

## Clinical trials with canine distemper vaccines in exotic carnivores

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### SUMMARY

Two types of killed canine distemper virus (CDV) vaccine and a modified-live CDV vaccine were clinically evaluated in four species of exotic carnivores. In 16 trials in which 13 red pandas (*Ailurus fulgens*) were given the killed vaccine, only 1 animal had a virus-neutralization titer that exceeded 1:100. A red panda given modified-live CDV vaccine deemed safe for gray foxes and ferrets died of bacterial pneumonia 16 days later. There was no pathologic evidence of canine distemper in that panda. The same modified-live vaccine proved to be immunogenic and safe in 12 bush dogs (*Speothos venaticus*), 5 maned wolves (*Chrysocyon brachyurus*), and 3 fennec foxes (*Fennecus zerda*) in which virus-neutralization titers often exceeded 1:512 and persisted for several months after vaccination.

CDV infection in a zoo setting affected 7 species in 2 families of carnivores, all of which had been vaccinated with a killed CDV vaccine.<sup>4</sup> A vaccination program, using modified-live CDV vaccine, was initiated after that outbreak and was recommended for immunization against CDV infection in exotic carnivores. Modified-live CDV vaccine also has been recommended by others<sup>5-7</sup> for use in certain species of carnivores.

Recently, CDV infection was induced by modified-live CDV vaccines in red pandas,<sup>8-13</sup> black-footed ferrets (*Mustela nigripes*),<sup>14,15</sup> kinkajous (*Potos flavus*),<sup>16</sup> and possibly in African cape hunting dogs (*Lycaon pictus*).<sup>17</sup> Modified-live CDV vaccine also will cause clinical distemper in gray foxes (*Urocyon cinereoargenteus*), but appears to be safe for use in red foxes (*Vulpes fulva*).<sup>18</sup> Because of the likelihood of disease induction in some of these carnivorous species with modified-live CDV vaccine, we have recommended that only killed vaccines be used.<sup>8</sup> However, serologic evidence has not been available to support the efficacy of killed CDV vaccine in any of these exotic carnivores.

The purpose of this report is to give the results of clinical trials in which 2 types of killed CDV vaccine and a modified-live CDV vaccine were used to vaccinate several species of exotic carnivores against CDV infection.

### History

For the past 20 years, all carnivores at the National Zoological Park, Washington, DC, have been vaccinated with a killed CDV vaccine. The vaccine in use since 1975 was prepared by one of the authors (MJGA; see Materials and Methods). The vaccine was administered to species in the following families of carnivores: Ailuridae, Ailuropodidae, Canidae, Hyaenidae, Mustelidae, Procyonidae, and Viverridae. Each animal was given 1 ml of the killed vaccine sc. Carnivores born in the zoo were vaccinated at 2-week intervals, 2 or 3 times after they reached 10 weeks of age, followed by annual or semiannual boosters. Adult animals were vaccinated at 6- to 12-month intervals, depending on the species and ease of restraint.

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FOR THE 11 SUBGROUPS of mammals currently considered to be families<sup>a</sup> of the order Carnivora,<sup>1</sup> species in 7 of these families have been reported to be susceptible to canine distemper virus (CDV) infection. These include Ailuridae,<sup>b</sup> Canidae, Hyaenidae, Mustelidae, Procyonidae, Viverridae,<sup>2</sup> and possibly Felidae.<sup>3</sup> Many of the reports of CDV infection in these species have been based on isolated instances in one or several animals in which the diagnosis was based on clinical signs and histopathologic identification of CDV inclusion bodies. The largest recent outbreak of

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<sup>a</sup>Canidae, Ursidae, Procyonidae, Ailuridae, Ailuropodidae, Mustelidae, Viverridae, Cryptoproctidae, Herpestidae, Hyaenidae, and Felidae.

