Evaluation of the Efficacy and Duration of Immunity of a Canine Combination Vaccine Against Virulent Parvovirus, Infectious Canine Hepatitis Virus, and Distemper Virus Experimental Challenges*

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The results of this study confirmed that dogs vaccinated subcutaneously with a commercially available multivalent vaccine containing modified-live canine distemper virus, canine adenovirus type 2, canine parvovirus type 2b, and canine parainfluenza virus antigens were protected against sequential experimental challenge 55 to 57 months after initial vaccination given at 7 to 8 weeks of age. All 10 vaccinates were protected against clinical diseases and mortality following parvovirus and infectious canine hepatitis experimental infections. All vaccinates were protected against mortality and 90% against clinical disease following distemper challenge. These data support at least a 4-year duration of immunity for these three “core” fractions in the combination vaccine.

INTRODUCTION

Canine parvovirus (CPV), infectious canine hepatitis (ICH), and canine distemper virus (CDV) infections are potentially fatal diseases of dogs and other related species. The etiologic agent for CPV infections is a nonenveloped DNA-containing virus, CPV type 2 (CPV-2). The virus is very stable in the environment and highly contagious.1 In the United States, the CPV-2b genotype, through mutations,2 has largely replaced previously isolated genotypes (CPV-2 and CPV-2a). In the Far East3,4 and
Europe, both CPV-2a and CPV-2b may be present, but current published information is not available. Canine distemper infection is caused by CDV, a contagious *Morbillivirus* belonging to the Paramyxoviridae family. CDV affects Canidae and other carnivores such as pandas, raccoons, and large felids. ICH is caused by canine adenovirus type 1 (CAV-1). The virus is stable in the environment and affects Canidae and Ursidae (bears).

Immunization of dogs through the use of vaccination during the past decades has greatly reduced the incidence of these infectious diseases in dog populations. Modified-live vaccines were shown to induce superior and longer-lasting immunity compared with inactivated vaccines. Safe and efficacious vaccines against CDV, CPV, and ICH infections are considered essential vaccines that every dog should receive and were recently designated as “core vaccines” by Schultz, the American Veterinary Medical Association Council on Biological and Therapeutic Agents (COBTA), and the American Animal Hospital Association (AAHA) Canine Vaccine Task Force.

Determination of vaccination frequency for the core vaccines was a subject of debate and differing views among experts in the field. Veterinarians have to make decisions regarding the frequency of vaccination based on safety, efficacy, and duration of immunity as well as such factors as animal susceptibility, animal environment, risk of exposure, breed involved, and other considerations. To determine the duration of immunity of “core” vaccines, some authors relied on serologic data obtained from field studies. Although serologic response is a good indicator of the level of protection for each of these three antigens, the potential for environmental exposure to these viruses under field conditions compromises the conclusion that protection is solely due to vaccine-induced antibodies.

This article presents vaccine efficacy and duration of immunity data obtained from direct animal vaccination and experimental challenge studies. The vaccinated animals were housed under high levels of biosecurity throughout the holding period to prevent extraneous virus exposure. Control nonvaccinated dogs were maintained in the same facilities throughout the period and were shown to remain antibody negative to the three viruses of interest.

**MATERIALS AND METHODS**

**Animals**

Ten beagle puppies seronegative to CPV-2b, CAV-1, canine adenovirus type 2 (CAV-2), and CDV were vaccinated at 7 to 8 weeks of age with two doses of vaccine given 3 weeks apart; the puppies were held in isolation until challenge: CPV-2b challenge was administered 55.1 months after vaccination, CAV-1 challenge at 55.8 months, and CDV challenge at 56.5 months. Seven age-matched unvaccinated puppies were held separately at the same facility as controls. Three of the seven controls were entered into the study at the time the other dogs were vaccinated, and the remaining four were introduced 5 months after vaccination. These controls are referred to as “adult control dogs” in this article. An additional three sets of five young beagle puppies were used as young susceptible controls to validate the virulence of each challenge (five per challenge) and are referred to as “young control puppies” in this ar-
article. All dogs in this study were treated according to animal welfare regulations outlined in 9 CFR, Subchapter A, Part 3, Subpart A. Animal care and use committee (IACUC) approval was obtained at each site before the study was conducted.

