

# Field evaluations of safety and efficacy of an Australian Marek's disease vaccine

RC KARPATY<sup>ab</sup>, GA FIRTH<sup>a</sup> and GA TANNOCK<sup>c</sup>

**Objective** To demonstrate the safety and efficacy of the Marek's Disease Virus-1 vaccine (strain BH 16) from field studies in comparison with the CVI 988 Rispens vaccine currently available in Australia.

**Study design** A small field trial was carried out on nine breeder flocks and a larger trial on 21 breeder flocks. All chickens were obtained from a commercial hatchery and each was vaccinated at hatch with cell-associated Herpes Virus of Turkeys vaccine. A group of chickens vaccinated with BH 16 vaccine was placed in one shed per property and the remainder were vaccinated with the Rispens vaccine and placed in the remaining sheds. At 25, 30, 35, and 40 weeks after hatch, the field veterinarian or farm manager examined all birds dying on two consecutive days in the designated placement sheds.

**Results** In the small trial there was a significantly lower incidence of MD in birds vaccinated with the MDV-1 vaccine compared with the Rispens vaccine ( $P < 0.001$ ). In a larger trial there was no difference in the incidence of MD between the treatment groups, due possibly to a lower rate of natural challenge. Egg production results and average weekly mortality results for both groups were similar.

**Conclusion** The present study describes an attenuated type 1 MD vaccine which is at least equivalent to a vaccine derived from the CVI 988 Rispens strain in terms of safety and efficacy when used in combination with HVT vaccine.

*Aust Vet J* 2003;81:222-225

HVT	Herpesvirus of turkeys
MD	Marek's disease
MDV	Marek's disease virus
TCID <sub>50</sub>	50% tissue culture infective dose

Marek's disease is a lymphoproliferative disease of commercial poultry caused by an alphaherpesvirus. Three serotypes of MDV are recognised. Serotype 1 viruses are pathogenic for chickens; serotype 2 and 3 viruses (including HVT) are naturally apathogenic viruses of chickens and turkeys, respectively. Viruses of serotypes 2 and 3 have been successfully used as vaccines throughout the world for over 30 years. Since the early 1990s, however, there has been a global increase in the virulence of serotype 1 viruses, which has led to the use of attenuated serotype 1 viruses as vaccines to provide optimum protection.<sup>1</sup> Until recently such vaccines were not available in Australia because of quarantine regulations. In a previous report an Australian attenuated MDV-1 vaccine (strain BH 16) was developed into a vaccine and shown to be

safe and efficacious under laboratory conditions.<sup>2</sup> This paper describes the results of two field trials of the vaccine.

## Materials and methods

### Vaccines

**Serotype 1 vaccines** – MDV-1 vaccine: Vaccine batch, passage level 49 of the attenuated strain BH 16<sup>2</sup> administered at the rate of 2500 TCID<sub>50</sub> per dose by subcutaneous injection. TMC Rispens: the Rispens CVI-988 strain<sup>3,4</sup> supplied by The Marek's Company, Bioproperties (Australia) Pty Ltd, Ringwood, Victoria. The vaccine was used according to the manufacturer's instructions.

**Serotype 3 (HVT) vaccine** – Cell-associated HVT, strain NSW 1/70, was supplied by Intervet Australia Pty Ltd, Black Hill, New South Wales and was used according to the manufacturer's instructions.

### Field trial design

Two trials were conducted. A small field trial consisting of nine breeder flocks was carried out from March to August 1998. In the larger trial 21 breeder flocks were placed from September 1998 to December 1999. All chickens were hatched at Bartter's Warabrook hatchery and each was vaccinated at hatch with HVT vaccine. Chickens sufficient for one shed per property were then vaccinated with MDV-1 vaccine and the chickens in the remaining sheds were vaccinated with TMC Rispens vaccine. In order to demonstrate efficacy, the field veterinarian or farm manager examined all birds dying on two consecutive days at 25, 30, 35 and 40 weeks of age, and total mortality and the mortality due to MD were reported. The results were analysed by estimating the proportion of the deaths due to MD per shed compared with that from other causes. The proportion was standardised to the total mortality at 40 weeks. That is, if 5000 birds placed had 10% mortality at 40 weeks, 20% of which was due to MD:

$$\text{Total MD deaths} = 5000 \times 0.1 \times 0.2 = 100$$

A field diagnosis of MD was made on the basis of the presence of lymphoid tumours in the viscera or skeletal muscle of birds not younger than 6 weeks. Lymphomatous lesions were typically found in the ovary, lung, mesentery, kidney, liver, spleen and proventriculus.

