

An attenuated strain of canine distemper virus for stoat and ferret control

Tao Zheng and Henry Chiang

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ABSTRACT

To evaluate the potential virulence of modified-live canine distemper virus (MLV-CDV) vaccines for mustelids, four commercial MLV-CDV vaccines that are currently available and licensed in New Zealand were tested. A CDV-neutralising antibody response was detected in the blood of 10 of 18 inoculated ferrets and daily checks revealed no signs of clinical distemper, indicating that these vaccines were avirulent for ferrets. To develop a strain of CDV for stoat control, we attempted to attenuate a virulent Snyder Hill distemper virus in embryonated eggs. However, the inoculum was only weakly virulent for ferrets, and those passages in embryonated eggs resulted in a complete loss of virulence for ferrets. A MLV-CDV vaccine reported to be virulent for ferrets and mink and licensed overseas was tested in four ferrets. Apart from one ferret which died following blood collection on day 1, the other three inoculated ferrets produced a strong antibody response and showed no signs of clinical distemper. Low titres of CDV were detected by RT-PCR from the ferrets' spleens. This vaccine was serially passaged 13 times in ferrets to enhance its virulence. Splenomegaly, a typical distemper pathology, was observed in ferrets after the ninth back passage. In summary, currently licensed MLV-CDV vaccines are not suitable for the biological control of mustelids in New Zealand. Serial back passage of a MLV-CDV in ferrets represents one of the most promising approaches to produce a MLV-CDV useful in the biological control of stoats and other mustelids.

Keywords: Canine distemper virus, CDV, attenuation, stoat, ferret, biological control, serological prevalence

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1. Introduction

Canine distemper virus (CDV) is a single stranded, negative sense RNA virus, and is a member of the genus *Morbillivirus*, subfamily *Paramyxovirinae*, in the family *Paramyxoviridae*. Other members in the *Morbillivirus* genus are measles virus, cetacean morbillivirus, peste-des-petit-ruminants virus, phocine distemper virus and rinderpest virus. CDV is the pathogen for canine distemper, an acute to subacute contagious systemic disease that can bring about dysfunction in the respiratory, gastrointestinal and central nervous systems. The main transmission route for CDV is by direct contact with aerosol respiratory secretions. Recovery from infection depends largely on the ability of the host to mount an early immune response to the virus (Appel 1969).

Natural infections of CDV have been reported in many different species including those belonging to the orders Carnivora (families Canidae, Mustelidae, Hyaenidae, Procyonidae, Ailuridae, Viverridae and Felidae), Pinnipedia (family Phocidae), Ungulata (family Tayassuidae) and Primates (family Cercopithecidae) (O'Keefe 1995). The pathogenicity of CDV infection varies greatly between species and ranges from asymptomatic infection to high mortality. Dogs and ferrets are highly susceptible to CDV infection. The reported mortality rate for dogs from experimental exposure to CDV aerosols or via the intravenous route was about 50% (Appel 1969), while the mortality rates of experimentally and naturally infected ferrets were estimated at 90–100% (Dunkin & Laidlaw 1926; Spooner 1938). A cyclic epizootic of distemper was noted in dogs (Gorham 1966).

Viruses have been used in a cost-effective manner to control vertebrate pests. For example, myxoma virus and rabbit haemorrhagic disease virus have been used to control wild rabbits. CDV is a possible candidate for the biological control of stoats. Ferrets are extremely susceptible to CDV infection, with high mortality rates (Dunkin & Laidlaw 1926). An natural outbreak of CDV in black-footed ferrets (*Mustela nigripes*) almost eliminated a colony in Wyoming, USA (May 1986; Williams et al. 1988) and stoats are also reported to be susceptible to CDV infection (Keymer & Epps 1969). An epizootic of CDV could target a large stoat population especially in areas where access (for other control methods) is difficult, providing a very cost-effective method of controlling mustelids in New Zealand. It is not clear if wild mustelids in New Zealand have had prior contact with CDV. However, a preliminary study of sera collected from of two sites in New Zealand indicated that CDV-neutralising antibodies were not present in feral ferrets (O'Keefe 1995).

There are potential negative impacts associated with the use of virulent CDV for the biological control of stoats and other mustelids in New Zealand. First, unvaccinated dogs are susceptible to virulent CDV infection and could become infected by contact with infected mustelids. Second, virulent CDVs may also pose a potential threat for marine mammals in New Zealand, as some marine mammals are susceptible to CDV infection and suffer high mortality rates (Osterhaus 1989; Osterhaus & Vedder 1988; Visser et al. 1990, 1993a, b). Third, there may be animal welfare concerns of ferrets and stoats dying of distemper virus infection (Dunkin & Laidlaw 1926; Spooner 1938).

