RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Marek's disease vaccine trials in commercial birds

A report for the Rural Industries Research and Development Corporation

Kelly M. Read, Maggie Cheong and G. A. Tannock
Department of Applied Biology and Biotechnology, RMIT

R. J. Condron and W. M. Forsyth
Department of Natural Resources and Environment
Victorian Institute of Animal Science, Attwood

June 1999

RIRDC Publication No 99/.....
RIRDC Project No. DAV-158A

ISBN *(RIRDC to allocate)*
ISSN 1440-6845

"Marek's disease vaccine trials in commercial birds"
Publication no …….
Project no. DAV-158A……..

Researcher Contact Details
Professor Greg Tannock
Department of Applied Biology and Biotechnology
Royal Melbourne Institute of Technology
GPO Box 2476V
MELBOURNE VIC 3001

Phone**: 03 992 53088**
Fax:03 96623421
Email:gtan@rmit.EDU.AU
Website:

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone**:** 02 6272 4539
Fax**:** 02 6272 5877
Email: rirdc@netinfo.com.au
Website: http://www.rirdc.gov.au

Published in …….. 1999
Printed on environmentally friendly paper by the DPIE Copy Centre

Foreword

In 1994 the RIRDC funded a project for the development of an Australian serotype 1 vaccine against Marek's disease (MD; De Laney & Tannock. 1999).

A suitable candidate for the RMIT vaccine was selected from a clone in its 60th cell culture passage after a pathogenicity trial was conducted (Morrow 1995). Several of these clones were tested for their relative protection to each other and 60/2 showed minimal pathogenicity and a high protection rate. This clone was further attenuated to overcome its residual pathogenicity and in 1997 large scale trials (De Laney *et al.* 1998) were held to test its safety and to compare its protective rate with other vaccines in specific pathogen-free (SPF) birds.

As an extension of the previous project, the RIRDC have funded a related project "The development of effective immunisation strategies against Marek's disease" (RIRDC Project No. RMI-6A). The following report addresses one of the project's objectives. To further evaluate the RMIT serotype 1 Marek's disease vaccine in comparison with currently used Australian vaccines in commercial chickens.

Contents

Abbreviations

B:B ratio	bursa:body weight ratio
CAV	chicken anaemia virus
g	grams
HVT	Herpes Virus of Turkeys
LSD	least significant difference test
MD	Marek's Disease
MDV	Marek's Disease Virus
MV	Maravac
PCR	polymerase chain reaction
PCV	packed cell volume
RMIT	Royal Melbourne Institute of Technology
SE	Standard error of the mean
SPF	specific-pathogen-free
TMC	The Marek's Company
VIAS	Victorian Institute of Animal Science

Executive summary

This report describes a trial conducted to assess the newly developed RMIT serotype 1 vaccine in a commercial line of chickens.

Birds were vaccinated with the RMIT vaccine, commercial vaccines or a combination of both and later challenged with a very virulent cell culture preparation of MDV (MPF 57). At the end of the trial, birds were assessed for presence of Marek's disease (MD) to determine the protection provided by the vaccines.

By a number of criteria, the RMIT vaccine did not perform as well as in specific-pathogen-free (SPF) birds. However, the results indicated that the RMIT vaccine when used in combination with The Marek's Company HVT produced comparable rates of protection to the Rispens vaccine when used alone.

1. Introduction

In a previous study, the RMIT serotype 1 Marek's Disease vaccine was shown to be relatively safe and efficacious, giving a comparable rate of protection in Specific Pathogen-Free (SPF) birds as the commercially available Rispens vaccine. However, the efficacy of the vaccine under field conditions may depend upon many other factors such as genetic characteristics of a chicken and its maternal antibody status.

Because of this, a trial involving commercial birds was undertaken to assess the RMIT vaccine and to compare it with commercially available vaccines.

2. Objectives

The research aims of this project were to develop effective immunisation strategies for the prevention of Marek's disease using newer vaccines (RMIT vaccine).

3. Materials and Methods

Sixty-three female day-old Cobb chickens (supplied by BAIADA Hatchery, Kootingal, NSW) were assigned to each of eight vaccine groups (Table 1.) and were identified by an aluminium tag inserted through the wing web. The parent flock was more than 40 weeks of age and had been vaccinated with the Rispens vaccine. Each bird was vaccinated at day-old subcutaneously in the back of the neck with 0.2 mL of the appropriate vaccine and dose (Table 2.). The vaccine was diluted in Cell Culture Medium; mixed vaccines were combined in the one 0.2 mL dose. The two control groups were inoculated with diluent alone.

