PRESENT STATUS OF THE PRACTICAL RESEARCHES ON MAREK'S DISEASE IN JAPAN

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Prevalence of Marek's disease in Japan had begun to increase significantly in the latter half of the nineteen-sixties.

Though the causal agent of Marek's disease had not been known for a long time, it was identified rapidly after the isolation of a herpesvirus from affected chickens by Churchill & Biggs[2] in 1967. In Japan, Yuasa *et al.*[22] also isolated the same herpes-type virus from affected chickens in 1969, and regarded it as the causal organism of this disease judging from its properties.

Developmental researches on vaccine have also been carried out actively resulting in the marketing of inactivated Marek's disease virus (MDV) vaccine in 1971, and attenuated MDV and herpesvirus of turkey (HVT) vaccines in 1972. Now the cell-associated HVT vaccine is used mostly.

Since the dissemination of the vaccine, the outbreaks of Marek's disease have decreased remarkably and only a few occasional and regional outbreaks have been reported lately.

In this paper, the trend of outbreaks, and research works on diagnosis and prevention during the last 10 years in Japan are described.

Outbreaks of Marek's disease in Japan

1 Status before application of Marek's disease vaccine

Striking increase in culling rate had been observed in Toyama in 1966, in Shizuoka in 1967 and in Hiroshima in 1968 according to the data of investigations which were carried out on a national scale by the Animal Hygiene Service Stations[7]. Consequently, it was considered that the occurrences of Marek's disease which was regarded as the principal cause of the rapid increase in culling rate took place during the period 1967 - 68 in Japan.

In those days, lymphomatosis including lymphoid leukosis had been recognized at the rate of 12.2 - 16.5% in 54 chicken flocks in 34 poultry farms located in 24 prefectures according to the results of investigations conducted by Horiuchi *et al.*[5] (Table 1).

The incidence of lymphomatosis increased rapidly after 90 days of age, reached a peak in birds 120 - 150 days of age and thereafter decreased gradually[5] (Fig. 1).

Table 1 Incidence of lymphomatosis during each experimental period
(Horiuchi et al. 1972)

Age of chickens investigated (in days)	No. of flocks	No. of chickens	Death and discard during invest. period (%)	
			Total	Lymphomatosis
61–240	54	24,048	18.7 (0.75–50.4)	13.4 (0.25–44.6)
61–300	29	15,605	18.9 (0.75–45.4)	13.8 (0.25–40.8)
61–360	16	9,960	23.2 (2.9–46.5)	16.5 (3.1–41.4)

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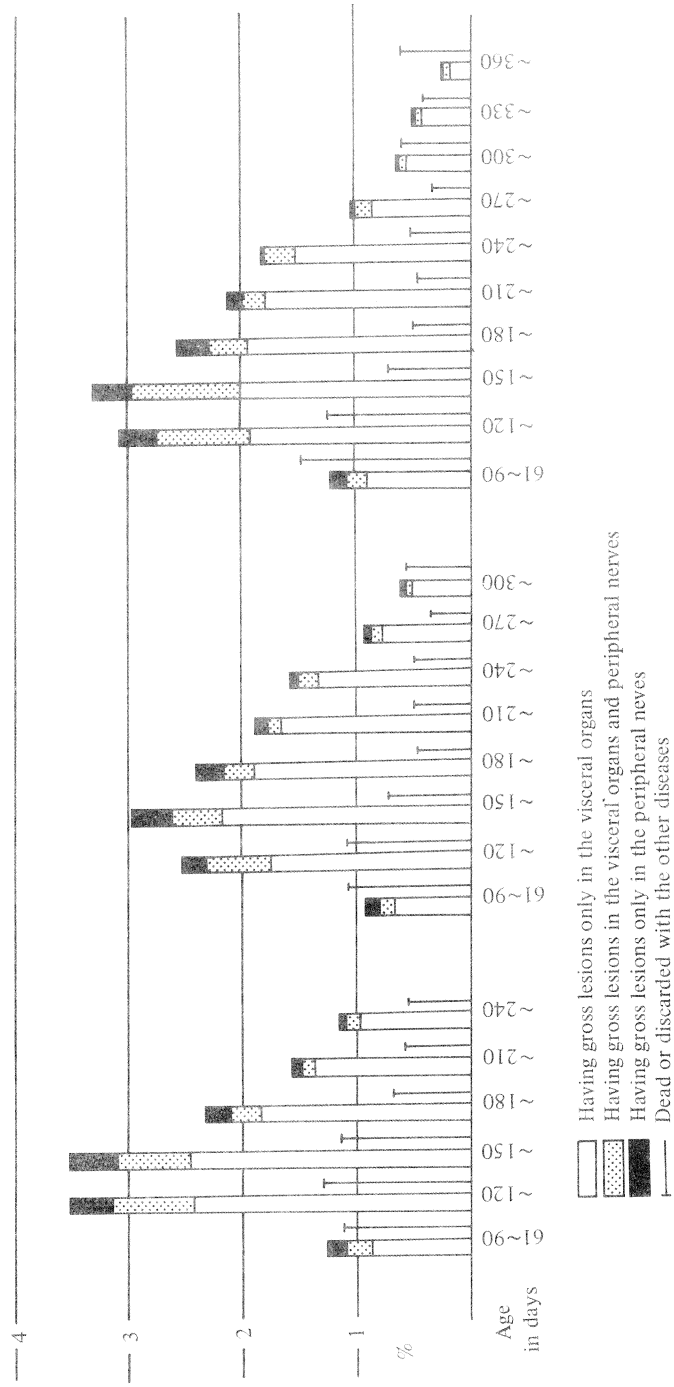


