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IMMUNE RESPONSE OF CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINES USING DIFFERENT VACCINATION PROGRAMS.

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SUMMARY

Six vaccination programs against Newcastle disease were evaluated. The criteria of evaluation were haemagglutination-inhibition (HI) test for humoral immunity, lymphocyte transformation (LT) test for cell-mediated immunity, ciliary activity (CA) test for local tracheal immunity and challenge test for overall vaccinal protection. The vaccines used are Hitchner B_1, LaSota and inactivated oil-adjuvant vaccines. The early vaccination with Hitchner B_1 vaccine gave a considerable immune response but not as high as later vaccination. While the use of inactivated oil-emulsion vaccine with living vaccines, specially if simultaneously used, afforded a solid immunity particularly if used at 5 day-old under our experimental conditions.

INTRODUCTION

Newcastle disease (ND) outbreaks are still exist, although mass immunization against ND has become commonplace (Siddique et al. (1986) due to high density of commercially reared poultry. Outbreaks of ND arise because effective immunization of chicks is difficult due to interference with vaccine virus replication by maternal antibodies, as well as the chicks have relatively low level of immune competence. This is compounded by variation in the maternal immunity and the period of waning among individuals (Giambrone, 1983). In addition, these outbreaks may arise due to appearance of new pathotypes or occurring of strain variation (Manal et al., 1997, & El-Kady and Madbouly 1997).

There are currently several NDV vaccination programs commonly used for broilers. As a rule, a single ND vaccination during the first two weeks of age doesn't provide adequate protection for the life of the broilers (Edison et al., 1976). Therefore, revaccination is common to stimulate higher, more durable level of immunity (Giambrone, 1985). Drinking water, intra-ocular,

intranasal and aerosol routes of vaccination are widely used in broilers against ND using live vaccines as Hitchner B_1 and LaSota strain which depend mainly in its protection on local and cellular type of immunity (Edison and Kleven, 1979). But inactivated vaccine of ND in endemic areas is recommended to be used for vaccination of chickens to obtain a better immune response which depends in its protection on the humoral type of immunity, (Kolbl, 1981).

Therefore, in out study we aimed to evaluate some different vaccination programs (including live and inactivated vaccines of ND) to conclude the reliable procedure which give good protection trying to minimize these storms of ND outbreaks. The evaluation of the immune response was done including humoral (by haemagglutination inhibition test), cell-mediated (by lymphocyte transformation test), local tracheal immunity (by ciliary activity), and resistance to velogenic viscerotropic strain of ND (by challenge test) at different intervals after vaccination.

MATERIAL AND METHODS

I- Material:

1.1- Embryonated chicken eggs (ECE):-

Fertile chicken eggs were obtained from a commercial source and embryonated to an age of 9 days

1.2- Experimental chickens:-

One day old commercial broiler chicks were used. The chicks were floor reared and f balanced commercial ration.

1.3- Newcastle disease virus strains:-

1.3.1- Vaccinal strains:

1.3.1.1- Lentogenic live vaccines: were 9 day old ECE and the EID_{50} was calc described by Reed and Muench (193 were used recommended by the producer

a- Hitchner B_1 strain vaccine: (batch n produced by Intervet Internation Boxmeer-Holland with virus titer $10^{9.3}$ E (1000 doses) was used for vaccin experimental chicks via eye drop.

b- LaSota strain vaccine: (batch n produced by Rhone Merieux 17, rue E 69002 Lyon-France with virus tit EID_{50}/vial (1000 doses) was used vaccination of experimental chicks via water.

1.3.1.2- Inactivated oil-emulsion vaccir no. 49031), produced by Intervet Inte B.V. Boxmeer- Holland was used for va of experimental chicks at a dose o through intramuscular (I/M) route.

1.3.2- Challenge strain: A local viscerotropic NDV (VVNDV) strain, iso identified by Sheble and Reda (1976) initial titre of 10^6 EID_{50}/0.1 ml was used

1.3.3- Antigen: HI antigen was according to Heinz, (1982) from pa

LaSota vaccine in allantoic sac 9 days old ECE.

