Protective Effect of Marek's Disease Vaccine and Its Problems on Application

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Though the aetiological agent of Marek's disease had not been known for a long time, it was elucidated immediately after the isolation of a kind of herpesvirus from affected chickens by Churchill & Biggs[9] in 1967.

In Japan, Yuasa et al. also isolated the same as the herpes-type virus from affected chickens in 1969, and regarded it as the viral origin of this disease judging from its properties.

Since it had been clarified that this disease is caused by a virus (Marek's disease virus: MDV), a great interest was taken in the development of vaccine to prevent the disease, and attenuated MDV (Churchill et al. 1969)[10], herpesvirus of turkey (HVT, Okazaki et al. 1970)[15] and avirulent MDV (Rispens et al. 1972)[18] were reported to have protective effect.

In Japan, developmental researches on vaccine were also carried out actively resulting in the marketing of inactivated MDV vaccine in 1971, and attenuated MDV and HVT vaccines in 1972, but now the HVT vaccine is used exclusively.

Since the popularization of the vaccine, the outbreaks of Mardk's disease have decreased remarkably and only a few occasional and regional outbreaks have been reported in late years.

The author investigated the problems of protective effects and application of HVT vaccine for three years from 1970 and hopes for its wide utilization in future.

The outline of my investigation is described here including some data on the effect of HVT vaccine in the field tests carried out by other laboratories in Japan.

Protective effect and safeness of herpesvirus of turkeys as vaccine for Marek's disease[22]

One-day old or three-week old chicks introduced at the same time were inoculated with cell-associated HVT of 520 or 57 plaque-farming unit (PFU) per bird, and were challenged with virulent MDV four weeks after the inoculation. They were observed until 26 weeks of age.

(1) Since no abnormality was observed in the group of birds inoculated with HVT at one day of age but not challenged afterwards as shown in Table 1, HVT was considered to be safe for birds.

(2) Since the protection rate was 86 percent in the group of chicks inoculated with HVT at one day of age and 100 per cent in that at three weeks of age, HVT was recognized to be effective for the protection of Marek's disease.

(3) Protective effect was also manifested with findings on body weight, clinical sign, egg production and maturation.

Degree of protective effect against Marek's disease by vaccination with a herpesvirus of turkeys

Results of field tests carried out at the time of raging prevalence of Marek's disease when HVT vaccine did not yet appear in the Japanese market are summarized in Table 2.

This table was made on the basis of data

Table 1. Protective effect and safeness of herpesvirus of turkeys (HTV) as vaccine for Marek's disease (MD)

Group of birds	Vaccination[1] (PFU/bird)	Challenge[2]	MD[3] positive (%)	Protection[4] rate (%)	Body weight (20-weeks-old g)	Clinical sign (20-weeks-old %)	Egg production (24~25-weeks-old %)	Ovary[5] maturation (26-weeks-old %)
Vaccinated at 1-day-old	520	+	10/100 (10.0)	86	1,420.7	5.0	34.3	78.5
	—	+	62/86 (72.1)		1,177.2	49.2	23.7	62.9
	520	—	0/8 (0)		1,408.1	0	41.5	75.0
Vaccinated at 3-weeks-old	57	+	0/18 (0)	100	1,495.4	0	54.1	100.0
	—	+	3/10 (30.0)		1,260.0	30.7	24.4	62.5
Unvaccinated-unchallenged control	—	—	0/3 (0)		1,468.0	0	44.4	100.0
Challenged at 1-day-old	—	+	8/11 (72.7)		978.0			

1) FC 126 strain of HVT, cell-associated, intramuscular inoculation
2) S strain-infective chick blood of MD Virus, intramuscular inoculation, 4 weeks after HVT inoculation, observed until 26 weeks of age
3) Birds which died with lymphomatous lesions determined grossly or histopathologically and those which survived with gross lesions at the end of experiment were considered to be MD-positive
4) $c=\frac{a-b}{a}\times 100$ a : MD positive rate of unvaccinated-challenged control group
b : MD positive rate of vaccinated group
c : Protection rate
5) Chickens having ovarian follicle of 10 mm or more in diameter

