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HUMORAL AND LOCAL IMMUNE RESPONSE TO DIFFERENT NEWCASTLE DISEASE VACCINES

BY

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INTRODUCTION

Newcastle disease virus is the main poultry pathogen severely endandering out of the poultry industry. In Egypt., ND is well established and widespread throughout the whole country commonly assuring on acute and subacute form (**Daubney and Mansy, 1947**). By the begining of the 1960, ND became endemic (**Sheble, 1962**). In view of the fact that vaccination is still the only mean for the control of ND (**Partadiredja et al., 1979**), there is increasing awarness of the value of used vaccines and vaccination programmes.

So, the aim of this stduy is to compare between the immune responses of one day old chicks vaccinated by intraocular method using the locally prepared inactivated aluminium hydroxide gel formalized vaccine and subcutaneous vaccination by importaed inactivated oil emulsion vaccine and also to asses the use of the highly immunogenic vaccine and the most suitable vaccination protocol.

MATERIAL AND METHODS

1. Chicks:

210 one day old Hubbard chicks obtained from immunized parents stocks and supplied by the General Poultry Company were used in this study.

2. Fertile chicken eggs:

9-10 days old embryonated chicken eggs (ECE) were supplied by General Poultry Company, the eggs were used for virus titration and virus strain propagation.

3. Viruses:

3.1. Virulent strain:

The virus used for challenging chicks throughout these experiments was a local field isolate, velogenic viscerotropic Newcastle disease virus VVNDV). It was iso-

lated and identified by **Reda and Sheble (1976)**. The infectivity titre was $10^{8.5}$ EID_{50}/ml.

3.2. Lentogenic strains:

3.2.1. Lentogenic Hitchner B_1:

HB_1 vaccine of ND was locally prepared in the Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, lot No. 1496- protection percent = 100%. Infectivity titre was $10^{10.5}$ EID_{50}/ml. and store at-20°C.

3.3. Inactivated NDV vaccine:

3.3.1. Inactivated aluminium hydroxide gel formalized ND vaccine:

This vaccine of NDV used in this study was locally prepared in the Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo lot No. 6591 - protection rate 90% and stored at refrigerator between +2 and + 8°C.

3.3.2. Inactivated oil emulsion ND vaccine:

The vaccine used in this study was manufactured in Holland Industries Hallondesa supplied by Intervert International Company B.V.Boxmeir-Holland, log No. 09028 protection rate 90% and stored in refrigerator between +2 and +8°C.

4. Serum samples:

5- Mucous samples (trachea swab samples):

Preparation of the trachea swabs were done after **Yoshido e al. (1971).**

6. Blood samples:

METHODS:

1. Propagation of the virus ii embryonated chicken eggs:

Virus propagation in embryo nated chicken eggs was applied ac cording to **Allan , W.A. (1974).**

2. Infectivity titration in em bryonated chicken eggs:

The titre was expressed in terms of the 50 percent end point. The 50% end point was estimated ac cording to **Reed and Muench (1938).**

3. Rapid plate haemagglutination test:

It was carried out according to the standard method described in (Methods for Examination of Poultry Biologics and for **Identifying and Quantifying Avian Pathogens, 1971).**

4.Haemagglutination-inhibition (HI) test:

The test was carried out accord-
ıg to the standard procedure de-
:ribed by Majiyabe and Hitch-
er (1977).

5. Challenge test:

All vaccinated birds were chal-
:nged intramuscularly with 0.5
l. of virulent VVNDV strain (10^6
ID_{50}/ml.) and were put for 15
ıys for observation. Birds died
ithin this period were collected
ıd subjected to detailed post mor-
m (P.M) examination for charac-
ristic lesions of NDV.

EXPERIMENTS AND RESULTS

Experiment (1):

**Estimation of maternal anti-
ıdies in chicks:**

2- one day old chicks were cho-
n randomly to estimate the level
maternal antibodies in their ser-
n before vaccination. Haemag-
ütination inhibition test (HI) was
ıplied for this purpose. The re-
lts of this experiment showed
at these chicks had HI antibody
res ranging from 2^4 to 2^7 with a
ain value of $2^{5.5}$.

Experiment (2):

Vaccination of chicks:
210-one day old chicks were di-
vided into six groups (35 in each). Each group of chicks was brooded in separate isolated brooder. This groups of chicks were vaccinated at one day of age as the following design.

Group I:
Vaccinated with inactivated aluminium hydroxide gel formalized ND vaccine by subcutaneous S/C injection of 0.2 ml. per bird in the dorsal side of the neck.

Group II:
Vaccinated with inactivated oil emulsion ND vaccine by S/C injection of 0.2 ml. per bird in the dorsal side of the neck.

