The study reported here investigated the efficacy of two commonly used modified-live virus vaccines to induce seroconversion against canine parvovirus (CPV) in 213 Rottweiler and Doberman pinscher pups with various titers of maternally derived CPV antibody. Beginning at 6 to 8 weeks of age, pups were given a subcutaneous vaccination every 21 days (range, 18–24 days) in the dorsal region of the neck or shoulder area. Pups vaccinated with vaccine A received three vaccinations and completed the vaccination series by 12 to 14 weeks of age. Pups vaccinated with vaccine B received four vaccinations and completed the vaccination series by 15 to 17 weeks of age. Antibody titers against CPV in both vaccine groups were similar before vaccination. Pups in the vaccine-A group seroconverted significantly earlier than those in the vaccine-B group. After the first vaccination, more pups with a CPV-2b hemagglutination inhibition (HI) titer of ≤1:80 responded to vaccine A than to vaccine B. In addition, CPV-2b HI titers after vaccination were also significantly (P ≤ 0.05) higher for the pups in the vaccine-A group after first, second, and third vaccinations, compared with those of pups in the vaccine-B group.

INTRODUCTION

Canine parvovirus (CPV)-2 infection in dogs was first reported in 1978\textsuperscript{1,2} and is caused by a single-stranded DNA virus of the parvovirus family (Paroviridae). As determined by DNA analysis, the virus mutated to CPV-2a in approximately 1979 and mutated to CPV-2b in approximately 1984.\textsuperscript{3} Despite development of many different vaccines, CPV is still an important cause of morbidity and mortality in young pups in many countries.\textsuperscript{4} Neonatal pups are protected from CPV infection mainly by passive transfer of maternal antibodies. However, as their maternal antibodies decline below a protective level, they become susceptible to infection, and if not adequately immunized, they become infected in the first year of life. Following infection with CPV, the severity of clinical signs depends on the pup’s age, stress level,
breed, and immune status, with the most severe cases occurring in pups 6 weeks to 6 months of age. Certain breeds, including Rottweilers, Doberman pinschers, Labrador retrievers, Springer spaniels, Yorkshire terriers, American pit bull terriers, and German shepherd dogs, have been identified as having an increased risk for developing parvovirus enteritis. 6–8

It has also been determined that maternally derived antibody titers that are too low to provide protection from naturally acquired infection are high enough to prevent immunization with low-titer modified-live CPV vaccines.9,10 Thus with the low-titer CPV vaccines, pups are susceptible to infection for as long as 10 weeks while passive (maternally derived) immunity wanes and before active immunity can be induced.11

Development of new generation, high-titer, low-passage CPV vaccines has, to some extent, overcome problems associated with maternal antibody interference.12,13 However, differences in vaccine efficacy still exist. The purpose of the study reported here was to determine the efficacy of two commonly used, modified-live virus vaccines to induce seroconversion against CPV in Rottweiler and Doberman pinscher pups with various titers of maternally derived CPV antibody.

MATERIALS AND METHODS

Eight private small animal veterinary practices located throughout the United States participated in the study. Privately owned, healthy, purebred Doberman pinscher and Rottweiler pups aged 6 to 8 weeks were eligible for entry into the study. Pups that were ill, debilitated, previously vaccinated against CPV, or treated within 14 days of entry with corticosteroid anti-inflammatory drugs, or pups that had a history of CPV infection were not eligible for inclusion. Owners were informed of the nature of the study and signed an informed consent form prior to their pups’ inclusion in the study.

Prior to entry all pups received a thorough physical examination, including fecal flotation for gastrointestinal parasites. Upon entry each pup was assigned an identification number and allocated to a treatment group by use of a random number technique. For each veterinary clinic, equal numbers of pups were allocated to each treatment group.