Fig. 1 Mortality from lymphomatosis in 3 age groups *(Horiuchi et al., 1972)*

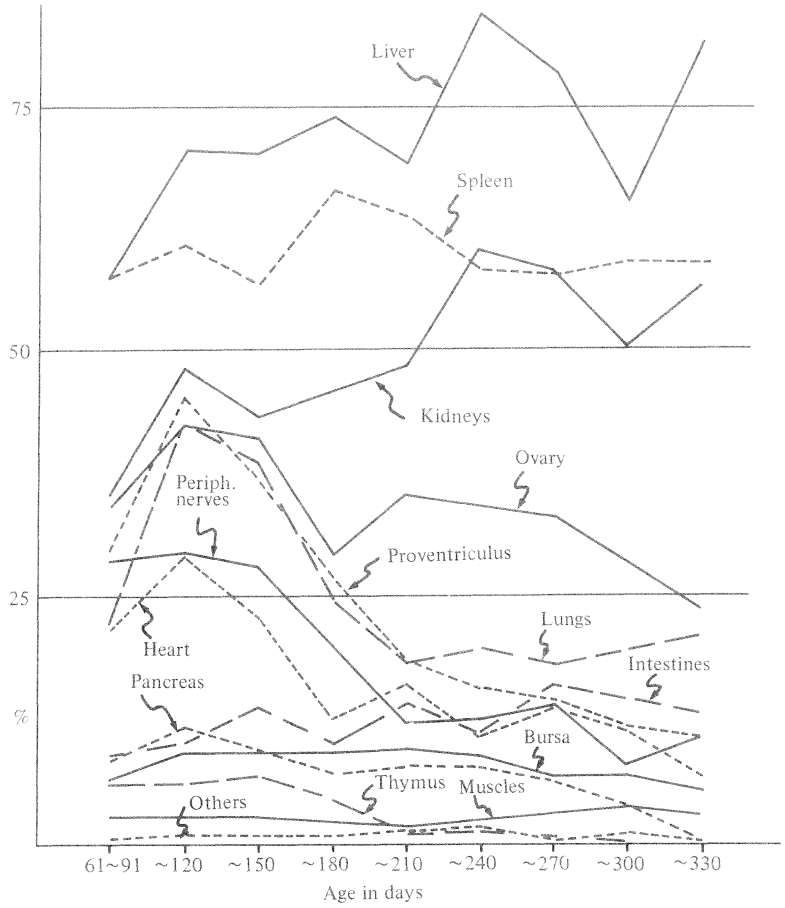


Fig. 2 Appearance of gross lesions in each organ according to age (*Horiuchi et al.* 1972)

Appearance of the lymphomatous lesions was particularly frequent in the liver, spleen and kidney, less frequent in the ovary, lung, proventriculus and peripheral nerves, and uncommon in the bursa of Fabricius[5] (Fig. 2).

