Serological Response of Puppies to the Selected Canine Vaccines and Vaccination Schedules against Canine Distemper Virus

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Abstract: This study was undertaken to compare the serological response of dogs to four commercially available combination vaccines and three different vaccination schedules to canine distemper virus (CDV). A total of 120 healthy puppies (20 puppies per group) at 6 weeks of age were randomly assigned to one of four vaccines [C, G, K, and V (or V3) groups] and one of vaccination schedules [V2 and V4 groups]. At six, nine, and 12 weeks of age, puppies in each group were vaccinated with one of four combination vaccines subcutaneously. And puppies in V2 and V4 groups were vaccinated with V vaccine every 2 weeks and 4 weeks, respectively. The serological responses to CDV component of the vaccines were determined by measuring SN titers. The immunogenicity of V vaccine was superior to the other vaccines and optimum vaccination schedule was 3 times vaccination with 3 weeks-interval starting vaccination at 6 weeks of age. Although puppies were vaccinated at 6 weeks of age, the geometric mean CDV titers of puppies in all groups by 9 weeks of age were under the protective level. Therefore, prophylactic measures should include isolation of young dogs from the dog population until vaccination can be expected to provide protection.

Key words: canine distemper virus, vaccine, vaccination schedule, SN titer

Introduction

Canine distemper (CD) is a highly contagious, acute, or subacute systemic viral disease which often has a high mortality rate in dogs and other carnivores. The effective measure to control CD is, at present, immunization by vaccination, even if classical hygienic measures can be applied in parallel.

Active immunization became very successful after live attenuated vaccines became available: ferret-passaged modified vaccine, modified hen egg virus vaccine, modified cell culture virus vaccines, heterotypic measles vaccine, and combined vaccines. Purified vaccines and recombinant DNA vaccines prepared over the past few decades remain at an experimental stage.

A number of factors influence the level of antibodies attained. And the most important of which are probably the intrinsic properties of the virus strain, including its epitope structure and its ability to multiply in the susceptible cells. The efficacy of a vaccine depends also on its method of production and, to some extent, on the number of attenuated virus particles in a dose. Further vaccine-related factors are the other antigens included in the vaccine, storage and handling of vaccine, the frequency of vaccinations and vaccination procedure.

Host-related factors include age, health, especially with respect to immunocompetence, and possibly breed. Early in life, maternal antibodies may interfere active immunization and the immune system may not be fully matured. Aging also leads to the impairment of humoral and cellular immunity. Pregnancy, immunocompromising diseases, and medication with cortisone may suppress the immune response. Gender is usually not known to affect the antibody levels induced.

The current recommended vaccination protocol is that puppies should be given a series of vaccinations to stimulate active immunity as maternally derived immunity declines. And a series of vaccination seems to be widely accepted. Morbillivirus vaccination failures are not uncommon. Several outbreaks of CD among vaccinated dogs have been reported in some countries. During the 1980s, outbreaks of distemper occurred in dogs throughout Europe and, in 1990s, the disease reappeared in Finland after an absence of 16 years. A previous study of dogs that succumbed to CD in spite of vaccination suggested that a critical decline in population immunity had contributed to severity of the outbreak. Although the level of neutralizing antibodies alone does not predict the protection of an individual against infection, the average level indicates the immune status of the population.

The recent outbreaks of CD were reported from mid-1990s in Korea as well as other countries even though modified live canine distemper virus (CDV) vaccine has been used as the same way as before. This study was undertaken to compare the serological response in puppies to four commercially available combination vaccines and three different vaccination schedules against CDV.

Materials and Methods

Experimental animals

1) Efficacy of vaccines

A total of 120 healthy puppies at 6 weeks of age were included in this study. These puppies were presented for vacci-
cination by owner in 9 local animal clinics between March, 2002 and October, 2002. After owners were in compliance with participation in this study, puppies at six weeks of age (20 puppies per group) were randomly assigned to one of six groups shown in Table 1. Each puppy was reared in owner’s house and managed according to the ownership. Antihelmintics were administered at beginning of the study.