Modified-live vaccine strains of CDV (MLV-CDV) could offer an alternative approach to virulent CDV. There is evidence that MLV-CDVs that were developed for domestic dogs are lethal in other species. The 'Green' MLV-CDV, widely used in dogs to prevent distemper in Europe and USA in the 1940s, was highly virulent for ferrets (Green 1939, 1945). Vaccine-related deaths were reported in red pandas (*Ailurus fulgens*), kinkajous (*Potos flavus*) and black-footed ferrets (Bush et al. 1976; Carpenter et al. 1976; Montali et al. 1987). Post-vaccination clinical distemper deaths have also been reported in grey foxes (*Urocyon cinereoargenteus*) and fennec foxes (*Fennecus zerda*) (Halbrooks et al. 1981; Montali et al. 1987). Two recent incidents of post-vaccination deaths from CDV in mink (*Mustela vison*) were reported in USA and Finland (Sutherland-Smith et al. 1997; Ek-Kommonen et al. 2003). An outbreak of post-vaccination distemper in farmed ferrets killed approximately 350 of 6000 young ferrets in 1985 in New Zealand (Gill et al. 1988). In this outbreak, many ferrets had no prior signs of disease, suggesting that MLV-CDV may cause a less painful clinical infection than virulent CDV infection (Gill et al. 1988). As MLV-CDVs are attenuated in dogs, they would pose minimal threat to the unvaccinated dogs in New Zealand.

As the variation of CDV pathogenicity from species to species ranges from 100% mortality in ferrets to 25-50% mortality in dogs (reviewed in Appel & Gillespie 1972), we proposed that MLV-CDV of certain passage (attenuation) levels could cause considerable mortality in mustelids but be innocuous for dogs and marine mammals. This could be a cost-effective control strategy for stoats and other mustelids in New Zealand.

The objectives of this project were to test the potential virulence for ferrets/ stoats of MLV-CDVs that are licensed for dogs in New Zealand, and to determine the infection status of CDV in stoats by assessing the prevalence of the CDV-neutralising antibody in feral stoats in New Zealand.

2. Materials and methods

2.1 Canine distemper viruses

Various strains of CDV were obtained including those in the multivalent commercial vaccines (Table 1). The Onderstepoort strain was propagated in Vero cells in the laboratory and used in serological tests. Onderstepoort and Snyder Hill strains were stored at -75°C , while CDV commercial vaccines were stored at $4-8^{\circ}\text{C}$.

2.2 Experimental design

Ferrets

Domestic ferrets (*Mustela putorius furo*) were obtained from AgResearch Invermay Research Centre and further stock was bred at Wallaceville. Ferrets were housed in a purpose-built ferret containment facility in free-range pens and were moved to individual cages for the challenge trials. The animals were provided with a commercial ferret meal and water *ad libitum*. All ferrets tested negative for CDV antibodies prior to challenge trials. All procedures involving the experimental use of the ferrets were approved by the Wallaceville Animal Ethics Committee, Upper Hutt.

Potential virulence of commercial CDVs in ferrets

Ten dog-doses of vaccines (Vanguard 5, CanVac 3, Canigen DH_{A2}PPi and NOBIVAC DHP) were reconstituted in 2.0 mL of the vaccine diluent and administered to ferrets in groups 1A, 1B, 2A and 2B (see Table 2). Approximately 1.5 mL was inoculated subcutaneously (s.c.) in the dorsal aspect

TABLE 1. CDV STRAINS USED IN THIS STUDY.

STRAIN	SOURCE	BATCH	DERIVATIVE	CDV TITRE (TCID ₅₀)	CONTAINED OTHER LIVE VACCINE VIRUSES
Onderstepoort	Massey University	NA	Vero Cell culture	10 ^{5.5} /mL	No
Snyder Hill CDV-AER [†]	USDA	90-18	Ferret spleen extract	Unknown	No
Snyder Hill CDV-IC [†]	USDA	98-07	Dog brain extract	Unknown	No
Vanguard 5*	Pfizer Animal Health	A123737A	Cell culture	Unknown	CAV-2, CPI, CPV
Canvac 3*	CSL (NZ) Ltd	0943-24701 & 0943-25001	Cell culture	Unknown	CAV-2, CPV-2
Canigen DH _{A2} PPi*	Virbac Laboratories (NZ) Ltd	V069	Cell culture	10 ³ /dose	CAV-2, CPI, CPV
NOBIVAC DHP*	Intervet International	405-0012	Cell culture	Unknown	CAV-1, CPV
SOLO-JEC-7 [‡]	Boehringer Ingelheim Vetmedica, Inc.	GG-775-302C	Cell culture	10 ^{3.6} /dose	CAV-1, CPI, CPV

Abbreviations used: CAV-1, -2 = canine adenovirus type 1, type 2; CPI = canine parainfluenza virus; CPV = canine parvovirus; NA = not applicable; TCID₅₀ = median tissue culture infectious dose; USDA = United States of Department of Agriculture.

* Vanguard 5, Canvac 3, Canigen DH_{A2}PPi, and NOBIVAC DHP were licensed vaccines in New Zealand.

[†] Permissions were obtained from MAF Biosecurity for the use of Snyder Hill CDV and SOLO-JEC-7 in ferrets held in the ferret containment facility at Wallaceville.

[‡] SOLO-JEC-7 was a licensed vaccine in USA.

of the neck and approximately 0.25 mL was injected intramuscularly (i.m.) to each back leg. A single dose of SOLO-JEC-7 was used in ferrets in trial 3 (Table 2). Control ferrets were injected similarly with the diluent of each vaccine (groups 1C, 1D, 2C and 2D).