Beginning at 6 to 8 weeks of age, pups were given a subcutaneous (SQ) vaccination every 21 days (range: 18–24 days) in the dorsal region of the neck or shoulder area. Pups vaccinated with vaccine A received three vaccinations and completed the vaccination series by 12 to 14 weeks of age. Pups vaccinated with vaccine B received four vaccinations and completed the vaccination series by 15 to 17 weeks of age. Pups also were vaccinated against rabies, according to state or federal government regulations, at the end of the study (15–17 weeks of age). Pups were not vaccinated against canine coronavirus, Bordetella bronchiseptica, or Borrelia burgdorferi during the study. Vaccine A contained a CPV-2 fraction at high titer (>10^7.0 TCID_{50}/dose) and was attenuated by low passage (35 passes from the canine isolate with a maximum of two additional passes allowed for production) on a canine cell line. Information was not available on the passage level or virus titer of the CPV-2 fraction of vaccine B.

Blood was collected from pups in each group on four occasions (before the first, second, and third vaccinations and 3 weeks after the third vaccination); 5 mL of blood was collected by jugular venipuncture into a 7-mL serum separator tube and allowed to clot. Tubes were centrifuged and serum was removed and frozen; samples were processed and frozen within 2 hours of collection. Every 2 weeks, frozen serum samples were shipped on ice to the Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Itha-
A CPV-2b hemagglutination inhibition (HI) geometric mean titer was determined for each sample after serum samples were treated with 2-mercaptoethanol to dissociate IgM antibody molecules. Diagnostic laboratory personnel were unaware of each pup’s assignment to treatment group.

For 24 hours after vaccination, pups were observed for anaphylactic or other adverse reactions attributable to the vaccination. If pups developed signs of gastroenteritis during the course of the study, the date of onset was recorded. Gastroenteritis was defined as multiple episodes of vomiting or diarrhea that persisted ≥24 hours without fever, or episodes of vomiting or diarrhea accompanied by signs of depression, dehydration, or fever of any duration. If signs of gastroenteritis developed, diagnostic tests were performed, including physical examination, complete blood cell (CBC) count, serum biochemical analyses, fecal examination for parasites (nematodes, Giardia spp, and coccidia), enzyme-linked immunosorbent assay (ELISA) for CPV in fecal samples, and abdominal radiography. If test results were negative or within reference ranges, further tests, including ELISA for Giardia spp in fecal samples, bacteriologic culture of feces for Salmonella spp, Clostridium spp, and Campylobacter spp, and viral culture of fecal samples for canine coronavirus were performed.

Pups were considered infected with CPV if findings included all of the following: clinical signs consistent with CPV, diarrhea, positive results for the ELISA performed on feces, leukopenia (<50% of lower limit of reference range), and rectal temperature <38.1°C (100.5°F) or >39.7°C (103.4°F). Pups were also considered infected with CPV if they died and histopathologic evidence of CPV infection was detected.

Pups that were vaccinated at inappropriate intervals or that developed enteritis caused by CPV were excluded from the study, and further data from these dogs were not recorded. Pups that developed severe signs of concurrent diseases (specific clinical signs accompanied by signs of depression, dehydration, and fever—rectal temperature >40°C [104°F]) were similarly excluded from further analysis.

ANALYSIS

Data were analyzed with mixed, frequency, and life-test procedures by using a computer software program.14 A repeated-measures mixed general linear model was used to analyze titer data and days between visits. X-fold changes in titer were estimated from the repeated-measures analysis of titer data. Pairwise comparisons between treatments were made for each data collection time point. Pairwise comparisons of X-fold changes between treatments were made for each interval for which the X-fold changes were estimated. Survival analysis was used to analyze days until seroconversion (fourfold increase in titer). Frequency distributions were used to summarize percentage of pups that seroconverted during intervals of the study. A Cochran-Mantel-Haenszel test was used to test for differences in the total percentage of enteritis cases between the two treatment groups, and for differences in the percentage of enteritis cases caused by CPV between the two treatment groups. A general linear mixed model was used to analyze age of pups at time of entry into the study. Titer data were transformed by the log base 2 (titer/10) before analysis and after analysis. Least squares means were back-transformed to geometric means for presentation.