2) Change of maternal antibodies

To observe the declining pattern of maternal antibodies of puppies, seven healthy mixed-breed puppies were used. These puppies were born from a bitch which was vaccinated 2 times with commercial combination vaccine containing CDV at 6 month interval before pregnant, and were reared for 7 weeks with the dam. Puppies were weaned at 7 weeks of age and managed in individual cage and provided with commercial dog feed and fresh water ad libitum by 17 weeks of age. Blood were collected every week during the experimental period. A serum neutralizing (SN) antibody titer of ≥ 1:16 against CDV was considered as a protective level\(^2\).

**Vaccines**

Two commercial combination vaccines (G and K vaccines) were produced in Korea and two vaccines (C and V vaccines) were imported from USA. Each vaccine contained modified-live CDV, canine adenovirus type 2, canine parvovirus (CPV), and canine parainfluenza virus in a lyophilized form and *Leptospira canicola-icterohaemorrhagiae* bacterin in a liquid form that was used as the vaccine diluent.

At six weeks of age, each puppy in each group was vaccinated with one of four vaccines. Revaccination was administered at 8, 9, 10, 12, and 14 weeks of age according to Table 1. Vaccines were administered subcutaneously in the dorsal aspect of the neck or thorax. Postvaccinal adverse effects were not observed in all vaccines.

**Serum neutralization test**

The serum neutralizing (SN) antibodies against CDV were determined with a minor modification of the microneutralization test described by Appel and Robson\(^2\). Briefly, two fold serum dilutions of 50 µl were prepared. A total of 50 µl of the minimum essential medium with 200 median tissue culture infective dose of the Ledler strain was added to each well. And then, plate was incubated at 37°C for one hour. A total of 100 µl of the Vero cell suspension (1 × 10^5 cells/well) was added to each well, and the titration plates were incubated at 37°C in 5% CO\(_2\) for 5 days. The test was read microscopically everyday for five days and check cytopathogenic effect (giant cell formation). A standard virus and a positive control serum were included in each round of test.

**Statistical analysis**

Prior to statistical analysis, all titers were converted to natural logarithms and geometric mean CDV SN titers were determined for each sample period. The week that each puppy seroconverted, the overall percentage of puppies in each group that had seroconverted at each sample period, and the mean and standard deviation of the week of seroconversion for each group were calculated.

In this study, seroconversion was defined as a four-fold increase in titer when compared to the titer prior to vaccination. A repeated-measures analysis of variance (ANOVA) was used to compare between-group titers or differences in regard to number of puppies that had seroconverted at the time of each vaccination, using Tukey’s multiple comparison test. A values of \(p\) less than 0.05 were considered significant. All analyses were performed with computer software package SAS (version 8.1 for Windows).

**Results**

**Change of maternal antibodies**

To observe the declining pattern of maternal antibodies of puppies, the 7 puppies ingested dam’s milk were examined for 17 weeks after birth. After ingestion of colostrum, the geometric mean titer of maternal antibodies against CDV of puppies were 105.5 / GB7 31.2. The maternal antibodies at 6 weeks of age were declined to 9.8 / GB7 3.9 which was under the protective level (Fig 1).

**Table 1.** Experimental groups designed in this study

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Number of dogs</th>
<th>Number of vaccination</th>
<th>Interval of administration (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>20 (18)*</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>G</td>
<td>20 (18)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>K</td>
<td>20 (17)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>V (or V3)</td>
<td>20 (20)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>20 (19)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>V4</td>
<td>20 (17)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120 (109)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The number in parenthesis is number of dogs which were provided all data and finally used for statistical analysis.

**Fig 1.** The change of geometric mean serum neutralization (SN) titer of maternal antibodies of 7 puppies against canine distemper virus. The SN titer were checked for 17 weeks after birth.