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

Doo Kim[1], So-jeo Ahn, Seok-young Jeoung, Jae-woong Hwang, and Son-il Pak

Department of Veterinary Medicine, Kangwon National University, Chunchon 200-701, Korea

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[5].

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[5,16,25,26,29]. Purified vaccines and recombinant DNA vaccines prepared over the past few decades remain at an experimental stage[5,6,8,18,21].

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 passage level, 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[15].

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[7,11,22,27]. Aging also leads to the impairment of humoral and cellular immunity[30]. Pregnancy, immunocompromising diseases, and medication with cortisone may suppress the immune response. Gender is usually not known to affect the antibody levels induced[28].

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[19,23,28,31].

Morbillivirus vaccination failures are not uncommon[3]. Several outbreaks of CD among vaccinated dogs have been reported in some countries[3,10,12,13,17,20]. 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[10,13]. 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[10]. 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[15].

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[1,34]. 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 vac-

[1]Corresponding author.
E-mail : kimdoo@kangwon.ac.kr

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 begining 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 $\geq 1:16$ against CDV was considered as a protective level[23].

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. And 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 ± 31.2. The maternal antibodies at 6 weeks of age were declined to 9.8 ± 3.9 which was under the protective level (Fig 1).

Table 1. Experimental groups designed in this study

Experimental groups	Number of dogs	Number of vaccination	Interval of administration (Weeks)
C	20(18)*	3	3
G	20(18)	3	3
K	20(17)	3	3
V (or V3)	20(20)	3	3
V2	20(19)	5	2
V4	20(17)	3	4
Total	120(109)		

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

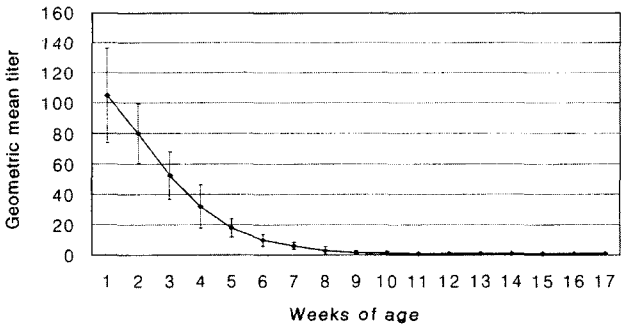

Fig 1. The change of geometric mean serum neutralization (SN) titer of maternal antibodies of 7 puppies against canine distemper virus. The SN titer were checked for 17 weeks after birth.

Comparison of seroconversion rate of puppies by four commercial vaccines

Geometric mean CDV SN titer prior to the first vaccination at six weeks of age (i.e., maternally derived antibody titer) was 6.1 ± 7.2 (range, 2 to 32), 7.4 ± 7.1 (range, 2 to 32), 8.3 ± 7.2 (range, 4 to 32), and 5.3± 3.3 (range, 0 to 16) in vaccine group C, G, K, and V, respectively.

All 20 puppies of vaccine V group seroconverted by 15 weeks of age. Eight (40.0%) seroconverted after the first vaccination at six weeks, and twelve puppies (60.0%) seroconverted after the second vaccination at nine weeks of age (Fig 2). However, 16 (88.9%) out of 18 puppies of vaccine C group seroconverted by 15 weeks of age. Two (11.1%) seroconverted after the first vaccination, six (33.3%) seroconverted after the second vaccination, and eight (44.4%) seroconverted after the third vaccination. Similarly, 15 (83.3%) out of 18 puppies of vaccine G group seroconverted by 15 weeks of age. None (0.0%) seroconverted after the first vaccination, eight (44.4%) seroconverted after the second vaccination, and seven (38.9%) seroconverted after the third vaccination. And 16 (94.1%) out of 17 puppies of vaccine K group seroconverted by 15 weeks of age. Two (11.8%) seroconverted after the first vaccination, seven (41.2%) seroconverted after the second vaccination, and seven (41.2%) seroconverted after the third vaccination. At each observation period after vaccination, the seroconversion rate of vaccine V group was significantly higher than those of vaccine C, G, and K groups ($p < 0.05$).

