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STUDIES ON THE EFEECT OF CRYPTOSPORIDIUM ON THE IMMUNE RESPONSE TO DUCK VIRUS HEPATITIS VACCINE

(With 2 Tables & 3 Figures)

By

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دراسات على تأثير الكربتوسبورديوم على الاستجابة المناعية للقاح الالتهاب الكبدى فى البط

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عند دراسه تأثير الاصابه بطفيل الكربتوسبوردیم على المناعه المتكونه نتيجه تحصين البط بلقاح الالتهاب الكبدى الوباتى تم اجراء تجربه على عدد ١٢٠ بطه بكينى قسمت الى خمس مجموعات متساويه كل مجموعه مكونه من ٢٤ بطه. المجموعه الاولى تم عدوتها صناعيا سن يوم بالطفيل وحصنت فى نفس اليوم بلقاح الالتهاب الكبدى. المجموعه الثانيه تم عدوتها صناعيا بالطفيل فى سن يوم وتحصينها بالقاح اليوم السابع. المجموعه الثالثه تم عدوتها بالطفيل صناعيا سن يوم ولم تحصن. المجموعه الرابعه تم تحصينها بالقاح فقط. المجموعه الخامسه لم يتم عدوتها ولم تحصن. وقد تم أخذ عينات دم عشوائيه حتى اليوم الاربعين بعد التحصين وتحديد كميه الاجسام المناعيه باختبار التلذن الدموى غير المباشر واختبار التعادل المصلى واختبار الترسيب فى الأجار. وقد تبين أن متوسط الاجسام المناعيه فى المجموعتين الاولى والثانيه أقل من متوسط الاجسام المناعيه فى المجموعه الرابعه (المحصن فقط). وقد سجلت المجموعه الاولى والثانيه ظهور أعراض ونسبة نفوق ونزول عدد كبير من حويصلات الكربتوسبوريديم فى البراز أعلى من المجموعه الثالثه (المعديه بالطفيل فقط) وكانت فترة الحضانه فى المجموعات المعديه ٥ أيام فى الجميع فى حين كانت فترة المرض مختلفه حيث كانت ٢٣، ٢٤، ٢١ يوم فى المجموعات الاولى والثانيه والثالثه على الترتيب.

SUMMARY

For studying the effect of cryptosporidiosis on the immune response of ducklings vaccinated with Duck Virus Hepatitis vaccine, 120 one day old, white pekin duckling were divided into 5 groups each of 24 ducklings. Two groups were infected at, one day old and vaccinated at either 1 and 7 days. The other 3 groups were kept as controls (one for infection, one for vaccination and the other as blank). It was found that duckling that were infected and vaccinated showed low level of antibody titer compared to the control group. The prepatent period was 5 days in the 3 experimentally infected groups, whereas the patent varied where it was 23, 24 and 21 days in group I, II and III respectively.

Key words: Cryptosporidium-Inmunity-Duck virus Hepatitis

INTRODUCTION

The production of poultry for meat has become the largest fact of the poultry industry which specialized in the production of chickens, turkeys and ducks. The chief advantages of ducks over other poultry are the dark meat, efficient converters of feed to flesh and less susceptibility to diseases and aliments.

Coccidiosis in ducks is the most important parasitic disease which is caused by small coccidian parasites of the genus cryptosporidium. It has been detected in ducks by *MASON (1986).*

Duck Virus Hepatitis (DVH) is a highly fatal rapidly spreading viral infection of young duckling characterized primairly by hepatitis. Protection against DVH may be attained by active immunization of duckling with a live attenuated vaccine in day old duckling *(CRIGHTON and WOOLLCOCK, 1978).*

The present work was designed to study the effect of coccidiosis on the immune system of ducklings vaccinated against DVH by evaluation of the antibody titers as well as recording the health condition of the experimentally infected and vaccinated ducklings.

MATERIAL and METHODS

Experimental Design:

120 white Pekin ducklings of a day old were divided into 5 equal groups each of 24 ducklings as follows:

Group I:

Inoculated with cryptosporidium oocysts at one day old and vaccinated against DVH at the same day.

Group II:

Inoculated as the first group but vaccinated against DVH on the seventh day.

Group III:

Only inoculated at one day old (unvaccinated).

Group VI:

Only vaccinated at one day old (uninoculated).

Group V:

Uninoculated and unvaccinated (Negative Control).

Inoculum:

Mucosal scrapings containing oocysts were obtained from the intestines and bursae of fabricius of naturally infected ducks, pooled and inoculated orally into five of 2 days old, coccidia free duckling. Faeces were collected from these inoculated ducklings after days 5 to 12 post infection and treated according to *LINDSAY et al. (1986)*. The number of oocysts in the concentrated stock inoculum was determined by using a haemocytometer and light microscope at 40X *(ZIERDT, 1984)*. The calculation was as follows:

Number of oocysts/ mm^3 = NX 100

(N= Number oocysts in 80 small squares).

