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Immunization of Puppies in the Presence of Maternally Derived Antibodies Against Canine Distemper Virus

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Summary

Vaccination of dams with modified-live canine distemper virus (CDV) vaccines will elicit high concentrations of colostral antibody, that although vital for protection of the pup during the first weeks of life, can interfere with active vaccination against the virus. In the present study, 12 pups, 7–9 weeks of age, with maternally derived immunity to CDV, were vaccinated with a canarypox-vectored CDV vaccine. These pups were protected against intravenous challenge with CDV. Three littermate pups that were unvaccinated all developed clinical signs of infection after challenge, and two of these control pups died.

Keywords: canine distemper virus; maternal antibody; canarypox-vectored vaccine

Introduction

The attenuation of the canine distemper virus (CDV) by passages in cell culture and in allantoic fluid of chicken embryos has enabled the production of CDV modified-live virus (MLV) vaccines and made mass vaccination of dogs possible since 1958 (Rockborn *et al.*, 1965). Inactivated vaccines have been used in exotic and very susceptible carnivores as the use of attenuated CDV is unsafe in these species (Appel and Summers, 1995). The canarypox-vectored CDV MLV (recCDV or rD) vaccine was licensed for use in dogs in 1997 in the USA (Pardo *et al.*, 1997) with the benefit that postvaccinal CDV encephalitis cannot occur with this vaccine. This vaccine is also being used in several Latin American countries where the field pressure of CDV infection is the greatest. Since 2001, the recCDV vaccine has also been available in the USA in monovalent presentation for use in domestic ferrets.

The CDV MLV vaccines are capable of eliciting high concentrations of colostral antibody in the progeny of healthy vaccinated dams. Although colostral antibody is vital for the protection of puppies during the first weeks of life, interference from this antibody can make a CDV vaccination program for puppies difficult to design. Newborn pups, given the opportunity to ingest colostrum, will acquire an initial CDV serum neutralizing antibody (SN) titre of up to 77 per cent of the serum titre of the dam (Greene and Appel, 2006). If the titre of passively acquired SN CDV antibodies in the pup is too high, these can interfere with active immunization, whereas if the titre is too low, the pup could be susceptible to CDV infection. In order to overcome this very practical issue, a range of alternative approaches has been investigated.

The first such approach involved the use of a measles virus vaccine. Measles virus is a paramyxovirus of the genus *Morbillivirus*, and intramuscular vaccination with experimental measles, or commercial measles/CDV vaccines, has been used to administer a first dose of vaccine to young pups in a kennel situation, in order to generate cross-reactive immunity (Appel *et al.*, 1984).

The use of CDV vaccines vectored with canine adenovirus-2 (CAV-2) has also been described. Intranasal administration of CAV-2 to pups in the face of CAV maternally derived antibodies is known to engender a

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good antibody response (Appel *et al.*, 1975). For this reason the potential of CAV-2 vectored CDV vaccines in puppies born to CDV and CAV-2 vaccinated dams was investigated (Fischer *et al.*, 2002). Two experimental replication-competent canine adenovirus type 2 (CAV-2)-based vaccines expressing CDV haemagglutinin (HA) and fusion (F) glycoproteins (vCA13 and vCA17, respectively), were tested in pups with maternally derived antibodies to CDV and CAV-2. CDV neutralizing antibodies were induced, and solid protective immunity against intracerebral challenge with virulent CDV was present, in all subcutaneously vaccinated pups, despite the presence of pre-existing systemic immunity to the CAV-2 vector. This approach may be an efficient strategy to overcome passively acquired immunity to CDV and CAV-2 in pups. However, when the same constructs were administered intranasally to pups with pre-existing systemic immunity to CAV-2, anti-CAV-2 vector interference was shown, suggesting limitations for the practical use of such recombinant viruses for intranasal vaccination in the face of maternally derived immunity.

CDV DNA vaccines have been tested by several researchers in specific pathogen-free (SPF) puppies and pups with maternally derived CDV antibody. We have demonstrated that vaccination of SPF puppies with a lipid-formulated DNA plasmid vaccine, encoding HA and F CDV membrane glycoproteins, protects against a severe CDV virus challenge (Fischer *et al.*, 2003). Researchers in Denmark subsequently confirmed the potential of CDV DNA vaccination, using plasmid DNA encoding the CDV HA and nucleoprotein antigens to protect against a lethal CDV challenge (Dahl *et al.*, 2004). The protective efficacy of experimental CDV DNA vaccines in pups with CDV maternally derived antibodies has been demonstrated by the University of Wisconsin and Mayo Clinic (Reed *et al.*, 2003) and the Institute of Virology and Immunoprophylaxis, Swiss Federal Veterinary Office (Griot *et al.*, 2004).

