2024

Serological Response to Canine Distemper Vaccination in Wild Caught Raccoons (*Procyon lotor*)

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DOI: 10.1638/2023-0078

Journal of Zoo and Wildlife Medicine 55(2): 462–465, 2024

SEROLOGICAL RESPONSE TO CANINE DISTEMPER VACCINATION IN WILD CAUGHT RACCOONS (*PROCYON LOTOR*)

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Abstract: Canine distemper virus (CDV) is a well-known RNA virus that affects domestic dogs and all families of wild terrestrial carnivores. Spillover infections from wildlife to domestic animals are mitigated by preventive vaccination, but there is limited information on the off-label use of veterinary vaccines for wildlife like raccoons (*Procyon lotor*). Twenty wild-caught raccoons were inoculated with a commercial recombinant DNA canarypox-vectored CDV vaccine, applying a regimen of two serial doses by SC route with an interval of 25-28 days between doses. The CDV serum virus neutralizing antibody (VNA) baseline titers and the postvaccination titers were measured at fixed time points. Forty percent (8/20) of the wild-caught raccoons had CDV VNA titers of 1:8 or greater upon intake, and all but a single individual were juvenile animals. Approximately one month following the first vaccine dose, 8% (1/12) of raccoons seronegative at baseline had serum CDV VNA titers of 1:24 or greater. Approximately one month following the booster vaccine dose, 67% (8/12) of raccoons seronegative at baseline had serum CDV VNA titers of 1:24 or greater. Among raccoons with CDV VNA titers greater than or equal to 1:8 at baseline, 13% (1/8) demonstrated a fourfold or greater rise in titer one month after the first vaccine dose, whereas 38% (3/8) reached the same threshold one month after the booster dose. The presence of naturally acquired CDV VNA in juvenile raccoons at the time of vaccination may have interfered with the humoral VNA response. A regimen of at least two serially administered SC vaccine doses may be immunogenic for raccoons, but further investigation of alternative routes, regimens, and CDV vaccine products is also warranted for this species.

INTRODUCTION

Canine distemper virus (CDV) (aka Canine morbillivirus) is a well-known RNA virus in the genus *Morbillivirus* (Family Paramyxoviridae)[9] that affects domestic dogs and all families of wild terrestrial carnivores.[6] It is primarily transmitted by aerosols and is highly contagious.[2] Multiple epizootics have been reported from free-ranging raccoons (*Procyon lotor*) across the United States,[13] and concerns have been raised identifying raccoons as a primary source of disease transmission to captive carnivores in zoological and conservation institution collections in North America.[3,10] With CDV's recognized negative impact on many threatened and endangered species and high mortality rate,[8] investigation of disease prevention strategies is warranted for both wild and captive populations of wildlife reservoir species.

One common disease prevention strategy for humans and animals is vaccination. Scientific study on the efficacy of CDV vaccination and refinement of protocols to improve efficacy in raccoons have been limited. Previous studies in raccoons[11,14] reported on performance of veterinary CDV vaccines, but the products evaluated in these studies are no longer commercially available (e.g. Galaxy D® [Merck Animal Health, Madison, NJ 07940, USA]; Fromm-D® [Solvay Animal Health, Incorporated, Kitchener, Ontario M5J 2T3, Canada]).[12] One commercial veterinary CDV vaccine is the recombinant DNA canarypox-vectored vaccine, labeled Purevax® ferret distemper vaccine (PFD; Boehringer Ingelheim Animal Health USA Inc., Athens, GA 30601, USA). PFD became commercially available in 2002 and has been tested in domestic and wild animals.[8] Our objective was to evaluate and summarize the CDV serum virus neutralizing antibody (VNA) response of wild-caught raccoons administered off-label PFD vaccine.

MATERIALS AND METHODS

In August 2021, 20 raccoons were captured locally and transported to the United States Department of Agriculture (USDA), National Wildlife Research Center's (NWRC) outdoor animal research facility in Fort Collins, Colorado, USA. Animals were housed individually in outdoor pens (3 × 3 × 2.5 m) with access to a den box, burlap hammock, and climbing log for enrichment. Seventeen individuals were aged by weight and tooth wear as juveniles (nine males, eight females) and three individuals as adults (two males, one female) at

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Table 1. The canine distemper virus serum virus neutralizing antibody (VNA) titers measured at intake, day (d), 25–28 post primary vaccination (pv), and d 52–55 pv (23 days post booster) for twelve raccoons seronegative at baseline sampling.*