Prior to inoculation, each animal was weighed and a blood sample was collected and rectal temperature measured. Following the inoculation, animals were observed daily, and the presence or absence of clinical signs consistent with CDV infection was recorded. The assessed parameters were appetite (based on daily food consumption), overall status (normal or less than normal inquisitive activity, curled up, less response, moribund), coat status and skin changes (normal or rash and erythema), eyelid status (normal or swollen, puffy, vesicles; left or right side or both), oculonasal discharge (none or watery, purulent, pus, sticky; left or right side or both), gastrointestinal function (normal or diarrhoea) and central nervous system status (normal or dazed, wobbly gait, convulsion, screaming). Post-inoculation (p.i.) blood samples were collected at approximately weekly intervals to test for the presence of CDV antibodies. Body weights were recorded at the times of blood sampling. Animals were euthanased on day 55 p.i for trials 1 and 2 and on day 34 p.i. for trial 3. Various tissue samples were collected at necropsy for virus detection.

2.3 Serological assay

Procedure

A virus neutralising test (VNT) was performed in 96-well flat-bottomed tissue culture plates using the constant virus and varied serum methodology. All test sera were heat-inactivated at 56°C for 30 min. Two-fold dilutions of serum samples (1:2 to 1:128 per 50 µL/well) were performed in a microtitre plate. An extra well of serum at 1:2 served as a serum control. CDV-neutralising antibody positive and negative control sera were included in the test. Fifty microlitres of approximately 100 TCID₅₀ (median tissue culture infectious dose) Onderstepoort CDV was added to each well, except for those of the serum controls, to which 50 µL/well (1 × 10⁵/mL) of culture medium was added. The final dilutions of test serum samples ranged from 1:4 to 1:256. Another 96-well plate was used for back titration of the virus. Following incubation at 37°C, 5% CO₂ in

TABLE 2. EXPERIMENTAL DESIGN FOR EVALUATING THE POTENTIAL VIRULENCE OF MLV-CDV VACCINES IN FERRETS.

TRIAL NO.	GROUP	NO. OF ANIMALS	INOCULUM	DOSE	INOCULATION ROUTE	OBSERVATION PERIOD (days)
1	1A	4	Vangard 5	10 doses in 2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	1B	4	Canvac 3	10 doses in 2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	1C	2	Diluent for Vangard 5	2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	1D	2	Diluent for Canvac 3	2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
2	2A	5	DH _{A2} PPi	10 doses in 2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	2B	5	NOBIVAC DHP	10 doses in 2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	2C	1	Diluent for DH _{A2} PPi	2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
	2D	1	Diluent for NOBIVAC DHP	2 mL	1.5 mL s.c.; 0.5 mL i.m.	55
3	3A	4	SOLO-JEC-7	1 mL	i.m.	34

a humidified incubator for 2 h, 50 mL of Vero cell suspension were added to each well of the plates and returned to the incubator. The result of the VNT was read on day 6 p.i. The test serum titre was the highest dilution that completely inhibited virus cytopathic effects. All samples were tested in duplicate.

Samples

All blood samples collected from experimental animals during the trials provided serum for assay. In addition, blood and serum samples from feral cats and mustelids were analysed. CDV-neutralising antibodies were tested for by VNT on blood samples from nine stoats and six weasels (*M. n. vulgaris*) collected from Te Puke in 2002, six stoats collected from Manawatu in 2001, and 32 stoats collected from North Canterbury in 2001. Many of the plasma/serum samples were toxic to cells and it was necessary to dilute some samples to 1:64 to remove toxic effects. The 32 Canterbury stoat samples were collected from various locations in the South Island including the West Coast (10 samples), Scargill/Greta Valley (6), Mount White (6), Reefton (3), Lewis Pass (1), Springs Junction (2), Tekapo (1) and Central Otago (1). The presence of CDV-neutralising antibodies were also assessed in 53 ferret serum/plasma samples collected in 1998 and 56 serum feral cat serum samples, all from North Canterbury.

2.4 CDV viral RNA detection by RT-PCR

RNA was prepared from approximately 50 mg of tissue sample, collected at necropsy, by homogenisation in TRI_{zol}[®] Reagent (Life-Technologies, Inc. Grand Island, NY, USA) and purified according to the protocol of the manufacturer. When RNA was extracted from the 10–20% tissue suspension, a commercial RNA purification kit, QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), was used following the manufacturer's instructions. All RNA samples were stored at -75°C.

CDV viral RNA was assayed by a RT-PCR method described by Frolich et al (2000), using CDV-specific primers, targeting the fusion protein gene of CDV (Frolich et al. 2000).

Precautions suggested by Kwok & Higuchi (1989) were taken to avoid false positives with PCR. PCR results were analysed by electrophoresis of 10 µl of a PCR product on a 1.5% agarose gel in the presence of ethidium bromide. Positive and negative controls were included in the tests. In addition, the authenticity of some of the PCR products was further confirmed by the use of either restriction endonuclease digestion test or DNA sequencing.

The sensitivity of the RT-PCR assay for CDV viral RNA was determined by testing RNA samples extracted from a serial 10-fold diluted Onderstepoort CDV virus stock using a QIAamp Viral RNA Mini Kit (QIAGEN). A clear positive result was obtained from RNA extracted from diluted samples containing 100 TCID₅₀/mL Onderstepoort CDV.

