Three-Year Rabies Duration of Immunity in Dogs Following Vaccination with a Core Combination Vaccine against Canine Distemper Virus, Canine Adenovirus Type-1, Canine Parvovirus, and Rabies Virus*

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INTRODUCTION

In recent years, the much-publicized debate among veterinarians regarding appropriate revaccination intervals in dogs has generated intense review of vaccination-related scientific data and information by major veterinary medical associations and academicians. As a result of this research, the American Veterinary Medical Association’s Council on Biologic and Therapeutic Agents (COBTA), the American

*Funding for this study was provided by Intervet Inc., Millsboro, Delaware.
Association of Feline Practitioners (AAFP), and the American Animal Hospital Association (AAHA) concluded that although traditional annual revaccination protocols were justified in some cases by the safety and efficacy limitations of earlier vaccines, such protocols have been based largely on historical precedent with minimal supporting scientific data.\textsuperscript{1–5} All of these associations have identified increasing evidence that vaccine-induced immunity lasts longer than 1 year for certain antigens.\textsuperscript{1–5}

In light of these findings, COBTA now recommends that veterinarians take an active role in determining the most appropriate vaccination protocols based on their assessment of individual patient needs.\textsuperscript{1} The AAHA Canine Vaccine Task Force determined that the evidence warranted revision of routine annual protocols and published its 2003 Canine Vaccine Guidelines and Recommendations, which recommended 3-year revaccination protocols for specified core antigens in dogs as appropriate based on veterinarians’ individual patient risk assessments.\textsuperscript{2} The AAHA updated these guidelines again in March 2006 to reflect the latest scientific data and build on the foundation of the American Veterinary Medical Association, the AAFP, and its own 2003 guidelines.\textsuperscript{1–5}

In its new 2006 Canine Vaccine Guidelines, the AAHA noted that its 2003 guidelines encouraging 3-year core revaccination protocols have been increasingly implemented by veterinarians who have seen no negative clinical ramifications.\textsuperscript{5} The AAHA also stated that at the time of publication of the 2006 guidelines, only one vaccine manufacturer had obtained a USDA approved license for a 3-year claim following an initial vaccination series.\textsuperscript{5}

The generation of additional data in conjunction with vaccine labeling, particularly related to duration of immunity, has been encouraged and requested by the profession.\textsuperscript{1,6,7} To provide more scientific data for practitioners, we previously published real-time challenge-of-immunity data supporting a 3-year duration-of-immunity claim for a combination canine distemper virus (CDV), canine adenovirus type-2 (CAV-2), and canine parvovirus (CPV) vaccine (in accordance with 9 CFR specifications).\textsuperscript{8,9}

Similarly, the objectives of the study reported here were to:

- Use challenge-of-immunity testing methodologies to demonstrate that the inactivated rabies virus component in the new test vaccine provides at least a 3-year duration of immunity in dogs when administered in combination with modified-live CDV, CAV-2, and CPV components
- Establish that the modified-live antigens do not interfere with the immunogenicity of the killed rabies virus component

**MATERIALS AND METHODS**

**Dogs**

Sixty-six antibody profile–defined beagles were enrolled in the study; however, before
vaccination, three dogs developed unrelated health conditions and were removed from the study and not included in test results reported for the remaining 63 dogs. These non–study-related conditions included a prolapsed rectum in two dogs and death from colonic rupture possibly associated with an inguinal hernia in one dog.

Pups were approximately 8 weeks of age or younger when they were purchased from a commercial breeder for the purpose of participating in this study. The average pup age was 54.5 days (standard deviation, 7.8 days), with a range of 43 to 67 days.

Randomization of test dogs was accomplished according to standard methods. Dogs were segregated by gender, and randomization was conducted separately for each gender group. After random number assignment, test puppies were blocked by litter and ranked within each litter from the lowest to the highest, and group numbers were allotted. Dogs were identified through use of unique numeric sequences marked on permanent individual ear tattoos.

To prevent any passage of maternal antibodies to dogs used in this study, their dams had been housed in highly secure, barrier-isolation (Animal Biosafety Level 2) facilities since birth and maintained free of vaccination against and exposure to bacterial and viral pathogens, including CDV, CAV type-1 (CAV-1), CAV-2, CPV, and canine parainfluenza virus (CPIV).