Group III:
This group of chicks was vaccinated at the same time with both inactivated aluminium hdyroxide gel formalized ND vaccine by S.C and with Hitchner B_1 (HB_1) by eye drop where each 10^6 EID_{50}/bird.

Group IV:
This group of chicks was vaccinated simultaneously with both inactivated oil emulsion ND vaccine by S/C injection of 0.2 ml. per bird and HB_1 live ND vaccine by eye dorp as in group III.

Group V:
They were vaccinated with HB_1 live ND vaccine by eye drop as before.

Group VI:

This group was kept without voccination as control birds.

Table (1) : Maternal HI antibody titer .

No of chicks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HI titer	4	5	6	7	7	6	6	5	4	5	4	6	6	5	7	7	6	5	4	5

HI titer Log 2

The mean value = $2^{5.5}$

Experiment (3):

Evaluation of humoral immune response in chicks after vaccination:

8 chicks were chosen randomly from each group every week post vaccination for 6 successive weeks to evaluate the humoral immune response of such birds to the used vaccine. Using HI test, the results are shown in table (2) and and fig. (1).

Table (2) : Challenge test for chicks six weeks post vaccination .

Groups	No. of birds	Protected birds	Protection %
I	8	4	50
II	8	5	62.5
III	8	7	87.5
IV	8	8	100
V	10	8	80
VI	8	0	00

Experiment (4):

Evaluation of local immne response in chicks post vaccination:

3 chicks were chosen randomly from each group weekly for six successive weeks post vaccination to detect the local antibodies in the tracheal and pharyngolaryngeal region using swabs. HI test was applied on the mucous samples obtained from these regions of bleeded birds.

The results of this experiment revealed that the vaccinated birds did not response locally to the applied vaccination.

Experiment (5):

Challenge test:

To evaluate the potency of the used applied vaccination, challenge

test was applied six weeks post vaccination. A locally isolated velogenic viscerotropic strain of NDV (**Reda and sheble, 1976**) was used for challenge in dose of 10^6 EID_{50} per bird injected intramuscularly in all gorups. All birds were bled for two weeks under observation after challenge.

DISCUSSION

Several types of ND vaccines, both killed and living were available. The antigenic similarity among NDV had greatly contributed to the simplification of vaccination and had probably been the major factor in allowing the poultry industry to expand world wide bases.

Thus any ND strain vaccine or combination of different vaccine strains if used in the suitable dose and route property applied for vaccination of susceptible chicks can lead to the desired protective effect.

From the results it had been found that, the used chicks had maternal immunity of mean HI titre of 5.5 $\log_2$ and decreased within three weeks and disappeared in the fourth week of age. These results

