Three-Year Serologic Immunity against Canine Parvovirus Type 2 and Canine Adenovirus Type 2 in Dogs Vaccinated with a Canine Combination Vaccine*

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CLINICAL RELEVANCE

A group of client-owned dogs and a group of dogs at a commercial kennel were evaluated for duration of antibody responses against canine parvovirus type 2 (CPV-2) and canine adenovirus type 1 (CAV-1) after receiving a combination vaccine containing recombinant canarypox-vectored canine distemper virus (CDV) and modified-live CPV-2, CAV-2, and canine parainfluenza virus, with (C6) or without (C4) two serovars of *Leptospira* (Recombitek C4 or C6, Merial). Duration of antibody, which correlates with protective immunity, was found to be at least 36 months in both groups. Recombitek combination vaccines can confidently be given every 3 years with assurance of protection in immunocompetent dogs against CPV-2 and CAV-1 as well as CDV. This allows this combination vaccine, like other, similar modified-live virus combination products containing CDV, CAV-2, and CPV-2, to be administered in accordance with the recommendations of the American Animal Hospital Association Canine Vaccine Task Force.

INTRODUCTION

Canine parvovirus type 2 (CPV-2) causes a highly contagious enteric disease that often results in severe morbidity and high mortality in unvaccinated dogs worldwide.[1–3] All naïve dogs (defined as CPV-2–antibody negative) are susceptible to infection with CPV-2. Dogs younger than 1 year have the highest risk of developing severe disease, leading to mortality in 50% or more of these young animals. Naïve dogs older than 1 year are highly susceptible to infection and will shed CPV-2 in feces, but they often develop inapparent or mild clinical disease with low mortality. However, these dogs pose a significant threat to susceptible puppies because the CPV-2 shed is virulent for

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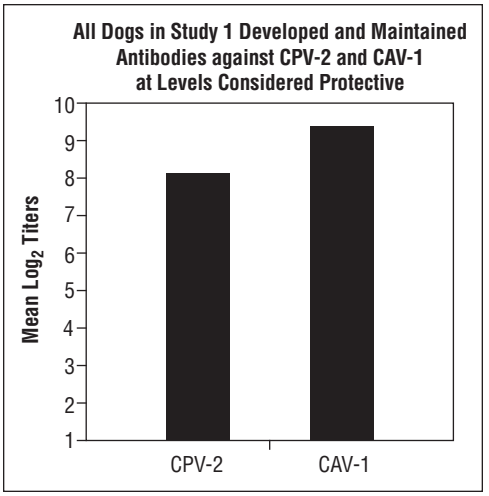

Figure 1. *Mean log_2 CPV-2 and CAV-1 antibody titers in 51 beagle dogs that received Recombitek C4 or C6. Note that the data reflect combined findings from all three subgroups. No differences were detected between the subgroups. Serum samples were collected more than 33 months after the last vaccination with Recombitek and more than 36 months after the primary vaccination series.*

young pups and may cause high morbidity and mortality. For these reasons, the American Animal Hospital Association (AAHA) Canine Vaccine Task Force strongly recommends that *all* puppies be vaccinated one or more times with a combination product containing antigens considered essential for all dogs—the "core" vaccines CPV-2, canine distemper virus (CDV), and canine adenovirus type 2 (CAV-2)—ensuring that the final dose is given at 14 to 16 weeks of age or older.[4] Rabies is also a core viral antigen, but it is not part of the combination vaccine. A monovalent product is administered to pups at approximately 12 weeks and again at 1 year of age.

Serologic immunity to CAV-1 virus is conferred by vaccination with a CAV-2 vaccine. CAV-1 is the cause of infectious canine hepatitis (ICH), which can be fatal in up to 20% of susceptible dogs. In the United States, ICH is rarely recognized as a clinical entity because almost all dogs in this country are immunized (through infection and/or vaccination) with the more common respiratory form CAV-2, which is antigenically similar to CAV-1.[5–8] Virulent CAV-2 is capable of causing pneumonia and is frequently associated with canine respiratory disease complex, also referred to as "kennel cough." When canine respiratory disease complex occurs, it is almost always in association with multiple viruses, bacteria, mycoplasmas, environmental problems (such as poor ventilation), and stress.[8–11] Although ICH caused by CAV-1 is uncommon in the United States, it might become more common if dogs are not vaccinated with CAV-2 because many dogs in Mexico and Central and South America as well as parts of Europe are infected with CAV-1.[12] CAV-1 is also present in wildlife species (e.g., foxes, wolves) in North America. Some of these species are highly susceptible to infection with the virus, and although they may not develop disease, they can be carriers.[13–15]

