

# Three-Year Duration of Immunity for Canine Distemper, Adenovirus, and Parvovirus After Vaccination with a Multivalent Canine Vaccine

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## ABSTRACT

A study was conducted to evaluate the 3-year duration of immunity claim for the canine distemper virus, canine adenovirus type 2, and canine parvovirus fractions in a commercially available modified-live virus vaccine (Duramune Adult, Fort Dodge Animal Health) that was specially formulated to provide a longer duration of immunity than currently available products that are labeled for 1 year of protection. Vaccinated puppies were protected against the virulent challenge with virus exposures 3 years after vaccination and had fewer clinical signs, lower body temperatures, and greater weight gains compared with nonvaccinated controls.

## INTRODUCTION

The development, licensing, commercial production, and marketing of veterinary biologi-

cal vaccines in the United States are rigidly controlled by the United States Department of Agriculture (USDA). Current testing guidelines for registration of a veterinary biological product include efficacy, purity, potency, and safety studies. The majority of vaccines are evaluated for efficacy only for a short duration (i.e., a few weeks or months). Until recently, licensure of veterinary biologicals has not required evaluations of the duration of immunity, except for rabies vaccines, which require 1- or 3-year studies, depending on the species and the product. USDA guidelines now require actual duration of immunity challenge studies for vaccines containing novel antigens for which no other products are available.

By tradition, manufacturers of veterinary biologicals with USDA-approved labels have recommended annual revaccinations. In recent years, there have been an increasing number of questions about the need for administering vaccines to animals annually. Scientific infor-

mation based on well-controlled study designs provide evidence that the recommendations for longer durations of protection has not been substantiated for any of the vaccines other than rabies and a few other products.

Canine distemper virus (CDV), canine adenovirus type 2 (CAV2), and canine parvovirus (CPV) are considered the core antigens recommended for routine vaccination of dogs. Recommendations made by the American Animal Hospital Association and the American Veterinary Medical Association's Council on Biologics and Therapeutic Agents support the concept of core vaccinations, with clinicians using judgment, experience, and the best science available to determine the vaccination requirements for each individual animal.<sup>1,2</sup> To provide additional information to the clinician on duration of immunity of a new canine vaccine, a study was conducted to determine whether the CDV, CAV2, and CPV antigen fractions in a modified-live multivalent product (Duramune Adult, Fort Dodge Animal Health) would provide protection against virulent challenge virus exposures for at least 3 years following the primary vaccination regimen.

## MATERIALS AND METHODS

### Test Animals

Forty specific pathogen-free puppies, 6 to 8 weeks of age that were seronegative to the antigen fractions in the test vaccine were obtained from commercial sources and randomly allocated to two groups. Thirty-two of the puppies were allocated to a vaccinated group (Group A) and were housed individually in isolation cages for the duration of the test period. The remaining eight puppies were assigned to a nonvaccinated control group (Group B). Control puppies also were individually caged, but in a separate isolation facility. Puppies in both groups were observed daily following vaccination and challenge.

### Vaccine

A commercial vaccine (Duramune Adult, Fort Dodge Animal Health) containing mod-

ified-live virus components against CDV, CAV2, and CPV at minimum immunizing dose levels that was specially formulated to provide a longer duration of immunity was used for the study.

### Vaccination

Thirteen of the puppies from Group A were vaccinated IM with 1 ml of the test vaccine on Day 0 and again 21 days later. Nineteen of the Group A puppies were similarly vaccinated SC. The control puppies in Group B were not vaccinated.

### Challenge Virus

The virulent Snyder Hill strain of CDV, 2b strain of CPV, and Mirandola strain of CAV1 were obtained from the Center for Veterinary Biological Laboratory (CVB-L), Ames, IA. The study animals were challenged with the Snyder Hill strain of CDV by intracranial administration of 0.5 ml of the standardized challenge virus. The challenge dose of the type 2b strain of CPV was standardized, and 3 ml was administered by intranasal and oral routes. The Mirandola strain of CAV1 was administered IV (1.0 ml).

