

SEROLOGIC RESPONSE TO A CANARYPOX-VECTORED CANINE DISTEMPER VIRUS VACCINE IN THE GIANT PANDA (*AILUROPODA MELANOLEUCA*)

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Abstract: The giant panda (*Ailuropoda melanoleuca*) is known to be susceptible to natural infection with canine distemper virus (CDV). Vaccination of giant pandas with conventional modified live CDV vaccines has been avoided due to the numerous carnivore species known to have become infected with CDV after vaccination. Serum-neutralizing antibodies to CDV were measured after s.c. and i.m. annual vaccination with a canarypox-vectored recombinant CDV vaccine in an adult male and female giant panda over the period of 2 yr. The vaccine proved to be safe, and serum-neutralizing antibody titers interpreted as protective against CDV disease were measured in each animal.

Key words: *Ailuropoda melanoleuca*, canarypox-vectored recombinant vaccine, canine distemper virus, giant panda, titer, vaccine.

BRIEF COMMUNICATION

Canine distemper virus (CDV) is a morbillivirus known to cause morbidity and mortality in a broad range of carnivore species.² Fatal cases of CDV in giant pandas (*Ailuropoda melanoleuca*) have been documented in Chinese breeding centers.⁶ Moreover, a serosurvey demonstrated neutralizing antibodies to CDV in one of five captive and one of three recently rescued giant pandas sampled within 24 hr of arrival to a giant panda breeding facility.³ Findings from these two studies suggest exposure of giant pandas in China in both captive breeding facilities and in the wild. Feral dogs as well as raccoons (*Procyon lotor*) and other wild carnivores are known to carry the virus in several regions of the world and are commonly found in urban areas where zoological parks and breeding centers are located.^{2,4}

Vaccine-induced infection after administration of modified live distemper vaccines of dog, primate, and avian cell origin have been documented in numerous carnivore species.² Vaccine-induced CDV infection has not been documented in the giant panda to date, but vaccination with a modified live vaccine in this species is generally not recommended due to the number of closely related exotic species in which vaccine-induced CDV infection has occurred.⁴

Vaccine studies have not been published for the giant panda. Current giant panda species survival plan recommendations include annual vaccination with the univalent canarypox-vectored recombinant distemper vaccine (Purevax Ferret[®], Merial, Athens, Georgia 30601, USA). The American Association of Zoo Veterinarians' Distemper Vaccine subcommittee furthermore recommends this vaccine for extra-label use in all susceptible exotic carnivore species.

The two giant pandas in this study, one male (born 27 August 1997) and one female (born 22 July 1998), arrived at Smithsonian's National Zoological Park (NZIP) as 3- and 2-yr-old adults, respectively, in late 2000 from the China Conservation and Research Centre for the Giant Panda, Wolong Nature Reserve, Sichuan, People's Republic of China. While at this facility, they received 1 ml of a polyvalent CDV vaccine labeled for domestic dogs in China in September 2000 to comply with Chinese export regulations, but further details concerning the vaccine were not available. They were boosted three times every 3 wk with 1 ml of the canarypox-vectored CDV vaccine i.m. via dart or s.c. in a training cage in December 2000 and January 2001 by NZIP veterinarians. The first booster was given immediately before transport to the United States, and the remaining boosters were given after arrival. A further s.c. vaccination was given 1 yr later in February 2002.

The period during which the current study occurred was from May 2003 to October 2005. The male received a 1-ml dose of the canarypox-vectored CDV vaccine on 7 May 2003 (s.c.) and on 13 May 2004 (i.m.). The female received the vaccination on 24 June 2003 (i.m.) and on 12 October 2004 (i.m.).

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For this prospective study, blood was drawn before administration of the vaccine, weekly for the first month postvaccination, twice monthly 1–3 mo postvaccination, and then once monthly until 1 yr after vaccination or until the next vaccine could be administered. However, blood sampling also was dictated by the willingness of the pandas to enter the training cage and participate in training, resulting in some missed samples, especially during breeding seasons each year (March–May) and during the pregnancy of the female in summer 2005. Vaccination was further delayed in the female to avoid administering the vaccine while possibly pregnant. Blood was drawn from the left cephalic vein in a training cage by using operant conditioning and food rewards. The blood was allowed to clot, and serum was separated and stored at -70°C until testing. Serum-neutralizing antibodies were measured at the New York State Veterinary Diagnostic Laboratory (Ithaca, New York, USA). Samples were considered positive at or above a dilution of 1:8.