Animal ID	Age	Sex	Intake	d 25–28 pv	d 52–55 pv	VNA response to primary/booster vaccination
2-086	Juvenile	Male	<1:8	<1:8	<1:8	N/N
2-871	Adult	Male	<1:8	1:16	1:32	N/Y
5-522	Juvenile	Female	<1:8	<1:8	1:128	N/Y
1-882	Juvenile	Female	<1:8	1:32	1:256	Y/Y
2-001	Juvenile	Male	<1:8	1:8	1:16	N/N
2-359	Juvenile	Female	<1:8	<1:8	1:8	N/N
2-634	Juvenile	Male	<1:8	<1:8	1:32	N/Y
4-308	Juvenile	Female	<1:8	<1:8	1:32	N/Y
4-618	Adult	Male	<1:8	<1:8	1:8	N/N
1-359	Juvenile	Male	<1:8	<1:8	1:256	N/Y
1-847	Juvenile	Male	<1:8	<1:8	1:64	N/Y
3-304	Juvenile	Female	<1:8	1:8	1:64	N/Y

* Animals were determined to be VNA responders if their titers both experienced a fourfold change, and the value was 1:24 or higher.

intake. The daily ration for raccoons during study consisted of 200 g of omnivore diet (Mazuri Omnivore-Zoo Feed "A," Richmond, Indiana, 47374, USA) and various commercially available food enrichment such as peanuts, vegetables, and eggs ad hoc. Animals had access to water *ad libitum*. Procedures used in this study followed the guidelines of the USDA NWRC Institutional Animal Care and Use Committee (QA-3359). The scientific collection permit to capture and house the raccoons was authorized under Colorado Parks and Wildlife scientific collection license 21TR4813.

For intake, examination, vaccination, and sample collection procedures, animals were anesthetized with isoflurane by inhalation as described elsewhere.[4] During intake, raccoons were examined, weighed, microchipped, dusted with permethrin (Prozap® Insectrin® [Neogen, Lexington, KY 40511, USA]), and administered ivermectin (0.05 mg/kg) (Noromectin® [Norbrook Laboratories, Lenexa, KS 66219, USA]). Raccoons were then vaccinated with 1.0mL of Purevax CDV vaccine SC at two timepoints: d 0 (baseline, primary dose) and d 25–28 (booster dose). A sample of whole blood (~3mL) was collected by syringe under anesthesia from the jugular vein of each raccoon immediately prior to primary and booster vaccination for d 0 and d 25–28, with an additional sample collected d 52–55 postvaccination.

Serum was separated from whole blood by centrifuge within hours of collection and stored at −80C until submitted to Colorado State University Veterinary Diagnostic Laboratory to estimate the CDV VNA titer. The VNA titer is reported as the highest dilution of serum in which antibodies were detected, tested in a twofold dilution series from 1:8 up to 1:8192. VNA cutoff thresholds from previous studies on CDV vaccination in dogs, wild felids, and captive raccoon pups were referenced.[8,11,15] In this study, VNA seronegative raccoons at intake were considered naïve and a VNA response to CDV vaccination documented when titers were greater than or equal to 1:24 pv. Raccoons with VNA titers of 1:8 or greater at intake were separately evaluated for a fourfold or greater rise in titer as evidence for response to CDV vaccination.

RESULTS

Of the 12 raccoons that were seronegative for CDV VNA (<1:8) at baseline, one raccoon increased in VNA titer after primary CDV vaccination (d 25–28 pv), whereas 11 raccoons had no or only a modest change in VNA titer (Table 1). After the booster CDV vaccination (d 52–55 pv) 11 animals demonstrated an increase in VNA titer, but only eight animals (67%) presented titers greater than or equal to 1:24. One seronegative raccoon did not demonstrate a detectable humoral VNA titer response to SC administration of two doses of the PFD vaccine.

Eight raccoons showed CDV VNA levels of 1:8 or greater at intake (Table 2). Among these eight individuals, one had an increased (>1:24) CDV VNA titer, two had no change, and five showed a decrease after primary vaccination (d 25–28 pv). Six raccoons showed an increase in CDV VNA titer (d 52–55 pv) after booster vaccination; however, only three (38%) showed a fourfold rise in CDV VNA titer compared to intake values.

Table 2. The canine distemper virus serum virus neutralizing antibody (VNA) titers measured at intake, day (d) 25–28 post primary vaccination (pv), and d 52–55 pv (23 days post booster) for raccoons with titers ≥1:8 at baseline.