Vaccination with the core antigens (CDV, CPV-2, CAV-2, and rabies virus) is a significant component of a comprehensive canine health program and is the single most important method to protect dogs from these viruses. Although annual revaccination against these viruses has been common during the past 25 years, many studies in our laboratory and by others have shown that the duration of immunity (DOI) for modified-live virus (MLV) vaccines from the major biologic manufacturers is many years and most dogs are likely to have lifetime immunity after vaccination with CDV, CPV-2, and CAV-2 antigens.[16–20] Because differences can exist among the various commercial vaccines or combinations available for dogs, specific studies have been and continue to be conducted to demonstrate a minimum DOI of 3 years for each product.[21]

We recently published a study showing that

the recombinant CDV (rCDV) component that is part of these canine combination vaccines (Recombitek C4 and C6, Merial) has a DOI of at least 3 years.[22] Recombitek C4 contains a lyophilized suspension of a recombinant canarypox vector expressing the HA and F glycoproteins of CDV and conventional modified-live CAV-2, canine parainfluenza virus, and CPV-2 vaccines. Recombitek C6 contains the viral components of C4 (above) with a liquid suspension of killed *Leptospira canicola* and *Leptospira icterohaemorrhagiae*. The goal of the present study was to demonstrate whether the CPV-2 and CAV-2 components of the Recombitek C4 and C6 vaccines also provide a minimum DOI of 3 years, similar to the rCDV, so that Recombitek products containing CPV-2, CAV-2, and rCDV can be used as recommended by the AAHA Vaccine Task Force.

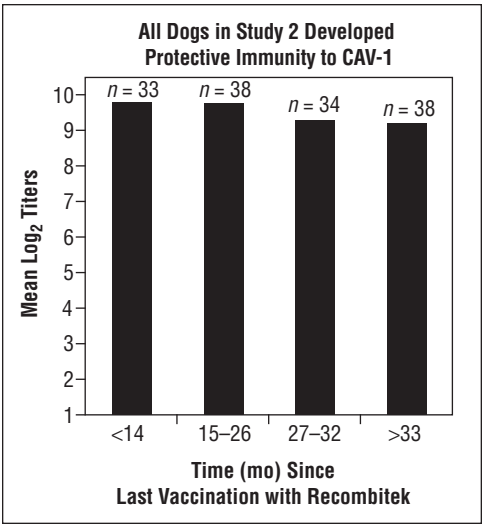

Figure 2. *Mean log_2 CAV-1 antibody titers in 143 client-owned dogs that received Recombitek C4 or C6.*

MATERIAL AND METHODS

Two separate and distinct serologic studies were performed. Institutional Animal Care and Use Committee approval was obtained before conducting Study 1, which included 51 beagle pups housed in a CPV-2 disease–free environment. CAV-2 virus is known to be present in the environment. Pups from multiple litters were randomly separated into three groups, designated A, B, and C. All groups of pups in Study 1 were vaccinated with two doses of Recombitek C4 approximately 4 weeks apart according to the manufacturer's label recommendations. All pups were 12 to 13 weeks of age at the first vaccination and 15 to 17 weeks at the second vaccination. Group A was subsequently revaccinated at 1 year of age, as recommended by the AAHA Vaccine Task Force; Group B was revaccinated at 6 months of age; and Group C was not revaccinated. Sera were collected from all dogs between 36 and 48 months after receiving their final vaccination and were assayed for the presence of antibody to CPV-2 that inhibited viral hemagglutination of porcine erythrocytes and virus-neutralizing CAV-1 antibody as previously described.[23,24]

Study 2 included 327 client-owned dogs of various breeds seen for routine care at veterinary clinics throughout the United States; all dogs had previously been vaccinated with Recombitek C4 and/or C6. The clinics selected to participate in Study 2 used Merial vaccines exclusively. Vaccination intervals for dogs included in Study 2 ranged from 10 to 48 months as determined through examination of medical records. All sera collected from these dogs were tested for CPV-2 antibodies as described above. A subset of 143 serum samples was randomly selected from each vaccination interval group (i.e., time since last vaccination: less than 14 months, 15 to 26 months, 27 to 32 months, and more than 33 months) and assayed for antibody against CAV-1 as described above. For both Study 1 and Study 2, serologic assays were performed in our laboratory. Serology technicians were blinded to study details and assayed the sera as part of ongoing routine serologic testing.

