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EFFECT OF INFECTION WITH EIMERIA NECATRIX ON THE RESPONSE OF CHICKENS TO ND VACCINES L. Using Mesogenic Komarov "K" Strain of NDV (With 7 Table)

By
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تأثير العدوي لطفيل الأبميريا نيكاتركس علي استجابة الكتاكيت للقاح فيروس النيوكاســـل (ـ استخدام لقاح النيوكاسل العضلي عترة كومـارون (ك)

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تم استخدام ١٥٠ كتكوت حيث تم اصابتهم بب ٢٠٠٠ حويصلة ايميريا نيكاتر كـــس وتم تحصيفهم بلقاح النيركاس العضلي عند من شهر وقد تم تقييمهم الي عثر مجموعـــات واصابتهم على فترات مختلفة بعد التحصين وأخدت عينات براز عثوائية يوميا من كـــل مجموعة لعد حويصلات الكوكسيديا واجراء اختبار التلا زن الدموي لكل الكتاكيت وكانـــت النتائج كالآتى : في المجموعة الأولي وجد أن هناك فرق ملحوظ بين بداية انتاج الإجــام المناعية مقارنة بمجموعة الكنترول ولكن وجد فارق في النهاية العظمي للوسط الهندسي للاجـام المناعية لهذه المجموعة والتي بلغ (٢٦٢) مقارنة بمجموعة الكنترول الذي بلغ (٢٠٢) ، وفــي المجموعة الثانية والثالثة أوضحت النتائج زيادة عدد الحويملات في هذه المجموعات مقارنـــة بمجموعة الكنترول ، وفي المجموعة الرابعة والخامسة كان هناك فرق واضح في النهاية العظمي والمغري للوسط الهندسي كان أقل نسبيا مقارنة بمجموعة الكونترول ، وفي المجموعة السادسة والسابعة لوحظ أن الوسط الهندسي كان أقل نسبيا مقارنة بمجموعة الكونترول ، وتوصــــــي هذه الدراسة باجراء فحص دوري لبراز الكتاكيت على فترات متباينة قبل وبعد عمليـــــــة هذه الدراسة باجراء فحص دوري لبراز الكتاكيت على فترات متباينة قبل وبعد عمليـــــــة التحصين بلقاح النيركاسل وذلك للحصول على النتيجة المتوقعة للتحصين ولتجنب حـــــــــدوث

SUMMARY

One hundred and fifty chicks were used to study the effect of Enecatrix infection on the immune response of Newcastle disease vaccine. The chicken were classified into 7 experimental groups and 3 control groups. They were vaccinated at age of 30 days of age. with komarov vaccine. They were infected with 2000 sporulated Enecatrix oocysts 3 days before vaccination in the first group. Similtaneously in the second group and after 3, 18, 30, 60 and 90 days after vaccination in the succeeding 5 groups. The serum haemagglutinating inhibition titre and the faecal oocysts output of the different groups were determined. The comparison between the results of the experimental groups and those of the control groups showed that ENecatrix infection of the vaccinated chicks resulted in suppression of the immune response to the vaccine.

INTRODUCTION

Infectious diseases whether viral, bacterial and parasitic are among the main problems facing poultry production (COCKRILL, 1971). Coccidiosis is one of the major parasitic diseases affecting poultry. It is a diseased condition usually of the digestive system caused by Eimeria species characterized by diarrhoea, high morbidity and mortality rtes beside poor growth and decrease in egg production (REID, 1978). Newcastle disease (ND) is a highly contagious disease that affects chicken and turkeys. The high virulence of some strains and its high contagiousness make this disease a serious problem to many countries. In Egypt, Newcastle disease is well established and widespread throughout the whole country. Prevention of ND is achieved by sanitation and vaccination programmes with live locally manufactured vaccines (B, F and Komarov). Regarding the role played by parasitic disease on the immune response by vaccination MASTSUEV (1974) studied the effect of Ascaridia galli infection on the immune response against ND. MOHAMMED (1980) studied the effect of coccidial parasite Estenella on the immune response against NDV. The present study was designated to study the effect of the protozoon parasite Eimeria necatrix on the immune response to NDV using mesogneic "K" strain of NDV.

