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THE IMMUNE SUPPRESSIVE EFFECT OF MAREK'S DISEASE VIRUS VACCINE (RISPEN STRAIN) ON THE IMMUNE RESPONSE OF CHICKENS TO NEWCASTLE DISEASE VIRUS VACCINE

By

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INTRODUCTION

Control of infectious disease in poutry depend on adequate flock immunity. Reduced immune reseonsiveness leading to increased disease losses can seriously damage the Poultry industry; immunosuppression is a term to describe this phenomena.

Many viral agants have been implaicated in impressing the immune system of chickens; these include Marek's disease virus "MDV" (Lu and Lapen, 1974; Thesis, 1977; Schat et al., 1977; Lee et al., 1978 and Powell, 1980); lymphoid leucosis "L.L" (Meyers et al. 1976; and Israel et al., 1980); Reticuloendotheliosis (Carpenter et al., 1977 and Carpenter et al., (1978); Infectious bursal disease virus IBDV (Sivanadan and Maheswarn, 1980) and other viruses.

Marek's disease is lymphoproliferative neoplasm of chickens caused by highly cell-associated herpes virus (MDV); T-lymphocytes act as target cells for MDV transformation and the effector cells which play role in controlling tumor development; it is known since long time for its immunosuppressive potentialities. This virus immunosuppression found to interfere with the immune response against other microbial vaccines and the degree of immunosuppression was found to be associated with the severity of disease (Purchase et al., 1968; Payne, 1970 and Sharma, 1987). Although several vaccines have been adopted for its control, the failure of vaccines to provide the expected protective level has been attributed to miscellenous causes and immunosuppression found to play a major role among these causes (Ravis, A. and J. Fabricant, 1988).

Therefore, this work was designed to determine if any of a series of parameters of immunosuppresssion were associated with one of the most wide applicable MDV Vaccines in Egypt (Respin-MDV).

MATERIAL AND METHODS

*Experimental Birds:

320 one day old chicks were

floor reared and fed on balanced ration free from mycotoxins and microbial agents the chicks were divided into 4 groups 80 each:

- Birds of group 1 were vaccinated with MDV vaccine at one day old.

- Birds of group 2 were given MDV vacine at one day old and NDV vaccine at 14th day of age.

- Birds of group 3 were vaccinated only with NDV vaccine at 14th day of age.

- Birds of group 4 were non-vaccinated and served as negative controls.

* Viruses

1. Vaccinal strain of MDV.

Cell associated fowl herpes virus CVI 988 (Rispen strain "R-MDV") was obtained from INTE-VET INTERNATION B.V. BOX-MEER-HOLLAND.

2. Vaccinal strain of Newcastle disease virus (NDV):

Lentogenic NDV vaccine laSota strain was obtained from INTER-VET INTERNATIONAL B.V. BOXMEER-HOLLAND.

3. Challenge strain of NDV.

A local velogenic viscerotropic strain of NDV (VVNDV74) was obtained from the Veterinary Laboratories for Biological Products and Vaccines (ABBASSIA, CAIRO, EGYPT).

In order to study the influenc of MDV vaccine on the immune system of chickens, evaluation of the imune response to NDV in MDV and/or NDV vaccinated chickens carried out as follows:

1. Quatitative determination of antibodies in chicks sera was performed using heamagglutination inhibition "HI" test (Majiyabe and Hitcher, 1977), and ELISA (Syunder et al., 1983 and Madboly, 1989).

2. Antibody secreting cells (ASC) in spleen of chicks were counted using the enzyme linked immunospot (ELI-spot) assay (Andrej Tarkowiski, et al., 1984).

3. Estimation of total serum protein levels and quantitive estimation of different serum proteins and globulins was determine by polyacrylamide gel electrophoresis. (Davis and Ornstein, 1964).

4. The blastogenic respose of chicken peripheral blood lymphocyte to T-cell mitogen was determined using the glucose consumption test principally as described by Decoke et al. (1986).

5. The phagocytic activity o peripheral blood moncyte wa measured using C. albicans (Richardson and Smith, 1981 an Barry et al., 1988).