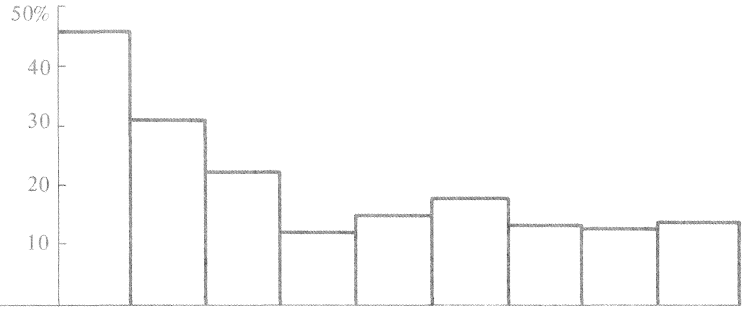
Remarkable low titer of hemagglutination-inhibition antibodies against Newcastle disease virus was observed in the chicken flocks having visceral lesions of Marek's disease compared with normal flocks[11]. It is suggested that the immunosuppressive responses had occurred in the chickens affected with Marek's disease.

Although the time since MDV has been present in Japan is not precisely known, it is presumed that MDV infection among chickens had already existed in the nineteen-fifties, since the agar gel precipitating antibodies against MDV were detected in the chicken sera collected from Hokkaido in 1958[23].

2 Status after application of Marek's disease vaccine

Since Marek's disease vaccines have been applied widely in the field, the systematic investigation of incidence of the disease has not been carried out. Yoshimura[16], however, showed the recent trend of the incidence of Marek's disease from the results of daily diagnosis at the Institute

for Field Crops and Livestock Hygiene, Aichi Prefectural Federation of Agricultural Cooperation. According to their data, after application of the vaccine, the number of chickens considered to be affected with Marek's disease among chickens submitted to their laboratory as materials for diagnosis has decreased remarkably (Fig. 3). Chickens showing leg paralysis, however, are still



Year		1970*	1971	1972	1973	1974	1975	1976	1977	1978
No. of chickens examined		420	623	446	328	370	317	242	360	405
MD positive	No. of birds	192	195	102	41	57	59	34	48	59
	%	45.7	31.3	22.9	12.5	15.4	18.6	14.0	13.3	14.6

* From May to Dec.

Fig. 3 Incidence of Marek's disease according to yearly diagnosis (*Goda et al.* 1979)

Table 2 Appearance of lesions in each organ of chickens affected with Marek's disease (*Goda et al., 1979*)

Year	No. of chickens examined	No. of chickens having lesions in each organ						
		Peripheral nerves	Proventriculus	Liver	Spleen	Kidney	Ovary	Others
1972*	41	16 (39.0%)	19 (46.3)	10 (24.4)	7 (17.1)	4 (9.8)	6 (14.6)	6 (14.6)
1973	47	39 (83.0)	16 (34.0)	8 (17.0)	9 (19.1)	6 (12.8)	3 (6.4)	2 (4.3)
1974	83	68 (81.9)	30 (36.1)	10 (12.0)	9 (10.8)	4 (4.8)	7 (8.4)	1 (1.2)
1975	92	85 (92.4)	9 (9.8)	2 (2.2)	1 (1.1)	0	0	0
1976	50	44 (88.0)	17 (34.0)	7 (14.0)	10 (20.0)	4 (8.0)	3 (6.0)	4 (8.0)
1977	69	50 (72.5)	40 (58.0)	18 (26.1)	13 (18.8)	8 (11.6)	10 (14.5)	5 (7.2)
1978	118	110 (93.2)	53 (44.9)	33 (28.0)	33 (28.0)	8 (6.8)	17 (14.4)	14 (11.9)

* From Aug. to Dec.

being observed from time to time in the field. Usually, the frequency of the incidence is below 1%, but sometimes it reaches 2 - 5%. Since 1973, the age of incidence of Marek's disease became lower and the peak of incidence has been observed at about 80 - 100 days of age during the past 5 years (Fig. 4). Frequency of the appearance of lesions in peripheral nerves became higher than that in visceral organs (Table 2).

