CANINE TECHNICAL REPORT

Distemper
Adenovirus
Parvovirus

Research & Development

Duramune® Adult
Three-Year Duration of Immunity Vaccination Challenge Studies

Fort Dodge Animal Health
CANINE TECHNICAL REPORT

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Dear Doctor,

Fort Dodge Animal Health is a leader in the industry of cutting-edge animal vaccine technology. Our pioneering efforts have produced recent groundbreaking firsts such as Fel-O-Vax® FIV and West Nile - Innovator™. In addition to these well-recognized scientific achievements, we have also continued to improve existing products with new field isolates, better adjuvants and additional duration of immunity data based on challenge studies.

More than four years ago, Fort Dodge initiated a long-term vaccination challenge study to determine if three main canine antigens, canine distemper virus (CDV), canine adenovirus type 2 (CAV2) and canine parvovirus (CPV), protected for three years. These studies were conducted under strictly controlled laboratory conditions to ensure the best possible evaluation of the product. The dogs in this study were isolated during the entire study period to eliminate any possible outside influences on their response to challenge. The purpose of this technical report is to review the latest information our Research and Development Department has generated on strains of CDV, CAV2 and CPV.

Results indicated that three years after vaccination, the antigens of CDV, CAV2 and CPV in Duramune® Adult all demonstrated a high degree of efficacy against virulent challenges of canine distemper virus, infectious canine hepatitis virus (CAV1) and canine parvovirus. The USDA has allowed Fort Dodge to reference this data on the vaccine label and include it on the package insert for Duramune® Adult.

The foundation of a sound preventative health program includes twice-a-year visits, comprehensive wellness exams and appropriate vaccinations. For most diseases and most dogs, annual protocols will continue to be the standard, based on a thorough assessment of disease risk. If risk assessment indicates a modified protocol is appropriate, use Duramune® Adult with confidence because it is backed by three-year challenge data to support your recommendations. The Responsible Healthcare for Pets (RHP) Program, developed by Fort Dodge, provides tools to assist you in educating your customers about vaccines. Your Fort Dodge Territory Manager can provide you with additional information about vaccination protocols and the RHP program.

Mike LaRosh, DVM
Director
Professional Services
Fort Dodge Animal Health
Evaluation of Three-Year Duration of Immunity of Canine Distemper Virus Antigen Fraction of Duramune® Adult in 6-Week-Old Puppies.

OBJECTIVE

The objective of this study is to confirm three-year duration of immunity of the CDV strain of Duramune® Adult. In this study, the three-year duration of immunity testing of the CDV fraction was done by challenging dogs with virulent CDV.

MATERIALS AND METHODS

A group of 13 healthy dogs, all approximately 6 weeks of age, were used in the study. The dogs were housed in isolation facilities and were under veterinary care for the duration of the study. The animals were fed a standard diet of water and commercial food.

Controls and vaccinates were housed separately in isolation facilities during the immunization period and until they were challenged to prevent exposure to vaccine virus shedding. During the baseline and challenge procedures, the controls and vaccinates were housed together in an isolation facility.

The seronegative 6-week-old puppies were randomly separated into three groups as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>Dose</th>
<th>Number of Puppies</th>
<th>Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>IM</td>
<td>1 mL</td>
<td>5 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>SC</td>
<td>1 mL</td>
<td>5 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Controls</td>
<td>N/A</td>
<td>N/A</td>
<td>3 puppies</td>
<td>Unvaccinated controls</td>
</tr>
</tbody>
</table>

Injection sites were in the left or right rear legs for IM administration, or in the dorsal aspect of the nape of the neck for SC administration.

**CDV Challenge and Observation Procedure**

Three years after the second vaccination, 10 vaccinates and three unvaccinated age-matched control dogs were challenged intracerebrally with the Snyder Hill strain of CDV. The animals were observed on –4 and from –2 to 0 DPC to establish a baseline, and from 1 to 21 DPC for clinical signs, including but not limited to nasal and ocular discharge, conjunctivitis, depression/lethargy, inappetence, vomiting and coughing.
RESULTS AND DISCUSSION

Protection provided by Duramune Adult was measured by post-challenge clinical scores. Clinical scores were based on morbidity, mortality, and signs of nasal and ocular discharge, conjunctivitis, depression/lethargy, inappetence, vomiting, coughing and death.