All birds, with the exception of the negative control group; were housed together on the floor of a single controlled environment room to inlet and outlet airflow at the Victorian Institute of Animal

Science (VIAS), Attwood. In accordance with standard commercial practice, birds were controlled fed to limit their growth to the industry standard.

One week after vaccination, 10 birds per group selected at random were killed and tested for chicken anaemia virus (CAV) and their packed cell volume (PCV) determined.

Nine days after vaccination all groups, except for the negative control, were challenged intra-peritoneally with the standard dose (50 PFU/0.2 mL) of a cell-culture preparation of MPF 57 (De Laney *et al.* 1998, Morrow *et al*. 1997).

Birds were maintained for 10 weeks after challenge and any that died or required euthanasia were examined for gross and histological lesions. After ten weeks, all remaining birds were euthanased, examined for gross lesions and assigned a thymus score; measurement of bursa and body weights were then taken. Thymus scores were graded 0-3 where a score of 3 was normal and one of 0 indicated total atrophy.

Histology was performed on 10 randomly chosen birds per group and on any suspect tissues; tissues examined included brachial, sciatic and caeliac nerves, left gonad, kidney, liver, heart, lung and brain. Assessment of lesions was by the criteria in Table 3, and scores of each bird were summed and averaged per group. The final histology score was derived by subtracting the mean score of the control group from the mean score of the vaccine group. Gross and histological examination was used to confirm the presence of MD for birds that died during the experiment.

Table 1. Vaccine groups for protection comparison using commercial Cobb birds challenged with MPF 57.

Vaccine	***No. of birds***
RMIT alone	52
RMIT + The Marek's Company (TMC) HVT	52
Rispens alone	52
Rispens + TMC HVT	52
TMC HVT alone	52
Maravac + TMC HVT	52
Negative control (nonvacc./non challenged)	52
Positive control challenge (nonvacc.)	52
Total	416

Table 2. Vaccine doses.

Vaccine	***Batch***	***Dose***
RMIT (Woodlands 60/2 passage 78)	02/06/97	4,000 PFU*
The Mareks' Company (TMC) Rispens	M7101	4,000 PFU*
TMC HVT	H7301	8,000 PFU*
Fort Dodge Maravac (MV)	70470	2,000 PFU

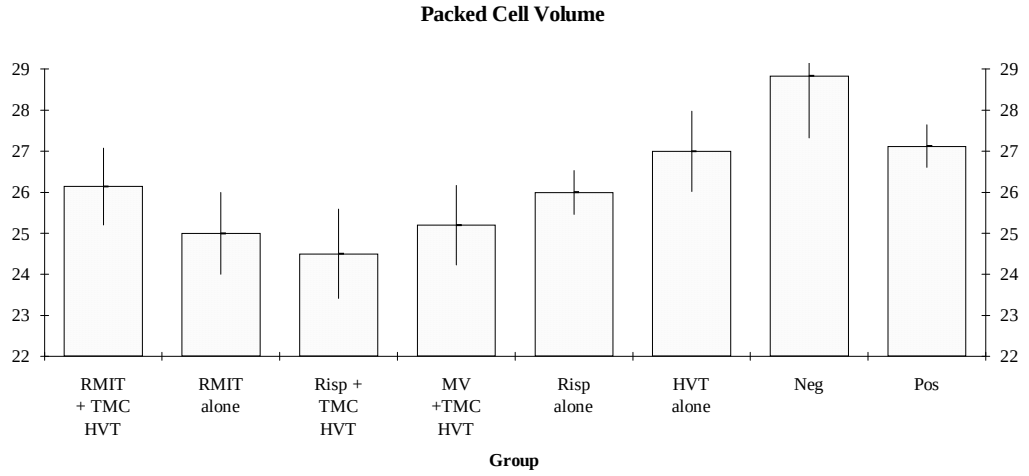
*Titre determined by the RMIT plaque-assay method and the vaccines administered at the minimum recommended dose.

Table 3. Histological scoring of lesions.