<sup>b</sup>Ailuridae, in which one species, the red or lesser panda (*Ailurus fulgens*) is represented, has recently been split from Procyonidae.<sup>1</sup>

Although naturally occurring distemper did not develop in any of our carnivores during these programs, distemper did develop in a red panda that had been vaccinated with modified-live CDV vaccine 2 weeks before its arrival at the National Zoo.<sup>8</sup> Because of our interest in propagating pandas,<sup>19</sup> and since naturally occurring distemper has been reported in a group of red pandas,<sup>20</sup> we decided to determine the CDV-immune status of our red pandas as well as the other exotic carnivores. In each case, an animal had been vaccinated with the killed CDV vaccine from 2 weeks to 6 months before obtaining the serum samples. The ages of animals tested ranged from 6 weeks to aged adults and included the following species: in the family Canidae, bush dog (*Speothos venaticus*), maned wolf (*Chrysocyon brachyurus*), fennec fox (*Fennecus zerda*), kit fox (*Vulpes velox*), crab-eating fox (*Cerdocyon thous*); in the family Ailuridae, red panda; in the family Ailuropodidae, giant panda (*Ailuropoda melanoleuca*), and in the family Viverridae, binturong (*Arctictis binturong*). With few exceptions, animals were seronegative at virus-neutralization (VN) titers of 1:25, 1:50, and 1:100; and when lower dilutions were used, most titers were less than 1:5, with an occasional one reaching 1:20. With the exception of 3 of 35 bush dogs tested, which developed titers up to 1:256, all remaining species tested (8 red pandas, 2 giant pandas, 3 fennec foxes, 3 kit foxes, 8 crab-eating foxes, 8 maned wolves, and 8 binturongs) had VN titers of less than 1:25, and usually less than 1:5.

A serum titer of 1:100 is used as the standard for indicating immunity against distemper in domestic dogs. Since this titer, considered to be protective, was derived from studies in dogs with passive immunity, it is recognized that some vaccinated dogs with titers lower than 1:100 may be immune.<sup>21</sup> Because the killed vaccine-induced titers only rarely exceeded 1:100 in the animals we surveyed, clinical trials were established subsequently to test 3 types of CDV vaccine in 4 species of exotic carnivores.

## Materials and Methods

Vaccine trials were performed in red pandas, maned wolves, bush dogs, and fennec foxes that were either at our breeding facility in Front Royal, Va, or on display at the zoo in Rock Creek Park, Washington, DC. Each animal received a diet formulated to meet its particular needs, and all were well nourished and healthy appearing at the time of the vaccination.

Two killed CDV vaccines were used. The first vaccine (CDVac 1) contained the Rockborn strain of CDV and was inactivated and lyophilized as previously described.<sup>22</sup> The second killed CDV vaccine (CDVac 2) was similarly prepared except that the Onderstepoort strain of CDV was used.<sup>23</sup>

Three protocols were used for the clinical trials. In protocol A, CDVac 1 was administered with an adjuvant<sup>c</sup> at a ratio of 1 part adjuvant to 4 parts vaccine. Five red pandas (No. 1 to 5) of both sexes, ranging in age from 9 to 12 months, were anesthetized with a combination of ketamine hydrochloride and xylazine. Blood samples were obtained for determination of prevaccination VN titers, and two to four 1-ml doses of the adjuvanted vaccine were given SC. Blood

samples also were obtained for determination of post-vaccination VN titers. In protocol B, CDVac 2 was administered similarly, and pre- and postvaccination blood samples for determination of VN titers were collected from 11 red pandas (No. 1, 2, 5, and 6 through 13), ranging from 6 to 40 months of age and of both sexes. Frequency and intervals of vaccinations and blood samples were somewhat varied: in protocol A, red pandas were vaccinated from 2 to 4 times at 1- to 2-week intervals. Blood samples usually were obtained 2 weeks after vaccination, and in some animals at monthly intervals for up to 6 months after vaccination. In protocol B, with the exception of panda 5 which was vaccinated 3 times, the remaining pandas were vaccinated twice, usually 2 weeks apart. Blood samples were taken from 9 to 18 days after the vaccinations.

