R. and Salt, S. (1996):*** "Preliminary studies on (emergency) foot and mouth disease vaccine formulated with metabolizable or semi-metabolizable oil adjuvants season of the research group of the standing technical committee". MaCale HA, hamisha, 6-12 September European Commission for the Control of FMD.

16.***Anon, (1971):*** Methods for examination of poultry biologics and for identifying avian pathogens. National Academy of Science, Washington, D.C., p. 83-87.

17.***Majujabe, K.A. and Hitchner, S.B. (1977):*** Antibody response to strain combination of Newcastle disease virus as measured by haemagglutination inhibition test. Avian Dis., 21: 576-584.

18.***Nam, S.; Ko, E.; Park, S.K.; Ko, S.; Jun, C.Y.; Shin, M.K.; Hong, M.C. and Bae, H. (2005):*** Bee venom modulates murine Th1/Th2 lineage development. Int. Immunopharmacol., 5 (9): 1406-1614.

19. ***Hamedani, M.; Vatanpour, H.; Saadat, F.; Reza Khorramizaheh, M. and Mirshafiey, A. (2005):*** Bee venom, immunostimulant or immunosuppressor? Insight into the effect on matrix metalloproteinases and interferons. Immunopharmacol. Immunotoxicol., 27 (4): 671-681.

20. ***Quercia, O.; Rafanelli, S.; Puccinelli, P. And Stefanini, G.F. (2001):*** The safety of cluster immunotherapy with aluminium hydroxide-adsorbed honey bee venom extract. J. Investig. Allergol. Clin. Immunol., 11 (1): 27-33.

21. ***Wyss, M.; Scheitlin, T.; Stadler, B.M. and Wuthrich, B. (1993):*** Immunotherapy with aluminium hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. Allergy, 48 (2): 81-86.

22. ***Ahlstedt, S.; Bjorksten, B. and Akerblom, E. (1983):*** Antibody response to honey-bee venom and monomethoxy-polyethylene glycol-modified honey-bee venom in mice. Int. Arch. Allergy Appl. Immunol.. 71 (3): 228-232.

23. ***Schroder, U. and Stahl, A. (1984):*** Crystallized dextran nanospheres with entrapped antigen and their use as adjuvants. J. Immunol. Methods. 70 (1): 127-132.

الملخص العربى

دراسة التأثير المحفز للمناعة لبعض الإضافات المختلفة المستخدمة فى لقاحات النيوكاسل المثبطة

لمياء محمد عمر

المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية-العباسية-القاهرة

مازال مرض النيوكاسل يعتبر من أهم الأمراض التى تصيب الدواجن فى مصر. ومازال يستخدم اللقاح المثبط فى السيطرة علـى المرض بكفاءة عن طريق تحفيز الجهاز المناعى لإنتاج مناعة سائلة humoral immunity بنسبة عالية وليس عن طريق إنتاج مناعة خلوية cellular immunity مثل اللقاح المستضعف.

لـذا كان الهدف من هذه الدراسة إضافة بعض المواد مثل سم النحل التى لها تأثير مناعى محفز يساعد على رفع الكفـاءة المناعية السائلة او الخلوية للقاح النيوكاسل المثبط سواء المحمل على زيت البارافين او المانتونيد. ولقد تم استخدام تركـيزات مخـتلفة مـن سـم النحل تتراوح بين ٢٥، ٥٠، ٧٥ ميكروجرام لكل مللى فى تحضير أنواع مختلفة من لقاح النيوكاسـل. ولقـد استخدم لقياس رد الفعل المناعى للدجاج المحصن بهذه اللقاحات تجربة التلازن الدموى المثبط وتجربة التحدى.

ومن النتائج لوحظ الآتى:

١. ارتفـاع مسـتوى المناعة مبكراً بعد التحصين (٢ اسبوع) كما أنها استمرت لفترة طويلة فى حالة اللقاحات التى تحتوى على سم النحل.
٢. زيـادة تركيز سم النحل فى اللقاح يزيد من كفاءة الجهاز المناعى وبالتالى يؤدى الى زيادة إنتاج الاجسام المضادة والخلايا المناعية فى الدم.
٣. اللقاحـات الـتى تحتوى على سم النحل مدمص فى الزيت سواء بارافين او مانتونيد تعطى رد فعل مناعى قوى بالمقارنة برد الفعل الناتج من حقن سم النحل فى صورة مائية.
٤. ارتفـاع مسـتوى المناعة واستمرارها لفترة طويلة فى الدجاج المحصن باللقاح الذى يحتوى على زيت المانتونيد بالمقارنة باللقاح المحضر من زيت البارافين.

وبالتالى ومن هذه النتائج نلاحظ وجود تأثير مناعى محفز لمادة سم النحل مما يؤدى الى استخدامها فى كثير من المجالات مثل مجال العلاج المناعى لكثير من الأمراض او لرفع كفاءة العديد من اللقاحات.