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A COMPARATIVE STUDY OF VACCINATION AGAINST MAREK'S DISEASE OF POULTRY (With 2 Tables)

By

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دراسة مقارنة للقاحات ضد مرض (ماريك) في الدواجن

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إستضعفت عترة فيروس مرض (ماريك) MDIP4 المعزولة محلياً في أنسجة الخلايا الحية المحضرة من أجنة بيض الدجاج وعند مستوى التمريرة العشرون إستخدم هذا الفيروس كعترة لقاح مع مقارنة الوقاية المعطاة من هذا اللقاح مع كل من لقاح مرض (ماريك) الحسي المستضعف (من المستحضرات البيولوجية للدواجن) واللقاح المحضر من عترة هربس الخاصة بالرومي (لقاح هربس فيروس الرومي عترة FC 126) وتبين حدوث حماية ملحوظة للطيور بدرجات متفاوتة عند إستخدام إختبار تحدى المناعة بعترة فيروس (الماريك) الكلاسيكية وكانت أعلى درجات الحماية (95%) بإستخدام لقاح (الماريك) المضعف في حين وصلت 92% للقاح هربس فيروس الرومي بينما لم تزد عن 87.5% عند إستخدام اللقاح المضعف لمرض (الماريك) المحضر في الفترة المعزولة محلياً وزاد وزن الطيور المحصنة وتلك التي أجرى لها إختبار تحدى المناعة بالمقارنة بالضوابط كما وقد عزلت عترة الفيروس بنسب أقل في الطيور المحصنة والتي أجرى عليها إختبار وتحدى المناعة .

SUMMARY

A marek's disease virus (MDV) strain isolated locally from an apparently healthy bird was attenuated in chicken embryo fibroblast cell culture. The virus at its 20th passage level was used as a vaccine and the protection offered was compared with MD attenuated vaccine (Poultry Biologicals) and Herpes virus of turkey (HVT) Fc₁₂₆ strain. All the 3 vaccines showed significant protection against challenge with the classical strain of MDV. The degree of protection was highest in MD attenuated (Poultry Biologicals) strain i.e. 95% followed by HVT vaccine (92%) and local isolate of MD attenuated vaccine (87.5%). The vaccinated as well as vaccinated challenged birds showed gain in body weight comparable to controls. The virus reisolation was less in the vaccinated challenged birds.

V.D.P. RAO, et al.**INTRODUCTION**

Experimental laboratory studies and field trials have shown that attenuated strains of Marek's disease virus (MDV) (CHURCHILL, et al. 1969; EIDSON and ANDERSON, 1971; BIGGS, et al. 1972; WITTER, 1987) and antigenically related herpes virus of turkey (HVT) strains (OKAZAKI, et al. 1970; PURCHASE, et al. 1972; PRUTHI, et al. 1987) have been able to offer a considerable degree of protection against virulent strains of MDV. Some of the MDV of low virulence may even fail to produce neoplastic lesions in chickens because of virus or host controlled factors or interactions or both. RAO, et al. (1984) isolated a moderately virulent MDV from apparently healthy bird. An attempt, therefore, was made to attenuate the local isolate (MDIP₄) by passaging in chicken embryo fibroblast (CEF) cell culture and at 20th passage it was used as a vaccine and the results were compared with that of known vaccine strains.

MATERIAL and METHODS**Vaccine strains:**

Herpes virus of turkey (HVT (Fc¹²⁶) strain and an attenuated live MDV vaccine were procured from M/S Bio-Med Pvt. Ltd., Ghaziabad, India and M/S Poultry Biologicals, Houghton, England, respectively, in freeze dried form. A virus isolated from apparently healthy bird (MDIP₄) passaged 20 times in CEF cell culture was also used as a vaccine strain.

Experimental chicks:

A total of 660 day old, unsexed, White Leghorn chicks, free from maternal MD antibodies were obtained from University Poultry Farm, Pantnagar. The chicks were individually numbered by wing bands and divided into different groups.

