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Evaluation for Protective Effect of CPV-2 and CPV-2b Vaccines against a Korean CPV-2a Isolate in Pups

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Abstract : The aim of this study was to determine if vaccines containing CPV-2 or CPV-2b provided protection against challenge with a recent Korean CPV-2a isolate. Twenty mongrel pups aged 9 weeks old were used. The commercial CPV-2 or CPV-2b vaccines were administered to each of the 8 pups thrice every 3 weeks, respectively. Two weeks after the last vaccination, all pups were challenged with CPV-2a (VR00174 strain) 1×10^6 $TCID_{50}$. Clinical signs, fecal excretion of challenged CPV, and serological response of pups were observed for 2 weeks after challenge. All vaccinated pups did not display any clinical signs of disease after challenge with Korean CPV-2a isolate, whereas all non-vaccinated pups exhibited mucoid or hemorrhagic diarrhea, vomiting and anorexia. In all non-vaccinated pups, the virus could be detected in feces from 4 days after challenge, whereas in vaccinated pups, no evidence of viral excretion could be detected. Two of 4 non-vaccinated pups died 6 days after the challenge. This study showed that the two commercial CPV-2 and CPV-2b vaccines were effective in preventing infection and/or disease caused by the Korean CPV-2a isolate.

Key words **:** canine parvovirus, CPV-2a, vaccines, protection, Korea.

Introduction

Canine parvovirus (CPV) infection in dogs has been associated with outbreaks of acute hemorrhagic enteritis characterized by bloody diarrhea, vomiting, depression, leukopenia, dehydration, and high mortality (18). The causative organism, CPV type 2 (CPV-2), was first identified in 1979 (1). Since then, 2 variants of the virus have been identified: CPV-2a in 1980 and CPV-2b in the mid 1980s (18). From 1997, CPV-2c strains have emerged in Europe and other countries (2,15,24). The original CPV-2 virus has now disappeared from the field and has been replaced by the 2a, 2b or 2c variants whose relative proportions vary from country to country. CPV-2a is the major variant in Germany and India, while CPV-2b is common in USA, Italy and Japan (7,9,12, 13,20,23). An epidemiological study has identified that the CPV-2a strains were the major isolates of CPV infection in Korea. Among the 31 field CPV isolates, 28 (90.3%) isolates were classified as CPV-2a and other 3 isolates as CPV-2b (16).

The mortality of dogs with CPV infection is high, even with adequate treatment (8,19). For this reason, extensive efforts have been directed at preventing the disease through vaccination. Many of the currently used vaccines are modified live virus vaccines based on the original virus type CPV-2 isolated during the end of the 1970's or early 1980's (3,4). They are very potent vaccines that prevent disease, and successfully transformed the CPV pandemic into a well-controlled endemic situation. However, a number of outbreaks of CPV enteritis were reported throughout the world in spite of intensive vaccination (10,14).

Although current CPV vaccines provided effective protection and long duration of immunity, the recent mutations of CPV-2 raises concerns on the efficacy of current CPV vaccines against the mutant viruses. The aim of this study was to determine if vaccines containing CPV-2 or CPV-2b provided protection against challenge with a recent Korean CPV-2a isolate.

Materials and Methods

Experimental animal

Twenty non-vaccinated mixed breed pups aged 9 weeks old were used. All the pups were declared fit and healthy on veterinary inspection. Eight pups were vaccinated with CPV-2 vaccine (Group 1, Novivac), 8 pups were vaccinated with CPV-2b vaccine (Group 2, Quantum), and 4 pups were injected with vaccine diluent (control group). Each pup was housed separately and given commercial meat-based dog food twice daily. Water was freely available at all times. All experiments were carried out in accordance with the guideline of Animal Ethics Committee of Kangwon National University.

Vaccines and vaccination

The two commercial modified-live combination vaccines

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were used. Novivac™ DHPPi+L (Intervet, Netherland), which contains canine parvovirus (CPV- 2, strain 154), canine adenovirus (type 2), distemper virus, and parainfluenza virus, Nobivac Lepto (inactivated leptospirosis vaccine-Intervet), and Nobivac Pi (live parainfluenza virus only). Quantum™ DA2PPvL (Schering-Plough, USA), which contains canine parvovirus (CPV-2b strain), canine distemper virus, canine adenovirus (type 2), canine parainfluenza virus, *Leptospira canicola*, *Leptospira icterohaemorrhagiae.*

At 9 weeks of age, each pup in the vaccine groups was vaccinated with one of the vaccines. Revaccination was conducted at 12 and 15 weeks of age. Vaccines were administered subcutaneously in the dorsal aspect of the neck. Adverse effects were not observed post-vaccination, in all vaccine groups.