The geometric mean SN titer against CDV of puppies of vaccine V group was increased sharply after the second vaccination, however, those of puppies of vaccine C, G, and K groups were increased slightly. The geometric mean SN titer was significantly higher for puppies of vaccine V group than those for puppies of vaccine C, G, and K groups at 3 weeks after the second and third vaccination ($p < 0.05$) (Fig 3). The mean titer peaked at three weeks after third vaccination for puppies of all vaccine groups. Although puppies were vaccinated at 6 weeks of age, the geometric mean SN titers of puppies in all groups (C, G, K, and V groups) by 9 weeks of age were under the protective level.

Comparison of seroconversion rate of puppies by three different vaccination schedules

Geometric mean SN titer against CDV prior to the first vaccination at six weeks of age (i.e., maternally derived antibody titer) was 6.7 ± 6.6 (range, 2 to 32), 5.3 ± 3.3 (range, 0 to 16), and 2.3 ± 1.1 (range, 0 to 4) for puppies in V2, V3 and V4 group, respectively.

All 19 puppies of V2 group seroconverted by 17 weeks of age. Four (21.1%) seroconverted after the first vaccination at six weeks, six (31.6%) seroconverted after the second vaccination at eight weeks of age, six (31.6%) seroconverted after the third vaccination at 10 weeks of age, and remaining three (15.8%) seroconverted after the fourth vaccination at 12 weeks of age (Fig 4). All 20 puppies of V3 group seroconverted by 15 weeks of age. Eight (40.0%) seroconverted after the first vaccination at six weeks, and twelve (60.0%) seroconverted after the second vaccination at nine weeks of age. Similarly, all 17 puppies of V4 group seroconverted by 17 weeks of age.

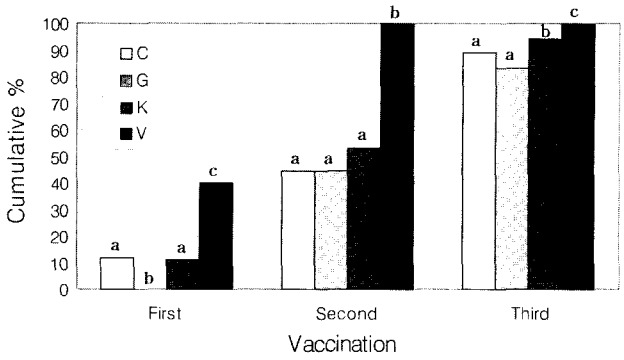

Fig 2. Cumulative percentages of puppies that seroconverted, determined on the basis of serum neutralizing titers against canine distemper virus. All puppies were vaccinated at six, nine, and 12 weeks of age with C, G, K, and V vaccines.

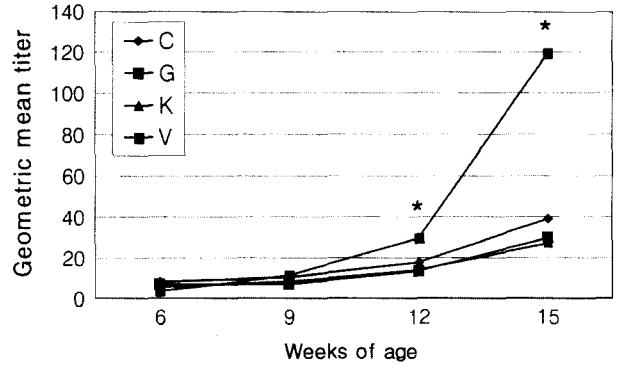

Fig 3. The changes of geometric mean serum neutralization (SN) titers against CDV in puppies in four vaccine groups. SN titer at 9 and 12 weeks of age were significantly ($p<0.05$) different between V group and other groups.