Fig. 4 Age of appearance of Marek's disease according to yearly diagnosis
(Goda et al., 1979)

Age in days	1972*	1973	1974	1975	1976	1977	1978
–28							00
35			0	0	0		00
42		00	0000	0000000	000	0	0000
49	0	0	0	00	00	0	0000
56		0		000000	00	0	000
63			000	0000000	000000	0	0000
70	0	0	000	0	0	00	00
77		0	0000	0000		00	000
84			000	0000		0000	000
91	0		0000	0000	00	00	0
98	0	00	000	00000	0	000	00
105			0	0	0	00	0
112	0	0	0				00
119			0	0	00	000	000
126	0	0000		00000	00	0	00
133	000	00	0	0	00	0	0
140		0	0		00		00
147		0	0			00	0
154	0	000	0			0	000
161	000	0		0	0	00	
168		00					0
175	0	0	0		0		0
182	00	00				0	000
189							0
196	0			0	0	0	
203		0			0	0	
210	00						
217							
224					0		
231							
238							0
245							0
Average	144.1	126.0	86.9	84.5	100.0	109.2	100.8

* From Aug. to Dec.
0: Indicate 1 case

Differential diagnosis of Marek's disease

Virus isolation or antibody detection is not a conclusive evidence for diagnosis of Marek's disease due to latent infection prevalent among chickens. Consequently, differential diagnosis of Marek's disease from lymphoid leukosis induced by avian leukosis virus is mainly based on histological study of these lymphomas.

Marek's disease tumor-associated surface antigen (MATSA) was first demonstrated by Witter *et al.*[15] using the indirect membrane immunofluorescence technique on three classes of Marek's disease tumor cells. Matsuda *et al.*[9] found MATSA on most cells of four Marek's disease lymphoma cell lines as well as on a certain number of cells of Marek's disease lymphomas. However, they failed to demonstrate MATSA on line cells derived from avian leukosis virus-induced lymphoma[4], MDV infected chick embryo fibroblasts or normal thymic, bursal and splenic lymphoid cells. Thus MATSA seems specific for MDV-transformed cells.

Sato *et al.*[12,13] found that demonstration of MATSA-positive cells in lymphoma tissues was the most specific and simple technique for differential diagnosis of Marek's disease from lymphoid leukosis in field work. Furthermore, they found that the detection of MATSA-positive cells in the peripheral blood of chickens with Marek's disease lymphomas was a useful means to diagnose Marek's disease in living chickens (Tables 3 & 4).

Table 3 **Relationship between Marek's disease tumor-associated surface antigen (MATSA), positive lymphomas and histopathological diagnosis in the chicken.**

(Sato et al., 1978)

Histopathological diagnosis	No. of cases	MATSA	
		Positive	Negative
MD*	4	4	0
MD**	7	7	0
LL**	14	0	14
Not certain MD or LL	2	1	1

* Experimental cases
** Field cases

Table 4 **Incidence of chickens with MATSA positive cells in the peripheral blood in chickens experimentally infected with MDV and naturally infected with MDV or ALV**

(Sato et al., 1976)

Case	No. of chickens examined	No. of chickens with	No. of chickens with MATSA positive cells in peripheral blood
MDV-infected*	10	MD lymphoma 8	4
		no lymphoma 2	1
Control*	10	no lymphoma 10	0
MD**	26	MD lymphoma 26	22
LL**	5	LL lymphoma 5	0
Control**	6	no lymphoma 6	0

* Experimental cases
** Naturally infected with MDV or ALV

Method for detection of MATSA in the peripheral blood:

MATSA was demonstrated by the direct fluorescent antibody technique using chicken anti-MSB-1 cell (MDV-induced lymphoma cell line) sera conjugated with fluorescein-isothiocyanate. Samples of 2 ml of blood from the wing vein of chickens were heparinized and centrifuged at 2,000 rpm for 5 min, and the buffy coat obtained was put into 2 ml of cold Earle's solution. These white cells were washed 3 times with Earle's solution, and then suspended in Earle's solution at a density of about 10^7 cells/0.05 ml. The cell suspension was mixed with an equal volume of conjugated anti-MATSA antibody and kept at 37°C for 30 min with occasional stirring. Then the cells were washed 3 times with Earle's solution. A drop of mounting medium (90% glycerol and 10% carbonate-bicarbonate buffer, pH 9.5) was added to the packed cells, and a drop of the suspension was placed on a microscopic slide, covered with a coverslip, and observed with a fluorescence microscope.