### Duration of Immunity

Approximately 3 years after the second vaccination, each of 13 puppies (5 vaccinated IM, 5 vaccinated SC, and 3 controls) was challenged intracranially with the virulent Snyder Hill strain of CDV. Similarly, each of 14 puppies (3 vaccinated IM, 9 vaccinated SC, and 2 nonvaccinated controls) was challenged IV with the virulent Mirandola strain of CAV1. The remaining 13 puppies (5 vaccinated IM, 5 vaccinated SC, and 3 controls) were challenged orally and intranasally with a virulent type 2b strain of CPV.

### Clinical Assessments

The 10 vaccines and 3 control puppies challenged with the virulent CDV 3 years after vaccination were observed 2 days before and daily 0 to 21 days after challenge for clinical signs associated with CDV infection, including lethargy, inappetence, ocular discharge, fecal consistency, retching, vomiting, and seizures or convulsions.

**Table 1.** Scoring System for Clinical Signs of Canine Distemper, Canine Adenovirus, and Canine Parvovirus in Dogs Challenged 3 Years After Vaccination with A Multivalent Modified-Live Virus Vaccine

Canine Distemper Virus	Canine Adenovirus	Canine Parvovirus
Normal = 0	Normal = 0	Normal = 0
Lethargy = 1	Lethargy = 1	Lethargy = 1
Inappetence = 1	Inappetence = 1	Inappetence = 1
Ocular discharge	Ocular discharge	White blood cell counts
Mild/moderate = 1	Mild/moderate = 1	40%–<50% of baseline = 1
Severe = 1	Severe = 2	30%–<40% of baseline = 2
		20%–<30% of baseline = 3
		<20% of baseline = 4
Fecal consistency	Conjunctivitis	Fecal consistency
Watery = 2	Mild/moderate = 1	Mucous = 1
Bloody = 3	Severe = 2	Watery = 2
		Bloody = 3
Retching = 1	Tonsillitis = 1	Dehydration = 1
Vomiting = 1	Buccal mucosal red = 1	Vomiting = 1
Seizures/convulsions = 3	Nasal discharge	
	Mild/moderate = 1	
	Severe = 2	
Temperature (°F)	Temperature (°F)	Temperature (°F)
103.0–103.9 = 1	103.0–103.9 = 1	103.4–104.4 = 1
104.0–104.9 = 2	104.0–104.9 = 2	104.5–105.5 = 2
≥105.0 = 3	≥105.0 = 3	≥105.5 = 3
Death = 20	Death = 20	Death = 20

Clinical signs were recorded, and a scoring system was applied to provide a quantitative assessment of the response to challenge (Table 1). Rectal temperatures for all animals were recorded from on the day of challenge and daily for 21 days after challenge. Body weights for all animals were determined 0, 7, 14, and 21 days after challenge.

The immunogenicity of the CAV2 fraction was evaluated by virulent CAV1 challenge exposure of 12 vaccinees and two controls. The challenged animals were observed from 2 days before and daily for 21 days after challenge (3 years after vaccinations were given) for clinical signs associated with CAV1 infection, including lethargy, inappetence, ocular discharge, conjunctivitis, nasal discharge, tonsillitis, and buccal mucosal redness. Clinical signs were recorded, and a scoring system was applied to arrive at a quantitative assessment of the response to challenge (Table 1). Rectal temperatures for all animals were recorded from

0 through 21 days after challenge. All animals were weighed 0, 7, 14, and 21 days after challenge.

The immunogenicity of the CPV fraction was evaluated in a controlled challenge of immunity by the virulent CPV exposure of 10 vaccinees and 3 control puppies. The challenged animals were observed daily from 2 days before through 14 days after challenge for clinical signs associated with CPV infection, including lethargy, inappetence, dehydration, vomiting, and fecal consistency. Clinical signs were recorded, and a scoring system was applied to arrive at a quantitative assessment of the response to challenge (Table 1). Rectal temperatures for all animals were recorded from 0 through 14 days after challenge. Daily blood samples for white blood cell counts and fecal samples for virus isolation were collected from all animals from 0 through 14 days after challenge. All animals were weighed 0, 7, and 14 days after challenge.