1.4- ND antiserum: It was used as positive control in HI test.

1.5- Washed chicken erythrocytes:- prepared as Alexander, et al. (1983)

1.6- Buffers and Media:-

a- Phosphate buffer saline (PBS, pH 7.2).

b- RPMI 1640 tissue culture medium (Gibco - limited, U.K.).

c- Ficoll-hypaque (Sigma, U.S.A.) was used for separation of lymphocytes from the blood.

d- Sterile fetal calf serum (Gibco-limited, U.K.).

1.7- Reagents:

a- Heparin solution ampoules (5000 i.u.) was used as anticoagulant for collection of blood for LT test.

b- Phytohaemagglutinin-P (PHA), Sigma, U.S.A. was used as non-specific mitogen in the lymphocyte transformation test.

1.8- Kits: Kits for glucose consumption (God Pab method) and enzyme kits for sugar consumption (Boehringer Mannheim GMBH Diagnostica, West Germany, Cat. No. 124-036) were used to detect the residual glucose in RPMI medium.

2- Mehtods:

2.1- Lymphocyte transformation test (LT): A modified method of Lucy (1977) and Charles. et al. (1978) was used.

2.2- Glucose consumption assay: The blastogenic response of peripheral blood lymphocytes was measured through biochemical estimation of residual glucose in culture medium using the glucose consumption test described by Shimakura et al. (1985).

2.3- Haemagglutination (HA) test: The test was carried out after Anon (1971).

2.4- Haemagglutination inhibition (HI) test: Micro- beta-procedures of the HI method (using 8 HA units of antigen) was done after Takatsy (1956). Interpretation of the obtained results were given titer reference numbers (TRN) as described by Kaleta and Sigmann, (1971).

2.5- Virus infectivity and tracheal immunity: Tracheas were removed and examined for ciliary activity to assess virus infectivity and local tracheal immunity. The ciliary reaction was given an activity score after Marquardt, et al. (1985).

2.6- Challenge test: The chickens were challenged I/M with 0.1 ml containing 10^6 EID_{50}/ of VVNDV/chick. The challenged birds were daily observed for symptoms and/or specific moralities for 2 weeks. Birds with symptoms and survived till the end of the observation period were considered as if dead (Giambrone, 1985). Percentages of survival and acutal protection due to vaccination were calculated and recorded. Actual vaccination protection percentages were calculated according to the following formula: Survival % of vaccinated-challenged birds minus survival % of non-vaccianted-challenged birds.

2.7- Statistical analysis: It was carried out after the method of Cochran and Cox (1960).

Experimental design:-

Eight hundreds and fifty day old chicks were divided into 8 groups, as follow:

Group 1: 120 chicks kept as control non-vaccinated non-challenged (blank control).

Group 2: 40 chicks kept as control non-vaccinated challenged.

Group 3: 135 chicks were vaccinated with Hitchner B1 (HB1) and inactivated vaccine at day-old, LaSota at 14 day-old, then LaSota at 28 day-old.

Group 4: 135 chicks were vaccinated with HB1 at day old, LaSota at 14 day-old, then LaSota at 28 day-old.

Group 5: 115 chicks were vaccinated with HB1 at 5-day old, LaSota at 19 day-old, then LaSota at 33 day-old.

Group 6: 100 chicks were vaccinated with HB1 at 5-day old, then LaSota and inactivated at 19 day-old.

Group 7: 100 chicks were vaccinated with HB1 and inactivated at 5 day-old, then LaSota at 19 day-old.

Group 8: 105 chicks were vaccianted with
8 day old, at 10 day-old, then L
25 day-old.

- To monitor antibody response (using HI
random chicks were bled at one-day
determine the level of maternal a
Another 15 chicks from each gorup w
weekly till 35 day-old.

- To evaluate the cell-mediated immuni
LT test), 5 chicks from each group wer
1, 3, 5, 8, 11, 14, 17, 19, 21, 25, 28
day-old.

