Development of a Vaccine Incorporating Killed Virus of Canine Origin for the Prevention of Canine Parvovirus Infection

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SUMMARY

A parvovirus of canine origin, cultured in a feline kidney cell line, was inactivated with formalin. Three pilot serials were produced and three forms of finished vaccine (nonadjuvanted, single adjuvanted and double adjuvanted) were tested in vaccination and challenge trials. A comparison was also made with two inactivated feline panleukopenia virus vaccines, one of which has official approval for use in dogs. The inactivated canine vaccine in nonadjuvanted, adjuvanted or double adjuvanted form was immunogenic in 20 of 20 vaccinated dogs. The double adjuvanted vaccine is selected as the one of choice on the basis of best and most persistent seriological response.

RÉSUMÉ

Développement d'un vaccin inactivé, à base de parvovirus canin, pour la prévention de la parvovirose du chien

Cette expérience consistait à inactiver, avec de la formaline, une souche de parvovirus canin cultivé sur cellules rénales de chat. Elle visait aussi à produire trois lots de vaccin pilote et les trois variétés suivantes d'un vaccin complet : sans adjuvant et avec un seul or deux adjuvants. On utilisa ces vaccins pour effectuer des vaccinations expérimentales et des infections de défi. On les compara aussi à deux vaccins inactivés contre la panleucopénie féline, dont l'un est officiellement approuvé pour la vaccination du chien. Les trois variétés du vaccin complet s'avérèrent immunogènes, chez les 20 chiens auxquels on les administra. Celle qui contenait deux adjuvants se révéla cependant la meilleure, parce qu'elle provoqua le développement du taux d'anticorps sériques le plus élevé et le plus durable.

INTRODUCTION

During the past three years infection of dogs with the parvovirus designated canine parvovirus-2 (CPV-2) has become recognized as a widespread occurrence in the dog population worldwide. The majority of infections are mild or subclinical, but a fatal myocardiopathy of puppies and acute mucohemorrhagic gastroenteritis in dogs of all ages are reported frequently.

Following recognition of the disease and cultivation of the causative virus it was quickly appreciated that close similarities existed between the canine parvovirus and the panleukopenia virus of cats (1,5,10,11,12,13). It was logical therefore to use already available vaccines, designed for the prevention of feline panleukopenia, in the hope of protecting dogs against CPV-2. Some experimental evidence has been presented (8) to support this use. Another closely related virus is mink enteritis, and mink enteritis vaccines have also been used in dogs. Limited evidence has been presented for the efficacy of such mink enteritis vaccines, and some preparations were shown to be incapable of stimulating a satisfactory immune response in dogs (7).

The development of an inactivated vaccine in nonadjuvanted, single-adjuvanted and double adjuvanted forms, using a canine isolate of parvovirus, is the subject of this paper.

MATERIALS AND METHODS

Dogs

A total of 49 beagles and crossbred dogs of between six and 16 weeks of age were used. All dogs were demonstrated free of hemagglutination-inhibiting (HI) antibody to CPV-2 in their sera prior to obtaining the animals. The dogs were housed in the isolation facilities of Ontario Veterinary College and rigid disinfection and protective clothing regimes were adopted to prevent accidental introduction of parvovirus.

Virological Procedures

These are detailed elsewhere (6). Briefly the Crandell feline kidney (CRFK) cell line, obtained originally from Dr. C. Fabricant, Cornell University, was used routinely for the assay and detection of canine parvovirus-2 in this study. Young (4-8 h) monolayers of cells were inoculated with material known or suspected to contain CPV. The culture medium was Eagles's basal medium with Hank's salts supplemented with 0.5% lactalbumin hydrolysate, 1% L-glutamine, 20 ml/L of 7.5% $NaHCO_3$, 125 000 units/L penicillin, 125 000 μg/L of streptomycin, and 6% fetal calf serum. Cultures were examined for hemagglutinating activity after five days of incubation at 37.5°C, by the addition of 50 μL of a chilled 0.5% suspension of African green monkey (AGM) red cells. The hemagglutination (HA) could be shown to be specific for CPV-2 by blocking with CPV antibody. Negative cultures were passaged by monolayer trypsinisation and replantation and reexamined for specific HA activity at five days.