**Vaccine**

The test vaccine is a commercially available multivalent freeze-dried vaccine (DA2PPv) containing modified-live CDV, CAV-2, canine parainfluenza virus (CPI), and CPV-2b antigens. The vaccine is marketed by Schering-Plough Animal Health in the United States, Canada, and South Africa under the trade name Galaxy and in Europe and other markets under the trade names Procyon Dog or Quantum Dog. The test vaccine was rehydrated with liquid vaccine containing killed *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, and coronavirus antigens at the time of vaccination and administered subcutaneously in 1-ml doses.

**Animal Trial Design**

Before vaccination, puppies were blocked by litter and gender and were randomly assigned as vaccinates or controls. Vaccinates (n = 10) received two doses of vaccine given 3 weeks apart and were held with the controls (n = 7) in an isolation facility in pens at Liberty Research (Waverly, NY) for 55 months. During this period, they were cared for and blood samples were collected regularly to monitor their antibody status. At the end of the holding period, the designated vaccinates and adult control dogs were transferred to the University of Wisconsin animal facility in Madison, Wisconsin, for experimental challenge. The dogs were acclimated for 8 days and determined to be fit before challenge inoculation. Five 12-week-old, CPV-2b-seronegative puppies (young control puppies) arrived at the facility at the same time for use in the CPV-2b challenge. Two additional sets of five young control puppies were used to validate CAV-1 and CDV challenges (five puppies per challenge).

**Experimental Challenge**

**Canine Parvovirus Type 2b Challenge**

The 10 vaccinates, four adult controls, and five young control puppies were all challenged oronasally (day 0) with virulent CPV-2b (obtained from the USDA Center for Veterinary Biologics). Each dog received $10^{4.1}$ TCID$_{50}$ of challenge virus. Inoculated dogs were observed for clinical signs, temperature, and mortality for 14 days after challenge. Whole blood (in EDTA tubes) and fecal swabs were collected daily to evaluate leukocyte counts and virus shedding, respectively.

**Canine Adenovirus Type 1 Challenge**

After the vaccinates were rested for 1 week, they and three adult controls were inoculated intravenously (day 21) with CAV-1 (obtained from the USDA Center for Veterinary Biologics). Five young control puppies were challenged in a similar manner to validate the challenge dose. Each dog received at least $10^{3.3}$ TCID$_{50}$ of challenge virus. The inoculated dogs were observed daily for clinical signs for 21 days following challenge.

**Canine Distemper Virus Challenge**

Three weeks following CAV-1 challenge (day 42), the 10 vaccinates and four adult controls (the same adult controls used in the parvovirus challenge) were physically examined and determined to be fit before they were inoculated intranasally and intravenously with virulent CDV Snyder Hill challenge virus (provided by R. S.). Five young (12-week-old) control puppies were challenged separately in advance to validate the challenge virus virulence and were observed for 15 days following challenge. Inoculated adult vaccinates and con-
pressed as the reciprocal of the dilution, which
neutralized 50% of the virus, as calculated by
the Spearman–Karber method. For purpose
of analysis, SN titers below 2 were given a val-
ue of 1. Antibody titers that were not end-
pointed at dilution 1:4,096 were given the val-
ue of 4,096. Geometric mean titers (GMTs)
obtained in these cases were indicated as
>GMT value.

Detection of Canine Parvovirus in Fecal
Samples by Hemagglutination Test

CPV in rectal swabs was quantified by the
hemagglutination assay (HA) using 96-well
plates. Briefly, the swabs were stored in tubes
containing 1 ml of phosphate-buffered saline
(PBS) and frozen until thawed for extraction.

Following vaccination, the vaccinates developed high levels of SN antibodies to all fractions.