Problems of prevention

1 Isolated rearing and environmental sanitation[19]

Relationship between age and appearance of lesions of Marek's disease was examined by inoculation with MDV at various ages, as shown in Table 5.

Table 5 Age susceptibility of chicks to Marek's disease (MD)

(Yoshida et al. 1975)

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 7

In the comparison with the positive rate of Marek's disease in chicks challenged at 4 weeks of age, the significance of difference was recognized in chicks challenged at 1 day of age in trial 1, and in chicks challenged at 1, 2 and 3 weeks of age in trial 2.

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

The susceptibility may vary with 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 cases.

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

2 Prevention by vaccination

1) Degree of protective effect against Marek's disease by vaccination with a herpesvirus of turkey[17]

Results of field tests carried out at the time of maximum prevalence of Marek's disease when HVT vaccine was not yet available in the Japanese market are summarized in Table 6. This table was made on the basis of data supplied by 8 private laboratories.

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

Therefore, the outbreak of Marek's disease cannot always be prevented completely even in the flocks inoculated with HVT vaccine, and a number of inoculated birds corresponding to 20 to 30% of the affected number of unvaccinated control flocks could not be freed from the disease. For example, under the conditions where Marek's disease can break out in 10% of unvaccinated birds, 2 to 3% of vaccinated birds may be affected. From this viewpoint, sanitation and isolation of young chicks should be carried out strictly to control properly Marek's disease even if they are vaccinated.

Table 6 Degree of protective effect against Marek's disease (MD) by vaccination with herpesvirus of turkey (HVT)

(Yoshida et al., 1974)

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 supplied by 8 laboratories.
2) Observations were performed until 21 weeks of age in all tests.

2) Dose of herpesvirus of turkey required for the appearance of protective effect[18]

The relation between the inoculation dose of HVT and the protective effect was examined with chicks inoculated at 1 day of age with cell-associated HVT in 6 grade-doses ranging from 7.5 to 17,300 PFU per bird and challenged 4 weeks after vaccination.

As shown in Table 7, even such a small dose of virus as 7.5 PFU afforded protection rate as much as a dose of 1,700 PFU, which can be regarded as the standard dose of vaccine. Moreover, a larger dose such as 17,000 PFU could not increase the protection rate further.

On the basis of this result and other reports[1,10], infection of HVT may be essential to develop the protective effect against Marek's disease while a large inoculation dose may not always be required for it.

Thus although immunity can be acquired even with a small dose of HVT, this phenomenon is observed only 4 weeks after the inoculation of HVT, and the time when the immunity is acquired is not considered.

Naturally, vaccine which causes immunity to develop earlier is a vaccine of good quality. Under such circumstances, we investigated the time when immunity is acquired.

Table 7 Protection of chicks against Marek's disease (MD) by vaccination with graded doses of herpesvirus of turkey (HVT)

(Yoshida et al., 1973)

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 challenged 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
   * $P < 0.05$
   ** $P < 0.01$

3) Time of appearance of protective effect[18]

One-day old chicks were vaccinated with a dose of 75 or 17,300 PFU of cell-associated HVT at various weeks before the simultaneous challenge.

As shown in Table 8, when a small dose of 75 PFU was administered, the chicks vaccinated until 2 weeks before the challenge showed a weak protective effect, whereas nearly complete protection was recognized in the chicks vaccinated 4 weeks before the challenge.

On the contrary, when a large dose of 17,300 PFU was administered, sufficient protective effect appeared even in the chicks vaccinated 1 week before the challenge.

