

The Quest for a Safe and Effective Canine Distemper Virus Vaccine for Black-footed Ferrets

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Abstract

Canine distemper virus (CDV) causes a systemic disease that is highly virulent to mustelids and other carnivore (Order Carnivora) species and is found worldwide. Endemic canine distemper in wild and domestic carnivores in the United States has made reintroduction of endangered black-footed ferrets (*Mustela nigripes*) difficult in the absence of safe and effective CDV vaccines and vaccination practices. Toward this end, researchers have explored appropriate animal models and vaccine preparations in highly susceptible species. Published studies involving domestic ferrets (*M. putorius furo*) using Galaxy-D[®] and evaluating a recombinant canarypox-vectored vaccine for oral administration are reviewed. In addition, we present new findings in domestic and black-footed ferrets and Siberian polecats (*M. eversmannii*) that have extended our understanding of CDV in the black-footed ferret and other at-risk carnivore species. Original research presented here includes trials that determined an effective challenge dose (by route) of virulent CDV in domestic ferrets and Siberian polecats; the low likelihood of collateral vaccination with Galaxy-D; the adverse effect of modified-live virus boosting in black-footed ferrets receiving killed vaccine previously and the response of Siberian polecats receiving canarypox-vectored recombinant CDV vaccine (reCDV); the absence of an effect of reCDV vaccination on conception, pregnancy, and neonatal growth in Siberian polecats; and the apparent inefficacy of active reCDV vaccination during the period of passive immunity in young Siberian polecats. In the final section, we discuss emerging concerns and avenues for disease intervention that may present new opportunities to solve problems in

vaccine safety, vaccine availability, field vaccine delivery, and other therapeutic modalities.

Keywords: black-footed ferret, canarypox, canine distemper, ferret, morbillivirus, oral vaccine, paramyxovirus, recombinant, Siberian polecat

Introduction

Canine distemper virus (CDV; family Paramyxoviridae, genus *Morbillivirus*) is a single-stranded, negative sense, 16-kilobase RNA virus encoding six genes (designated N, P, M, F, H, L) and eight protein products. The N gene has been used for diagnostic CDV identification (Wimsatt and others, 2001; Rzezutka and Mizak, 2002) while the M and P genes have been used in phylogenetic analyses (Barrett and others, 1993; Saliki and others, 2002) and subtype identification (Roelke-Parker and others, 1996; Carpenter and others, 1998; van de Bildt and others, 2002; Bronson and others, 2003), respectively. Phylogenetic analysis using other genes has repositioned CDV within the paramyxoviridae (Westover and Hughes, 2001). Vaccine developers have focused on hemagglutinin (HA) and fusion (F) gene product antigens, which appear to confer highly protective immunity when antibodies are successfully raised in response to vaccination.

Canine distemper virus is found worldwide. The hallmarks of CDV-induced disease are the result of primary host tissue tropisms for the cutaneous (maculopapular rash, erythema), respiratory (increased respiratory rate or labored respirations, dyspnea, cyanosis), gastrointestinal (diarrhea), and central and peripheral nervous systems. While respiratory and gastrointestinal manifestations of this disease can cause considerable morbidity and mortality, it is often the central nervous system manifestations that portend death during its clinical expression (Leisewitz and others, 2001). Nervous signs attributed to CDV include seizures, tremors, depression, and myoclonia (peripheral nervous signs). While some tissue tropism differences in CDV are expected, the Center for Veterinary Biologics (CVB; Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture [USDA]) virulent challenge strain ultimately leads to neurological disease; nervous signs can also dominate in

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previously vaccinated mustelids that ultimately succumb to CDV infection (J. Wimsatt, unpub. data, 1996–98).

Canine distemper primarily affects carnivores (Order Carnivora), but may opportunistically infect other taxa (Appel and others, 1991; Svansson and others, 1993; Appel and Montali, 1994; Appel and Summers, 1995; Kennedy and others, 2000; Pollack, 2001; Noon and others, 2003). In terms of its risk to endangered carnivores, CDV is the most significant pathogenic virus known, and the black-footed ferret (*Mustela nigripes*) reintroduction program must address this ongoing threat to captive breeding and wild population stability (Williams and Thorne, 1996).

It is the general intent of this paper to accomplish two somewhat disparate goals. First, we chronicle what research on canine distemper virus prophylaxis in mustelids has revealed, the roles of various animal models and vaccine preparations in the quest, and where new discoveries could likely lead these pursuits in the future. Second, we present new findings of black-footed ferret responses to CDV vaccination and studies using CDV vaccines in surrogate animals to find a practical approach for CDV prophylaxis in susceptible *Mustela* species.

The Ecology of Canine Distemper Virus and the Risk It Presents to the Black-footed Ferret

Canine distemper virus is enzootic in urban and rural settings (Grinder and Krausman, 2001). Canine distemper virus becomes rapidly inactivated once in the environment (Fox and others, 1998) but is readily spread by aerosol, even under dry, hostile conditions (Williams and others, 1988, 1997). In the wild, transfer can occur at carnivore food (e.g., burrow entrances) and water sources. Wildlife epizootics may emerge as a consequence (Noon and others, 2003).

Traditionally, the primary reservoir and ultimate source of CDV outbreaks in the wild is assumed to be unvaccinated domestic dogs that infect wildlife with CDV during chance encounters. The potential role of wild carnivores (especially young) as primary reservoirs of CDV is difficult to discount (Guo and others, 1986; Gese and others, 1991, 1997; Williams and Thorne, 1996; Williams and others, 1997; Cypher and others, 1998; Grinder and Krausman, 2001; Arjo and others, 2003) since high CDV seroprevalence rates, suggestive of high levels of exposure, are found in several wild species (Guo and others, 1986; Gese and others, 1991, 1997; Williams and others, 1997; Cypher and others, 1998; Dunbar and others, 1998; Truyen and others, 1998; Grinder and Krausman, 2001; Ikeda and others, 2001). During a recent outbreak of CDV at an urban zoo, wild raccoons (*Procyon lotor*) were found to harbor a unique CDV variant (Lednicky and others, 2004), and they appeared to serve as a distinct reservoir. Most dogs are vaccinated for CDV (Greene and Appel, 1998); as a result,

wild carnivores may be of greater infective potential to high-risk species, such as the black-footed ferret, than are domestic dogs. However, resident CDV in domestic dogs is under strong vaccine-induced selection pressure (Mochizuki and others, 1999; Hashimoto and others, 2001; Lednicky and others, 2004) and thus cannot be discounted as an emergent source in the future.

One area of growing relevance to captive and exotic carnivores is the possibility of CDV persistence and later viral shedding (elaboration and release of virus by renewed replication from the host at a later date) after the primary infection has subsided. This issue is of great concern where modified-live virus (MLV) vaccines are used in nontarget species.

Persistence of morbillivirus infections has led to such diseases as subsclerosing panencephalitis in humans (Dyken, 2001; Garg, 2002; Schneider-Schaulies and others, 2003), Paget's disease (Cartwright and others, 1993; Fraser, 1997; Mee and others, 1998; Friedrichs and others, 2002; Hoyland and others, 2003), and canine orthopedic conditions (Mee and others, 1993; Harrus and others, 2002). Autoimmune-mediated demyelination associated with measles or CDV infection has been studied in relation to its possible association with multiple sclerosis (Anonymous, 1978; Appel and others, 1981; Cook and others, 1986; De Keyser and others, 2001; Hernan and others, 2001). A link between infectious obesity and CDV has been proposed as well (Dhurandhar, 2001; Verlaeten and others, 2001).

