EVALUATION OF TWO CANINE DISTEMPER VIRUS VACCINES IN CAPTIVE TIGERS (PANTHERA TIGRIS)


Source: Journal of Zoo and Wildlife Medicine, 47(2):558-563.
Published By: American Association of Zoo Veterinarians
DOI: http://dx.doi.org/10.1638/2015-0223.1
URL: http://www.bioone.org/doi/full/10.1638/2015-0223.1
EVALUATION OF TWO CANINE DISTEMPER VIRUS VACCINES IN CAPTIVE TIGERS (PANTHERA TIGRIS)


Abstract: Canine distemper virus (CDV) has caused clinical disease and death in nondomestic felids in both captive settings and in the wild. Outbreaks resulting in high mortality rates in tigers (Panthera tigris) have prompted some zoos to vaccinate tigers for CDV. In this study, six tigers received a recombinant canarypox-vectored CDV vaccine (1 ml s.c.) and were revaccinated with 3 ml s.c. (mean) 39 days later. Blood collection for CDV antibody detection via serum neutralization was performed on (mean) days 0, 26, and 66 post–initial vaccination. No tigers had detectable antibodies at days 0 or 26, and only two tigers had low (16 and 32) antibody titers at day 66. Eight additional tigers received a live, attenuated CDV vaccine (1 ml s.c.) on day 0 and were revaccinated with 1 ml s.c. (mean) 171 days later. Blood collection for CDV antibody detection via serum neutralization was performed on (mean) days 0, 26, 171, and 196. Seven of eight tigers receiving the live, attenuated vaccine had no detectable titers prior to vaccination, but all animals had titers of >128 (range 128–1,024) at day 26. At 171 days, all tigers still had detectable titers (geometric mean 69.8, range 16–256), and at 196 days (2 wk post-revaccination) all but two showed an increase to >128 (range 32–512). To determine safety, an additional 41 tigers were vaccinated with 2 ml of a recombinant vaccine containing only CDV components, and an additional 38 tigers received 1 ml of the live, attenuated vaccine, administered either subcutaneously or intramuscularly; no serious adverse effects were noted. Although both vaccines appear safe, the live, attenuated vaccine produced a stronger and more consistent serologic response in tigers.

Key words: Canine distemper virus, Nobivac DP®, Panthera tigris, Recombitek®, tiger, vaccine.

INTRODUCTION

Canine distemper virus (CDV) has caused clinical disease and death in nondomestic felids in both captive settings and in the wild. Historical outbreaks of CDV in wild lions (Panthera leo) have resulted in major mortality events.4,9,13 Outbreaks in zoos and sanctuary facilities affecting both lions and tigers (Panthera tigris)14 combined with serologic surveys showing exposure of Panthera species to CDV have resulted in growing concern about felid CDV infections and have prompted the Tiger Species Survival Plan (SSP) to recommend vaccination for CDV, beginning in the early 1990s (Armstrong, pers. comm.). In addition, CDV has caused mortality in wild Amur tigers (Panthera tigris altaica) in the Russian Far East, and vaccination of wild tigers has the potential to provide protection against CDV in select wild populations.19,23

Previous vaccination efforts of nondomestic species against CDV are described in the literature but have resulted in variable protection and adverse effects. Use of live, attenuated CDV vaccines has been associated with the development of clinical disease in exotic carnivores, such as red pandas (Ailurus fulgens), black-footed ferrets (Mustela nigripes), South American bush dogs (Speothos venaticus), and kinkajous (Potos flavus).2,3,5,7,10,18 Killed CDV vaccines have generally resulted in poor serologic responses, although in some cases these vaccines may possibly provide protection.11 Following CDV outbreaks in the Maasai Mara National Reserve in Kenya, captive lions were vaccinated with a live, attenuated CDV vaccine and had strong antibody responses.8 A canarypox-vectored recombinant CDV vaccine was widely used in domestic ferrets (Mustela putorius furo) and anecdotally was applied to many nondomestic carnivores after recommendation by various SSPs. Using an antigenic portion of CDV inserted into a canarypox vector, this product allowed for the development of sustained, cell-mediated immunity without inoculation with a whole, live virus. However, the recombinant vaccine product that was widely used in nondom-
mestic species (Purevax® Distemper, Merial, 69007 Lyon, France) was recently removed from commercial availability, thus limiting the options for its use in nondomestic species.

