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Authors: Paré, Jean A., Barker, Ian K., Crawshaw, Graham J., McEwen, Scott A., Carman, P. Susy, et al.

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HUMORAL RESPONSE AND PROTECTION FROM EXPERIMENTAL CHALLENGE FOLLOWING VACCINATION OF RACCOON PUPS WITH A MODIFIED-LIVE CANINE DISTEMPER VIRUS VACCINE

Jean A. Paré,[1,6,7] **Ian K. Barker,**[1] **Graham J. Crawshaw,**[3] **Scott A. McEwen,**[2] **P. Susy Carman,**[4] **and Roger P. Johnson**[5]

[1] Department of Pathobiology and [2] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[3] Toronto Zoo, 361A Old Finch Avenue, Scarborough, Ontario M1B 5K7, Canada
[4] Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario N1H 6R8, Canada
[5] Health Canada, 110 Stone Rd West, Guelph, Ontario N1G 3W4, Canada
[6] Present address: Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA
[7] Corresponding author: (e-mail: ParеJ@svm.vetmed.wisc.edu)

ABSTRACT: Eight 8-wk-old raccoon pups (*Procyon lotor*) with maternal canine distemper virus (CDV) neutralizing antibodies (NAb) and 24 8-wk-old seronegative pups were administered a commercial modified-live CDV vaccine (Galaxy D®, Solvay Animal Health, Inc., Kitchener, Ontario, Canada). All 24 seronegative raccoons had detectable serum CDV NAb titers 14 days after the initial dose. Titers rose to maximum levels 4 wk post-vaccination. Mean titers for groups of vaccinated seronegative pups were maintained between 1:256 and 1:2,048 for the remainder of the 3 mo observation period. Geometric means of the serum CDV NAb titer of eight seronegative pups given a single vaccine dose at 8 wk of age did not differ significantly from those of eight pups that were given serial doses at 8, 12, and 16 wk of age, or from those of eight pups vaccinated once at 16 wk of age. Seven unvaccinated 8-wk-old raccoon pups used as controls remained seronegative throughout the trial. Seven out of eight 8-wk-old pups with maternal antibodies, vaccinated at 8, 12, and 16 wk of age, failed to develop a rise in their CDV NAb titers until at least 18 wk of age, 2 wk after the third vaccination. Titers in eight unvaccinated raccoons with maternal antibodies declined steadily to undetectable levels at 20 wk of age. A half-life of 10.55 days was calculated for maternally-derived CDV NAb in raccoon pups. Sixteen vaccinated raccoons were protected from clinical disease following experimental oronasal challenge with a virulent raccoon strain of CDV, 13 to 23 wk after vaccination. Serum CDV NAb titers at the time of challenge ranged from 1:12 to 1:384 and increased during the period of observation. Three of four unvaccinated seronegative raccoons used as controls failed to mount any detectable CDV NAb and were euthanatized after developing clinical signs of canine distemper 26, 29, and 30 days post-challenge (PC). Necropsies confirmed the diagnosis. The fourth control raccoon exhibited transient equivocal clinical signs, mounted a sluggish humoral response, but was clinically normal when euthanatized 42 days PC. In this raccoon, there was focal non-suppurative encephalitis with intranuclear inclusion bodies typical of CDV infection.

Key words: Canine distemper virus, challenge, humoral response, *Procyon lotor*, raccoon, vaccination.

INTRODUCTION

The susceptibility of raccoons (*Procyon lotor*) to canine distemper virus (CDV) infection has long been established (Hemboldt and Jungherr, 1955; Kilham et al., 1956). The disease is endemic in raccoon populations across North America (Hoff et al., 1974; Roscoe, 1993). Wild raccoons have been incriminated as the source of epizootics in captive carnivores in zoological collections and conservation parks (Sedgwick and Young, 1968; Appel et al., 1994). Vaccination of captive animals at risk is not always practical or reliable (Sedgwick and Young, 1968; Montali et al., 1983). Data from a recent study (Schubert-Kuehner, 1995) suggests that a trap-vaccination-release (TVR) program targeting raccoons on zoo or park grounds could enhance local population immunity and lower pressure of infection, helping to minimize risks of CDV transmission to the collection. However, studies of efficacy of CDV vaccination in raccoons are scarce, and protocols that would yield optimal protection have not been established. Ear-

ly CDV vaccination trials and challenges (Kilham et al., 1956; Robinson et al., 1957) were conducted on raccoons of undetermined CDV-immune status, therefore careful interpretation of results is warranted. More recently, a study suggested that raccoons administered a now discontinued avianized modified-live virus (MLV) vaccine were protected from canine distemper (CD) disease (Evans, 1984). In the present trial, the humoral antibody responses of raccoon pups of known CDV immune status to Galaxy D® (Solvay Animal Health, Inc., Kitchener, Ontario, Canada), a commercial CD MLV vaccine, were investigated. Interference of maternal antibodies with active immunization was examined, and a CDV neutralizing antibody (NAb) decay curve was established. A controlled challenge study was conducted in vaccinated raccoons to assess efficacy of the vaccine in providing protection from disease.