- Half the number of each group (from
was challenged at 28 day-old, the other
challenged at 35 day-old. The challen
were daily observed for 15 days t
clinical signs, post-mortem lesions and

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challenged at 35 day-old. The challen
were daily observed for 15 days t
clinical signs, post-mortem lesions and

- To assess virus infectivity and local
immunity (using ciliary activity), 3 ch
each group were suffocated and thei
were examined daily post-vacciantio
chicks were examined per group
post-challenge.

RESULTS

Table (1) Stimulation index of lymphocytes of vaccinated and non-vaccinated chickens with different programs.

Age of testing/ days	Lymphocyte stimulation index							
	Gp 1 C. B.	Gp 2 C. Ch.	Gp 3 H&Iat1d L.at14d L.at28d	Gp 4 H.at1d. L.at14d, L.at28d.	Gp 5 H.at5d. L.at19d, L.at33d.	Gp 6 H.at5d. L.at19d, I.at19d.	Gp 7 H&Iat5d L.at19d.	Gp 8 H.at8d. I.at10d, L.at25d.
1	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62
3	1.61	1.61	2.05	2.00	1.61	1.61	1.61	1.61
5	1.61	1.61	2.15*	2.01*	1.61	1.61	1.61	1.61
8	1.63	1.63	1.94*	1.89*	2.14**	2.13**	2.16**	1.63
11	1.62	1.62	1.82*	1.72	1.98*	1.97*	2.00**	2.02**
14	1.62	1.62	1.82*	1.67	1.72	1.82*	1.90*	1.92*
19	1.61	1.61	2.12**	2.02**	1.62	1.72	1.82*	1.83*
21	1.61	1.61	1.93*	1.83*	2.00*	2.18**	2.26***	1.83*
25	1.60	1.60	1.86	1.73	1.93*	2.19**	2.02**	1.82*
28	1.60	1.60	1.75	1.65	1.77	2.05**	1.89*	2.14**
32	1.59	1.59	2.15**	2.09*	1.68	1.89*	1.70	1.91*
35	1.58	1.58	1.94*	1.89*	2.00*	1.73	1.63	1.74*

* significant difference at $p < 0.05$.(*** : highly significant compared to other vaccinated groups, ** moderate significant and * low significant).

Gp: Group. d: day. C. B: Control Blank. C.Ch.: Control Challenged.

H: Hitchner B1vaccine I: Inactivated vaccine L: LaSota vaccine.

Statistical analysis of lymphocyte transformation test results in Table (1):

Revealed that stimulation index of gorup 6 was significantly higher from 8 to 32 day-old, as well as that of group 7 which ended this increase at 28 day-old. stimulation index of gorup 3 was significantly higher from 5 to 21 days old, then returned to increase from 32 to 35 day-old, and that of gorup 8 was increased from 11 to 35 days old. while that of group 4 & 5 showed interrupted moderate increase from 5 to 8 till 35 day-old

Statistically, the results of HI test in table (2) showed that HI antibody mean titer of group 3 was significantly higher from beginning of the experiment till 35 day-old, and that of group 7 which significantly increased at 14 days, then that of group 6 & 8 which significantly increased from 21 days old. While HI titer of group 5 increased from 21 to 28 days old then declined, and that of gorup 4 significantly increased at 35 days old.

Table (2) Haemagglutinating inhibiting (HI) antibodies titers in sera of chickens vaccinated with different programs.

Age of testing /days	Arithmetic mean of HI titers							
	Gp 1 C. B.	Gp 2 C. Ch	Gp 3 H&Iat1d L.at14d L.at28d	Gp 4 H.at1d. L.at14d. L.at28d	Gp 5 H.at5d. L.at19d. L.at33d.	Gp 6 H.at5d. L.at19d. I.at19d.	Gp 7 H&Iat5d L.at19d	Gp 8 H.at8d. I.at10d. L.at25d.
1	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02
7	2.21	2.21	2.05*	2.30	2.21	2.21	2.21	2.29
14	1.42	1.42	3.15*	1.51	3.00	3.00	3.4*	2.61
21	0.63	0.63	3.94***	2.89	3.1*	3.13*	3.76**	3.63*
28	0	0	5.1***	3.12	3.38*	3.38*	5.11***	4.59**
35	0	0	6.2***	3.67**	3.00	4.32***	6.10**	5.5***

* significant difference at p< 0.05. *** : highly significant compared to other vaccinated groups, ** moderate significant and * low significant).

