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STUDIES ON THE PROTECTIVE EFFICACY OF DIFFERENT TYPES AND COMBINATION OF MAREK'S DISEASE VIRUS VACCINES*

BY

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INTRODUCTION

Marek's disease (MD) is one of the most known clinical neoplastic conditions of chicken . The disease was first recognized as clinical entity by Marek in 1907.

In Egypt, the disease was first recorded by Shebl et al. (1975) and it has been incriminated for heavy losses due to lymphatic tumors it induces among layer and breeder flocks.

The advantage of MDV vaccines and its world wide use was reported in disease decline substantially. The increasing number of available types and combination of MDV vaccines has created a greater need to conduct critical comparisons of the protective efficacy of MDV vaccines. This was the aim of the present investigations where the protective efficacy of different types and combinations of MDV vaccines in

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chickens were comparativily evaluated by experimental challenge using local isolate of MD- virus and also by field challenge using MD./virus infected litter.

MATERIALS AND METHODS

Materials:

1. Chicks:

3360 one day old lohman broiler breeder chicks were kindly supplied by Lohman Company, West Germany.All chicks contained maternal antibodies against chicken herpes viruses (CHV). The chicks were separated into seven groups (480 chicks for each group) and were vaccinated I/M at one day old with different Marek's disease virus vaccines as shown in Table 1.

Table. 1: Various types of MDV -vaccines used for vaccination of different chicken groups

Chicken group	Type of MD-vaccines
A	HVT (monovalent vaccine)
B	HVT + CVI 988 (Bivalent vac.).
C	HVT + SBI (Bivalent vac.).
D	CVI 988 (monovalent vac.).
E	Clone C (monovalent vac.).
F	Non vaccinated negative control.
G	Non vaccinated SPF, as a neg. SPF control.

2. Marek's disease virus strains:

a. Vaccinal strains:

HVT (TAD Marek VACT).

CVI 988 (TAD Marek VAC forte).

Clone C (TAD Marek VAC).

SB1 (TAD Marek VAC-Compositium).

b. Locally isolated MD virus:

The virus was propagated in one day-old SPF chicks and then harvested 14 days post infection from their blood. The virus was titrated in embryonated chicken eggs (ECE and EID_{50} was calculated according to Reed and Muench (1938). This virus strain was used for experimental challenge according to Bulow (1977).

3. MD-Viral antigen used for Agar Gel Precipitation (AGP):

a. Standard positive AGP antigen of Marek's disease virus containing (A) antigen(prepared from feather follicles of infected SPF birds) was kindly provided by Dr. Von Below, Free Univ., Berlin.

b. The MDV-infected chorioallantoic membranes with different pock lesions (of either different vaccinal strains or the locally isolated MD virus strain) were harvested, treated with triton X-100, then freezed and thowed 3 times and used in AGP tests.

c. Treated buffy coats obtained from blood of vaccinated chickens in different groups and from blood of SPE chicks naturally infected with MD virus.

d. Non infected chorioallantoic membranes harvested from non inoculated ECE were prepared as previously discussed and used as negative control in AGPT. Also buffy coats from blood of non infected SPF chicks were used as negative control in AGPT.

4. Sera:

a. Standard MDV positive antiserum was provided kindly by Dr. Vielitz, Lohman Company, Germany.

b. Negative sera were collected from one day old SPF chicks.

5. Embryonated chicken eggs (ECE):

A total number of 1170 ECE of six day's old were used either for isolation or reisolation of different vaccinal strains of MD virus. Also these ECE were used for isolation and titration of local field isolate of MD virus.

EXPERIMENTS

1. Detection and isolation of Marek's disease virus (MDV) vaccinal strains from chickens 14 days post vaccination:

Ten heparinized blood samples were collected from each chicken group (A to G) 14 days post vaccination with different Marek's vaccines. Using Ficoll - isopaque density gradient centrifugation technique (Payne, et al. 1976) lymphocytes were separated, homogenized and tested for the presence of MDV-strains by means of AGP using known standard MDV-positive serum. Also, the prepared lymphocyte cell lysates were tested for the presence of MDV-vaccinal strains by being inoculated in 6 days's old ECE via yolk sac route.