MATERIALS AND METHODS

Vaccination trial

Forty-seven clinically healthy raccoon pups, all between 4 and 6 wk of age, were collected from the wild in the greater Toronto (43°50′N, 79°10′W) and the Barrie (44°25′N, 79°45′W) areas in the province of Ontario (Canada) between May 9th and June 19th 1996. Age determination was based primarily on teeth eruption pattern (Montgomery, 1964). Pending determination of their CDV immune status, raccoon pups were housed with littermates in pens or stainless steel cages at the Toronto Zoo (TZ). Some of the younger pups were bottle- or syringe-fed with KMR® milk replacer (Pet-Ag, Inc., Hampshire, Illinois, USA), progressively thickened with rice cereal for babies (Rice Cereal®, H. J. Heinz Company of Canada Ltd., North York, Ontario, Canada). Once they were eating from a dish, weaning was quickly achieved, first on a beef-based carnivore mix prepared at the zoo, then onto commercial dry cat food. The pups were examined, sexed, weighed, ear-tagged, and prophylactically given ivermectin (Ivomec®, Merck Agvet, Merck and Co., Inc., Whitehouse Station, New Jersey, USA, 0.2 mg/kg SC) and a killed feline panleukopenia vaccine (Fel-O-Vax®, Ayerst Veterinary Laboratories, Division of Wyeth-Ayerst Canada Inc., Guelph, Ontario, Canada; 1 ml SC). Blood was collected from the jugular vein under isoflurane anesthesia (AErrane®, Ohmeda Pharmaceutical Products, Division of BOC Canada Ltd., Mississauga, Ontario, Canada) for determination of CDV maternal antibody status. All pups were weaned at 8 wk of age, at which time they were moved to the isolation units of the Ontario Veterinary College (OVC; University of Guelph, Guelph, Ontario, Canada), and entered the trial.

Thirty-one CDV-seronegative 8-wk-old raccoon pups were randomly allocated to one of four experimental groups: Group A ($n = 7$) pups were controls that were administered 1 ml of sterile saline subcutaneously (SC) at 8, 12, and 16 wk of age; Group B ($n = 8$) pups were given 1 ml of a commercial MLV vaccine, Galaxy D®, SC between the scapulae, using aseptic technique, at 8, 12, and 16 wk of age; Group C ($n = 8$) pups received a single dose of Galaxy D® at 8 wk of age; and Group D ($n = 8$) pups received a single dose of Galaxy D® at 16 wk of age.

Sixteen seropositive 8-wk-old raccoon pups with serum CDV NAb titers ranging from 1:40 to 1:1536 were randomly allocated to one of two groups: Group E ($n = 8$) in which animals were administered one dose of Galaxy D® at 8, 12, and 16 wk of age, and Group F ($n = 8$) in which animals received 1 ml of sterile saline SC at 8, 12, and 16 wk of age.

Blood was collected for serology weekly from all raccoons from 8 to 20 wk of age (12 wk postvaccination (PV)) except for raccoons of Group D, which were sampled until they were 24 wk of age (8 wk PV).

At the OVC, raccoons were housed in pairs in spacious stainless steel cages. Raccoons from groups A, D, and F were kept in a room separate from group B, C, and E raccoons. Once Group D pups reached 16 wk of age, they were moved in the same room as those of groups B, C, and E.

On the day of their arrival at the OVC at 8 wk of age, and weekly thereafter, all pups were anesthetized using a combination of ketamine hydrochloride (Ketaset®, Ayerst Laboratories, Division of Wyeth-Ayerst Canada Inc., Montreal, Quebec, Canada; 10 mg/kg) and xylazine (Rompun®, Bayer Inc., Agriculture Division, Animal Health, Etobicoke, Ontario, Canada; 2mg/kg), injected intramuscularly. Animals were weighed, examined, and blood was collected. All pups were given ivermectin and Fel-O-Vax® on the week of arrival at the OVC and every second week thereafter, for 4 and 6 wk respectively.

Blood samples were centrifuged, and the sera separated and refrigerated within 24 hr of collection. Each serum sample was divided in

two equal volumes and frozen at −70 C until submission to the laboratory. Virus neutralization assays for the detection of CDV were performed by the Animal Health Laboratory (University of Guelph) in microtiter format using standard techniques (Mahy and Kangro, 1996), 100 $CCID_{50}$ Onderstepoort strain CDV, Vero cells and known positive and negative sera. Sera were serially diluted two-fold in duplicate, with antibody titers determined as the 50% endpoint for cytopathic effect after 5 days incubation at 37 C in 5% CO_2. Except for week 12 samples (week 8 for group D), all samples from individual animals were assayed simultaneously to eliminate between-batch test variation. Seronegativity and seropositivity were defined by the absence or presence of detectable serum CDV neutralizing antibodies at a 1:2 dilution of serum. Serologic results were recorded as the reciprocal of the end point dilution and transformed to the $\log_2$. Group means and standard deviations of transformed data were calculated and presented graphically. Statistical analysis was performed with PC-SAS 6.12 for Windows® (SAS Institute Inc., Cary, North Carolina, USA) using a repeated measures ANOVA (Motulsky, 1995). Data for week 12 (group A, B, C, E, and F) and week 8 (group D) were excluded from the graphs, but used in statistical comparison between groups. The decay curve was established by standard least squares linear regression analysis of the data using the Corel Quattro Pro 7® analytical statistics package (Corel Corporation, Ottawa, Ontario, Canada). The slope was calculated using the equation: $y = \beta_0 + \beta_1 x$, where y is the antibody titer at time x, β_0 is the Y intercept and β_1 is the slope of the regression line.