Serum-neutralizing antibody titers to CDV are shown in Fig. 1 during the 2-yr period of this study. Both animals developed peak titers of 1:384–1:1538 by 7–14 days postvaccination, and antibody levels slowly returned to a lower level (1:12–1:64) by 7–14 wk postvaccination and remained at this level throughout the year until the next booster vaccination. During the first year of the study in the male panda, the initial elevated spike in titer levels was probably present in the first weeks, but the animal was not sampled until 5 wk postvaccination, when the titers were probably beginning to decrease. In the first year of the study in the female panda, the initial spike in the first and second weeks postvaccination was lower than that in the second year, but the animal also was not sampled between weeks 3 and 7, so a further increase in titer may have occurred during this time but was not documented.

Serum-neutralizing CDV antibody levels are considered fully protective against challenge in domestic dogs if $>1:100$ and partially protective if titers are $<1:100$ after vaccination with a modified live vaccine. However, without challenge experiments, which are not feasible in endangered species, it is difficult to compare antibody levels measured at different laboratories at different times. Furthermore, studies testing recombinant vaccine titers have found lower levels of antibody postvaccination than with modified live vaccines but effective protection against challenge in dogs⁵ and Siberian polecats (*Mustela eversmanni*).⁷ It is thought that not only humoral but also cellular immune fac-

tors play a role in viral protection with recombinant vaccines.

Avipox-vectored recombinant vaccines have been developed for use in humans and domestic animals to protect against various infectious diseases in recent years. They are produced by inserting a gene for specific immunogenic proteins into the genome of the vector. In the canarypox-vectored CDV vaccine, the hemagglutinin and fusion glycoproteins are inserted into the canarypox vector, which is unable to replicate in nonavian host cells but will express the target antigens and not the entire virion in the host.⁵ Therefore, it should be impossible for the distemper virus to revert to virulence or be shed in body excretions by the vaccinated animal.

Limited studies have shown the vaccine to be efficacious in inducing humoral immunity, cellular immunity, or both in various species.^{1,5} On the basis of these results and the paucity of reports of CDV infection from natural challenge in the numerous at-risk species vaccinated in North American zoological institutions since the vaccine was marketed in 2001, it can only be assumed that the vaccine provides sufficient protection from CDV infection. Exposure to wild carnivore species, including raccoons and red foxes (*Vulpes vulpes*), known to carry CDV infection in North America, has been noted in the giant panda enclosures at the National Zoological Park during this study. In a survey completed during 2005, three of 14 (21%) raccoons and one of four (25%) red foxes captured for routine monitoring on zoo property had low-positive CDV titers (1:8) (Viner, unpubl. data).

Both giant pandas had received s.c. and i.m. administration of this vaccine before the study. During the study period, the male received an s.c. vaccine the first year and an i.m. vaccine in the second year due to clinician preference. The female panda received her vaccine i.m. both years of the study. Although i.m. vaccination is often recommended for extra-label use in exotic animals, this vaccine has been shown to be effective in domestic dogs, ferrets, and Siberian polecats when given subcutaneously.^{5,7} A correlation between antibody titers and route of administration were not found in this study, although this cannot be definitively determined due to incomplete sampling during the first year. However, based on these limited data, adequate antibody levels were attained with both routes of administration in the two pandas in this study.

This study demonstrates the safety and adequate humoral response to vaccination with a commercially available, canarypox-vectored recombinant distemper vaccine labeled for use in domestic ferrets. When this vaccine is given extra-label to the