Table 2. Degree of protective effect against Marek's disease (MD) by vaccination with herpesvirus of turkeys (HVT)

MD % of control group	Cell-associated HVT			Cell-free HVT		
	No. of flocks	Protection rate (%)	Average (%)	No. of flocks	Protection rate (%)	Average (%)
20–	2	79, 90	85	1	75	75
10–	6	61, 70, 89, 89, 91, 92	82	4	67, 70, 87, 96	80
5–	12	62, 74, 82, 83, 83, 85, 87, 87, 88, 88, 92, 94	84	3	26, 93, 96	72
2.5–	14	37, 69, 82, 82, 82, 83, 87, 88, 89, 90, 92, 94, 94, 100	84	2	45, 78	62
1.3–	9	20, 71, 75, 82, 87, 93, 94, 95, 96	79	1	63	63
Total	43		83	11		72
0.6–	3	0, 71, 94				
0.3–	5	20, 60, 67, 100, 100				
<0.3	2	0, 100				

1) The table shows the summary of the data of field tests offered by 8 laboratories
2) Observations were performed until 21 weeks of age in all tests

Table 3. Influence of inoculation route of herpesvirus of turkeys (HVT) on the protective effect

Inoculation route of HVT	Number of birds tested	MD positive*3)		Protection rate (%)
		Number of birds	%	
Intramuscular*1)	30	4	13.3	63
Oral*1)	30	10	33.3	7
Contact*2)	20	7	35.0	2
Uninoculated control	28	10	35.7	

*1) 750 PFU/birds of HVT were inoculated at one day of age
*2) Kept in contact with the oral inoculation group during 4 weeks from one day of age
*3) Observed during 20 weeks after intramuscular challenge inoculation with S strain-infective chick blood of MD Virus. See the footnote of Table 1 for determination of Marek's disease

offered from eight private laboratories.

Except the tests in which the morbidity (positive rate of Marek's disease) of the unvaccinated control flocks was less than 1.3 per cent*, protection rate was 83 per cent in average with cell-associated HVT vaccine and 72 per cent with cell-free HVT vaccine respectively.

Therefore, the outbreak of Marek's disease cannot always be prevented completely even in the blocks inoculated with HVT vaccine, and a number of inoculated birds which corresponds 20 to 30 per cent of the affected number of unvaccinated control flocks cannot be rid of the affection. For example, under the condition where Marek's disease can break out on 10 per cent of unvaccinated birds, two to three per cent of vaccinated birds may be affected. From this viewpoint, sanitation and isolation of young chicks should be managed seriously to control properly Marek's disease even if they are vaccinated.

Influence of inoculation route of herpesvirus of turkeys on the protective effect

Though Marek's disease vaccine is usually inoculated to birds through intraperitoneal, subcutaneous or intramuscular route, an experiment was carried out with three flocks of chicks to find-out the effect of inoculation through other routes.

That is, the first flock was inoculated with 750 PFU of cell-associated virus through intramuscular route at one day of age, the second was also inoculated in the same way as the first but through oral route and the third was not inoculated but was kept in contact with the second flock, and then all of them were challenged with MDV at the end of four weeks after the inoculation and were observed during 20 weeks.

As shown in Table 3, the protective effect was scarcely found except the inoculation through the intramuscular route.

Relationship between the inoculation dose of herpesvirus of turkeys and the protective effect

Though the practical dose of HVT as a vaccine settled to be more than 1,000 PFU per bird, the minimum effective dose, the relation between dose and effect and the term needed for the appearance of effect are not yet clarified completely. These relationships were examined by changing inoculation dose of HVT and the term before challenge.

1) Dose of herpesvirus of turkeys required for the appearance of protective effect

The relation between the inoculation dose

* It is not proper to judge protective effect with the value of protection rate alone when the morbidity of unvaccinated control is not high because the variability of protection rate becomes large.