Challenge study

Twenty raccoons were selected from the Galaxy D® vaccination trial to enter a challenge study. All animals were 7- to 8-mo-old by then, and were housed individually in stainless steel cages in a single room at the OVC. Four unvaccinated raccoons, randomly selected from groups A and F, had no detectable serum CDV NAb and were used as control animals. The 16 seropositive raccoons were selected randomly from Group C ($n = 4$), Group D ($n = 4$), and groups B and E ($n = 8$). They had CDV NAb titers ranging from 1:12 to 1:384. They were inoculated via the oculonasal route with a virulent raccoon CDV isolate (day 0) and followed for a period of 42 days. On days 0, and 3, 7, 10, 14, 21, 28, 35, 42 post challenge (PC), blood was collected under ketamine hydrochloride/xylazine, and serum virus NAb titers were measured, as described in the vaccination trial. Group means and standard deviations of transformed data were calculated and presented graphically.

The challenge virus, California Raccoon Isolate A92-27/14, generously provided by M. J. G. Appel (Cornell University, Ithaca, New York) was amplified by passage in two 21-wk-old seronegative raccoons, which were given 0.75 ml of the virus tissue suspension intravenously. Lymphoid tissues were collected aseptically from both raccoons immediately following euthanasia 6 days later. Tissues were trimmed, placed in tissue culture medium, homogenized and centrifuged, pooled, then further clarified by centrifugation. Aliquots of undiluted, 1:10, and 1:100 dilution of the virus suspension were stored at −70 C until use.

A pilot trial was conducted to determine an effective challenge dose using six seronegative 24-wk-old raccoons. Two animals were each administered 1 ml of undiluted CDV suspension that had been thawed on ice. Five drops were placed in the conjunctival sac of each eye, five drops were instilled in each nostril, and the remainder of the dose was sprayed onto the oropharyngeal mucosa. This procedure was repeated with the 1:10 and the 1:100 dilutions of the CDV suspension, using two raccoons for each dose. The raccoons that had received the undiluted suspension were euthanatized 28 days PC showing classical signs of disease (mucopurulent blepharoconjunctivitis and rhinitis). Those that had received the 1:10 dilution of the inoculum were euthanatized at 26 and 33 days PC respectively, with clinical CD. Both raccoons administered the 1:100 dilution were euthanatized 33 days PC, with one showing clinical CD.

On day 0 of the challenge study, the 20 raccoons were anesthetized using ketamine hydrochloride and xylazine, and inoculated with the CDV suspension as described above. Raccoons were inspected daily by one of two observers who were both blinded to the vaccination status of the animals. Alertness, responsiveness and general demeanor were evaluated. Food consumption, as well as fecal output and consistency were also assessed. Animals were monitored for cutaneous erythema, pustules, foot pad and muzzle skin thickening and depigmentation, ocular and nasal discharge, sneezing, coughing, and any behavioral abnormality or neurologic disturbance. Criteria for disease and euthanasia were established prior to the challenge: Anorexia, severe lethargy, ocular and/or nasal mucoid discharge, vomiting, diarrhea, pustules or blisters, and neurologic signs of any kind were deemed indicative of disease. Raccoons convincingly demonstrating any of the above clinical signs for more than 3 consecutive days, or sooner if deemed appropriate on hu-

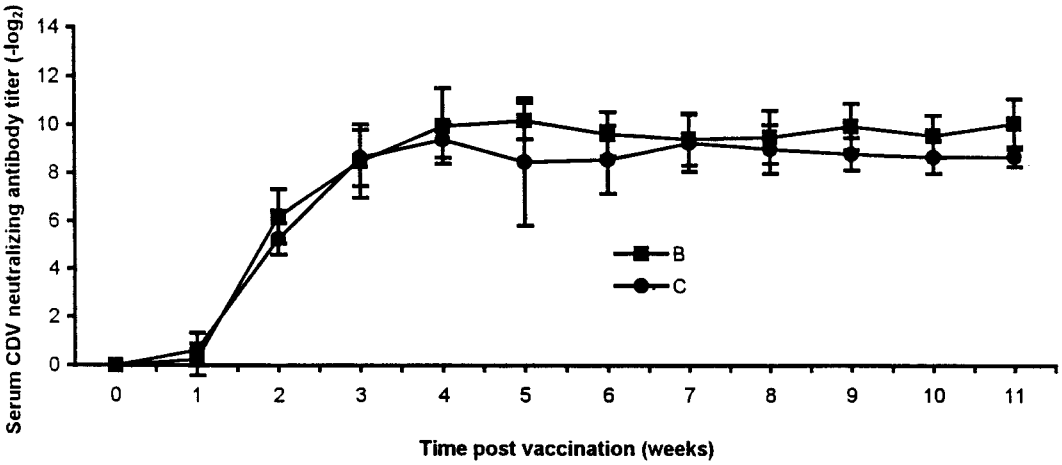

FIGURE 1. Antibody titers ($-\log_2$) of initially seronegative raccoon pups following a single dose of Galaxy D® at 8 wk of age (Group C) or three doses at 8, 12, and 16 wk of age (Group B). Each curve represents the geometric mean of the $\log_2$ of the reciprocal of serum CDV neutralizing antibody titers of an experimental group of raccoons ($n = 8$) versus time post-vaccination (±SD).

mane grounds, were euthanatized. Surviving animals were euthanatized on day 42. A thorough necropsy was conducted on all animals immediately after death. Tissue samples were collected and fixed in 10% buffered formalin. The presence of characteristic intracytoplasmic and intranuclear acidophilic inclusion bodies in epithelial, lymphoid, or glial cells was considered diagnostic for CD (Dungworth, 1993), whether or not other compatible lesions were present.

