THE SUSCEPTIBILITY OF SCALELESS MUTANT CHICKENS TO VERY VIRULENT MAREK'S DISEASE VIRUS

J. A. Lin[1], J. J. Liu Tai[2], Y. S. Lu[3], P. P. Liou[4] and C. Tai[5]

Department of Veterinary Medicine, National Chung Hsing University, Taichung 40227, Taiwan, R.O.C.

Summary

This study evaluates the susceptibility of scaleless mutant chickens to very virulent Marek's disease virus (vvMDV) inoculation. One day old chickens were inoculated subcutaneously with Taiwanese isolates of LTB-1 and LTS-1 strains, and standard strain of Md/5. Compared with the non-inoculated group, the vvMDV-inoculated chickens showed decreased body weights and atrophy of lymphoid organs before 35 days old. These results indicate that scaleless chickens show the same susceptibility as the wild type chickens to vvMDV infection. Furthermore, the protective effect of herpesvirus of turkey (HVT) vaccination at 1 day old against vvMDV challenge was evaluated. Scaleless mutant chickens of treated groups showed 20-30% early death, and 85.7-100% and 12.5-14.2% had lymphomatous lesions in visceral organs and peripheral nerves, respectively. No significant lesions were observed in non-challenged chickens of the control group. The HVT vaccination did not provide an effective protection against vvMDV infection. It is concluded that scaleless mutant chickens are susceptible to vvMDV infection.

(**Key Words** : Susceptibility, Scaleless Mutant Chicken, Marek's Disease Virus)

Introduction

Marek's disease (MD) is an important contagious infection of chickens, caused by Marek's disease virus (MDV) of α-herpesvirus (Calnek and Witter, 1991). Although many serotypes of MD vaccines (e.g. CVI 988 of serotype 1; SB-1 of serotype 2; herpesvirus of turkey, HVT of serotype 3) are available (Rispens et al., 1972; Schat and Calnek, 1978, Witter et al., 1970), yet vaccine failures and severe MD outbreaks occur in vaccinated chickens. Occurrence of very virulent MDV (vvMDV) infection is considered to be one of the main factors responsible for MD outbreaks in vaccinated flocks in USA, Italy and Japan (Witter et al., 1980; Witter, 1983; Powell and Lombardini, 1986; Imai et al., 1992). High incidence of vaccine outbreaks have been recorded in vaccinated chickens in Taiwan, not only in the latter growing stage of local chickens, but also in prelaying stage of layer pullet with the isolation of vvMDV (Lin and Chen, 1996). One of Taiwanese isolates of the vvMDV (strain LTB-1) caused early death and lymphomatous formation in experimentally inoculated broilers and layers.

The scaleless mutant chickens used in the present study were derived from New Hampshire breed of chickens, possessing a pair of recessive genes (sc/sc) (Abbott and Asmundson, 1957).

The feathers and their feather follicles are very few, and distributed only at some specific areas of body (Abbott and Asmundson, 1957; Goetinck and Abbott, 1960; Sawyer and Abbott, 1972). The defect is due to an outmost excessive genetic mutation in epidermal formation. These birds require adequate brooding and grow poorly compared to wild type chickens. However, some researchers from Cornell University, USA indicated that the scaleless mutants would get more body weight, better feed conversions and more protein content in carcass with less fat, when they were raised at a temperature of 38℃ (Somes and Johnson, 1982). The

[1] Address reprint requests to Dr. J. A. Lin, Department of Veterinary Medicine, National Chung Hsing University, Taichung 40227, Taiwan, R.O.C.

[2] Department of Animal Physiology, Taiwan Livestock Research Institute, Tainan, Taiwan, R.O.C.

[3] Department of Epidemiology, Taiwan Provincial Research Institute for Animal Health, Taipei, Taiwan, R.O.C.

[4] Director General, Taiwan Povincial Research Institute for Animal Health.

[5] Director General, Taiwan Livestock Research Institute.