Protocol C involved the vaccination of a 6-month-old female red panda (No. 14) with a modified-live CDV vaccine<sup>d</sup> (CDVac 3). This vaccine consists of the Onderstepoort strain of CDV that has been attenuated in cultured chicken cells. It has been deemed safe for gray foxes<sup>18</sup> and ferrets.<sup>24</sup> Blood samples were obtained for determination of VN titers before vaccination and on the 7th and 11th days after vaccination. Sterile swabs were used to obtain nasal secretions from the red panda from 4 days before vaccination and for 11 days after, at 3- to 5-day intervals. The nasal secretions were frozen at -70 C until they were examined for vaccine virus. The red panda died 16 days after vaccination and was necropsied. Specimens from the cerebrum, cerebellum, mesenteric lymph nodes, lung, thoracic lymph nodes, liver, and spleen were frozen at -70 C and later examined for CDV. Heart blood and lungs were cultured for bacteria. Sections of all organs were fixed in buffered 10% formalin, processed routinely, and stained with hematoxylin and eosin.

In protocol D, CDVac 3 was given in 1-ml doses, SC, to 12 bush dogs of both sexes and ranging from 3 to 30 months of age, 5 maned wolves of both sexes and ranging from 5 to 13 months of age, and 3 fennec foxes of both sexes and ranging up to 16 months of age. Blood was obtained for determination of pre- and postvaccination VN titers, and each animal was then vaccinated 1 to 4 times. Intervals of vaccinations and blood collections for the bush dogs are in Table 1, and for maned wolves and fennec foxes in Table 2. Sterile swabs were used to obtain nasal secretions from 4 bush dogs (No. 1 to 4), daily for 6 days and at days 12 and 13 after vaccination, and these were examined for CDV.

The microneutralization test was used to determine VN titers.<sup>23,25</sup> Nasal secretions and tissues were processed and used to inoculate Vero cells that were recently trypsinized. The mixture was cultured in Eagles minimal essential medium with 10% bovine fetal serum, 300 mg of L-glutamine/ml, and 10 µg of kanamycin/ml in glass cell culture tubes. Monolayers were observed for cytopathic effect for 3 weeks, with weekly changes of medium. Subpassage was done after 3 weeks and cells were observed daily for 3 more weeks. If cytopathic effect was not evident after 42 days, the cultures were discarded. The CDVac 3 was used as a positive virus control and gave typical cytopathic effect after 6 days' growth under the same conditions.

## Results

**Red pandas**—Prevaccination VN titers in animals vaccinated with CDVac 1 and CDVac 2 were all less than 1:10 in 16 trials. Postvaccination VN titers were 1:256 in 1 of 16 trials, 1:20 in 1 of 16 trials, 1:16 in 2 of 16 trials, and less than 1:10 in 12 of 16 trials after multiple vaccinations.

<sup>c</sup>Obtained from Fort Dodge Laboratories, Fort Dodge, Iowa.

<sup>d</sup>Fromm D, Fromm Laboratories Inc, Grafton, Wis.

TABLE 1—Virus-neutralization antibody titers to CDV in bush dogs given modified-live vaccine, chicken tissue culture origin (CDVac 3)

