

Studies on Immunization against Marek's Disease:  
Development of Herpes Virus of Turkey Vaccine

Hideo Fujikawa

(Chemo-Sero-Therapeutic Research Institute)

Marek's disease (MD) is an avian infectious disease caused by an avian herpes virus. The first description of the disease was shown in 1907 by a Hungarian scientist, Marek who found the evidence, in the pathological studies on avian leukosis, that there was a lymphomatosis with the neural enlargement which was distinguished from other leukosis. From 1950s to 1960s, MD appeared in Europe and the United States of America, but the disease spreads in all over the world at present time. In Japan, MD was recognized in 1963 to 1964, and nowadays the disease is prevalent in all over the country. MD is one of the most principal diseases causing decrease in weight gain and feed conversion or increase in the rate of dead and culled birds.

The chickens exposed to MD virus infection die at 30 to 120 days of age, which has been causing a great deal of economic loss in all areas of intensive poultry production. The discovery of a viral agent closely associated with MD was done by Churchill and Biggs in 1967, and the virus was found to be a highly cell associated herpes virus (MDHV). As soon as MDHV was isolated, studies on the control of MD were started almost simultaneously with the investigation on the properties of MDHV, and three methods of control of MD were available. They were the filtered air positive pressure system of management, selection for genetically controlled resistance and vaccination. Some success in reducing the incidence of MD was obtained by the former two methods, but it was too expensive to put them in practical use in poultry farms, therefore, the development of MD vaccine was strongly desired. In Europe, two types of MD vaccines were developed. One was prepared from an attenuated MDHV and another was from a naturally avirulent MDHV isolated from a normal flock. On the other hand, in the United States of America, an another type of MD vaccine was produced, which was

prepared from a herpes virus of turkey (HVT) being no pathogenic for chickens and related to MDHV in its antigenicity.

All of these vaccines were found to be effective in the field. In Japan, as MD vaccine had not been developed, the author began to study for the purpose of producing the vaccine. At first time, isolation of a virulent MDHV needed as a challenge virus was attempted, and from the birds closely associated with MD, 6 viral agents were isolated. These isolates were identified to be MDHV by the examination of their properties. The epizootiological surveys on MD in Kyushu district were taken by the agar gel precipitation test, from which it was evident that MD had already appeared in the district in 1964. On the other hand, the blood of the turkeys bred in Japan were examined for isolation of HVT, as which is effective in reducing the incidence of MD, and viral agents were isolated from the 2 flocks of the 7 ones examined. Some properties of these isolates were examined, and as there was no difference in their properties, an isolate was selected as a representative strain among them (YT-7). Further investigations on the properties of strain YT-7 were done in detail, and this virus proved to be a HVT. The requirements for developing MD vaccine were studied with the YT-7 strain of HVT. After the fundamental studies, 2 types of HVT vaccines were prepared, namely one was cell-associated HVT and another was cell-free HVT. The safety and immunogenicity for chickens of these vaccines were tested in detail in several poultry farms. Additionally for the purpose of decrease in vaccination times, a Newcastle disease (ND)-MD combined vaccine was developed. From the studies in the laboratory and field, the combined vaccine was considered to be effective for controlling these disease.

It is the intention of this paper to show the summary of the studies on MD vaccines.

#### 1) Isolation of MDHV and it's properties

In August, 1970, Some birds of flock consisted of 120-day-old chickens in a poultry farm in Saga prefecture showed the clinical signs associated with MD, and from the 6 cases of 9 ones examined, viral agents were isolated. A few properties of strain SD-3, which was selected from these isolates as a representative virus, were examined, and the isolate was identified to be a MDHV. The primary cells in culture of chicken, duck and turkey were susceptible to MDHV infection, and these infected