Recently, evidence of CDV persistence has been documented in domestic dogs in which selected strains of the virus survived without detection by the host immune system (Lincoln and others, 1971; Povey, 1986; Leisewitz and others, 2001). A major requirement for chronically persistent CDV infection involves the selection of a cell-associated strain with limited capability for antigen presentation (Vandeveldel and Zurbriggen, 1995) and conferring only limited antibody diversity (Rima and others, 1987); this latter strain differs in its pathogenesis from more virulent forms causing acute disease (Vandeveldel and others, 1980). One key site of CDV persistence may be dendritic cells, reflecting a change in CDV cell tropism (Wunschmann and others, 2000). The condition "old dog encephalitis" is one presentation of chronic CDV infection (Lincoln and others, 1971; Hall and others, 1979; Tobler and Imagawa, 1984; Evans and others, 1991; Axthelm and Krakowka, 1998). Moreover, a tropism for epithelial cells (in addition to the typical tropism for macrophages) in culture suggests that persistent strains behave more akin to vaccine strains (Evans and others, 1991). A recent case report highlighted the risk of CDV persistence from vaccine strains when a red panda (*Ailurus fulgens*) vaccinated 3 years earlier with a commercial MLV CDV vaccine developed progressive CDV-induced neurological disease and subsequently died (Bronson and others, 2003). Gene typing (P gene) demonstrated that the offending CDV isolate was actually the original vaccine strain. Another recent paper suggested that incomplete CDV expression of fusion (F) protein may facilitate persistent viral infection; likewise, hemagglutinin (HA) heterogeneity of new

emerging strains could lead to more widespread CDV persistence if F protein immunity becomes the primary source of protection following vaccination (Meertens and others, 2003).

Animal Models for Testing CDV Vaccines Destined for the Black-footed Ferret

Historically, guidelines for vaccinating free-ranging and captive wild carnivores were derived from those used for vaccines in domestic dogs, mink (Hagen and others, 1970), and domestic ferrets (*M. putorius furo*) (Hagen and others, 1970; Farrell and others, 1971). Interestingly, while domestic dogs are commonly vaccinated, they are not among the most CDV-susceptible carnivore species. One study estimated that up to 70 percent of urban dogs that were exposed to natural CDV infection never developed overt disease signs although they seroconverted, suggesting occult infection (Rockborn, 1957). Likewise, experience has shown that vaccines developed for high efficacy in dogs (and also sometimes used safely in some wild canids) may be too virulent for more susceptible species (Fox and others, 1998) such as red pandas (Bush and others, 1976; Itakura and others, 1979; Montali and others, 1983; Appel and Summers, 1995), gray foxes (*Urocyon cinereoargenteus*) (Halbrooks and others, 1981), and selected *Mustela* species (Carpenter and others, 1976; Montali and others, 1983, 1994; Sutherland-Smith and others, 1997). Canine cell line origin passaged vaccines were quickly realized to be pathogenic to domestic ferrets, commonly vaccinated as pets against CDV (Fox and others, 1998). Early MLV CDV vaccines intended for ferrets utilized primary chick embryo passage. These procedures were expensive, and assuring product uniformity was an ongoing concern (Fox and others, 1998).

An immune deficiency in black-footed ferrets that may be of prime importance in explaining the unique, extreme susceptibility of this species to CDV and other infectious diseases is the diminished production of the proimmune cytokine interleukin-6 (Stoskopf-Kennedy and others, 1997). In contrast, Siberian polecats (*M. eversmannii*) appear to produce greater amounts of interleukin-6 (S. Wisely, oral commun., 2004). Homozygosity among Wyoming black-footed ferrets is recognized from genetic comparisons to historical populations from Kansas and to Siberian polecats (Wisely and others, 2002); this limited diversity may have contributed to the unique susceptibility of black-footed ferrets to natural and vaccine strains of CDV. Further investigations will reveal whether other highly susceptible species exhibit the same predisposition to diminished interleukin-6 production. Other cytokines need to be explored in this light as well (Bencsik and others, 1996; Grone and others, 2002).

A recent refinement in the production of one widely used CDV vaccine strain involved serial passage of the virus on an

immortal primate Vero cell line (rather than chick embryo) and a more controlled process of vaccine attenuation. These procedures appear to improve product reliability, but highly susceptible species still succumb to vaccine-induced viral disease (Sutherland-Smith and others, 1997).

The characterization of appropriate models for the study of CDV vaccines in susceptible species has been a high priority. Based on taxonomy, domestic ferrets appeared to provide a close model for interpreting the likely CDV responses of black-footed ferrets as compared with other carnivores; more closely related Siberian polecats (O'Brien and others, 1989) and black-footed ferret × polecat hybrids helped to further define the likely impact and efficacy of existing vaccine strategies destined for the black-footed ferret (Williams and others, 1996). Recently, surplus black-footed ferrets have sometimes been available for CDV vaccine studies (J. Kreeger, oral commun., 2004), but definitive challenge studies may still rely heavily on other mustelid models.

Vaccines: the Past, Present, and Future

Traditionally, killed virus (KV) vaccines were reserved for species and situations where MLV vaccines were considered unsafe. Potential disadvantages of KV vaccines include: unreliable inactivation; short-lived immunity (in addition, adjuvants that may cause some side effects may be required); the need for high antigenic doses (possible side effects if redosed); variable protection in poor responders; and finally, the induction of humoral (antibody production) rather than cell-mediated (i.e., T cell-mediated cellular) immunity (Schultz and Zuba, 2003). Thus, KV vaccines may not protect against overwhelming exposures to wild-type CDV; protection in such instances likely requires both robust humoral and cell-mediated immune responses. A nonadjuvanted KV vaccine was produced for use in highly susceptible species such as the black-footed ferret and red panda by Dr. Max Appel, of the Baker Institute at Cornell University; this vaccine was provided until a more favorable vaccination strategy became available.

Commercial CDV vaccines are primarily modified-live products incorporating carefully selected wild strains that respond favorably to serial passage and graded attenuation. Of these, the Onderstepoort strain has been most extensively used for vaccination in the domestic ferret and exotic carnivores in zoological collections, first as the chick-embryo product Fromm-D (Solvay Co., Mendota Heights, Minn.; no longer produced) and later as the primate Vero cell line attenuated vaccine, Galaxy-D[®] (Schering-Plough Animal Health, Inc., Union, N.J.). As a rule, modified-live products do not supply sufficient antigenic load to confer immunity unless active infection is engendered by vaccination (Schultz and Zuba, 2003). A recent study on the efficacy of Galaxy-D in domestic ferrets demonstrated, by polymerase chain reaction (PCR) amplification, the presence of CDV vaccine virus in the blood

5 days following the first of two inoculations. A primary vaccination series led to protective immunity as defined by virulent strain challenge (Wimsatt and others, 2001). Modified-live CDV vaccines have been shown to provide substantial and long-lived immunity following a primary vaccination series that invokes both cell-mediated and humoral immunity in dogs and domestic ferrets (Gorham, 1966, 1999). In the past, Fervac-D[®] (United Vaccines, Inc., Madison, Wis.) and other modified-live CDV vaccines (Fromm-D and Galaxy-D) routinely used in domestic ferrets were tested in surrogate species and were found unsuitable for black-footed ferrets. Either primary (CDV-induced) or secondary immunosuppression-related disease ensued when black-footed ferrets and black-footed ferret hybrids were vaccinated with these formulations (E. Williams, oral commun., 1995). Lymphocyte apoptosis accompanies CDV infection leading to its immunosuppressive effects (Moro and others, 2003a,b). As with natural infection, the immunosuppressive fallout of CDV infection from modified-live vaccination can lead to significant secondary morbidity and mortality in stressed or particularly susceptible individuals. The closely related measles and CDV viruses directly inactivate lymphocytes by virus-dependent and independent means (Krakowka, 1982) whereas more “adapted” strains do not inhibit lymphocyte proliferation (Schultz, 1976; Schlender and others, 1996) or T cell-mediated cytotoxicity (Tipold and others, 1999), and lead to the elaboration of immune-modulatory substances (Krakowka and others, 1987; Tipold and others, 1999).