Table 4. Protection of chicks against Marek's disease (MD) by vaccination with graded doses of herpesvirus of turkeys (HVT)

Trial No.	Dose of HVT (PFU/bird)	Number of birds tested	MD positive		Protection rate (%)
			No. of birds	%	
1	7.5	30	2	6.7**	81
	75	29	3	10.3*	71
	750	30	4	13.3*	63
	Unvacc.	28	10	35.7	
2	173	26	1	3.8**	88
	1,730	29	1	3.4**	89
	17,300	31	2	6.5*	79
	Unvacc.	29	9	31.0	

1) HVT was administered intramuscularly at one day of age
2) All chickens were chalenged intramuscularly with infective chick blood of MD virus at 4 weeks of age. The viruses were S strain in Trial 1 and V-1 strain in Trial 2
3) Observation periods after challenge were 20 weeks in Trial 1 and 22 weeks in Trial 2
4) Birds which died with lymphomatous lesions determined grossly or histopathologically and those which survived with gross lesions at the end of experiment were considered to be MD-positive
5) Significance of difference was calculated by the chi-square test in comparison with controls challenged in parallel
   * $P<0.05$
   ** $P<0.01$

of HVT and protective effect was examined with chicks inoculated at one day of age with cell-associated HVT dosed in six grades from 7.5 to 17,300 PFU per bird and challenged four weeks after vaccination.

As shown in Table 4, even a small dose of virus such as 7.5 PFU manifested the protection rate as much as the dose of 1,730 PFU, which can be regarded as the practical dose of vaccine, did, and moreover, a large dose such as 17,300 PFU could not elevate the protetcion rate further.

Churchill et al.[8] recognized viremia at a week after the inoculation of HVT with a dose more than 50 PFU and also at two weeks after the inoculation even with a dose of 5 PFU.

Patrascu et al.[16] observed, in inoculation test of HVT with a small dose of less than 10 PFU, that most of the chicks which showed sure virema three weeks after the vaccination resisted the challenge of MDV but the chicks in which no virema was recognized scarcely showed the protective effect.

In consideration of these results, infection of HVT may be essential to develop the protective effect against Marek's disease but a large inoculation dose may not always be required for it.

Thus immunity can be acquired even with a small does of HVT but, in strict meaning, this is only a phenomenon observed three to four weeks after the inoculation of HVT, and the time when the immunity is acquired is not considered.

Naturally, vaccine which makes immunity developed earlier is a vaccine of good quality. In consideration of this point, the author followed up the investigation on the time when immnuity is acquired.

2) Time of appearance of protective effect

One-day old chicks were vaccinated with a dose of 75 or 17,300 PFU of cell-associated HVT at one day, one week, two weeks, three weeks and four weeks before the simultaneous challenge.

As shown in Table 5, when vaccinated with a small dose of HVT 75 PFU, the chicks vaccinated until two weeks before the challenge showed a little protective effect, and nearly

Table 5. Appearance of protective effect in chicks vaccinated with herpesvirus of turkeys (HVT) at one day of age

Trial No.	Dose of HVT (PF/Ubird)	Vaccination status	Time of challenge after vaccination	Number of birds tested	MD positive		Protection rate (%)
					No. of birds	%	
1	75	Uacc.	1 day	29	23	79.3	5
		Unvacc.		30	25	83.3	
		Uacc.	1 week	26	23	88.5	−50
		Unvacc.		22	13	59.1	
		Vacc.	2 weeks	33	16	48.5	15
		Unvacc.		30	17	56.7	
		Vacc.	3 weeks	30	8	26.7	43
		Unvacc.		32	15	46.9	
		Vacc.	4 weeks	29	3	10.3*	71
		Unvacc.		28	10	35.7	
2	17,300	Vacc.	1 week	30	3	10.0**	83
		Unvacc.		29	17	58.6	
		Vacc.	2 weeks	30	4	13.3**	82
		Unvacc.		28	21	75.0	
		Vacc.	3 weeks	31	3	9.7**	83
		Unvacc.		30	17	56.7	
		Vacc.	4 weeks	31	2	6.5*	79
		Uuvacc.		29	9	31.0	

For remarks see footnote of Table 4

complete protective effect was recognized in the chicks vaccinated four weeks before the challenge.

