

# Effect of Recombinant Canine Distemper Vaccine on Antibody Titers in Previously Vaccinated Dogs\*

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## CLINICAL RELEVANCE

Two canine distemper virus (CDV) vaccine types are currently commercially available: modified-live virus (MLV) vaccines and a canarypox recombinant CDV (rCDV) vaccine (Recombitek, Merial). This study compared the ability of the rCDV vaccine and MLV vaccines to significantly enhance (boost) the antibody response of previously immunized adult and juvenile dogs. A significant (four-fold or greater) increase in titer occurred in significantly more dogs revaccinated with Recombitek C-4 or Recombitek C-6 than with the MLV-CDV vaccines. This study demonstrates that Recombitek, the only vaccine for dogs containing rCDV, is more likely to significantly boost the CDV antibody response in previously vaccinated dogs than are the MLV-CDV vaccines. Because rCDV vaccine can boost the antibody titer of dogs previously vaccinated with an MLV vaccine, it can and should be used when core vaccines are readministered.

## INTRODUCTION

Canine distemper occurs worldwide and affects all members of the Canidae family as well as other animals, such as ferrets, mink, pandas, raccoons, and large cats.<sup>1,2</sup> A single-stranded, negative-sense RNA virus, canine distemper virus

(CDV) belongs to the genus *Morbillivirus* in the Paramyxoviridae family and is closely related to the human measles virus and bovine rinderpest virus.<sup>3</sup> Disease caused by CDV is found primarily in young unvaccinated or vaccinated pups. Vaccinated pups sometimes fail to develop an immune response because of interference of active immunity by passively acquired maternal CDV antibody. Disease is also seen in unvaccinated adult animals. CDV infection in unprotected animals results in significant morbidity

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and mortality due to respiratory, gastrointestinal, and neurologic abnormalities. CDV-related morbidity in unvaccinated animals ranges from 25% to 75%, and the fatality:case ratio can be as high as 90%.<sup>4</sup>

Transmission of CDV occurs primarily by aerosolization of droplets from body secretions of infected animals. Acutely infected dogs start shedding virus about 7 days after exposure; virus is shed in all body secretions regardless of their clinical appearance. Only one CDV serotype exists; however, there are many biologically divergent CDV strains (biotypes) that cause differential clinical signs after a postinfection incubation period of approximately 2 to 4 weeks.<sup>3,5</sup>

Infected pups may be febrile and leuko-

genic proteins of CDV (HA and F genes) necessary to elicit a protective immune response against CDV in dogs.<sup>7</sup> Thus, it is not possible for the vaccine to produce CDV disease in vaccinated animals, which is possible with the MLV-CDV vaccines in certain dogs and certain species.<sup>3</sup> Studies have demonstrated that previous vaccination with one recombinant form of canarypox does not interfere with development of an immune response to a different recombinant vaccine using the same canarypox vector.<sup>8</sup> Canarypox-vectored rCDV vaccine was proven safe and effective in stimulating immunity.<sup>7</sup>

The 2003 American Animal Hospital Association (AAHA) Canine Vaccine Guidelines stated that all puppies should be vaccinated

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***This study assessed the efficacy of rCDV vaccine in stimulating an anamnestic antibody response in dogs previously immunized with MLV vaccine.***

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penic. Clinical signs of CDV include conjunctivitis, rhinitis, coughing, diarrhea, vomiting, anorexia, dehydration, weight loss, and often encephalitis. Secondary bacterial infections may occur as a result of CDV's ability to cause severe immunosuppression.<sup>6</sup>

Modified-live virus (MLV) vaccines to control distemper have been available since the 1950s, and a canarypox-vectored recombinant CDV (rCDV) vaccine (Recombitek C-4 or C-6, Merial) was approved for use in April 1997. Research was initiated in our laboratory in 2003 to determine how the rCDV vaccine compares to currently available MLV vaccines. The canarypox-vectored rCDV vaccine contains only CDV genes for the hemagglutinin (HA) and fusion proteins (F) and neither the complete CDV genome nor infectious CDV. The canarypox viral vector expresses the anti-

with a CDV vaccine.<sup>9</sup> At the time the guidelines were written, data on rCDV vaccine were incomplete. Therefore, rCDV vaccine was differentiated from the other major distemper vaccines. The purpose of this study was to assess the efficacy of rCDV vaccine in stimulating an anamnestic (booster) antibody response to CDV in dogs previously actively immunized with MLV vaccine.