Received August 28, 1995
Accepted July 30, 1996

susceptibility of scaleless mutants to MDV remains unknown. The scaleless mutants, however, may be of economical importance in the hot environment, especially in tropic area. Scaleless chickens were obtained from UC Davis and bred in the Taiwan Livestock Research Institute in 1990 (Tai et al. 1991). In this study, we tried to investigate the pathogenic susceptibility of scaleless mutants from the Taiwan Livestock Research to vvMDV of Taiwanese strain.

Materials and Methods

Strains of MDV

Four MDV strains were used in these experiments.

1. VvMDV of Taiwanese isolates: Strains of LTB-1 and LTS-1 isolated from lymphomatous lesions of Leghorn layers and fighting cocks, respectively, from Taiwan (Lin and Chen, 1996) were used.

2. Reference strains: Md/5 strain (Purchase and Biggs, 1967), belonging to vvMDV, was kindly provided by Dr. Mikami, Professor of Department of Veterinary Medicine, Tokyo University.

3. Vaccine strain: Vaccines of HVT were purchased from INTERVET Co. (Boxmeer, Netherlands) for inoculating 1-day-old chicks.

Replication of virus

The chicken embryo fibroblasts (CEFs) were made from trypsining specific pathogen-free (SPF) chicken embryos, and incubated to grow into monolayers by adding minimum essential medium (MEM) with 2% of fetal calf serum (FCS) (Lin et al., 1990). The CEF monolayers were used to replicate the above MDVs. After the appearance of cytopathic effect (CPE), the infected cells were trypsinized, then suspended in MEM with 10% FCS and DMSO, and stored in liquid nitrogen until used.

Experimental chickens

The scaleless mutant chicks were delivered from the experimental farms of the Taiwan Livestock Research Institute. They were inoculated with vaccines or vvMDV (of strains LTB-1, LTS-1 and Md/5) at 1 day old, and moved to SPF cages for further rearing and observation. The SPF cages were ventilated by filtrating the air through filter of 0.4 μm. The feeds and drinking water were sterilized with formalin fumigation and by autoclaving, respectively, before supplied to birds.

Experimental design

The experimental chickens were divided into groups for the following purposes.

1. To study the effects of acute infectious stage on immune organs: Forty experimental chickens were divided into 8 groups (Groups A-H). Six treated groups (Groups A-C and E-G) were inoculated subcutaneously at 1 day old with 2,000 plaque forming units (PFU) of strains LTB-1, LTS-1 and Md/5 as shown in tabel 1. The control groups (Group D and H) were inoculated with un-infected CEFs subcuataneously at 1 day old. All the experimental chickens were necropsized to measure the body weights and the weights of lymphoid organs (spleen, bursa of Fabricius and thymus) at 11 and 35 days old. The suspected specimens were fixed in 10% neutral formalin, and examined for acute cytolysis and other histoligical changes in paraffin sections and stained by haematoxylin and eosin.

2. To assess the lymphomatous lesions: Thirty 1-day-old chickens were inoculated with HVT subcutaneously in the dose of 1,000 PFU before separated into three experiment groups (10 for each group). Chickens in groups A-C were challenged with LTB-1, LTS-1 and Md/5 by intraperitoneal injection of 2,000 PFU at 10 days old, respectively. Ten chickens of control group D were not inoculated with either vvMDV or HVT. All chickens were raised in different SPF cages in order to examine the lymphomatous formation and the preventive effects on vvMDV. The chickens were necropsized at 10 weeks of age, and examined for gross lesions of the visceral organs. The histological examination of the suspected specimen used by the same method described previously.