## Serology

Blood samples for serological evaluation of virus neutralization (VN) antibody titers were collected from each dog before each vaccination, at selected times after vaccination, at the time of challenge exposure, and 21 days after challenge.

Serum neutralizing antibody titers for CDV, CAV1, and CPV were determined by micro-neutralization test procedures. Briefly, serum samples were heat-inactivated at  $56 \pm 2^\circ\text{C}$  for 30 minutes. Assays were performed by combining 0.05 ml of serum in serial twofold dilutions with an equal volume of a virus suspension containing approximately 100 tissue culture infective dose 50 (TCID<sub>50</sub>) of the test virus. Serum-virus mixtures were incubated for 90 minutes at  $36 \pm 2^\circ\text{C}$ , after which they were inoculated into the wells of a microtiter tissue culture plate with monolayers of canine kidney cells for CDV, CAV1, or freshly planted canine kidney cells for CPV. The plates were incubated in a humidified carbon dioxide chamber at  $36 \pm 2^\circ\text{C}$  for 4 to 6 days, fixed in cold acetone, stained with a fluorescein-conjugated specific anti-serum, and observed under an immunofluorescence microscope. Serum neutralization 50% end-point titers were calculated by the method of Reed and Muench.<sup>3</sup>

## Virus Isolation

Isolation of CPV from fecal samples was performed by inoculating fecal filtrates onto a feline kidney cell line. Fecal samples were collected daily from the cage of each challenged dog for 14 days after challenge. A 10% fecal suspension was prepared by mixing fecal material with cell growth medium and adding an equal volume of chloroform. The suspension was mixed by vortexing, clarified by low-speed centrifugation and the aqueous phase collected. Suspension cultures of the feline kidney cells in the wells of a microtiter tissue culture plate were inoculated with 0.1 ml/well of appropriate 10-fold dilutions of the fecal supernatants and the cultures incubated at  $36 \pm 2^\circ\text{C}$  for 4 to 6 days. The presence of CPV was determined by

immunofluorescence staining with fluorescein-conjugated antibody. Results of the isolation were calculated according to the method of Reed and Muench and expressed as TCID<sub>50</sub> per mg of feces.

## Statistical Analysis

PROC MIXED procedures of SAS (SAS Institute) were used for all analyses of variance and covariance. The PROC FREQ procedure was used for all categoric data analyses. Differences were considered significant at  $P < .05$ .

The experimental unit was the individual animal. The primary outcome was prevention of clinical disease. Clinical disease was assessed by fever and clinical signs. These studies tested the null hypothesis that there was no difference in clinical disease in vaccinated animals compared to the non-vaccinated control group. The number of dogs with positive clinical signs was compared between each vaccinated group and the control group by Fisher's Exact test (PROC FREQ).

Fever, body weights, leukopenia, and VN antibody titers were compared between groups by repeated measures analysis of variance (ANOVA-PROC MIXED). The best-fit covariance structure was selected using the Akaike Information Criterion (AIC) fit statistic. The best-fit covariance structure was modeled as compound symmetry. Group comparisons were made using Tukey's test for multiple comparisons.

Evaluation of the reduction in frequency of clinical signs was accomplished by calculating the frequency of positive clinical signs over the observation period after challenge. The number of observations of clinical signs was compared between groups by Wilcoxon rank sum test using PROC NPAR1WAY. Additionally, the fecal CPV titer was assessed. The cumulative amount of virus shed in the feces was calculated. The maximum amount of virus shed on a single day was determined using the MAX function. The cumulative fecal virus titer and the maximum fecal virus titer were compared between each vaccinated group and the non-

vaccinated control group by Wilcoxon Rank Sum test (PROC NPAR1WAY).

