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Utility of two modified-live virus canine distemper vaccines in wild-caught fishers (Martes pennanti)

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Background: Canine distemper virus (CDV) infects families in the order Carnivora. As a preventive measure, vaccinations against CDV are frequently given to mustelids in captive environments.

Objectives: Our objectives were to compare the utility between two modified-live virus canine distemper vaccines (MLV CDVs), Fervac-D® (no longer manufactured) and Galaxy-D® (now manufactured by MSD Animal Health as part of a multivalent vaccine), in developing an immune response in wild-caught fishers.

Animals and methods: The Pennsylvania Fisher Reintroduction Project (PFRP) used 14 wild-caught fishers during one year of the project to evaluate the utility of vaccinations against CDV as part of any reintroduction project. Fishers were injected subcutaneously in the nape of the neck with their designated vaccine.

Results: Fervac-D® did not effectively stimulate development of a serologic antibody response, whereas Galaxy-D® had adequate seroconversion or rise of titer levels to suggest that the general use of MLV CDV may be suitable in fishers pending further studies.

Conclusion: We recommend that future studies be conducted, evaluating the use of currently produced vaccines in fishers. Future research should also focus on the length of days required between administration of primary and booster vaccines to achieve sufficient immune response.

Clinical importance: If only primary doses are required, then hard-release reintroduction projects for fishers could be recommended. If primary and booster vaccines are required then soft-release reintroduction projects should be recommended that include captive management periods, allowing for appropriate vaccination intervals needed to maximize the probability of protection against CDV.

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Fisher; Martes pennanti; Morbillivirus; Canine distemper virus; vaccination

1. Introduction

Canine distemper virus (CDV) is a Morbillivirus in the family Paramyxoviridae (Bernard et al. 1984; Greene & Appel 1990). CDV is very contagious, and well established for infecting families in the order Carnivora: Canidae, Mustelidae, Procyonidae, Hyaenidae, Ursidae, Viverridae, Felidae, Otariidae, Phocidae, and Mephitidae (Grachev et al. 1989; Mamaev et al. 1996; Kennedy et al. 2000; Williams 2001; Deem et al. 2000; Barrett et al. 2004). CDV also has been reported in families of the order Cetartiodactyla [Artiodactyla; Cervidae and Suidae (Kameo et al. 2012)] and Primates [Cercopithecidae (Sun et al. 2010)]. CDV is transmitted mainly through aerosolization of respiratory exudate containing the virus. Other bodily secretions may be contagious if aerosolized (e.g., urine; Greene & Appel 1990), and at specific periods during infection also can be transmitted through fecal material (Brown et al. 2006).

The respiratory, gastrointestinal, integumentary, and central nervous systems are most commonly affected by CDV.

Members of the family Mustelidae are presumed to be susceptible to a variety of infectious diseases and are among the species most susceptible to CDV (Deem et al. 2000), with a high mortality rate that approaches 100% in some species (e.g., ferrets [Mustela putorius]; Duplaix-Hall 1975; Wallach & Boever 1983; Bernard et al. 1984; Stephensen et al. 1997). Based on information from other Mustelidae, fishers (Martes pennanti) could likewise be presumed to experience high mortality rates if exposed to CDV. However, relatively little is known about the epizootiology of CDV in fishers. Among four fishers suspected to have died from CDV in California, one had clinical signs of CDV but no antibody titers were detected and antibody titers were reported in three that had died before being recovered.
for examination for clinical signs of the disease (Keller et al. 2012).

Vaccinations against CDV are frequently given to mustelids in captive environments to prevent infection. However, the immunogenicity and duration of immunity provided by such vaccinations has not been documented well for mustelids or other non-domestic carnivores (Jacobson et al. 1988; Petrini 1992). The Minnesota Zoo has used Fromm-D, Galaxy-DA2L, and Galaxy-DA2PL (Solvay Animal Health, Inc., Mendota Heights, Minnesota USA), modified-live virus (MLV) vaccines on captive mustelids. No untoward effects were observed in any of the vaccinated fishers, but seroconversion had not been evaluated (Petrini 1992). As part of a river otter (Lontra canadensis) reintroduction project in Pennsylvania, Galaxy-D® and Fervac-D® were used as part of the associated captive management program. These vaccines demonstrated an adequate rise in titer level or seroconversion (Peper et al. 2014).