Our interest in modified-live CDV vaccination in the black-footed ferret arose in exploring the possibility that a reliable, less virulent, modified-live vaccine might be used to booster black-footed ferrets that had been vaccinated previously with a KV vaccine. A modified-live CDV booster would be expected to last for the reproductive life of the animal, thus obviating the need for vaccination in the wild after reintroduction. Experimental KV vaccine (inactivated Onderstepoort strain) was widely used by zoos to protect high-risk species such as lesser pandas and black-footed ferrets (R. Montali, oral commun., 1996), but a vaccinated cohort had never been extensively challenged under controlled conditions to determine efficacy because of the scarcity and inherent value of these species. Use of a CDV modified-live booster following repeated KV vaccination served as a mild challenge. Boosting efficacy was further tested by subsequent virulent strain challenge. Based on experience gleaned from studies on surrogate species and hybrids with various candidate vaccines, current vaccine trials now focus primarily on safer subunit vaccines for genetically “bottlenecked” or exquisitely susceptible species.

More recently, the advent of vectored vaccines employing a wide range of different vectors and supplying antigens for many diseases affecting many species (Tartaglia and others, 1990, 1992, 1993; Paoletti and others, 1993, 1994, 1995; Taylor and others, 1994; Pincus and others, 1995) has fostered new optimism about the potential to find a safe and effective CDV vaccine for use in highly susceptible species.

Recent Studies Guiding Use of CDV Vaccine in Mustelids

All animals undergoing vaccine and challenge trials described below were housed in a biosafety level-2 room in modified rabbit cages and fed a high quality cat (Siberian polecats or domestic ferrets) or mink (black-footed ferret) chow; water was provided free choice. Animals were randomly assigned to treatment groups unless otherwise specified and grouped in cage racks by treatment. All animals were supplied with 40.6-cm (10.2-cm diameter) PVC hide tubes with fixed end caps. Animals were anesthetized without restraint by placing a second end cap with an inhalant anesthetic delivery port over the opposite end while the animal was inside.

Anesthesia was induced using 5 percent isoflurane in 3 L/min oxygen. After approximately 2 minutes, the animal was transferred from the PVC chamber to a face mask, and anesthesia was maintained at 1–2 percent isoflurane in 1.5 L/min oxygen. Care was taken to anesthetize the controls before the vaccines in all cases. Blood samples (1 mL) were collected from the cranial vena cava or from an external jugular vein into serum tubes, and serum was frozen until assayed. Under anesthesia, vaccination was accomplished by subcutaneous injection (Galaxy-D, following the manufacturer’s instructions, and canarypox-vectored recombinant canine distemper virus [reCDV] vaccine), or by the oral route (reCDV), spraying the reconstituted vaccine in the back of the mouth.

Serology and Challenge Strain Dose Validations

An adapted standard serum microneutralization test was used to assess CDV titers (Appel and Robson, 1973). All virulent CDV challenge studies employed the CVB USDA Snyder Hill virulent challenge strain (Lot # 90-18). This same strain is used for vaccine challenge studies required for USDA licensing of commercial CDV vaccines. Dose selection for these studies was validated as described below.

Initial challenge dose-response studies using six domestic ferrets per group and five dose groups (J. Wimsatt, unpub. data, 1996) established a minimal 100 percent lethal intraperitoneal dose of CVB Lot # 90-18 challenge strain ferret spleen suspension in domestic ferrets as a dilution of 1:1,000 (pH 7.0, delivered in 1 mL total volume). Thus, for all subsequent challenge studies, regardless of the *Mustela* species tested, a 1-mL volume of challenge strain diluted to 1:250 in phosphate buffered saline (same pH and total volume) was used. This final lethal dose selected for challenge studies was confirmed in four Siberian polecats (J. Wimsatt, unpub. data, 1996) and was also found to be 100 percent effective (lethal) when used in challenge controls in subsequent studies. Later investigations extended these initial determinations to suggest that combined oral/intranasal instillation yielded the same results as intraperitoneal administration in Siberian polecats (J. Wimsatt, unpub. data, 1997) and domestic ferrets (Wimsatt

and others, 2001). The only exception was that CDV-induced skin erythema or maculopapular rash usually occurred first at the site of challenge strain inoculation.

Challenge studies still remain the best available means to test vaccine efficacy. The significance of different routes of challenge, like those influencing vaccination, may be of considerable importance and requires careful study (Schultz and Zuba, 2003). While intracerebral and intraperitoneal challenge are commonly used, mucosal (intranasal/oral) challenge more closely mimics natural infection. Mucosal immunity is often considered the first line of defense against infectious agents (Ogra and others, 1980). In our studies, using survival as the endpoint, the intraperitoneal and oral/intranasal routes yielded similar results. This is of interest since CDV has a tropism for mucosal tissue (Jozwik and Frymus, 2002), and mucosal presentation to dendritic cells may stimulate cell-toxic lymphocytes (Etchart and others, 2001) early in the disease pathogenesis. Likewise, active CDV mucosal immunization may minimize disease-induced immunosuppression (Liashenko and others, 1999) or bypass maternal passive immunity (Fischer and others, 2002), leading to qualitatively different outcomes during challenge and vaccination. During challenge, such differences were not evident.

Modified-live Vaccine Studies in Domestic Ferrets

A chick embryo origin product (Fromm-D) using an attenuated Onderstepoort strain was found to be safe and effective when tested in black-footed ferret \times Siberian polecat hybrids (Williams and others, 1996) and domestic ferrets (Fox and others, 1998). Galaxy-D was tested in male domestic ferrets vaccinated and challenged as described previously (Wimsatt and others, 2001). Briefly, eight randomly selected CDV-seronegative male domestic ferrets (Marshall Farms, Rose, N.Y.) were subcutaneously vaccinated twice 4 weeks apart with Galaxy-D according to the manufacturer's instructions. Eight control animals received saline injections. Challenge followed 21 days after the last vaccination (Wimsatt and others, 2001).

Virulent virus challenge produced 100 percent mortality in the controls, with prolonged presence of virus nucleoprotein in the blood detected by CDV-specific nucleoprotein reverse transcriptase PCR (RT-PCR). All Galaxy-D vaccinates ($n = 8$) survived following a primary two vaccine series although one first-time and two second-time vaccinates expressed viral nucleoprotein in their blood following challenge (Wimsatt and others, 2001). After active infection, this MLV vaccine induced a robust immune response protective against lethal CDV challenge, indicating that domestic ferrets responded with protective adaptive immunity to this same CDV strain, originally packaged in the avian embryo passaged Fromm-D vaccine.