Before vaccination, negative serology status for rabies virus was confirmed in each test dog via rapid fluorescent focus inhibition test (RFFIT) evaluation of individual serum samples. Dogs were considered seronegative for rabies virus–neutralizing antibodies if serum neutralization (SN) titers measured less than 5 as determined by the RFFIT results.

The general health of all study dogs was monitored and recorded daily for 27 days after administration of the second vaccination. Throughout the study period, dogs were fed standard growth or maintenance rations, and water was available ad libitum. Dogs remained sexually intact—neither spayed nor neutered. Dogs were maintained throughout the 36-month postvaccination period by their breeder in Animal Biosafety Level 2 facilities until 1 week before the challenge. At that time, all test dogs were transferred to an animal handling facility at Intervet (Millsboro, DE) for the rabies virus challenge.

Test Vaccine

The new test vaccine (Continuum DAPP-R, Intervet) included CPIV as well as the four components in Continuum DAP-R (Intervet):

- High-titer Onderstepoort strain of CDV
- Manhattan strain of CAV-2, which confers cross-protection against canine infectious hepatitis caused by CAV-1 without the adverse reactions associated with CAV-1, such as corneal edema
- Attenuated, high-titer patented CPV STRAIN 154 (Intervet, US Patent No. 4,810,494) of canine origin
- Inactivated rabies virus produced from the highly immunogenic Pasteur rabies virus strain

Modified-live viral vaccine components were formulated at maximum virus passage level from the master seed virus. The vaccine serial was formulated at minimum protection titers and stored at 4°C until use. The test vaccine was presented in a desiccated form, with monovalent rabies vaccine used as the diluent for reconstitution.

The vaccine also contains a non–aluminum-salt adjuvant. The adjuvant enhances the immune response by encapsulating the vaccine antigen and providing both a slow release and
depot effect to improve antigen presentation to effector cells. In comparison with conventional aluminum-based adjuvants, the non–aluminum-salt adjuvant’s mode of action offers the potential to produce higher levels of humoral and cell-mediated immunity, more rapid immune response, and enhanced protection with a single vaccine dose. The non–aluminum-salt adjuvant suspension has been used in veterinary vaccines since the 1970s and has proven to be safe and effective when used in all animal species. Each batch of the adjuvant is safety tested in mice before release.

The potency of the inactivated rabies vaccine component was tested via the National Institutes of Health (NIH) Rabies Potency Test using the National Veterinary Services Laboratories reference strain in accordance with the guidelines described in the Mammalian Virology Supplemental Assay Method.\textsuperscript{12}

**Vaccination Protocol**

Sixty-three seronegative pups were randomly divided into two test groups:

- **Group 1** (n = 32)—Pups were vaccinated at 8 weeks of age with CDV–CAV-2–CPV–CPIV vaccine (Continuum DAPP) and at 12 weeks of age with a CDV–CAV-2–CPV–CPIV–rabies virus vaccine (Continuum DAPP-R). At each vaccination, group 1 pups received 1 ml total volume of reconstituted vaccine administered SC in the scruff of the neck. For the initial vaccination, sterile diluent was used to reconstitute the vaccine; for the booster vaccination, monovalent rabies vaccine was used as the diluent.

- **Group 2** (n = 31)—Dogs served as age-matched, nonvaccinated controls.

**Serologic Assays**

As required by 9 CFR § 113.209 (rabies vaccine, killed virus), before vaccination, test animals must have no neutralizing antibodies for rabies virus as determined by SN tests.\textsuperscript{13} Individual serum samples from all dogs were tested for neutralizing antibodies using RFFIT analysis. This testing was performed on sera from all test dogs just before the first vaccination at 8 weeks of age and immediately before challenge 36 months after administration of the test vaccine. In addition, during the 3-year postvaccination isolation period, RFFIT titers were measured randomly in five to 10 control dogs every 1 to 6 months. Neither vaccinated nor control dogs received any other vaccines or drugs within 3 weeks before any serum antibody test or within 3 weeks before or at any time after challenge with virulent rabies virus.