RESULTS

Eight veterinary clinics located across the United States participated in the study, with the mean number of pups entered into the study per clinic being 27 (range: 3–73 pups). Two hundred and thirteen pups (178 Rottweilers
and 35 Doberman pinschers), of which 108 were male and 105 were female, were entered into the study. One hundred and six pups (89 Rottweilers and 17 Doberman pinschers) were assigned to the vaccine-A group and 107 pups (89 Rottweilers and 18 Doberman pinschers) were assigned to the vaccine-B group.

Of the 213 pups entered into the study, 106 were excluded from final analysis because they were the incorrect age when first vaccinated, failed to return for additional vaccinations, or developed CPV enteritis. Ten of the 213 pups that were entered into the study developed clinical signs of gastroenteritis during the study. Four pups (Rottweilers) were in the vaccine-A group and six pups (five Rottweilers and one Doberman pinscher) were in the vaccine-B group.

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One hundred and seven pups completed all four visits successfully. Fifty-seven pups (49 Rottweilers and eight Doberman pinschers; mean age at entry: 46.1 days [range: 42–56 days]) were in the vaccine-A group and 50 pups (37 Rottweilers and 13 Doberman pinschers; mean age at entry: 45.2 days [range: 42–56 days]) were in the vaccine-B group. Before vaccination, CPV-2b geometric mean titer for pups in the vaccine-A group was 1:34.3 (range: 1:10 to 1:640); titer for pups in the vaccine-B group was 1:32.6 (range: 1:10 to 1:320).

Of the 57 pups in the vaccine-A group, seroconversion was detected in 33 pups after the first vaccination, in 13 pups after the second vaccination, and in seven pups after the third vaccination; seroconversion was not detected in four pups after all three vaccinations (Table 1). Of the 50 pups in the vaccine-B group, seroconversion was detected in 13 pups after the first vaccination, in 11 pups after the second vaccination, and in eight pups after the third vaccination; seroconversion was not detected in 18 pups after three vaccinations with vaccine B.

Survival analysis was used to analyze days until seroconversion for each group. Pups in the vaccine-A group had evidence of seroconversion significantly ($P = 0.001$) earlier than pups in the vaccine-B group. The proportion of pups responding to vaccination was inversely related to the pups’ antibody titer at the time of vaccination; a greater percentage of pups with low titers seroconverted after each vaccination than did pups with higher titers (Tables 2 and 3). After the first vaccination, more pups with a CPV-2b HI titer of ≤1:80 responded to vaccine A than to vaccine B. Overall, CPV-2b HI titers after vaccination were also significantly ($P \leq 0.05$) higher for the pups in the

**TABLE 1. Number (%) of Pups that Seroconverted (≥ Fourfold Increase in Titer) After the First, Second, or Third Vaccination with One of Two Canine Parvovirus Vaccines**

<table>
<thead>
<tr>
<th>Seroconversion</th>
<th>Vaccine A (n = 57)</th>
<th>Vaccine B (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>33 (58)</td>
<td>13 (26)</td>
</tr>
<tr>
<td>Second</td>
<td>46 (81)</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Third</td>
<td>53 (93)</td>
<td>32 (64)</td>
</tr>
</tbody>
</table>

*Numbers and percentages are cumulative.
M. J. Coyne

vaccine-A group after first, second, and third vaccinations, compared with those of pups in the vaccine-B group (Table 4).