The average percent weekly mortality was calculated for each week of rearing and from weeks 20 to 40 in lay. The number of eggs per hen housed was a production statistic recorded for each shed. These two parameters were chosen to compare the safety of the MDV-1 vaccine with that of the TMC Rispens vaccine under field conditions.

## Results

The results of the small field trial are summarised in Table 1. The combined data indicate that there was a statistically lower incidence of MD in the birds vaccinated with MDV-1 compared with those vaccinated with the TMC Rispens vaccine (Student's *t*-test;  $P < 0.001$ ). The incidence of MD in the TMC

<sup>a</sup>Intervet Australia Pty Ltd, Vaccine Production Laboratory. PO Box 285, Beresfield, New South Wales 2322

<sup>b</sup>These findings are taken from thesis by the senior author, in part fulfilment of requirements for a PhD degree at RMIT University, Melbourne, Victoria.

<sup>c</sup>Department of Biotechnology and Environmental Biology, RMIT University, PO Box 71, Bundoora, Victoria 3083

Rispens-vaccinates was higher on farms Vic-1 (6.04%) and NSW-3 (9.03%). Chickens in nearby sheds on the same farms that were vaccinated with MDV-1 had lower levels of MD (2.28% and 1.32%, respectively). No MD was observed on four farms and the incidence of MD in the other three was similar for both treatment groups.

Table 2 presents the results for the large field trial. Five of the 21 farms had no reported observations of MD. There was no significant difference in either the observed or calculated incidence of MD ( $\chi^2$ :  $P < 0.05$ ) or between the average calculated % MD (Student's t-test;  $P < 0.05$ ) in birds given either vaccine. The overall incidence of MD following natural challenge was

**Table 1. MD mortality and calculated MD deaths and % MD for the small field trial.**

Farm	MDV-1 vaccinates					TMC Rispens vaccinates				
	No. placed	No. with lesions <sup>a</sup>	% lesions	Total MD deaths <sup>b</sup>	% MD <sup>c</sup>	No. placed	No. with lesions	% lesions	Total MD deaths	% MD
NSW-1	5087	4/31	12.9	103	2.03	5095	8/29	27.6	181	3.54
VIC-1	6106	7/26	26.9	139	2.28	6642	33/63	52.4	401	6.04
NSW-2	7522	7/35	20	157	2.08	7521	5/33	15.1	103	1.37
VIC-2	6987	10/36	27.8	210	3.01	6641	11/39	28.2	190	2.86
NSW-3	7642	4/20	19	101	1.32	7610	31/80	38.7	687	9.03
VIC-3	7356	0/24	0	0	0	7352	0/40	0	0	0
QLD-1	6066	0/50	0	0	0	6057	0/48	0	0	0
SA-1	6448	0/22	0	0	0	6524	0/24	0	0	0
QLD-2	5179	0/18	0	0	0	5177	0/30	0	0	0

<sup>a</sup>Observed MD incidence from field reports.

<sup>b</sup>Total MD deaths = number of chickens placed x % mortality to 40 weeks x % observed MD from field reports.

<sup>c</sup>% MD = total MD deaths ÷ number placed.