2.5 Passage of CDV on chorioallantoic membrane (CAM) of embryonated fowl's eggs

20% (w/v) spleen suspension preparation

A 20% spleen suspension was prepared in virus transport medium (VTM) (Eagles MEM, Gibco, Invitrogen Corp, Carlsbad, CA, USA) containing 0.5% bovine serum albumin, 0.03% gentamycin, 0.01% kanamycin, 0.01% streptomycin and 0.06% penicillin) by grinding a piece of spleen tissue in a Dounce homogeniser. This suspension was centrifuged at 1000 g for 10 min at 4°C. The supernatant was transferred to a fresh tube and used as the inoculum for the passage trials. The suspension was stored at -75°C.

Embryonated fowls' eggs

Special pathogen-free (SPF) eggs (Charles River Laboratories, North Franklin, CT, USA) were obtained either as fertile eggs or 6-7-day-old embryonated eggs from the National Centre for Diseases Investigation, Upper Hutt. The eggs were incubated at 37°C in a humidified incubator and were candled daily to observe the viability and development of the embryos.

Passage procedure

Embryonated SPF eggs 6-7 days old were used to passage CDV samples on CAMs. Approximately 0.1 mL (spleen suspension) or 0.2 mL (CAM preparation) of inoculum was inoculated on the CAM of each embryonated egg. The inoculated eggs were incubated at 37°C in a humidified incubator. A total of 3-10 embryonated eggs were inoculated at each passage. The inoculated eggs were observed daily to monitor embryo viability. The eggs were chilled to 4°C after 5 days' incubation and CAMs were harvested and pooled from the inoculated eggs and homogenised in a Dounce homogeniser with an equal volume of VTM. The homogenate was centrifuged in a 15-mL Falcon tube at 1000 g for 10 min at 4°C. The supernatant was used either as an inoculum for the succeeding passage or stored at -75°C. All procedures involving the experimental use of embryonated fowls' eggs were approved by the Wallaceville Animal Ethics Committee, Upper Hutt.

2.6 Back passage of CDV vaccine strain in ferrets

The spleen of a ferret that had received one dog-dose of SOLO-JEC-7 i.m. in the back legs was collected on day 7 p.i., at necropsy. A 20% spleen suspension was made as described previously. Prior to any succeeding passage, the presence of CDV in a sample was confirmed by testing for CDV virus RNA by RT-PCR. For subsequent passages, a total of 1.0 mL of the 20% spleen suspension was inoculated into a ferret by injecting 0.5 mL intraperitoneally (i.p.) and 0.5 mL i.m. The ferrets (three in total) were killed on days 5 to 7 p.i., and spleens were collected.

3. Results

3.1 Potential virulence of multivalent CDVs

Clinical distemper signs were not observed in ferrets inoculated with Vanguard 5, CanVac 3, Canigen DH_{A2}PPi, NOBIVAC DHP and SOLO-JEC-7 during the observation period after challenge. An antibody response against CDV was detected in a proportion of inoculated ferrets; antibody titres ranged from 1:32 to 1:256 (Table 3). CDV-neutralising antibodies were not detected in controls. CDV viral RNA was detected only from the spleens of three SOLO-JEC-7 inoculated-ferrets.

3.2 Potential virulence of CAM-passaged distemper virus

The initial attempt to passage the original virulent Snyder Hill CDV on CAMs of embryonated eggs was not successful. No plaque was observed on the CAMs during 11 passages nor was CDV viral RNA detected by RT-PCR in CAM preparations. The possibility of the existence of low titres of CDV in CAMs was ruled out because neither sero-conversion nor clinical distemper signs were observed in the CAM-inoculated ferrets.

The titres of viable CDV in the obtained Snyder Hill CDV-IC inoculum were low; when it was used at a 1:10 dilution to inoculate two ferrets, no distemper occurred during 35 days observation period, though CDV-neutralising antibodies (1:64 and 1:256) were detected from serum collected on day 20 p.i. CDV viral RNA was not detected by RT-PCR from spleens collected on day 34 p.i. However, distemper did occur in ferrets inoculated with undiluted inoculum.

TABLE 3. CDV-NEUTRALISING ANTIBODY AND CDV VIRAL RNA IN SPLEENS OF FERRETS IN MLV-CDV EVALUATION TRIALS.

TRIAL NUMBER	GROUP*	ANTIBODY TITRE†					CDV IN SPLEEN‡
		< 1:16	≥ 1:32	≥ 1:64	≥ 1:128	≥ 1:256	
1	1A	3/3	-	-	-	-	0/4 [§]
	1B	2/4	1/4	-	-	1/4	0/4
	1C	2/2	-	-	-	-	0/2
	1D	2/2	-	-	-	-	0/2
2	2A	-	1/5	2/5	-	2/5	0/5
	2B	3/5	-	-	-	2/5	0/5
	2C	1/1	-	-	-	-	0/1
	2D	1/1	-	-	-	-	0/1
3	3A	-	-	-	-	3/4 [¶]	3/4 [¶]

* For details of Groups, see Table 2.