## RESULTS

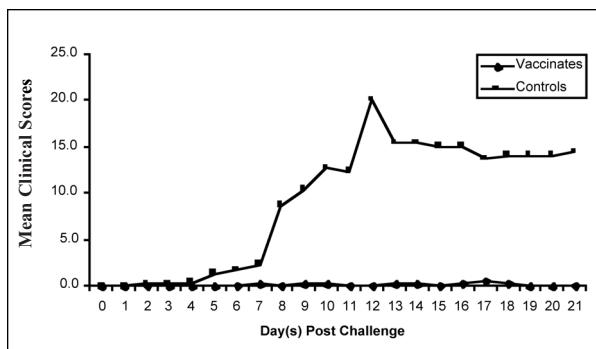
All puppies had VN antibody titers less than 2 for the viral fractions in the test vaccine before the vaccine was administered.

### Canine Distemper Virus Challenge

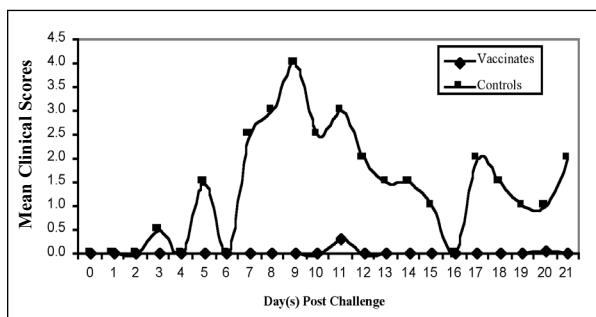
Following CDV challenge, the three control dogs were highly susceptible to CDV infection. One control dog exhibited clinical signs of inappetence and mild to moderate conjunctivitis, and the dog died from acute systemic distemper disease 8 days after challenge. Another control dog exhibited mild to severe clinical signs of distemper illness (mild to moderate conjunctivitis, mucopurulent ocular discharge, depression, mucous and bloody stools, retching, and seizures) and was humanely euthanized 12 days after challenge.

The third control survived the CDV challenge but exhibited clinical signs of distemper illness, including mild to severe conjunctivitis, mucopurulent ocular discharge, inappetence, depression, and bloody stools from 2 through 21 days. In contrast, all 10 vaccines remained healthy and active following the virulent CDV exposure, with no mortality or apparent signs of clinical distemper illness. There was a significant ( $P = .0385$ ) difference in mortality rate between vaccines and controls. Mean clinical scores following virulent CDV challenge are shown in Figure 1. Scores for dogs vaccinated IM and SC have been pooled for this plot.

Mean clinical scores for the control group showed a marked increase beginning 5 days after challenge. The vaccinated puppies exhibited protection against the severe challenge, and mean clinical scores in this group never fluctuated from the baseline



**Figure 1.** Mean clinical scores for dogs challenged with canine distemper virus 3 years after vaccination with a multivalent modified-live virus vaccine.



**Figure 2.** Mean clinical scores for dogs challenged with canine adenovirus 3 years after vaccination with a multivalent modified-live virus vaccine.

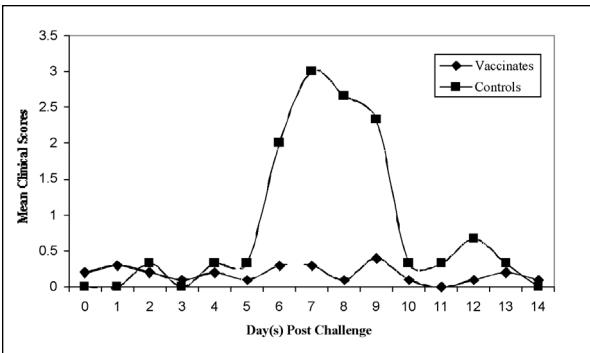
value. Two of the three control dogs exhibited elevated temperatures for 1 to 2 days after challenge, whereas none of the vaccinated dogs had elevated temperatures. The control dog that survived the CDV challenge had a net loss of 0.5 kg in body weight during the 21-day observation period.