Gp. Group. d: day. C. B: Control Blank. C.Ch.: Control Challenged.
H. Hitchner B1vaccine I: Inactivated vaccine L: LaSota vaccine.

Table (3) Results of challenge test at 28 & 35 day-old in vaccinated and non-vaccinated chickens with different programs.

Gp No.	Survival and vaccinal protection percentages of challenged birds						
	No. of challenged birds at each interval	At 28 days dead birds	Survival %	old vaccinal protection %	At 35 days dead birds	Survival %	old vaccinal protection %
Gp 1 C. B.	-	-	-	-	-	-	-
Gp 2 C. Ch	15	14	6.6	-	14	6.6	-
Gp 3 H.&I. at 1d. L. at 14d.& L. at 28 d.	15	0	100	93.4***	0	100	93.4***
Gp 4 H. at 1d. L.at 14d. &L.at 28d	15	3	80	73.4	2	86.6	80*
Gp 5 H. at 5d., L.at 19d.& L.at 33 d.	15	2	86.6	80*	3	80	73.4
Gp 6 H. at 5d. L.at 19d.& I. at 19d	15	2	86.6	80*	0	100	93.4***
Gp 7 H.&I. at 5 d L. at 19 d.	15	1	93.3	86.7**	1	93.3	86.7**
Gp 8 H. at 8 d. I.at 10 d &L. at 25 d.	15	2	86.6	80*	0	100	93.4***

* significant difference at p< 0.05. *** : highly significant compared to othe vaccinated groups, ** moderate significant and * low significant).

Gp. Group d: day. C. B. Control Blank. C.Ch.: Control Challenged.
H. Hitchner B1vaccine I: Inactivated vaccine L: LaSota vaccine

Statistical analysis of results of challenge test (table 3) revelaed that:

At 28 days, vaccinal protection % of group 3 is higher%, group 7 followed by groups 5,6 and 8 finally that of group 4 which showed the one. At 35 days, groups 3,6 and 8 significant higher protection %, then g followed by group 4 and group 5.

Table (4)Ciliary activity in tracheas of vaccinated and non-vaccinated chickens with different programs.

Age of testing /days	Ciliary activity							
	Gp 1 C. B.	Gp 2 C. Ch.	Gp 3** H&Iat1d L.at14d L.at28d	Gp 4** H.at1d. L.at14d, L.at28d.	Gp 5* H.at5d. L.at19d, L.at33d.	Gp 6* H.at5d. L.at19d, I.at19d.	Gp 7* H&Iat5d L.at19d.	Gp 8 H.at8d. I.at10d, L.at25d.
1	4@	-	4	4	4	4	4	4
2	3	-	3	3	3	3	3	3
3	2	-	3	2	2	2	2	2
4	1	-	2	2	1	1	1	1
5	±	-	2	1	±	±	±	±
6	0	-	1	1	1	1	1	-
7	0	-	±	±	1	1	1	-
8	0	-	1	1	1	1	1	-
9	0	-	2	2	1	1	1	±
10	0	-	3	3	2	2	2	1
11	0	-	4	4	3	3	3	2
12	0	-	4	4	3	4	4	2
13	0	-	4	4	3	4	4	2
14	0	-	4	4	4	4	4	3
15	0	-	3	4	4	4	4	3
16	0	-	3	3	4	4	4	3
17	0	-	3	3	4	4	4	4
18	0	-	3	3	4	4	4	4
19	0	-	2	2	4	4	4	4
20	0	-	2	2	3	3	3	4
21	0	-	2	3	2	2	2	4
22	0	-	3	3	2	2	2	4
23	0	-	4	4	1	1	1	4
24	0	-	4	4	1	3	3	4
25	0	-	4	4	3	4	4	4
26	0	-	4	4	3	4	4	3
27	0	-	4	4	4	4	4	2
28	0	-	4	4	4	4	4	3
29+	0	0	3	3	4	4	4	3
30	0	0	3	4	4	4	4	4
31	0	0	4	4	4	4	4	4
32	0	0	4	4	4	4	4	4
33	0	0	4	4	4	4	4	4
34	0	0	4	4	3	4	4	4
35	0	0	4	4	1	4	4	4
36	0	0	4	4	1	4	4	4
37	0	0	4	4	2	4	4	4
38	0	0	4	4	3	4	4	4
39	0	0	4	4	3	4	4	4
40	0	0	4	4	3	4	4	4