Domestic Ferret Collateral Vaccination of Cage Mates

In a second study, randomly selected pair-housed male CDV-seronegative domestic ferrets were subcutaneously vaccinated with a single dose of Galaxy-D. Blood sampling for serology and challenge were performed as indicated in fig. 1. Unvaccinated CDV-naïve cage mates were blood-sampled for seroconversion to assess for collateral vaccination.

None of the six male co-housed domestic ferrets seroconverted in response to a single Galaxy-D delivered to their (CDV-naïve) cage mate up to 25 days after vaccination. All vaccinated ferrets (six of six) survived challenge following the single Galaxy-D dose. Serology values for unvaccinated cage mates, vaccinates, and unvaccinated controls are shown in fig. 2; titers for unvaccinated cage mates housed contemporaneously with Galaxy-D vaccinates remained low and indistinguishable from those of seronegative controls (fig. 2), suggesting that if primary vaccine shedding or contamination following vaccination occurred, it was insufficient to produce a MLV-induced immune response in the CDV-naïve cage mates.

Subcutaneous vaccination of CDV-naïve domestic ferrets with Galaxy-D did not appear to present a sufficient antigenic dose for collateral vaccination of co-housed cage mates and thus did not lead to seroconversion. This is not surprising since modified-live virus load is typically too low to induce an immune response in the absence of a host infection (i.e., host infection replicates more virus, thus increasing its antigenic load) caused by the vaccine strain (Schultz and Zuba, 2003). However, the timeframe was not sufficient to conclude that shedding of the Galaxy-D CDV virus from vaccinates would not have occurred eventually from virus replication in the host.

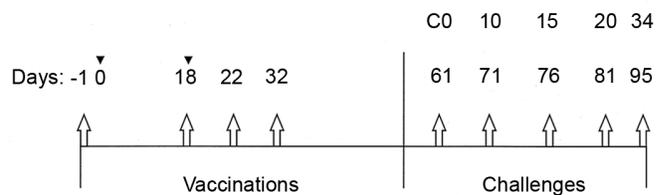


Figure 1. Timeline for the black-footed ferret (*Mustela nigripes*) Galaxy-D booster and virulent canine distemper virus challenge study. Seronegative domestic ferrets (*M. putorius furo*) in the same room served as challenge strain controls, and another cohort of pair-housed domestic ferrets had one member of the pair randomly selected for Galaxy-D vaccination at the same time; vaccinates were later challenged with the others while the unvaccinated member of the pair was removed just prior to challenge. Triangles indicate days of vaccination. Arrows indicate days when blood samples were drawn.

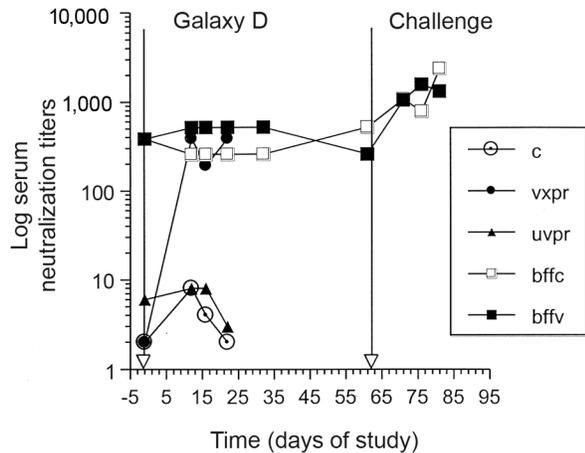


Figure 2. Serum neutralization titers for domestic ferret (*Mustela putorius furo*) controls (c), vaccinated domestic ferrets (vxpr), pair-housed unvaccinated domestic ferrets (uvpr), previously vaccinated older black-footed ferrets (*M. nigripes*) receiving Galaxy-D boosting prior to challenge (bffv), and previously vaccinated older black-footed ferrets challenged with canine distemper virus (bffc). Controls and unvaccinated pair-housed domestic ferrets did not exhibit significant titer increases. Black-footed ferrets started with high median titers from previous vaccinations, but boosting with Galaxy-D had no significant effect on their titers. Challenge caused elevated titers in the survivors.

Black-footed Ferrets

Nonreproductive, older (6–8 years), mixed-sex black-footed ferrets (culled from the breeding program) that had previously received one or more experimental KV vaccinations (an Onderstepoort strain-origin experimental vaccine produced by M. Appel, Baker Institute, Cornell University) were randomly assigned to one of two treatment groups after being matched for CDV serum neutralization titer across groups prior to study. At the beginning of the study, the first group ($n = 8$) received a single dose of Galaxy-D subcutaneously while the second group ($n = 7$) served as controls. Surviving vaccinates ($n = 6$) and controls ($n = 5$) were challenged 61 days later. The timeline for the experiment is shown in fig. 1. The primary endpoint of interest was survival although necropsies were performed to determine pathological changes following challenge as well as the cause of death.

Serum neutralization titers in surviving black-footed ferrets revaccinated with Galaxy-D and previously vaccinated (with the M. Appel killed CDV vaccine) black-footed ferret controls were comparable to those observed in newly vaccinated domestic ferrets receiving Galaxy-D for the first time. As expected, these titers contrasted sharply with those of unvaccinated seronegative domestic ferret controls (fig. 2). Prior to challenge, one black-footed ferret with a titer of 1:8 from prior vaccination succumbed (one of eight) to vaccine strain CDV 15 days after vaccination, and another died from

a secondary infection, likely related to CDV-induced immunosuppression (*Clostridium* sp. was isolated from this case of vascular sepsis). In addition, a control black-footed ferret (unvaccinated during the present trial) succumbed to CDV (one of seven; it succumbed 32 days after vaccine delivery and had an initial titer of 1:64) although it was housed in a separate rack of cages adjacent to the black-footed ferret vaccinates. Following challenge, three of six vaccinates died, one 17 days after challenge (1:512). Of black-footed ferret controls, when they were finally challenged, one died 11 days later, and another died in response to a secondary infection (*Enterobacter faecalis*-induced sepsis). All black-footed ferret challenge survivors developed elevated CDV titers.

Previously, CDV-naïve black-footed ferrets were shown to be highly susceptible to the development of canine distemper even when the virus (canine passaged) was supplied by vaccination as a modified-live CDV strain (Carpenter and others, 1976). The presence of high titers from the KV vaccine appeared protective for black-footed ferrets exposed to live attenuated CDV in vaccine (Galaxy-D) or to the challenge strain; nevertheless, high titers alone were not always indicative of protection, as illustrated by one animal with a high titer (1:512) that still succumbed to CDV. From this series, MLV boosting of black-footed ferrets with high circulating CDV titers was of marginal value, most likely due to the blocking effect of these antibodies on the vaccine strain. There is no evidence that cell-mediated immunity was enhanced from boosting. Even so, overall, titers above 1:64 in this series appeared to confer protection against CDV challenge. Perhaps more important was the observation that protection against CDV did not necessarily ameliorate the likelihood of immunosuppression and death from secondary invaders. Finally, of those succumbing to CDV, the precipitous onset of neurological signs, without other prodromal signs, was the hallmark of disease development in prior vaccinates. This has been explained as a persistence of F protein-directed immunity with waning HA protection and is qualitatively similar to the outcome observed when CDV-infected vaccinates encounter novel CDV strains where HA antigenicity has shifted (Stern and others, 1995).