Score	***Features***
1	No infiltration with lymphoid cells
2	Slight/any infiltration
3	Moderate infiltration
4	Massive infiltration (Tumour)
5	Gross lesion

3. Results

All birds tested negative for CAV by the PCR at eight days of age. The remaining results are presented in table and graph form.



Analysis of variance (ANOVA) results:

Group effects were not significant (p0.087)

PCV

	RMIT+HVT	RMIT	Rispens+HVT	MV+HVT	Rispens	HVT	Pos	Neg
RMIT+HVT								
RMIT								
Rispens+HVT								
MV+HVT								
Rispens								
HVT								
Pos								
Neg		*	*	*	*			

*Indicates significant differences (p< 0.05) between groups by the least significant difference (LSD) test.

Figure 1. Packed Cell volume. One week after vaccination, blood samples were taken from 10 random birds per group, heparinised and loaded into capillary tubes. Tubes were then placed in a microhaematocrit centrifuge and then the PCV read by placing the capillary tube against the appropriate sized segment of a haematocrit grid and the percentage of packed cells was determined. The results are presented as the mean percentage haematocrit reading +/- the standard error of the mean (SE).

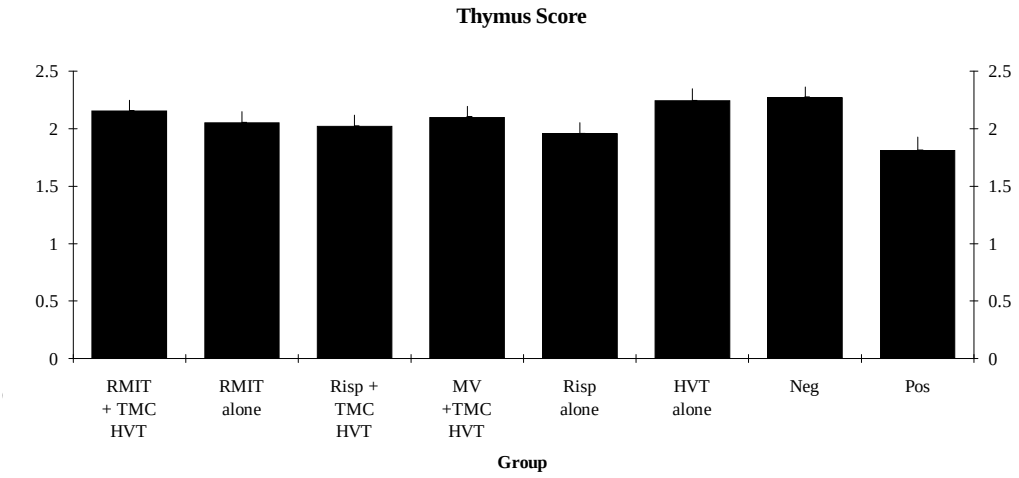
Table 4. Protection results for large-scale comparison of RMIT and commercial vaccines in commercial chickens challenged with MPF 57. Birds that died prior to challenge were not included in the protection calculations.

Group	MD			Group size	MD Total %	Protective Index[b] (PI)%
	Deaths	Tumours[a]	Total			
RMIT alone	11	1	12	50	24.0	66.5
RMIT + TMC HVT	1	1	2	49	4.1	94.3
Rispens alone	1	2	3	49	6.1	91.5
Rispens + TMC HVT	1	0	1	45	2.2	96.9
TMC HVT alone	0	4	4	45	8.9	87.6
MV + TMC HVT	0	6	6	49	12.2	83.0
Negative control	0	0	0	51	0.0	100
Positive control (Challenge only)	18	15	33	46	71.7	

[a] Tumours do not include perivascular cuffs and do not include tumours of birds that died during experiment (ie. these are represented under deaths).

[b] Protective Index (PI%)= $\frac{\%\text{MD Positive control} - \%\text{ MD observed group}}{\%\text{MD Positive control}} \times 100$

Bird deaths during the experiment and their MD status are listed in Appendix 1.