2. Isolation and titration of local strain of MDV from SPF chicks 14 days after their exposure (at one day old) to known MDV infected litter:

In this experiment 65 one day old chicks (group G) were kept on MDV infected litter for 2 weeks. Then 25 chicks were killed, their blood was collected, heparinized (10 1U heparin/ml), pooled together and the lymphocytes were separated using sterile Ficoll-isopaque solution. The collected lymphocytes were disrupted by freezing and thawing several times, then the cell suspension was centrifuged at 1200 xg for 10 minutes and the supernatant was collected and used for virus isolation by intra yolk sac inoculation in 6 days old ECE. The inoculated eggs were candeled daily and chorioallantoic membranes showing pock lesions were harvested and tested for MDV specificity using known standard MDV positive serum. The EID_{50} was calculated according to Reed and Muench, (1938).

3. Evaluation of the protective efficacy of different types of MDV-vaccines by challenge test:

a. Experimental challenge with the local isolate of MDV:

Thirty five chicks from each group from A to G were kept in an open house, then each chick was injected with 1000 EID_{50} (Bulow, 1977) of the MDV-local isolate prepared from infected chorioallantoic membrane homogenate. The chickens were observed daily and mortalities due to MDV were recorded up to 20 weeks. At the end the protective index was calculated for the different types of MD vaccines as follows:

Protective index=

$$= \frac{\text{\% of MD mortalities in control} - \text{\% of MD mortalities in vaccinated chicken}}{\text{\% MD mortalities in control chickens.}}$$

b. Field challenge of chicken by MDV infected litter:

From chicken groups (A to E) vaccinated with different types of MDV vaccines and also from the non vaccinated control groups (F and G), the following numbers of chicken were gathered together 14 days post vaccination and kept in an open house; 411 chicks from groups A,B,C,D and E, 345 chicks from group F and 299 from group G.

Infected litter obtained from a farm with long history of MDV infection was introduced to the above mentioned chicks. Then all chicks were kept under observation , the MD mortality was calculated from each group daily up to 20 weeks and the protective index was calculated.

RESULTS

As shown in Table 2, the homologous monovalent MDV vaccines namely the CVI- 988 and HVT vaccines showed better viraemic response compared to other tested vaccines. The highest rate of viral detection was reported in chicks vaccinated with CVI-988 vaccine (70.9%)followed by HVT vaccine (60%). Other vaccines, however, showed a detection rate of 50%.

The rate of isolation of the MDV- vaccinal straims during viraemic stages caused by different MD vaccines ranged from 80-100%, where in HVT vaccinated chicks (group A), it reached to 100% followed by CVI-988 vaccinated chicken (group D) which showed

an isolation rate of 90%. In the other tested vaccines, however, the isolation rate was 80%. No MDV was isolated or detected from chickens in the non vaccinated groups F and G.

The MDV isolation on ECE was more sensitive and accurate in detection of viraemia than the AGP test .

The results of challenge test for evaluation of the protective efficacy of different MDV-vaccines in chickens are demonstrated in Table 3 and 4.

Results shown in Table 3, revealed that the best protection of chicks against experimental challenge with the local isolate of MDV was achieved by CVI-988 vaccine (group D) which showed a protective index of 87.76% followed by a protective index of 83.67% in chickens vaccinated with HVT plus CVI-988 bivalent vaccine (group. B). The least protection was observed in chicken group vaccinated with the monovalent HVT vaccine (prot. index = 30.61).

In Table 4, (experimental challenge with infected litter) the highest protective index was obtained in chicken group vaccinated with CVI-988 (Prot. index = 90.45%). The protective index in chickens vaccinated with other MDV vaccine was relatively lower, where it was 75.50% in chickens vaccinated with HVT vaccine; 79.24 in chickens vaccinated with HVT + SBI bivalent vaccine and 77.16% in clone c vaccinated group.

Table 2: Comparison between AGP and isolation on chorio toic membrane of E C E for detection of viraemia induced by different vaccinal strains of MDV in chick-ens 14 days post vaccination.

Group No.	Type and combina-tion of MDV-vaccines	Detection by AGP.Positive/ Total	Isolation on CAM of ECE* postive/Total
A	HVT	6/10	10/10*
B	HVT + CVI-988	5/10	8/10
C	HVT + SBI	5/10	8/10
D	CVI-988	7/10	9/10
E	Clone-C	5/10	8/10
	Controls:		
F	Non-vaccinated	0/10	0/10
G	SPF	0/10	0/10

* Each sample was inoculated on 10 ECE (6 days old) by intra yold SAC route.