Serological Procedures

Serum antibody to CPV-2 was assayed using a microhemagglutination-inhibition test. The hemagglutinating antigen was a local isolate of CPV-2, designated CPV-Rae, which had been cultured from a fatal case of canine gastroenteritis in Ontario in October 1978. Initially isolated in canine kidney cells, the virus was then

adapted to feline cells. The hemagglutinin used in this study was prepared from 8th passage virus with an infectivity titer of $10^{5.2}$ cell culture median infective dose ($CCID_{50}$) per mL and a hemagglutinating (HA) titer of 1:1024 at 4°C using a 0.5% suspension of AGM red cells with 1% fetal calf serum in phosphate buffered saline (PBS) pH 7.2. The HA antigen was stored frozen at -90°C and sonicated immediately prior to use.

The hemagglutination-inhibition (HI) test was performed in 96 well (round bottom) microtiter plates. Sera were heat inactivated at 56°C for 30 minutes and adsorbed overnight at 4°C with an equal volume (100 μL) of 0.5% AGM red cell suspension in duplicate in the first row of wells. Adsorbed sera with the red cells settled out were then diluted in twofold steps in PBS. Four to eight HA units of CPV-Rae 8 were then added to each well. Plates were incubated for 1 h at 37.5°C, and 50 μL of chilled 0.5% AGM red cell suspension added to each well. Further incubation of the plates was at 4°C for 2-18 h. Positive and negative serum, red cell and HA antigen controls were included in each test. A 50% end point for inhibition of hemagglutination was determined by observation or extrapolation.

Vaccines

The canine parvovirus-2, CPV-Rae, from its second passage in dog kidney cells was adapted to CRFK cells and the eighth passage used as seed stock for vaccine production. Aliquots of this stock have been tested and found free of adventitious agents by culture in canine, feline and porcine cells and by electron microscopy.

Three separate lots of vaccine virus were produced and used in the present study. These were designated lots 5,7 and 9. Hemagglutinating titers at harvest were 64, 2048 and 384 respectively. Virus titers (- log 10) of lots 7 and 9 were 5.4 and 6.2 $CCID_{50}$/mL respectively. Harvested virus was inactivated with formalin and completeness of inactivation was tested by neutralization of residual formalin by sodium metabisulphite, dialysis and inoculation of CRFK cultures. These were trypsinized twice at five day intervals and supernatants checked for CPV hemagglutinin. Standard safety tests were also performed in bacteriological media and mice.

Three forms of finished vaccine were made up. These were nonadjuvanted (vaccines 5 and 9), single adjuvanted with aluminum hydroxide gel[1] (vaccine 7A), or double adjuvanted with aluminum hydroxide gel and another adjuvant L80[2] (vaccines 7B and 9B).

For comparative purposes two inactivated feline panleukopenia virus vaccines[3,4] (one of them licensed for use in dogs for protection against CPV-2), were included in two of the trials.

Vaccination

All dogs received a 1 mL dose of vaccine. This dose represented HA titers of CPV-2 prior to inactivation of 64, 1640, 1624, 384 and 346 for vaccines 5, 7A, 7B, 9 and 9B respectively. The subcutaneous and intramuscular routes were used as indicated (Table I).

The inoculation was repeated at an interval of two or three weeks.

Challenge

For Trial 1 (see Table I) all pups were challenged orally with either 1 mL of cell culture virus (10^5 $CCID_{50}$ Rae-5) or 1 mL of a 1:10 dilution in PBS of fecal suspension from a fatal case of CPV. For reasons given in the results a second challenge of fecal suspension from a separate case of CPV was given 11 days later.

For trials 2 and 3, challenge material was a gut mucosal homogenate (10% W/V) from natural fatal cases of CPV, supplemented for trial 2 only with 25% V/V cell culture harvest fluids of CPV-Rae at the eighth passage, in an attempt to increase the virulence of the challenge.

The gut mucosal homogenate was screened for bacterial pathogens by cultural methods and for other viruses by cell culture and electron microscopy. Additionally, the material was heat treated at 56°C for 30 minutes. Each dog was starved for 48 hours prior to challenge with 3 mL of the

TABLE I
EXPERIMENTAL DESIGN OF CANINE PARVOVIRUS VACCINATION AND CHALLENGE TRIALS

Trial	Vaccine	Date First Vaccination	Dose Interval (Wk)	Route	No. of Dogs	Date of Challenge
I	Killed CPV-2 Lot 5 Non adjuvanted	79 03 29	3	SC[c]	4	79 05 14 & 79 05 25
	Felocine®[a]	79 03 29	3	SC	4	79 05 14 & 79 05 25
	None	—	-	-	4	79 05 14 & 79 05 25
II	Killed CPV-2 Lot 7A Single adjuvant	80 07 28	3	SC[c]	4	80 09 08
	Killed CPV-2 Lot 7B Double adjuvant	80 07 28	3	SC	4	80 09 08
	Parvocine®[b]	80 07 28	3	SC	3	80 09 08
	None	—	-	—	4	80 09 08
III	Killed CPV-2 Lot 9 No adjuvant	80 12 05	2	IM[d]	4	81 01 08
	Killed CPV-2 Lot 9B Double adjuvant	80 12 05	2	IM	4	81 01 08
	None	—	-	—	2	81 01 08