The Pennsylvania Fisher Reintroduction Project (PFRP) was implemented in 1994 to restore fishers to regions of their historic range in Pennsylvania. As part of PFRP, fishers were trapped from New Hampshire and New York and relocated to Pennsylvania. Prior to release, wild-caught fishers underwent a captive management program designed to evaluate their health and condition upon arrival at The Pennsylvania State University (PSU), treat pre-existing injuries or diseases, and provide pre-release conditioning, including a high caloric diet (Mitcheltree 1996; Mitcheltree et al. 1997). The Pennsylvania Fisher Reintroduction Project (PFRP) was used to evaluate the use of two different CDV vaccines. To the best of the authors’ knowledge, this study is the first to investigate whether fishers are able to develop an immune response after being vaccinated against CDV. Our objectives were to compare the effectiveness between two MLV CDV, Fervac-D® and Galaxy-D®, in developing an immune response in wild-caught fishers.

### 2. Methods

In 1996, 14 fishers were live-trapped from New Hampshire for inclusion in the PFRP. Fishers from New Hampshire were captured by fur trappers during the legal fisher-trapping season and sold to PFRP through arrangements coordinated with the New Hampshire Fish and Game Department. New Hampshire Fish and Game Department facilitated the establishment of protocols with trappers, the collection of fishers from trappers, and transport of the fishers to PSU, which typically occurred for each fisher within 24 hrs of its capture (Mitcheltree et al. 1997). All 14 fishers were assigned a unique alpha-numeric identity code and were used to evaluate titer development to vaccinations against CDV (n = 14, Table 1). The use of all fishers and the procedures of this study were reviewed and approved by the PSU Institutional Animal Care and Use Committee (IACUC #94RO57A1).

All fishers underwent a pre-release management regimen administered by PFRP at PSU to evaluate general health (Mitcheltree 1996; Mitcheltree et al. 1997). Initial focus of the exam was directed toward the detection of trap-related injuries to teeth and digits. Fishers were immobilized upon arrival with an intramuscular injection of 100 mg ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) to enable initial examination, blood collection for pre-vaccination titers and serum banking.

<table>
<thead>
<tr>
<th>Sex ID</th>
<th>Vaccine</th>
<th>Serum 1</th>
<th>Days</th>
<th>Serum 2</th>
<th>Days</th>
<th>Serum 3</th>
<th>Days</th>
<th>Serum 4</th>
<th>Days</th>
<th>Serum 5</th>
</tr>
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<tr>
<td>F-120</td>
<td>Fervac-D</td>
<td>1:8</td>
<td>6</td>
<td>1:8</td>
<td>9</td>
<td>Toxic</td>
<td>11</td>
<td>1:4</td>
<td>15</td>
<td>1:4</td>
</tr>
<tr>
<td>F-116</td>
<td>Galaxy-D</td>
<td>1:16</td>
<td>6</td>
<td>1:4</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
<td>1:4</td>
<td>14</td>
<td>1:4</td>
</tr>
<tr>
<td>F-172</td>
<td>Control</td>
<td>1:8</td>
<td>6</td>
<td>1:4</td>
<td>11</td>
<td>1:8</td>
<td>9</td>
<td>1:4</td>
<td>15</td>
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</tr>
<tr>
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<td>Control</td>
<td>1:8</td>
<td>6</td>
<td>1:4</td>
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<td>14</td>
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<tr>
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<td>Control</td>
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<td>6</td>
<td>1:8</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
<td>1:4</td>
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</tr>
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<td>6</td>
<td>1:4</td>
<td>9</td>
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<td>1:4</td>
<td>14</td>
<td>1:4</td>
</tr>
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<td>6</td>
<td>1:4</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
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<td>14</td>
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<td>M-186</td>
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<td>6</td>
<td>1:4</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
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<td>14</td>
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</tr>
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<td>6</td>
<td>1:4</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
<td>1:4</td>
<td>14</td>
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</tr>
<tr>
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<td>6</td>
<td>1:4</td>
<td>9</td>
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<td>12</td>
<td>1:4</td>
<td>14</td>
<td>1:4</td>
</tr>
<tr>
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<td>Fervac-D</td>
<td>1:8</td>
<td>6</td>
<td>1:4</td>
<td>9</td>
<td>1:8</td>
<td>12</td>
<td>1:4</td>
<td>14</td>
<td>1:4</td>
</tr>
</tbody>
</table>