RESULTS

Vaccination trial

There were no discernible local or systemic adverse reactions to Galaxy D® in any of the 24 raccoons vaccinated in this trial. None of the initially seronegative unvaccinated raccoons (Group A) developed a detectable serum CDV NAb titer, while all initially seronegative vaccinated raccoons (groups B, C and D) had measurable serum CDV NAb titers by week 2 PV ($P = 0.0001$) (Figs. 1 and 2). Five of these 24 raccoons had developed titers as early as 1 wk PV. In all vaccinates, titers climbed abruptly between weeks 2 and 4 PV, and remained high throughout the follow-up period (group means between 1:256 and 1:2,048).

There was no significant difference ($P < 0.05$) between the PV geometric mean CDV NAb titers at each sampling of initially seronegative 8-wk-old raccoons vaccinated sequentially at 8, 12, and 16 wk of age (Group B) and those that received a single dose at 8 wk of age (Group C) (Fig. 1).

There was no significant difference ($P < 0.05$) between the mean antibody titers following a single dose of vaccine in initially seronegative 8-wk-old raccoons (Group C) and initially seronegative 16-wk-old raccoons (Group D) (Fig. 2).

Four of eight initially seropositive 8-wk-old control raccoons (Group F) had no detectable antibody by 16 wk of age, suggesting strongly that the antibodies detected initially were passively acquired from the dam rather than a product of active immunity. Standard least squares linear regression gave a slope of -0.663 with an intercept of 13.35 on the Y axis when the X axis is projected back to birth (Fig. 3). The half-life of maternal antibodies was calculated at 10.55 days.

There was no significant difference ($P < 0.05$) between the mean titers of 8-wk-old unvaccinated raccoons with maternal antibodies (Group F) when compared to mean titers of 8-wk-old raccoons with maternal antibodies vaccinated at 8, 12, and

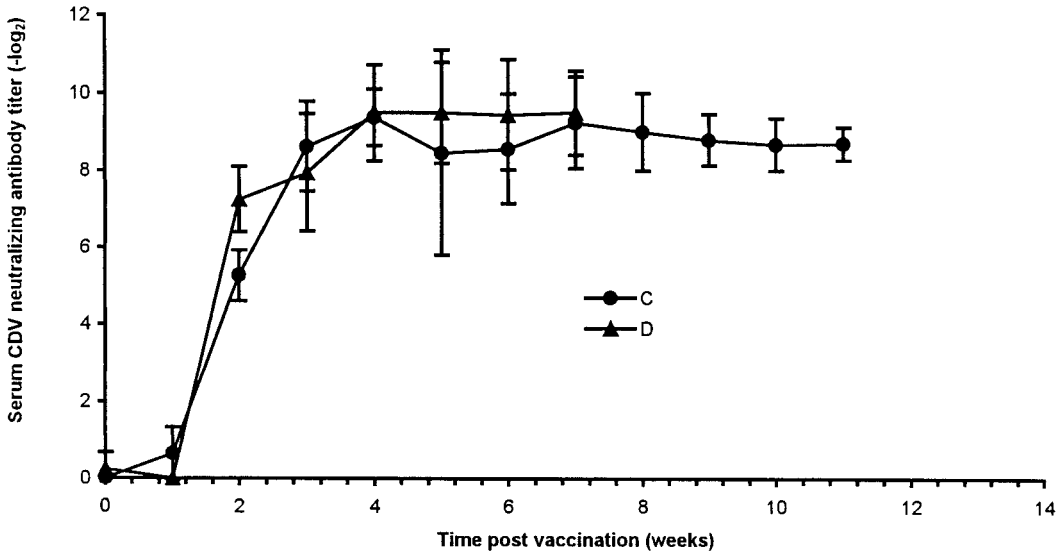

FIGURE 2. Effect of age at vaccination with Galaxy D® on antibody titers ($-\log_2$) in initially seronegative raccoons vaccinated at 8 wk of age (Group C) or 16 wk of age (Group D). Each curve represents the geometric mean of the $\log_2$ of the reciprocal of serum CDV neutralizing antibody titers of an experimental group of raccoons ($n = 8$) versus time post-vaccination (±SD).

16 wk of age (Group E), until week 10 PV, or 2 wk after the third vaccination at 16 wk of age (Fig. 4). From that point on, vaccinates (Group E) developed significantly ($P = 0.023$) higher titers than the controls over the remainder of the observation period. Vaccination failed to elicit a humoral response before the third vaccination (16 wk of age) in all but one of the eight raccoons that possessed maternal antibodies. The exception, one of the three with the lowest titer at 12 wk of age, experienced a rise in antibody titer 2 wk after the second vaccination, while two other raccoons with similar titers at the time of the second vaccination, one of them a litter mate, failed to respond until 16 wk of age. One raccoon from Group E did not respond to the third vaccine dose, in spite of a relatively low titer (1:3) at 16 wk of age.

Challenge study

All 16 vaccinated raccoons survived the challenge, and none met the criteria defined for clinical disease over the 42 day period of observation. These raccoons experienced a statistically significant rise ($P < 0.05$) in their antibody titer levels between challenge and 10 d PC (Fig. 5).

Of the four seronegative controls, one developed disease on day 29 and was euthanatized on day 33 (#1). A second raccoon showed clinical signs on day 30, and was euthanatized on day 33 PC (#2). These two animals exhibited marked depression, and had bilateral ocular and nasal mucopurulent discharge and crusting. A third raccoon (#3) was euthanatized on day 30 PC, the day it developed seizures. These three raccoons never developed a detectable serum CDV NAb titer.