CDV [distemperoid strain]) containing 50 to
300 log_{10} TCID_{50} was added to each well. After
30 to 60 minutes of incubation at 36 ± 2°C,
cell substrate (dog kidney cell in the case of
CPV, CAV-1, and CAV-2; Vero cells in the case
of CDV) was added to each well (seeded at ap-
proximately 1 to 2 × 10^4 cells/0.1 ml/well in
DMEM supplemented with 2 mm L-gluta-
mine and gentamicin [50 µg/ml], and 5% fetal
bovine serum). Plates were incubated in a hu-
midified carbon dioxide (4% to 6 %) incubator
for 3 days (CPV) or 5 to 7 days (CAV-1, CAV-
2, and CDV) at 36 ± 2°C.

At the end of the incubation period, SN an-
tibody response was determined by examina-
tion of wells for typical CDV or CAV cyto-
pathic effect. For CPV, plates were fixed with
80% acetone (100 µl/well) and stained with
CPV-specific fluorescein-labeled antibody con-
jugate (75 µl/well). SN antibody titers were ex-
At extraction, tubes were vortexed, swabs were
removed, and 500 µl chloroform was added. Tubes were vortexed intermittently for 15 min-
utes and centrifuged, and the supernatant was
removed and stored frozen until tested. The sample was twofold serially diluted (in PBS
with bovine serum albumin) in duplicates of
wells of a round-bottomed 96-well plate. After
dilution were made, 1% of washed porcine erythrocytes was added to each well
and plates were refrigerated for 4 to 8 hours.
Titers were calculated as the reciprocal of the
highest dilution of sample that produced com-
plete agglutination. Positive (sample with
known titer) and negative (sample with no
virus) controls were used to monitor the assay.

Leukocyte Counts

Leukocyte counts were determined using an
ADVIA 120 Hematology System (Bayer
HealthCare, Tarrytown, NY). The EDTA blood sample was mixed with ADVIA 120 BASO solution containing acid and surfactant. After erythrocytes were hemolyzed, the leukocyte counts were analyzed using two-angle laser light scatter signals.

RESULTS

Antibody Response

All puppies were confirmed to be seronegative to CPV, CAV-1, CAV-2, and CDV before vaccination (Table 1, Figure 1). Following vaccination, the vaccinates developed high levels of SN antibodies to all fractions. The control dogs remained seronegative throughout the holding period, indicating lack of extraneous exposure to these agents.

Canine Parvovirus Type 2b Response

All 10 vaccinates (100%) responded with very high SN titers, with the highest GMT obtained 1 month after vaccination (GMT >3,956). The GMT was maintained above 1,078 during the entire postvaccination period. GMT was above 2,656 before challenge (55.1 months after vaccination). The hemagglutination inhibition (HI) antibody GMT for the vaccinates at this point was 844 (data not shown).

Canine Adenovirus Type 1 Response

All 10 vaccinates (100%) responded with high SN titers after the first vaccination (GMT = 388). The SN GMTs were maintained at high levels during the entire postvaccination period and until challenge (55.8 months after vaccination), when the GMT was 274.

Canine Adenovirus Type 2 Response

All 10 vaccinates (100%) responded with very high SN titers within 1 month of vaccination (GMT > 3,385). The high SN titers were maintained through the entire postvaccination period and until challenge (GMT = 152).

Canine Distemper Virus Response

Nine of the 10 vaccinates (90%) responded with high SN titers following the second vaccination. One dog did not develop neutralizing antibodies after vaccination. The highest SN GMT was obtained 14 days after the second vaccination (GMT >846). CDV-specific antibodies were maintained for 56.5 months after vaccination and until challenge (GMT = 24).

Canine Parvovirus Type 2b Challenge

Clinical Evaluation

Starting 5 days after CPV-2b challenge, the young unvaccinated controls developed severe clinical signs typical of parvovirus, including diarrhea, bloody diarrhea, vomiting, dehydration, and depression. One of the puppies died on day 7 after challenge, and the remaining four were euthanized on the same day for humane reasons, resulting in 100% mortality (Table 2, Figure 2). Two of the adult controls showed sporadic clinical signs, including inappetance, diarrhea, depression, and vomiting, between days 5 and 8 after challenge. Total average clinical score was 37.4 for the young controls and 1.8 for the adult controls. By contrast, the vaccinates remained in good health and none showed any clinical signs (clinical score = 0).