Vaccination:

A total of 200 chicks were administered with HVT (Fc¹²⁶ strain), each receiving 0.2 ml dose by I/P route (Group I). Another 200 chicks were vaccinated with attenuated MD vaccine (Poultry Biologicals) in 0.2 ml dose/chick by I/P route (Group II). One hundred chicks were vaccinated with attenuated MDV (MDIP₄) and each chick received 1000 PFU of virus (0.5 ml dose) by I/P route (Group III). Controls included 80 chicks inoculated with 0.2 ml of whole blood collected from birds suffering from classical form of MD, by I/P route (Group IV) and 80 chicks were kept in strict isolation to serve as uninoculated healthy control (Group V).

Challenge:

Three weeks post-vaccination, 100, 100, 40 and 50 chicks were selected randomly from Groups I, II, III and V respectively, and challenged by injecting 0.2 ml of whole blood/bird, collected from ND infected birds showing symptoms of MD. These chicks were then grouped as Group VI, VII, VIII and IX respectively.

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The following observations were recorded for 14 weeks of experimental period:

- 1 - The chicks of individual groups were weighed from 3 weeks onward at weekly intervals to determine growth rate.
- 2 - All the chicks of different groups were observed for appearance of clinical symptoms of MD.
- 3 - The mortality due to MD and non specific causes was recorded in all birds died during the period of experimentation. The visceral organs and sciatic nerve were examined for gross and microscopic changes. In addition 2 chicks from each group were sacrificed at weekly intervals to examine the organs as above.
- 4 - Twenty five chicks from each group were examined at weekly interval for the detection of feather follicle antigen and serum antibodies by agar gel precipitation test as per the method of OKAZAKI, *et al.* (1970).
- 5 - After 10 weeks the birds in each group were tested for virus reisolation. Blood samples from 5 birds were collected in antibiotic-EDTA solution and from the pooled sample the buffy coat was separated and inoculated into primary CEF cultures for observation of plaques (PURCHAST, *et al.* 1971).

RESULTS

The chicks vaccinated with different vaccines and challenged with classical strain of MDV at 21 days post-vaccination resulted in varying degrees of protection. The birds vaccinated with MD attenuated (Poultry Biologicals) vaccine showed 95% protection. The birds vaccinated with HVI (1c₁₂₆ strain) and MD attenuated (MDIP₄) vaccine offered 92% and 87.5% protection respectively (Table 1). Unvaccinated and control challenged birds (Group IX) showed 58% mortality as a result of Marek's disease.

There was no significant difference in gain in body weight between 3 vaccinated groups. The gain in body weight was poor in MD infected (Group IV) and control challenged (Group IX) birds with mean body weight of 506.5 g. and 582 g. respectively. However, the gain in body weight was significant in vaccinated and challenged (Group VIII) birds (850 g) as compared to controls (506.5 g).

Feather follicle antigen was detected in MD infected (Group IV) in 3.2% of birds at 3 weeks of age and by 8th week all the birds showed positive MD antigen. In vaccinated and challenged birds (Groups VI, VII, VIII) the antigen appeared at 6 weeks of age. Likewise, the MD precipitins could be detected at the age of 6 weeks in groups II, III and IV.

Eight of the 20 birds (40%) showed paralytic signs in the MD infected (Group IV) birds at 9 weeks of age. Likewise, 3 out of 29 birds (10.9%) showed paralytic signs in control challenged (Group IX) birds at 12 weeks of age. The birds showed uni-/bilateral paralysis of limbs and wings. No clinical symptoms were observed in group I, II, III, V, VI, VII and VIII through out the experiment.

The mortality due to MD and causes other than MD is presented in Table 2. The gross lesions appeared at 8 and 12 weeks post-infection in MD infected (25.4%) and

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control challenged (18%) group respectively. The lesions included enlargement of visceral organs with white grey foci and sciatic nerve showed increase in diameter with focal or complete loss of cross striations. Atrophy of bursa of Fabricius was also observed. The microscopic changes were first seen in MD infected group at 3 weeks post-infection and characterized by varying degrees of infiltration and proliferation of small to large lymphocytes, lymphoblasts and reticulum cells which were either focal or diffuse in distribution. In vaccinated groups, the birds showed slight congestion of sinusoids and lymphofollicular reaction in the liver and slight degeneration and rarification of follicles of bursa fabricius at 2 to 3 weeks post vaccination. By 5th week post-vaccination all the organs were found normal.