Virus and challenge

CPV-2a strain (Korean CPV-2a isolate, VR00174, kindly provided by Animal and Plant Quarantine Agency, Korea) was used as challenge virus. Virus was propagated and titrated in Crandell Rees feline kidney cells (CrFK) as described by Mochizuki *et al* (21) using Dulbeco's modified Eagle's medium (GIBCO™, Invitrogen Corporation, NY, USA) supplemented with 5% fetal bovine serum containing penicillin and streptomycin.

Two weeks after the last vaccination, all pups were challenged orally with CPV-2a 1×10^6 $TCID_{50}$ in a volume of 1.0 ml. Prior to challenge, all pups were confirmed for no pre-existing CPV-2 infection.

Efficacy study

Clinical signs of pups were recorded daily for 2 weeks after challenge. And fecal swab samples were collected daily during the first week after challenge and every other day during the second week. Fecal samples were tested for virus excretion with the SNAP Parvo Antigen Test Kit (IDEXX Laboratories, USA). Blood samples for viral serology were collected every 3 weeks during vaccination period and every week after challenge.

Hemagglutination inhibition (HI) test

Serum samples were assayed for antibodies to canine parvovirus using HI test. The HI test was based on the method described by Carmichael *et al* (5). Briefly, after heat inactivation at 56°C for 30 minutes, the sera were treated with pig erythrocytes to remove non-specific inhibitors of viral hemagglutination. Serial 2-fold dilutions of the treated sera were prepared in U-bottomed microtiter plates and then an equal volume (50 μl) of virus suspension, containing 8 hemagglutination units of CPV, was added to each well. When the serum-virus mixtures had been incubated for 60 minutes at room temperature, 50 μl of 0.5% pig erythrocytes was added to each well and the plates were kept at 4°C for 4 hours. The antibody titer was considered to be the reciprocal of the highest dilution that completely inhibited hemagglutination. In each test, known negative and positive sera with moderate and high titers were included. Results were only accepted when the titers of the standard sera fell within specific limits (a 2-fold change for the positive sera).

Statistical analysis

A repeated-measures analysis of variance (ANOVA) was used to compare between vaccinated group titers at the time of each observation, using Tukey's multiple comparison test. A values of $p < 0.05$ were considered significant. All analyses were performed with the computer software package SAS (version 8.1 for Windows).

Results

Clinical observation

After challenge with Korean CPV-2a isolate, all 16 vaccinated pups did not display any clinical signs of disease during the experimental period, whereas 3 out of 4 non-vaccinated pups started to show clinical signs (such as anorexia, dullness, mucous diarrhea, and mild dehydration) from 4 days after challenge. All non-vaccinated pups exhibited anorexia, vomiting, and mucoid or hemorrhagic diarrhea by 5 days after challenge. One (C1) of the non-vaccinated pups exhibited severe clinical signs that continued up to 14 days after challenge. One (C4) of the non-vaccinated pups exhibited less severe signs that disappeared by 9 days after challenge. Two (C2 and C3) of the non-vaccinated pups died 6 days after challenge (Table 1).

Viral excretion in feces after challenge

No evidence of viral excretion could be detected after challenge in vaccinated pups, whereas in non-vaccinated pups, the virus could be detected in feces from 4 days after challenge. Viral excretion in non-vaccinated pups was not detected from 5 days after challenge in C4 pup and 11 days in C1 pup. Two pups was not test viral excretion from 6 days after challenge because of death (Table 2).

Serological responses

All pups in the vaccinated groups had 10~80 HI antibody titers to CPV prior to vaccination. At 3 weeks after the first CPV vaccination, pups had developed HI titers ranging from 160-5,120. At the time of challenge, all the vaccinated pups had developed 5,120 HI titers (Table 3). There were no observable differences in HI titer between the 2 vaccination groups. Following challenge, the vaccinated pups did not show anamnestic immune response to CPV.

In non-vaccinated control pups, pups had 10~40 HI titers to canine parvovirus at the start of the experiment and pups remained sero-negative at the time of challenge. However, after challenge the control pups did mount an antibody response. Pups who subsequently died could not be tested for HI titer because of death within 1 week after challenge (Table 3).

