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EFFECT OF THE INFECTION WITH CRYPTOSPORIDIUM ON THE IMMUNE RESPONSE OF CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINES AND/OR GUMBORO DISEASE VACCINES

(With 7 Tables, 4 Figures and 2 Photos)

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تأثير الإصابة بطفيل الكربتوسبورديم على استجابة الكتاكيت للقاحات النيوكاسل والجمبورو

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لدراسة تأثير الإصابة بطفيل الكربتوسبورديوم على الإستجابة المناعية للقاحي النيوكاسل والجمبورو تم إجراء تجربة على عدد ٢٨٠ كتكوت قسمت إلى ١٤ مجموعة كل مجموعة مكونة من ٢٠ كتكوت . وقد تم إستخدام لقاح النيوكاسل الحي وكذا لقاح الجمبورو الميت وذلك في وجود ضوابط ، وقد تم أخذ عينات دم عشوائية من كل مجموعة لمدة ٤ أسابيع لقياس المناعة الخلطية للقاحي النيوكاسل والجمبورو وذلك بإستخدام تثبيط التلازن الدموي للقاح النيوكاسل وإختبار التعادل المصلى والترسيب في الأجار للقاح الجمبورو. ولقياس المناعة الخلوية تم إجراء إختبار تحور الخلايا الليمفاوية (ب ، ت) لكل من فيروسي النيوكاسل والجمبورو وكذا إختبار تحور الحساسية المتأخرة لفيروس مرض النيوكاسل وقد أظهرت النتائج حدوث تثبيط مناعي معنوي على كل من الإستجابة الخلطية والخلوية لكل من لقاحي النيوكاسل والجمبورو نتيجة الإصابة بطفيل الكربتوسبوريدم وكذلك في المجاميع المصابة والمحصنة مقارنة بالمجاميع المحصنة فقط. وتوصي هذه الدراسة بتربية الدواجن في ظروف صحية جيده ، وفحص دوري لعينات براز الدجاج للتأكد من خلوها من أي طفيليات خاصة الكبربتوسبورديم خاصة قبل عملية التحصين.

SUMMARY

To study the efficacy of Newcastle disease and infectious bursal disease vaccines in chickens infected by cryptosporidium, 280, seven days old chicks were used and divided into 14 groups. Chickens were vaccinated with live or inactivated Newcastle disease vaccines and live or inactivated Gumboro disease vaccines or both vaccines. Random blood samples were collected for four weeks post vaccination for investigation of humoral immune response against Newcastle disease vaccine by the haemagglutination inhibition (HI) test, and by serum neutralization and agar gel precipitation tests for Gumboro disease vaccine. Investigation of the cellular immune response was carried out using lymphocyte transformation test for both Newcastle and Gumboro disease vaccines and delayed hypersensitivity for Newcastle disease vaccine only. Significant difference was found in both humoral and cellular immune response between groups that infected and vaccinated either with Newcastle or Gumboro disease vaccines or both compared to the control groups that vaccinated only. From the obtained results, the following recommendation should be put in consideration : (1) Rearing the birds in good hygienic measures, (2) Faecal examination of the birds specially for cryptosporidium before vaccination.

Key words: *Chickens - Newcastle - Gumboro - Vaccines - Cryptosporidium*

INTRODUCTION

Spreading of infectious diseases (viral, bacterial and parasitic) are among the main problems facing poultry production. Parasitic diseases cause great losses in poultry industry as they may not only be the direct cause of death but also affect the general condition of the birds inducing a decrease in their productivity. Infection of a variety of mammalian and avian hosts with coccidian parasites of the genus cryptosporidium may be associated with severe, life threatening disease in both immunodificient and normal hosts (Michael et al., 1988).

The effect of cryptosporidium on the immune response to Newcastle disease vaccines have been studied by Blagburn et al. (1987), Badawy (1989) and Nassef (1996) and its effect on duck virus hepatitis vaccine by Abd El-Waniss (1995) (no available literature about cryptosporidium infection and

Gumboro disease vaccination) . The purpose of this investigation was to determine the effect of exposure to cryptosporidium on the immune status of chicken vaccinated with Newcastle and/or Gumboro disease vaccines.

MATERIAL and METHODS

Experimental birds:

Two hundred and eighty, one day old, Hubberd chicks which proved to be free from coccidia, were obtained and reared under complete hygienic measures in isolated and disinfected wire floor net.. The birds were fed on commercial broiler ration which was requested not to contain anticoccidial drugs.