MATERIAL and METHODS

1 - Experimental birds: One hundred and fifty white huppered one-day-old chicks which proved to be free from coccidia were obtained and reared under compelte hygienic measures.

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- 2 Ration: Commercial broile ration was obtained from El-Salam poultry Company.

 This ration was requested not to contain any coccidiostats.
- 3 Seed oocysts of <u>Eimeria necatrix</u> strain: A pure strain of <u>E.necatrix</u> was kindly suplied by Dr. Notron (Central Vet. Lab. New Haw, Weybridge, U.K.). The oocysts were kept in 25% potassium dichromate till use.
 - a) Preparation of culture and inoculation of birds: These were performed as sescribed by BARWICK, et al. (1970).
 - b) Determination of the infective doser four groups of 10 one-week-old chicken were infected with 10,000, 20,000, 30,000 sporulated E.necatrix oocysts respectively. The results were recorded to determine the dose of oocysts that will be able to induced clinical disease.
 - c) Counting of vocysts output per gram of faeces by use of mac Master slide: The technique was described by GORDON and WHITELOOK (1939).
- 4 Vaccine used: The komarov strain vaccine used was prepared locally in Vet Serum and Vaccine Res. Inst., Abbassia, Cairo, lot No. (3178). The titer was 10 ml EID given by intramuscular (I/M) route with a dose of 0.5 ml.
- 5 Haemagglutination test (HA): It was carried out according to the standard method described in (Methods for examination of poultry Biologies, 1963).
- 6 Haemagglutination inhibition test (HI): The beta procedure was carried out using 4 HA units according to ANNON (1971) of the sera of chicken.

Experimental design:

One hundred and fifty chicks were used in this group. The chicks were infected each with the infective dose which contained 20,000 sporulated Enecatrix oocysts and vaccinated with "K" Strain vaccine of NDV when they 30 days old. The chicken were divided into ten groups, each group consisted of 15 chicks and were treated as followed:

- Group 1: Members were inoculated with the infective dose 3 days before vaccination.
- Group 2: Members were inoculated with the infective dose on the day of vaccination.
- Group 3: Members were inoculated with the infective dose 3 days post vaccination.
- Group 4: Members were inoculated with the infective dose 18 days post vaccination.
- Group 5: Members were inoculated with the infective dose 30 days post vaccination.
- Group 6: Members were inoculated with the infective dose 60 days post vaccination.
- Group 7: Members were inoculated with the infective dose 90 days post vaccination.
- Group 8: Members were uninoculated with the infective dose and unvaccinated.
- Group 9: Members were inoculated with the infective dose and unvaccinated.
- Group 10: Members were only recieving the vaccine.

Random faecal samples were collected daily from each group until the end of the experiment (up to 4 months post vaccination). Each sample was examined using the technique of DAVIS, et al. (1963) and the number of oocysts/gm faeces was estimated. Blood samples were taken by heart punture 4 days post vaccination and then daily from chicken of each group for estimating HI antibody response to ND vaccine. The sera were separated from the blood and inactivated at 56°C for 30 minutes.

RESULTS

Table 1, showed the relation between the number of oocysts output/gm faeces and geometric mean of HI antibody titre in group No. 1. The results showed that the infected, chicken shed the unsporulated Enecatrix oocysts 6 days post infection and continued to shed the oocysts for 25 days post infection. The minimum oocysts output (85) was recorded on the 25th day post infection when the GMT was at its peak 5.53 at the 22th day post vaccination. Chickens in the control group No. 9, infected (non-vaccinated) begin to shed the 5 days post infection and continue to shed for 18 days post infection. The number of oocysts output/gm faeces varried from 65 on the 18th day to 75700 on the 5th day post infection. The GMT of the control group 10, varried from 2.8 on the 7th day to 7.2 on the 21st day post vaccination. The infected hirds of the first group showed mild symptoms of coccidiosis which appeared 5 days post infection and on the 15th day 2 birds died which the post mortum examination revealed multiple petechial haemorrhage over large area of the intestine and the intestinal content consists of blood and mucous.