Each duckling was inoculated orally with 1 ml inoculum containing 2X 10^4 oocysts.

Vaccination:

The Vaccine used was a modified egg adapted mild virus vaccine. The vaccine was obtained from RHONE MERIUX Company with a titer of 10^8/ ml EID_{50}. It was reconstituted in 1000 ml distilled water and the recommended dose was 0.5 ml containing 10^3 EID_{50} while the route of vaccination was subcutaneous.

Procedure:

For evaluation of the antibodies titer in ducklings before the experiment (Maternal immunity), 10 randomly blood samples were collected, and the sera were titrated to determine the level of maternal immunity. Faecal samples were collected just before the experiment and examined for cryptosporidium with floatation technique

Blood samples were randomly collected from the vaccinated groups at weekly intervals till the 40 th day post vaccination to test development of resistance against DVH vaccine.

Each serum was tested separately with both agar gel precipitation test and indirect haemagglutination test, while a pooled serum sample was used for the neutralization test.

Faecal samples were collected and examined daily for detecting the oocyst output. The clinical symptoms and mortality percentage as well as post mortem lesions were also recorded.

Serological Examination:

Virus Titration:

Titration of the virus was carried out according to *ANON (1971).*

Calculation of EID_{50} was carried out after the method of *REED and MUENCH (1938).*

Serological tests:

Indirect Haemagglutination test (IHA): (Tannic Acid Method). This test was carried out according to the method described by *TRIPATHY et al. (1971).*

To 10 ml of 5% tanned sheep red cells, suspension were added 10 ml of partially purified concentrated antigen and 40 ml of phosphate buffer saline (PBS) (pH 6.4). The mixture was incubated after thorough mixing in a water bath at 37°C for 30 minutes and the cells were sedimented by centrifugation at 1000 r.p.m. for 15 minutes. Two washes with PBS (pH 6.4) and sedimentation by centrifugation at 1000 r.p.m for 15 minutes were carried out. The supernatant was decanted and the sedimented RBCs were washed with 20 ml of 1% normal rabbit serum and stored at + 4°C till used (within 3 hours).

Serial two fold dilutions of the pretreated duck sera (diluted 1·5) were prepared in 0.025 ml volumes of PBS (pH 7.2) containing 1% inactivated normal rabbit serum using the microtiter plates. Equal volumes (0.025 ml) of virus sensitized sheep red cells were then added. Two control wells were included, one containing the prepared serum to be tested plus 1% tanned sheep red cells and the other contained 1% tanned sheep red cells plus the diluents. The end point was determined by recording the highest serum dilution at which red cells were agglutinated.

Serum Neutralization Test (SNT):

This test was used for screening duck sera for DVH neutralizing antibodies. The test was carried out using constant-serum- variable virus technique as described by *HWANG (1969)* and recommended by *TOTH (1975).*

Calculation of 50% end point of virus infectivity was carried out according to the method of *REED and MUENCH (1938)*. The criteria used for virus infectivity were usually the embryo mortality in conjunction with characteristic lesions of DVH infection in dead and surviving embryos *(TOTH, 1975)*.

Agar Gel Precipitation Test (AGPRT):

The micro-procedure of the AGP test was used according to the method of *MURTY and HANSON (1961)* for detection of DVH precipitating antibodies in duck sera. The agar media was prepared by dissolving 1.5 gm of Nobel agar powder 0.8 gm of sodium chloride and 0.01 gm of merthiolate in 100 ml distilled water. The melted agar was distributed in 6.0 ml volumes into sterilized plastic petri-dishes. Before use, wells were cut with a special appliicance which produced a hexagonal pattern consisting of six outer wells surrounding a central one. In testing sera for precipitating antibodies the central well in each pattern was filled with known DVH antigen and the peripheral wells with the sera to be tested. The agar plates were incubated at room temperature for 2 days and the reaction was observed daily by an indirect light. The final readings were recorded after 2 days.

RESULTS

Results are presented in Tables (1 & 2), Fig. (1, 2 & 3).

DISCUSSION

The studies dealing with cryptosporidiosis among ducks are little and obscure and the present work aimed to clarify the effect of cryptosporidium infection on the immune response of the ducklings vaccinated with Duck Virus Hepatitis and vice versa.

IHA, SNT and AGP tests were carried out for recording the development of resistance against DVH.