6. Relative weights of lymphoi organs "Bursa, Thymus an

Spleen" were also recorded. (Rivas and Fabricant 1988 and Giambrone, 1989).

7. The protection capability of vaccinated and control chicks to challenge with virulent strain of NDV was determined.

* Statistical analysis:

Student's t-test was applied to analyze the difference in the results of the above tests with p-values <0.05. (Snedecar and Cochran, 1976).

RESULTS

A. EFFECT OF MDV VACCINE ON ANTIBODY LEVEL AGAINST NDV IN CHICKEN SERA.

Statistical significant depressions in HI antibodies were observed in group 2 at 25, 28 and 35 day of age compared with group 3 as clearly illustrated in Fig. (1), while in ELISA statistical significant depressions were detectable at 17, 25, 35 and 42 day in that geoup (2) in relation to group 3 (table 1).

B. EFFECT OF MDV VACCINE ON TOTAL SERUM PROTEIN AND ALBUMIN/ GLOBULIN (A/G) RATIO:

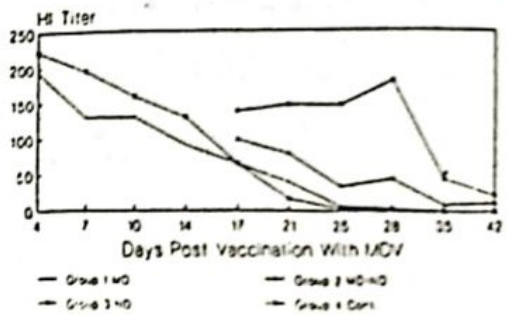
Statistical significant decrease in total serum proteins has been expressed in group 1 at 4 and 10 days post vaccination with MDV in relation to control chickes (group 4), such a decrease also was occurring at 25 and 42 day of age in gorup 2 in camparable with group 3. Marked increase in A/G ratio with prominant decrease in Globulin content was determined at 7,25 and 28 days of age in group 1 in comparison with gorup 4, while in group 2, prominant increase in A/G ratio were occurred at 25 and 28 day of age in relation to group 3 as clearly shown in (Fig. 2).

D. EFFECT OF MDV VACCINE ON LYMPHOCYTE RESPONSE TO PHA MITOGEN STIMULATION:

Table (1): Results of Solid-Phase ELISA to NDV expressed by Mean Titer.

Day Post Vaccination	Group 2	Group 3
17	32±0	265±2
21	128±0	128±2
25	46±2	512±2
28	128±2	256±2
35	8±0	46±2
42	11.3±1.4	45.2±1

Fig. 1 HI Response to NDV in Chickens Vaccinated With MDV (Rispen)

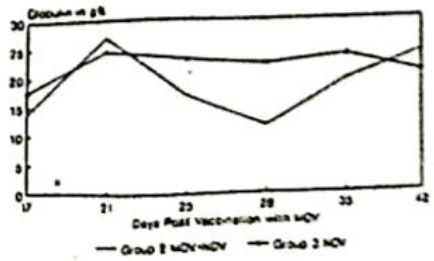


Statistical significant depressions were observed in group 1 at 7, 10, 14, 25, 28 and 35 days of age when compared with group 2. These depressions also were occurring in group 2 at 17, 25, 28, 35 and 42 day of age if compared with gorup 3 (Table 2 and Fig. 4, 5).

Table (2): Suppression of Mitogen Induced blastogenesis by vaccination with MDV (rispen strain).

Days post vaccination	Percentage of suppression		
	Group 1	Group 2	Group 3
4	8.0 %		
7	53.2 %		
10	35.0 %		
14	16.0 %		
17	7.0 %		
21	0.0 %	18.0 %	0.0 %
25	25.0 %	25.0 %	9.0 %
28	25.0 %	25.0 %	0.0 %
35	57.2 %	20.0 %	14.0 %
42	23.0 %	48.0 %	21.0 %
		22.0 %	0.0 %

Fig. 2 Globulin % in chicken Sera Obtained from MDV and/or NDV Vaccinated groups.