The birds tested for reisolation of MDV at 10 weeks of age showed that frequency of virus isolation was more in MD infected (Group IV) and control challenged (Group IX) groups (100%) than the vaccinated challenged (60%) birds.

DISCUSSION

The vaccine prepared from 20th passage level of local MD isolate (MDIP₄) gave comparatively less protection than the MD attenuated (Poultry Biologicals) and HVT vaccines. Similarly, CHURCHILL, et al. (1969) and BIGGS, et al. (1972) used classical MD virus as vaccine after 60 passages in CK cell culture and observed 84.8% protection which is comparable to protection offered in the present study with the local strain. In the present study using HVT (Fc₁₂₆ strain) the mortality due to MD has been reduced from 38% to 8% and degree of protection averaged to 92%. PURCHASE, et al. (1972) while conducting field trials with HVT vaccine also reported 88.8% protection after challenge with virulent strain of MD. The results are also in agreement with HONEGGER, et al. (1972) where 7% mortality had been experienced with HVT vaccine.

The significant gain in body weight in the vaccinated as well as vaccinated challenged birds compared to the controls may be due to beneficial effect of vaccination which prevented the development of MD lesions is in agreement with that of BIGGS, et al. (1972) and HONEGGER, et al. (1972).

The appearance of gross as well as microscopic lesions in few vaccinated challenged birds may be due to apparent delay in the establishment of vaccine virus in the inoculated chicks. Similar observations were also made by GREWAL and SINGH (1977) in birds vaccinated and challenged with MD virus.

The frequency of virus reisolation in vaccinated challenged birds was less in comparison to MD infected and control challenged birds which indicate that the vaccine virus reduces the multiplication of challenge virus as reported also by CHURCHILL, et al. (1969); PURCHASE, et al. (1972) and PRUTHI, et al. (1987).

It may be concluded from the present study that the vaccine prepared from local MD isolate (MDIP₄) can be improved further by passaging in CEF cell culture to achieve sufficient attenuation and to afford better protection against MD.

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Table (1)

Showing percentage of protection with different vaccines against Marek's disease

Treatment	No. of birds challenged at 3 weeks of age	Percent of MD mortality	Percent of protection against MD
HVT (Fc ₁₂₆) vaccine (Bio-Med)	100	8.2	92.0
MD attenuated vaccine (Poultry Biologicals)	100	5.0	95.0
MD attenuated local (MDIP ₄) vaccine	40	12.5	87.5
Control	50	38.0	-

Table (2)
Showing the mortality (Gross and microscopic lesions) due to MD in different groups

Treatment	No. of birds at 3 weeks	No. sacrificed		No. positive for MD		No. died (3-14) weeks	No. died due to NSD	Mortality due to MD		Cumulative positive (%) of MD
		At weekly interval	At the end	Gross	Micro			Gross	Micro	
HVT vaccinated (Group I)	88	12	63	-	-	13	13	-	-	-
MD attenuated (Poultry Biologicals) (Group II)	85	12	54	-	-	19	19	-	-	-
MD attenuated (MDIP ₄) vaccine (Group III)	45	10	34	-	-	1	1	-	-	-
MD infected (Group IV)	59	14	10	8	16	35	24	7	4	59.3
Control Unchallenged (Group V)	44	13	26	-	-	5	5	-	-	-
HVT vaccinated & challenged (Group VI)	100	12	61	4	-	27	23	-	4	8.0
MD attenuated (Poultry Biol.) & challenged (Group VII)	100	12	61	2	3	27	27	-	-	5.0
MD attenuated (MDIP ₄) & challenged (Group VIII)	40	11	20	3	-	9	7	-	2	12.5
Control challenged (Group IX)	50	12	14	5	4	24	14	4	6	38.0