Thus the time when the protective effect appears seems to depend on the dose of HVT, but it remains to be known how many days are necessary for the appearance of protective effect in the chicks inoculated with the standard dose of vaccine. Though it may vary, according to conditions such as the dose of challenge virus, virulence, infection route and the maternal antibody titers of chicks, Fujikawa et al.[3] reported that sufficient protective effect appeared 10 days after the inoculation of a 1,500 PFU dose of HVT.

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

4) Effect of maternal immunity on development of Marek's disease and protective ability of vaccine[19]

Following widespread application of HVT vaccine, most of the breeding hens have acquired immunity against HVT and the chicks produced from such hens appear to inherit maternal antibodies. Thereafter, the effect of maternal immunity on development of Marek's disease and protective ability of vaccine were evaluated.

The chicks used were the progeny produced by 4 parent stocks selected from the birds used in

the former experiment; namely, the parent stocks, as shown in Table 9, were the ones inoculated with HVT and MDV, those inoculated with only HVT, and those inoculated with only MDV as well as the uninfected control parent stock.

Table 8 Appearance of protective effect in chicks vaccinated with herpesvirus of turkey (HVT) at one day of age

(Yoshida et al., 1973)

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	Vacc.	1 day	29	23	79.3	5
		Unvacc.		30	25	83.3	
		Vacc.	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
		Unvacc.		29	9	31.0	

For remarks see footnote of Table 7.

Table 9 Infection status of parent stocks

(Yoshida et al., 1975)

Parent stock	Infection history				
	HVT (FC 126)			MDV (V-1)	
	Viral material	Dose (PFU/bird)	Age at inoculation	Viral material	Age at 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) Fertilized eggs were collected at 30-35 weeks of age.

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

Table 10 shows the results which may be summarized as follows: Chicks (HVT-MDV group and MDV group) derived from the parent stock which had a history of post infection with MDV showed resistance against Marek's disease at 3 days of age and the HVT-MDV group showed stronger resistance than the MDV group. However the resistance of the MDV group declined at 10 days of age and that of HVT-MDV group at 21 days of age, and the differences of positive rate of Marek's disease between these and that of control group (derived from uninfected stock) could not be regarded as significant.

Resistance against Marek's disease was not found regardless of the age in the group of chicks (HVT group) derived from the parent stock with a history of infection with HVT only.

Many other researchers have also observed the resistance against Marek's disease in the chicks derived from the parent stock which had been infected with MDV.

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

Table 11 shows the results which are summarized as follows: The control group and MDV group of chicks vaccinated with cell-free or associated HVT showed sufficient protective ability at 10 days of age.

Limitation of the effect of HVT vaccine was recognized in the HVT group, and was particularly striking in the group vaccinated with cell-free virus.

Vaccination effect of both types of HVT virus was attenuated in the HVT-MDV group against the challenge at 10 days of age. The effect was improved to some extent 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 decreased in the chicks derived from the parent stock with a history of infection with HVT.

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

Table 10 Influence of infection history of parent stocks on the susceptibility of their progeny to Marek's disease (MD)

(Yoshida et al., 1975)

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 7.

Table 11 Influence of infection history of parent stocks on the protective effect of turkey herpesvirus (HVT) vaccines in progeny

(Yoshida et al., 1975)

Source of progeny	Cell-free HVT					Cell-associated HVT				Unvaccinated control		
	Age challenged	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 10.

5) Accident caused by Marek's disease vaccine contaminated with a reticuloendotheliosis virus (REV)

During the period from spring to fall of 1974, in various parts of Japan, a disease characterized by delayed growth, anemia, abnormal feathers, and leg paralysis as main symptoms broke out in flocks of chickens inoculated with Marek's disease vaccine.

The disease was found to have been caused by REV, which contaminated the vaccine, since REV could be isolated from the affected chickens and from the vaccine lot, and a similar disease could be produced in chickens inoculated with the isolated virus and the vaccine lot[8,24] (Tables 12, 13 & 14).

The virus persisted in the body for a long time and induced horizontal infection[24]. A vertical transmission was also recognized at low rate[14] (Table 15). It was presumed that cells used for the preparation of the vaccine might have been contaminated.