Canarypox-vectored Vaccination and the Potential for Oral Vaccine Delivery

A dose-response study was performed to define the minimum protective dose and chronicle possible side effects of an experimental canarypox-vectored recombinant CDV vaccine (reCDV) in Siberian polecats, as described in detail elsewhere (Wimsatt and others, 2003). Briefly, subcutaneous dose groups received $10^{5.5}$, $10^{5.0}$, or $10^{4.5}$ plaque-forming units (PFU, a measure of vector and therefore vaccine concentration), and oral dose groups received $10^{8.0}$ and $10^{5.5}$ PFU. The timeline used for vaccination, blood sampling, and challenge is shown in fig. 3; challenge was performed 61 days after the

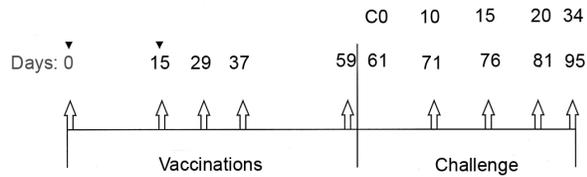


Figure 3. Timeline for canarypox-vectored recombinant canine distemper virus vaccine (reCDV) trials in Siberian polecats (*Mustela eversmannii*) and in the reCDV-Galaxy-D boosting study where the same timeline was used except that Galaxy-D was substituted for the second reCDV vaccination. Triangles indicate days of vaccination. Arrows indicate days when blood samples were drawn.

first vaccination. For standardization purposes, only vaccine expressing >95 percent expression-capable canarypox vaccine vector was used. Outcomes included CDV-associated clinical sign development, survival of virulent challenge postvaccination, and antibody development; only the latter two outcomes will be recounted here.

As previously reported, oral reCDV vaccination of Siberian polecats with $10^{8.0}$ PFU vaccine was protective for five of six vaccinates, or 83.3 percent effective in protecting Siberian polecats against lethal CDV challenge (Wimsatt and others, 2003). A difference in survival following challenge was noted in groups receiving the same vaccine dose ($10^{5.5}$ PFU) by different routes (oral vaccine, none of six survived challenge; subcutaneous vaccine, three of six survived) indicating that the parenteral route was superior to oral delivery. The difference in challenge survival between the $10^{5.5}$ PFU (three of six survived) and $10^{5.0}$ PFU (three of five survived) subcutaneous dose groups was not significant, suggesting the minimal protective CDV PFU dose is higher than $10^{5.5}$.

A Kaplan-Meier survival analysis was performed with dose and route of reCDV administration as predictors (fig. 4) (Wimsatt and others, 2003). Protective titers in response to reCDV were typically lower than those measured following vaccination with Galaxy-D in naïve animals; higher relative titers in response to reCDV were associated with greater protective value of the vaccine, and generally predictive of vaccine efficacy overall, as was the case for the modified-live vaccine. Even so, some challenge survivors that received reCDV had titers low enough that they would have been predicted to succumb to the challenge if modified-live vaccine protective titers were used as a guideline (e.g., 1:50–100; see fig. 5). It seems plausible that the protective titer differential between reCDV and modified-live vaccines in challenge survivors reveals that cell-mediated immunity conferred by the reCDV vaccine is a major aspect of its protective effect.

Starting in the early 1990s, interest was developing among black-footed ferret conservationists for the identification of a safe and effective CDV vaccine to use in this endangered species. The potential to safeguard the black-footed ferret using a canarypox-vectored subunit vaccine led to a

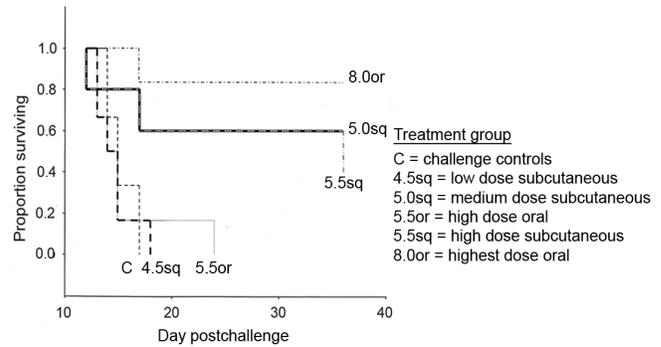


Figure 4. A survival curve is shown for canarypox-vectored recombinant canine distemper virus vaccine trials with Siberian polecats (*Mustela eversmannii*). Animals receiving two $10^{8.0}$ PFU vaccinations orally (8.0or) survived. Those receiving lower subcutaneous doses (e.g., 5.0sq) had lower survival as did those receiving lower oral doses (e.g., 5.5or), which fared even worse. (From Wimsatt and others, 2003. Reprinted with permission of the *Journal of Zoo and Wildlife Medicine*.)

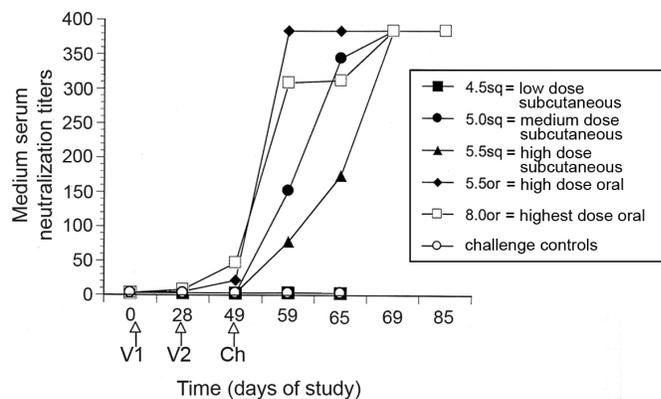


Figure 5. Serology results from the canarypox-vectored recombinant canine distemper virus (CDV) vaccine dose-response study where varied doses were administered subcutaneously or orally under isoflurane anesthesia. These results indicated that survivors mounted CDV serum neutralization titers above those of nonsurvivors. An exception was noted in the case of the oral $10^{5.5}$ group, where nonsurvivors mounted elevated titers, but these titers were insufficient for protection against challenge. Thus, a cell-mediated component of immunity, mounted at higher protective vaccine doses, must be important for vaccine efficacy with vectored subunit vaccines against CDV. V1 = first vaccination, V2 = second vaccination, Ch = challenge. (Adapted from Wimsatt and others, 2003. Reprinted with permission of the *Journal of Zoo and Wildlife Medicine*.)