† CDV-neutralising antibody was detected by a virus-neutralising test from serum samples collected at necropsy. Tested positive animals/total tested animals.

‡ CDV viral RNA was detected by RT-PCR from spleen samples collected at necropsy.

§ One animal died following blood collection on day 9.

¶ One animal died following blood collection on day 1 and CDV RNA was not detected from its spleen.

To further test the viability of CDV in the original Snyder Hill inoculum, undiluted CDV-IC and CDV-AER were used to challenge five ferrets. Ferret A was inoculated i.p. with 1.0 ml undiluted CDV-AER, Ferret B had 0.05 ml undiluted CDV-AER instilled into its nasal cavities. Similarly, Ferret C and D were inoculated with undiluted CDV-IC i.p. and via the nasal route, respectively. Ferret E was housed in the same room with the inoculated ferrets as a sentinel animal. Ferret A was less inquisitive on day 2 p.i.; showed puffy eyes and anorexia on day 3 p.i.; had diarrhoea and a wobbly gait on day 4 and died on day 9 p.i. Necropsy revealed lesions including small ulcers on the lips, a congested and enlarged spleen, a few haemorrhagic mesenteric lymph nodes and five 1-mm-diameter white, spotted lesions on the left kidney. During the infection period, Ferret A lost 25% of its body weight. For Ferret C, mild diarrhoea and anorexia were noted on day 7 p.i., puffy eyes and less inquisitive behaviour on day 9 p.i., a pink chin (erythema) on day 14 p.i. and the animal was wobbly on day 16. It died on day 18 p.i. Ferret D displayed signs of distemper on day 7 p.i. and was put down on 14 p.i. Ferret E displayed the distemper signs of anorexia and less inquisitive behaviour on day 26 p.i. and it died on day 31 p.i. Distemper virus was detected from samples of spleen, liver, lung, brain, mesenteric lymph nodes, kidney and bladder collected from ferrets A, C, D, and E by RT-PCR. No signs of distemper were observed from ferret B and distemper virus was not detected from its spleen collected on day 50 p.i., at necropsy.

The distemper virus strain CDV-AER from the spleen of Ferret A was passaged two more times in ferrets in an attempt to increase viable CDV titres in the spleen (see Table 4). CDV-AER third ferret-passage was used for 24 passages in CAMs of embryonated eggs. Macroscopic changes of the CAM were not observed until the sixth passage, when CAMs became thick and cloudy and often with plaques. CDV viral RNA was also detected by RT-PCR from the CAM preparations indicating the growth of the virus. The fifteenth and the third CAM-passaged virus tested avirulent for ferrets (Table 4). The third ferret-passaged virus, which was the starting inoculum for CAM passage, was only weakly virulent in two inoculated ferrets. Mild diarrhoea was observed from day 10 p.i. and lasted 2 days for one ferret and 6 days for the other. No further abnormalities were observed. CDV viral RNA was not detected in the spleens (Table 4).

TABLE 4. VIRULENCE OF CAM- AND FERRET-PASSAGED SNYDER HILL VIRUSES IN FERRETS.

TRIAL	NO. OF ANIMALS	INOCULUM	DOSE/ROUTE	CLINICAL DIS-TEMPER	CDV ANTIBODY TITRE*	CDV IN SPLEEN†	OBSERVATION PERIOD (days)
A	2	Fifteenth CAM-passage	1.0 mL/i.m.	No	> 1:256 and > 1:256	0/2‡	35
B	2	Third CAM-passage	1.0 mL/i.m.	No	> 1:32 and > 1:64	0/2	35
C	2	Third ferret-passage	1.0 mL/i.m.	Yes	> 1:32 and > 1:64	0/2	55

* CDV-neutralising antibody was detected by a virus-neutralising test from serum samples collected on day 18 p.i. for trial A, and on day 16 p.i. for trials B and C.

† CDV viral RNA was detected by RT-PCR from spleen samples collected at necropsy.

‡ Tested positive animals/total tested animals.

3.3 Back passage of vaccine CDV to enhance its virulence for ferrets

Pulmonary congestion was observed in one of the four ferrets inoculated with SOLO-JEC-7, which had been back passaged in ferrets eight times. Ferrets infected with viral strains from the ninth back passage showed congested spleens with evidence of splenomegaly (spleen 2–3 times the size of control spleens) These pathological findings suggest that the virus became more virulent for ferrets.

3.4 CDV-neutralising antibodies in feral stoats, ferrets and cats

Many of the stoat and weasel plasma/serum samples from 2001 and 2002 were toxic to cells and it was necessary to dilute some samples to 1:64 to remove toxic effects. The test result for Te Puke samples was inconclusive because of the poor quality of the samples. The six Manawatu stoat samples were diluted 1:16 to remove cell toxic effects and the CDV antibody was not detected above this dilution level. Of the 32 Canterbury stoat samples, only two of the three samples collected from Reefton were positive for CDV-neutralising antibodies (1:128 and 1:192). Of the 53 ferret serum/plasma samples collected from North Canterbury in 1998, 28 had detectable levels of CDV antibodies, with titres ranging from 1:16 to 1:256. Of the 56 feral cat serum samples from North Canterbury, seven of 46 collected in 1998 and one of seven collected in 2000 had detectable levels of CDV antibodies, with titres ranging from 1:16 to 1:128. The CDV antibody was not detected in three feral cat samples collected from the North Canterbury in 2001.