cells showed refractile and rounded CPE. The infectivity of strain SD-3 was highly cell-associated. The infected cells showed syncytial formation, and Cowdry type A intranuclear inclusion bodies were recognized among them. While a thorough attempt to propagate strain SD-3 in a wide variety of mammalian primary cells of rabbit, guinea pig, mouse, swine and bovine kidneys, bovine testicle and mouse embryo and cell lines of green monkey kidney (GMC), swine kidneys (PK-15, PS), hamster lung(HmLu), hamster kidney (BHK-21), and human amnion (FL) was unsuccessful. Strain SD-3 propagated in chorio allantoic membrane (CAM) and formed pocks on CAM, and the pock formation was inhibited by the treatment of IUDR. In addition, the cells infected with strain SD-3 was examined by electron microscopy, and herpes virus particles were seen in the infected cells. The hemoagglutination (HA) test was done with the erythrocytes from chicken, guinea pig, rabbit, cattle and horse, but strain SD-3 agglutinated none of these erythrocytes. When one-day-old SPF chickens were inoculated intraperitoneally with strain SD-3, the clinical symptoms, growth lesions and microscopic lesions of MD were recognized in 2, 10 and all cases respectively among the 33 birds tested at 70 days after the inoculation. The lesions of MD appeared chiefly in the organs of liver, spleen, kidney, lung and gizzard, but very few cases of the neural lesions were observed.

MDHV was isolated from all of the chickens in the inoculated and contact exposed groups, but the gel precipitating antibody was detected in only 50% cases among the chickens.

## 2) The epizootiological survey on Marek's disease

Both of the sera taken from the 544 birds in 1964 in Kyushu district (A group) and the 871 ones in 1968 to 1971 in the same district (B group) were investigated for the gel precipitating antibody to MDHV. In A group, the antibody was detected in the sera from the chickens of all of the prefectures surveyed, and the positive rate were 15.4 to 44.8%. Meanwhile, in B group, the positive rate were 0 to 84.3%. This showed that MD had already spread widely in Kyushu district in 1964. The appearance of the gel precipitating antibody

to MDHV was depended on the age of birds. The positive rate of the antibody in 1 to 30-day-old chickens was 1%, but it increased to 72.4% at 90 to 120 days of age. There was a variation in the appearance of the gel precipitating antibody among the genetically different chickens. Incidentally, the positive rate in Handless and Dekalb were 10 and 68.8% respectively. Seven groups consisted of 1 to 3-day-old chickens in Kumamoto prefecture were examined for the maternal antibody to MDHV by the agar gel precipitation test. The antibody was detected in the sera from the 6 groups except one group, and the positive rate were 55 to 83%. The maternal antibody was detectable until 12 days of age, but at 15 or more days of age, it become undetectable. Antibody response by MDHV infection occurred at 30 to 100 days of age. No evidence of the gel precipitating antibody to MDHV was detected in the sera from turkey, duck, pigeon, cattle, dog or human being. Virus isolation tests from the chickens being hatched and bred under the hygienic and no disinfectant circumstances were done. MDHV was isolated at 28 to 33 days of age under the hygienic condition, and at 9 days of age under the dirty condition.

### 3) Isolation of HVT and it's Properties

An attempt to isolate HVT from the materials of blood, kidney and feather follicles from the normal turkeys reared in Kyushu and Yamaguchi districts was done. Virus was isolated from the blood from 39 of 43 turkeys raised in Yamaguchi prefecture, but no virus was detected in the materials from Kumamoto prefecture. A virus strain was selected from these isolates as a representative strain (YT-7), and strain YT-7 was proved to be a HVT from it's properties. Strain YT-7 produced refractile CPE in chicken embryo fibroblast (CEF) and duck embryo fibroblast (DEF), and syncytial formation and Cowdry type A intranuclear inclusion bodies were seen in the infected cells. The infectivity of strain YT-7 was highly cell associated. Strain YT-7 propagated in chicken embryonated eggs and made pocks on the CAM. These propagation of strain YT-7 were inhibited by adding IUDR in the medium. The cells infected with strain YT-7 were examined by electron microscopy, and in which a number of herpes virus particles were seen. HA activity of strain YT-7 was tested

with the erythrocytes prepared from chicken, guinea pig, rabbit, cattle and horse, but none of them used was agglutinated by the stain. A thorough attempt to propagate strain YT-7 in a wide variety of small animals (suckling and adult mice, suckling guinea pig and rabbit), mammalian primary cells (rabbit, guinea pig, mouse, swine and bovine kidneys, mouse embryo fibroblast and bovine testicle) and mammalian cell lines (GMC, PK-15, PS, BHK-21, HmLu and FL) was unsuccessful. Twenty one-day-old SPF chickens were inoculated intraperitoneally with strain YT-7, and these chickens were observed for the clinical signs for 70 days postinoculation. During the observation, none of the 20 chickens showed any clinical signs, and neither gross lesions nor microscopic ones at necropsy were observed, but antibody response to HVT was detected in the sera from all the chickens.