series of studies in Siberian polecats with the ultimate goal of applying this vaccine to the black-footed ferret; this work became a major focus starting in 1996. At the same time, it was recognized that this work could serve as a guide for other highly CDV-susceptible species. This vectored vaccine type,

sometimes referred to as a type III recombinant vaccine (Van Kampen, 2001), used a canarypox vector to infect local (at the site of delivery) host cells, which then present HA and F antigens to T cells and macrophages, initiating cell-mediated and humoral responses (Schultz and Zuba, 2003). The canarypox vector was chosen because pox viruses do not use cell receptors for cell uptake during cellular endocytosis, the avian virus is avirulent at mammalian body temperatures, the pox genome is large enough to allow sizable vaccine-related gene substitutions, and pox vectors potentially reduce the risk of host genomic splicing (Tartaglia and others, 1992, 1993; Perkus and others, 1995a,b; Adams and others, 1997). Optimal recombinant vaccines are constructed to obtain high gene expression rates in host cells. Ideally, the immune system recognizes these cells and presents them to the humoral and cell-mediated arms of the immune system to develop a broad immune response with protective attributes somewhere between those of a modified-live vaccine and a KV vaccine (Schultz and Zuba, 2003). Advantages of this approach are that (1) no intact infectious agent is used, (2) pox virus products are more durable than modified-live CDV, and (3) adjuvants are not required. Vaccinated domestic cats (*Felis silvestris*) (Macy and Couto, 2001) appear to be at risk of developing injection site-associated sarcomas; this issue has also been raised with domestic ferrets, which appear at lower risk with recombinant vaccines (Merial Technical Services, oral commun., 2000). Another concern seen in domestic ferrets following repeated vaccination with approved modified-live products has been the increased risk of anaphylaxis (Fox and others, 1998). In one study surveying the risk of side-effects of vaccination in domestic ferrets, adverse reactions were reported approximately 5 percent of the time, particularly in older, previously vaccinated ferrets (Greenacre, 2003). This appears to be rarer with some products than others (Fox and others, 1998) and may be less likely with vectored vaccines although they have not been evaluated long enough to answer this question conclusively at this time. Repeated vaccination increased glomerular immune-complex deposition in mink receiving a multivalent vaccine that included CDV; unfortunately, the potential risk of glomerular disease was not studied (Newman and others, 2002). Recent anecdotal reports suggest that even the commercially available vectored CDV vaccine (PureVax[®] Ferret Distemper Vaccine; Merial, Inc., Athens, Ga.) is not without some risk in black-footed ferrets. Recently, several deaths in black-footed ferrets have been linked to its use in zoos (D. Garelle, oral commun., 2004).

Another important objective was to determine the efficacy of reCDV vaccine when delivered orally, so it could ultimately be used for wild black-footed ferrets in baits. Raboral V-RG[®] (Merial, Inc., Athens, Ga.), a vaccinia-vectored rabies subunit vaccine had been successfully packaged and broadcasted in baits to curtail fulminant rabies outbreaks in several wild carnivore populations (Fearneyhough and others, 1998; Hanlon and others, 1998; Olson and Werner, 1999). As demonstrated in domestic ferrets, vaccinia likely represents a better vector for oral administration than canarypox based

on vaccination and challenge by enteric instillation (Welter and others, 1999). However, the risk of human infection when encountering the vaccinia vector remains of potential concern, particularly for immunocompromised individuals; a vectored-vaccine, bait-induced vaccinia infection was documented in a pet owner when she tried to remove a bait from her dog's mouth and was bitten in the process (Rupprecht and others, 2001). The appearance of a vaccinia strain from Brazil pathogenic to cattle and humans (Palca, 2005) may ignite a debate about the persistence of this virus, or of genetic constructs of this virus when used as a vector in the future.

Vaccination Effect on Humoral Immunity

In this study, pokeweed blastogenesis (pokeweed is a nonspecific B lymphocyte mitogen) was performed on blood samples from Siberian polecats collected immediately prior to and 14 days after a single reCDV vaccination ($10^{5.5}$ PFU) and coincidentally from unvaccinated saline control polecats.

Changes in blastogenesis responses of B lymphocytes in primary culture between vaccinates and controls were not statistically different (fig. 6). Hence, reCDV vaccination did not appear to cause significant suppression of B cell lines (immunosuppression) expected during sequelae of CDV modified-live vaccination and natural CDV infection.

In this study, we hypothesized that the immunosuppression associated with modified-live vaccination would not occur when using vectored CDV vaccines, a major

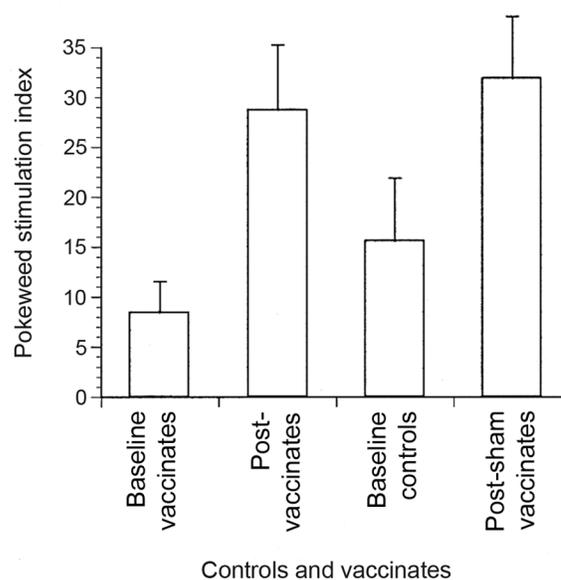


Figure 6. Pokeweed blastogenesis was performed on two samples of peripheral lymphocytes before and 14 days after canarypox-vectored recombinant canine distemper virus (reCDV) vaccination or saline control injections. The change in lymphocyte blastogenesis between controls and vaccinates was not significantly different and suggests that reCDV was not immunosuppressive as compared to live CDV exposure or modified-live virus vaccination.

advantage of the latter type. These results confirmed that the reCDV vaccine did not appear to cause a blunted B lymphocyte blastogenic response to pokeweed mitogen, typical of immunosuppression seen with modified-live CDV vaccines.

MLV Vaccine Boostering Following Vectored Vaccine

Onderstepoort strain origin genes for F and HA were used during construction of the reCDV vaccine and are expressed in Galaxy-D. To assess the potential for interference or synergy expected from use of reCDV followed by modified-live (Galaxy-D) vaccination, Siberian polecats that received a single reCDV dose ($10^{5.5}$ PFU) were subsequently boosted with Galaxy-D subcutaneously. These animals were challenged 61 days later. The timeline employed for blood sampling, vaccination, and challenge is depicted in fig. 3.

Five of five mixed sex Siberian polecats that received a single reCDV dose boosted with Galaxy-D survived challenge whereas six of six seronegative challenged controls succumbed.

This study in Siberian polecats showed that a single reCDV vaccination using the F and HA proteins from the Onderstepoort strain did not interfere with a single Galaxy-D vaccination that followed, in effect using the same antigens from this strain in both cases; likewise, during the challenge that followed, this combination provided 100 percent survival, and, in our hands, provided protection equivalent to that of a single Galaxy-D vaccination in domestic ferrets, as mentioned previously. The use of a MLV vaccine to booster the commercial reCDV vaccine (PureVax) is of interest to domestic ferret owners, and this practice has been shown to be effective in pet ferrets when using the currently USDA approved MLV (Fervac-D) vaccine (Merial Technical Services, oral commun., 2001). The production of low (blocking) titers and immune priming conferred by recombinant vectored vaccines may make them ideal candidates for MLV boosting that is expected to confer long-term immunity.