Results as shown in Table (1) and Fig. (1) revealed that the IHA antibody was detected in the 3 vaccinated groups and started nearly with the same level on the 4 th day post-vaccination. This may be due to the neutralization of the virus vaccine by the maternal antibody transmitted from the parent stock to the young ducklings via the yolk sac as reported by *ASPLIN (1961)*. It is also noticed that the IHA antibodies gradually increased in the 3 vaccinated groups and reached its maximal level on the 40 th day post vaccination. However the pattern of the mean IHA in the sera of control group was significantly higher than those of the two infected groups. The lower IHA level in the sera of infected groups is most probably due to the

deleterious effect of the parasite on the immune system of duckling which was more evident in ducklings vaccinated on the 7 th day (group II). Similar results were obtained by *BLAGBURN et al.(1987).*

Regarding the neutralizing antibody, results as shown in Table (1) and Fig. (2) revealed that the neutralizing antibody could be detected by SNT from the 4 Th day post vaccination in the 3 vaccinated groups, then the titres increased gradually reaching the maximal level on the 40 th day post vaccination (3.05) with a mean of 1.43 neutralizing indices. This result agreed with that obtained by *HUSSIN et al. (1993).*

The significant decrease in the level of neutralizing antibody in both two infected groups (I and II) compared with that obtained in the control one could be similarly attributed to the destructive effect of cryptosporidial infection on the immune system responses; similar results were obtained by *BLAGBURN et al. (1987).*

As regards the agar gel precipitation test, the results as shown in Table (1) and Fig. (3) revealed that the precipitating antibody could be detected in the 3 infected groups 4 days post vaccination. These results agreed with those obtained by *MURTY and HANSON (1961) and HUSSIN et al. (1993).*

It was noticed that the mean of precipitating antibody after 40 days in the control group (group IV) was higher than those of either the first or second group. The sharp elevation in the precipitating antibody specially in the first group may be due to depression of the immune system of ducklings caused by the cryptosporidial infection. These results agreed with those obtained by *BLAGBURN et al. (1987) and BADAWY (1989).*

It was noticed that the recorded symptoms of the experimentally infected ducklings were confined mostly to the intestinal tract and some birds showed conjunctivitis and oculonasal discharge. This may be due to the type of infection since it was acquired orally. This presumption agreed with that of *MA et al. (1984).*

The present results showed that ducklings of group I suffered from severe clinical symptoms comparing to the other two infected groups and this may be attributed to the over stress on a one day old duckling by vaccination accompanied with infection in the same day. On the other hand, ducklings of group III showed mild symptoms of cryptosporidiosis since this group was not subjected to the vaccinal factor and infected with a dose of the inoculum.

The prepatent period was 5 days in the experimentally infected ducklings. This observation was previously noted by *BLAGBURM et al. (1987).* The longer patent period as well as shedding period of oocysts in group I and II may be due to stress effect of vaccination on the infected ducklings. Similar results were obtained by *NACIRI et al. (1989).*

Regarding the mortality percentage in the infected ducklings, it was 33.3%, 20.8% and 8.33% in groups I, II and III respectively and in other words the infected vaccinated ducklings groups showed higher percentage of mortality than the only infected group. These results agreed with that obtained by *NACIRI et al. (1989).*

In conclusion, the obtained results revealed that the infected vaccinated duckling showed lower levels of antibody titer compared with the control group (vaccinated non- infected). It was also noticed that IHA antibody titer of group of ducklings which was infected and vaccinated at a day old was higher than that of the infected ducklings but vaccinated on the 7 th day. Meanwhile, the ducklings of the former group showed severe clinical symptoms, high mortality rate and shedding large number of oocysts output due to the double stress effect of infection and vaccination in the same day.

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Table (1): Serum antibody levels of vaccinated ducklings by three different serological tests.

Exp.	Group I			Group II			Group IV		
DPV	IHA	SNT	AGP	IHA	SNT	AGP	IHA	SNT	AGP
M.I.	0.25	1.06	20 %	0.25	1.06	20 %	0.25	1.06	20 %
4	3.80	1.04	20 %	3.60	1.75	50 %	3.60	1.75	100 %
10	3.85	0.72	50 %	2.35	1.03	50 %	4.56	1.13	100 %
17	2.90	0.52	20 %	1.80	0.21	80 %	4.12	1.41	100 %
24	2.60	0.98	80 %	2.25	1.06	80 %	4.62	1.25	80 %
30	3.80	0.83	60 %	2.05	1.28	30 %	5.12	2.82	90 %
35	3.33	0.72	30 %	2.06	0.34	100 %	5.05	2.15	100 %
40	3.44	0.15	20 %	3.04	0.13	80 %	6.00	3.05	100 %
Mean S.E.	++ 3.34 ± 0.183	+ 0.70 ± 0.114	+++ 40.0 ± 0.106	+++ 2.52 ± 0.232	0.82 ± 0.231	+ 67.14 ± 0.091	4.52 ± 0.302	1.53 ± 0.304	95.71 ± 0.029
T. test	- 3.226	- 2.248	- 5.068	- 5.243	- 1.597	- 2.991			

IHA = Indirect Haemagglutination test.
SNT = Serum Neutralization Test.
AGP = Agar Gel Precipitation Test.
DPV = Days Post Vaccination.
Group I = Infected and vaccinated in the same day.
Group II = Infected at day old vaccinated at 7 days.
Group IV = Uninoculated vaccinated.
+++ = Significant 99.9 %.
++ = Significant 99 %.
+ = Significant 95 %.
0 = Non significant.