Table 2, showed that the vaccinated chicken shed the unsorulated Enecatrix oocysts 5 days after infection and continue shedding for 19 days. The minimum oocysts output/gm faeces (160) was recorded on the 23rd days post infection when the GMT was at its peak (6.8) at the 23rd day post vaccination. The maximum oocyst output (48750) was recorded on the 8th day post infection when the GMT was (1). The chicken of this grou showed severe symptoms of coccidiosis, 3 birds died one on each of the 8th, 9th day post infection. P.M. lesions revealed multiple petechnial haemorrhages over large area of the intestine with bloody faeces in the intestine.

Table 3, showed that chicken infected with Enecatrix 3 days post vaccination began to shed the unsporulated Enecatrix oocyst 7 days post infection and continued to shed for 16 days. The minimum oocyst output (120) was recorded on the 22th day post infection whenthe GMT was at its peak (5) on the 25th day post vaccination.

Chickens of this group revealed high mortality and morbidity as well as severe symptoms of coccidia. Five birds died; two on the 5th day post inoculation while the other 3 died one on each of the 8th, 9th and 12th day post infection. P.M. lesions revealed multiple petechial haemorrhages over large areas of the intestine with necrotic foci on the intestinal wall.

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Table 4, showed the results of the chicken infected with Enecatrix 18 days post vaccination. The results revealed that chicken started to shed the oocysts after 7 days and continued to shed for 17 days. The minimum oocysts output (160) was recorded on the 23rd day post infection when the GMT was 4.66 at the 41st day post vaccination. The maximum oocysts output (21400) was recorded on the 9th days post infection when the GMT was 4.91. The chicken of this group showed no clear symptoms of coccidiosis. Concerning mortality 2 birds died on the 9th post infection. The R.M. lesions revaled multiple petechial haemorrhage over the intestinal wall and bloody faeces in the intestine.

Table 5, revealed the results of chicken infected with E.necatrix 30 days post vaccination. The results showed that chicken started to shed oocysts after 7 days and continued to shed for 15 days. The minimum oocysts output (110) was recorded on the 21st day post infection when the GMT was (5.69%). The maximum oocysts output (6000) was recorded on the 16th day post infection when the GMT was (5.75%). The GMT of control group (10) varied from 7.2 on the 31st day to 5.2 on the 57th day post vaccination. Significant difference was found when this control group was compared with the group No. 5, when the GMT was at its peak at the time of infection (6.1) on the 31st day post vaccination and reached to 4;53 on the 58th day post vaccination. Chicken of this group showed mild symptoms on inappetance and ruffled feathers. Only one bird died on the 9th day post infection and the P.M. examination revealed petechial haemorrhage over the intestinal wall.

The results in Table 6, whowed that the chickens infected with Enecatrix 60 days post vaccination began to shed the oocysts after 7 days and continued to shed for 14 days. The minimum oocysts output (150) was recorded on the 20th day post infection when the geometric mean of HI titre was 3.66. The maximum oocysts output (32000) was recorded on the 10th day post infection when the GMT 4.26. The GMT of the control group 10, varied from 5.13 on the 61st day post vaccination to 4;4 on the 87th day post vaccination whereas the GMT of the group 6, was (5.2) at the time of infection and reached 3.4 on the 87th day post vaccination. The chicken of this group showed no clear symptoms of coccidiosis but there was slight inappetance with no mortality.

The table 7, showed the results of chicken infected with E.necatrix 90 days post vaccination. The chicken shed the occysts 7 days post infection and continued to shed for 16 days. The minimum occysts output (350) was recorded on the 13th day post infection when the GMT was 2.86. The maximum occysts output (12700) was recorded on the 9th day post vaccination when the GMT was 2.86. The GMT in the control group 10, varied from 4.13 on the 9th day post vaccination (at the time of infection) to 3.2 at 120 days post vaccination. A significant difference was observed when this control group was compared with group 7, when the GMT of the later was 4.13 at the time of infection and 1.8 at the end of the experiment (120 days).

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The chicken of group 7 showed no symptoms of coccidiosis but they suffered from stunted growth with no mortality.

DISCUSSION

The present study was designed to study the effect of the protozoon parasite Enecatrix on the immune response of NDV vaccine (Komarov). Chickens we vaccinated at the age of 30 days of age with komarov ND vaccine and experimentally infection with Enecatrix 3 days before vaccination in the first group 1, and simultaneously at vaccination in the second group and 3, 18, 30, 60 and 90 days post vaccination in the succeeding groups.