Table 3: Results of evaluation of the protective efficacy of different type of MDV vaccines by experimental challe-nge of the vaccinated chicks with the local MDV isolate

Group No.	Types of MDV	Mortality rate		PM-lesions**		Protc-tive index
		No.dead/ Total	%	No. posi-tive/ Total	%	
A	HVT	23/35	65.71	17/35	48.57	30.61
B	HVT+CVI-988	16/35	45.71	4/35	11.43	83.67
C	HVT + SBI	16/35	45.71	9/35	25.71	63.27
D	CVI-988	13/35	37.14	3/35	8.57	87.76
E	Clone-C	19/35	54.29	12/35	34.29	51.02
F	Non-vaccinated infected	35/35	100.0	22/35	62.86	-
G	Non-vaccinatd SPF	35/35	100.0	27/35	77.14	-

** The virus was detected from PM lesion in the proventriculus by AGP using known standard positive MDV antiserum.

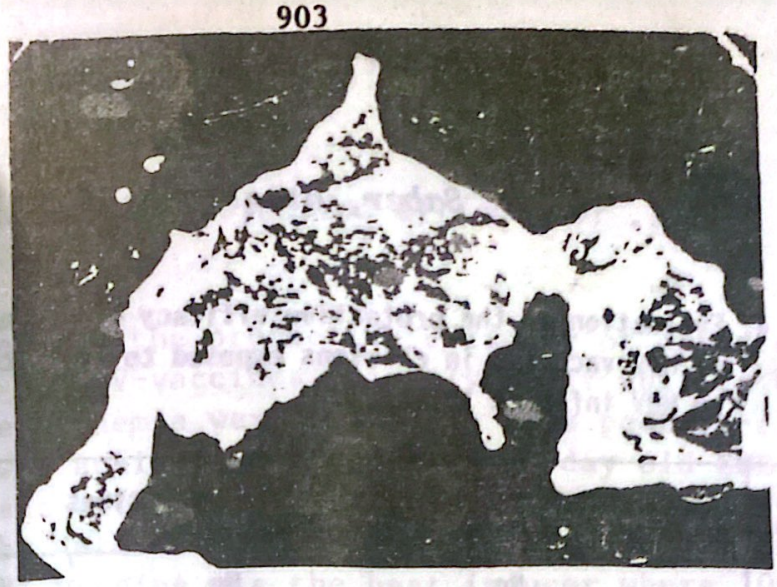

Fig. 1: Pock lesions on CAM of ECE, 6 days post inoculation with HVT strain of MD vaccine: Note the white pin head pock lesions.

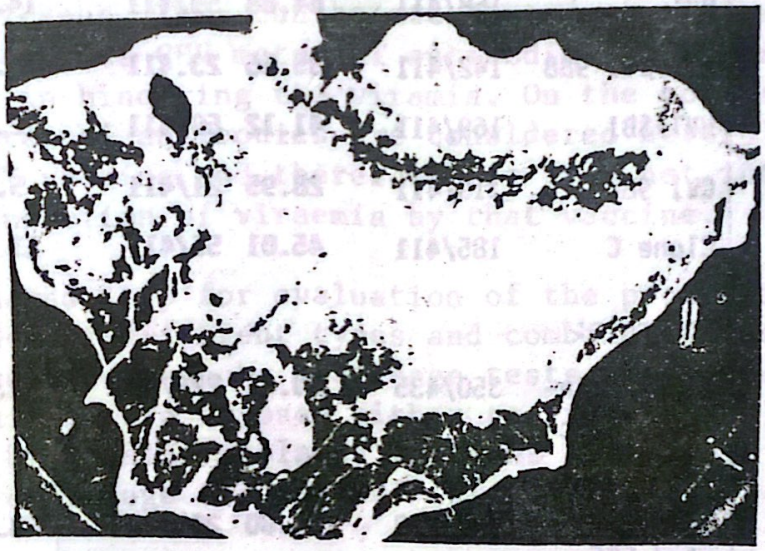

Fig. 2: Pock lesions on CAM of ECE, 6 days post inoculation with Rispen strain (CVI-988) of MD-vaccine. Note the proliferative nature of the pock lesions.

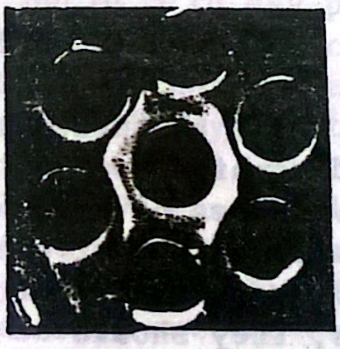

Fig. 3: Agar gel preceipitation test showing complete line of identity between buffy coat lysate from MDV vaccinated chicks and standard MD-antigen in peripheral wells against the MD positive standard antiserum in the central well.