Animal ID	Age	Sex	Intake	d 25–28 pv	d 52–55 pv	VNA response to primary/booster vaccination
6-043	Juvenile	Male	1:8	<1:8	1:8	N/N
6-623	Juvenile	Male	1:64	<1:8	1:8	N/N
1-090	Juvenile	Female	1:16	<1:8	1:32	N/N
2-823	Adult	Female	1:16	1:256	1:256	Y/Y
4-263	Juvenile	Female	1:16	1:8	1:32	N/N
9-089	Juvenile	Male	1:8	1:8	1:64	N/Y
9-318	Juvenile	Male	1:8	<1:8	1:256	N/Y
1-839	Juvenile	Female	1:8	1:8	1:16	N/N

* Animals were determined to be VNA responders if their titers both experienced a fourfold change, and the value was 1:24 or higher.

DISCUSSION

Following the booster dose, 67% (8/12) of the naive raccoons and 38% (3/8) of raccoons with baseline VNA titers demonstrated a measurable response to CDV vaccination as defined in the study parameters. No consistent VNA correlate of protection against CDV infection in the previous wildlife studies reviewed was identified.[8,11,15] Since the study did not involve a CDV challenge to evaluate vaccine efficacy, it is unclear whether any of the vaccinated raccoons in this study would be considered protected against a CDV infection.

Fold change and absolute change are two commonly used metrics of antibody response. It can be challenging to interpret results since the change in metric can have an impact on the data analysis outcome.[17] This study observed both the fold change and the absolute change to report immunogenicity in raccoons. A fourfold increase in titer is a widely accepted metric of antibody response,[17] but previous studies have challenged the fourfold metric[1] or have opted to not use fold-change at all, especially when pre-vaccination titers are present.[5]

One potential limitation is the timing and nature of the vaccine regimen employed in the study. The label for the PFD vaccine recommends a three-dose regimen and using a three-week spacing interval between doses, yet the spacing interval between doses in the study was slightly longer at four weeks and the regimen in this study consisted of two rather than three vaccine doses. The stark differences in the proportions of raccoons responding to primary versus booster vaccination supports the importance of multiple doses of this CDV vaccine product for raccoons. Despite multiple doses, one raccoon (8%) never showed evidence of any serum CDV VNA titer following two SC doses of the vaccine. In ferrets, the suggested veterinary practice is three serial SC injections at three-week intervals.[12] The study results indicate that a regimen of at least two SC injections separated by 25-28 d demonstrated moderate immunogenic responses among captive raccoons. However, a multiple-dose regimen could be challenging and impractical for free-ranging wild raccoons, given that free-ranging raccoons may be difficult to recapture. Paré et al treated raccoons with a single vaccine dose (Galaxy D®) and the vaccinated animals survived CDV challenge, supporting the conclusion that a single vaccination could benefit seronegative raccoons captured as part of trap-vaccinate-release program.[11] A limitation of this study is that the Galaxy D vaccine used by Paré et al is no longer commercially available and none of the vaccinated raccoons with CDV were challenged to determine efficacy of the PFD vaccine.

Since this study evaluated vaccination in mostly juvenile wild-caught raccoons, maternal antibodies might have been detected among some individuals with CDV VNA titers at intake. The presence of maternal antibodies could have interfered with the response to immunization and/or may partly explain the lack of VNA response detected among some vaccinated raccoons with titers of 1:8 or greater at baseline.[11] Previous studies have also shown that maternal antibodies may interfere with CDV and rabies vaccination, respectively.[7,11] Prior exposure to CDV could also explain positive VNA titers at intake and CDV infections have been documented in raccoons from the area of collection.[16]

The study data support an off-label multi-dose SC regimen to administer the PFD vaccine in captive raccoons. However, further research is needed to refine regimens for the off-label use of veterinary CDV vaccines for wild carnivores, including efficacy testing, in order to document a consistent VNA correlate of protection against CDV infection. Studies might continue to take into consideration

the potential for baseline VNA titers in wild-caught animals and the interaction between naturally acquired antibodies and the response to off-label use of commercial veterinary CDV vaccines in wildlife.

Acknowledgments: The authors thank the US Department of Agriculture National Wildlife Research Center and National Rabies Management Program, A. Barbee, L. Rader, C. Ellis, D. Spock, M. Allira, A. Walker, K. Bird, and M. McBride. Funding was provided by the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, and the National Rabies Management Program.

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Accepted for publication 22 January 2024