**Table 2. MD mortality and calculated MD deaths and % MD for the large field trial.**

Farm	MDV-1 vaccinates					TMC Rispens vaccinates				
	No. placed	No. with lesions <sup>a</sup>	% lesions	Total MD deaths <sup>b</sup>	% MD <sup>c</sup>	No. placed	No. with lesions	% lesions	Total MD deaths	% MD
NSW-4	8867	6/22	27.3	235	2.65	8809	2/30	6.7	98	1.11
QLD-3	9404	3/40	7.5	47	0.50	9528	1/26	3.8	16	0.16
NSW-5	6944	21/41	51.2	335	4.82	6960	1/31	3.2	13	0.19
VIC-4	5550	4/24	16.7	47	0.84	5504	8/23	34.8	133	2.42
VIC-5	6528	0/21	0	0	0	6528	0/20	0	0	0
NSW-6	8897	0/25	0	0	0	9347	6/41	14.6	101	1.08
QLD-4	7514	0/36	0	0	0	7534	0/31	0	0	0
VIC-6	7158	1/12	8.3	22	0.31	7174	1/14	7.1	19	0.27
SA-1	6991	1/31	3.2	9	0.14	7234	1/25	4.0	8	0.11
NSW-7	4821	1/25	4.0	18	0.37	4833	0/29	0	0	0
QLD-4	10191	0/45	0	0	0	10601	1/40	2.5	15	0.14
NSW-1	4690	0/58	0	0	0	4962	1/43	2.3	27	0.54
NSW-8	7150	1/48	2.1	22	0.31	7150	5/45	11.1	101	1.42
NSW-2	6846	7/36	19.4	201	2.93	6979	2/33	6.1	43	0.61
VIC-2	7390	0/18	0	0	0	7408	0/11	0	0	0
VIC-1	6978	0/97	0	0	0	7035	0/30	0	0	0
NSW-3	6912	0/29	0	0	0	6912	4/59	6.8	66	0.95
SA-1	6485	2/28	7.1	40	0.61	6424	1/37	2.7	15	0.24
QLD-1	6107	6/31	19.3	65	1.06	6106	7/42	16.7	99	1.62
VIC-3	7538	0/39	0	0	0	7589	0/44	0	0	0
NSW-9	6617	2/41	4.9	44	0.66	6618	6/41	14.6	127	1.92

<sup>a</sup>Observed MD incidence from field reports.

<sup>b</sup>Total MD deaths = number of chickens placed x % mortality to 40 weeks x % observed MD from field reports.

<sup>c</sup>% MD = total MD deaths ÷ number placed.

lower in the large field trial. A summary of the results of both trials is presented in Table 3.

The average percent weekly mortality was calculated for all sheds within a test group for each week during rearing, and up to 40 weeks of age in lay. In the small field trial, the rearing mortality for both groups followed a similar pattern, except for a peak at 5 weeks in the MDV-1 vaccinates resulting from a coccidiosis outbreak on the SA-1 farm in which the weekly mortality in the affected shed reached 13.1% (Figure 1). The mortality on the laying farms was also similar (Figure 2). The higher average weekly mortality seen in TMC Rispens-vaccinates between 25 and 32 weeks was due to the higher mortality on the NSW-3 farm (see Table 1).

Mortality during rearing and lay was similar for both treatment groups in the large field trial (Figures 3 and 4). The higher mortality in the MDV-1-vaccinates between 27 and 31 weeks was due to reproductive problems induced by early over-stimulation with light and feed at point-of-lay on the NSW-1 farm where weekly mortality was 2 to 3% over this time (T. Ryan, personal communication).

Tumours of the ovary interfere with reproductive function<sup>5</sup> as can the nonspecific debilitating signs such as weight loss and anorexia<sup>6</sup> and therefore, the eggs per hen housed results for the two vaccines were compared. With the exception of the NSW-3 farm, where there was a high incidence of MD in the TMC

Table 3. Comparison of field trial results.

	Field trial 1		Field trial 2	
	MDV-1	TMC Rispens	MDV-1	TMC Rispens
Total observed MD	32	88	55	47
Total observed deaths	263	386	747	695
Total number placed	58,393	58,619	149,578	151,235
Average % cumulative mortality at 40 weeks	8.43%	10.11%	9.54%	8.53%
% observed MD	11.84%	18.00%	8.15%	6.53%
Calculated total MD deaths	710	1562	1084	881
Average calculated % MD	1.22% <sup>A</sup>	2.67% <sup>B</sup>	0.72% <sup>C</sup>	0.61% <sup>C</sup>

<sup>ABC</sup>Different superscripts within the same trial indicate significant differences (Student's t-test, P < 0.001).

Table 4. Eggs per hen housed for both field trials.