Bush dog No.	Sex	Age (mo)	Vaccination dates	VN titer*, postvaccination day													
				0	12	14	17	18	28	30	49	87	150	198	207		
1	M	3	8/19/82	8													
			9/30/82	32													
			12/10/82	16	16												
			1/7/83	32		128											
2	F	3	8/19/82														
			9/30/82	64		64											
			12/10/82	32		64											
			1/7/83	64			64										
3	F	3	8/19/82	8		32										200	
			9/30/82	32	64												
			12/10/82	64		64											
			1/7/83	64													
4	F	3	8/19/82	8	8	32										100	
			9/30/82	256	512												
			12/2/82	<4													
			1/7/83	<4													
5	F	6	12/2/82	<4						512					512		
			1/7/83	<4													
6	F	6	12/2/82	<4						>512							
			1/7/83	<4													1,600
7	F	6	12/2/82	<4						>512						>3,200	
			1/7/83	<4													>3,200
8	F	6	12/2/82	<4						512			512			>3,200	
			1/7/83	<4													1,600
9	M	4.5	3/8/83	<4						>512							
			4/7/83	<4													
10	F	4.5	3/8/83	<4						>512			>3,200				
			4/7/83	<4													
11	M	30	12/10/82	4		256											
			1/7/83														
12	F	12	12/10/82	64		256											
			1/7/83														

\*Expressed as reciprocal of dilutions of serum that neutralized CDV. Nasal secretions of bush dogs 1 to 4 were negative for vaccine virus shedding.

Red panda 14, which was given CDVac 3, had a VN titer of less than 1:4 before vaccination, with no change on the 7th day, but with a titer of 1:50 by the 11th day after vaccination. There was no evidence of vaccine viral shedding in any of the nasal secretions obtained after vaccination, nor was there any vaccine virus evident in any of the tissues obtained at necropsy. There was extensive suppurative bronchopneumonia with acute necrotizing inflammation in several visceral organs and lymph nodes. *Pseudomonas aeruginosa* was isolated from the lungs and heart blood. None of the histologic hallmarks of canine distemper was evident in any of the tissues examined.

*Bush dogs, maned wolves, fennec foxes*—There was a good antibody response to CDVac 3 in these 3

species of canids studied. Ten of 12 vaccinated bush dogs had VN titers equal to or exceeding 1:512. In the 4 bush dogs (No. 1 to 4) that were vaccinated beginning at 3 months of age, antibody titers greater than 1:100 were not detected until the 3rd or 4th vaccination, whereas in bush dogs 6 months or older, titers greater than 1:100 were detected 2 to 3 weeks after the first vaccination (Table 1). Bush dogs 1 to 4 had no evidence of vaccine viral shedding.

Maned wolves and fennec foxes also developed titers greater than 1:100 after vaccination with CDVac 3 (Table 2).

With the exception of red panda 14, which died 16 days after CDVac 3 was administered, none of the other carnivores vaccinated with this modified-live product had any untoward responses that could be attributed to the vaccine.

TABLE 2—Virus-neutralization antibody titers to CDV in maned wolves and fennec foxes given modified-live vaccine, chicken tissue culture origin (CDVac 3)

Animal No.	Sex	Age (mo)	Vaccination date	VN titer*, postvaccination day					
				0	17	25	44	45	
<b>MANED WOLF</b>									
1	M	13	1/8/83	<25					
			2/5/83	<25					
2	M	13	3/23/83	>512					
			1/8/83	<25					
3	F	4.5	2/5/83						
			3/23/83	256					
4	F	4.5	5/11/83	4					
			5/21/83			1,600			
5	F	4.5	5/11/83						
			5/21/83			>3,200			
5	F	4.5	5/11/83	<4					
			5/21/83			400			
<b>FENNEC FOX</b>									
1	F	60	1/8/83	8					
2	F	48	2/5/83						
			1/8/83	<4			>512	800	
3	M	(Adult)	2/5/83						
			1/8/83	32			>512	400	
			2/5/83					>64	200

\*Expressed as reciprocal of dilutions of serum that neutralized CDV.