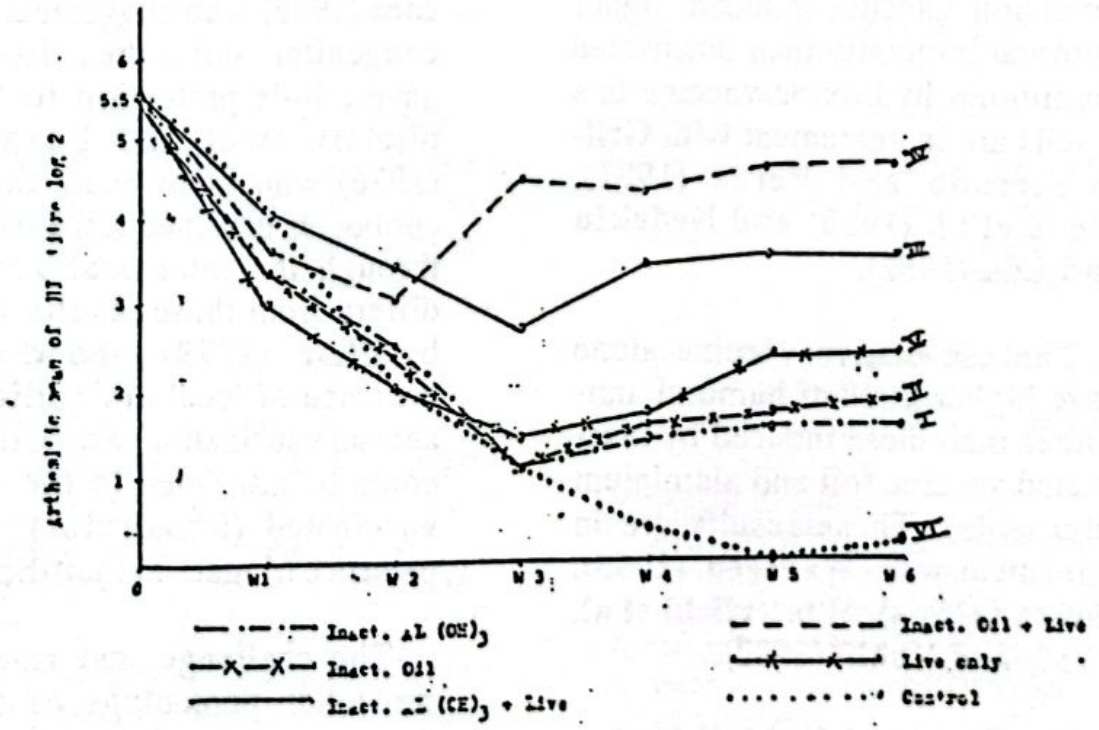

Fig.(1): Evaluation of humoral immunity by HI titer for chicks post vaccination

are in agreement with **Brandly et al. (1946), and Eissa (1979).**

The results of evaluation of humoral immunity which are shown in figure (1), revealed that in all groups except group IV the HI titre declined till the third week post vaccination then increased up to the sixth week.

In group IV, the decline in HI titre was to the second week and reincrease on the third week up to the sixth week. This result could be attributed to the neutralizing action of maternal antibody to the antigen of vaccines which is oberved clearly in goup V vaccinated with live strain vaccine only.

It was clear that inactivated oil emulsion vaccine induced higher humoral immunity than inactivated aluminium hydroxide vaccine this results are in agreement with **Grillo Terrado and Perez (1971), Stone et al. (1980) and Nedelciu and Edu (1982).**

The use of live vaccine alone gave higher titres of humoral antibodies than those induced by inactivated vaccine (oil and aluminium hdyroxide). These results are in agreement with **Waveren (1955), Hofstad (1964), Winterfield et al. (1980) and Kahlil (1982).**

Vaccination of chicks using both live and inactivated oil vaccine gave higher titres of humoral antibodies than in case of vaccination using liver inactivated aluminium hydroxide. Thes results are in agreement with **Box and Furminger (1975) and Kim et al. (1989).**

It could be suggested that the use of both live and inactivated ND vaccine induced best results than the use of each type alone. These results are in agreement with that reported by **Darderi et al. (1961), Box and Furminger (1975), Warden et al. (1975) and El-Sayed (1981).**

The experiment for detection of local immunity resulted in negative local immune response to the intraocular route of vaccination using the live vaccine; these results could be agreed with **Levine and Fabricant (1950)** who suggested that the congenital antibodies in chicks gave a little protection to the respiratory tract and **Levy et al. (1976)** who mentioned that local antibodies confined immunity only through the intranasal route and differn from those results reported by **Eissa (1979)** who detect the presence of local antibodies using aerosal vaccination. So, our results could be attributed to the route of vaccinated (intraocular) and the presence of maternal antibodies.

The challenge test resulted in protection percentage of 50% for inactivated aluminium hydroxide ND vaccine, 62.5% for inactivated oil emulsion vaccine, 87.5% for al-

uminium hydroxide vaccine with live HB_1, 100% for oil vaccine with live HB_1 and 80% for live alone. These results are agreed with those recorded in humoral immunity.

From this work it could be concluded that the use of both live and inactivated oil emulsion Newcastle disease vaccine is the best vaccination to protect birds against the disease during the early stage of live.

SUMMARY

This study was planned to assess the use of highly immunogenic vaccine. Comparing between live and killed ND vaccine and between two types of killed vaccine were used: HB_1 live NDV vaccine, inactivated aluminium hydroxide gel formalized ND vaccine and inactivated oil ND vaccine.

210 one day old chicks having maternal immunity of mean HI titre 5.5 $\log_2$ were sujected to vaccination with the mentioned ND vaccines and the following results were obtained:

1. The humoral immune response in chikcs after vaccination with live vaccine alone resulted in higher mean HI titre (2.375 $\log_2$ in 6th weeek post vaccination) than the use of killed vaccine alone. Inactivated oil emulsion ND vaccine alone induced higher level of humoral HI antibodies titre (1.75 $\log_2$ in 6th week post vaccination) than aluminium hydroxide gel formalized ND vaccine alone (1.5 $\log_2$ in 6th week post vaccination). Also, the use of both live with oil vaccine produced higher humoral immune response (4.5 $\log_2$ in 6th week post vaccination) than the use of live with aluminium hydroxide vaccine (3.5 $\log_2$ in 6th week post vaccination).

2. Study of the local immunity in vaccinated chicks for all groups revealed that these chicks have no local immunity.

3. The challenge test applied 6 weeks post vaccination indicated that the protection percent was 50%, 62.5%, 87.5%, 100% and 80% for the aluminium hydroxide vaccine, oil vaccine, aluminium hydroxide vaccine with live vaccine, oil vaccine with live vaccine and liver vaccine alone respectively.

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