4. Discussion

Because it is difficult to obtain sufficient numbers of stoats for CDV trials, ferrets (readily available and also pests) were used in the initial phase of the study. It is intended that potential control products be validated later in stoats.

Given reports of post-vaccination distemper in mink and ferrets, it was worthwhile testing commercial MLV-CDV vaccines for virulence in ferrets. In the current study, no post-vaccination distemper was observed in the four or five inoculated ferrets. The observation period was 35–55 days, as the incubation times for post-vaccination distemper in ferrets and mink have been reported as 7–30 days p.i. (Gill et al. 1988; Sutherland-Smith et al. 1997; Ek-Kommonen et al. 2003). High mortality rates of post-vaccination distemper in mink and black-footed ferrets have been reported in various studies: four of six black-foot ferrets died within 21 days after vaccination (Carpenter et al. 1976); all four vaccinated European mink (*Mustela lutreola*) died on days 16–20 (Sutherland-Smith et al. 1997); 350 of 6000 ferrets died in New Zealand (Gill et al. 1988); and one of two European mink died (Ek-Kommonen et al. 2003). In the current study, ferret virulent MLV-CDV was not identified in the tested vaccines.

There are several essential requirements for a vaccine strain of CDV to be used for the biological control of stoats and other mustelids in New Zealand. First, a relatively high mortality rate in infected stoats or other mustelids is desirable for CDV vaccines intended as lethal agents. Second, a relatively long incubation period is desirable, to enable the agent to be effectively transmitted among cohorts. Third, the agent should be innocuous to unvaccinated dogs and marine mammals. Three approaches could be used to produce a vaccine CDV that would meet all three requirements: modification of a virulent CDV through limited passages in embryonated fowls' eggs or cell cultures (see Cabasso & Cox 1949; Haig 1956; Rockborn 1959); reversion to virulence for ferrets of the vaccine CDV by rapid serial passage in ferrets (see Gorham & Goto 1998); and attenuation of a CDV virulent for dogs by passaging it in ferrets (see Green 1939, 1945).

Currently, there are no virulent CDV strains or field CDV isolates available in New Zealand. An attempt was made to isolate CDV from a ferret with suspected clinical distemper from Woodville, but it was not successful (data not shown). Therefore, it was necessary to import virulent CDV for attenuation. The Snyder Hill strain of CDV is virulent for dogs, ferrets and mink and it is widely used as a challenge strain for testing animal immunity against distemper (Gillespie & Rickard 1956; Castle et al. 2001; Wimsatt et al. 2001). In the current study, stocks of Snyder Hill strain of CDV were obtained from the USDA for modification.

Modification of Snyder Hill CDV by passage in embryonated eggs was the approach adopted because of the method's proven history (Cabasso & Cox 1949; Haig 1956) and the ease of obtaining SPF eggs. Direct passage of the original Snyder Hill CDV in embryonated eggs was unsuccessful. The inoculation of 1:10 diluted Snyder Hill CDV stock did not kill the ferrets, but undiluted stock did. The two original Snyder Hill CDV stocks were prepared in 1990 and 1998. When they arrived at Wallaceville, the vials were frozen on dry ice and were then stored at -75°C . The inoculum of Snyder Hill CDV-AER, a ferret spleen suspension, had been used at 1:5-1:10 dilutions to challenge mink, with a mortality rate close to 100% (Dr L.A. Wibur from USDA, pers. comm.). The inoculum was passaged three times in ferrets in an attempt to increase the virus titre prior to CAM passage. The attenuation of virulent CDVs by egg-passage was noted at the twenty-sixth passage in one study (Cabasso & Cox 1949), and at the one-hundred-and-tenth in the other (Haig 1956); the difference probably reflecting the fact that the CDV strains for attenuation were different. In the current study, virulence of the CDV was lost at the third egg-passage because the starting CDV, the third ferret-passaged CDV, was only weakly virulent in inoculated ferrets.

Although we have obtained another stock of Snyder Hill CDV from American Tissue Culture Collection (ATCC, Manassas, VA, USA), its virulence for ferrets cannot be tested because the test results for extraneous viruses are not available from ATCC, which is a MAF biosecurity requirement for *in vivo* usage. It will be difficult to obtain further archive vaccine strains of CDV that meet the MAF biosecurity requirements for testing in animals in New Zealand. However, the virulence for ferrets of attenuated CDV can be restored through serial back passage in ferrets (Gorham & Goto 1998), while ferret-passaged CDV should

remain innocuous to dogs (Green 1939, 1945). SOLO-JEC-7 strain was selected for back-passage trials because the vaccine manufacturer stated that it had been virulent for ferrets and was not recommended for use in ferrets and mink. Indeed, it was the only vaccine in which CDV persisted at low levels in the spleen, as detected when the ferrets were killed on day 34 p.i. We noted congested enlarged spleens (consistent with CDV pathology) from ferrets inoculated with the eighth to thirteenth back passages, the highest back-passage level so far. This pathological change coincided with the diminished ability of the RT-PCR assay to detect virus RNA. The passage of CDV in ferrets favours the selection of a CDV that is virulent for mustelids. The viral sequences of this quasi-species at the RT-PCR primer areas may be different from those of the original CDV, which decreases the sensitivity of the RT-PCR test. Another possible reason for the negative RT-PCR result was that the titres of the selected ferret-favourable quasi-species of CDV in the spleen were very low, below the RT-PCR threshold of detection. A substantial decrease in CDV titres in various tissues during back passage in ferrets has been noted previously (Gorham & Goto 1998).