In the chickens inoculated with REV at the neonatal stage, immune response against infection with other viruses was inhibited, resulting in subsequent enhancement of the infection[20,21] (Table 16).

The direct damage to chicken flocks by infection with the isolated REV was not so striking, unless a large dose of the virus was inoculated artificially into baby chicks. Further studies, however, are needed to clarify the aspect of infection with REV combined with other factors, and the influence of infection upon immunosuppression.

Recently, a similar accident caused by Marek's disease vaccine has also been reported in Australia[6].

Table 12 Results of virus isolation and antibody test with field materials

(Yuasa, Yoshida & Taniguchi, 1976)

Material*1 No.	Prefecture in which materials were collected	Date of collection of material (1974)	Chicks examined			Virus isolation								Antibody against*6			Marek's disease vaccine used
			Age in days	Leg paralysis	Abnormality of peripheral nerves	MDV*2	HVT*3	REV*4				Other*5		MDV	HVT	REV	
								Li	Sp	BF	Bl	Li	Sp				
1	Aichi	4.27	45	7/ 9	9/ 9	–	+	•	•	•	•	•	•	0/ 9	0/ 9	0/ 9	A
2	Gifu	5.2	35	9/13	12/13	–	+	•	•	•	1/1	•	•	0/13	0/13	0/13	A
3	Gifu	5.18	78	0/ 2	•	–	+	0/ 2	0/ 2	•	•	1/ 2	0/ 2	0/ 2	2/ 2	1/ 2	A
4	Kyoto	5.24	58	7/ 7	7/ 7	–	+	2/ 2	1/ 2	1/2	•	0/ 2	0/ 2	0/ 7	0/ 7	0/ 7	A
5	Gifu	5.29	60	6/11	7/11	•	•	9/11	8/11	•	•	1/11	0/11	0/11	0/11	0/11	A
6	Gifu	6.12	77	6/ 8	7/ 8	•	•	•	•	•	•	•	•	0/16	2/16	2/16	A
7	Akita-1	9.8	45	4/11	6/11	•	•	3/ 9	•	•	•	0/ 9	•	0/ 9	0/ 9	0/ 9	B
8	Akita-2	9.8	60	0/ 5	0/ 5	•	•	1/ 5	•	•	•	0/ 5	•	0/ 5	0/ 5	1/ 5	B

MDV: Marek's disease virus, HVT: Herpesvirus of turkey, REV: Reticuloendotheliosis virus.

Li: Liver, Sp: Spleen, BF: Bursa of Fabricius, Bl: Blood

*1 Nos. 2, 3, 5, and 6 were derived from the same poultry farm and Nos. 2, 5, and 6 from the same flock.

*2 Materials from two chicks were pooled into one sample, which was subjected to direct kidney culture.

*3 Material was inoculated into fibroblasts of SPF chick embryos and subjected to detection of virus by the direct fluorescent antibody method with fluorescent antibody against REV-T strain.

*4 Material was inoculated into chicken kidney cell cultures. All the isolates were avian adenovirus.

*5 Agar gel precipitation test.

In each fraction, the numerator indicates the number of positive materials and the denominator that of materials examined.

•: Not examined

Table 13 Results of isolation of virus from vaccine
(Yuasa, Yoshida & Taniguchi, 1976)

Vaccine	Number of vials examined	Number of vials positive for virus isolation*1
A	10	6
B	3	3

*1 Material was inoculated into the yolk sac of 6-day-old SPF chick embryos. Liver emulsion originated from embryos immediately before hatching was inoculated into fibroblasts of chick embryos. The culture inoculated was examined for antigen with fluorescent antibody against REV-T strain.