Results

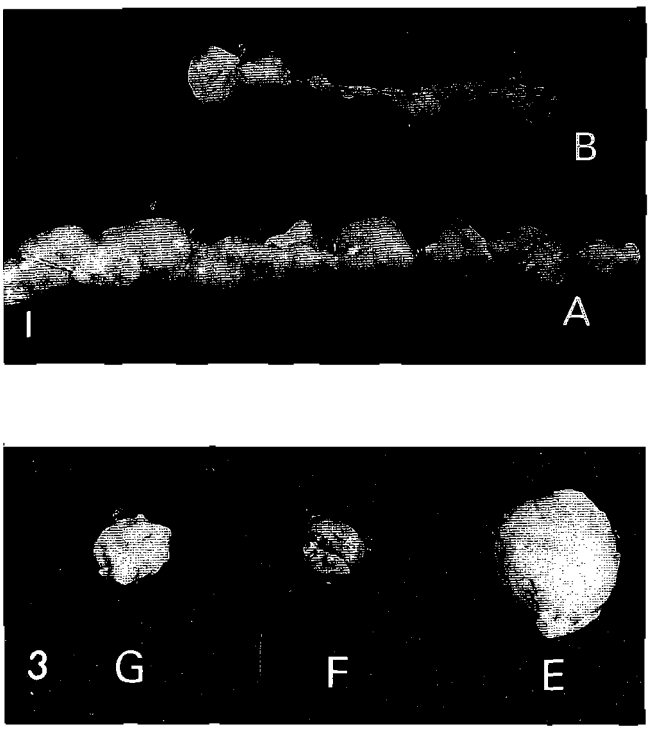
1. Effects of acute infectious stage on immune organs:

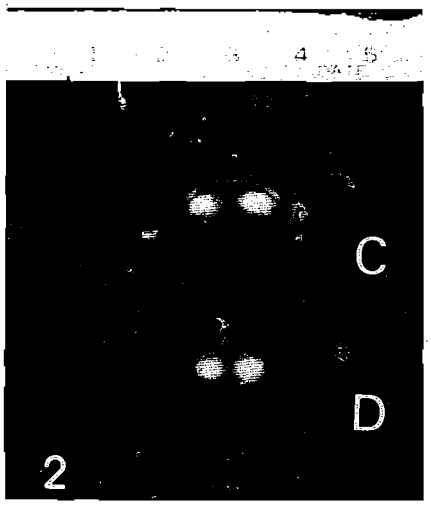
The weights of various lymphoid organs and the body weights of the inoculated chickens are shown in table 1. Besides the spleens of 35-day-old chickens in Groups F and G, the lymphoid organs of inoculated groups were atrophic as seen at 11 or 35 days old. Infiltration and aggregation of pleomorphic lymphoblasts were found by histological examination in the spleens of 35-day-old chickens of Groups F and G. While the lymphoid organs revealed depletion of lymphocytes and atrophy of lymphoid follicles in Groups. A, B, C and E. Thus, all the vvMDV strains caused acute infectious stage and atrophy of lymphoid organs of scaleless mutants. It is obvious that the virus will affect the development of organs associated with immune system. Figures 1-3 showed the decreased size of lymphoid organs in LTB-1 inoculated chickens of 11 days old.

TABLE 1. COMPARISON OF INFLUENCE OF STRAINS LTB-1, LTS-1 AND MD/5 ON BODY WEIGHTS AND LYMPHOID ORGANS OF SCALELESS MUTANT

Group	DPI[1]	No. of birds	Inoculation[2]	Body weight		Bursa / BW (%)	Spleen		Thymus	
				Wt (g)	Ratio (%)		Wt (g)	Ratio (%)	Wt (g)	Ratio (%)
A	11	5	LTB-1	58.7	90.7	0.48	0.14	41.1	0.30	32.6
B	11	5	LTS-1	47.5	73.4	0.38	0.16	47.0	0.20	21.7
C	11	5	Md/5	55.5	85.8	0.42	0.18	52.9	0.22	23.9
D	11	5	Non	64.7	100.0	0.68	0.34	100.0	0.92	100.0
E	35	5	LTB-1	282.0	73.4	0.14	0.87	93.5	0.53	12.4
F	35	5	LTS-1	267.0	69.5	0.11	1.04	111.8	0.49	11.5
G	35	5	Md/5	289.0	75.2	0.15	0.97	104.3	0.56	13.1
H	35	5	Non	384.0	100.0	0.37	0.93	100.0	4.26	100.0

[1] DPI : days post-inoculation.
[2] Dose : 2,000 PFU.