*aInitial vaccination was given after collection of Serum 1.
*bNumber of days from initial vaccination until collection of Serum 2.
*cNumber of days from collection of Serum 2 until collection of Serum 3.
*dBooster vaccination was given after collection of Serum 3.
*eNumber of days from booster vaccination until collection of Serum 4.
*fNumber of days from collection of Serum 4 until collection of Serum 5.
primary vaccination, and subsequently for administration of booster vaccines when possible, and/or blood collection. Blood samples were collected by jugular venipuncture. Fishers were injected subcutaneously in the nape of the neck with MLV CDVs (1 cc reconstituted): Fervac-D® (United Vaccines, Madison, Wisconsin, USA), a chick embryo-tissue culture-origin vaccine approved for use in the domestic ferret (Mustela putorius furo) or Galaxy-D® (Solvay Animal Health, Inc., Mendota Heights, Minnesota, USA), a mammalian cell-line of monkey origin vaccine approved for use in the domestic dog (Canis lupus familiaris).

As part of PFRP, 14 fishers were included with the intent of evaluating the safety and utility of Fervac-D® and Galaxy-D®, MLV CDVs, and were held for 6 weeks. Manufacturer instructions for these vaccines recommended a 2–4 week delay between administration of the primary and booster vaccination and these time-frames were followed throughout this study. Each of the 14 fishers was randomly assigned to one of the three study groups that received a series of two injections of either Fervac-D® (n = 5), Galaxy-D® (n = 6), or a physiological 0.9% saline solution (n = 3) (sodium chloride injection, Baxter Healthcare Corporation, Deerfield, Illinois, USA).

Each of the 14 fishers were assigned to be housed in one of the four animal holding rooms following a randomly stratified design: Fishers in Room 1 were randomly assigned to receive injections of Fervac-D® (n = 1), Galaxy-D® (n = 2), or saline (n = 0); in Room 2, Fervac-D® (n = 1), Galaxy-D® (n = 2), or saline (n = 1); in Room 3, Galaxy-D® (n = 2) or saline (n = 1); and in Room 4, Fervac-D® (n = 3) or saline (n = 1).

The primary vaccination or saline injection was administered immediately after the pre-vaccination serum sample was collected (Serum 1). Blood for evaluating serology for the primary vaccination was collected at 6 days (Serum 2) and 15 days (Serum 3) after the primary vaccination was administered. Serum 3 was collected immediately before the booster vaccination was administered. Blood was then collected at approximately biweekly intervals (Serums 4 and 5).

2.1. Analysis

Sera from all fishers were separated from clotted blood samples and kept frozen at −20 °C. Sera samples were shipped on dry ice to the New York State Veterinary Diagnostic Laboratory at Cornell University (Ithaca, New York 14850, USA) for serum neutralization (SN) testing (Appel & Robson 1973) to detect presence of canine distemper antibodies.

Serum samples were described as Serums 1, 2, 3, 4, and 5 (Serum 1 = pre-vaccination titer levels; Serum 2 = post-vaccination titer levels; Serum 3 = continued post-vaccination titer levels; Serum 4 = post-booster titer levels; Serum 5 = continued post-booster titer levels). Descriptive statistics were used to portray the percent of fishers that had positive post-vaccination evidence of seroconversion. Seroconversion was defined as a fourfold increase in titer level from the pre-vaccination titer level (Coyne 2000).