Currently, two vaccines approved for use in domestic dogs show the greatest promise for use in zoo and wildlife species: a live, attenuated CDV vaccine (Nobivac® series, Merck, Kenilworth, New Jersey 07033, USA) and a recombinant canarypox-vectored vaccine (Recombitek series, Merial). However, data on the use of these vaccines in CDV-sensitive species are limited. Nobivac has potential for use in nondomestic carnivores, as it is attenuated through VERO cells and therefore should have a low possibility of virulent reversion. Recombitek, though antigenically similar to Purevax (both use CDV H and the F proteins vectored by canary pox), may have less plaque-forming units than the Purevax product and therefore may result in less antigenic response. Given these benefits and drawbacks of both potential vaccines, further investigation is needed in order to provide information to both zoologic and wildlife professionals regarding prevention of CDV in Panthera species.

This study investigated the safety and serologic responses to both commercially available CDV vaccines in captive tigers. The results provide information important in designing protocols when considering CDV vaccination in captive or wild tigers.

MATERIALS AND METHODS

Animals

Captive tigers (n = 102) of unknown subspecies were used in these studies. All tigers were held at a large exotic felid sanctuary and were deemed healthy based on visual examination and lack of current, major medical issues. The tigers had not been held by the sanctuary for their entire lives and therefore lacked complete medical records. To the authors' knowledge, none of the tigers had a record of being previously vaccinated against CDV. All tigers were kept in similar chain-link enclosures and were fed the same meat-bone diet throughout the study period. The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee (2220-1113).

Serology study

Fourteen tigers were enrolled in the serology study. Immediately before initial vaccination, each of these tigers had a physical examination and blood samples taken for a complete blood cell count, plasma chemistry analysis, and serology. The tigers were then divided into two groups: a recombinant canarypox vectored vaccine (RCP, n = 6) group, which consisted of four castrated males and two intact females, ages 2–4 yr; and a live, attenuated vaccine (NDP, n = 8) group consisting of four castrated males, one intact male, two spayed females, and one intact female, ages 2–6 yr.

Each tiger was immobilized for examination and initial venipuncture with a combination (target dosages) of i.m. ketamine (3 mg/kg), midazolam (0.2 mg/kg), and medetomidine (0.02 mg/kg). Each additional sampling point listed below used this same anesthesia protocol. Blood was obtained from the medial saphenous, tail, or jugular vein. Each animal was monitored throughout the study period for signs of local or systemic vaccine reactions, such as lameness, anorexia, vomiting, or other signs, including anaphylaxis or death.

The RCP group tigers received 1 ml of a canarypox-vectored CDV vaccine (Recombitek C3, Merial) along the cranial aspect of the right thigh (s.c., day 0). In addition to the CDV component, this vaccine also contains live, attenuated canine adenovirus-2 (CaV2) and canine parvovirus type 2 components. Blood samples for the first post–initial vaccination (PIV) titers were collected between days 19 and 28 (mean = 26 days). Following review of the serology results, each animal was revaccinated, without immobilization, between days 35 and 42 (mean = 39 days) PIV with 3 ml i.m. of the recombinant vaccine by hand-injection or pole syringe. Each tiger was bled for serologic testing again between days 61 and 70 (mean = 66 days) PIV.

The NDP group tigers were vaccinated with 1 ml of a live, attenuated CDV vaccine (Nobivac DP, Merck) s.c. along the cranial aspect of the right thigh on day 0 and between days 167 and 175 (mean = 171 days) PIV. This vaccine contained the attenuated Onderstepoort CDV strain (≥10⁴ TCID₅₀) and attenuated canine parvovirus 2a strain (strain 154, ≥10⁴ TCID₅₀). The NDP tigers were again immobilized and bled for serologic testing between days 23 and 28 (mean = 26 days) PIV, immediately prior to revaccination on days 167 to 175 PIV (mean = 171 days), and on days 191 to 200 (mean = 196 days) PIV.

The CDV serum neutralization titers were determined by a standard protocol used in the Clinical Virology Laboratory at the University of Tennessee Veterinary Medical Center. Briefly,
sera were incubated at 56°C for 30 min, and twofold serial dilutions were prepared and tested in triplicate. A CDV Onderstepoort strain (200 TCID₅₀ per well) was incubated with the serum dilutions for 60 min at 37°C and 5% CO₂. Vero SLAM cells (kindly provided by Dr. Edward Dubovi, Cornell University) were added to the wells. Plates were incubated for 5 days, and wells were evaluated for the presence of viral cytopathic effect (CPE). Titers were reported as the reciprocal of the last dilution prior to the one with CPE in each of the replicates, and all titer means listed are calculated as the geometric mean titer.