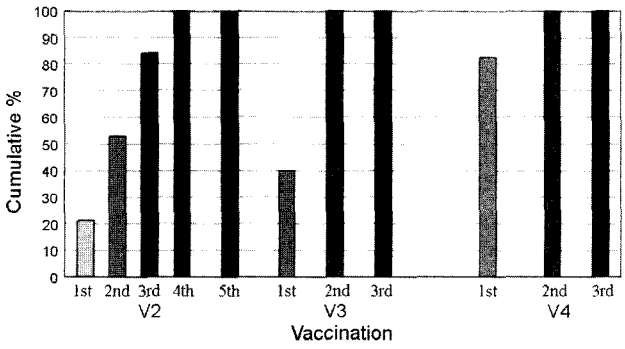

Fig 4. Cumulative percentages of puppies that seroconverted, determined on the basis of serum neutralizing titers against canine distemper virus, after vaccination with vaccine V at different weeks of age, interval and frequency according to protocol of Table 1.

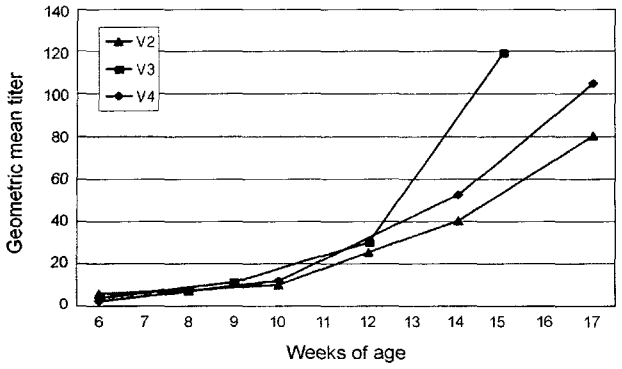

Fig 5. The changes of geometric mean serum neutralizing titers against canine distemper virus in puppies after vaccination with vaccine V at different weeks of age, interval and frequency according to protocol of Table 1.

14 (82.4%) seroconverted after the first vaccination at six weeks and remaining three (17.7%) seroconverted after the second vaccination at 10 weeks of age.

The geometric mean SN titers against CDV of puppies in all three groups (V2, V3, and V4 group) were increased sharply after 10 weeks of age. However, the geometric mean CDV titer of puppies in V3 group was increased rapidly and higher than those in V2 and V4 groups (Fig 5). Although puppies were vaccinated at 6 weeks of age, the geometric mean SN titers against CDV of puppies in all three groups (V2, V3, and V4 group) by 9 weeks of age were also under the protective level.

Discussion

Passively derived maternal antibodies not only inhibit neonatal immunoglobulin synthesis but also prevent the successful vaccination of young animals. This refractory period may persist for many months, and its length depends on the amount of antibody transferred to the neonate and the half-life of the immunoglobulins involved[32]. On the average, the level of passively acquired antibodies to CD in puppies will have declined to insignificant levels by about 10 to 12 weeks, although in extreme cases they may persist for as long as 16 to 20 weeks[32]. Therefore, investigating how maternal antibody concentration in dogs in the field setting relates to the age at which most puppies are vaccinated is essential for the development a vaccination protocol. In this study, the maternal antibodies of seven puppies against CD were declined to under the protective level at 6 weeks of age. The time declined to under the protective level was earlier than those of other study results[31,32]. And this decline in maternal antibody titer often coincides with the time that puppies are separated from their dams, which increase the risk of being exposed to the virus and becoming infected[7]. Therefore, it was considered that vaccination of puppies for CDV in Korea should be started at 6 weeks of age.

The use of a vaccine should decrease the number of puppies that become infected with CDV. In addition, use of a vaccine that protects puppies earlier after vaccination should help compensate for those owners who do not ensure that their puppies complete the recommended initial vaccination schedule[33]. The most of puppies in four vaccine groups in this study seroconverted to CDV after vaccination. However, at each observation period, the seroconversion rate of puppies in vaccine V group was significantly higher than those of puppies in vaccine C, G, and K groups. In addition, puppies in vaccine V group seroconverted earlier than puppies in vaccine group C, G, and K groups regardless of SN titers. Thus, results of this study suggest that puppies vaccinated with vaccine V would be protected earlier than those of puppies vaccinated with other vaccines.