■ **MATERIALS AND METHODS**

Institutional Animal Care and Use Committee approval was obtained before conducting this study.

Trial 1 used 100 beagles (male and female; various ages) separated into five groups of 20 dogs each. Trial 2 used 135 beagles (male and female; all approximately 2 years of age) separated into nine groups of 15 dogs each. Physi-

**TABLE 1. Vaccination Types for Each Dog Group: Trial 1**

<i>Group</i>	<i>No. of Dogs</i>	<i>Vaccination Type</i>	<i>Vaccine</i>
A	20	rCDV	Recombitek C-4 (Merial)
B	20	MLV-CDV	Galaxy DA2PPv (Schering-Plough Animal Health)
C	20	MLV-CDV	Duramune Max 5 (Fort Dodge Animal Health)
D	20	MLV-CDV	Vanguard 5 (Pfizer Animal Health)
E	20	MLV-CDV	Progard-5 (Intervet)

**TABLE 2. Vaccination Types for Each Dog Group: Trial 2**

<i>Group</i>	<i>No. of Dogs</i>	<i>Vaccination Type</i>	<i>Vaccine</i>
A	15	rCDV	Recombitek C-6 (Merial)
B	15	rCDV	Recombitek C-4 (Merial)
C	15	MLV-CDV	Galaxy DA2PPv (Schering-Plough Animal Health)
D	15	MLV-CDV	Galaxy DA2PPvL (Schering-Plough Animal Health)
E	15	MLV-CDV	Progard-5 (Intervet)
F	15	MLV-CDV	Duramune Max 5 (Fort Dodge Animal Health)
G	15	MLV-CDV	Duramune Max 5/4L (Fort Dodge Animal Health)
H	15	MLV-CDV	Vanguard 5 (Pfizer Animal Health)
I	15	MLV-CDV	Vanguard 5/L (Pfizer Animal Health)

cal examinations revealed no abnormalities. The dogs were housed in individual pens, with some pens having two dogs. Each dog had been previously immunized with two doses of Duramune Max 5/4L (Fort Dodge Animal Health), an MLV-CDV vaccine, at 13 and then at 15 to 17 weeks of age and were revaccinated approximately 1 year before initiation of the present study.

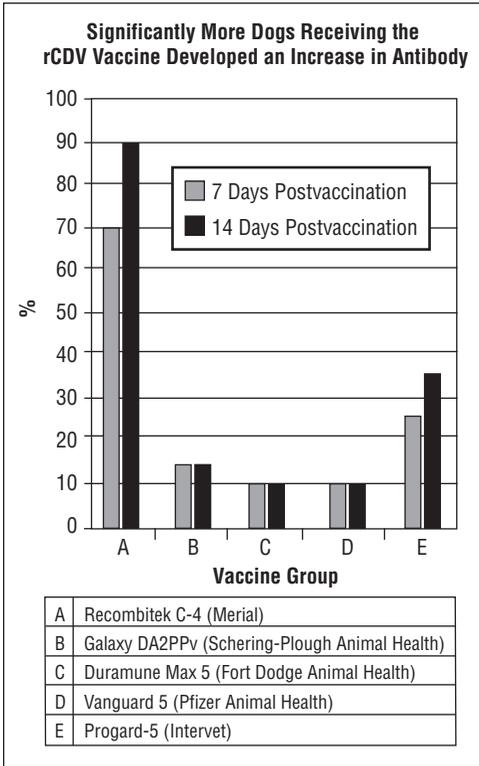
In Trial 1, blood was collected and dogs were vaccinated SC with one of five vaccines (Table 1); blood was collected again 7 and 14 days after vaccination to determine antibody titer to CDV. In Trial 2, blood was collected and dogs were vaccinated SC with one of the vaccines listed in Table 2; blood was collected again 7 days after vaccination to determine CDV antibody titers. All serum samples were batched by trial and tested for antibody using the viral

neutralization test described previously.<sup>10</sup>

Statistical analysis to compare MLV vaccinates with recombinant vaccinated groups of dogs was performed by entering data from all dogs in the "Statcalc" Module of EpiInfo (v.3.3.2, Centers for Disease Control and Prevention, Atlanta, GA) for calculation of chi-square comparison of the two proportions. Chi-square (Yates corrected) value obtained was 57.17, giving a *P* value < .00000001.