**DISCUSSION**

Rottweiler and Doberman pinscher pups were used in the study reported here because

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**TABLE 2. Number (%) of Pups with Various Titers Before Vaccination that Seroconverted After the First, Second, or Third Vaccination with a Combination Modified-Live Canine Parvovirus Vaccine (vaccine A)**

<table>
<thead>
<tr>
<th>Vaccine Titer</th>
<th>First Vaccination</th>
<th>Second Vaccination</th>
<th>Third Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pups Vaccinated</td>
<td>Number of Pups (%) Responding</td>
<td>Number of Pups Vaccinated</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>18 (90)</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>10 (83)</td>
<td>4</td>
</tr>
<tr>
<td>40</td>
<td>7</td>
<td>3 (43)</td>
<td>9</td>
</tr>
<tr>
<td>80</td>
<td>8</td>
<td>2 (25)</td>
<td>3</td>
</tr>
<tr>
<td>160</td>
<td>4</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>320</td>
<td>5</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>640</td>
<td>1</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>33 (58)</td>
<td>24</td>
</tr>
</tbody>
</table>

*Data for 57 pups that received a first vaccination.
†Data for 24 pups that did not seroconvert after the first vaccination.
‡Data for 11 pups that did not seroconvert after the first or second vaccination.

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**TABLE 3. Number (%) of Pups with Various Titers Before Vaccination that Seroconverted After the First, Second, or Third Vaccination with a Combination Modified-Live Canine Parvovirus Vaccine (vaccine B)**

<table>
<thead>
<tr>
<th>Vaccine Titer</th>
<th>First Vaccination</th>
<th>Second Vaccination</th>
<th>Third Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pups Vaccinated</td>
<td>Number of Pups (%) Responding</td>
<td>Number of Pups Vaccinated</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>11 (58)</td>
<td>14</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>2 (25)</td>
<td>10</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>0 (0)</td>
<td>9</td>
</tr>
<tr>
<td>80</td>
<td>6</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>160</td>
<td>7</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>320</td>
<td>2</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>640</td>
<td>0</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>13 (26)</td>
<td>37</td>
</tr>
</tbody>
</table>

*Data for 50 pups that received a first vaccination.
†Data for 37 pups that did not seroconvert after the first vaccination.
‡Data for 26 pups that did not seroconvert after the first or second vaccination.
dogs of these breeds appear to be more susceptible to CPV infection than dogs of many other breeds.\(^6,7\) Causes for this increased susceptibility have not been determined with certainty. Von Willebrand’s disease and hereditary influences (i.e., common ancestry) may play a role in Doberman pinschers, as may inherited immunodeficiency in Rottweilers.\(^5,7,15\)

Substantial differences have been detected among various CPV vaccines in their efficacy to induce antibody production\(^13\) or to protect against challenge exposure with virulent virus.\(^9\) A major factor contributing to immunization failure is maternal antibody that interferes with a vaccine’s ability to induce production of protective antibodies in the pup.\(^10,16–19\) Most adult dogs have protective immunity against CPV, and approximately 5.7% of a dam’s antibodies against CPV are passed transplacentally to their offspring. With the addition of antibodies absorbed from colostrum, neonatal pups may acquire as much as 60% of their dam’s titer to CPV.\(^20\) Maternally derived CPV HI titers ≥1:80 protect pups from infection, but titers between 1:10 and 1:80 are not protective and may interfere with vaccination.\(^21\) Over time, as maternally derived antibody titers decrease, pups become susceptible to natural infection despite repeated vaccinations.\(^22\)

Both vaccines were administered to the pups according to the manufacturer’s recommendation. The administration of three doses of vaccine is recommended by the manufacturer for vaccine A, whereas four doses are recommended for vaccine B. Because the author was interested in comparing the performance of the two vaccines, he measured only the response to the first three vaccines administered. The additional administration of a fourth dose of vaccine to pups in the vaccine-B group would likely have resulted in seroconversion in additional pups in that group.

Two pups in the vaccine-A group and four pups in the vaccine-B group developed parvovirus enteritis and did not complete the study. Although all of these pups received two or three vaccinations prior to infection with CPV, none showed serologic evidence of responding to the vaccine and all had CPV-2b HI titers of ≤20 prior to infection.

