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## INTRODUCTION

Parasitic diseases cause great losses in poultry industry, as they may not only be the direct cause of death but also deffect the productivity of the birds. Coccidiosis is one of the major parasitic diseases affecting poultry. It is caused by *Eimeria* species characterized by diarrhea, high morbidity and mortality (REID, 1978). Newcastle disease is a highly contagious speticaemic disease which attacks chiefly chickens and turkeys usually in a acute form (HANSON and BRANDLY, 1958).

In Egypt, Newcastle disease (ND) is well established and wide spread through the whole country assuring an acute and subacute form (DOUBNEY and MANSI, 1947). Prevention of ND is accomplished by sanitation and vaccination programmes against the disease with live locally produced vaccines (B<sub>1</sub>, F and Komarov).

The present study was disignated to study the effect of the protozoon parasite (*Eimeria naecatrix*) on the immune response using HB<sub>1</sub> NDV vaccine.

## MATERIAL and METHODS

### 1 - Experimental birds:

Eighty five one-day-old white huppered chicks which proved to be free from coccidia were obtained and reared under complete hygienic measures.

### 2 - Ration:

Commercial broiler ration was obtained from El-Salam Poultry Company, this ration requested not to contain any coccidiostats.

### 3 - Seed oocysts of *Eimeria necatrix* strain:

A pure strain of *E.necatrix* was kindly supplied by Dr.Norton (Central Vet. Lab. New Haw, Weybridge, U.K). The oocysts were kept in 2.5% potassium dichromate till use.

### 4 - Preparation of culture and inoculation of birds:

As described by BARWICK, et al. (1970).

### 5 - Determination of infective dose:

To ascertain the number of oocysts necessary to cause consistent rate of morbidity among susceptible birds, four groups of 10 one-week old chicks were infected with 10,000-20,000-30,000 and 50,000 sporulated *E.necatrix* oocysts respectively. The effect was recorded to determine the dose of oocyst that will be able to induce clinical disease.

### 6 - Counting of oocysts out put/gm of faeces by use of Mac-Master slide:

The technique of GORDON and WHITLOCK (1939) was used. Calculation was

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made by using the formula adopted by GORDON and WHITLOCK (1939):

$$\frac{\text{Number of oocysts in two chambers}}{2} \times 100 = \frac{\text{Number of oocysts out put/gm faeces}}{\text{faeces}}$$

**7 - Vaccine used:**

The lentogenic living HB<sub>1</sub> strain was used. It is prepared locally in Vet. Serum & Vaccine Res. Inst., Abbassia, Cairo. Lot No. 5988. This lot was titrated in the Inst. and its titre was 10<sup>9.5</sup>/ml. EID<sub>50</sub> (BARHOUMA, et al. 1987).

**8 - Haemagglutination test (HA):**

It was carried out according to the standard methods described in (Methods for examination of poultry biologics, 1963).

**9 - Haemagglutination inhibition (HI):**

The beta procedure was carried out using 4 HA units according to ANON (1971) on the sera of chickens.

**Experimental design:**

Eighty five chicks were used. The chicks were infected with 20,000 sporulated *E.necatrix* oocysts and vaccinated with HB<sub>1</sub> vaccine. The chicks were classified into five groups each of 17 chicks, each subgroup was as follows:

- Group 1:** Inoculated with 20,000 sporulated *E.necatrix* oocysts each 3 days before vaccination.
- Group 2:** Inoculated with 20,000 sporulated *E.necatrix* oocysts each on the day of vaccination.
- Group 3:** Uninoculated and unvaccinated.
- Group 4:** Inoculated with 20,000 sporulated *E.necatrix* oocysts each and unvaccinated.
- Group 5:** Uninoculated and vaccinated.

Random faecal samples were collected daily from each group until the time of challenge with the velogenic strain of ND (21 days post vaccination). The faeces was examined using the concentration floatation technique as described by DAVIS, et al. (1963) and the number of oocysts out put/gm faeces was estimated using Mac Master technique after GORDON and WHITLOCK (1939).

One ml. chicken blood was collected every 48 hours by heart puncture four days post vaccination from each chick of the different subgroups for estimating the HI antibody titre.

**10- Virulent virus:**

The virulent velogenic viscerotropic (V.V) locally isolated field strain of NDV was employed for challenge (SHEBLE and REDA, 1978). A dose of 10<sup>4</sup> EID<sub>50</sub>/bird was used.