On the contrary, when vaccinated with a large dose of 17,300 PFU, sufficient protective effect appeared even in the chicks vaccinated one week before the challenge.

Thus the appearing time of proctective effect seems to depend on the dose of HVT, but how many days are necessary for the appearance of protective effect in the chicks inoculated with the practical dose of vaccine, then? Though it may vary, according to the conditions such as the dose of challenge virus, virulence, infection route and the maternal antibody titers of chicks, it is presumed to be one or two weeks because of the results described above and in Table 9 in which sufficient protective effect appeared ten days after the inoculation with HVT 5,000 PFU dose.

In any event, attention must be paid not to cause any small loss of HVT to be inoculated because the less the dose of virus, the later the appearance of effect.

Effect of maternal immunity on development of Marek's disease and protective ability of vaccine[23)]

According to the wide spread of the application of HVT vaccine, most of the breeding hens have acquired immunity against HVT and the chicks produced from such hens inherit maternal antibody.

Then the author experimented principally on the influence given to the protetcive effect caused in such chicks by the inoculation of HVT vaccine, especially by that of cell-free virus.

The material chicks were the progeny produced by four parent stocks selected from the birds used in the former experiment on the relationship between the inoculation dose of HVT and the protective effect; namely, the four parent stocks, as shown in Table 6, were the ones inoculated with HVT and MDV, the one inoculated with only HVT virus, the one inoculated with only MDV virus and the uninfected control parent stock.

The antibody titers on different parent

Table 6. Infection Status of Parent Stocks

Parent stock	Infective history				
	HVT (FC 126)			MDV (V-1)	
	Viral material	Dose (PFU/bird)	Age of inoculation	Viral material	Age of inoculation
HVT-MDV	Cell-associated	17,300	1 day	Infected blood	4 weeks
HVT	Cell-associated	17,300	1 day	Not done	
MDV	Not done			Infected blood	4 weeks
Uninfected control	Not done			Not done	

1) All viral materials were inoculated through intramuscular route
2) Breeding hens were inseminated artificially using male (6BR line) from Okayama Poultry Experimental Station
3) Fertilize eggs were collected during 30–35 weeks of age

Table 7. Antibody titers of different parent stocks before collection of fertilize eggs

Parent stock	Number of birds tested	Antigen	Agar gel precipitin titers (geometric mean)	Neutralizing antibody titers (geometric mean)
HVT-MDV	12	HVT MDT	2.4 1.9	52.6
HVT	15	HVT MDT	3.3 0.6	220.0
MDV	13	HVT MDT	1.2 1.5	5.9
Uninfected control	16	HVT MDV	<0.5 <0.5	3.5

1) Antibody titers were estimated at 26 weeks of age by the serum dilution method
2) Agar gel precipitin test was performed using the concentration of infected chick kidney cell culture fluid as an antigen
3) Neutralization test conducted by the 50 percent plaque reduction method on chick embryo cell culture Antigen was prepared from HVT-infected chick embryo cells disrupted urtrasonically

stocks before the collection of fertilize eggs shown in Table 7 suggested HVT stock was not infected with MDV and uninfected control stock was not infected with HVT and MDV. Therefore, this material design seemed proper for experiment.

Chicks were vaccinated at one day of age with cell-free or cell-associated virus of HVT in a dose of 5,000 PFU per bird, and were challenged at 10 days of age with infected blood of MDV. The vaccination and challenge were performed through intramuscular inoculation.

Another group of chicks was taken out from HVT-MDV stock and was challenged at 21 days of age to examine the remaining maternal immunity.

Unvaccinated challenge control groups which correspond to HVT inoculation groups were set. Morevore, challenge groups at three days of age were set in chicks derived from the stocks to examine the influence of maternal immunity on the development of Marek's disease immediately after hathcing.