Fecal Virus Shedding

Results of fecal virus shedding are shown in Table 3 and Figure 3. All dogs in the two control groups (100%) shed challenge virus between days 4 and 8 after challenge. The highest shedding was scored on day 6 (GMT = 4,096) for the young controls (one day before death/euthanasia) and on day 7 (GMT = 512) for the adult controls (Table 3, Figure 3). None of the vaccinates shed virus in feces as determined by HA.

Leukopenia

Measurement of total leukocytes (data not shown) has shown that leukopenia was ob-
served in three of the five young controls (60%) on day 7 after challenge. While no clinical leukopenia (i.e., greater than 50% diminution of circulating leukocytes from baseline values) occurred in the adult controls, one dog experienced significant reduction in leukocyte count (37% below baseline value) on day 7 after challenge. None of the vaccinates developed leukopenia following challenge.

**Rectal Temperature**

One of the adult controls and two of the young controls developed rectal temperatures above 103.1°F. None of the vaccinates showed temperatures above 103.1°F (data not shown).

**Canine Adenovirus Type 1 Challenge Clinical Evaluation**

Average daily clinical scores are shown in Table 4 and Figure 4. Following CAV-1 challenge, four of the five young unvaccinated control dogs developed moderate clinical signs typical of canine hepatitis. The fifth young control developed severe clinical signs followed by death on day 6 after challenge. All three adult controls (100%) developed clinical signs following challenge, and two of three (66%) showed severe clinical signs, including depression, dehydration, conjunctivitis, oral hemorrhages, and/or petechiae. One of the adult controls was euthanized on day 6 after challenge. The total average daily score was 25 for adult controls and 17 for young controls. By contrast, vaccinates remained in good health and showed no clinical signs (clinical score = 0).

**Rectal Temperature**

None of the dogs developed clinical fever after challenge (data not shown).

**Canine Distemper Virus Challenge Clinical Evaluation**

Average daily clinical score results are shown in Table 5 and Figure 5. Following CDV challenge, all five young unvaccinated control dogs...
(100%) developed severe clinical signs typical of distemper. Clinical signs displayed included ocular discharge, conjunctivitis, bloody diarrhea, dehydration, depression, and vomiting. One dog developed nervous signs. Four of the five young controls (80%) were either euthanized or died. The remaining control had severe signs, and was killed.

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**Figure 1.** Serum-neutralizing antibody response to CPV, CAV-1, CAV-2, and CDV after vaccination. Refer to Table 1 for detailed description and antibody responses to the three antigens for the postchallenge (PC) time point. AC = adult unvaccinated controls; C = challenge time point.

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### Months After Vaccination

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Challenge Point</th>
<th>Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.5</td>
<td>&gt;3,866</td>
<td>&gt;3,327</td>
</tr>
<tr>
<td>19.6</td>
<td>&gt;3,888</td>
<td>&gt;3,504</td>
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<tr>
<td>25.7</td>
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<td>31.7</td>
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<td>36.7</td>
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<td>50.4</td>
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<td>2,327</td>
</tr>
<tr>
<td>53.0</td>
<td>152</td>
<td>3,158</td>
</tr>
</tbody>
</table>

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**Note:**
- Postchallenge time point = 14 days for CPV-2b and 21 days for ICH and CDV.
- Unvaccinated adult controls (AC) used for CPV-2b challenge (four animals), ICH challenge (three animals), and CDV challenge (four animals) remained seronegative (SN < 2) until challenge.
- Postchallenge antibody response of AC was >3,922 against CPV-2b, >4,096 against CAV-1, and 181 against CDV. ND = no data.
### Table 2: Average Daily (±SD) Clinical Score Following CPV-2b Challenge

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Days After Challenge</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–2 –1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14</td>
<td></td>
</tr>
<tr>
<td>Adult controls</td>
<td>0 0 0 0 0 0 0.5 ± 1 0 0.5 ± 1 0.8 ± 1.5 0 0 0 0 0</td>
<td>1.8 ± 2.4</td>
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<tr>
<td>Vaccinates</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Young controls</td>
<td>0 0 0 0 0 0 4.8 ± 0.8 7.6 ± 1.3 25 ± 0</td>
<td>All puppies died or were euthanized 37.4 ± 2.1</td>
</tr>
</tbody>
</table>