Cryptosporidium oocysts :

Oocysts of cryptosporidium were isolated and identified by *Abd El-Waniss (1995)* from ducks. The identified oocysts were spherical in shape with a mean dimension of 3.6 um and it was found identical to *C. melagridis*.

Preparation of fresh inoculum :

To obtain fresh culture of cryptosporidium oocysts, the isolated oocysts were inoculated orally into five of two days old coccidia free chicks. Faeces were collected from these inoculated chicks from day 5 to 12 post inoculation whereas mucosal scrapings containing oocysts were obtained from the intestine and bursa of Fabricius at the day 12 post inoculation. Isolation and purification of oocysts from faeces and scrapings was carried out according to Lindsay et al. (1986).

Estimation of number of oocysts in the concentrated stock and the infected dose was carried out using the haemocytometer and the light microscope at 40 X (***Zierdt, 1984***). Each chicken was inoculated orally with inoculum containing 5 X 10^6 oocysts.

Vaccines used :

I. Newcastle Disease Vaccine :

1. The live lentogenic LaSota vaccine against Newcastle disease prepared in Vet. Serum and Vaccine Research Institute as freeze dried vials, Lot No. 1896 with a titre of $10^{10.5}$ EID_{50} / ml) was administrated as drinking water.
2. Inactivated alum hydroxide gel vaccine against Newcastle disease locally prepared in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Lot No. 2796 with HA log 2^8 was used at a dose of 0.5 ml S/C.

II. Gumboro Disease Vaccine :

1. **Gumboro vaccine Bursa Vac. strain :** Prepared locally in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Lot No. 2796 with a titre of $10^{7.5}$ EID_{50} / ml. Each bird was vaccinated with 1 ml intramuscular.
2. **Inactivated Gumboro disease vaccine :** Obtained from Intervet Company, Batch No. 50L802. The recommended dose was 0.5 ml intramuscular.

Experimental design :

Two hundred and eighty chicks were used. 30 random serum samples were obtained at 2, 7 and 14 days old and tested for NDV by haemagglutination test (HI) and serum neutralization test (SNT) against gumboro disease to evaluate the maternal antibodies that recorded 5, 3, 0.5 log_2 HI antibody titre against Newcastle disease respectively and 16, 8, > 2 SNT antibody titre against gumboro disease respectively. Chicks were proved to be susceptible to both diseases. The chicks were classified into fourteen groups of 20 chicks. Chicks of infected groups were inoculated orally with 1ml inoculum containing 5 X 106 cryptosporidium oocysts.

Group 1: Inoculated with cryptosporidium oocysts and vaccinated with live Newcastle disease vaccine.

Group 2: Vaccinated with live Newcastle disease vaccine only non inoculated.

Group 3: Inoculated with cryptosporidium oocysts and vaccinated with inactivated Newcastle disease vaccine.

Group 4: Vaccinated with inactivated Newcastle disease vaccine only non inoculated.

Group 5: Inoculated with cryptosporidium oocysts and vaccinated with live Gumboro disease vaccine.

Group 6: Vaccinated with live Gumboro disease vaccine only non inoculated.

Group 7: Inoculated with cryptosporidium oocysts and vaccinated with inactivated Gumboro disease vaccine.

Group 8: Vaccinated with inactivated Gumboro disease vaccine only non inoculated.

Group 9: Inoculated with cryptosporidium oocysts and simultaneously vaccinated with live Newcastle and live Gumboro disease vaccines.

Group 10: Simultaneously Vaccinated with live Gumboro and live Newcastle disease vaccines non inoculated.

Group 11: Inoculated with cryptosporidium oocysts and simultaneously vaccinated with inactivated Newcastle and inactivated Gumboro disease vaccines.

Group 12: Simultaneously Vaccinated with inactivated Gumboro and inactivated Newcastle disease vaccines non inoculated.

Group 13: Inoculated with cryptosporidium oocysts non vaccinated.

Group 14: Non inoculated non vaccinated.

The immune response of the vaccinated chicks was evaluated by measuring the following :

A. Humoral immune response :

1. The Haemagglutination inhibition test for estimation of Newcastle disease antibodies was carried out according to Majijabe and Hitchner (1977).
2. Serum neutralization test (Weisman and Hitchner, 1978). Serum neutralization test (B-procedure) was used to quantitate neutralizing antibodies against Gumboro vaccines in serum samples as detected by screening test in microtiter tissue culture plate.
3. Agar gel precipitation test (AGPT) was used to detect the antibodies against Gumboro disease vaccine, the micro-procedure of the AGP test was used according to Murty and Hanson (1961).