Figures 1, 2 and 3 : At 11 days post-inoculation, the LTB-1 inoculated chickens showed markedly decreased sizes of thymus (B), spleen (D) and bursa of Fabricius (F and G). A, C and E are the normal sizes of lymphoid organs in control chickens.

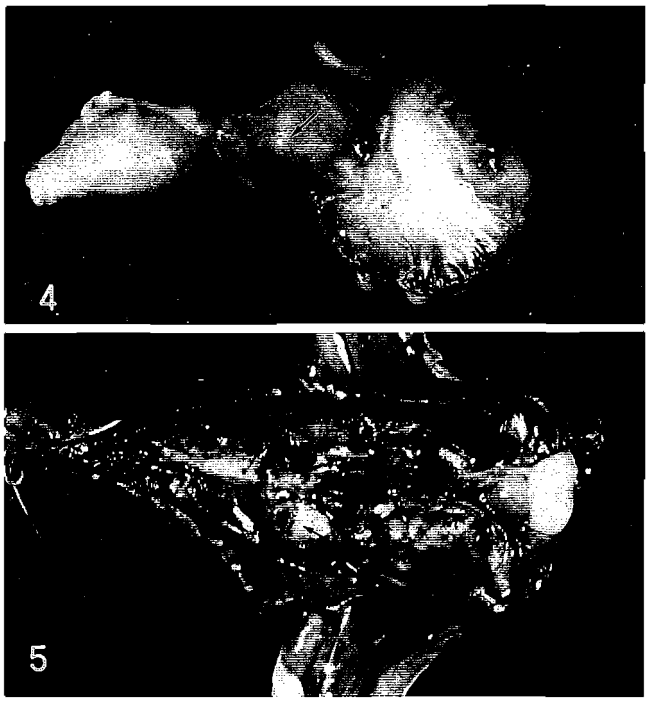
2. The formation of lymphomatous lesions :

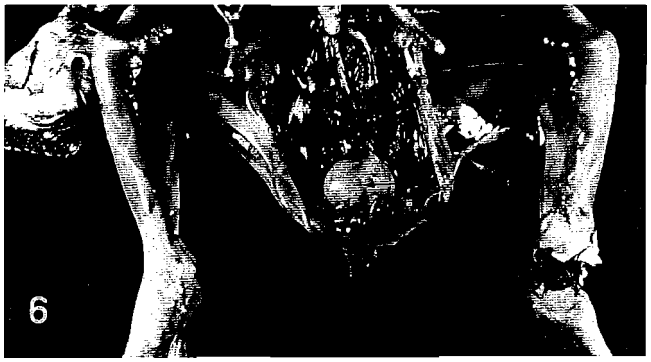
Table 2 shows percentage mortalities and lymphomatous lesions of vvMDV challenged groups before 5 weeks old and at 10 weeks old, respectively, as compared with the control group of Group D. The early mortalities were 20-33.3% in Groups A-C, compared with none in Group D. All birds in the treatment group had lymphomatous lesions compared with none in the control group. Furthermore, the rate (%) of lesion distribution in various organs were 85.7-100%, compared with 12.5-14.2 % of sciatic nerves. The lymphomas were found in the liver and spleen of all treated birds. Lymphomas in gizzards, kidneys, bursas, and heart are shown in figures 4-7. Same gross findings of the tumors were gray/white, firm with a granule cut surface, and ranged in size from 2-5 mm in diameter. By histological examination, aggregates of lymphoblastoid cells of liver and sciatic nerve were found in figures 8 & 9, respectively.