Analysis of variance (ANOVA) results:

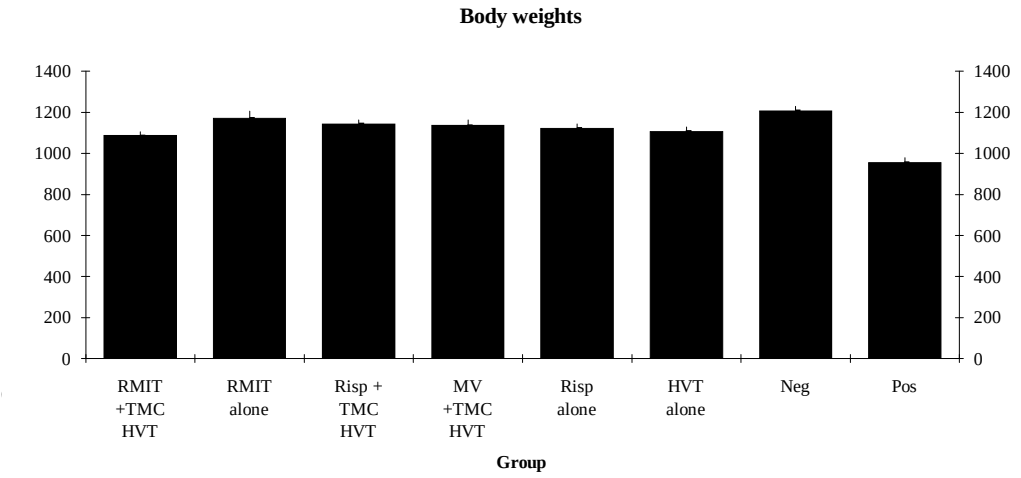
Group effects were not significant (p0.064)

Thymus score

	RMIT+HVT	RMIT	Rispens+HVT	MV+HVT	Rispens	HVT	Pos	Neg
RMIT+HVT								
RMIT								
Rispens+HVT								
MV+HVT								
Rispens								
HVT					*			
Pos	*					*		
Neg					*		*	

*Indicates significant differences (p< 0.05) between groups by the least significant difference (LSD) test.

Figure 2. Thymus Score. At ten weeks after challenge all remaining birds in the experiment were euthanased and assigned a thymus score. Thymus scores were graded 0-3 where a score of three was normal and one of zero indicated total atrophy. The results are presented as the mean thymus score per group +/- the standard error of the mean (SE).



Analysis of variance (ANOVA) results:

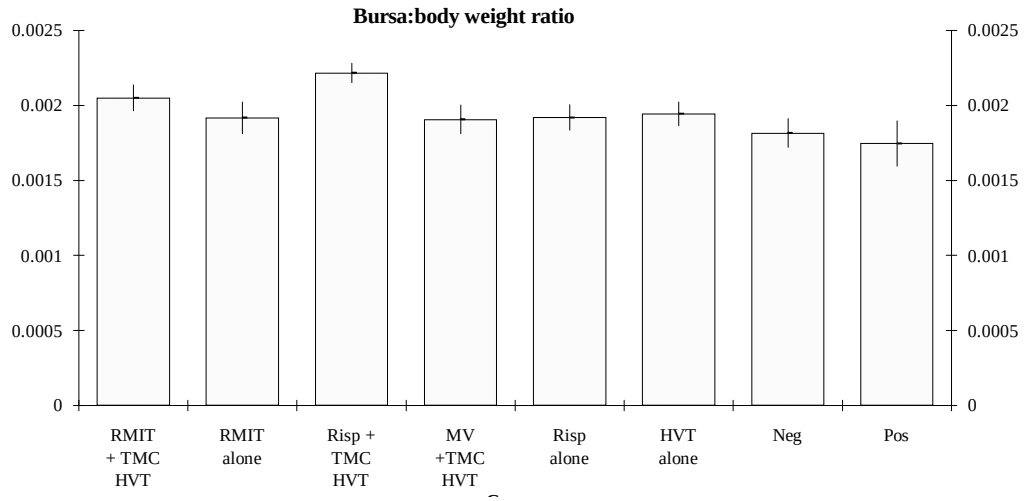
Group effects were significant (p0.00)

Body weights

	RMIT+HVT	RMIT	Rispens+HVT	MV+HVT	Rispens	HVT	Pos	Neg
RMIT+HVT								
RMIT	*							
Rispens+HVT								
MV+HVT								
Rispens								
HVT								
Pos	*	*	*	*	*	*		
Neg	*		*	*	*	*	*	

* Indicates significant differences (p< 0.05) between groups by the least significant difference (LSD) test.

Figure 3. Body weights. At the end of the experiment the body weights of each bird in each group were taken and averaged. The results are presented as the mean bird body weight +/- the standard error of the mean (SE).