[a] Killed feline panleukopenia virus. Norden Laboratories, Lincoln, Nebraska.
[b] Killed feline panleukopenia virus. Dellen Laboratories, Omaha, Nebraska.
[c] Subcutaneous inoculation.
[d] Intramuscular inoculation.

[1] Rehsorptar, Reheis Chemical Co., Berkeley Heights, New Jersey.
[2] Kindly supplied by Langford Laboratories, Guelph, Ontario.
[3] Felocine, Norden Laboratories, Lincoln, Nebraska.
[4] Parvocine, Dellen Laboratories, kindly supplied by Dr. D. Moont, Bayvet, Mississauga, Ontario.

challenge material, given orally on each of two successive days (trial 2) or by a single 5 mL oral dose (trial 3).

Observations and Samples

All dogs were observed daily for one week following vaccination and daily for ten to 14 days following challenge. Serum samples were collected prior to vaccination, at intervals following vaccination, and two weeks after challenge. Fecal samples were collected at least three times weekly following challenge and were processed in cell culture for CPV detection.

Challenge Trial Designs

These are summarized in Table I.

Compatibility Trial

To be of use in the field a canine parvovirus vaccine has to be compatible with other antigens used in routine canine prophylactic programs. The compatibility of the newly developed vaccine with a widely used live modified canine vaccine[5] containing canine distemper, adenovirus and parainfluenza antigens was tested as follows. Pups eight to ten weeks old, were housed in three groups of four and vaccinated with double adjuvanted CPV-2 vaccine alone, or with the two vaccines combined using the double adjuvanted CPV-2 vaccine to reconstitute the other vaccine. Appropriate vaccinations were repeated after two weeks. Serum samples were obtained prior to and following vaccination and assayed for antibody titers. Adenovirus antibody was assayed by an HAI test using four HA units of canine adenovirus-1 using human "O" red cells at 4°C, and canine distemper serum neutralizing antibody was titrated in a microtitration system (3) using 100 $CCID_{50}$ of distemper virus and vero cells.

RESULTS

Trial 1

There was no observed local or systemic ill effect of vaccination. Following initial challenge several control dogs vomited and had occasional loose stools. More marked evidence of gastroenteritis had been expected in these unvaccinated dogs and a decision to rechallenge was made. This rechallenge did not have any observable clinical effect on any of the dogs. Retrospective examination of fecal samples (Table II) showed that the initial challenge had established infection. Canine parvovirus was recovered from the feces of three unvaccinated dogs and on single occasions from three of the vaccinates. The remaining control, and three of the dogs which had received the feline panleukopenia vaccine, Felocine®, shed virus following the rechallenge. Only one of the four pups which had received the unadjuvanted CPV vaccine (vaccine 5), shed virus. This was on a single occasion, the day after challenge.

The serological responses are shown in Table III. Dogs vaccinated with Felocine® showed no serological response to the initial dose but three of the four had HI titers of 1:8 to 1:16 three weeks after the second dose. All pups receiving unadjuvanted CPV vaccine developed titers after a single dose (1:8 to 1:48) and these were boosted (1:24 to 1:256) following the second inoculation. Antibody titers were further increased following challenge.

Trial 2

There were no detectable clinical reactions to vaccination in dogs receiving the single adjuvanted CPV vaccine '7A' or Parvocine®. Following initial vaccination with the double adjuvanted CPV vaccine "7B", the dogs

TABLE II

TRIAL 1. RECOVERY OF CANINE PARVOVIRUS FROM FECES

Vaccine	Dog #	Days Postchallenge: Challenge 1 0	1	2	3	4	5	6	7	8	9	Challenge 2 0	1	2	3	4
Killed CPV-2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lot 5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No adjuvant	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	11	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Felocine®[a]	1	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
	4	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
	7	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-
	10	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
None	3	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-
	6	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
	12	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-

+= Canine parvovirus recovered in cell culture.