The fourth seronegative control raccoon (#4) exhibited vague signs of illness (inappetence, lethargy, cutaneous erythema) on days 21, 22, and 29 but recovered and appeared clinically normal 42 days PC. In this individual, detectable serum NAb titers appeared at 21 days PC and rose slowly over the remainder of the observation period (Fig. 5).

On post-mortem examination, two of the three raccoons with distemper (controls #1 and #2) had bilateral mucopuru-

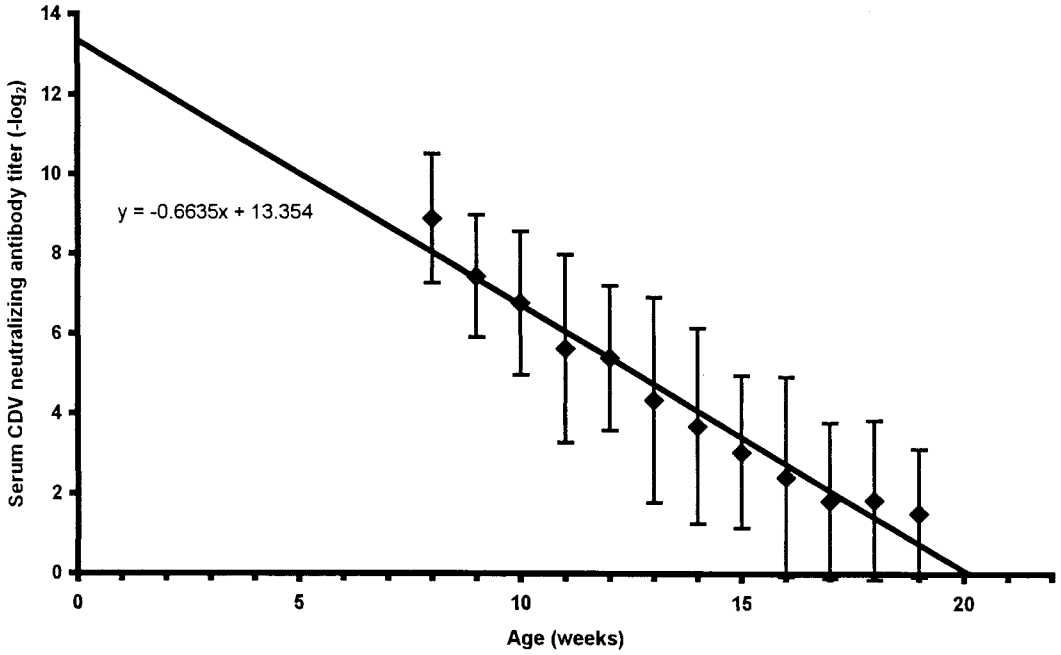

FIGURE 3. Maternal antibody decay curve in raccoons 8 to 20 wk of age (Group F). The curve represents a least squares linear regression line of best fit through the geometric means of the $\log_2$ of the reciprocal of serum CDV neutralizing antibody titers of raccoons (n = 8) plotted against age (±SD).

lent blepharoconjunctivitis and rhinitis. One of these developed depigmentation of the muzzle and footpads, while these tissues were hyperkeratotic in the other. Histologically, in both animals, intracytoplasmic and intranuclear acidophilic inclusion bodies were identified in the epithelial cells of the bladder, bronchioles, epidermis of the footpad, tongue, Meibomian glands, and seminiferous tubules. Inclusion bodies were also observed in the splenic reticuloendothelial cells of one raccoon.

The third raccoon (control #3), euthanatized because of seizures, had a mild bilateral conjunctivitis. Microscopically, intranuclear inclusion bodies were identified in the hippocampal neurons and in the epithelial cells of the bladder mucosa, with no associated inflammation.

The only unvaccinated raccoon that survived the challenge (control #4) had no remarkable gross lesions when necropsied on day 42 PC, except a chronic cystitis with pyuria. Histologically, a single but conspicuous focus of non-suppurative encephalitis was observed in the medulla oblongata. There was malacia and glial activation with moderate perivascular cuffing. Inclusion bodies were readily identifiable in surrounding neurons.

There were very few gross external or internal lesions in the 16 vaccinated raccoons. Histologically, 10 of 16 raccoons had mild to moderate, patchy or diffuse interstitial pneumonia, but giant cells and inclusion bodies were not detected.

DISCUSSION

The dynamics of the humoral response in this trial are consistent with investigations of humoral response to MLV vaccination in other species (Halbrooks et al., 1981; Montali et al., 1983; Hoover et al., 1989; Goodrich et al., 1994; Williams et al., 1996) but quantitative comparison of the magnitude of the response to other studies is precluded due to inter-laboratory variations in methodology.