*Clinical signs observed following CPV-2b challenge in unvaccinated controls included vomiting, bloody diarrhea, dehydration, depression, and inappetance. No clinical signs were observed in the vaccinates. Animals were observed for 14 days after challenge.*

### Table 3: Geometric Mean (±SD) Fecal Virus Shedding Titers Determined by Hemagglutination Assay Following CPV-2b Challenge

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Days After Challenge</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10 11 12 13 14</td>
<td></td>
</tr>
<tr>
<td>Adult controls</td>
<td>0 0 0 0 0 2 ± 16 23 ± 2,037 512 ± 2,328 45 ± 1,008 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Vaccinates</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Young controls</td>
<td>0 0 0 0 256 ± 194 891 ± 1,984 4,096 ± 0</td>
<td>All puppies died or were euthanized</td>
</tr>
</tbody>
</table>

*Fecal samples were evaluated for parvovirus challenge virus by hemagglutination assay. Titers were obtained for each group for each day after challenge.*

*Young controls had 100% mortality on day 7 after challenge.*

0 = no virus detected at the lowest sample dilution (1:10).
nized or died between days 11 and 14 after challenge. All four adult controls developed typical distemper. Two of four (50%) died (one on day 13 and one on day 16 after challenge). By contrast, only one of the 10 vaccinates showed significant distemper clinical signs, and it recovered. Except for a mild and transient ocular/nasal discharge for 1 day in one
TABLE 4. Average (±SD) Daily Clinical Score Following CAV-1 Challenge

<table>
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<th>Treatment Group</th>
<th>Days After Challenge</th>
<th>Total Score</th>
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<tr>
<td></td>
<td>–2 –1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14</td>
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<tr>
<td>Adult controls</td>
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<tr>
<td>Vaccinates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young controls</td>
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</tbody>
</table>

*Clinical signs observed following CAV-1 challenge in unvaccinated controls included nasal/ocular discharges, vomiting, oral hemorrhages, dehydration, depression, edema, conjunctivitis, corneal opacity, and icterus signs. One of the three adult controls was euthanized on day 6 after challenge. One of the five young unvaccinated controls was found dead on day 6 after challenge. No clinical signs were observed in the vaccinates. Animals were observed for 21 days after challenge.

TABLE 5. Average (±SD) Daily Clinical Score Following CDV Challenge

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Days After Challenge</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17</td>
<td></td>
</tr>
<tr>
<td>Adult controls</td>
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<tr>
<td>Vaccinates</td>
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<td></td>
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<tr>
<td>Young controls</td>
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</tbody>
</table>

*Clinical signs observed following CDV challenge in unvaccinated controls included ocular discharges; vomiting; bloody diarrhea; dehydration; depression; central nervous system signs; conjunctivitis; and emaciation. Two of the four adult controls (50%) died after the challenge (one on day 13 and one on day 16). Four of the five (80%) young unvaccinated controls died between days 11 and 14 after challenge. One of the 10 vaccinates developed clinical signs for 7 days after challenge. A second vaccinate showed mild ocular discharge for 1 day. ND = no observations were made for the surviving young control beyond day 15 after challenge.
dog, the remaining nine vaccinates (90%) were normal throughout the postchallenge period. The total average daily score was 45 for the adult unvaccinated controls and 2.1 for the vaccinates (Table 5).

**Rectal Temperature**

One young control puppy developed a rectal temperature of 103.4°F after challenge. None of the vaccinates developed a temperature above 102.5°F (data not shown).

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Figure 4. Average daily clinical score following CAV-1 challenge; also see Table 4. AC = adult unvaccinated controls; SC = subcutaneously vaccinated dogs; YC = young unvaccinated controls.