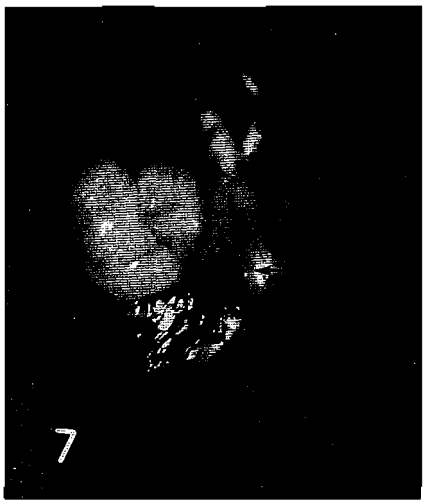
TABLE 2. COMPARISON OF DISTRIBUTION OF LYMPHOMA LESIONS ON SCALELESS MUTANTS BY STRAINS LTB-1, LTS-1, MD/5 AT 10TH WEEK POST-INOCULATION

Groups[1]	No. of birds	% of early MD mortality	% of total birds with lesion	% of lesion distribution						
				Liver	Spleen	Kidney	Heart	Nerve	Others[2]	Total[3]
A	10	20.0	100.0	100.0	100.0	87.5	87.5	12.5	87.5	90.9
B	10	33.3	100.0	100.0	100.0	85.7	100.0	14.2	85.7	90.9
C	10	33.3	100.0	100.0	100.0	85.7	85.7	14.2	85.7	90.9
D	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[1] Groups A, B and C were inoculated by strains LTB-1, LTS-1, and Md/5, respectivley. Group D was non-inoculated, as negative control.
[2] Including proventriculus, intestine and lung.
[3] Including all visceral organs, except for nerve.



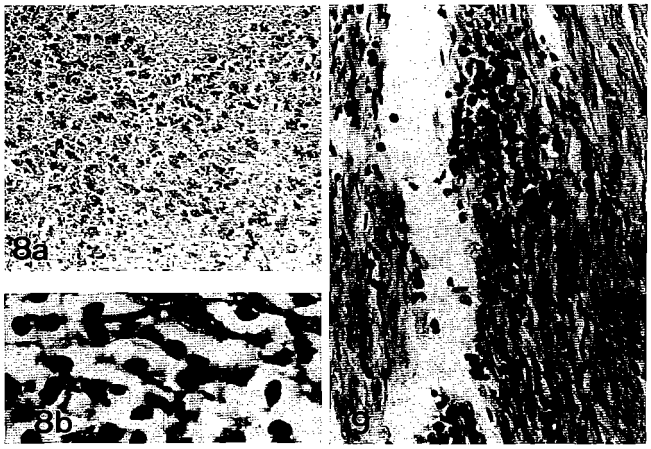




Figures 4, 5, 6 and 7 : At 10 weeks post-inoculation, the LTB-1 inoculated chickens showed whitish lymphomatous lesions of gizzard, kidney, bursa of Fabricius and heart, as indicated by arrows.

Discussion

The bird species infected naturally by MDV belong to three genera of *Galliformes,* especially the *Gallus* genus (Kenzo et al., 1969; Lin et al., 1990; Witter et al., 1971). Scaleless mutant chickens are *Gallus* genus having few feathers and feather follicles on their body surface (Abbott and Asmundson, 1957; Goetinck and Abbott, 1960; Sawyer and Abbott, 1972). Tai et al. (1991) investigated the effects of hot stress on growing performance of the scaleless chickens, silky chickens (Ukokkei), and their crosses. They concluded that body increments would be improved due to increased body heat releasing in scaleless chickens. Therefore, it is interesting to study the virus replication and pathogenecity of vvMDV in scaleless mutants.



Figures 8 and 9: At 10 weeks post-inoculation, infiltration and aggregation of pleomorphic lymphoblasts were found in liver (figure 8a), H&E, X100, and sciatic nerve (figure 9), H&E, X430. Higher magnification of liver was shown in figure 8b, H&E, X700.