**Challenge Protocol**

Challenge protocol procedures for killed rabies vaccine virus were established and followed in accordance with 9 CFR § 113.209.\textsuperscript{13} All test dogs were strictly isolated for 3 years after receiving the second vaccination and then challenged with a virulent rabies virus challenge strain (New York City Street Virus) obtained from the Center for Veterinary Biologics–Laboratory. One week before challenge, all test dogs were transferred to challenge facilities at Intervet (Millsboro, DE) and housed in individual cages in an isolation facility (six rooms with 10 dogs each and one room with three dogs).

On challenge day, each dog was anesthetized and received a total of 1 ml of diluted challenge virus administered with a needle and syringe via the intramuscular route (0.5 ml/masserter muscle). All dogs were challenged within 4 hours after the challenge virus was thawed. The challenge virus titer was confirmed via NIH mouse potency tests, and titers were calculated using the Reed–Muench method. A USDA representative was present on the day of challenge to witness dilution of challenge
stock, challenge administration, and verification of test dog identity, as well as to inspect Intervet facilities and study-related records.

After the challenge, dogs were observed daily for a minimum of 90 days for clinical signs typical of rabies virus infection, including but not limited to excessive salivation or urination, anorexia, adipsia, dysphagia, depression, vicious behavior, paralysis, coma, and death.

At the end of the postchallenge observation period, brain tissue from each test animal that died after the challenge was examined for the presence of rabies virus using the fluorescent antibody (FA) test. All challenge survivors were humanely euthanized after the postchallenge observation period. Brain tissue taken posthumously from all challenge survivors was examined to confirm the absence of rabies virus through FA testing and by intracerebral inoculation of laboratory mice with homogenized brain tissue.

### RESULTS

#### Serologic Tests

Serologic tests were performed to determine the presence of rabies virus antibodies in the vaccinated dogs. As required by 9 CFR § 113.109, all dogs were confirmed seronegative for neutralizing antibodies to rabies virus on the day of initial vaccination as demonstrated by RFFIT results from individual sera samples. After vaccination, serum antibody titers for rabies virus were measured every 1 to 6 months throughout the 36-month postvaccination isolation period using RFFIT analysis (Table 1). RFFIT titer results indicated that all vaccinated dogs in the study responded serologically to the test vaccine.

#### Clinical Signs of Disease Evaluated

Clinical signs of disease evaluated during the 90-day postchallenge observation period included but were not limited to excessive salivation or urination, anorexia, adipsia, drinking, dysphagia, depression, vicious behavior, paralysis, coma, and death.

### CDV, CAV-1, and CPV Challenge Results

Challenge-of-immunity efficacy study re-

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**TABLE 1. Geometric Mean Rabies Virus Antibody Titers in Dogs Following Vaccination against CDV, CAV-2, CPV, and Rabies Virus**

<table>
<thead>
<tr>
<th>Virus Fraction (Assay)</th>
<th>Prevaccination</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>30</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies (RFFIT)</td>
<td>&lt;5</td>
<td>116</td>
<td>34</td>
<td>111</td>
<td>28</td>
<td>46</td>
<td>18</td>
<td>17</td>
<td>35</td>
<td>15</td>
</tr>
</tbody>
</table>

*Dogs were initially vaccinated at 8 weeks of age with CDV, CAV-2, CPV, and CPIV and received a booster vaccination with CDV, CAV-2, CPV, CPIV, and rabies virus at 12 weeks of age.*
sults for CDV, CAV-1, and CPV were reported previously. These results indicated 100% protection in vaccinates against clinical signs of disease for a duration of at least 3 years after virulent challenge. Additional study results reported here exceeded 9 CFR requirements to establish rabies vaccine efficacy and indicated that no immunogenic interference occurred between the modified-live vaccine components and the killed rabies virus component.

Canine Rabies Virus Challenge Results

During the postvaccination observation period, all dogs remained healthy and active. None of the dogs exhibited systemic reactions or elevated temperatures (≥103.4°F). During injection-site palpation, some of the vaccinated test dogs had thickening or lumps detectable only on palpation of the affected area. All of the observed thickening or lumps were painless and dissipated within 23 days following vaccination.