Table (2): The cryptosporidial oocysts out put in the experimentally infected ducklings.

Days post Inoculation	Mean number of oocysts out put		
	Group I	Group II	Group III
5	650	350	850
6	5250	1250	2100
7	28500	8750	5800
8	32300	9300	7300
9	50200	12500	10350
(10)	42750	29000	23000
11	34500	24500	26000
12	30200	23300	21000
13	32800	22900	14300
14	31300	20220	11250
15	18300	17350	9800
16	16350	17000	9500
17	12330	16450	8750
18	9050	14600	3000
19	8750	13400	6100
20	8320	12150	4640
21	630	11100	1350
22	4220	11000	745
23	2540	10200	560
24	1200	7320	350
25	550	6420	300
26	500	2250	0
27	500	850	0
28	0	320	0

Group I = Infected and vaccinated in the same day.
Group II = Infected at day old and vaccinated at 7 days.
Group IV = Uninoculated vaccinated.

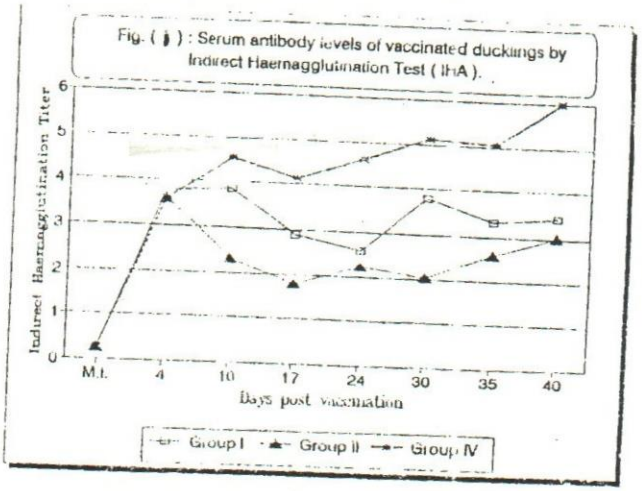

Fig. ( ) : Serum antibody levels of vaccinated ducklings by Indirect Haemagglutination Test (IHA).

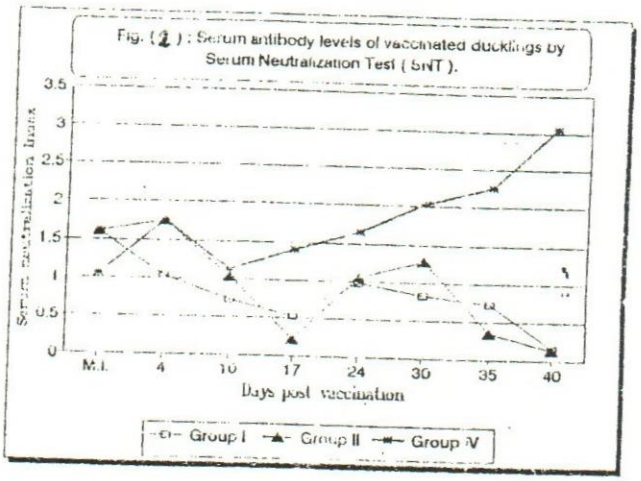

Fig. ( ) : Serum antibody levels of vaccinated ducklings by Serum Neutralization Test (SNT).

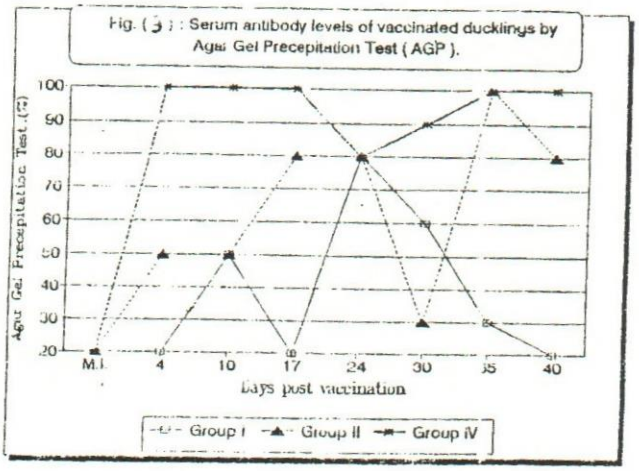

Fig. ( ) : Serum antibody levels of vaccinated ducklings by Agar Gel Precepitation Test (AGP).