[a] Killed feline panleukopenia virus. Norden Laboratories, Lincoln, Nebraska.

TABLE III

TRIAL 1. SEROLOGICAL RESPONSES (RECIPROCAL HI TITER) TO VACCINATION AND CHALLENGE

Vaccine	Dog #	Prevacc. (Day 0)	2nd Vacc. (Day 21)	1st Chall. (Day 42)	2nd Chall. (Day 53)	Postchall. (Day 63)
Killed CPV-2	2	<8	48	256	196	96
Lot 5	5	<8	32	128	128	>512
No adjuvant	8	<8	16	96	64	>512
	11	<8	8	24	32	>512
	Mean	<8	21	126	105	>408
Felocine®[b]	1	<8	<8	12	24	>512
	4	<8	<8	8	12	>512
	7	<8	<8	16	12	>512
	10	<8	<8	<8	>512	NT[a]
	Mean	<8	<8	9	>140	>512
None	3	<8	<8	<8	>512	>512
	6	<8	<8	<8	>512	>512
	9	<8	<8	<8	<8	>512
	12	<8	<8	<8	>512	>512
	Mean	<8	<8	<8	>384	>512

[a] NT=Not tested.

[b] Killed feline panleukopenia virus. Norden Laboratories, Lincoln, Nebraska.

[5] Vanguard DA_2P, Norden Laboratories, Lincoln, Nebraska.

developed transient firm subcutaneous nodules 0.5 to 1.0 cm diameter at the inoculation site. These nodules resolved within five to eight days and did not recur after the second vaccination.

Apart from one dog which had a raised rectal temperature immediately prior to challenge and which remained slightly elevated at 24 hours, only the nonvaccinated control dogs showed a postchallenge pyrexia ($\geq 39.6°C$). This pyrexia occurred between days 3 and 8 postchallenge, with a second fever spike (40.2° C) in one control dog at day 11. The onset of fever in the control dogs coincided with loss of appetite and looseness of stools. Passage of large clots of clear mucus also occurred in one control dog. In contrast, none of the dogs vaccinated with either Parvocine® or inactivated CPV showed pyrexia or loss of appetite. One dog, vaccinated with Parvocine®, had a soft stool. The stools of the CPV vaccinated dogs remained normal throughout the experiment.

Virus was regularly recovered (Table IV) from fecal samples of all controls between days 4 and 9 postchallenge. Three of the vaccinated dogs (2 Parvocine®, 1 vaccine 7B) shed virus, but each on only a single occasion.

The serological response to vaccination (Table V) indicated that the double adjuvanted vaccine 7B produced consistently higher titers than the single adjuvanted vaccine 7A, which in turn stimulated superior titers to those induced by Parvocine®. After challenge, dogs vaccinated with vaccine 7B showed little or no boosting compared with the very marked increases in antibody titer in the dogs receiving Parvocine®, and in two of the dogs receiving vaccine 7A.

One control dog and one from each of the groups vaccinated with CPV vaccines 7A and 7B were removed to separate isolated accommodation prior to challenge of the other dogs in order to observe the duration of the serological response.

The results for the 36 weeks of this study are given in Table VI. The dog which had received the double adjuvanted vaccine, 7B, showed a persistence of antibody throughout the period, at levels which following rapid decline in the weeks immediately after

TABLE IV
TRIAL 2. DETECTION OF CPV IN FECES OF DOGS POSTCHALLENGE

Vaccine	Dog #	Days Postchallenge 0	2	4	7	9	11	14
Killed CPV-2	2	—	—	—	—	—	—	—
Lot 7A	6	—	—	—	—	—	—	—
Single adjuvant	10	—	—	—	—	—	—	—
Killed CPV-2	1	—	—	—	—	—	—	—
Lot 7B	5	—	—	—	—	—	—	—
Double adjuvant	9	—	—	—	+[a]	—	—	—
Parvocine®[b]	3	—	—	—	—	—	—	—
	7	—	—	—	+[a]	—	—	—
	11	—	—	—	—	+[a]	—	—
None	4	—	—	+[a]	+[a]	+[a]	—	—
	8	—	—	+[a]	+[a]	+[a]	—	—
	12	—	—	+[a]	+[a]	—	—	—

[a]=Canine parvovirus recovered in cell culture.
[b]Killed feline panleukopenia virus. Dellen Laboratories, Omaha, Nebraska.