Analysis of variance (ANOVA) results:

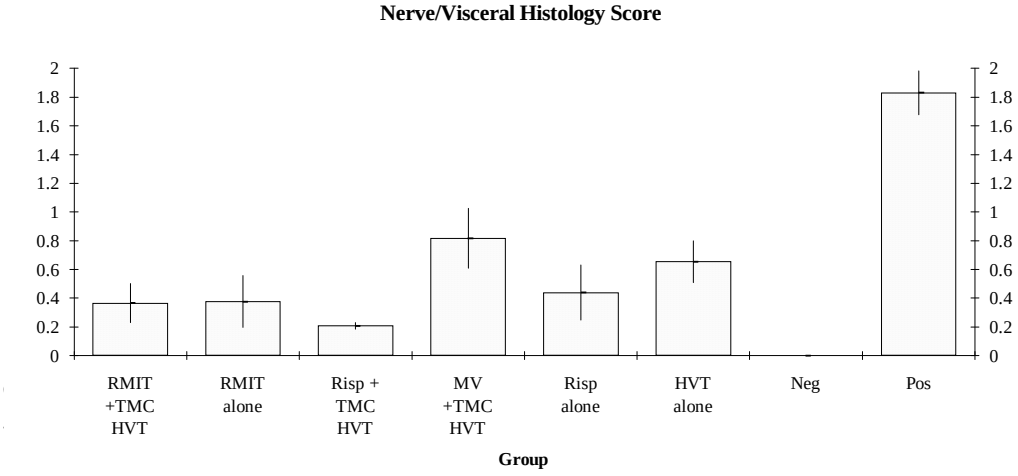
Group effects were significant (p0.035)

B:B weight ratios

	RMIT+HVT	RMIT	Rispens+HVT	MV+HVT	Rispens	HVT	Pos	Neg
RMIT+HVT								
RMIT								
Rispens+HVT		*						
MV+HVT			*					
Rispens			*					
HVT			*					
Pos	*		*					
Neg			*					

*Indicates significant differences (P< 0.05) between groups by the least significant difference (LSD) test.

Figure 4. Bursa:body weight ratios of commercial birds of various vaccine groups. After euthanasia at ten weeks after challenge, the body and bursa weights of the birds were taken and graphed as a mean ratio +/- the standard error of the mean (SE).



Analysis of variance (ANOVA) results:

Group effects were significant (p0.002)

Histology Score

	RMIT+HVT	RMIT	Rispens+HVT	MV+HVT	Rispens	HVT	Pos	Neg
RMIT+HVT								
RMIT								
Rispens+HVT								
MV+HVT								
Rispens								
HVT								
Pos	*	*	*	*	*	*		
Neg				*			*	

*Indicates significant differences (p< 0.05) between groups by the least significant difference (LSD) test.

Figure 5. Nerve/Visceral Histology Score. Histology was performed on 10 randomly chosen birds per group and on any suspect tissues. The assessment of the lesions was by the criteria in Table 3, and scores of each bird was summed and averaged for the group. The final histology score (as plotted) was derived by subtracting the mean score of the negative control group from the mean score of the vaccine group +/- the standard error of the mean (SE).

4. Discussion

CAV could not be detected by the polymerase chain reaction (PCR) in the blood lymphocytes of birds during the trial. CAV can account for increases in the virulence and in the number of deaths in a challenge experiment. However, because it was not detected, it can be assumed that observed lesions and other clinical signs were the result of MD challenge alone.

The packed cell volume of a blood sample is a useful objective correlate of the incidence of MD in challenged birds. However, the PCV results of this trial are only indicative of the residual virulence in the vaccines. From Figure 1, the PCV of birds following vaccination was reduced, although the difference with unvaccinated birds was not significant.

Table 4 indicates the highest rate of protection was obtained by the Rispens vaccine when used in combination with The Marek's Company HVT. RMIT in combination with HVT produced a comparable rate of protection. The RMIT vaccine used alone appeared to be not as protective as the other vaccines. The incidence of MD in the positive control, which was derived from a vaccinated flock, was lower than obtained for SPF birds (De Laney & Tannock 1999).