Figure 5. Average daily clinical score following CDV challenge; also see Table 5. No further observations were made for the surviving young unvaccinated controls (YC) beyond day 15 after challenge. AC = adult unvaccinated controls; SC = subcutaneously vaccinated dogs.
DISCUSSION

Results obtained from this study provided evidence that vaccination of young (7- to 8-week-old) susceptible puppies with a commercially available combination vaccine containing modified-live CPV-2b, CDV, and CAV-2 antigens provided and maintained adequate immunity for at least 4 years following vaccination. Except for one dog’s response to CDV, the three antigens induced high levels of SN antibodies following vaccination, and those levels persisted at protective levels \(^{21-24}\) for the entire postvaccination period until challenge (Table 1, Figure 1). HI antibody titers for CPV were also high (GMT = 844) at 55 months after vaccination (data not shown). The adult unvaccinated control dogs housed in the same facility remained seronegative, indicating lack of extraneous exposure to these agents and confirming that the immunity induced in the vaccinates was from the vaccine. The CPV challenge was confirmed adequate to measure the efficacy of the vaccine because it resulted in 100% morbidity and mortality in the young unvaccinated controls.

Adult controls experienced transient clinical signs, including diarrhea, vomiting, and depression. The difference in the severity of disease between young and adult controls is expected because of the difference in age susceptibility to parvovirus infection. \(^{26}\) All vaccinates (100%) were protected against clinical disease and mortality. Furthermore, vaccinates were completely protected against fecal virus shedding (Table 3, Figure 3) and leukopenia (data not shown) in contrast to unvaccinated controls that shed significant amounts of virus and developed leukopenia. These findings collectively indicate that the vaccine was able to induce immunity to CPV in dogs that lasted for at least 55 months following vaccination.

The subsequent CAV-1 challenge was confirmed to have sufficient virulence to judge the efficacy of the vaccine because it caused 100% morbidity and 33% mortality in the adult unvaccinated controls (Table 4, Figure 4). Vaccinates remained in good health and showed no clinical signs. The postchallenge antibody response (GMT = 3,327 to CAV-1 and 3,158 to CAV-2 [Table 1, Figure 1]) suggests the presence of effective immunologic memory.

The experimental distemper challenge resulted in 100% morbidity in the combined adult and young controls, 80% mortality in the young controls, and 50% mortality in the adult controls, validating the severity of the challenge and its adequacy to judge vaccine efficacy. Nine of the 10 vaccinates (90%) were protected against clinical signs associated with distemper except for mild transient conjunctivitis and ocular discharge in one dog for 1 day. One of the vaccinates developed clinical signs of distemper, including bloody diarrhea, depression, and nervous signs, but fully recovered by day 14 after challenge. This dog was a nonresponder and did not mount an antibody response following vaccination. Nonresponse to CDV vaccination is not uncommon in the field, and nonresponders constitute approximately 1% of cases (R. S., personal observation).
In summary, this report provided evidence for the efficacy and duration of immunity of this combination vaccine based on validated vaccination and experimental challenge studies. It is the first published report in which dogs were maintained in a known virus-free environment that supports more than 4 years of immunity based on challenge evaluation under controlled conditions. Data obtained from challenge evaluation are far more reliable than serologic response evaluation from field studies previously described.\textsuperscript{19,20} Although serologic response is a good correlate of protection for each of these three antigens,\textsuperscript{21–24} extraneous virus exposure to these agents is likely to occur in dogs in the field, thereby compromising findings. This report provides veterinarians with critically needed information regarding duration of immunity of commercial “core” vaccines to help in assessing and designing appropriate vaccination programs according to the recently issued recommendations by AAHA,\textsuperscript{15} COBTA,\textsuperscript{14} and published literature.\textsuperscript{16}

\section*{CONCLUSION}

The modified-live CPV-2b, CDV, and CAV-2 fractions in the multivalent vaccine evaluated in this study induced immunity in 7- to 8-week-old susceptible puppies that persisted for more than 4 years after vaccination. The immunity in vaccinated dogs was evaluated against virulent experimental sequential parvovirus, infectious hepatitis, and distemper challenges. All 10 vaccinates (100%) were protected against clinical diseases and mortality following parvovirus and infectious hepatitis experimental infections. All vaccinates (100%) were protected against mortality, and 90% were protected against clinical disease following distemper challenge. The data obtained support at least 55 months’ duration of immunity to the CPV-2b, CDV, and CAV-2 fractions in the combination vaccine.

\section*{REFERENCES}