CDV-neutralising antibodies were detected from two of three stoat samples collected from Reefton in 2001, suggesting previous exposure of stoats at Reefton to CDV. The prevalence of CDV in populations of stoats throughout New Zealand remains to be established. Although CDV-exposed stoats were identified in only one of eight locations in Canterbury, the sample sizes from each location were very small. An extensive serological survey needs to be conducted to gather data on the prevalence of CDV in stoats. An outbreak of distemper in a stoat colony (Keymer & Epps 1969) indicated that stoats are susceptible to CDV infection.

In the current study, CDV-neutralising antibodies were detected from approximately 53% (28/53) of ferret blood samples collected in North Canterbury in 1998. This indicated that CDV infection was enzootic in feral ferrets in North Canterbury at that time. In addition, CDV-neutralising antibodies were detected from 15% (7/46) of serum samples collected from feral cats in North Canterbury in 1998. In an earlier study (1993), appreciable levels of CDV-neutralising antibodies were not detected from 54 serum samples collected from ferrets in North Canterbury (Amuri) (O'Keefe 1995), suggesting that CDV had probably been absent in this population of feral ferrets. There are few reports on the epidemiology of CDV in feral ferrets. CDV antigen was detected from 37% (54/146) of mustelid fixed brain tissue collected in Germany between 1989 and 1991, including 50 of 132 stone martens (*Martes foina*), one of five badgers (*Meles meles*), one of four weasels (*Mustela* spp.) and two of five polecats (*Mustela putorius*) (van Moll et al. 1995). The prevalence of CDV antibody in free-ranging foxes in Germany was approximately 5% (30 of 591) (Frolich et al 2000). It is not known what strain(s) was enzootic in feral ferrets in North Canterbury in 1998. The finding of 15% sero-positive feral cats from the same area at same time allows us to speculate a possible transmission model between these animals or common source of infection. It is known that cats are susceptible to CDV infection without developing disease (Appel et al. 1974). Cats could acquire a transmissible CDV from CDV-shedding dogs and maintain the virus. The passage of CDV in cats may alter CDV virulence for ferrets.

5. Future directions and recommendations

- Back passage of vaccine CDV in ferrets to regain its virulence for ferrets represents the best opportunity to develop a CDV strain that is lethal for ferrets but innocuous for dogs and other marine mammals.
- CDV antibody prevalence was low (2 of 38) in stoats. Testing of a larger number of stoat samples, from different geographic areas, is desirable to gain a better understanding of the epidemiology of CDV infections in stoats.
- An integrated control strategy including conventional and biological control measures will be required for stoat and ferret management. Conventional control methods would be essential for controlling animals that are immune to CDV infection.

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7. References

- Appel, M.J. 1969: Pathogenesis of canine distemper. *American Journal of Veterinary Research* 30(7): 1167-1182.
- Appel, M.J.; Gillespie, J.H. 1972: Canine distemper virus. Pp. 1-96 in Gard, S.; Hallauer, C.; Meyer, K.F. (Eds): *Virology Monographs* 11. Springer-Verlag, New York.
- Appel, M.J.; Sheffy, B.E.; Percy, D.H.; Gaskin, J.M. 1974: Canine distemper virus in domesticated cats and pigs. *American Journal of Veterinary Research* 35: 803-806.
- Bush, M.; Montali, R.J.; Brownstein, D.; James, A.E., Jr.; Appel, M.J. 1976: Vaccine-induced canine distemper in a lesser panda. *Journal of the American Veterinary Medical Association* 169(9): 959-960.
- Cabasso, V.; Cox, H.R. 1949: Propagation of canine distemper virus on the chorio-allantoic membrane of embryonated hen eggs. *Proceedings of the Society for Experimental Biology and Medicine* 71: 246-250.