**11- Challenge test:**

Twenty one days after vaccination, the group of birds to be challenged transferred to Animal Health Research Institute. Each chicken was inoculated I/M with challenge virus, watched daily for clinical symptoms for 14 days. Mortality and P.M. lesions were recorded.

**RESULTS**

The results showing the relation between the number of oocysts out put/gm faeces and geometric mean of HI antibody titre against NDV (HB<sub>1</sub>) were studied in 5 groups.

**Group 1:**

Chicks infected 3 days before vaccination. Table (1) showed that the vaccinated chicks shed the sporulated *E.necatrix* oocysts 8 days post infection and continued to shed oocysts for 32 days post infection. The number of oocysts varied from zero to 61100 oocysts/gm faeces. The minimum oocysts out put zero was recorded on the 15<sup>th</sup> day post infection when the geometric mean titre (GMT) was (2.05) on the 21<sup>st</sup> day post vaccination. The maximum oocysts out put (61100) was recorded on the 8<sup>th</sup> day post infection when the GMT was zero on the 5<sup>th</sup> day of vaccination.

The control chickens (infected non vaccinated) in group (4) began to shed the unsporulated *E.necatrix* oocysts after prepatent period 6 days with patent period of 13 days. The number of oocysts varied from 450 to 73250 on the 14<sup>th</sup> day and 10<sup>th</sup> post infection respectively. The GMT varied from (2.52) on the 7 days to (6.94) on the 21<sup>st</sup> day post vaccination in control chickens in group (5). Infected birds of this subgroup showed no clinical symptoms of either coccidiosis or NDV, only one bird died after challenge. The protection % was 94.11%.

**Group 2:**

Chickens infected with *E.necatrix* oocysts and simultaneously vaccinated. Chicks infected with 2000 sporulated *E.necatrix* oocysts on the same day of vaccination shed the unsporulated *E.necatrix* oocysts after 6 days with patent period of 15 days post infection. The number of oocysts out put per gram faeces varied from 100 to 13850 oocysts. The minimum oocysts out put "100" was recorded on the 15 day post infection where the GMT was 40 on the 15<sup>th</sup> day post vaccination. The maximum oocysts out put 13850 was recorded on the 10<sup>th</sup> day post infection when the GMT was (2.35) on the 10<sup>th</sup> day post vaccination.

When the GMT was at its peak (4) on the 21<sup>st</sup> day post vaccination, the number of oocysts out put per gram was 1600. Chicks in the control group (4) (infected non vaccinated) began to shed the unsporulated *E.necatrix* after patent period 6 days

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with patent period 13 days. The number of oocysts out put/gm faeces varied from 450 to 73250 on the 14th and 10th day post infection respectively. The GMT of the control group (5) (vaccinated non infected) varied from 2.52 on the 7th day to 6.94 on the 21st day post vaccination. A great difference was found when this control subgroup was compared with group (2) where the GMT of HI antibody titre varied from (1.76) on the 6th and group (4) on the 21st day post vaccination Table (2). The chicks in this subgroup showed severe symptoms of coccidiosis started 3 days post infection. Two birds died 9 and 10 days post infection. P.M examination revealed multiple peticheal haemorrhages over large area of the intestine. After challenge 2 birds died and the protection was 86.66%.

### DISCUSSION

Group (1) was infected with *E.necatrix* oocysts 3 days before vaccination to study the effect of the 1st generation schizonts which appear 72 hours post infection (DAVIS, 1956) on the elicitation of haemagglutinins after B<sub>1</sub> vaccination. The results showed clearly that the maximum number of oocysts out put (61100) was recorded on the 8th day post infection. The greatest number of oocysts out put in this time might be due to the stress effects as the chicks were passing the period of post vaccinal reaction.

On the other hand, the GMT in the 7th day post vaccination was (1.47) whereas the GMT in the control group was (2.52). This might be due to the stress effect of the inoculated parasite. It was also noticed that the peak of HI antibody titre (4.53) was lower when compared with that (6.94) of the control subgroup. There is no other reason which could be responsible for that except the inoculated parasite (MOHAMMED, 1980 & 1982). The death of only one bird out of 16 chicks after challenge might be attributed to immune factors after vaccination which protected other birds of this group.