4) A field experiment of Marek's disease vaccine (freeze-type)

A preparation of Marek's disease vaccine (freeze-type) was made with strain FC 126 of HVT. The vaccine was tested for safety and immunogenicity for layers and broilers in the field. The field experiment of the vaccine for layers was done in 5 poultry farms in Kumamoto prefecture. Each of 10,174 chickens was inoculated intraperitoneally or subcutaneously with 1,000 PFU of the vaccine (inoculated group), and 4,972 chickens were applied to the control group. These chickens were observed for clinical symptoms for 180 days postinoculation. No birds showed any clinical signs due to the inoculation. All of the birds which died or were culled during the observation were examined histopathologically for the cause of them. The rate of death and culling in the vaccinated and control groups were 3.3 to 10.1% (7.5% on the average) and 6.1 to 23.2% (14.4% on the average) respectively. The rate of diseased birds in the vaccinated group compared with that in the control group was decreased by 48.1% ( $P < 0.001$ ). The causes of death and culling were weakness, accident, cannibalism, bad growth, MD, avian leucosis, chronic respiratory disease and so forth. The number of these causes except weakness, accident and ovarian disease in the vaccinated group was fewer than that in the control group.

The rate of MD occurrence in the vaccinated and control groups were 1.1 and 7.1% respectively, and the rate in the former compared with that in the latter was decreased by 84% ( $P < 0.001$ ). The vaccinal virus was reisolated from 91.4 to 100% of the vaccinated birds and from the control ones though it was just a few rate. MDHV was isolated even from the vaccinated birds, and that showed the vaccine could not prevent MDHV infection in the field. Between the vaccinated and control groups, there were no significant differences in a variety of MD-gel precipitating, IB-neutralizing, Mycoplasma gallisepticum-agglutinating and Newcastle disease-hemagglutinating antibody titers and the rate of weight gain and egg laying.

In addition, the field test of the vaccine for broilers was done in the 4 poultry farms in Kumamoto prefecture. A total number of 103,509 chickens were inoculated subcutaneously with a variety of 1 (1,000 PFU), 1/2 and 1/4 doses of the vaccine (vaccinated group) and a total number of 36,021 chickens were applied to the control group. Observation was done for 49 to 70 days postinoculation, and at the time of slaughter in the processing plants, they were examined for MD lesions.

There was no significant difference in the rate of death and culling in both of the groups, but the rate of tumor lesions was lower in the vaccinated group than that in the control one. The decreased rate in the former compared with the latter was 88.7% ( $P < 0.001$ ). Fifty to 150 birds were sampled at random at a proper interval from the vaccinated and control groups. The weight of the birds in the vaccinated group was heavier by 10 to 100 gs than that in the control group, and the variance in weight in the former was smaller than that in the latter. The vaccinal virus was reisolated from the vaccinated birds at 17 or more days of age, and gel precipitating antibody response to HVT was detected at 33 or more days of age. The ratio of feed demand in vaccinated group was lower by an average of 0.09 (0.06 to 0.11) than that in the control group, and economic income in the vaccinated group was larger by 14.38 yen/chicken than that in the control group.

These results indicate that this vaccine is highly useful for the prevention of MD and decrease in feed demand.

#### 5) Studies on a lyophilized HVT vaccine

Strain YT-7 of HVT was passed serially in DEF and CEF, and with undergoing the passage, cell free virus increased. The titer of cell free virus at the passage level of DEF 12-CEF40 was  $6.5 \times 10^6$  PFU/ml, and cell free HVT had the same properties with cell associated HVT. A preparation of cell free HVT was lyophilized. One-day-old SPF chickens were inoculated with 200 to 9000 PFU of the vaccine. None of them showed any clinical symptoms due to the vaccination. Gel precipitating antibody to HVT was detected in 80% of the sera from the vaccinated birds at 15 weeks postinoculation, and the vaccinal virus was reisolated from all of the birds. The vaccinated chickens were challenged with a pathogenic MDHV at 3 weeks of age. None of them had the lesions of MD, but the lesions were shown in 80% of the control group. Contact transmission of HVT from the donor to the pen mate was not evident. In addition, the effect of the maternal antibody to MDHV on immunization with the vaccine was tested with one-day-old chickens. The vaccinal virus was reisolated from the maternal antibody negative chickens even when they were inoculated with only  $10^{1.0}$  PFU of the vaccine, but the recovery rate was lower than that when they were inoculated with  $10^{2.0}$  to  $10^{5.0}$  PFU of the vaccine. On the other hand, when the maternal antibody positive chickens were inoculated with  $10^{3.0}$  PFU of the vaccine, it needed more days to reisolate the vaccinal virus from them.