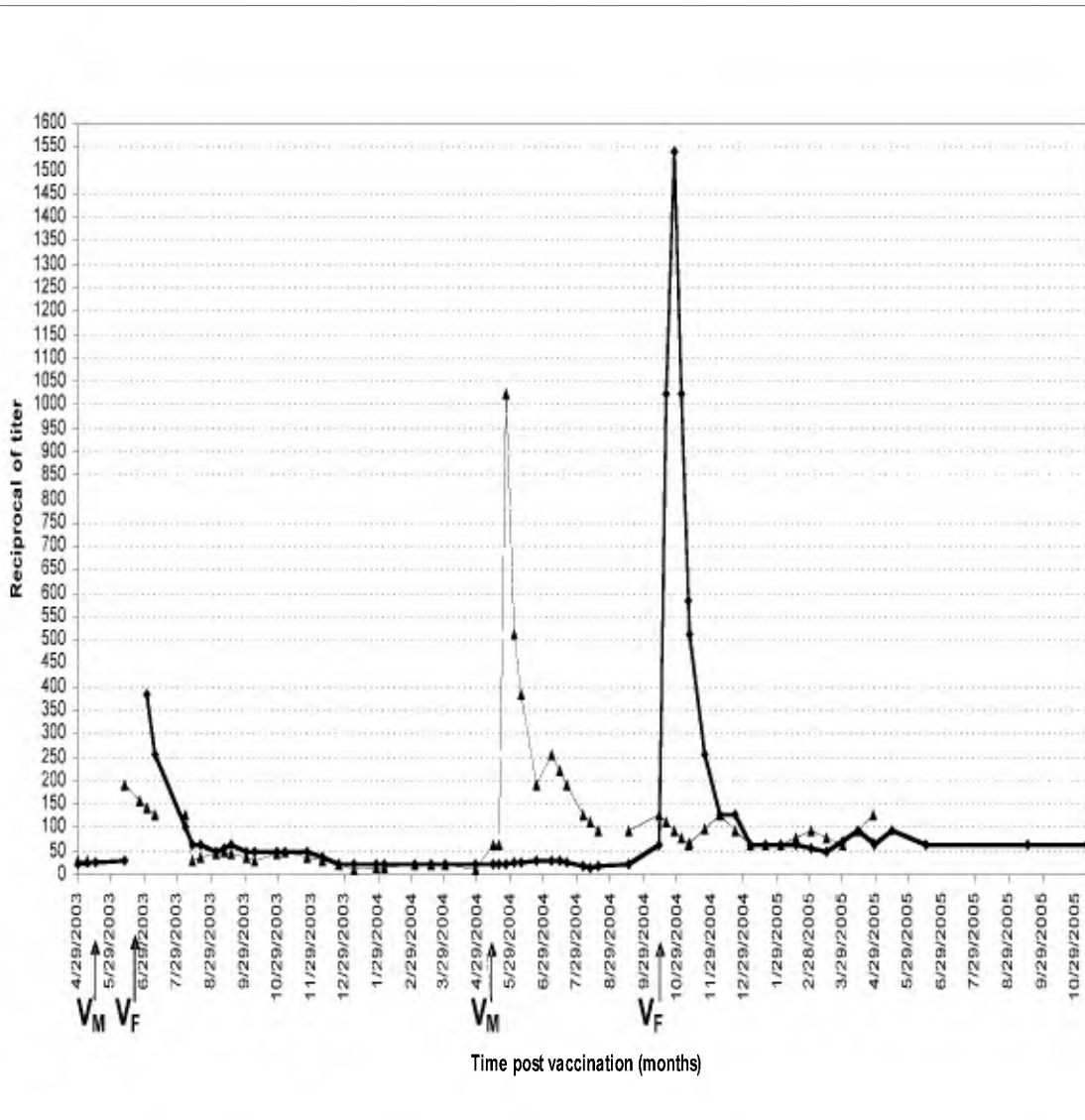


Figure 1. Serum-neutralizing antibody levels as a reciprocal of the titer in a male and female adult giant panda after annual administration of a canarypox-vectored recombinant CDV vaccine measured over a 2-yr period. In the male, the vaccine was given s.c. the first year and i.m. the second year; the vaccine was given i.m. both years in the female. V_M , date of vaccination of male giant panda. V_F , date of vaccination of female giant panda.

giant panda, it is suspected that adequate protection against natural challenge with CDV is provided for at least 1 yr after vaccination. Due to the improved safety of this vaccine over all modified live CDV vaccines and the superior humoral response over inactivated CDV vaccines in the giant panda and other species,⁴ it is recommended that giant pandas be vaccinated annually using the canarypox-vec-tored recombinant distemper vaccine.

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