1) Influence of infective history of parent stocks on the susceptibility of their progeny to Marek's disease

Table 8 shows the results which may be summarized as follows:

(1) Chicks (HVT-MDV group and MDV group) derived from the parent stock which has infective history of MDV showed resistibility against Marek's disease at three days

Table 8. Influence of infective history of parent stocks on the susceptibility of their progeny to Marek's disease (MD)

Source of progeny	Incidence of MD in chicks challenged at 3 days			10 days			21 days		
	No. of birds tested	MD positive		No. of birds tested	MD positive		No. of birds tested	MD positive	
		No. of birds	(%)		No. of birds	(%)		No. of birds	(%)
HVT-MDV	30	8	26.7**	27	5	18.5*	27	7	25.9
HVT	23	16	69.6	22	10	45.4			
MDV	29	13	44.8*	25	11	44.0			
Uninfected control	11	9	81.8	15	9	60.0	22	11	50.0

1) All chickens were challenged intramuscularly with JM strain-infective blood of MDV
2) Observation periods after challenge were 23 weeks in groups challenged at 3 and 10 days of age, and 21 weeks in group challenged at 21 days of age
3) Birds which died and survivors at the end of observation periods with lymphomatous gross lesions were considered to be MD positive
4) For significance of difference see footnote of Table 4

of age. The HVT-MDV group showed stronger resistibility than the MDV group. But the resistibility of the MDV group declined at 10 days of age and that of HVT-MDV group did at 21 days of age, and their differences of positive rate of Marek's disease from that of control group (derived from uninfected stock) could not be regarded as a significant value.

(2) No resistibiilty against Marek's disease was found in any age of the group of chicks (HVT group) derived from the parent stock which possesses infective history of HVT only.

Many researchers recognized the resistance against Marek's disease in the chicks derived from the parent stock which were infected with MDV, as shown in Table 10, and the author also obtained the same result. But Eidson et al.[12] reported, contrary to my results, that the chicks derived from the HVT infected parent stock restrained the development of Marek's disease more effectively than the chicks derived from the MDV infected parent stock. This might be caused by the different degree of maternal immunity because the parent stock used by Eidson was inoculated three times with HVT while my stock was inoculated only once.

2) Influence of infective history of parent stocks on the protective effect of turkey herpesvirus vaccines in progeny

Table 9 shows the results which are summarized as follows:

(1) The control group and MDV group of chicks vaccinated with cell-free or associated HVT showed sufficient protective ability at 10 days of age.

(2) Restraint of the effect of HVT vaccine was recognized in the HVT group, especially remarkably in the group vaccinated with cell-free virus.

(3) Vaccination effect of both types of HVT virus was restrained in the HVT-MDV group against the challenge at 10 days of age. The effect was improved in some degree at 21 days of age though cell-associated virus was not tested in this case.

In general, the effect of vaccine by cell-free HVT is restrained in the group of chicks derived from the parent stock which possesses HVT infective history. But, sometimes, opposite data are also reported (Table 10). In such cases, the time of challenge was late, that is, three or seven weeks after the inoculation with HVT, resulting in the obscurity of appearance status of vaccination effect.

The negative data, therefore, cannot be compared directly with my results.

Table 9. Influence of infective history of parent stocks on the protective effect of turkey herpesvirus (HVT) vaccines in progeny

Source of progeny	Age challenged	Cell-free HVT				Cell-associated HVT				Unvaccinated control		
		No. of birds tested	MD positive		Protection rate	No. of birds tested	MD positive		Protection rate	No. of birds tested	MD positive	
			No. of birds	(%)			No. of birds	(%)			No. of birds	(%)
HVT-MDV	21 days	30	4	13.3	48.6					27	7	25.9
	10 days	29	10	34.5**	−86.5	30	4	13.3	28.1	27	5	18.5*
HVT	10 days	30	8	26.7**	41.2	29	6	20.7	54.4	22	10	45.4
MDV	10 days	30	1	3.3	92.5	30	2	6.7	84.8	25	11	44.0
Uninfected control	21 days	28	3	10.0	78.6					22	11	50.0
	10 days	25	0	0	100.0	26	3	11.5	80.8	15	9	60.0

1) 5,000 PFU of HVT per bird were inoculated intramuscularly at one day of age
2) Protection rate was calculated in comparison with unvaccinated controls
3) For other remarks see footnotes of Table 8