The vvMDV (LTB-1 and LTS-1 strains) used in this experiment were isolated from layers or fighting cocks (*Gallus* genus) with lymphomatous lesions (Lin and Chen, 1996). The source of reference virus of Md/5 strain is also chickens (Purchase and Biggs, 1967). The experiment reported here found that scaleless mutant chickens inoculated with vvMDV had decreased body weight, and atrophy lymphoid organs of bursa, spleen and thymus before 15 days old. The early mortalities also revealed that vvMDV of serotype 1 killed scaleless mutants as it did other birds. Atrophied lymphoid organs in vvMDV infected chickens results immune depletion against various infectious diseases (Calnek et al., 1991; Calnek, 1986; Schat et al. 1981). This study shows that scaleless mutant chickens are susceptible to vvMDV infection.

MDV of serotype 1 not only causes acute infection, but also induces lymphomatious formation of various visceral organs, body surface and peripheral nerves in chickens (Calnek et al., 1991). The study shows that vvMDV causes lymphomatous lesions on scaleless mutant chickens (as shown in table 2). MHC-related alleles (Cole, 1972) and Ly-4 alleles (Fredericksen et al., 1977; Fredericksen et al., 1985) are considered to be involved in genetic resistance to MD transformation. This study shows that the scaleless gene which effects the expression of feather on body surfaces and scales on shanks does not confer resistance to MD. Scaleless mutant fowls are as normal chickens as susceptible to vvMDV infection.

Althrough one day old chickens are vaccinated with HVT as a MD prevention program (Witter, 1972), outbreaks of MD in chicken flocks vaccinated with HVT have been attributed to vvMDV infections (Witter et al., 1980; Witter, 1983; Powell and Lombardini, 1986; Imai et al., 1992). The results given in table 2 also indicate HVT vaccines failed to protect scaleless mutant chickens against vvMDV. It is important to use the same vaccine program as is used in regular birds such as Leghorns. Combinations of different serotypes of MDV strains made up as bivalent or trivalent vaccines should be used in chicken flocks of scaleless mutants.

The most likely major source of infection is through desquamated epithelial cells and moulted feathers (Carrozza et al., 1973), because the feather follicle epithelium is the only site where enveloped and cell-free infectious virus is formed (Calnek et al., 1970). Shedding of infectious material occurs about 2 to 4 weeks after infection, prior to the appearance of clinical signs, and can continue throughout the life of the chickens (Carrozza et al., 1973; Kenzy and Cho, 1969; Witter et al., 1971). However, virus shedding from the only few feather follicles in scaleless mutant requires further investigation.

Acknowledgements

This study was supported by the National Science Council, Republic of China (NSC-80-0409-B-061-02, NSC-81-0409-B-061-05 and NSC-84-3221-B-005-059). The authors would like to express their appreciation to Dr. Mikami (Department of Veterinary Microbiology, Faculty of Agriculture, Tokyo University) for providing the Md/5 virus, and Mr. Lee J. S. (Institute of Veterinary Midicine, National Chung Hsing University) for his technical help.

Literature Cited

Abbott, U. K. and V. S. Asmundson. 1957. Scaleless, an inhereited ectodermal defect in the domestic fowl. J. Heredity. 48:63-70.

Calnek, B. W., H. K. Adldinger and D. E. Kahn. 1970. Feather follicle epithelium: A source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Dis. 14:219-233.

Calnek, B. W. 1986. Marek's disease - a model for herpesvirus oncology. pp. 273-320. In: the CRC Critical Review in Microbiology, vol. 12 (O'Leary, W. M., ed.). CRC Press, Florida.

Calnek, B. W. and R. L. Witter. 1991. Marek's disease. pp. 325-360. In: Diseases of Poultry, 9th ed. (Hofstad, M. S., H. J. Barnes, B. W. Calnek, W. M. Reid and

Jr., H. W. Yorder, eds). Iowa State University Press, Ames, Iowa.

Carrozza, J. J., T. N. Fredricksen, R. P. Prince and R. E. Luginbuhl. 1973. Role of desquamated epithelial cells. Avian Dis. 17:767-781.

Cole, R. K. 1972. The genetics of resistance to Marek's disease. pp. 123-128. In: On-cogenensis and Herpesviruses (Biggs, P. M., G. de-The, and L. N. Payne, eds.). IARC. Lyon.