B. Cell mediated immune response :

1. Assay of lymphocyte blastogenesis:

It was applied according to the method adopted by Lucy (1974 and 1977) and modified by Charles et al. (1978). Evaluation of the test using (MTT) according to Mosmann (1983). This test was used for evaluation of cell mediated immune response to both Newcastle and Gumboro disease vaccine. Results of this test was expressed as Delta Optical Density.

2. Delayed hypersensitivity (Skin test) :

It was used for chicken vaccinated with Newcastle disease vaccines. This test was adopted according to Cheville and Beard (1972).

Challenge Test :

This test was carried out 28 days post vaccination for both viruses.

A. To Newcastle Disease Vaccine :

The VVNDV strain containing 10^6 EID_{50} was used as a challenge virus for vaccinated birds with Newcastle disease vaccine. Each bird received 0.5 ml I/M and all groups were observed for 15 days after challenge.

B. To Gumboro Disease Vaccine :

Local virulent Gumboro disease virus kindly supplied by Hala M.M. Makaky (1996) was used in this study.

Histopathological examination :

This test was carried out on different parts of intestine and bursa of Fabricius according to the technique described by Luna (1968).

RESULTS

Results are presented in Tables (1, 2, 3, 4, 5, 6 and 7), Fig. (1, 2, 3, 4, 5 and 6).

DISCUSSION

Regarding the effect of cryptosporidium infection to the humoral immune response to live and inactivated Newcastle disease vaccine expressed by mean log_2 HI titres (Table 1, Fig. 1) showed that significant reduction in the immune response in groups infected with cryptosporidium and vaccinated either with live or inactivated Newcastle disease vaccine (group 1, 3, 9, 11) where it was (1.5, 1, 3, 2.6) respectively. Compared to the control groups that were vaccinated only (group 2, 4, 10, 12) where they recorded (2.6, 2.6, 3.5 and 5) respectively. The low HI level in the sera of infected groups compared with that of the control may be due to the adverse effect of the parasite on the immune system. Similar results was obtained by Blagburn et al. (1987), Badawy (1989) and Nassef (1996) where they found decrease in the antibody titre in infected chickens with cryptosporidium oocysts and vaccinated with Newcastle vaccines compared to the control groups which were vaccinated only.

Dealing with the results of Gumboro disease vaccines, Table (2) Fig. (2) showed that significant difference was found in the level of neutralizing antibodies between groups that infected and vaccinated either with live or inactivated vaccine (group 5, 7, 9, 11) where it was (0, 2, 4, 4) at the end of the 4th week respectively compared to the control groups that vaccinated

only (group 6, 8, 10, 1) where their neutralizing titers at the end of the 4th week was (4, 4, 8, 4) respectively. The significant decrease in the level of neutralizing antibodies in infected groups compared to the control groups could be attributed to the effect of cryptosporidial infection as well as its destructive effect on the immune system. These results agreed with that obtained by Blagburn et al. (1987) and Tarwid et al. (1985) where they concluded that alternatively infection with E. coli and cryptosporidia both could be secondary to unidentified immunosuppressive agent.

In the same time, it was noticed that the neutralizing titer of group that vaccinated with inactivated Newcastle and Gumboro vaccines was higher during the first three weeks compared to that group vaccinated with inactivated Gumboro only and these results agreed with that obtained by Nedelciu and Sofei (1990) where they found that inactivated vaccines either bivalent against Newcastle and IBD induce higher immunogenicity against the different antigens than with single vaccine.

Dealing with the precipitating antibody to Gumboro vaccine results as shown in Table (3) Fig. (3) showed significant difference between groups that infected and vaccinated (5, 7, 9, 11) where it was (75 %, 60 %, 33 % and 66 %), respectively compared to the control groups (6, 8, 10, 12) which recorded (80 %, 100 %, 80 % and 75 %), respectively. The noticeable difference between infected and non infected groups could be due to the effect of cryptosporidium infection. Parallel results was obtained by Blagburn et al. (1987) and Badawy (1989) and Nassef (1996).