Table 1, showed that the GMT 7 days after vaccination was (1.2) in the first group whereas the GMT in the control group 10, was (2.8). This decrease may reflect the depressive effect of the first generation schizont. It was also noticed that the peak of the GMT (3.32) of the first group was lower than that (7.2) of the control group. The possible reason for that is the inoculated parasite (MOHAMMED, 1980, 1982). In the second group, the result displayed in Table 2, whowed that the GMT recorded on 7 days post vaccination was very low (1) compared to that (2.8) of the control group. This may reflect the depressive effect of the third generation schizont which occurred on the 6th to 7th day (DAVIS, 1956). Also the GMT at 21 days post vaccination dropped from (7.2) to (4); this result agrees with that obtained by HEGAZY, et al. (1986) who stated that infection with Estenella lead to impression of the immune response to ND vaccines. From table 3, the results showed that the GMT (4.2) was lower than that of control group on 21 days. Also, group 4, in table 4, showed that the peak of HI titre (5.85) recorded on 21 days post vaccination was lower as compared with that (7.2) of the control group 10. This might reflect the depressive effect of the first generation schizont. It was also noticed that the GMT (4.5) recorded at the end of this experiment was lower than that of the control group which was (6). Most probably there are reasons to consider other than that of the inoculated parasite (MOHAMED, 1980 & 1982 and HEGAZY, 1986). The relatively large number of oocysts in this groups might be due to stress effect of vaccination. From table 5, it is shown that there was a noticeable decline in the HI titre of the fifth group which was at its maximum value after one month, while the control group 10, did not show any decline during the same period. This might be due to the loculated parasite (MOHAMED, 1980 & 1982). On the other hand, it was noticed that there was a great difference between the number of oocysts output in this group and that of the control group 9. It was noticed that the maximum occysts output in this group (6000) was lower than that of the control which reached (75700). This may be due to age resistance as the birds were 2 months old when they were infected. This result agrees with that

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of MICHEAL and HODGES (1972) who stated that age factor might have great influence on the pathogenicity of coccidiosis and this also could explain the low mortality and morbidity in this group. Concerning groups 6 and 7, it was noticed that the GMT was significantly lower than that ofthe control group 10. On the other hand, there were neither morbidity nor mortality inspite of detection of cocysts in the faeces. This might be due to the age resistance of the infected birdes (90–120 days). This result agrees with that obtained by DAVIS (1956) and MICHAEL and HODGES (1972) as they stated that the age of the birds might have a great influence on the pathogenicity to coccidiosis.

From these results it could be concluded that Enecatrix infection in ND vaccinated chicken resulted in supperssion of the immune response. So, it is recommended that periodical faecal examination should be done specially after before vaccination to obtain the expected results of the 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	Number of oocysts out put per gram faeces		Days post	Geometric mean of HI antibody titre	
	gr.No. 1	gr.No.9		gr.No. 1	gr.No.10
1234567891011231456789910112322345678991011	0 0 0 0 0 0 0 0 0 0 12000 15000 15000 700 450 550 2000 1720 2500 1720 2500 1720 2500 1720 2500 1720 2500 1720 2500 1720 2500 2000 1000 2000 2000 2000 2000 20	0 0 0 75707 63225 9200 1650 450 3600 3700 2500 150 200 350 320 180 65 0	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 28 28 29 20 20 20 20 20 20 20 20 20 20 20 20 20	0 0 1.2 1.2 1.2 1.2 1.2 1.3 3.16 4.38 4.38 5.53 5.53 5.53 5.53 5.53 5.53 5.53 5	000 888 888 3366 222244.888 3366 44.9966 55.2222222222222222222222222222222222

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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.

Days post	Number of occysts out put per gram faeces.		Days post	Geometric mean of HI antibody titre	
INoculation	gr.Nc. 2	gr.NO.9	vaccination	gr.No.2	gr.No 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28 29 20 31	0 0 0 0 2250 4735 13500 48750 2600 31500 650 4530 22250 44060 1300 2200 1300 2200 1300 225 160 0 0	0 0 0 75700 63225 9200 1650 450 3600 3700 2500 150 2500 150 0 0 0	1 2 3 4 5 6 7 8 9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0 0 0 0 0 1 1.64 1.64 1.22 1.61 1.63 3.61 1.33 5.33 5.33 5.00 6.88 6.86 6.00 6.00 6.83 4.83	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Relation between the number of oocysts output per gram faeces and geometric mean of HI antibody titre in group 3.