@ Composite score based upon the ciliary activity in tracheas of 3 chickens examined at each date. Ciliary activity graded as follow: 4= 100%, 3= 75%, 2= 50%, ± = < 25% to zero activity, - = not done.

+ Tracheas of 2 chickens from each gp were examined post-challenge.

Gp: Group. d: day. C. B: Control Blank. C.Ch.: Control Challenged.
H: Hitchner B1vaccine I: Inactivated vaccine L: LaSota vaccine.

Results of table (4): Showed the ciliary activity which observed microscopically, began to decline at 1 day to reach the lowest degree at 6 days post first vaccination. While the activity was declined shortly for 2-4 days post second vaccination. Recovery began at 7 days post 1st vaccination. While at 8 days post 1st vaccination, normal ciliary activity was again restored. When vaccinated chickens were challenged, no ciliostasis was occurred, whereas non-vaccinated challenged control chickens showed characteristic ciliostasis. Statistically; examined birds of groups 3 and 4 showed higher ciliary activity rates, followed by groups 5,6 and 7 while gorup 8 showed the lowest rate.

DISCUSSION

Because of the economic importance of Newcastle disease to the poultry industry it is desirable to establish immunity by vaccination as early in the life of birds as possible. In order to properly immunize young chickens against ND, certain requirements must be met. Quaglio et al. (1975) have determined the choice of vaccine type to be very important, the age of the birds at time of vaccination, the time elapse between vaccinations and the maternal antibody titers also influence the efficacy of the ND vaccine.

Under field conditions severe outbreaks occur despite of vaccination with various vaccines in different vaccination schedules. Some of the currently used vaccination programs were assayed in our study.

The first program used in this study (R ocular instillation of HB1 simultaneously injection of oil-inactivated vaccine at one and oral administration of LaSota at 14 days old; LT test results revealed stimulation index of chicks from 1-21 (table 1), because the cell-mediated imm not affected by maternal antibody and wa by 3 days post-vaccination (Hofacre et al So HB1 vaccination at 1 day old induced stimulation for 7 days. Then re-stimulat revaccination with LaSota at 14 days and in addition to the oil-inactivated vaccine w as a continuos stimulus to cellular im (Allan, 1971). Jayawardane and Spr (1995) detected CMI 9 days after vaccination by eye drop, while only a second dose of vaccine delivered into th While alexander, 1997 stated that the immune response to infection with N cell-mediated immunity and may be detec early as 2-3 days after infection with live strain, this presumably explains the protection against challenge that has recorded in vaccinated birds before a consi level of antibody response is seen. On co Chen and Liang, (1985) found that no sig reaction to lymphocyte transfor micro-assay of chickens vaccinated with LaSota vaccines.

Maternal HI titer showed transient decreas at 7 days old, followed by gradual incre reached to highly significant level (6.2) at 5 old (table 2). High levels of maternal antib partially neutralize live vaccine as w inactivated oil-emulsion ND vaccine (Qu

al., 1975 and Edison et al. , 1982). The presence of maternal NDV antibody reduced the response to vaccination, (Bell et al., 1991). As well as Warden et al. (1975) showed that the inhibitory effect of maternal antibodies is partially alleviated if B1 vaccine is given at the same time as the oil-emulsion vaccine. Seham (1997) observed that solid immunity could be achieved when vaccination with live ND vaccine was followed by an inactivated oil-emulsion ND vaccine. The results of cell-mediated immunity (CMI) were correlated to the presence of the serologic immune response till 21 day-old but differed quantitatively as the results of Agrawal and Reynolds (1991) in which the quantitative level of CMI response didn"t always carrespond to the quantitative level of the serologic immune response, so we were unable to predict humoral antibody level on the basis of a CMI response and vice versa. On the other hand, Jayawardane and Spradbrow (1995) observed no obvious correlation between antibody and CMI responsiveness.