Obtained results showed that live gumboro disease vaccination (strain bursal vac) has no immunosuppresive effect on chickens vaccinated with live Newcastle disease vaccine. These results agreed with those obtained by Abou-Zeid et al. (1995) where they found that chicken vaccinated with gumboro disease vaccine prepared from the imported attenuated (bursa vac) strains has no immunosuppresive effect on the birds vaccinated with Newcastle disease vaccine.

Regarding the results of the cell mediated immunity data presented in Table (4) and Fig. (4) showed significant immuno - suppression in groups infected and vaccinated with either live or inactivated Newcastle vaccine (group 1, 3, 9, 11) where it was (0.8, 0.51, 0.32, 0.9), respectively compared with the control groups (2, 4, 10, 12) that vaccinated only where it was (1.05, 1.3, 0.96, 0.8), respectively. These results agreed with those obtained by Nassef (1996) and explained by Saltenova et al. (1991) who concluded

that cryptosporidium infection was the cause of depression in both humoral and cellular immune response.

It was found also that the stimulation wattle index (SWI) was significantly higher in chickens of groups (2, 4) that vaccinated with Newcastle vaccine alone where it was (1.05, 1.3), respectively compared to that of the control groups (9, 10, 11, 12) that vaccinated with both Newcastle and Gumboro disease vaccines where it was (0.32, 0.96, 0.9, 0.8), respectively. These results agreed with the suggestion of Yamamoto et al. (1979) which was explained as the effect of both inactivated vaccines induce over stimulation and proliferation of the T8 suppressor cells resulting in a lower cell mediated immune response.

Regarding the results of lymphocyte blastogenesis, results as shown in Table (5) indicated significant differences between infected vaccinated groups compared with the vaccinated groups only in both (T) and (B) cells. This result is supported by Saltenova et al. (1991) who found that *C. baileyi* oocysts infection in broiler chickens induced decrease in numbers of B and T lymphocyte in the peripheral blood.

Concerning the protection percentage against the virulent Newcastle virus in groups vaccinated with Newcastle vaccine and infected with cryptosporidium (Table 6) (group 1, 3, 9, 11), it was 90 %, 90 %, 80 %, 90%, respectively while it was 100 %, 100%, 90 %, 100 % in the control groups (group 8, 10, 12, 13) that vaccinated only. However, the protection percentage against Gumboro disease virus in infected and vaccinated groups (Table 7) (group 2, 4, 10, 11) was 70 %, 80%, 80 %, 90 %, respectively while in the control groups that vaccinated only, the recorded percentage was 100%, 100 %, 90% and 100 % in groups (9, 11, 12, 13). Similar results was obtained by Blagburn et al. (1991) where they stated that cryptosporidiosis may enhance the severity of diseases caused by other avian pathogens.

Pathological changes observed showed the cryptosporidium oocysts as small spherical basophilic stained bodies embedded on the brush border and epithelial surface of the cloaca and large intestine of infected chickens (Fig. 5 and 6). Similar observation was found by Goodwin (1988) and Badawy (1989).

In conclusion, significant reduction in both humoral and cellular immune response was found between groups that infected and vaccinated

either with Newcastle or Gumboro disease vaccine or both compared with the control group that vaccinated only.

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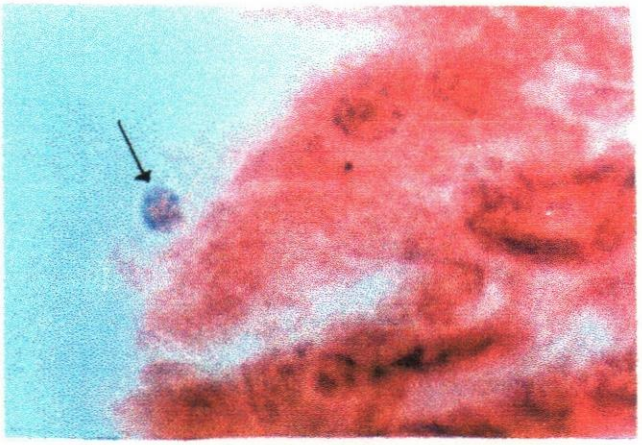

Photo 1: Showing development stage of cryptosporidium on the lining epithelium of cloaca (H & E) (X 1000)