The vaccine was tested for safety and immunogenicity with a total number of 13,965 chickens in 5 poultry farms in Kumamoto prefecture. A total number of 10,384 chickens were inoculated subcutaneously with 1600 PFU/0.2 ml of the vaccine, and the remainder 3,581 chickens were applied to the control group. Observation was done for 150 days post-inoculation, and the birds which died or being culled during the observation were examined histopathologically for the causal agents. None of the vaccinated chicken showed any clinical symptoms due to the inoculation. The rate of death and culling in the inoculated and control groups were 4.5 and 7.6% respectively. MD occurred in 117 cases (1.13%) of the vaccinated birds, and in 133 ones (3.71%) of the control birds.

The rate in the former compared with the latter was decreased by 69.7% ( $P < 0.001$ ). The vaccinal virus was reisolated from 79 to 90% of the vaccinated birds for 150 days postinoculation, and contact transmission of HVT from the donors to the pen mates was recognized in only one group among the 5 groups. Gel precipitating antibody response to HVT at 121 to 150 days of age was detected in 72% of the sera from the vaccinated birds. MDHV infection occurred in the vaccinated groups, and HVT and MDHV persisted in the same host for long time. These results indicate that the lyophilized HVT vaccine has the same level of safety and efficacy for the prevention of MD in the field.

#### 6) Studies on MD (HVT)-ND combined live virus vaccine

A MD-ND combined vaccine was prepared from HVT and the B<sub>1</sub> strain of Newcastle disease virus. The vaccine was examined for safety and immunogenicity with conventional and SPF one-day-old chickens. They were inoculated with the combined vaccine involving 1200 to 2200 PFU of HVT and  $10^{6.1}$  to  $10^{6.5}$  EID<sub>50</sub> of NDV per chicken by the routes of hypoderm, muscle and abdominal cavity. Between the combined vaccine inoculated group and MD vaccine inoculated group, there were no differences in the rate of HVT recovery, neutralizing antibody response to HVT and protective efficacy against the challenge with a pathogenic MDHV. Similarly, between the combined vaccine inoculated group and ND vaccine inoculated group, there were no differences in ND-HI antibody response and protective efficacy against the challenge with a pathogenic NDV. For the route of vaccination, intraperitoneal inoculation was better than other methods. HVT was reisolated at 6 days of age in 86 to 100% of the vaccinated chickens, and the recovery rate was almost equal to that of the chickens inoculated with MD vaccine. The combined vaccine inoculation gave no inhibition to the secondary antibody response to NDV. Contact transmission of ND vaccinal virus from the donors inoculated intranasally with ND vaccine to the pen mates was more detectable than that from the donors inoculated subcutaneously with the combined vaccine to the pen mates. Safety for conventional and SPF chickens of the combined vaccine was tested. None of the conventional chickens inoculated with the vaccine showed any clinical symptoms, but



some SPF chickens inoculated with the vaccine showed slight temporary respiratory symptoms (1 to 2 sneezes per 5 to 10 minutes), though such signs were recognized when SPF chickens were vaccinated with ND vaccine, which gave no significant influences on the growth of the chickens.

In addition to these experimental studies, a field experiment of the combined vaccine was done with a total number of 5,092 chickens in 2 poultry farms in Kumamoto prefecture. A total number of 3014 chickens were inoculated subcutaneously with the combined vaccine, and a total number of 2078 ones were applied to the MD vaccine inoculated group. None of the chickens inoculated with these vaccines showed any clinical symptoms due to the vaccination. Observation was done for 120 days postinoculation. The rate of death and culling at 120 days of age were 1.2 to 3.9% in the combined vaccine inoculated groups and, almost same level of the rate was recognized in the MD vaccine inoculated group. HVT was reisolated from all of the vaccinated birds, and ND-HI antibody response was detected in all of them. In vitro assay on interference between HVT and NDV was done, but no evidence of the interference was detected. From these results, it can be said that the combined vaccine is sufficiently and practically usable in the field.