## Discussion

The clinical trials with the killed CDV vaccines (CDVac 1 and 2) supported our earlier serologic observations in that they indicated a minimal VN antibody response against CDV in all but 1 of the 13 red pandas vaccinated. The decision to try the modified-live CDV vaccine (CDVac 3) in red panda 14 was based on the unreliability of the killed CDV vaccines in red pandas and the proved safety of CDVac 3 in gray foxes and ferrets, which are also sensitive to modified-live CDV vaccines of canine origin.<sup>18</sup> The cause of death in red panda 14 was bronchopneumonia attributed to *Pseudomonas aeruginosa*. Although CDVac 3 did not cause distemper, it may have caused immunosuppression in the red panda. This, together with the stress of handling and anesthetizing the red panda during periods of specimen collecting, may have rendered the animal susceptible to the bacterial infection. In a recent study, immunosuppression has been induced and measured in ferrets given modified-live CDV vaccine.<sup>26</sup> In that study, 3 of 4 ferrets vaccinated died of gram-negative bacterial pneumonia, with no histologic evidence of CDV infection. The death of 1 of 4 gray foxes vaccinated with CDVac 3 occurred under similar circumstances as red panda 14, and the vaccine was subsequently approved for use in gray foxes.<sup>18</sup> Further use of CDVac 3 in red pandas, however, would not be advisable. Because of the potential threatened status of this species, it would not be feasible now to conduct attenuation studies that require challenge of immunity with virulent CDV. The lack of any less valuable taxonomic family member (since the red panda is the sole genus in the family Ailuridae) also precludes such types of distemper vaccine trials.

Until a better inactivated vaccine becomes available, we will continue to vaccinate red and giant pandas with CDVac 2 to induce a primary antibody response. This might provide some protection, even though not measurable, in the event a vaccinated animal is exposed to virulent CDV.<sup>27</sup>

Although these vaccine trials were, of necessity, limited in scope, modified-live vaccine (CDVac 3) proved to be immunogenic and safe in bush dogs, maned wolves, and fennec foxes. The onset of seroconversion to the CDVac 3 varied somewhat, both within and between the species, but there was a consistent difference between the 3-month-old and 6-month-old bush dogs in that the younger animals required multiple vaccinations and had longer intervals between vaccination and development of protective titers (Table 1). These differences were attributable to interference of the vaccine virus with maternal antibodies. Bush dogs 1, 2, and 3 were littermates whose dam was vaccinated with CDVac 2 twenty days before giving birth and had a titer of 1:20 on the day of birth; all pups had titers of 1:8 at the initial vaccination, whereas bush dogs 5 to 10, which were vaccinated from 4½ to 6 months of age and had prevaccination titers of less than 1:4, all developed protective titers after the first vaccination. The dam of bush dog pup 4 also was vaccinated less than 1 month before whelp-

ing, but her titer was not available at the time of whelping. Until nomographs for immunity against CDV infection can be developed for these exotic carnivores, it is important to monitor titers in the young vaccinated animals.

Another concern when vaccinating exotic carnivores with modified-live vaccines is the possibility that vaccinated animals may shed the vaccine virus and infect potentially susceptible species housed nearby. Overt distemper has been shown to occur in susceptible gray foxes that had contact with other gray foxes vaccinated with canine-origin attenuated CDV vaccine.<sup>18</sup> In our case, the 4 bush dogs (No. 1 to 4) tested did not shed vaccine virus from the upper respiratory tract for 2 weeks after vaccination. The fennec foxes and maned wolves vaccinated with CDVac 3 were not tested for shedding but were housed well away from the red and giant pandas so that direct contact or contact through the keeper staff or via fomites was not possible.

The threat of a distemper outbreak at a zoo has perhaps become lessened by the decline of this disease in the pet canine population because of the availability of good immunization procedures. The greatest threat of introducing distemper into a zoo, however, is by infected wildlife such as raccoons, skunks, and foxes, which are known to develop this disease. In our case, the zoo grounds teem with wild raccoons, and an outbreak of distemper in them could have serious consequences for our zoo carnivores that are not optimally protected against the disease. Careful surveillance and trapping of wildlife and limiting exposures of the zoo carnivores to potential contacts with nonzoo inhabitants, of course, would be helpful in preventing introduction of the disease, but vaccination with a reliable CDV vaccine would be the only way of protecting an irreplaceable collection of animals.

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