Field Trial	Age (w)	Vaccine	
		MDV-1	TMC Rispens
1	64.3	134.16	129.05
2	40	67.17	69.75

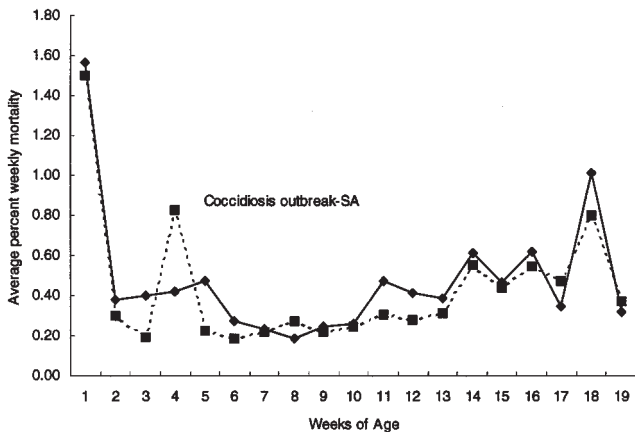


Figure 1. Overall average percent weekly mortality in the small field trial during rearing. Broken lines show results for the MDV-1 group, solid lines the TMC Rispens group.

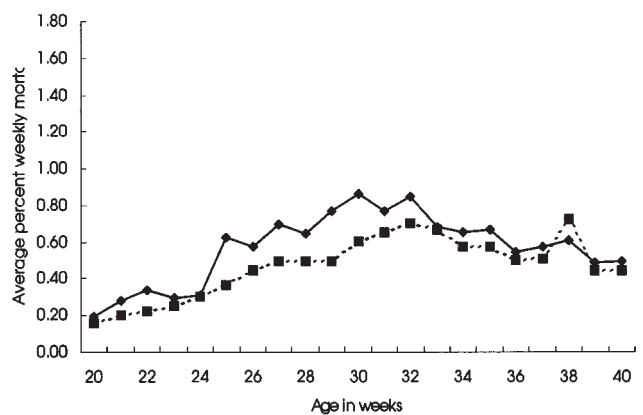


Figure 2. Overall average percent weekly mortality in the small field trial during lay up to 40 weeks. Broken lines show results for the MDV-1 group, solid lines the TMC Rispens group.

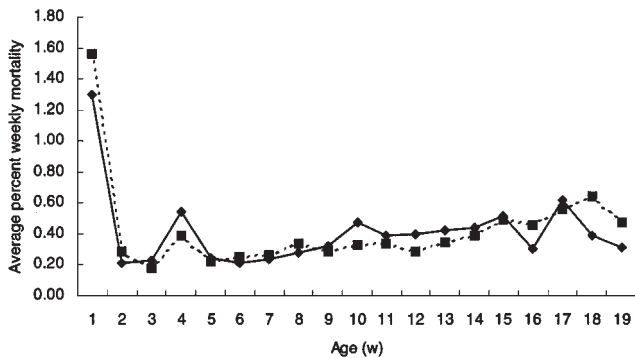


Figure 3. Overall average percent weekly mortality in the large field trial during rearing to 19 weeks. Broken lines show results for the MDV-1 group, solid lines the TMC Rispens group.

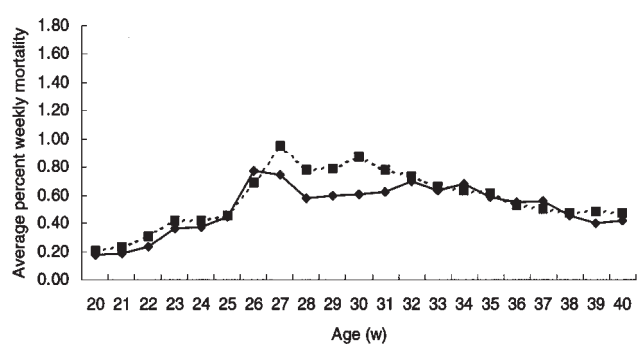


Figure 4. Overall average percent weekly mortality in the large field trial during lay to 40 weeks. Broken lines show results for the MDV-1 group, solid lines the TMC Rispens group.

Rispens-vaccinates, egg production results from both groups were similar in both field trials where there was no significant difference between treatment groups ( $\chi^2$ :  $P > 0.05$ ; Table 4).

## Discussion

The results of laboratory studies of efficacy and safety for the MDV-1 vaccine previously reported<sup>2</sup> were confirmed in the two field trials. Birds vaccinated with MDV-1 and HVT had significantly fewer MD lesions ( $P < 0.001$ ) than those vaccinated with TMC Rispens and HVT in the first field trial. The overall incidence of MD in these groups was 12.2% and 22.8%, respectively. The incidence of MD was the only parameter measured to show a difference between treatment groups. Egg production and average weekly mortality were similar with the exception of one farm (NSW-3) where birds vaccinated with TMC Rispens and HVT had exceptional MD mortality (Table 1). As the NSW-3 farm was the site of the original vaccine isolate, it seems likely there may have been greater antigenic similarity between the MDV-1 vaccine and the field strain on that site. There was no difference between the treatment groups in the second trial, possibly explained by a lower level of natural challenge as indicated by the fall in the overall incidence of MD. The MDV-1 vaccine therefore appears safe when used under field conditions.