Following virulent rabies virus challenge 36 months after administration of the last vaccination, 30 of 31 (97%) control dogs developed signs of rabies, including excessive salivation and urination, anorexia, dysphagia, depression, paralysis, coma, and death (Table 2). In contrast, only four of 32 (12%) vaccinates developed clinical signs of rabies following virulent challenge. Similarly, just one (3%) control dog survived virulent challenge, whereas 28 (88%) vaccinates were protected from clinical signs of disease. There was a significant difference (P < .001) in disease incidence between vaccinates and controls. These results also exceeded the 9 CFR requirements that at least 80% of controls must die as a result of rabies infection following challenge while at least 87% of vaccinates survive.

Rabies infection was confirmed via FA testing of brain tissue, as sanctioned by 9 CFR. Direct FA testing of brain tissue is considered to be the definitive diagnostic test to detect the presence of rabies virus.

TABLE 2. Infection and Death Rates in Dogs Following Virulent Rabies Virus Challenge

<table>
<thead>
<tr>
<th>Test Group</th>
<th>No. of Dogs</th>
<th>Postchallenge Fluorescent Antibody–Positive for Rabies Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>32</td>
<td>28 (88%)</td>
</tr>
<tr>
<td>Controls</td>
<td>31</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Study results demonstrated that one dose of the killed rabies component in the test vaccine provided protection against virulent rabies challenge in dogs 12 weeks of age or older for a minimum of 3 years after vaccination. In a normal clinical situation, rabies vaccines are administered to animals at 3 months of age or older, with a repeat dose given 1 year later. Therefore, it is expected that the efficacy of the vaccine described in this study should be further enhanced with a prime-boost regimen. Furthermore, challenge-of-immunity study results indicated that no immunogenic interference occurred between the modified-live virus and killed rabies virus vaccine components when used together in a four-way core combination vaccine. To our knowledge, this is the first time that the duration of immunity of a multivalent vaccine including all four core canine antigens specified by the AAHA has been critically evaluated via 3-year challenge-of-immunity studies and subsequently approved by the USDA for use at triennial revaccination intervals.

These study results are especially significant to the veterinary medical profession in light of the following facts:

![Vertebrate Therapeutics](image.png)
• The 2006 AAHA Canine Vaccine Guidelines continue to encourage appropriate incorporation of 3-year core revaccination protocols.5

• The 2003 AAHA Canine Vaccine Guidelines, which first recommended extended core revaccination intervals, were criticized by some as lacking sufficient scientific support.5

• The new 2006 AAHA guidelines in general offer more scientific data supporting extended vaccine-induced duration of immunity. AAHA also indicates that only one non–rabies vaccine has achieved a USDA duration-of-immunity claim for 3-year use. AAHA does not recommend particular brands or vaccines and specifically indicates that the task of vaccine selection is best left to clinicians. This selection process presents a challenge for practitioners who would like to implement 3-year protocols.

The particular relevance of these study data lie in the real-time challenge-of-immunity methodologies used. Challenge data are considered to be the gold standard when evaluating vaccine efficacy and immunity conferred in dogs.1,6 Alternatives to challenge studies, such as analyses of serologic data alone, are generally considered unreliable and not acceptable for establishing the efficacy of a vaccine.1,6 In particular, although laboratory tests have improved greatly in the past several years and titers can be used to determine whether an animal has responded to vaccination,5 rabies has been cited as one of the diseases for which titers provide less useful data regarding the level of disease protection.18 In fact, in the past, some manufacturers were permitted to market rabies vaccines based on serologic evidence alone. Unfortunately, it was found that serologic test results were not predictive of resistance to challenge exposure, and those vaccines had to be withdrawn.18