E. EFFECT OF MDVVACCINE ON THE PHAGOCYTIC ACTIVITY:

Dealing with group 1, statistical significant depressions have already been obtained at 4, 14 and 28 days of age, while the activity significantly increased at 7 and 21 days in that group (1) in relation to group 4. On the other side, group 2 showed statistical significant depressions at 7, 21, 35 days of age compared with the activity given by group 3 (Table 3).

F. EFFECT OF MDV VACCINE ON THE RELATIVE WEIGHT OF LYMPHOID ORGANS:

Table (3): Influence of vaccination with MDV (Rispen strain) on the phagocytic activity of PBM of chickens using C. albicans.

Days post vacination	Phagocytic Index			
	Group 1	Group 2	Group 3	Group 4
4	0.3 ± 0.07			0.57 ± 0.04
7	0.67± 0.05			0.056± 0.05
10	ND			ND
14	0.32± 0.09			0.50 ± 0.01
17	0.78± 0.05	0.81± 0.08	0.91± 0.00	0.75 ± 0.01
21	0.71± 0.07	0.66± 0.09	0.91± 0.05	0.33 ± 0.04
25	0.52± 0.04	0.51± 0.06	0.43± 0.06	0.64 ± 0.09
28	0.43± 0.02	0.57± 0.04	0.49± 0.08	0.53 ± 0.01
35	0.79± 0.08	0.63± 0.01	0.82± 0.05	0.07 ± 0.04
42	0.40± 0.09	0.46± 0.06	0.44± 0.02	0.40 ± 0.01

ND = Not done.

Fig. (3): Detectable Number of ASC in chicken spleen as measured by ELI - spot Assay.

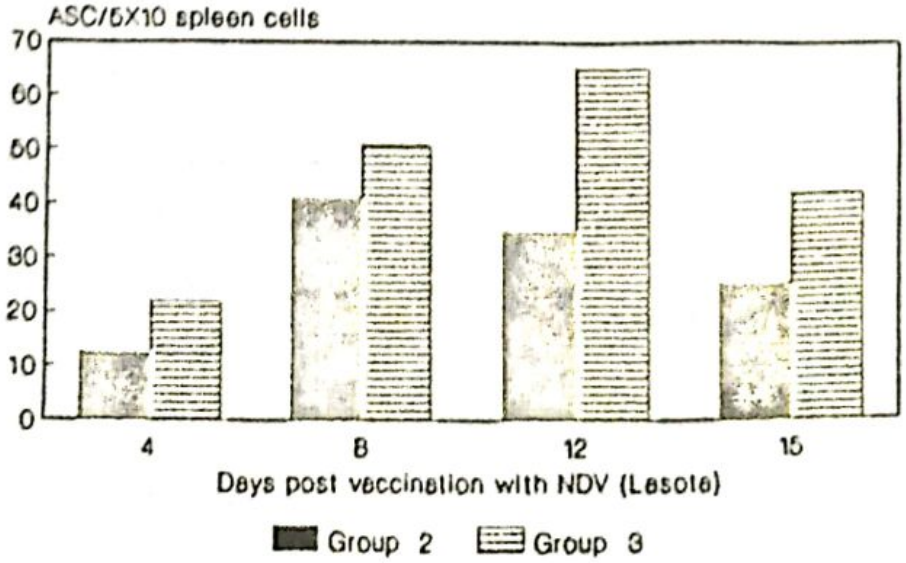


Fig. (4) : The plastogenic response of chicken PBL to PHA in MDV vaccinated and control Gs.

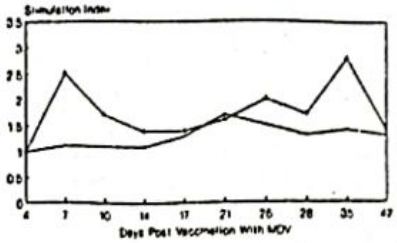
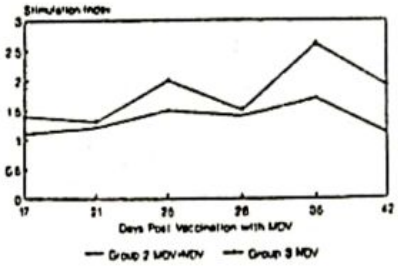


Fig. (5): The plastogenic response of chicken PBL to PHA in MDV and/or NDV vaccinated Gs.