Using recombinant technology, genes encoding two immunogenic CDV or measles virus antigens (HA and F) were inserted into the genome of a vaccinia (Taylor *et al.*, 1991) or ALVAC® canarypox (Stephensen *et al.*, 1997) vector virus. We have previously demonstrated that a canarypox CDV vectored vaccine protects SPF pups against lethal intracerebral CDV challenge (Pardo *et al.*, 1997). The aim of the present study was to determine the efficacy of the canarypox vectored CDV vaccine in pups with maternally derived antibodies to CDV.

Materials and Methods

A vaccination challenge study was carried out in 15, 7–9-week-old pups born to CDV immune dams. The CDV SN antibody titres of the three dams at whelping were high (600, 906 and 1520, respectively). Fifteen puppies with CDV SN antibody titre $\geqslant 30$ were selected for the study. At least two litters were represented in each vaccine group as shown in Table 1.

The canarypox-vectored CDV antigen was delivered in a combination lyophilized vaccine containing

Table 1
Experimental design

Group	*Vaccine*	*Route of administration*	*Number of pups*	*Pup ID (dam ID)*	*Mean CDV SN titre 1 week before first vaccination*
1	rDACPiP	s/c	6	1652 (12777)	60
				1659 (12777)	60
				1701 (11927)	30
				1702 (11927)	30
				1703 (11927)	30
				1704 (11927)	30
2	rDACPiP	i/m	6	1641 (44025)	30
				1642 (44025)	30
				1651 (12777)	30
				1653 (12777)	30
				1654 (12777)	50
				1705 (11927)	30
3	ACPiP	s/c	3	1643 (44025)	30
				1656 (12777)	30
				1706 (11927)	30

rDACPiP, canarypox-vectored CDV, adenovirus type 2, coronavirus, parainfluenza, and parvovirus; ACPiP, adenovirus type 2, coronavirus, parainfluenza, and parvovirus; s/c, subcutaneous; i/m, intramuscular; ID, identification number; SN, serum neutralizing antibody.

Table 2
CDV serum neutralizing antibody titres

Vaccine given	*Mean SN titre 6 days before vaccination*	*Mean SN titre 14 days after second vaccination (day 35)*	*Mean SN titre before CDV challenge (day 51)*
rDACPiP	34	77	103
ACPiP	32	⩽3	⩽3

canine adenovirus, canine coronavirus, canine parainfluenza and canine parvovirus (rDACPiP) antigens. Six pups received two doses of vaccine, administered subcutaneously, 3 weeks apart (Group 1). Six pups recived two doses of vaccine, administered intramuscularly, 3 weeks apart (Group 2). The remaining three pups received a vaccine identical to those animals in Groups 1 and 2, but devoid of canarypox-vectored CDV antigen (ACPiP). These three pups constituted the negative control group (Group 3). All pups were co-housed in pens regardless of group assignment.

In order to evaluate protection against disease, CDV USDA NVSL challenge stock was administered intravenously to all pups 21 days after the second vaccination. All animals were observed twice daily for 21 days after challenge to record morbidity and mortality. The staff performing clinical observations and laboratory analyses were "blind" to the dog/group assignments.

Results

After vaccination, active CDV seroconversion was demonstrated in the pups (Table 2). All three negative control pups started to show signs of CDV-related illness 7–8 days after challenge. By 10 days after challenge they presented with prostration, dehydration, respiratory, enteric and nervous signs. Two pups were humanely destroyed on day 10 after challenge, due to the severity of the disease. One control pup was kept alive and recovered from disease.

All 12 vaccinated pups survived intravenous challenge with CDV uneventfully; they were alert and healthy during the 21 day observation period. No marked clinical abnormalities were observed in any of the vaccinated dogs after challenge. Three vaccinated pups were recorded to have either serous nasal discharge or to have loose stool for 1 day only.

Conclusion

Canarypox-vectored canine distemper vaccine immunized and protected pups with maternally derived immunity to CDV against an intravenous challenge with higly virulent CDV. Other independent studies performed by University of Wisconsin confirm that the use of this vaccine in pups with maternal antibodies to CDV induces protective immunity to CDV (Haase *et al.*, 2006).

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