Days post Incoulation	No of oocysts out p put per gram faeces		Days post vaccination	Geometric mean of	
	gr.No 3	gr. No 9		gr, No. 3	gr. No 1
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28 29 30 31	0 0 4000 21000 23500 11300 9250 7745 6540 3350 1540 650 300 250 400 450 200 120 0	0 75700 63225 9200 1650 450 3600 3700 2500 150 200 350 320 180 65 0	7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28 30 31 33 34	1.92 1.92 2.45 3.45 3.45 3.6 3.6 3.6 3.6 4.4 4.9 4.9 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0	2.8 2.8 2.85 2.45 4.8 4.93 4.93 4.99 6.8 7.2 7.2 7.2 7.2 7.2 7.2 7.2 7.2 7.2 7.2

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Table 4
Relation between the number of pocysts output per gram faeces and geometric mean of HI antibody titre in group 4.

Days post	No of oocysts out p but per gram faeces		Days post vaccination	Geometric mean of hI antibody titre.	
	gr.No 4	gr. No 9		gr.No. 4	gr. No 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28	0 0 0 0 1500 3500 21400 2500 3500 4520 10860 6300 3800 1420 800 600 350 160 0	0 0 0 76700 63225 9200 1650 450 3600 3700 2500 150 200 350 65 65 6	199012345678901234567890123454545454545	4.993555551111133555555666666 4.993885555111133355555666666666666666666666	4.96 9.88 9.22 9.22 9.88

Table 5
Relation between the number of oocysts output per gram faeces and geometric mean of HI antibody titre in group 5.

	No of cocysts out p put per gram faeces		Days post	Geometric mean of HI antibody titre.	
	gr.No 5	gr. No 9		gr.No. 5	gr. No 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28	0 0 0 0 0 0 3900 4100 4250 1700 1900 1600 1500 4500 6000 3400 1200 500 320 110 0	0 0 0 76700 63225 9200 1650 450 3600 3700 2500 150 200 350 350 350 350 350 350 350 350 350 3	30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57	6.1 6.1 6.1 6.11 6.11 6.11 6.11 6.11 6.	7.2 2.2 2.8 6.8 6.8 6.4 6.4 6.4 6.6 6.6 6.6 6.6 6.6 6.6 6.6

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Table 6

Relation between the number of cocysts output per gram faeces and geometric mean of HI antibody titre in group 6.

Days post	No. of oocysts out put per gram faeces.		days post	Geometric mean of HI antibody titre	
inoculation	gr.NO 6	gr.No 9		gr.No. 6	gr.No.10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28	0 0 0 0 0 2390 3600 7200 32000 32500 24520 1 2030 9730 646 540 420 300 200 150 0	0 0 0 75700 63225 9200 1650 450 3500 2500 150 200 350 320 180 63 0 0	60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 67 77 80 81 82 83 84 85 86 87	5.224444466633333333333333333333333333333	5.13 5.13 5.13 5.13 5.0 5.0 4.86 4.86 4.86 4.86 4.86 4.86 4.86 4.86

Table 7
Relation between the number of occysts output per gram faeces and geometric mean of HI antibody titre in group 7.

Days Dost inoculation	No . of oocysts out put per gram faeces		days post	Gedmetric mean of KI antibody titre	
	gr.No 7		vaccination	gr.NO 7	gr.No.10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 27 28 29 30	0 0 0 0 0 2300 3610 12700 9700 3400 1250 350 270 4350 9730 1056 5500 3600 1800 700 400 0	0 0 0 0 0 75700 63225 9200 1650 450 3600 3700 2500 150 200 350 320 180 65 0 0	90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119	4.13 4.13 3.66 3.66 3.66 3.33 3.33 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.86 2.87 2.13 2.13 2.13 2.13 1.93 1.93 1.93 1.88	4.13 4.13 4.13 4.13 4.13 4.13 4.13 4.10 4.00 4.00 3.773 3.77