In the present study, vaccination protocols yielded similar antibody responses in

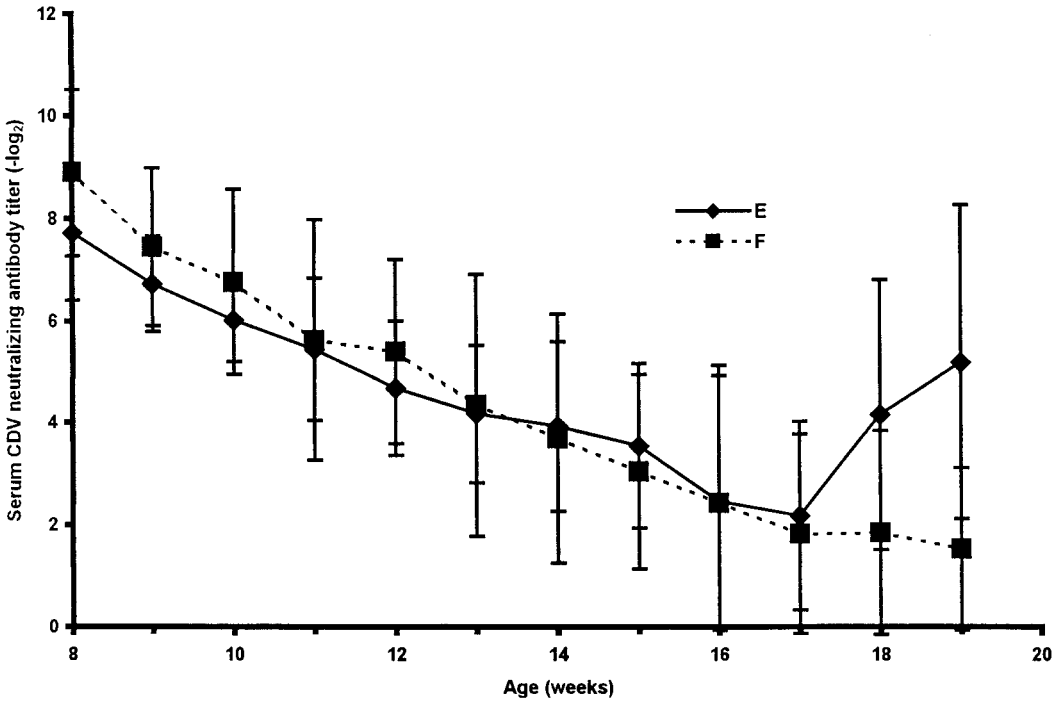

FIGURE 4. Antibody levels of raccoons with maternal antibodies following vaccination with Galaxy D® at 8, 12, and 16 wk of age (Group E) compared with unvaccinated controls with maternal antibodies (Group F). Each curve represents the geometric mean of $\log_2$ of the reciprocal of serum CDV neutralizing antibody titers of an experimental group of raccoons (n = 8) plotted against age (±SD) from 8 to 19 wk.

seronegative raccoon pups. There was no significant difference over the period of observation between titer levels of raccoons receiving single or multiple vaccinations. Seronegative raccoons captured as part of a TVR program, in which booster vaccination is unfeasible or impractical, should therefore benefit from a single vaccination.

Eight-week-old pups were as capable of responding to vaccination as 16-wk-old pups. Raccoons can probably mount an immune response to CDV very early in life, as is the case in the dog (1 day of age) and ferret (*Mustela putorius furo*) (8 days of age) (Ott and Gorham, 1955).

Maternal antibodies in all seropositive control raccoon pups declined gradually to negligible levels by the time they had reached 20 wk of age. The half-life of maternal antibodies, estimated at 10.55 days was similar to that reported for dogs (8.5 days, Gillespie et al., 1958) and ferrets (9.4 days; Appel and Harris, 1988). This decay reflects normal protein catabolism and is comparable for various antibodies against other pathogens (e.g., 9.7 days for parvovirus antibodies in dogs (Pollock and Carmichael, 1982)).

The present study demonstrated that maternal antibodies will nullify or seriously interfere with active immunization in 8-wk-old raccoon pups until they reach up to 14 to 16 wk of age. Examination of individual data from raccoons of Group E reveals differences in the ability of pups to respond to vaccination in the presence of similar maternal antibody titers. The raccoon in group E that failed to respond to the third vaccination, could have benefited from a fourth dose at 18 to 20 wk of age. It is difficult to determine a threshold antibody titer below which an animal's maternal antibodies will not interfere with vaccination. From Figure 4, it appears that a titer of 1:32, but not 1:8, will nullify any