Table 4: Evaluation of the protective efficacy of different types of MDV vaccines in chickens exposed to field challenge by MDV infected litter.

Group No.	Types of MDV vaccine	Mortality rate		PM lesions		Protective index
		No. Positive/ Total	%	No. positive*/ Total	%	
A	HVT	158/411	84.88	59/411	14.36	75.50
B	HVT+CVI 988	142/411	35.55	23.411	5.60	90.45
C	HVT+SBI	169/411	41.12	50/411	12.17	79.24
D	CVI 988	119/411	28.95	21/411	5.11	91.28
E	Clone C	185/411	45.01	55/411	13.38	77.16
	Controls:					
F	Non vaccinated infected	350/435	80.46	226/435	51.95	-
G	Non vaccinated SPF. infected.	229/299	100.00	204/299	68.23	-

DISCUSSION

In the present study the protective efficacy of different types and combinations of the commercially available MDV vaccines have been evaluated. All tested vaccines were applied at one day-old according to Payne (1985) who stated that for maximal efficacy of MDV vaccines, they should be given as soon as possible after hatching in order to counteract the very early exposure of newly hatched chicks to virulent field strain of MDV of inoculated eggs.

To evaluate the protective efficacy of different types of MDV-vaccines, firstly their ability to induce viraemia were tested 14 days post vaccination. This was achieved by inoculating 6 day old ECE via the yolk sac with lymphocyte lysates (Sharma, 1976) followed by detection of pock lesions developed on CAM. The HVT vaccine was the best inducer where 100% of tested chickens were viraemic. In chickens vaccinated with other types and combination of MDV-vaccines, viraemia were proved in only 80-90% of the vaccinated chickens. This can be attributed to the presence of homologous CHV maternal antibodies which may play a role in hindering the viramia. On the contrary the CHV maternal antibodies are considered heterologous for HVT vaccine and therefore, it does not interfere with induction of viraemia by that vaccine.

The second step for evaluation of the protective efficacy of different types and combination of MDV-vaccines was made by challenge tests where vaccinated chicks were exposed either to experimental challenge by locally isolated virulent MDV strain or field challenge through MDV infected litter.

According to Writter (1984) the vaccinal immunity can be overwhelmed by early exposure to virulent MDV strains, also protection is unsatisfactory in case when challenge occurs simultaneously with the vaccination or at 2nd or 3 rd day post vaccination. However, good protection by MDV vaccines of all the 3 serotypes resulted when challenge with virulent serotypes was delayed 5-8 days post vaccination. On the other hand, Vielitz (1984) stated that early challenge by virulent MDV can easily induce vaccine break. Also, vaccinal immunity takes at least 7 days to develop and may be delayed by another 7 days in the presence of maternal antibodies.

For the above-mentioned reasons in our experiment challenge was delayed to 14 days post vaccination to ensure better protection.

From the challenge experiment it was clear that the protection was recorded by Rispen CVI 988 alone or combined with HVT vaccine where protection index was 87.75% and 83.67% respectively. Similar results were also observed in case of field challenge experiments. However, the protection index value was relatively higher reaching to 91.27% in CVI 988 vaccine and 90.45% in case of combined vaccination with CVI-988 and HVT-vaccines.

In general the lower protection rate in case of experimental challenge can be attributed to the high dose of virulent MDV given directly to challenged birds which is not the case in field challenge where virus infection comes as aerosol with undetermined virus contact.

In conclusion it is clear that the Rispen CVI-988 vaccine alone produced the strongest protective effect against MDV-infection.

SUMMARY

This study was tried to evaluate the efficacy of different vaccinal strains of Marek's disease virus (single or in combined form) in protection of chickens against natural and experimental challenge with MDV.

Five groups (each contained 480 one day old chicks)were vaccinated separately with the following types and combinations of MDV-vaccines; HVT; CVI-988; HVT + CVI-988; HVT + SBI, and Clone-C. Two additional groups were used as non vaccinated controls, one of them contained SPF chicks.

The 7 groups (vaccinated and controls) were challenged 14 days post-vaccination with virulent MDV either through experimental infection by local isolate of virulent MDV or by natural infection through MDV infected litter.

The highest protection rate was recorded in chicks group vaccinated with CVI-988 either alone or combined with the HVT-vaccine, the lowest protection rate, however, occurred in chicken gorups vaccinated with HVT, SBI and clone C vaccines.

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