Determination of HI titers is currently the most widely used procedure for determining antibody status of dogs vaccinated against CPV, and HI titers are a useful measure of immunity to infection.\(^23\) When antibody titers are low, HI titers may not be as useful as serum neutralization (SN) titers for predicting when pups would be susceptible to infection or responsive to vaccination.\(^23\) However, a recent vaccine study showed that results obtained when HI titers were analyzed were similar to those obtained when SN titers were analyzed.\(^24\)

The ability of a vaccine to overcome the inhibitory effects of maternal antibodies and induce seroconversion is attributable, to a large degree, to its inherent immunogenicity and to the virus titer in the vaccine.\(^17,23\) High-titer, low-passage CPV vaccines containing a canine-origin, attenuated virus are currently the vac-

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**TABLE 4. Geometric Mean Hemagglutination Inhibition Titer Against Canine Parvovirus-2b in Pups Vaccinated with One of Two Combination Modified-Live Canine Parvovirus Vaccines**

<table>
<thead>
<tr>
<th>Vaccination Status</th>
<th>Vaccine A</th>
<th>Vaccine B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vaccination*</td>
<td>34.3</td>
<td>32.6</td>
</tr>
<tr>
<td>After first vaccination</td>
<td>192.9(\dagger)</td>
<td>53.0</td>
</tr>
<tr>
<td>After second vaccination</td>
<td>441.0(\dagger)</td>
<td>116.8</td>
</tr>
<tr>
<td>After third vaccination</td>
<td>612.3(\dagger)</td>
<td>224.1</td>
</tr>
</tbody>
</table>

\*Pups were vaccinated every 21 days (range: 18–24 days).
\(\dagger\)Significantly (\(P \leq 0.05\)) different from vaccine B.
The use of these high-titer, low-passage CPV vaccines consistently results in the seroconversion of ≥90% of pups, irrespective of breed, when the final vaccine of a two- or three-dose vaccination regimen is administered at 12 weeks of age.9,12,13, 23, 24, 26–28 This earlier immunization of pups by using the high-titer, low-passage CPV vaccines would be expected to reduce the risk of infection, and in susceptible breed pups, the risk of severe or life-threatening disease. However, no vaccine or vaccination regimen can reduce the risk of infection to zero. As seen in this study, cases of clinical CPV infection were observed in a small number of pups in both vaccine groups. Infection developed prior to completion of the vaccination series, occurring prior to the pups’ response to the vaccine. Optimally, all pups, but especially pups from susceptible breeds, should be kept isolated until the vaccination series is completed and the pup is fully immunized.

**CONCLUSION**

The study reported here examined the efficacy of two combination vaccines that contained modified-live canine parvovirus. Antibody titers against CPV in both vaccine groups were similar before vaccination, but pups in the vaccine-A group had significantly higher titers after the first, second, and third vaccinations than did pups in the vaccine-B group. In addition, pups in the vaccine-A group seroconverted significantly earlier than those in the vaccine-B group. A greater percentage of pups with prevaccination titers ≤1:80 seroconverted following vaccination with vaccine A, compared with that for pups vaccinated with vaccine B. Pups with higher titers (≥1:80) failed to respond to either vaccine, revealing the inhibitory effects of maternal antibody on response to vaccination. These seroconversion results are consistent with those reported for the same vaccines in previous studies that used different breeds of dogs.9,13, 23, 24, 26–28

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Participating Veterinarians: Michelle Fordham, Rehm Animal Clinic, Mobile, Ala; William Losch, St. Paul Veterinary Clinic, Milwaukee, Wis; Istvan, Tanko, VetSmart Veterinary Services, Clearwater, Fla; William Padilla, VetSmart Veterinary Services, Vista, Calif; Joseph Quinn, Town East-Galloway Clinic, Mesquite, Tex; Lawrence Rebbeccchi, Rebbeccchi Animal Hospital, Philadelphia, Penn; Kristine Hare, Mangrove Veterinary Hospital, Chico, Calif; Beverly Porter, VetSmart Veterinary Services, St. Petersburg, Fla.

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