Among the vaccinated dogs in this study, some did not reach sufficient titer to protect CD. This does not necessarily mean that all these dogs were unprotected, because some dogs do not respond to vaccination in a normal manner. Whether a vaccination program should be designed to protect all dogs or merely to protect a sufficiently large part of the population to minimize the risk of an epizootic has not been determined. The latter goal probably is reached if between 20 to 30% of the canine population is unprotected[24].

Vaccination at 8 weeks of age and again at 12 weeks will effectively protect the majority of puppies when risk of infection is low. Where a higher risk exists, earlier and more frequent vaccination may be appropriate, e.g. 6, 9, and 12 weeks. And puppies should be kept away from potential sources of infection for a week after their 12 week inoculation[9].

Interference with maternal antibodies or improper handling of the vaccine (for example, vaccine stored too cold, too warm or for too long) are probably the most common causes of vaccine failure[4]. This could explain the lack of antibody titres in some of the vaccinated dogs. Maternally derived CDV titers of 1:16 or greater was considered to protect puppies from infection, but titers between 1:10 and 1:16 are not protective and may interfere with vaccination. In this study, the authors used puppies with a wide range of maternal SN antibody titer, including some puppies with titers of 1:64 or greater. There were some puppies in vaccine C, G, and K groups which were not seroconverted by 15 weeks of age. However, all puppies vaccinated with V vaccine had seroconverted by 15 weeks of age, indicating that V vaccine was able to overcome interference by maternal antibodies.

It is impossible to eradicate CDV essentially because of the reservoir in the fauna. Thus a more extensive use of CDV vaccines should be encouraged in order to reduce the impact of virulent infections in the dog population. Furthermore, as no immunization strategy can eliminate the protective gap between passive maternal immunity and actively acquired immunity, prophylactic measures should include isolation of young dogs from the dog population until vaccination can be expected to provide protection[3].

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CDV 함유 혼합백신과 예방접종 스케줄에 따른 강아지의 면역반응

김 두[1] · 안소저 · 정석영 · 황재웅 · 박선일
강원대학교 수의학과

요 약 : 본 연구에서는 우리나라 실정에 맞는 개 디스템퍼에 대한 예방접종 프로토콜을 마련하기 위하여, 국내에서 사용 중인 4 종류의 상업용 백신과 3 가지의 예방접종 스케줄에 따른 강아지의 면역형성 능력을 비교 평가하였다. 생후 6주령에 예방접종을 실시하기 위하여 동물병원에 내원한 120두의 강아지를 4 종류의 백신[C, G, K, V (또는 V3) 군]과 예방접종 스케줄(V2, V4 군)에 따라 20두씩 임의배치하여 C, G, K, V (또는 V3)군은 3주 간격으로 3회(6, 9, 12주), V2 군은 5회(6, 8, 10, 12, 14주), V4 군은 3회(6, 10, 14주)에 피하로 예방접종을 실시하였다. 초유를 섭취한 7마리 강아지의 모체이행 항체의 소장상태를 확인한 결과 모체이행항체는 생후 6주령에 방어수준 이하로 떨어졌다. 백신에 따른 면역형성능에서 V 백신의 면역형성 능력이 다른 백신보다 우수하였으며 백신 종류에 따라 면역형성 능력에 차이가 인정되어 사용되는 백신의 효능을 주기적으로 평가하여야 할 것으로 판단되었다. 그리고 가장 효과적인 예방접종 스케줄은 6주령에 예방접종을 시작하여 3주 간격으로 3회 접종하는 것으로 판단되었다. 그러나 예방접종을 실시한 모든 군의 대부분의 강아지는 생후 9주령까지 항체가 수준이 방어수준 이하로 나타나 개 디스템퍼의 감염을 예방하기 위하여 이 시기까지는 감염위험성이 높은 곳에 노출되는 것을 피해야 할 것으로 생각되었다.

주요어 : 개 디스템퍼 바이러스, 백신, 접종 스케줄, 중화항체가