Discussion

Historically, outbreaks of CPV enteritis have been difficult to control. The virus is ubiquitous, can survive for more than 6 months at room temperature, and is readily transported among dogs *via* cages, soiled bedding, or humans. Good hygienic practices, including vigilant disinfection of all exposed surfaces and personnel in kennels, are paramount for

Table 1. Clinical observations of pups challenged with CPV-2a isolate

Group	Pup	Clinical observation (Days after challenge)										
		0	1	2	3	4	5	6	7	9	11	14
Group 1, CPV-2 vaccinate	N1	N	N	N	N	N	N	N	N	N	N	N
	N2	N	N	N	N	N	N	N	N	N	N	N
	N3	N	N	N	N	N	N	N	N	N	N	N
	N4	N	N	N	N	N	N	N	N	N	N	N
	N5	N	N	N	N	N	N	N	N	N	N	N
	N6	N	N	N	N	N	N	N	N	N	N	N
	N7	N	N	N	N	N	N	N	N	N	N	N
	N8	N	N	N	N	N	N	N	N	N	N	N
Gruop 2, CPV-2b vaccinate	Q1	N	N	N	N	N	N	N	N	N	N	N
	Q2	N	N	N	N	N	N	N	N	N	N	N
	Q3	N	N	N	N	N	N	N	N	N	N	N
	Q4	N	N	N	N	N	N	N	N	N	N	N
	Q5	N	N	N	N	N	N	N	N	N	N	N
	Q6	N	N	N	N	N	N	N	N	N	N	N
	Q7	N	N	N	N	N	N	N	N	N	N	N
	Q8	N	N	N	N	N	N	N	N	N	N	N
Control Group	C1	N	N	N	N	M,D,Dh	V,H,D,Dh	A,V,H,L,Dh	A,V,H,L,Dh	A,V,H,D,Dh	A,M,D,Dh	M,D,Dh
	C2	N	N	N	N	A,M,D,Dh	A,V,H,L,Dh	Di	Di	Di	Di	Di
	C3	N	N	N	N	A,M,D,Dh	A,V,H,L,Dh	Di	Di	Di	Di	Di
	C4	N	N	N	N	N	A,V,Dh	A,V,Dh	A,V,M,Dh	N	N	N

N = normal, A = anorexia, D = dullness, Dh = dehydration, Di = died, H = hemorrhagic diarrhea, L = lethargy, M = mucoid diarrhea, V = vomiting

Table 2. Viral excretion in feces after challenge with CPV-2a isolate

Group	Pup	Viral excretion (Days after challenge)										
		0	1	2	3	4	5	6	7	9	11	14
Group 1, CPV-2 vaccinate	N1	N	N	N	N	N	N	N	N	N	N	N
	N2	N	N	N	N	N	N	N	N	N	N	N
	N3	N	N	N	N	N	N	N	N	N	N	N
	N4	N	N	N	N	N	N	N	N	N	N	N
	N5	N	N	N	N	N	N	N	N	N	N	N
	N6	N	N	N	N	N	N	N	N	N	N	N
	N7	N	N	N	N	N	N	N	N	N	N	N
	N8	N	N	N	N	N	N	N	N	N	N	N
Gruop 2, CPV-2b vaccinate	Q1	N	N	N	N	N	N	N	N	N	N	N
	Q2	N	N	N	N	N	N	N	N	N	N	N
	Q3	N	N	N	N	N	N	N	N	N	N	N
	Q4	N	N	N	N	N	N	N	N	N	N	N
	Q5	N	N	N	N	N	N	N	N	N	N	N
	Q6	N	N	N	N	N	N	N	N	N	N	N
	Q7	N	N	N	N	N	N	N	N	N	N	N
	Q8	N	N	N	N	N	N	N	N	N	N	N
Control Group	C1	N	N	N	N	+	+	+	+	+	N	N
	C2	N	N	N	N	+	+	ND	ND	ND	ND	ND
	C3	N	N	N	N	+	+	ND	ND	ND	ND	ND
	C4	N	N	N	N	+	N	N	N	N	N	N

N = negtive, ND = non-determined, + = positive

Table 3. Hemagglutination inhibition titer of pups after challenge with CPV-2a isolate

Group	Pup	Hemagglutination inhibition titer (Week)					
		0	3	6	8 (C^a + 0)	9 (C + 1)	10 (C + 2)
Group 1, CPV-2 vaccinate	N1	10	160	5120	5120	5120	5120
	N2	20	1280	5120	5120	5120	5120
	N3	10	1280	5120	5120	5120	5120
	N4	10	1280	5120	5120	5120	5120
	N5	20	640	5120	5120	5120	5120
	N6	10	1280	5120	5120	5120	5120
	N7	80	1280	5120	5120	5120	5120
	N8	10	160	5120	5120	5120	5120
Gruop 1, CPV-2b vaccinate	Q1	20	320	5120	5120	5120	5120
	Q2	40	2560	5120	5120	5120	5120
	Q3	40	2560	5120	5120	5120	5120
	Q4	20	2560	5120	5120	5120	5120
	Q5	40	1280	2560	5120	5120	5120
	Q6	0	2560	5120	5120	5120	5120
	Q7	80	5120	5120	5120	5120	5120
	Q8	80	320	5120	5120	5120	5120
Control Group	C1	10	0	0	0	1280	1280
	C2	40	0	0	0	ND^b	ND
	C3	20	20	0	0	ND	ND
	C4	40	20	0	0	320	5120

C^a = challenge, ND^b = non-determined

prevention of nosocomial transmission of viral infection (6,11).