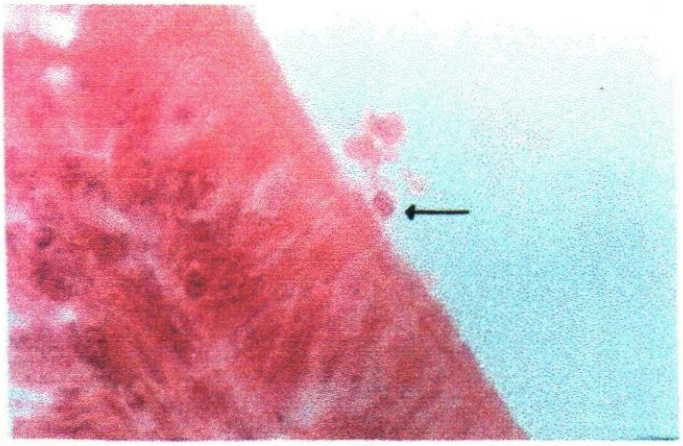

Photo 2: Showing development stage of cryptosporidium on the brush border of large intestine (H & E) (X 100).

Fig. (1) : The mean log2 of HI titre to ND induced by live and inactivated Newcastle disease vaccine compared with control groups.

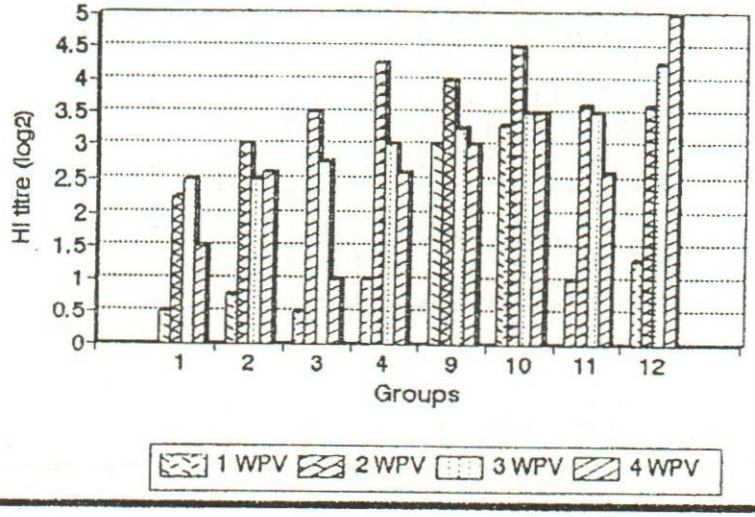

Fig. (2) : Results of neutralizing antibodies in groups of birds vaccinated with live and inactivated Gumboro disease vaccines and infected with cryptosporidium oocysts compared with control groups vaccinated only.

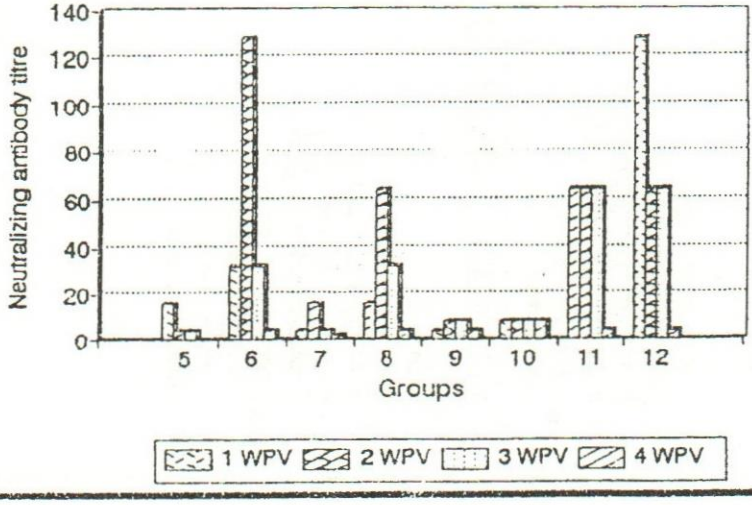

Fig. (3) : Results of precipitating antibodies in groups of birds vaccinated with live and inactivated Gumboro disease vaccines and infected with cryptosporidium oocysts compared with control groups vaccinated only.