Statistical significant decrease in relative weight of thymus was recorded at 10 days post vaccination in gorup 1 in comparable with control chicks. In contrast, R-MDV did not influence the relative weights of bursa and spleen.

G. EFFECT OF MDV VACCINE ON THE RESISTANCE OF MDV AND/OR NDV VACCINATED CHICKENS TO CHALLENGE WITH $VVNDV_{74}$:

Birds of group 1 and 4 showed 0.0% protection against $VVNDV_{74}$, while birds of group 2 showed 50% protection in contrast to birs of group 3 which expressed 80% protection against $VVNDV_{74}$.

DISCUSSION

MDV is known for its immunosuppressive potentialities (Rivas and Fabricant, 1988) and inteferes with the humoral and cell-mediated immune response of chickens against other microbial agents and vaccines. Therefore, the present study was devoted to investigate the effect of MDV vaccine (R-MDV) on the immune response of chickens to NDV.

Statistical depression in the level of antibodies to NDV vaccine was detected by using HI and ELISA as shown in Fig. (1) and Table (1); this documents the adverse immunosuppressive effect of R-MDV on the humoral immune response, which might be attributed to the destructive effect of the virus on the bursa of fabricius. The noticeable decrease in antibody secreting cells (ASC) obtained from the ELI-spot assay (Fig. 3) support such finding which are found to agree with those of **Lui and Lee (1983) and Rivas and Fabricant (1988).**

Concerning the obtained result of total serum protein and A/G ratio; our finding in such a respect agree with those obtained by **Grandbock-Jusko et al., (1985)** who found that total protein content was slightly lowered in MDV infected birds than in non infected birds and **Jurazdoa and Napravink (1974)** who recorded a level of globulin below normal in chicks infected at one day with NDV. Our data reflect and confirm the adverse effect of R-MDV vaccine on the humoral side of immune response of chicken.

R-MDV showed its immunodepressive effect on the cell-mediated immune response, where two detectable phases of immunosuppression were obtained at the 7th and 35th days post vaccination as clearly shown from results of lymphocyte blastogenesis (Fig. 4). This suppressive effect was clearly reflected on the response to NDV with statistical significant depression in the blastogenic response to PHA (Fig. 5); thus supporting the kinetic reported by **Lui and Lee (1983).**

The phagocytic activity of PBM of chicks vaccinated with MDV and/or NDV was significantly depressed as shown in table (3); this depression could by attributed to the suppressor factors released by macrophages as also reported by Lee et al. (1978) and **Lee (1984).**

R-MDV caused significant decrease in relative weight of thymus at 10 days post vaccination which might lead us to the conclusion that the reproductive replication of R-MDV in the thymus and bursa induce slight degenerative changes in these organs. In contrast, R-MDV did not adversly influence the relative weights of bursa and spleen.

Alteration in the humoral and cell mediated immune response induced by R-MDV vaccinated birds showed 80% protection to VVNDV $_{74}$; while R-MDV and NDV vaccinated birds showed 50% protection. Birds vaccinated only with R-MDV and the control unvaccinated birds could not show any protection.

These observations suggest a direct relationship between R-MDV vaccination and the obtained immunodepression, supporting previous reports **(Liu and Lee, 1983;**

Lee, 1984 and Rivas and Fabricant, 1988) in dicating that fieled losses due to Marek's disease vaccinated birds might be related to immunodepression.

SUMMARY

In the present study the effect of MDV vaccine Rispen Strain (R-MDV) on the immune response of chicken to NDV vaccine (Lasota strain) was studied. 3 major components were determined; humoral immunity, cellmediated immunity and macrophage function. The obtained results revealed that MDV vaccine (R-MDV) has a clear immunosuppressive effect manifested by: significant depression of antibody production to NDV, decrease in the number of ASC in the spleens of MDV vaccinated chicks, slight influence of the total serum protein with decrease in globulin percentage, significant depression in lymphocyte blastogenesis induced by PHA, decrease in macrophage activity and percentage, slight changes in the weight of lymphoid organs and clear reduction in the protection rate as measured by intramuscular challenge with velogenic viscerotropic strain of NDV.

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