Figure 2 shows The Marek's Company HVT vaccine performed better than the others with respect to thymus score. According to thymus score, the RMIT vaccine when used in combination with HVT also performed better. The positive control group showed an unusually high thymus score with only two vaccinated groups (HVT and RMIT + HVT) significantly higher.

Body weights (Figure 3) for the challenge group were less than the negative controls, which were housed separately. They were the highest for the group vaccinated with the RMIT vaccine and were the lowest for the RMIT vaccine when used in combination with HVT. These results do not correlate with the thymus score or protection data obtained when the RMIT vaccine was used in combination with HVT. Birds vaccinated with Rispens + HVT also performed well by this criterion. These results do correlate with results from other sections of the experiment.

Statistically, body weights for the RMIT vaccine and Rispens + HVT were not significantly different than those from other vaccine groups.

The bursa:body weight ratios (Figure 4) show a similar trend to that reflected by the protective index figures. Once again, the RMIT vaccine in combination with HVT performed well. Rispens in combination with HVT performed best. Groups receiving these two vaccines also performed well and are the only two groups that differ significantly from the positive control.

As expected, the positive control group had the highest histology score (Figure 5), indicating that those birds had a greater number of lesions than any vaccinated group. Birds vaccinated with Rispens in combination with HVT had the least lesions followed by RMIT in combination with HVT. From histological scores alone, the RMIT vaccine when used alone gave the highest rates of protection. Scores from all vaccine groups were not statistically different from one another.

In conclusion, these experiments indicate that the RMIT vaccine when used in combination with The Marek's Company HVT was relatively efficacious, although the Rispens vaccine in combination with HVT performed marginally better. When used alone, the RMIT vaccine provided poor protection.

Commercial birds usually possess maternal antibodies which may influence the performance of individual vaccines and the incidence of infection in control birds. The birds in the experiment were presumed to possess maternal antibodies to the Rispens vaccine which had been administered to their parents. The success of Rispens and RMIT vaccines when given simultaneously with HVT could indicate an immunogenic contribution from other antigens to overall MD immunity. The contribution of genetic susceptibility could also be a factor.

5. Appendices

Appendix 1. Bird deaths during the experiment

[a]Marek's disease

Vaccine group	Wing tag	Gross lesions	Histological lesions	MD[a] status
ORANGE RMIT + HVT	41	+	+	+
GREY RMIT	1	+	+	+
	3	+	+	+
	8	+	+	+
	12	+	+	+
	21	+	+	+
	26	+	+	+
	58	+	+	+
	60	+	+	+
	62	+	+	+
	65	+	+	+
	68	+	+	+
GREEN RISPENS + HVT	6	-	-	-
	45	N/A	N/A	N/A
	61	+	+	+
BLUE MV + HVT	33	-	-	-
	56	-	-	-
RED RISPENS	45	-	-	-
	26	+	+	+
WHITE HVT	7	-	-	-
	23	-	-	-
	26	-	-	-
	75	-	-	-
	61	-	-	-
YELLOW POSITIVE CONTROL	2	-	-	-
	12	-	-	-
	23	-	-	-
	8	+	+	+
	10	+	+	+
	11	+	+	+
	14	+	+	+
	26	+	+	+
	35	+	+	+
	36	+	+	+
	39	+	+	+
	40	+	+	+
	41	+	+	+
	42	+	+	+
	43	+	+	+
	49	+	+	+
	57	+	+	+
	61	+	+	+
	63	+	+	+
	66	+	+	+
	68	+	+	+
NEGATIVE	NO DEATHS			

6. References

DE LANEY, D. B., MORROW, C. J., READ, K. M. & TANNOCK, G.A. (1998). The development and evaluation of two tissue culture-grown Marek's disease challenge viruses. *Avian Pathology* **27**, 472-477.

DE LANEY, D. B. & TANNOCK, G. A. (1999). Development of Marek's disease serotype 1 vaccine. Final report to Joint Chicken Meat and Egg Industry Research and Development Council, RMIT-12E.

MORROW, C. J. (1995). Assessment of attenuation of Marek's disease vaccine seeds. Final report to Joint Chicken Meat and Egg Industry Research and Development Council, DAV 41 CM.

MORROW, C. J., CONDRON, R. J., DE LANEY, D. B. & TANNOCK, G. A. (1997). Development of standard MDV challenge viruses in tissue culture. Final report to Joint Chicken Meat and Egg Industry Research and Development Council, DAV 115 AJ.