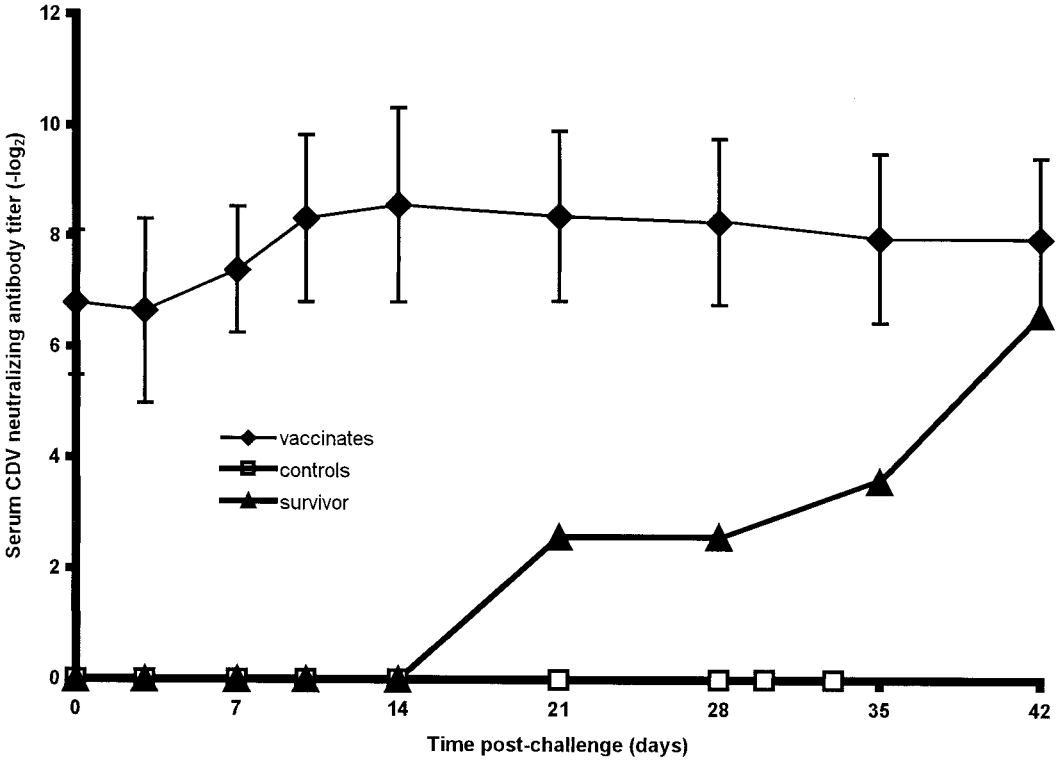

FIGURE 5. CDV neutralizing antibody titers ($-\log_2$) ± SD in vaccinated (n = 16), euthanatized unvaccinated control (n = 3), and surviving control (n = 1) raccoons following challenge with CDV.

vaccination attempt. However, in the field, the immune status of a raccoon pup is rarely, if ever, known.

Modified-live virus vaccines stimulate a cell-mediated immune response, which acts in concert with the humoral arm of the immune system to protect against CDV infection and disease (Appel, 1987; Greene, 1990). Although not assessed in this study, cell-mediated immunity may have contributed to protection of vaccinates since titers in several animals were quite low (<1:32) at the time of challenge. Fourteen of the 16 previously vaccinated raccoons experienced a rise in antibody titer in the 10 days following challenge. While in many animals this increase was slight, in others it was substantial. In one raccoon, the titer climbed from 1:12 on the day of challenge to 1:1,536 on day 10 PC, demonstrating a strong anamnestic response. Conversely, three of four unvaccinated seronegative control animals developed clinical signs characteristic of CD that were severe enough to warrant euthanasia. These three animals did not develop virus neutralizing antibody titers and typical CDV inclusion bodies were observed histologically in their tissues. This is in accordance with the accepted concept of CD pathogenesis, in which an early vigorous humoral response is mandatory for recovery from distemper infection (Appel, 1987).

The fourth control raccoon developed equivocal signs of illness during the observation period, but appeared clinically normal when euthanatized. This raccoon had a sluggish humoral response to challenge. A measurable but low titer (1:6) was first detected at 21 days PC and climbed very slowly. At necropsy, this raccoon had cystitis with pyuria, a common finding in raccoons with CD (Monson and Stone, 1976).

More convincingly, focal non-suppurative encephalitis, with typical CDV inclusion bodies in glial cells, was identified histologically. Low-grade or sluggish humoral responses are often associated with chronic progressive encephalitis in CDV-infected animals (Appel, 1987). It is likely that this raccoon would have developed neurological signs over a longer period, although similarly affected animals may recover (Appel, 1987).

In this study, the time between the last CDV vaccination and the day of challenge ranged from 13 to 23 wk. Three raccoons were vaccinated only once at 8 wk of age, and did not develop CD when challenged 23, 22 and 22 wk later respectively, suggesting that a single vaccination in a seronegative pup confers protection for a minimum of 5.5 mo. This is consistent with the notion that immunity conferred by MLV CD vaccines is long-lasting (Appel, 1987).

In the present vaccination trial, Galaxy D® proved effective in promoting a humoral response in that all seronegative animals developed measurable serum CDV NAb titers by the second week PV. The lack of local or systemic reactions suggests sufficient attenuation of this vaccinal strain for use in raccoons. Finally, while only three of four control raccoons developed clinical signs over the 42 day follow-up period, all four had lesions due to CD. Sixteen vaccinated raccoons, with titers at challenge ranging from 1:12 to 1:384, remained free of clinical signs of disease over a 42 days follow-up period, and none had gross or microscopic lesions of CD when euthanatized at that time. Statistically significant protection from clinical or subclinical infection was therefore achieved with Galaxy D® (0/16 vs. 4/4).

Although all seropositive pups were from wild unvaccinated mothers, some had very high titers (1:1,536) when initially tested at 4 to 6 wk of age, suggesting that the antibody titer in their dam was even higher (Gillespie et al., 1958). In Figure 3, linear regression allows estimation of a mean titer of 1:10,440 in pups of Group F immediately after colostral intake. This corroborates the observation that CDV is circulating in the Scarborough and Barrie populations of free-ranging raccoons.

In addition to naïve adults, the susceptible subsets of raccoons in an endemic population would consist of animals born to naïve dams, plus juvenile raccoons 16 wk or older in which maternal immunity has waned, leaving them vulnerable to infection. Results from this vaccination trial suggest that in raccoon orphanages, in captive animals, or whenever practical or feasible, a vaccination protocol extending to 16 or even 18 wk of age is prudent, particularly in a canine distemper endemic area. Immunization of pups at 8, 12, and 16 or 18 wk of age is therefore recommended. However, to boost local free-ranging raccoon population immunity, the benefits from an annual TVR program would be optimal if conducted 4 to 5 mo after the main whelping season, since all susceptible population subsets should then be responsive to a single vaccination.

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LITERATURE CITED

APPEL, M. J. G. 1987. Canine distemper virus. *In* Virus infections of carnivores, M. J. Appel (ed.). Elsevier Science Publishers B. V., New York, New York, pp. 133–159.