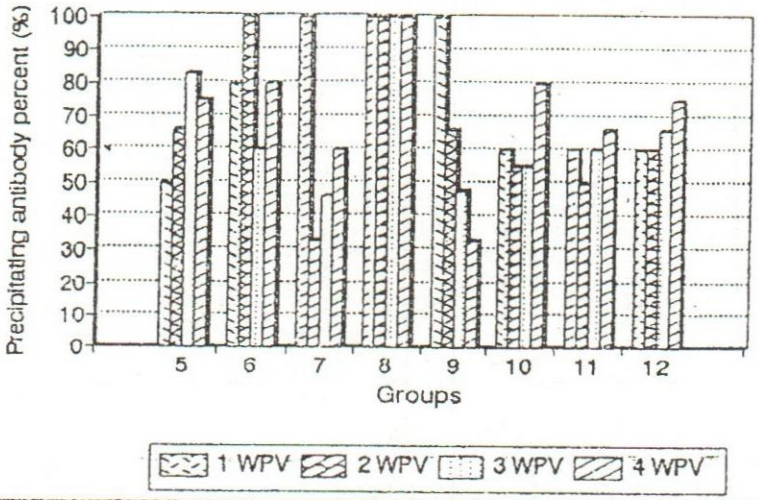

Fig. (4) : Evaluation of cell mediated immune response in chickens vaccinated with live and inactivated Newcastle disease vaccines and infected with cryptosporidium by DHR expressed by SWI.

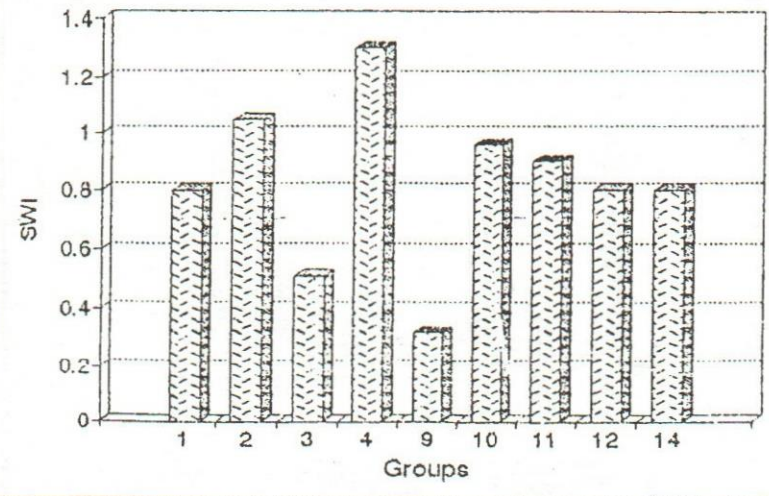

Table (1) : The geometric mean log2 of HI titre to ND induced by live and inactivated Newcastle disease vaccine compared with control groups.

Groups \ WPV	1	2	3	4
1	0.50	2.25	2.50	1.50
2	0.75	3.00	2.50	* 2.60
3	0.50	3.50	2.75	** 1.00
4	1.00	4.25	3.00	2.60
9	3.00	4.00	3.25	3.00
10	3.30	4.50	3.50	3.50
11	1.00	3.60	3.50	2.60
12	1.30	3.60	4.25	** 5.00
14	0.00	0.00	0.00	0.00

WPV : Weeks Post Vaccination.
Group 1 : Cryptosporidium + live ND.
Group 2 : Live ND only.
Group 3 : Cryptosporidium + Inactivated ND.
Group 4 : Inactivated ND only.
Group 9 : Cryptosporidium + live ND + live Gumboro.
Group 10 : Live ND + live Gumboro only .
Group 11 : Cryptosporidium + Inactivated ND + inactivated Gumboro.
Group 12 : Inactivated ND + inactivated Gumboro only.
Group 14 : Non infected non vaccinated.

Table (2) : Results of neutralizing antibodies in groups of birds vaccinated with live and inactivated Gumboro disease vaccines and infected with cryptosporidium oocysts compared with control groups vaccinated only.

Groups \ WPV	1	2	3	4
5	16	4	4	0
6	32	** 128	32	* 4
7	4	16	4	2
8	16	* 64	32	4
9	4	8	8	4
10	8	8	8	* 8
11	64	64	64	4
12	128	64	64	4
14	0	0	0	0

WPV : Weeks Post Vaccination.
Group 5 : Cryptosporidium + live Gumboro.
Group 6 : Live Gumboro only.
Group 7 : Cryptosporidium + Inactivated Gumboro.
Group 8 : Inactivated Gumboro only.
Groups 9, 10, 11, 12 and 14 as in table (1).

* Significant at P > 0.05.
** Significant at P > 0.01.

Table (3) : Results of precipitating antibodies in groups of birds vaccinated with live and inactivated Gumboro disease vaccines and infected with cryptosporidium oocysts compared with control groups vaccinated only.