3. Results

None of the fishers vaccinated with Fervac-D® (n = 5) seroconverted at any of the serum samples (Serums 2, 3, 4, or 5). One fisher (F288) that was vaccinated with Fervac-D® had an increased titer level (1:64) at Serum 3, but dropped to 1:4 at Serums 4 and 5. Two of the Serum 3 samples of fishers (F120, M228) vaccinated with Fervac-D® were toxic to the cell cultures used in the serum-neutralization assays, therefore, titer levels were not reported (Table 1).

None of the fishers vaccinated with Galaxy-D® (n = 6) seroconverted at Serum 2. Two of the six (33%) seroconverted at Serum 3 and remained seroconverted through Serums 4 and 5. Two of the remaining four (50%) non-seroconverted Galaxy-D® fishers seroconverted at Serum 4 and remained seroconverted through Serum 5. One of the remaining two non-seroconverted fishers seroconverted at Serum 5. Thus, by Serum 5, five of the six (83%) fishers vaccinated with Galaxy-D® had seroconverted. The remaining non-seroconverted fisher had increased titer levels, but never reached a fourfold increase from its pre-vaccination titer level. Two of the Serum 2 samples of fishers (F206, F116) vaccinated with Galaxy-D® were toxic to the cell cultures used in the serum-neutralization assays, therefore, titer levels were not reported (Table 1).

None of the fishers injected with a physiological saline solution (n = 3) seroconverted at any of the serum sampling times (Table 1). Untoward reactions, such as the development of clinical disease to CDV, immunosuppression, or death, did not occur in any fisher (n = 11) vaccinated with either vaccine, nor in three fishers that were injected with saline.

4. Discussion

In the 1920’s, Laidlaw and Dunkin prepared the first effective vaccine against CDV using killed virus obtained from domestic dogs previously infected with CDV (Cabasso et al. 1951). These vaccines resulted in unpredictable or short-lived protection. Killed vaccines are slow to develop an immune response and do not result in a sustained elevated antibody response (Bernard et al. 1984; Fowler 1986; Petrini 1992; Schultz & Zuba 2003). MLV vaccines are now recommended over killed vaccines because they provide long-lived protection (Petrini 1992; Williams et al. 1996) and, for the most part, have not shown any untoward effects.
after administration (Petrini 1992). However, MLV vaccines derived from chicken embryo cell cultures have had adverse effects on some mustelids. For example, four black-footed ferrets (Mustela nigripes) died within 21 days post-vaccination with such a vaccine (Carpenter et al. 1976), and Garner et al. (2007) reported myofasciitis in 17 domestic ferrets. None of the five fishers in our study experienced any untoward reactions after being vaccinated with Fervac-D®, a chick embryo-tissue culture-origin MLV CDV. However, Fervac-D® did not effectively stimulate development of a serologic antibody response for CDV. In contrast, three of the five river otters (a closely related species Lontra canadensis) seroconverted after receiving a primary vaccination and a booster vaccination (Peper et al. 2014). Serologic testing procedures in this study were identical to those in Peper et al. (2014). None of the six fishers vaccinated with Galaxy-D®, a mammalian cell-line of monkey origin MLV CDV, experienced any untoward reactions during this study. Galaxy-D® proved to be a more effective vaccine as five of these six (83%) fishers seroconverted after receiving a primary vaccination and a booster vaccination.

An SN titer of $\geq 1:100$ serves as the standard for protective immunity against CDV in domestic dogs (Budd 1981; Montali et al. 1983). Although seroconversion is ideal (i.e., a fourfold increase in pre-vaccination titer level), titers $\leq 1:100$ have been shown to sometimes provide protection from CDV infection (Montali et al. 1983; Wimsatt et al. 2003), and for lack of better information, we applied this standard for fishers. However, the lack of seroconversion or minimal rise in titer level in the five fishers vaccinated with Fervac-D®, emphasized the need for further research with other chick embryo-tissue culture-origin MLV CDVs, or it may be that fishers require a greater antigen concentration (2 ml vaccine dose compared to 1 ml that was administered) to elicit a sufficient rise in titer level.