TABLE V
TRIAL 2. SEROLOGICAL RESPONSES (RECIPROCAL HI TITER AGAINST CPV-2) TO VACCINATION AND CHALLENGE

Vaccine	Dog #	Prevacc. (Day 0)	2nd Vacc. (Day 21)	Postvacc. (Day 31)	Challenge (Day 42)	Postchallenge (Day 56)	(Day 63)
Killed CPV-2	2	<2	32	96	48	3074	512
Lot 7A	6	<2	12	128	192	64	24
Single adjuvant	10	<2	12	48	32	2048	768
	13	<2	96	192	96	(NT)[a]	(96)
	Mean	<2	38	116	92	1729	434
Killed CPV-2	1	<2	128	128	48	64	32
Lot 7B	5	<2	384	768	256	512	128
Double adjuvant	9	<2	384	384	128	128	96
	14	<2	384	768	192	(NT)[a]	(96)
	Mean	<2	320	512	156	235	85
Parvocine®[b]	3	<2	16	64	12	6000	1536
	7	<2	48	64	24	2048	354
	11	<2	16	64	24	3072	768
	Mean	<2	27	64	20	3710	886
None	4	<2	<2	<2	<2	512	354
	8	<2	<2	<2	<2	1024	256
	12	<2	<2	<2	<2	2048	256
	15	<2	<2	<2	<2	(NT)[a]	(<2)
	Mean	<2	<2	<2	<2	1194	289

[a]Not tested (13, 14, 15). These dogs were not challenged but held in separate isolation. They are excluded from mean titers postchallenge.
[b]Killed feline panleukopenia virus. Dellen Laboratories, Omaha, Nebraska.

TABLE VI
TRIAL 2. DURATION OF SEROLOGICAL RESPONSE (RECIPROCAL HI TITERS) TO CANINE PARVOVIRUS VACCINE

Dog	Vaccine	Weeks Postvaccination 0	3	4.5	6	9	13	20	24	28	32	36
13	Killed CPV Lot 7A Single adjuvant	<2	96	192	96	96	32	24	12	<4	<4	<4
14	Killed CPV Lot 7B Double adjuvant	<2	384	768	192	96	96	96	96	64	64	64
15	(Nonvacc.)	<2	<4	<4	<4	<4	<4	<4	<4	<4	1536[a]	768[a]

[a]Because of the sero conversion of the control dog, the study was discontinued at 36 weeks.

vaccination, were almost stable. However, the dog which had received the single adjuvant vaccine had lost detectable serum antibody to CPV-2 by 28 weeks postvaccination. The sentinel control dog remained healthy throughout the nine month period, but serum samples taken at 32 and 36 weeks showed a strong seroconversion to CPV-2 in the dog. No illness had been noted in the vaccinated dogs, and they did not show an antibody boost. It was concluded that the control dog had been accidentally exposed to CPV-2 and that probably the vaccinated dogs had also been exposed, although they had resisted infection. The three dogs, together with a newly introduced antibody free dog, were challenged with the challenge material used in trial 3. The three dogs on the duration of immunity study completely resisted challenge and there was no boosting of serum antibody. The newly introduced dog had mucoid diarrhea, vomited on several occasions over three days and developed an HI titer of 1:1536. A second duration of immunity study with ten dogs on double adjuvanted vaccine, four on nonadjuvanted vaccine and with four unvaccinated controls, is in progress.

Trial 3

No reaction to vaccination, local or systemic, was detected in any of the pups and they remained completely healthy until the time of challenge exposure to CPV. Between days 5 and 10 postchallenge both unvaccinated dogs became very depressed, anorexic, vomited and passed blood and mucus in an unformed stool. They rapidly weakened and became dehydrated. However, from ten days postchallenge onwards, they began a rapid recovery without treatment. In contrast, the vaccinated dogs remained bright and alert, without fever. Their appetite remained normal. Although six of the group sporadically passed a soft stool these did not have the mucohemorrhagic characteristics of the diarrhea seen in unvaccinated dogs. Canine parvovirus was recovered from the feces of both controls (Table VII) between days 7 and 10 postchallenge, but not from any of the vaccinated pups.