Tracheal immunity revealed normal ciliary activity in newly hatched chicks while after HB1 vaccination it ranged from decrease in the activity of the cilia to complete ciliostasis (table 4), as the results obtained by Marquardt et al. (1985). Regeneration of the cilia has been took place 6 days post 1st vaccination and 2 days post 2nd vacciantion. These results correlated with the appearance of moderate levels of humoral antibody and considerable level of stimulation of lymphocytes. However, Marquardt et al. (1985) reported that ciliary activity in immune birds was established as a similar marker in experimental NDV infection and it could serve as a suitable criterion for associating resistance to NDV challenge with serological titers. Local immunity is stimulated by vaccination or infection with live viruses and protection occurs very soon after application. The exact function of local immunity in protection is not clear although its role in protection of the respiratory tract independent of humoral immunity has been proposed (Alexander, 1997).

The chickens responded to antigenic stimulation and were protected against challenge virus; so vaccinal protection % was 94.4% (table 3) due to solid immunity (cellular, humoral and local immunity). As results of Cajavec et al. (1995) who showed protection of chickens vaccinated with live and killed vaccines against ND 90, 80% at 42nd and 74th day after vacciantion, respectivley. Moreover, Ratanasethakul et al (1985) obtained 80 - 100% protection of chickens vaccinated with LaSota, F, HB1 and/or M.P.

In the second program (group 4); ocular vaccination of HB1 at one day old, oral vaccination of LaSota at 14 and 28 days old, stimulation index of lymphocytes recorded transient increase 7 days post-each vaccination. A result which accords with those reported by Alexander, (1997) and Jayawardane and Spradbrow (1995). However; Chen and Liang, (1985) disagree with these results. Obtained results may be attributed bo absence of the action of inactivated vaccine which noticed in the first program (table 1).

HI antibodies decreased at the 7th day of age despite of HB 1 vaccination at 1 day-old however,

it began to increase till reached to moderate level (3.12) at 28 days old (table 2). A finding which may reflected to revaccination because antibodies are usually detectable in the serum within 6-10 days and peak response is achieved at 3-4 weeks post vaccination (Alexander, 1997). These HI levels are reverse to those obtained in the first program in which the oil-adjuvant vaccine was contributed.

Tracheal immunity showed no significant difference than that of gorup 3 table 4). But the survival % of group 4 (73.4%) was lower (94.4%) than that of group 3 at 28 days old, while it was 80% at 35 days old (table 3). Yalcin et al. (1984) showed that antibody titers has protective titers 15 days after the first vaccination, and showed 50% resistance against VVND after 15 days from vaccination. These may be due to the weak effect of live vaccines to induce strong humoral immunity and the resistance of chickens against VVND depends on CMI and local immunity. Secretory antibodies are induced on the respiratory mucosal surface by intranasal vaccination, so protected chickens from lethal infection by inhibiting virus replication at entry of the virus, (Takada and Kida, 1996).

The third program (group 5); intra-ocular vaccination with HB1 at 5 days old, oral vaccination with LaSota at 19 & 33 days old, resulted in increase in blastogenic response of lymphocytes 6 days post each vaccination as occurred in gorup 4 (table 1). Detected humoral immunity (HI) gradually decreased then started to increase 9 days post- vaccination till reached 3.38 at 28 days old. Significant increase in HI antibodies 3.1, 3.38 at 21, 28 days ol indicate transient increase (table 2). (1984). Mentioned that high level of in lasting up to 5 weeks was develope vaccination with B1 and LaSota str intra-ocular route.

Tracheal immunity showed depletion post-vaccination (table 4), due to the o vaccine on the cilia of trachea) then re normal. So this activity play a great challenge test.

Protection rate was 80% and 73.4% at 28 days old; respectively (table 3). the decreas resistance of this program may be due to a of continuos stimulus to immunity achie the oil adjuvant vaccine used in the first pr Whereas both stimulation index and an level showed transient increase at 21 & 2 old.