Vaccinated dogs had an average weight gain of 1.1 kg during the same period. The difference in weight gain was significant ( $P = .0136$  for IM and  $P = .044$  for SC).

All puppies were seronegative at the time of the first vaccination. At the time of challenge, all vaccines showed a high VN antibody response for CDV (294 for the IM group and 416 for the SC group) (Table 2). All vaccinated dogs remained seropositive throughout the entire 3-year period, and titers were similar for the SC and IM routes.

### Canine Adenovirus Type 2 Challenge

As early as 4 days following CAV1 challenge, the two control puppies developed



**Figure 3.** Mean clinical scores for dogs challenged with canine parvovirus 3 years after vaccination with a multi-valent modified-live virus vaccine.

signs of CAV1. Signs persisted as late as 16 days after challenge. Signs included repeated ocular discharge, severe conjunctivitis, severe nasal discharge, lethargy, inappetence, tonsillitis, and reddening of buccal mucosa. No mortality was observed in the controls, probably because these animals were somewhat resistant to CAV1 infection. These dogs were approximately 3 years of age at the time of challenge. All vaccinated dogs remained clinically normal following the challenge exposure. There was a significant difference in incidence ( $P = .018$ ) and frequency ( $P = .0182$ ) of clinical signs between vaccines and controls. Mean clinical scores in the vaccinated (pooled) and control groups following virulent CAV1 challenge exposure are shown in Figure 2. Mean clinical scores for the control group were markedly increased beginning 3 days after challenge. Vaccinated dogs exhibited protection against the severe challenge, and the mean clinical scores were similar to the baseline value.

Both control dogs had a body temperature reading of 103.8°F or higher on one or more days after challenge, whereas none of the vaccinated dogs showed any increase in body temperature during the 21-day observation period after challenge. Body temperatures were significantly different between vaccines and controls ( $P = .0062$  for IM and  $P = .0155$  for SC).

Control dogs showed significant body weight loss (mean = 3.1 kg) between Days 0 and 21 after challenge. Both vaccine groups gained an average of 0.6 kg. Weight gain was significantly higher in vaccines ( $P = .0112$  for IM and  $P = .0021$  for SC) than in controls.

All puppies were seronegative at the time of first vaccination. All vaccines developed CAV1 VN antibody titers by the time of second vaccination and all remained seropositive throughout the entire 3-year period after the second vaccination. The mean VN titer was 138 in IM vaccinated puppies and 126 in SC vaccinated puppies at the time of challenge. Twenty-one days after challenge, the VN titer was 2048 in the IM group and 2630 in the SC vaccinated group (Table 2).

#### Canine Parvovirus Challenge

Following challenge, all three control dogs were weak and exhibited various clinical signs of CPV infection (anorexia, lethargy, depression, dehydration, vomiting, and watery or bloody stools). These clinical signs in the controls were more severe from 2 to 10 days after challenge. However, all vaccines appeared healthy and active. Only limited transient mild clinical signs of anorexia and dehydration were noted in seven of the 10 vaccines. Two vaccines vomited one time each during the 14 days after challenge. Mean clinical scores for the control group showed a marked increase beginning 5 days after challenge, whereas vaccines exhibited only minor fluctuations in the mean clinical scores from the baseline value (Figure 3). Values for dogs vaccinated IM and SC have been pooled for this plot. Two control dogs exhibited leukopenia for 1 to 3 days following challenge, with values less than 50% of normal values. Leukocyte values for the vaccines were normal.