Additional scientific data, particularly challenge data, have been called for by the veterinary community to give practitioners more concrete information to evaluate when determining the best options for their patients.1,6,7 Studies show that data from one killed or modified-live vaccine cannot be used to demonstrate the postchallenge efficacy of another vaccine—there are simply too many differences among vaccines.18,19 Individual vaccines within a product category may differ in the duration of immunity they elicit as a result of antigen quantity and quality, the adjuvant used (if any), degree of attenuation, the master seed used, and methods of antigen preparation.6 COBTA has indicated that duration-of-immunity claims, in terms of both minimum and maximum length, are best answered by scientific study rather than arbitrary summation.1

In the past, the dearth of vaccines with specific 3-year challenge-of-immunity data and corresponding USDA label claims meant that veterinarians who decided to follow association recommendations for extended core-antigen revaccination intervals had to either use sometimes inconvenient protocols (e.g., separate 3-year DAP vaccines or annual DAP vaccines combined with 3-year rabies virus vaccine) or use vaccines on an extralabel basis. Although it is certainly an option for veterinarians to administer a vaccine in a manner inconsistent with its label recommendations, to do so may carry increased risks of litigation.19,20 Also, the most responsible approach to deviations from product labeling would be to base them on immunologic and scientific evidence, the preven-
tive care needs of the patient, and informed consent from the client.\textsuperscript{19,21}

Another concern veterinarians have expressed regarding extended revaccination protocols has been the possibility of fewer patient visits and decreased quality of care. Veterinarians can avoid this possibility by stressing the importance of all aspects of comprehensive personalized health care as the basis for annual visits.\textsuperscript{5} Practitioners should emphasize physical examinations and an individual patient focus on dental care, nutrition, appropriate diagnostic testing, parasite control, and any specific behavior or age-related concerns.\textsuperscript{5}

The major veterinary associations and peer-reviewed literature encourage veterinarians to develop vaccination schedules based on individual patient risk-assessment factors in the context of the veterinarian–client–patient relationship rather than on routine protocols. Vaccination needs should be assessed yearly as part of regular wellness visits based on the dog’s age, breed, health status, environment, lifestyle, and travel habits.\textsuperscript{5} Additional appropriate considerations include probable disease susceptibility, severity of the disease in question, vaccine efficacy and safety, public health concerns, and owner preferences.\textsuperscript{1} Based on these individual risk assessments, practitioners may determine that many of their canine patients require additional non-core antigens to protect against likely exposure to \textit{Leptospira} spp, \textit{Bordetella bronchiseptica}, \textit{Borrelia burgdorferi}, or canine parainfluenza virus.

COBTA sums it up best: “Currently, recommendations reflect what has always been true—vaccination is the complex use of medically powerful agents for which important medical decisions on relative risks and benefits must be individualized to the needs of the animal.”\textsuperscript{21}

\section*{CONCLUSION}

Using real-time challenge-of-immunity methodologies, the study results met or exceeded 9 CFR requirements for 3-year rabies vaccine licensing to demonstrate that the killed rabies component in the test vaccine provided protection against virulent rabies challenge in dogs 12 weeks of age or older for a minimum of 3 years following virulent challenge. Both challenge-of-immunity and serologic titer measurements after vaccination also indicated that no immunologic interference occurred between the modified-live viral components and the killed rabies virus. These results provide additional new scientific evidence in the form of gold standard challenge data, coupled with corresponding USDA labeling, to aid practitioners in making decisions concerning extended revaccination intervals and related vaccine selection.

These results also offer another protocol option and complete support for practitioners who choose to implement the AAHA’s continued recommendation for triennial revaccination intervals for all four core canine antigens. The profession asked that the AAHA guidelines recommending triennial core revaccination be enhanced and reinforced as based in science; this study and its previously published predecessor (providing 3-year DAP data) are the only studies that have followed 9 CFR guidelines to deliver the real-time gold standard challenge data requested by the profession.

\section*{ACKNOWLEDGMENTS}

We thank Intervet employees Scott Banks, BS, for his technical contributions, and Michael J. Garrison, BS, RLATG, and Cathy McCabe for their assistance in procuring animals and study facilities.

\section*{REFERENCES}


9. Animals and animal products, 9 CFR §§ 113.305 (canine hepatitis and adenovirus type 2 vaccine), 113.306 (canine distemper vaccine), and 113.317 (parvovirus vaccine [canine]), 2004.