Clinical Observations

Following CDV challenge, all 10 vaccinates appeared healthy and active following virulent CDV challenge, with no mortality or apparent signs of distemper. Only mild and isolated transient clinical signs were observed in some of the vaccinated dogs.

In contrast, all unvaccinated dogs in the control group were highly susceptible to CDV infection. One dog exhibited signs of inappetence and mild to moderate conjunctivitis, and died eight days post challenge. Another dog exhibited mild to moderate conjunctivitis and mucopurulent ocular discharge followed by severe conjunctivitis, depression, mucous and bloody stool, retching and seizures, and was euthanized on 12 DPC based on humanitarian grounds. The third control dog survived the CDV challenge, but exhibited clinical signs of distemper, including mild to severe conjunctivitis, mucopurulent ocular discharge, inappetence, depression and bloody stool from 2 to 21 DPC.

The results of clinical observations showed significant difference in clinical manifestation of CDV infection between vaccinated and control group dogs following virulent CDV challenge. Acute canine distemper illness with significantly high mortality was observed in two out of three control animals, compared to no mortality in the vaccinated dogs.

Study Conclusion

The results of the long-term duration of immunity study with Fort Dodge Animal Health’s Duramune Adult vaccine demonstrate a modified-live CDV strain is efficacious to protect puppies 6 weeks of age and older against virulent CDV infection for up to three years following second vaccination.
OBJECTIVE

The objective of this study is to confirm the three-year duration of immunity of the CAV2 strain of Duramune® Adult against infectious canine hepatitis virus (ICHV). In this study, the three-year duration of immunity testing of the CAV2 antigen fraction was performed by challenging dogs with a virulent ICHV strain (CAV1).

MATERIALS AND METHODS

A group of 14 healthy dogs, all approximately 6 weeks of age, were used in the study. The dogs were housed in isolation facilities and were under veterinary care for the duration of the study. The animals were fed a standard diet of water and commercial food.

Controls and vaccinates were housed separately in isolation facilities during the immunization period and until they were challenged to prevent exposure to vaccine virus shedding. During the baseline and challenge procedures, the controls and vaccinates were housed together in an isolation facility.

The seronegative 6-week-old puppies were randomly separated into three groups as follows:

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<th>Number of Puppies</th>
<th>Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>IM</td>
<td>1 mL</td>
<td>3 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>SC</td>
<td>1 mL</td>
<td>9 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Controls</td>
<td>N/A</td>
<td>N/A</td>
<td>2 puppies</td>
<td>Unvaccinated controls</td>
</tr>
</tbody>
</table>

Injection sites were in the left or right rear legs for IM administration, or in the dorsal aspect of the nape of the neck for SC administration.

ICHV Challenge and Observation Procedure

Three years after the second vaccination, 12 vaccinates and two unvaccinated age-matched control dogs were challenged with ICHV challenge material. The animals were observed on –4 and from –2 to 0 DPC to establish a baseline, and from 1 to 21 DPC for clinical signs of infectious canine hepatitis, such as depression, lethargy, inappetence, ocular discharge, conjunctivitis, nasal discharge, tonsillitis, redness of the buccal mucosal membranes and death.
RESULTS AND DISCUSSION

Protection provided by Duramune *Adult* was measured by post-challenge clinical scores. Clinical scores were based on morbidity, mortality, and signs of depression, lethargy, inappetence, ocular discharge, conjunctivitis, nasal discharge, tonsillitis, redness of the buccal mucosal membranes and death.

Clinical Observations

Following ICHV challenge, only three vaccinates showed mild transient depression, mild conjunctivitis or tonsillitis for one day on 11 DPC. The rest of the dogs appeared healthy and active following virulent ICHV challenge.

In contrast, both puppies in the control group were sick and weak throughout the 21-day post-challenge observation period. In addition to high fever, the control puppies exhibited clinical signs, such as ocular discharge, severe conjunctivitis, severe nasal discharge, lethargy, inappetence, tonsillitis and reddening of buccal mucosal for four to 16 days.

A significant difference was seen in the number of days of clinical signs observed between the vaccinates and controls. The controls were found to be significantly more susceptible to ICHV infection as compared to vaccinates in the same age group. No mortality was observed in the control or vaccinate group, probably due to increased resistance to ICHV infection, as the dogs were more than 3 years of age at the time of challenge.