WPV / Groups	1		2		3		4	
	+ve / Total	%	+ve / Total	%	+ve / Total	%	+ve / Total	%
5	10/20	50	10/15	66	10/12	83	9/12	75
6	16/20	80	15/15	100	9/15	60	12/15	80
7	20/20	100	5/15	33	7/15	46	9/15	60
8	20/20	100	15/15	100	15/15	100	15/15	100
9	20/20	100	10/15	66	8/17	48	5/15	33
10	12/20	60	11/15	55	11/20	55	12/15	80
11	12/20	60	7/15	50	9/15	60	10/15	66
12	12/20	60	9/15	60	10/15	66	9/12	75
14	0/20	0	0/15	0	0/15	0	0/15	0

WPV : Weeks Post Vaccination.
Group 5 : Cryptosporidium + live Gumboro.
Group 6 : Live Gumboro only.
Group 7 : Cryptosporidium + Inactivated Gumboro.
Group 8 : Inactivated Gumboro only.
Group 9 : Cryptosporidium + live ND + live Gumboro.
Group 10 : Live ND + live Gumboro only .
Group 11 : Cryptosporidium + Inactivated ND + inactivated Gumboro.
Group 12 : Inactivated ND + inactivated Gumboro only.
Group 14 : Non infected non vaccinated.

Table (4) : Evaluation of cell mediated immune response in chickens vaccinated with live and inactivated Newcastle disease vaccines and infected with cryptosporidium by DHR expressed by SWI compared with control groups.

Wattle / Groups	Control left wattle (mm)	Infected Right wattle (mm)	SWI
1	1.40	2.20	0.80
2	1.40	2.45	1.05
3	1.75	2.26	0.51
4	1.50	2.80	1.30
9	1.43	1.75	0.32
10	1.40	2.36	0.96
11	1.60	2.40	0.90
12	1.40	2.20	0.80
14	2.40	3.20	0.80

SWI : Stimulation Wattle Index.

Table (5) : Blastogenesis test for the evaluation of the cell mediated immunity for Newcastle and Gumboro disease vaccines in groups vaccinated and infected with cryptosporidium compared with control groups expressed as Delta Optical Density.

WPV	1		2		3		4	
Groups	PHA	PWM	PHA	PWM	PHA	PWM	PHA	PWM
1	0.065	0.083	0.088	0.097	0.054	0.090	0.032	0.078
2	0.073	0.087	0.089	0.119	0.067	0.085	0.053	0.062
3	0.053	0.072	0.072	0.089	0.047	0.080	0.041	0.073
4	0.052	0.099	0.075	0.129	0.060	0.011	0.037	0.054
5	0.095	0.110	0.124	0.137	0.082	0.135	0.053	0.127
6	0.043	0.050	0.055	0.059	0.043	0.046	0.033	0.032
7	0.082	0.097	0.110	0.121	0.073	0.110	0.060	0.085
8	0.080	0.053	0.065	0.055	0.047	0.042	0.035	0.062
9	0.051	0.061	0.072	0.072	0.045	0.056	0.035	0.082
10	0.055	0.090	0.062	0.120	0.055	0.115	0.040	0.062
11	0.090	0.095	0.112	0.117	0.085	0.115	0.045	0.084
12	0.011	0.015	0.015	0.012	0.013	0.011	0.014	0.017
13	0.040	0.065	0.065	0.080	0.055	0.072	0.040	0.054
14	0.009	0.007	0.007	0.005	0.008	0.003	0.010	0.007

Table (6) : Results of challenge test in groups of chicken vaccinated with Newcastle disease vaccine.

Groups	Total Number	Dead birds	Live	Protection %
1	10	1	9	90 %
2	10	-	10	100 %
3	10	1	9	90 %
4	10	-	10	100 %
9	10	2	8	80 %
10	10	1	9	90 %
11	10	1	9	90 %
12	10	-	10	100 %
14	10	10	-	0 %

Table (7) : Results of challenge test in groups of chicken vaccinated with Gumboro disease vaccine.

Groups	Total Number	Birds with PM lesion	Normal	Protection
5	10	3	7	70 %
6	10	-	10	100 %
7	10	2	8	80 %
8	10	-	10	100 %
9	10	2	8	80 %
10	10	1	9	90 %
11	10	1	9	90 %
12	10	-	10	100 %
14	10	10	-	0 %