———, AND W. V. HARRIS. 1988. Antibody titers in domestic ferret jills and their kits to canine distemper virus. Journal of the American Veterinary Medical Association 193: 332–333.

———, R. A. YATES, G. L. FOLEY, J. J. BERNSTEIN, S. SANTINELLI, L. H. SPELMAN, L. D. MILLER, L. H. ARP, M. ANDERSON, M. BARR, S. PEARCE-KELLING, AND B. A. SUMMERS. 1994. Canine distemper epizootic in lions, tigers, and leopards in North America. Journal of Veterinary Diagnostic Investigation 6: 277–288.

DUNGWORTH, D. L. 1993. The respiratory system. *In*

Pathology of domestic animals, 4th Edition, Volume 2, K. V. F. Jubb, P. C. Kennedy, and N. Palmer (eds.). Academic Press, Inc., Harcourt Brace Jovanovich, Publishers, Toronto, Canada, pp. 539–699.

EVANS, R. H. 1984. Studies of a virus in a biological system: naturally occurring and experimental canine distemper in the raccoon (*Procyon lotor*). M.Sc. Thesis, Southern Illinois University, Carbondale, Illinois, 135 pp.

GILLESPIE, J. H., J. A. BAKER, J. BURGHER, D. ROBSON, AND B. GILMAN. 1958. The immune response of dogs to distemper virus. Cornell Veterinarian 48: 103–126.

GOODRICH, J. M., E. S. WILLIAMS, AND S. W. BUSKIRK. 1994. Effects of a modified-live virus canine distemper vaccine on captive badgers (*Taxidea taxus*). Journal of Wildlife Diseases 30: 492–496.

GREENE, C. E. 1990. Immunoprophylaxis and immunotherapy. *In* Infectious diseases of the dog and cat, C. E. Greene (ed.). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Toronto, Ontario, Canada, pp. 21–54.

HALBROOKS, R. D., L. J. SWANGO, P. R. SCHNURRENBERGER, F. E. MITCHELL, AND E. P. HILL. 1981. Response of gray foxes to modified-live virus canine distemper vaccines. Journal of the American Veterinary Medical Association 179: 1170–1174.

HEMBOLDT, C. F., AND E. L. JUNGHERR. 1955. Distemper complex in wild carnivores simulating rabies. American Journal of Veterinary Research 16: 463–469.

HOFF, G. L., W. J. BIGLER, S. J. PROCTOR, AND L. P. STALLINGS. 1974. Epizootic of canine distemper virus infection among urban raccoons and grey foxes. Journal of Wildlife Diseases 10: 423–428.

HOOVER, J. P., C. A. BALDWIN, AND C. E. RUPPRECHT. 1989. Serologic response of domestic ferrets (*Mustela putorius furo*) to canine distemper and rabies virus vaccine. Journal of the American Veterinary Medical Association 194: 234–238.

KILHAM, L., R. T. HABERMANN, AND C. M. HERMAN. 1956. Jaundice and bilirubinemia as manifestations of canine distemper in raccoons and ferrets. American Journal of Veterinary Research 17: 144–148.

MAHY, B. W. J., AND H. O. KANGRO (EDITORS). 1996. Virology methods manual. Academic Press, Harcourt Brace & Company, Publishers, Toronto, Ontario, Canada, 374 pp.

MONSON, R. A., AND W. B. STONE. 1976. Canine distemper in wild carnivores in New York. New York Fish and Game Journal 23: 149–154.

MONTALI, R. J., C. R. BARTZ, J. A. TEARE, J. T. ALLEN, M. J. G. APPEL, AND M. BUSH. 1983. Clinical trials with canine distemper vaccines in exotic carnivores. Journal of the American Veterinary Medical Association 183: 1163–1167.

MONTGOMERY, G. G. 1964. Tooth eruption in preweaned raccoons. The Journal of Wildlife Management 28: 582–584.

MOTULSKY, M. D. 1995. Intuitive biostatistics. Oxford University Press, Inc., New York, New York, 386 pp.

OTT, R. L., AND J. R. GORHAM. 1955. The response of newborn and young ferrets to intranasal administration with egg-adapted distemper virus. American Journal of Veterinary Research 16: 571–572.

POLLOCK, R. V. H., AND L. E. CARMICHAEL. 1982. Maternally derived immunity to canine parvovirus infection: Transfer, decline and interference with vaccination. Journal of the American Veterinary Medical Association 180: 37–42.

ROBINSON, V. B., J. W. NEWBERNE, AND D. M. BROOKS. 1957. Distemper in the American raccoon (*Procyon lotor*). Journal of the American Veterinary Medical Association 131: 276–278.

ROSCOE, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. Journal of Wildlife Diseases 29: 390–395.

SCHUBERT-KUEHNER, C. A. 1995. The effects of disease management on urban wildlife populations: An experimental approach. Ph.D. Thesis, University of Guelph, Guelph, Ontario, Canada, 100 pp.

SEDGWICK, C. J., AND W. A. YOUNG. 1968. Distemper outbreak in a zoo. Modern Veterinary Practice 49: 39–44.

WILLIAMS, E. S., S. L. ANDERSON, J. CAVENDER, C. LYNN, K. LIST, C. HEARN, AND M. J. G. APPEL. 1996. Vaccination of black-footed ferrets (*Mustela* nigripes) × Siberian polecat (*M. eversmanni*) hybrids and domestic ferrets (*M. putorius furo*) against canine distemper. Journal of Wildlife Diseases 32: 417–423.

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