Safety study

Forty-one additional tigers (19 females, 22 males; ages 4 mo to 15 yr) were subsequently vaccinated with 2 ml of a monovalent, recombinant canarypox-vectored CDV vaccine (Recombi-trek CDV, Merial) i.m. by hand (n = 17) or by pole syringe (n = 24). Thirty-eight different tigers (19 females, 19 males; ages 3–17 yr) were similarly vaccinated with 1 ml NDP by either hand syringe (n = 8) or pole syringe (n = 30). In contrast to the tigers in the serology study, these tigers were not immobilized or monitored serologically; they were vaccinated only to assess each vaccine’s safety. Each tiger was observed daily, postvaccination, for adverse effects, such as lameness, anorexia, vomiting, nasal or ocular discharge, respiratory difficulty, neurologic abnormalities, or other signs, including anaphylaxis or death, for a period of at least 60 days.

RESULTS

Serology

No RCP group tiger had a titer to CDV prior to initial vaccination, nor did any develop measurable titer 19 to 28 days PIV. Four of these tigers still had no measurable titers to CDV 26 to 28 days postrevaccination (PRV), while the other two developed titers of 16 and 32 at 28 days PRV. These were the two smallest and youngest tigers in this group. No adverse effects of vaccinations were seen in any tiger in the RCP group.

Seven of eight tigers in the NDP group were sero-negative for CDV prior to vaccination, but one had a prevaccination titer of 64. All tigers had titers of ≥128 at 23 to 28 days PIV, with one having a titer of 1,024 (geometric group mean = 304.4; range = 128–1,024). At 171 days PIV, all tigers had slightly lower but still detectable titers (group geometric mean = 69.9; range = 16–256). Three tigers’ titers had not increased when sampled (mean) 26 days PRV (mean = 191 days PIV). The remainder of the tigers had a one- to twofold increase in their CDV titers (geometric mean = 128; range = 32–512) by (mean) 26 days PRV (mean = 191 PIV). No adverse effects of vaccinations were observed in the NDP group animals.

Safety study

No serious adverse effects directly related to vaccination were noted in any tiger. Minor twitching was seen in one immobilized animal’s leg from the RCP group immediately following initial vaccination in that leg, and mild transient lameness was noted 3 wk PIV in an NDP group tiger; both effects resolved in minutes to hours without intervention, respectively. No vomiting, diarrhea, lethargy, evidence of anaphylaxis, or death was noted following vaccination in any animal.

DISCUSSION

Canine distemper virus has long been known to cause disease outbreaks in both captive and wild nondomestic felids. Though riddled with significant logistical hurdles, vaccination programs for wild felids and reservoir species allow for the possibility of providing a population partial protection for disease. While parenteral inoculation of wild tigers may be impractical in some cases, knowledge of immune response and safety of vaccines provides important information in developing management plans.

In the current study, a poor serologic response was noted in each tiger vaccinated with the RCP vaccine. However, previous work has demonstrated that vaccination with a recombinant CDV vaccine in domestic dogs and Siberian polecats (Mustela eversmanni) resulted in protection to virus challenge, despite these animals developing lower titers than were noted in those vaccinated with a live attenuated CDV vaccine. In one of these studies, polecats receiving lower antigen doses of recombinant CDV material showed greater mortality when challenged. The authors concluded that a titer of ≥16 in polecats was predictive of survival. Interestingly, serologic response to a commercial combination CDV, CaV2, and CPV vaccine was significantly better in a group of lighter weight dogs compared to groups of heavier dogs, suggesting an antigen- or dose-dependent response. This variability in protective serum titer levels, combined with dose-dependent serologic response, may indicate
that while a humoral response is suggestive of CDV protection, other mechanisms, such as cell-mediated immunity (CMI), play an important role in protecting susceptible species. Increased antigen per vaccination or increased frequency of vaccination, combined with investigations of a vaccine’s induction of cell-mediated immunity, may allow for further understanding of protection against CDV infection in target species.