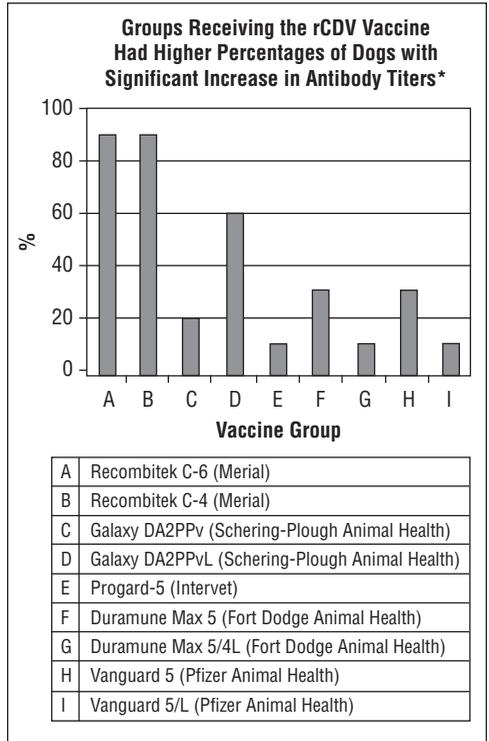
## ■ RESULTS

Figure 1 (Trial 1) shows the percentage in each group of 20 dogs that had a significant increase in CDV antibody titer 7 and 14 days after vaccination with the five vaccines. A significant increase in titer was defined as a fourfold or greater increase in antibody titer detected within 7 to 14 days after vaccination. Thus, if the initial titer



**Figure 1.** Percentage of dogs with significant antibody response to CDV 7 and 14 days after revaccination (Trial 1).

was 128, the postvaccination titer must be 512 or greater to be considered a significant increase. The study was designed to test the hypothesis that rCDV vaccine boosts the antibody titer of dogs previously vaccinated with MLV-CDV vaccine. Showing the percentage of dogs that had a boost is important when one wants to compare the percentage of dogs receiving different vaccines that showed a significant boost in titer, which is universally defined as a fourfold or greater increase in antibody titer when the viral neutralization serologic test is used to measure antibody titer. As can be seen in Figure 1, significantly more dogs in Trial 1 Group A (i.e., dogs that received recombinant canarypox-vectored



**Figure 2.** Percentage of juvenile dogs with significant antibody response to CDV 7 days after revaccination (Trial 2). \*Except for Group D, which contained a high percentage of pups with lower initial titers.

CDV vaccine) developed an increase in antibody after vaccination than did dogs in Groups B through D, all of which received combination vaccines containing MLV-CDV.

The study was then repeated in a second group of 135 juvenile dogs with nine vaccines (Trial 2). In the first study (Trial 1), it was noted that the titers were similar between 7 and 14 days postvaccination; therefore, blood samples were collected only on day 7 after vaccination in Trial 2. In Trial 2, groups receiving combination vaccines containing rCDV had higher percentages of dogs with significant increases in antibody titers than the MLV-vaccinated groups, with the exception of Group D. Group

D was found to have a lower initial mean antibody titer and more individual dogs with lower titers than the other groups of dogs (Figure 2).

## ■ DISCUSSION

The 2003 AAHA Canine Vaccine Guidelines state that all dogs should be vaccinated with a CDV vaccine.<sup>9</sup> At the time the guidelines were written, data on rCDV vaccine were incomplete, resulting in differentiation of rCDV vaccine from the other MLV major distemper vaccines. MLV-CDV vaccines had been an industry standard until the advent of recombinant technology and, specifically, canarypox-vectored rCDV vaccine (Recombitek

vaccinated groups ( $P < .00000001$ ). The MLV products stimulated a significant increase in CDV antibody in approximately 10% to 30% of the group. One exception was found in Trial 2, in which one combination vaccine containing MLV-CDV stimulated an increased response in 60% of the group; however, it was found that Group D had more dogs with low CDV titers than any of the other groups. It is important to understand that the lower the antibody titer is at time of revaccination, the more likely it is that an MLV vaccine will stimulate a significant increase, regardless of the type of MLV vaccine; this is because the lower the titer is, the more likely the MLV vaccine

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*This study demonstrates that Recombitek rCDV vaccine was more likely than the current MLV-CDV vaccines to boost an antibody response in dogs previously vaccinated with an MLV vaccine.*

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C-4, C-6). It was unknown whether dogs previously immunized with an MLV-CDV vaccine could successfully generate an anamnestic response when administered a subsequent rCDV vaccine. The purpose of this study was to assess the efficacy of rCDV vaccine in stimulating an anamnestic response to CDV in dogs actively immunized with conventional MLV-CDV vaccines; in addition, the response from rCDV was compared with the booster response seen with all MLV-CDV vaccines.