![Duramune Adult 3-Year D0I: ICHV Clinical Scores](image)

Study Conclusion

The results of the long-term duration of immunity study with Fort Dodge Animal Health’s Duramune *Adult* vaccine demonstrate a modified-live CAV2 strain is efficacious to protect puppies 6 weeks of age and older against virulent ICHV infection for up to three years following second vaccination.

Infectious canine hepatitis is a virulent virus and causes more serious disease than CAV2. A vaccine with CAV2 that demonstrates cross-protection against ICHV (CAV1), is the indicator of efficacy against either a CAV1 or CAV2 challenge. These results support the CAV2 efficacy against both a CAV1 and a CAV2 challenge.
**Parvovirus**

**Evaluation of Three-Year Duration of Immunity of Canine Parvovirus Antigen Fraction of Duramune® Adult in 6-Week-Old Puppies.**

**OBJECTIVE**

The objective of this study is to confirm three-year duration of immunity of the canine parvovirus (CPV) strain in Duramune® Adult. In this study, the three-year duration of immunity testing of the parvovirus fraction was done by challenging dogs with virulent CPV.

**MATERIALS AND METHODS**

A group of 13 healthy dogs, all approximately 6 weeks of age, were used in the study. The dogs were housed in isolation facilities and were under veterinary care for the duration of the study. The animals were fed a standard diet of water and commercial food.

Controls and vaccinates were housed separately in isolation facilities during the immunization period and until they were challenged to prevent exposure to vaccine virus shedding. During the baseline and challenge procedures, the controls and vaccinates were housed together in an isolation facility.

The seronegative 6-week-old puppies were randomly separated into three groups as follows:

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<tr>
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<tbody>
<tr>
<td>Vaccinates</td>
<td>IM</td>
<td>1 mL</td>
<td>5 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>SC</td>
<td>1 mL</td>
<td>5 puppies</td>
<td>1st vaccination at 6 weeks of age or less; 2nd vaccination at 21 DPV1.</td>
</tr>
<tr>
<td>Controls</td>
<td>N/A</td>
<td>N/A</td>
<td>3 puppies</td>
<td>Unvaccinated controls for CPV</td>
</tr>
</tbody>
</table>

Injection sites were in the left or right rear legs for IM administration, or in the dorsal aspect of the nape of the neck for SC administration.

**CPV Challenge Procedure**

Three years after the second vaccination, 10 vaccinates and three unvaccinated age-matched control dogs were challenged orally and intranasally with the CPV-2b strain. The animals were observed on –4 and from –2 to 0 DPC to establish a baseline, and from 1 to 14 DPC for various clinical signs of CPV disease, such as fever, leukopenia, diarrhea, mucous/bloody/watery feces and death.
RESULTS AND DISCUSSION

Protection provided by Duramune Adult was measured by post-challenge clinical scores. Clinical scores were based on morbidity, mortality, and signs of fever, leukopenia, diarrhea, mucous/bloody/watery feces and death.

Clinical Observations

Following CPV challenge, all 10 vaccinates appeared healthy and active. Only mild and transient clinical signs of anorexia and dehydration were seen in seven of 10 vaccinates for one to six days between 0 DPC and 14 DPC. Two dogs vomited one time during the post-challenge observation period.

All unvaccinated dogs in the control group were sick and weak with mild (anorexia, lethargy/depression, dehydration, coughing and vomiting) to severe (watery to bloody stool) clinical signs for two to 10 days between 2 DPC and 13 DPC. CPV was consistently isolated in the feces of all control dogs for two to five days between 4 and 9 DPC. Only one IM vaccinate shed the virus for one day on 8 DPC. No CPV shedding was seen in the SC vaccinates during the post-challenge observation period. The differences in the average of total virus shedding and the number of days of virus shedding between the vaccinate group and control dogs were significant following virulent CPV challenge.

No mortality was seen in any of the treatment groups. However, the severity of CPV disease-specific clinical signs following challenge was highly marked in the control group.

Study Conclusion

The results of the long-term duration of immunity study with Fort Dodge Animal Health’s Duramune Adult vaccine demonstrate the modified-live CPV is protective and efficacious in 6-week-old puppies against virulent CPV challenge for up to three years following second vaccination.