Fredericksen, T. L., B. M. Longenecker, F. Pazderka, D. G. Gilmour and R. F. Ruth. 1977. A T-cell antigen system of chickens : Ly-4 and Marek's disease. Immunogenetics. 5:535-552.

Fredericksen, T. L. and D. G. Gilmour. 1985. Influence of genotypes at a non-MHC B lymphocyte alloantigen locus bursal lymphocytes. J. Immunol. 134:754-756.

Goetinck, P. F. and U. K. Abbott. 1960. Transplantation studies with the scaleless mutant. Poult. Sci. 39:1231.

Imai, K., N. Yuasa, H. Iwakiri, K. Nakamura, H. Hara, T. Ishita, A. Inamoto, I. Okamoto, K. Ohta and M. Maeda. 1992. Characterization of very virulent Marek's disease viruses isolated in Japan. Avian Pathol. 21:119-126.

Kenzo, S. G. and B. R. Cho. 1969. Transmission of classical Marek's disease by affected and carrier birds. Avian Dis. 13:211-214.

Lin, J. A., H. Kitagawa, M. Ono, I. Rita, H. Kodama and T. Mikami. 1990. Isolation of serotype 2 Marek's disease virus from birds belonging to Genus *Gallus* in Japan. Avian Dis. 34:336-344.

Lin, J. A. and C.-P. Chen. 1996. First isolation and characterization of very virulent Marek's disease virus in Taiwan. J. Vet. Med. Sci. 58:1011-1015.

Powell, P. C. and F. Lombardini. 1986. Isolation of very virulent pathotypes of Marek's disease virus from vaccinated chickens in Europe. Vet. Rec. 118:688-691.

Purchase, H. G. and P. M. Biggs. 1967. Characterzation of five isolates of Marek's disease. Res. Vet. Sci. 8:440-449.

Rispens, B. H., H. Vloten, van, N. Mastenbroek and H. J. L. Mass. 1872. Control of Marek's disease in the Netherlands. I. Isolation of an avirulent Marek's disease virus (strain CVI 988) and its use in laboratory vaccination trials. Avian Dis. 16:108-125.

Schat, K. A. and B. W. Calnek. 1978. Characterization of an apparently oncogenic Marek's disease virus. J. Natl. Cancer Inst. 60:1075-1082.

Schat, K. A., B. W. Calnek and J. Fabricant. 1981. Influence of bursa of Fabricius on the pathogenesis of Marek's disease virus. Infect. Immun. 31:199-207.

Somes, R. G. and S. Johnson. 1982. The effect of the scaleless gene, sc, on growth per-formance and carcass composition of broilers. Poultry Sci. 61:414-423.

Tai, J. J. L., C. Tai, S. H. Yeh and S., C. Huang. 1991. The effects of scaleless gene and hookless gene on the economic performance of chickens under the environment of Taiwan. J. Che. Soc. Anim. Sci. 20 (supplement) : 1.

Witter, R. L., K. Nazerian, H. G. Purchase and G. H. Burgoyne. 1970. Isolation from turkeys of a cell-associated herpesvirus antigenically related Marek's disease virus. Am. J. Vet. Res. 31:525-538.

Witter, R. L. and J. J. Solomon. 1971. Epidemiology of a herpesvirus of turkeys : possible source and spread of infection in turkey flocks. Infect. Immun. 4:356-361.

Witter, R. L., J. J. Solomon, L. R. Champion and K. Nazerian. 1971. Long-term studies of Marek's disease infection in individual chickens. Avian Dis. 15:346-365.

Witter, R. L., J. M. Sharma and A. M. Fadly. 1980. Pathogenicity of variant Marek's disease virus isolates in vaccinated and unvaccinated chickens. Avian Dis. 24:210-232.

Witter. R. L. 1983. Characterization of Marek's disease virus isolated from vaccinated commercial chicken flocks : association of viral pathotype with lymphoma frequency. Avian Dis. 27:113-132.