Control dogs lost an average of 0.4 kg of weight, whereas vaccines had an average weight gain of 1.0 kg during the 14 day

**Table 2.** Serological Responses of Laboratory Dogs to Vaccination with a Multivalent Modified-Live Virus Vaccine

Vaccine Component	Vaccinate Group	Vaccination		Days After Challenge*		
		1	2	0	14	21
Canine Distemper	IM	Neg	3821	294	ND	2896
	SC	Neg	4385	416	ND	1783
	Control	Neg	Neg	Neg	ND	2896 <sup>†</sup>
Canine Adenovirus	IM	Neg	11	138	ND	2048
	SC	Neg	5	126	ND	2630
	Control	Neg	Neg	Neg	ND	3332
Canine Parvovirus	IM	Neg	45260	8481	8477	ND
	SC	Neg	59064	15826	18574	ND
	Control	Neg	Neg	Neg	12264	ND

\*Dogs were challenged with virus in isolation rooms 3 years after vaccine was administered.

<sup>†</sup>Value for one dog; two controls died before this evaluation.

ND = Not done.

observation period after challenge. Tests for recovery of CPV challenge virus revealed moderate to high CPV titers in the feces of all control dogs 4 to 9 days after challenge. No virus shedding was detected in any of the challenged vaccines, except for one dog in the SC vaccinated group that shed virus for 1 day. There were significant differences between vaccines and controls for the cumulative amount of virus shedding ( $P = .0357$  for IM and  $P = .0179$  for SC) and the maximum amount of virus shed ( $P = .0179$  for both IM and SC).

All vaccines had long-lasting VN antibody titers detectable at the various time points following the second vaccination. The mean VN titer was 8481 in the IM vaccinated puppies and 15826 in the SC vaccinated puppies at the time challenge, and 8477 and 18574 in the IM and SC vaccinated group, respectively, at 14 days after challenge (Table 2).

## DISCUSSION

More than 4 years ago, Fort Dodge Animal Health initiated a long-term vaccination challenge study to determine whether the three main canine antigens (CDV, CAV2, and CPV) in a specially formulated vaccine would provide 3 years of immunity in vaccinated dogs. These studies were conducted under strictly controlled laboratory condi-

tions to ensure the best possible evaluation of the product. The 40 dogs in the present study were isolated during the study to eliminate any possible accidental environmental viral exposure.

The results of this 3-year duration of immunity study demonstrated that the CDV fraction in the vaccine was efficacious against virulent CDV challenge. All 10 vaccines remained healthy and active following the virulent CDV exposure, with no mortality or apparent signs of clinical distemper illness. Two of the 3 control puppies died or became moribund during the 21-day observation period. The morbidity and mortality rates in controls affirmed that the experimental CDV challenge was a severe test of the vaccine's immunizing properties.

The CAV2 antigen in the vaccine was protective against CAV1 disease for 3 years. All vaccines remained clinically healthy following the challenge exposure, whereas control dogs had high fever and multiple clinical signs associated with CAV1 infection.

The vaccine provided significant protection against CPV infection following virulent challenge exposure that induced typical clinical signs of parvovirus enteritis in controls. There were significant differences following virulent CPV challenge between vaccinated and control groups for fecal

virus shedding, weight loss/gain, and post-challenge antibody responses. Most of the control dogs had mucous/bloody feces, with significant amounts of CPV shedding during the 14-day period after challenge.

The persistence of immunity conferred by this vaccine was demonstrated by the continued detection of antibody, which was confirmed by the challenge for each antigen fraction in the multivalent product. Serological responses in vaccinated animals have been shown to correlate reasonably well with protective immunity for CDV, CAV2, and CPV, but challenge studies are considered the most reliable measure of the true immunogenicity of a vaccine in dogs.<sup>4</sup>

The present study clearly confirms the 3-year duration of immunity for CDV, CAV2, and CPV fractions in a commercial multivalent modified-live virus vaccine. There was no difference between the IM and SC routes of administration in the level of protection for each component of the vaccine when dogs were challenged 3 years after vaccination. The USDA has approved reference to these data on the vaccine label and its inclusion on the package insert for

this vaccine (Duramune Adult). The efficacy of other vaccines developed in the future for protection over a 3-year period will depend on viral strains, attenuation levels, and product formulations that may affect the outcome of the duration of immunity elicited by each specific product.

These data indicate that this product is particularly well suited as a booster vaccination for adult dogs when following an extended vaccination interval.

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