While not specifically tested, modified-live CDV boosting in black-footed ferrets suggests that modified-live vaccination following limited reCDV vaccination may be quite risky. Studies are in progress to establish the duration of titrated immunity expected in black-footed ferrets over time following a primary two-vaccination series with PureVax (J. Kreeger, oral commun., 2004). Some investigators believe that three primary vaccinations will be warranted to provide a longer duration of immunity and higher protective titers (R. Montali, oral commun., 2003). Whether boosting with Galaxy-D or another relatively safe modified-live vaccine following some type of primary recombinant vaccination in black-footed ferrets will ever be worth the risk remains unclear. One important aspect of modified-live vaccination remains attractive; that is, the likely provision of life-long immunity in modified-live vaccinates. Immunity following a primary modified-live vaccination series with chick embryo attenuation was protective

against lethal challenge at 6 years of age in mink and domestic ferrets (Burger and Gorham, 1964), and 5.5 years after similar vaccination in another domestic ferret study (Cabasso and Cox, 1953); this same result was reported in dogs 6.5 years after vaccination (L. Carmichael, personal commun., 1997, as reported by Gorham, 1999, p. 559). If repeated recombinant vectored vaccine vaccination does not confer life-long immunity, a trial to determine if MLV boosting following a full reCDV primary series may be warranted in black-footed ferrets destined for release, since it is highly unlikely they can be caught again for revaccination once in the wild. Alternatively, an effective oral baiting program with recombinant vaccine may be developed.

Vectored Vaccine Safety During Pregnancy

The timeline for vaccination, blood sampling, and challenge for evaluation of vectored vaccine safety in pregnant Siberian polecat females is shown in fig. 7 (upper timeline). Twelve treatment-randomized, unvaccinated Siberian polecat jills were compared to 12 reCDV vaccinates. Vaccination of CDV-naïve, reproductively intact polecat jills with a moderate reCDV dose ($10^{5.5}$ PFU subcutaneously) immediately prior to conception was followed by a second vaccine dose during the last 10 days of pregnancy.

Initial vectored vaccination had no significant effect on conception rates. Following a second vaccination at 29 days of gestation, birth outcomes such as litter size and kit rate of weight gain (measured from 17 to 35 days of age) were not significantly different from those in unvaccinated controls.

Canine distemper virus has been demonstrated to be capable of crossing the placental barrier of infected pregnant bitches and infecting their unborn puppies (Krakowka and others, 1974, 1977). Most reproductive-age bitches are either

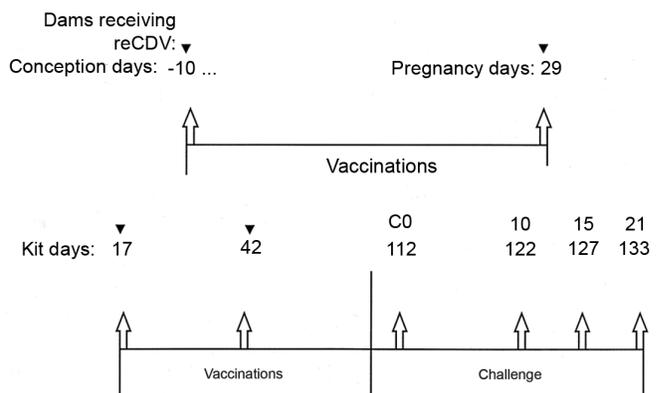


Figure 7. Timeline for the canarypox-vectored recombinant canine distemper virus (reCDV) immunization of Siberian polecat (*Mustela eversmannii*) dams to assess conception and pregnancy safety. Also shown is the timeline for vaccination of their kits in the passive immunity study. Triangles indicate days of vaccination. Arrows indicate days when blood samples were drawn.

vaccinated or exposed to CDV prior to pregnancy, conferring immunity; thus, it is likely that the potential for naïve dams of wild species or domestic canids to pass CDV transplacentally is underestimated (Krakowka and others, 1974), and the potential impact of CDV on reduced fecundity has not been well characterized in wild carnivores. Gorham (1999) conducted studies exploring the potential ill effects of vaccination before conception and during pregnancy employing a modified-live vaccine in mustelids. In those studies, modified-live vaccination influenced neither litter size nor apparent fertility; these results are similar to ours employing reCDV and suggest that high virus loads may be required to see transplacental disease.

Because the reCDV vaccine uses a novel vector, we tested the safety of this vaccine on reproductive polecat jills before conception, during pregnancy, and on kit growth 17–35 days postpartum as a prelude to vaccine use in reproductive black-footed ferrets. For 3 years, the National Black-footed Ferret Conservation Center has been vaccinating reproductive black-footed ferrets with PureVax starting several months prior to the breeding season. This practice has not caused any identifiable adverse effects on fecundity and overall production (P. Marinari, oral commun., 2004).

Vectored Vaccine Use in the Face of Passive Immunity

In 1997, 12 randomly selected Siberian polecat kits from mothers vaccinated twice with reCDV before conception and delivery (fig. 7, lower timeline) were themselves vaccinated at 4 and 6 weeks of age; kits received a standard challenge at 19 weeks of age.

All kits challenged at 19 weeks of age died with characteristic signs of CDV postchallenge. At this age, maternal protective immunity has disappeared in domestic ferrets (Gorham, 1999; Welter and others, 2000), suggesting that active immunization for CDV with reCDV (at $10^{5.5}$ PFU subcutaneously) in the presence of passive immunity, as tested in the present series, was without benefit.

Indirect evidence has suggested that antigen presentation to the cell-mediated arm of the immune system and particularly to T lymphocyte-induced cytotoxicity can lead to cell-mediated immunity independent of humoral responses (Siegrist and others, 1998a,b). It has been demonstrated in puppies (Taylor and others, 1994) that vectored vaccination with rabies glycoprotein results in active immunization in the face of blocking passive maternal antibodies. Here, we hypothesized that reCDV vaccine might actively protect young Siberian polecats postnatally even though they carried passive immune protection from circulating maternal antibodies generated against the same vaccine. According to this line of reasoning, active immunity would develop during postnatal vaccination with reCDV by independently augmenting active (mostly T cell-mediated) immunity. This possible application was attractive because maternal immunity typically blocks conventional vaccines during this period, and the actual trajec-

tory of waning maternal immunity is unpredictable in mustelid kits (Gorham, 1999), leaving susceptible young unprotected. Welter and others (2000) challenged domestic ferrets at 12 weeks of age after parenteral vaccination with canarypox and vaccinia-vectored CDV vaccines for F and HA. In their study, vector-origin antigens had little effect on survival in early vaccinates, which was not significantly different from that of CDV-naïve controls. These results are similar to ours for the Siberian polecat, where early vaccinates, like CDV-naïve controls, succumbed to CDV during challenge. In their study, Welter and others (2000) attributed this vaccination failure to immaturity and nonresponsiveness of the immune system of the domestic ferret, a relatively altricial species. Our results support their observation; however, a lower dose of a canarypox-vectored vaccine was used in our study in Siberian polecats, complicating the final interpretation.

Canarypox cross-vaccination was not observed in unvaccinated Siberian polecats housed in adjacent cages. Thus, reCDV does not appear to be prone to cross-vaccination in this species. Similarly, reCDV vaccinated pregnant Siberian polecat jills adjacently caged with CDV and reCDV vector-naïve polecat jills never seroconverted following reCDV vaccination (J. Wimsatt, unpub. data., 1997).

Discussion

The ability of a vaccine to protect against differing CDV strains depends on how close the HA and F proteins are to the vaccine's Onderstepoort-origin proteins expressed by the vector. In this regard, Galaxy-D and the vectored (reCDV) vaccine are similar in the qualitative aspects of their protection. For the vectored vaccine, it is too early to assess the long-term effects of injecting canarypox into foreign species. In theory, the nature of recombinant vaccines and the limited antigens they express may require that they be updated more frequently to keep pace with strain changes, if other antigens can contribute to immune protection during modified-live infection and immunity development. If so, verified failure of antigenic protection with reCDV vaccines may potentially serve as a more exacting measure of evolving antigenic shifts in wild strains in the future.