The objective of this work was to develop a serotype 1 MD vaccine from a highly virulent MDV pathotype with demonstrated safety and efficacy against early challenge with very virulent field strains in the presence of maternal antibodies. The

vaccine developed (MDV-1, strain BH 16), fulfils these requirements and complies with the safety and efficacy criteria specified in the European Pharmacopoeia.<sup>7</sup> Additionally, the vaccine performed at least as well as TMC Rispens vaccine in laboratory<sup>2</sup> and field trials when used together with HVT vaccine. Vaccines derived from the CVI 988/ Rispens strain are currently considered most suitable for protection against vvMDV. The vaccine developed here may have a significant role in the control of MD.

## References

1. Witter RL, Lee LF, Fadly AM. Characteristics of CVI 988/ Rispens and R2/23, two prototype vaccine strains of serotype 1 Marek's disease virus. *Avian Dis* 1995;39:269-284.
2. Karpathy RC, Firth GA, Tannock GA. Derivation, safety and efficacy of a Marek's disease vaccine developed from an Australian isolate of very virulent Marek's disease virus. *Aust Vet J* 2002;80:61-66.
3. Rispens BH, van Volten HJ, Mastenbroek N, Maas HJL, Schat KA. 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* 1972;16:108-125.
4. Rispens BH, van Volten HJ, Mastenbroek N, Maas HJL. Control of Marek's disease in The Netherlands. II. Field trials on vaccination with an avirulent strain (CVI 988) of Marek's disease virus. *Avian Dis* 1972;16:126-138.
5. Purchase HG. Clinical disease and its economic impact. In: *Marek's disease: Scientific basis and methods of control*. Martinus Nijhoff Publishing, Boston, 1985:17-42.
6. Calnek BW, Witter RL. Marek's disease. In: Calnek BW, editor. *Diseases of poultry*. 10th edn. Iowa State University Press, Iowa, 1997:369-413.
7. Anonymous. Marek's disease vaccine (live). In: *European Pharmacopoeia*. 3rd edn. Council of Europe, Strasbourg, 1997:1144-1146.

(Accepted for publication 2 September 2002)

---

## Effects of diet restriction on life span and age-related changes in dogs

**F**orty-eight Labrador Retrievers were paired at birth on the basis of sex and bodyweight. From 8 weeks of age one of the pair was fed a nutritionally balanced diet ad libitum, while the following day the other was fed 75% of the amount consumed by its pair-mate. The diet was changed from a growth to an adult formulation at 3.25 years of age. At the same time the intake of the ad libitum group was regulated to prevent any insidious obesity, with the 25% differential food intake maintained.

Forty-six of the dogs were eventually euthanased on humane grounds. The study continued until 90% of the dogs had died (13 years) and found food restriction produced significant increases in median life span as well as delaying the first appearance of chronic disease.

Kealy RD et al. *J Am Vet Med Assoc* 2002;220:1315-1320.

## Effects of postoperative rehabilitation on limb function after cranial cruciate surgery in dogs

**T**wenty-five dogs were subjected to a regimen of measured active exercise for a recuperative period of 16 weeks after unilateral cranial cruciate surgery. Concurrently, 26 dogs that underwent the same surgery were allowed only restricted exercise for 16 weeks.

At presentation all the dogs weighed between 20 and 40 kg and had suffered ruptured CCL and torn medial meniscus. One surgeon operated upon all the cases, using the same technique, which involved removal of the ruptured ligament, medial meniscectomy and lateral retinacular stabilisation of the stifle joint.

The authors conclude that a postoperative rehabilitation program is preferable to exercise restriction and will most likely result in better limb function. While their program was focussed on swimming, they do not suggest that this is the only regimen that could be successful, and emphasise that any program should be tailored to the individual patient.

Marsolais GS et al. *J Am Vet Med Assoc* 2002;220:1325-1330.

---