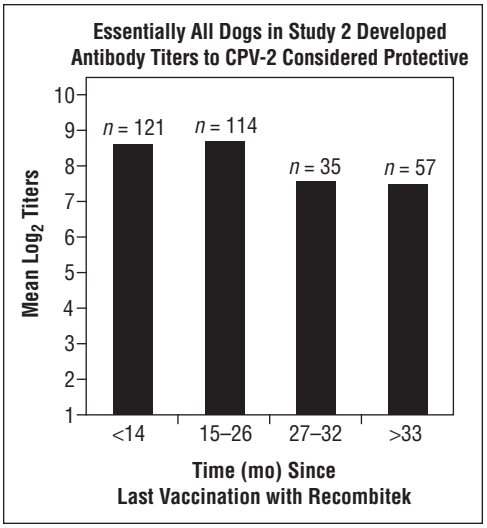

Figure 3. *Mean log_2 CPV-2 antibody titers in 327 client-owned dogs that received Recombitek C4 or C6.*

RESULTS

Mean log_2 antibody titers to CAV-1 and CPV-2 for beagle pups (Study 1) are shown in Figure 1. Mean log_2 antibody titers to CAV-1 and CPV-2 for client-owned dogs (Study 2) are shown in Figures 2 and 3.

Regardless of subgroup, dogs in Study 1 developed and maintained antibodies against CPV-2 and CAV-1 at levels considered protective for the duration of the study. All dogs assayed in Study 2 developed protective immunity to CAV-1. Essentially all dogs developed antibody to CPV-2 that was considered protective; however, a few dogs in each of the vaccination interval groups did not develop antibody to CPV-2.

DISCUSSION

The results demonstrated that dogs vaccinated with Recombitek C4 and/or C6 had serologic responses to CPV-2 and CAV-1 for up to 42 months in the Study 1 beagle dogs maintained in a colony and for up to 48 months in the Study 2 client-owned pet dogs. Multiple studies using other commercially available MLV CDV, CPV-2, and CAV-2 vaccines have demonstrated similar findings.[17–20] A difference was noted with regard to CPV-2 antibody between Study 1 and Study 2: All dogs in Study 1 developed antibody to CPV-2, whereas a few dogs in each of the subgroups in Study 2 were CPV-2 antibody negative. It must be assumed that these dogs either had maternally derived antibody that blocked the response to CPV-2, were nonresponders incapable of developing an antibody response to CPV-2, or were improperly vaccinated and thus did not achieve adequate circulating antibody titers because of a lack of immune stimulation (i.e., for dogs in age groups that should have been revaccinated at an age when maternally derived antibody was absent [e.g., at least 16 weeks]). It is also important to note that Study 1 animals were a homogenous population of beagle dogs while Study 2 dogs were not.

In one of our previous studies, which did not investigate Recombitek, when dogs at our veterinary medical teaching hospital received a variety of MLV vaccines, we also found dogs with no or low antibody to CPV-2 and CDV but none that lacked antibody to CAV-1.[25] More than 500 dogs were tested in that study, and approximately 10% of them failed to develop antibody to CPV-2 or had exceptionally low titers. When those dogs were revaccinated, the titers either did not increase (i.e., remained low or negative) or increased but returned to very low levels within 6 months. Also in that study, about 5% of dogs were noted to have low or no antibody to CDV. Low- or nonresponders were present in all groups of dogs, regardless of time since last vaccination, similar to findings in the present study. In both the study described here and our earlier study, very few dogs had exceptionally low antibody for CAV-1 and none was antibody

negative for CAV-1. Based on our extensive experience with vaccines and responses to vaccination, we estimate that approximately 0.1% to 0.2% of dogs are nonresponders (i.e., genetically incapable of responding) to CPV-2 vaccines and about 0.05% to 0.075% are unable to respond to CDV vaccines. We have never found an animal unable to respond to CAV-2 vaccine but presume it is possible that some may exist; thus, we estimate that less than 0.001% to 0.002% may be nonresponders to CAV-2 vaccines.

In contrast, low-responders are much more common. As reported in our previous study,[25] 10% of the dogs had very low responses to CPV-2, 5% had very low responses to CDV, and an estimated less than 0.5% to 1% had very low responses to CAV-1. Many researchers believe that the levels of antibody to CDV, CPV-2, and CAV-1 are important, and it has been reported that certain minimal antibody titers to these viruses are required for protective immunity.[26,27] Our evidence, gathered from challenge studies[16–18,21,22] in more than 1,000 dogs over the past 5 years, indicates that a dog with any detectable level of active antibody as a result of vaccination will be protected from the development of clinical disease when challenged. Some dogs may become transiently infected as demonstrated by a significant increase in their antibody levels; however, when such dogs are tested, they are not shedding virus and no significant clinical signs are seen.

This study, as with previous studies in our laboratory and by others, shows that the core antigens as present in Recombitek, including CDV, CAV-2, and CPV-2, when given as recommended in the AAHA Guidelines (i.e., beginning no earlier than 5 to 6 weeks of age, the last dose in the puppy series administered at 14 to 16 weeks, revaccination a year later or at 1 year of age, and then revaccination no more frequently than 3-year intervals), will provide excellent immunity in dogs that are immunologically competent to respond to these vaccines.[17–20]

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