Table 14 Inoculation test on chicks with isolated virus

(Yuasa, Yoshida & Taniguchi, 1976)

Group No.	Inoculum			Method of inoculation	Number of chicks	Abnormality of feathers (30 days of age)	Death (up to 65 days of age)	Abnormality of legs (up to 65 days of age)	Average body weight g (65 days of age)
	Virus strain	Passage	Virus dose ($TCID_{50}$/capita)						
1	DE	Chick*1 embryo liver-CEF2	$10^{4.5}$	Intraperitoneal	17	17	0	1	528.1**
				Contact in the same cage	19	0	0	0	879.5n.s.
2	CE	Chick embryo liver-CEF2	$10^{4.5}$	Intraperitoneal	19	19	0	6	725.7**
				Contact in the same cage	18	1	1	1	921.1n.s.
3	T	Chick liver-CEF2	$10^{5.5}$	Intraperitoneal	14	13	1	7	540.0**
				Contact in the same cage	18	2	0	1	975.9n.s.
4	Uninfected CEF			Intraperitoneal	19	0	0	0	943.7
				Contact in the same cage	19	0	0	0	986.8

One-day-old SPE chicks were inoculated with each viral material or placed in the same cage with inoculated chicks. Those chicks inoculated with the same virus strain were kept separately from those inoculated with any other strains and held under observation over a period up to 65 days of age.

The test of significant difference in body weight was carried out on each intraperitoneal inoculation group or contact infection group, as compared with uninfected CEF inoculated group or uninoculated group placed with this group. The difference between two mean values was tested (t-test).

*1 See the footnote of Table 2.
CEF: Chick embryo fibroblast
** $P < 0.01$. n.s.: not significant

Table 15 Virus isolation from CEF prepared from chick embryos derived from hens infected with REV persistently
(Wakabayashi & Kawamura 1975)

Virus isolation	
Number of positive/ Number of attempted	Number of positive/ Number of infected hens
3/98	2/13

Table 16 Periodical observation of immunosuppression estimated by NDV (Bl strain) infection in chickens inoculated with REV at neonatal stage

(Yoshida et al., 1977)

Chicken group	Inoculation		No. of chickens examined	Death*3 (%)	Antibody titer				NDV(Bl) recovery (%)	
	REV*1 Route	NDV*2 Age			2 weeks*4		4 weeks			
					HI	SN	HI	SN	2 weeks	4 weeks
a	IM		10	60	13.0**	−0.1*	38.1**	1.8	14.3	0
b	Contact*5	0 week	10	0	64.0		111.4		0	0
c	–		9	0	94.1	1.5	101.8	3.1	0	0
d	IM		10	20	7.0**		22.6		30.0	0
e	Contact	1 week	10	0	48.3		39.4		0	0
f	–		10	0	64.0		48.3		0	0
g	IM		8	0	12.3		34.8		12.5	0
h	Contact	2 weeks	11	0	46.7		64.0		0	0
i	–		10	0	51.8		64.0		0	0
j	IM		9	0	10.0**		10.0*		0	0
k	Contact	3 weeks	9	0	29.4		18.5		0	0
l	–		10	0	45.4		34.2		0	0
m	IM		8	0	10.8**	0.9	9.6	2.3	12.5	0
n	Contact	4 weeks	8	0	107.5		22.7		0	0
o	–		10	0	68.4	1.8	18.4	3.0	0	0

*1 REV CE strain E-CE$_5$ $10^{4\cdot2}$ TCID$_{50}$/bird ** $p < 0.01$

*2 NDV Bl strain E$_3$ $10^{6\cdot9}$ TCID$_{50}$/bird, orally * $p < 0.05$

*3 Observed up to 4 weeks after inoculation with Bl strain

*4 After inoculation with Bl strain

*5 Placed in the same cage with chickens of the group inoculated from IM route

HI: Hemagglutination inhibition titer, serum dilution method, geometric mean

SN: Serum neutralizing titer, virus dilution method, arithmetic mean

IM: Intramuscular

NDV recovery: From oral swab

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Discussion

Joseph P.G. (Malaysia): 1. Could you elaborate on the Marek's disease tumor-associated surface antigen (MATSA) technique for the differentiation of lymphoid leukosis from Marek's disease? 2. Was the contamination of the herpes virus of turkey (HVT) vaccine with reticulo-endotheliosis virus (REV) only found in the cell-associated HVT vaccine?

Answer: 1. The MATSA technique is described in detail in my paper. Please refer to it. 2. We isolated REV from both HVT vaccines, the cell-associated and the cell-free ones.