In contrast to modified-live vaccination, vectored vaccine presentation to the mucosal membranes may yield different results from parenteral administration, reflecting limited vector invasiveness of mucosal surfaces, particularly in regards to the canarypox vector (Welter and others, 1999). Whether this will have a practical outcome, say in the heterogeneity of host responses across species following oral administration, remains to be determined. The long-term impact of live virus vectors and their potential to revert to virulence remains a matter of speculation, but careful monitoring is warranted, since poxviruses generally have the potential to mutate and adapt to new species. While replication of the canarypox virus in hosts appears to be minimal, the period of retention of the

virus has not been as well characterized in varied species, and the large number of species receiving this vaccine leaves open the possibility of specific species predispositions and alterations in strain virulence over time, if persistence occurs. The recent emergence of a pathogenic variant of vaccinia virus may exemplify this concern (Palca, 2005).

What the Future May Hold

Considering the wide range of related morbilliviruses affecting diverse orders and classes of animals, and the demonstrated transfer of distemper and other morbilliviruses to bystander species (Stallknecht and others, 1991; Jacobson and others, 1992, 1997, 2001; Visser and others, 1993; Appel and Montali, 1994; Duignan and others, 1995; Richter and others, 1996; Karesh and others, 1997, 1999; Longbottom, 1997; Barrett, 1999; Jauniaux and others, 2000; Bossart and others, 2001; Lam and Chua, 2002; Johnson, 2003), the potential for cross-species movement and *de novo* creation of mutated variants of CDV seems high. For example, recent focus on HA variability among sympatric CDV strains (Gemma and others, 1996) suggests that commercial vaccine preparations may become inadequate for protection against CDV in the future (Mochizuki and others, 1999). However, caution is always warranted when documenting a vaccine failure because of the possibility of other causes. These other causes include incomplete dosing, genetic or ill-defined causes of host nonresponse (Leisewitz and others, 2001), administration during occult periods of host immunosuppression, and suboptimal product handling prior to use. Vaccine nonresponders have been documented for more than one canine disease (R. Schultz, oral commun., 2003).

A recent canine distemper outbreak at a zoo was associated with exposure to wild raccoons in the Chicago area (Lednicky and others, 2004). The appearance of this distinct strain has introduced some uncertainty about the ability of current commercial CDV vaccines to protect against new or emerging wildlife strains (Lednicky and others, 2004). Recent CDV disease outbreaks involving novel strains have raised the suspicion of vaccine failures although without controlled challenge studies these suspicions are difficult to prove (Bohm and others, 1989; Maes and others, 2003). Even so, this proposed causal relationship between novel strains, possibly from wildlife reservoirs, and the potential for vaccine failures has not been investigated adequately, employing careful ecological study techniques, modern molecular tools, and strain-specific challenge studies in vaccine-protected animals. An outbreak of naturally occurring CDV in black-footed ferrets highlights the need for safe and effective vaccines to protect them following reintroduction and as the threat continues into the future (Williams and others, 1988). Large cats and other carnivores would likely benefit as well (Blythe and others, 1983; Davidson and others, 1992; Appel and others, 1994; Harder and others, 1995; Roelke-Parker and others, 1996; Leisewitz and others, 2001).

The emergence of vaccine-resistant virus variants, like the analogous emergence of antibiotic-resistant bacteria, may be facilitated when vaccination is widely used and selection pressure is high. Even so, CDV vaccines have been surprisingly reliable over the last 50 years; this may relate to the observation that negative sense RNA viruses are less prone to recombine than other viruses (Chare and others, 2003).

Outbreaks of canine distemper in distant parts of the world have highlighted the significance of domestic and wildlife reservoirs as purveyors of distemper-induced disease worldwide (Bohm and others, 1989). Recent investigations surrounding CDV outbreaks in Japan (Mochizuki and others, 1999), Denmark (Blixenkroner-Moller and others, 1993), Poland (Jozwik and Frymus, 2002), and the United States (Lednicky and others, 2004) have brought into the focus the possible emergence of CDV strains no longer optimally immunized with commercial vaccine products. For the most part, such strains have shown characteristic heterogeneity in the HA gene, while the F component of current wild strains has remained surprisingly uniform across strains. This situation is analogous to using measles vaccination to cross-protect against CDV (Chalmers and Baxendale, 1994). When CDV passes across species, the possibility of variability at all sites, including the F protein gene, seems highly likely as new hosts tend to cause selection for greater virus diversity (Woolhouse and others, 2001). In related paramyxoviruses affecting other species, F gene heterogeneity has been noted and may influence species predilections, disease phenotypy, and vaccine efficacy in the future, especially under strong selection pressure (Collins and others, 1998; Ning and others, 2002; Ujvári and others, 2003).

The Promise of New Vaccine Strategies

A recent efficacy study using an adenovirus-vectored vaccine demonstrated the development of significant active immunity against CDV with the absence of mucosal immunity against the adenovirus vector in domestic puppies (Fischer and others, 2002). None of the other available vectored CDV vaccines are satisfactory for immunization of very young carnivores, and the adenovirus vector appears superior in this regard.

DNA vaccines are relatively safe, simple, and cheap to produce. They consist of DNA-encoding genes capable of producing vaccine antigens in host cells and mammalian promoters leading to selected gene expression (Liu, 2003). Recently, new DNA vaccines administered intramuscularly were shown to be highly effective against severe CDV challenge in mice (Sixt and others, 1998) and dogs (Fischer and others, 2003).

Unfortunately, nonparenteral methods of DNA vaccine and vectored vaccine delivery have low efficiency in producing a protective immune response. The low oral efficiency of the canarypox vector (Wimsatt and others, 2003) limits the potential use of commercial products now available

(Meril Technical Services, oral commun., 2004). Even so, a significant serological response was observed following oral administration (T. Vickers, oral commun., 2005) of two Pure-Vax vaccine doses at once in a recent study of Channel Island gray foxes (*Urocyon littoralis*) (Vickers and others, 2004). Vaccinia-vectored CDV constructs exist for research use (J. Taylor, oral commun., 1998). Vaccinia constructs appear to have greater enteric efficiency for bait delivery, as has been demonstrated during the use of Raboral V-RG in public health programs to vaccinate wild carnivores against rabies and experimentally with a vaccinia-vectored CDV vaccine (Welter and others, 1999). Mucosal delivery of DNA vaccines via new designer carriers will likely provide new opportunities for oral DNA vaccine delivery in the future (Hobson and others, 2003). With the advent of antiviral drugs, viral inhibitors of virus-host cell F are being developed to moderate paramyxovirus-induced disease progression, providing a new therapeutic approach (De Clercq, 2002).

The relatively homozygous (genetically depauperate) black-footed ferret is at risk of CDV-induced disease with the use of any currently available modified-live products. With the advent of designer vaccines for the concurrent delivery of immunostimulatory genes in concert with immunogens, the ability to stimulate the immune system (e.g., to express immunostimulatory levels of interleukin-6) while vaccinating will offer new possibilities in the future. Even the ability to correct an identified interleukin-6 deficiency in the black-footed ferret may be on the horizon through the use of gene therapy via vectored vaccine or naked DNA approaches. Such methods could eventually serve to enhance the resistance of this and other sensitive species to the ravages of infectious diseases, if germ line incorporation becomes practical.

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