The initial serologic response in the tigers receiving the live, attenuated vaccine in the present study was consistent with previously reported levels of protective titers in African lions, which received 1 ml of a similar live, attenuated Onderstepoort CDV vaccine. In that study, eight young domestic cats and four adult lions were each vaccinated once. The domestic cats demonstrated a moderate response (mean titer = 58; range 0–256), and all four lions showed strong humoral responses (titers ≥1,024); however, neither the lions nor the domestic cats were challenged postvaccination. When investigating revaccination strategies for CDV in domestic dogs, a “population titer” of >80 was suggestive of protection. In domestic ferrets vaccinated twice over a 4-wk period with a similar live, attenuated Onderstepoort strain vaccine, titers at 4 wk post–initial vaccination had a large range (8–2,084). Neutralizing titers in these ferrets increased, ranging from 512 to 4,096, following challenge with CDV. In young raccoons vaccinated with a similar live, attenuated Onderstepoort strain vaccine, protective serum neutralization titers ranged from 12 to 384. The amount of virus included in each of these vaccines, including the one used in the present study, was similar (>10^4 plaque-forming units). While controlled challenge studies have not been performed in tigers, the wide range of protective titers seen in other investigations indicates that protection is difficult to predict based on humoral immunity alone. Serologic assessments of captive vaccinated tigers from populations that have experienced CDV outbreaks would provide additional information about vaccine efficacy and protection in tigers.

In the current study’s population of tigers, no serious adverse effects were seen with either commercially available CDV vaccine. Furthermore, the NDP vaccine has also been investigated in domestic cats, and no adverse effects were observed in those cats (Ramsay, unpubl. data). However, all animals in both this and the domestic cat study were healthy, young adult or adult animals. Young, pregnant, or immunocompromised animals might experience adverse reactions not seen in either study population, and additional testing in these demographic groups would be of value. Another concern, particularly in wild tigers, is the potential for in utero infection of cubs when inoculating a pregnant female tiger with vaccines containing live, attenuated viruses. Feline panleukopenia infection in pregnant female domestic cats can result in cerebellar hypoplasia in the offspring, and therefore caution should be used when vaccinating pregnant felids with a live, attenuated parvovirus. The authors are unaware of any adverse reproductive effects of CDV vaccination in any species.

Given that vaccination of a large portion of a wild population can be logistically difficult, protection of a population may still be achieved without 100% vaccination. Vaccination of a portion of a free-ranging population might provide the opportunity to prevent extinction of localized groups or even species. Estimates of the proportion of a population requiring immunization in order to protect the group varies between 60% and 90% and depends upon many variables specific to that population. Novel methods of delivering CDV vaccines, such as via oral baits or aerosolized traps, could reach larger numbers of a target population and could potentially also vaccinate wildlife reservoirs for CDV.

A significant limitation of this study is the limited ability to assess true protection of each of the two vaccines in this population. Given the intracellular nature of viral pathogens and the significant role of CMI, it is difficult to fully assess the protection of these vaccines with viral neutralizing titers alone. In addition, neutralization titers reflective of protection may be variable between viral strain, population, laboratory used to perform the serology, and host immune status, among other variables. Given these limitations, however, serology has been used in other species in lieu of a readily available commercial CMI measurement or viral challenge and therefore may be a valuable, if somewhat limited, indicator of possible protection for a population, or at least a representation of the antigenicity of a particular vaccine.

Another significant limitation of this study is the relatively low incidence of vaccine-induced disease in relation to the study’s population size. Adverse effect prevalence in response to vaccination in veterinary patients is complicated by many factors, such as surveillance technique, owner participation, time period of surveillance, and data management, but the signs can vary from...
nonspecific systemic illness to reversion to virulence in the case of modified live vaccines.\textsuperscript{6,12} While this study’s tiger population was large compared to a typical zoological institution, when compared to the relatively uncommon occurrence seen in veterinary medicine it is possible that this sample size was not large enough to adequately assess for possibly fatal outcomes. However, given the relatively small wild population of Amur tigers, compared to this study population, it is possible that the incidence of a significant complication with either vaccine is low.

Based on these findings, both the recombinant CDV and the live, attenuated CDV vaccines appear to be safe for use in tigers. The live, attenuated vaccine produced a much greater humoral response compared with the recombinant vaccine. Though the recombinant vaccine did not show much humoral response, only the trivalent product was used in the serology portion of this study, and therefore the monovalent product may be more effective. Regardless, the amount of protection afforded by either vaccine in tigers is unknown in the absence of challenge studies, and further research is needed in order to identify effective protective vaccination programs for both captive and wild tigers.

Acknowledgments: The authors would like to thank Tiger Haven for providing both the tigers and project support, Martin Gilbert for assistance with the project, the Wildlife Conservation Society for advisory and financial support, and Misty Bailey and Elizabeth Carter for assistance with manuscript preparation.

LITERATURE CITED


Received for publication 21 September 2015