Table 10. References concerning effects of the maternal immunity on Marek's disease virus (MDV) and herpesvirus of turkey (HVT) infection

Maternal immunity	Virus inoculated into progeny		Inhibition by maternal immunity (Researchers)	
			Positive result	Negative result
Antibody to MDV	MDV		Ball et al.[2,3] Burgoyne & Witter[4] Calnek[5] Chubb & Churchill[7] Jakowski et al.[13] Payne & Rennie[17] Spencer & Robertson[19] Yoshida et al.[23]	Eidson et al.[12]
	HVT	Cell-associated		Eidson et al.[12] Yoshida et al.[23] Zygraich & Huygelen[27]
		Cell-free		Patrascu et al.[16] Yoshida et al.[23] Zygraich & Huygelen[27]
Antibody to HVT	MDV		Eidson et al.[12]	Yoshida et al.[23]
	HVT	Cell-associated		Eidson et al.[11,12]
		Cell-free	Calnek & Smith[6] Churchill et al.[8] Eidson et al.[11] Patrascu et al.[16] Yoshida et al.[23]	Kilgore & Brokken[14] Zygraich & Huygelen[26]
Antibody to HVT & MDV	MDV		Yoshida et al.[23]	
	HVT	Cell-associated	Yoshida et al.[23]	
		Cell-free	Churchill et al.[8] Yoshida et al.[23]	

The protective effect by HVT could be recognized when the time of challenge was delayed in the chicks which had maternal antibody against HVT. This may be related to later rising of recovery rate of HVT from these chicks compared to ones which had no antibody[8),21)].

Though resistance against MDV and restraint of the effect of vaccine were recognized strongly and continuously in the chicks derived

from the parent stocks which possessed HVT-MDV infective history, it is not clear whether super-infection in the parent stock has some meaning in itself or not. Most of the chicks in the field might have been derived from the parent stocks of such super-infection, therefore, the degree of their maternal immunity must be examined.

Even if the effect of HVT vaccine is restrained by the maternal immunity, actual outbreak of the disease caused by insufficient effect of vaccine may be seldom under the good sanitary management because the effect of vaccine could appear later and the resistance against Marek's disease might exist too.

It may be a most practical method to make the sufficient development of protective effect of HVT in commercial chicks that attenuated MDV vaccine would be inoculated to the parent stock instead of HVT vaccine. This point should be investigated further.

Relationship between age and appearance of lesions of Marek's disease[24)]

Comparative data on positive rate of Marek's disease of the control group of chicks uninfected with HVT were obtained in the course of challenge tests performed at various days of ages to know the appearing time of the protective effect described above. Table 11 shows the results.

In the comparison with the positive rate of Marek's disease in chicks challenged at four weeks of age, the significance of difference was recognized in chicks challenged at one day of age in trial 1, and in chicks challenged at one, two and three weeks of age in trial 2.

Consequently, it was manifested that older chicks were less susceptible to Marek's disease than younger ones.

As the chicks used in this experiment possessed the maternal antibody which is capable of inhibiting the development of Marek's disease at younger age, the susceptibility of the chicks without maternal antibody may be different among ages.

The susceptibility may differ by breed and stock, as hereditary properties influence the development of Marek's disease. But resistance seems to increase according to the growth of chicks in many case[1),20),24)].

Therefore, it seems important, from the viewpoint of disease control, to delay the time of MDV invasion as far as possible.

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Table 11. Age susceptibility of chicks to Marek's disease (MD)

Age when MD virus was inoculated	Trial 1		Trial 2	
	Number of birds tested	MD (%)	Number of birds tested	MD (%)
1 day	30	83.3**		
1 week	22	59.1	29	58.6*
2 weeks	30	56.7	28	75.0**
3 weeks	32	46.9	30	56.7*
4 weeks	28	35.7	29	31.0

1) Chicks were derived from dams having antibody to MD virus.
2) Significance of difference was calculated by the chi-square test in comparison with chicks inoculated at 4 weeks of age
3) For other remarks see footnote of Table 4.

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