In the group (2), the chicks were infected with *E.necatrix* oocysts and simultaneously vaccinated, it was found that the GMT was lower (1.76) when compared with the control group (2.52). This may reflect the depressive effect of the third generation schizonts which occurs on the 6th to 7th day post infection (DAVIS, 1956). This depressive effect on development of HI antibodies was more clear at 21 days post vaccination where GMT dropped from 6.94 to 4.0. This might be due to the inoculated parasite (MOHAMMED, 1980 & 1982 and HEGAZY, *et al.* 1986).

Regarding the effect of vaccination on the out put, the results (table 2) showed a relatively larger number of oocysts in the first 4 days in this subgroup when compared with that of the control. This might be attributed to the stress effect of vaccinat-

ion, a result which agreed with that of UGOCHUKU (1982) who concluded that post vaccination reaction interfered with resistance to caecal coccidiosis. On the other hand, the chicks of this subgroup showed severe symptoms of coccidiosis and two birds died. The results agreed with that obtained by HEGAZY, et al. (1986) who found that chicks infected with *E.tenella* and vaccinated with B<sub>1</sub> NDV revealed high morbidity and relatively higher mortality. Challenge results obtained in this subgroup showed death of two birds and development of specific signs. The lower protection obtained after challenge could be attributed to the influence of the development stage of *E.necatrix* infection.

Generally, the present results concerning the effect of ND vaccination on susceptibility of chickens to coccidiosis agreed with that obtained by MOHAMMED (1980) who reported that subclinical infection with coccidial parasite *E.tenella* had a depressive effect on the immune response of chickens vaccinated with lentogenic vaccine F of NDV. Also, MOHAMMED (1982) stated that *E.tenella* infection lead to obvious decrease of GMT of ND. Similar findings were obtained by HEGAZY, et al. (1986) who found that *E.tenella* infection especially to one day old chicks lead to suppression of the immune response to ND vaccine.

From the aforementioned results, it could be concluded that *E.necatrix* infection in ND vaccinated chicks resulted in suppression of immune response especially in young chicks. So, it is recommended that periodical faecal examination should be done before and after vaccination to obtain expected results of vaccine and to avoid any coccidial outbreaks.

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Table (1)  
Relation between the number of oocysts output per gram faeces  
and geometric mean of HI antibody titre in group (1)

Days post inoculation	No. of oocysts output per gram faeces		Days post vaccination	Geometric mean of HI antibody titre.	
	Gr.No. 1	Gr.No. 4		Gr.No. 1	Gr. No. 5
4	0	0			
5	0	0			
6	0	0			
7	0	600	4	0	0
8	61100	1000	5	0	0
9	30350	1650	6	0	0
10	39500	8450	7	1.47	2.52
11	29300	73250	8	1.47	2.52
12	27000	9650	9	1.58	2.64
13	2650	3100	10	1.58	2.64
14	150	600	11	2.05	3.11
15	0	450	12	2.05	3.11
16	500	750	13	2.05	3.17
17	3450	1150	14	2.05	3.17
18	1150	3350	15	3.23	3.82
19	1200	1050	16	3.23	3.82
20	900	0	17	3.23	3.94
21	200	0	18	3.23	3.94
22	300	0	19	4.29	6.76
23	150	0	20	4.29	6.76
24	100	0	21	4.35	6.94



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Table (2)  
Relation between the number of oocysts output per gram faeces  
and geometric mean of HI antibody titre in group (2)

D a y s p o s t i n o c u l a t i o n	No. of oocysts out put per gram faeces.		Days post vaccination	Geometric mean of HI antibody titre.	
	Gr.No.2	Gr.No. 4		Gr.No. 2	Gr.No. 5
4	0	0	4	0	0
5	0	0	5	0	0
6	0	0	6	0	0
7	650	600	7	1.76	2.52
8	2150	1000	8	1.76	2.52
9	7250	1650	9	2.35	2.64
10	13850	8450	10.	2.35	2.64
11	6250	73250	11	2.47	3.11
12	2550	9650	12	2.47	3.11
13	1450	3100	13	3.64	3.17
14	550	600	14	3.64	3.17
15	100	450	15	3.70	3.82
16	850	750	16	3.70	3.82
17	3300	1150	17	3.70	3.94
18	7550	3350	18	3.70	3.94
19	6800	1050	19	3.66	6.76
20	3500	0	20	3.66	6.76
21	1600	0	21	4.0	6.94