- Carpenter, J.W.; Appel, M.J.; Erickson, R.C.; Novilla, M.N. 1976: Fatal vaccine-induced canine distemper virus infection in black-footed ferrets. *Journal of the American Veterinary Medical Association* 169(9): 961-964.
- Castle, K.T.; Biggins, D.; Carter, L.G.; Chu, M.; Innes, K.; Wimsatt, J. 2001: Susceptibility of the Siberian polecat to subcutaneous and oral *Yersinia pestis* exposure. *Journal of Wildlife Diseases* 37(4): 746-754.
- Dunkin, G.W.; Laidlaw, P.P. 1926: Studies in dog-distemper: 1. Dog-distemper in the ferret. *Journal of Comparative Pathology and Therapeutics* 39: 201-212.
- Ek-Kommonen, C.; Rudback, E.; Anttila, M.; Aho, M.; Huovilainen, A. 2003: Canine distemper of vaccine origin in European mink, *Mustela lutreola*—a case report. *Veterinary Microbiology* 92(3): 289-293.
- Frolich, K.; Czupalla, O.; Haas, L.; Hentschke, J.; Dedek, J.; Fickel, J. 2000: Epizootiological investigations of canine distemper virus in free-ranging carnivores from Germany. *Veterinary Microbiology* 74(4): 283-292.
- Gill, J.M.; Hartley, W.J.; Hodgkinson, N.L. 1988: An outbreak of post-vaccinal suspected distemper-like encephalitis in farmed ferrets (*Mustela putorius furo*). *New Zealand Veterinary Journal* 36: 173-176.
- Gillespie, J.H.; Rickard, C.G. 1956: Encephalitis in dogs produced by distemper virus. *American Journal of Veterinary Research* 17: 103-108.
- Gorham, J.R. 1966: The epizootiology of distemper. *Journal of the American Veterinary Medical Association* 149: 610-618.
- Gorham, J.R.; Goto, H. 1998: Reversion to virulence of an attenuated mink distemper virus vaccine induced by rapid serial passage in ferrets. *Scientificur* 22(2): 149-156.
- Green, R.G. 1939: Modification of the distemper virus. *Journal of the American Veterinary Medical Association* 95: 465-466.
- Green, R.G. 1945: Zoologic and histologic modification of the distemper virus by ferret passage. *Journal of American Hygiene* 41: 7-24.
- Haig, D.A. 1956: Canine distemper-immunisation with avianised virus. *Onderstepoort Journal of Veterinary Research* 27(1): 19-53.
- Halbrooks, R.D.; Swango, L.J.; Schnurrenberger, P.R.; Mitchell, F.E.; Hill, E.P. 1981: Response of gray foxes to modified live-virus canine distemper vaccines. *Journal of the American Veterinary Medical Association* 179(11): 1170-1174.
- Keymer, I.F.; Epps, H.B. 1969: Canine distemper in the family Mustelidae. *Veterinary Record* 85(7): 204-205.
- Kwok, S.; Higuchi, R. 1989: Avoiding false positives with PCR. *Nature* 339(6221): 237-238.
- May, R.M. 1986: The cautionary tale of the black-footed ferret. *Nature* 320: 13-14.
- Montali, R.J.; Bartz, C.R.; Bush, M. 1987: Canine distemper virus. Pp. 437-443 in Appel, M.J. (Ed.): *Virus infection of carnivores*. Elsevier Science Publishers, Amsterdam.
- O'Keefe, J.S. 1995: Canine distemper and ferrets. Literature review prepared for MAF Policy New Zealand (unpublished). 45 p.
- Osterhaus, A. 1989: A morbillivirus causing mass mortality in seals. *Vaccine* 7(6): 483-484.
- Osterhaus, A.D.; Vedder, E.J. 1988: Identification of virus causing recent seal deaths [letter]. *Nature* 335(6185): 20.
- Rockborn, G. 1959: An attenuated strain of canine distemper virus in tissue culture. *Nature* 184: 822.
- Spooner, E.T. 1938: A disease resembling distemper epidemic among ferrets. *Journal of Hygiene* 38: 79-89.

- Sutherland-Smith, M.R.; Rideout, B.A.; Mikolon, A.B.; Appel, M.J.; Morris, P.J.; Shima, A.L.; Janssen, D.J. 1997: Vaccine-induced canine distemper in European mink, *Mustela lutreola*. *Journal of Zoo and Wildlife Medicine* 28(3): 312-318.
- van Moll, P.; Alldinger, S.; Baumgartner, W.; Adami, M. 1995: Distemper in wild carnivores: an epidemiological, histological and immunocytochemical study. *Veterinary Microbiology* 44(2-4): 193-199.
- Visser, I.K.; Kumarev, V.P.; Orvell, C.; de Vries, P.; Broeders, H.W.; van de Bildt, M.W.; Groen, J.; Teppema, J.S.; Burger, M.C.; UytdeHaag, F.G.; et al. 1990: Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in north west Europe and Siberia. *Archives of Virology* 111(3-4): 149-164.
- Visser, I.K.; van Bresseem, M.F.; Barrett, T.; Osterhaus, A.D. 1993a: Morbillivirus infections in aquatic mammals. *Veterinary Research* 24(2): 169-178.
- Visser, I.K.; Van Bresseem, M.F.; de Swart, R.L.; van de Bildt, M.W.; Vos, H.W.; van der Heijden, R.W.; Saliki, J.T.; Orvell, C.; Kitching, P.; Kuiken, T.; et al. 1993b: Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. *Journal of General Virology* 74(Pt 4): 631-641.
- Williams, E.S.; Thorne, E.T.; Appel, M.J.; Belitsky, D.W. 1988: Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. *Journal of Wildlife Diseases* 24(3): 385-398.
- Wimsatt, J.; Jay, M.T.; Innes, K.E.; Jessen, M.; Collins, J.K. 2001: Serologic evaluation, efficacy, and safety of a commercial modified-live canine distemper vaccine in domestic ferrets. *American Journal of Veterinary Research* 62(5): 736-740.