Evidence of seroconversion in five of the six fishers vaccinated with Galaxy-D® and the increased titer level in the remaining fisher indicates that this vaccine was effective for stimulating antibody production against CDV infection. Although seroconversion was not as rapid as observed in ferrets (48 hr post-vaccination with MLV; Baker et al. 1952), fishers seroconverted between 15 and 41 days post-vaccination in this study.

Aside from the river otter (Hoover et al. 1985; Peper et al. 2014) and the domestic ferret (Mustela putorius furo), little research has been conducted assessing the humoral immune response of other mustelids, including fishers, to vaccines, or other antigens. Our study adds considerably to the body of knowledge relating to such immune responses in this family of mammals and specifically to Martes pennanti.

At the time this study was conducted, Fervac-D® and Galaxy-D® were commercially available and recommended for use. However, at this time, these MLV CDVs are either no longer manufactured (Fervac-D®) or no longer available as previously labeled (Galaxy-D®). Galaxy-D® is now manufactured as part of a multivalent vaccine (Nobivac® Canine 1) by MSD Animal Health (Walton Manor, Walton, Milton Keynes, Buckinghamshire, MK7 7AJ, UK). Nobivac® Canine 1 vaccines are manufactured using the same cell-line as Galaxy-D®. Regardless, evaluating the utility (ability to seroconvert) of these MLV CDVs in wild fishers may indicate the effectiveness of MLV CDVs in general. This becomes even more relevant given that the canary pox-vectored CD vaccine, currently being recommended for non-domestic carnivores, is off the market. Our study represents an ‘extra label’ use of MLV CDVs. Thus, our outcomes serve to inform future ‘labeled use’ of similar vaccines. Related work was published showing the effectiveness of these MLV CDVs (Fervac-D® and Galaxy-D®) in river otters (Peper et al. 2014), of the same family (Mustelidae) as the fisher. Data such as those generated in this study in conjunction with Peper et al. (2014), and other published data, are valuable as a basis for the Food and Drug Administration and United States Department of Agriculture in deciding the justification of future labeled use of similar, currently produced MLV vaccines in fishers or other mustelids. Data derived from our study likewise form a foundation for similar, future studies involving vaccination of fishers against CDV and perhaps other viral diseases. Our results with Galaxy-D® are particularly relevant given that the vaccine is now part of the multivalent Nobivac® Canine 1 vaccines currently being produced.

Based on the adequate levels of seroconversion, rise in titer levels, and apparent safety of Galaxy-D®, the general use of MLV CDVs may be suitable in fishers, pending further studies. Based on the outlook of this study, benefits of this vaccine are (1) the relatively rapid response of fishers to the initial vaccine (seroconversion occurred by 15 days post-vaccination), and (2) the lack of adverse reactions or complications in vaccinated fishers (as of the 41 days post-vaccination observance prior to release). We recommend that future studies be conducted, evaluating currently produced CDV vaccines, particularly Nobivac® Canine 1 vaccines. The risk of infection must be weighed against the risk of the vaccine causing an untoward reaction. To know if vaccines provide fishers effective protection against CDV, vaccinated animals must be challenged with a virulent strain of CDV (Pavlack et al. 2007). However, because the fishers in this study were intended for reintroduction, it would not have been appropriate to challenge them with a virulent strain of CDV prior to release.

Future research should also focus on the length of days required between the administration of primary vaccinations and booster vaccinations to achieve an immune response. If seroconversion is demonstrated to occur sufficiently with one dose of vaccine then vaccination against CDV should be included in
hard-release (i.e., when animals are released without a captive management holding period) reintroduction projects for fishers. If two doses of vaccine are required for seroconversion, then soft-release (i.e., when animals are released after a captive management holding period) reintroduction projects should be recommended that allow for a captive management period to enable the appropriate vaccination timeframes needed to achieve proper protection against CDV for fishers, particularly when CDV is endemic in release areas.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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