Approximately 90% of the dogs vaccinated with a combination vaccine containing rCDV demonstrated a significant increase in CDV antibody response after vaccination. The rCDV vaccinated group had significantly greater numbers of dogs that showed a significant increase in their titers (fourfold or greater) after the booster vaccination than did the MLV

virus will replicate to form more viral antigens. This newly formed antigen is required to obtain enough antigen to boost the antibody response. The canarypox-vectored rCDV vaccine is not affected in the presence of antibody to CDV, and thus the canarypox enters the receptive host cells and expresses the HA and F gene products, resulting in an anamnestic response.

This study demonstrates that Recombitek rCDV vaccine was more likely than the current MLV-CDV vaccines to boost an antibody response in dogs previously vaccinated with an MLV vaccine. In an unpublished study with a small number of dogs, it was also found that rCDV was more likely to increase CDV titers in dogs previously vaccinated with rCDV vaccine than were the MLV-CDV vaccines.<sup>11</sup> This would be expected since antibody to canarypox virus does not inhibit infection with canarypox virus.

## ■ CONCLUSION

This study demonstrates for the first time that rCDV vaccine boosts the immune response in already actively immunized dogs more often than conventional MLV vaccines. This should not be a surprise since MLV vaccines must infect and replicate to cause an increase in titer and the existing antibody to CDV often prevents infection, whereas canarypox infection and expression of CDV antigens are not affected by CDV antibody.

This study does not show or suggest that the dogs did or did not have a protective immune response before and/or after revaccination. In fact, all the dogs in the present study would be expected to be immune from challenge with virulent virus.

## ■ ACKNOWLEDGMENTS

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## ■ REFERENCES

1. Appel MJ, Yates RA, Foley GL, et al: Canine distemper epizootic in lions, tigers, and leopards in North America. *J Vet Diagn Invest* 6(3):277–88, 1994.
2. Harder TC, Kenter M, Vos H, et al: Canine distemper virus from diseased large felids: Biological properties and phylogenetic relationships. *J Gen Virol* 77:397–405, 1996.
3. Appel MJ: Canine distemper, in Barlough J (ed): *Manual of Small Animal Infectious Diseases*. New York, Churchill and Livingstone, 1987, pp 49–60.
4. Swango LJ: Canine viral diseases, in Ettinger SJ (ed): *Textbook of Veterinary Internal Medicine*, ed 3. Philadelphia, WB Saunders, 1989, pp 298–311.
5. Appel MJ: Pathogenesis of canine distemper. *Am J Vet Res* 30:1167, 1969.
6. Krawkoka S: Mechanisms of in vitro immunosuppression in canine distemper virus infection. *J Clin Lab Immunol* 8:187–196, 1982.
7. Pardo MC, Bauman JE, Mackowiak M: Protection of dogs against canine distemper by vaccination with a canarypox virus recombinant expressing canine distemper virus fusion and hemagglutinin glycoproteins. *Am J Vet Res* 58(8):833–836, 1997.
8. Taylor J, Meignier B, Tartaglia J, et al: Biological and immunogenic properties of a canarypox-rabies recombinant, ALVAC-RG (vCP65) in non-avian species. *Vaccine* 13(6):539–549, 1995.
9. Paul MA, Appel M, Barrett R, et al: Report of the American Animal Hospital Association (AAHA) Canine Vaccine Task Force: 2003 canine vaccine guidelines, recommendations, and supporting literature. *JAAHA* 39:119–131, 2003.
10. Appel M, Robson DS: A microneutralization test for canine distemper virus. *Am J Vet Res* 34:1459–1463, 1973.
11. Larson LJ, Hageny TL, Schultz RD: Unpublished data, University of Wisconsin–Madison, 2004.