In the fourth program (group 6); vaccination with HB1 5 days old an vaccination with LaSota simultaneously wit injection of inactivated vaccine at 19 day revealed that stimulation index signifi increased at 8 and highly significant at 2 old (table 1). This induced a good CMI.

HI antibodies gradually increased vaccination and became considerably h 21-35 day-old (table 2). This gave an appre immune response.

Ciliary activity was highly reacted 4 days vaccination and still active till the end

experiment (table 4).

Collectively, Challenge test showed 80%, 93.4% vaccinal protection % at 28, 35 day-old, respectively (table 3). This solid immunity represented by LT, HI antibodies and ciliary activity is due to persistent activation of immune response.

The fifth program (group 7) where HB 1 was given ocularly with i/m. injection at 5 day-old followed by orall vaccination with LaSota at 19 days old., resulted in highly signifcant increase in blastogenic response of lymphocytes form 8 to 28 day-old (table 1).

HI antibodies were significantly high, 3.76 to 6.1 form 14 to 35 day-old (table 2)

These serological response correlated to CMI as shown in group 3.

Ciliary activity was not significantly different from other vaccinated groups (Table 4).

Challenge test revealed good protection 80 and 93.4% at 28 and 35 days old; respectively. The same protection rates were obtained in group 3 (Table 3).

The Sixth program where birds of group 8 received ocular vaccination with HB1 at 8 days old, i/m. injection of inactivated at 10 days old and oral vaccination with LaSota at 25 day-old, resulted in significant increase in stimulation index from 11 to35 days old, which was not high as in groups 3 and 6 (Table 1) and this began to increase later.

HI antibodies were significantly increase from 21 to 35 days old but not as high as groups 3 and 6 (Table 2).

Ciliary activity showed a good activation for a long period 14 to 35 days old (Table 3). The challenge test showed that vaccinal protection rate was 80 and 93.4% at 28 and 35 days old, respectively (Table 3). While Bastami,et al (1986) recorded 46.67, and 83.33% protection rates in chickens ocularly vaccinated with HB1 and inactivated vaccine by I/M route; respectively.

In conclusion, Live vaccines which used in vaccination of chickens against ND gave a considerable immune respons, but early vaccination may interfere with maternal antibodies. Allan, et al (1978) emphasized that the efficacy of a live virus vaccine depends on its invasiveness and its power to multiply sufficiently within the chickens to set up an adequate immune response. These are dependable on CMI and local immunilty but they have little stimulation of humoral immunity. While The use of inactivated oil-emulsion vaccine with live vaccines (specially if used simultaneously) gave a solid immunity. Its use at 5 day-old gave a best result. As described by Quaglio et al. (1975) who concluded that vaccination at 8 day-old more effective than vaccination at 5 days old and at 5 day-old is better than at one day old.

Oil-emulsion-inactivated vaccine have proven to be highly effective and are not as adversely affected by maternal immunity as live vaccines and can be used in day old chicks, (Alexander,1997). Oil emulsion vaccine have been shown to induce high titers of HI antibodies in chickens more than 5 weeks (Warden et al.,1975). The long duration of these titers suggests that the antigen persists in the tissue and continues to stimulate antibody production. If oil emulsion were given to maternally-immunee chickens shortly after hatching, the antigen might similarly persist, inducing an immune response

when the bird had reached full immunological competence as the maternal antibody titers declined.Quaglio et al.(1975) demonstrated that in the first 2 weeks of life the physiological capacity of chicks to respond to immunogenic stimulation is inadequate and to obtain solid immunity against ND at least two vaccinations are necessary. Moreover, Combination of live and inactivated vaccine is more efficacious for preventing ND than either live or killed vaccine alone (Giambrone and Clay, 1986).

As the immune response increases as pathognicity of the live vaccine increases. Therefore, to obtain the desired level of protection without serious reaction, vaccination programs are needed that involve sequential use of progressively more virulent viruses, or live virus followed by inactivated vaccine (**Alexander, 1997**).

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