More important than good hygiene for the prevention of CPV infection is assurance of strong individual-dog immunity *via* adoption of effective immunization protocols. The type of vaccine utilized also affects success in immunization. The vaccines of choice are canine origin attenuated vaccines with high-titer, low-passage CPV (6).

Outbreaks of CPV enteritis in Korea were mainly caused by CPV-2a isolates which had a unique mutation in the CPV VP2 gene and the common mutations in some amino acids of the CPV VP2 gene with the new strains from other countries (16). Because outbreaks of CPV enteritis occur generally during primary vaccination, a more extensive use of CPV vaccines should be encouraged in order to reduce the impact of virulent CPV infections in the canine population. This study was conducted to determine if vaccines containing CPV-2 or CPV-2b provided protection of pups against challenge with a recent Korean CPV-2a isolate.

In this study, after challenge with the CPV-2a isolate, all the non-vaccinated control pups started to show clinical signs such as reduced appetite, vomiting, mucous diarrhea, and dehydration from 4 days after challenge, and 3 of 4 control pups showed severe hemorrhagic diarrhea. The Korean CPV-2a field isolate can cause a severe enteritis in non-vaccinated pups, demonstrating similar pathogenicity to other CPV strains (18). In the vaccinated groups, pups did not display any clinical signs of disease at any stage during the experiment. Virus could be detected in feces taken from the control pups from day 4 to day 9 after challenge in this study, whereas no evidence of viral excretion could be detected in any of the vaccinated pups. Analysis of rectal swabs revealed that the vaccinated pups were not only protected from clinical disease but also that vaccination prevented shedding of challenge virus. This finding was in line with the efficacy of CPV-2 and CPV-2b vaccines not only in preventing the development of clinical signs in pups but also in the prevention of shedding of CPV-2a virus following the challenge (12,17,22).

In this study, all the vaccinated pups developed over protective (80) HI antibody titers 3 weeks after the first vaccination with CPV-2 and CPV-2b vaccines, and > 5,120 HI titer after the third vaccination. Moreover, there was no change in HI titers after challenge in vaccinated pups. This non-anamnestic response following challenge in the vaccinated pups indicated that pups had sterilising immunity to CPV (17,22). However, the 2 control pups, which survived from the challenge, were able to mount an immune response.

In conclusion, this study showed that the 2 commercial CPV-2 and CPV-2b vaccines are effective in preventing infection and/or disease caused by the recent Korean CPV-2a isolate. This study also demonstrated that a recent Korean CPV-2a isolate was able to induce severe clinical disease in non-vaccinated pups.

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국내에서 유행하는 CPV-2a 분리주에 대한 CPV-2와 CPV-2b 백신의 방어효능 평가

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요 약 : 본 연구는 상용화되어 판매되고 있는 CPV-2 백신과 CPV-2b 백신이 국내에서 주로 유행하고 있는 CPV-2a 분리주에 대해 방어능력을 가지는 지를 평가하기 위하여, CPV에 대한 예방접종을 실시하지 않은 생후 9주령의 잡종 강아지 20두를 예방접종 미실시군(대조군) 4두와 예방접종군 16두로 편성하였다. 예방접종 군의 강아지 8두씩은 3주 간격으로 3회에 걸쳐 CPV-2 또는 CPV-2b 백신을 각각 예방접종하였다. 3차 예방접종 2주 후에 모든 강아지에 1×10^6 $TCID_{50}$의 CPV-2a (VR00174 strain) 바이러스를 경구접종하였으며 강아지들의 임상증상, CPV의 변 내 배출, CPV에 대한 혈청학적 반응에 대하여 2주 동안 관찰하였다. 국내 분리 CPV-2a를 공격접종한 후에, 예방접종을 실시한 모든 강아지에서는 어떠한 임상증상도 나타나지 않았지만 예방접종을 실시하지 않은 강아지들은 식욕부진, 우둔, 구토, 점액 또는 혈액성 설사를 나타내었으며 2두는 공격접종 6일째에 폐사하였다. 예방접종을 실시하지 않은 강아지들에서는 공격접종 4일 후부터 변에서 CPV가 검출되었지만 예방접종을 실시한 강아지에서는 변에서 CPV가 검출되지 않았다. 그리고 예방접종 실시한 강아지들은 1차 예방접종에 의하여 방어수준 이상으로 CPV에 대한 항체를 형성하였다. 본 연구는 현재 국내에서 시판 중인 CPV-2와 CPV-2b 백신이 최근 국내에서 주로 분리되는 CPV-2a에 대하여 교차 방어력을 나타내는 것으로 확인되었다.

주요어 : 개파보바이러스, CPV-2a, 백신, 방어, 대한민국