The serological results (Table VIII) indicated a strong primary response to

TABLE VII

TRIAL 3. RECOVERY OF CPV-2 FROM FECES OF DOGS FOLLOWING CHALLENGE EXPOSURE

		Days Postchallenge							
Vaccine	Dog #	1	4	6	7	8	9	10	14
Killed CPV-2	LM1	—	—	—	—	—	—	—	—
Lot 9	LM2	—	—	—	—	—	—	—	—
No adjuvant	LM3	—	—	—	—	—	—	—	—
	LM4	—	—	—	—	—	—	—	—
Killed CPV-2	LM5	—	—	—	—	—	—	—	—
Lot 9B	LM6	—	—	—	—	—	—	—	—
Double adjuvant	LM7	—	—	—	—	—	—	—	—
	LM8	—	—	—	—	—	—	—	—
None	LM9	—	—	—	+[a]	+[a]	+[a]	+[a]	—
	LM10	—	—	—	+[a]	+[a]	+[a]	+[a]	—

[a] Canine parvovirus recovered in cell culture.

TABLE VIII

TRIAL 3. SEROLOGICAL RESPONSE (RECIPROCAL HI TITERS AGAINST CPV-2 TO VACCINATION AND CHALLENGE

Vaccine	Dog #	Prevacc. (Day 0)	2nd Vacc. (Day 14)	Challenge (Day 35)	Postchallenge (Day 42)
Killed CPV-2	LM1	<2	96	192	384
Lot 9	LM2	<2	192	512	512
No adjuvant	LM3	<2	192	64	512
	LM4	<2	192	128	512
	Mean	<2	168	224	480
Killed CPV-2	LM5	<2	192	512	1536
Lot 9B	LM6	<2	64	256	768
Double adjuvant	LM7	<2	128	384	512
	LM8	<2	128	512	2048
	Mean	<2	128	416	1216
None	LM9	<2	<2	<2	3072
	LM10	<2	<2	<2	4096
	Mean	<2	<2	<2	3584

CPV vaccination in all vaccinated pups with a boosting, secondary response in all but two dogs. Three weeks after the second vaccination titers in the group receiving double adjuvanted vaccine 9B were twofold greater than in the nonadjuvanted vaccine group (mean 1:416 versus 1:224).

Compatibility Trial

By five days after initial vaccination all pups receiving double-adjuvanted CPV-2 vaccine had antibody to CPV-2 at titers of 1:48 to 1:96, and there was no significant difference between dogs that had received only CPV-2 vaccine or those receiving both CPV-2 and distemper-adenovirus parainfluenza vaccine. However two weeks after the second vaccination the mean titer was higher in the group which received the CPV-2 vaccine alone (Table IX). Response to distemper antigens was slightly better in the group that had received both vaccines rather than the distemper-adenovirus-parainfluenza vaccine alone. All pups showed prevaccination adenovirus antibody which might have been maternally derived. All pups which received the vaccine containing adenovirus showed a significant (> fourfold) rise in adenovirus antibody following vaccination. The mean titer was slightly higher for the group which had received both vaccines.

DISCUSSION

The efficacy of this inactivated canine parvovirus vaccine has been demonstrated by its ability in three separate pilot production lots, and in nonadjuvanted, single adjuvanted, and double adjuvanted form, to produce an immunological response in 100 percent (20/20) of vaccinated dogs after just one inoculation. Furthermore, upon challenge none of these vaccinated dogs showed clinical signs of

TABLE IX

COMPATIBILITY BETWEEN INACTIVATED, DOUBLE-ADJUVANTED CPV-2 VACCINE AND A LIVE-MODIFIED CANINE DISTEMPER-ADENOVIRUS-PARAINFLUENZA VACCINE

	Group (n = 4) mean antibody titers of vaccinated dogs					
	CPV-2[b]		CD[c]		CAV[d]	
Vaccine	Prevacc.	Postvacc.[e]	Prevacc.	Postvacc.	Prevacc.	Postvacc.
CPV-2	<8	2432	<2	<2	12	12
CPV-2 + DA_2P[a]	<8	448	<2	63	21	256
DA_2P[a]	<8	<8	<2	43	17	144

[a] Vanguard-DA_2P, Norden Laboratories, Lincoln, Nebraska.
[b] Reciprocal hemagglutination-inhibiting antibody titer against CPV-2.
[c] Reciprocal median serum neutralizing antibody titer against canine distemper.
[d] Reciprocal hemagglutination-inhibiting antibody titer against canine adenovirus-1.
[e] Two weeks after second vaccination, four weeks after first vaccination.

CPV infection. Only from two of the 20 vaccinates, and on one occasion each, was CPV recovered from fecal samples taken after challenge. In contrast CPV was recovered from every one of the nine unvaccinated control dogs mainly during the period four to ten days postchallenge. In trials two and three, where a successfully developed challenge method (6) was employed, all of the controls showed prominent clinical signs consistent with CPV disease.

The commercial inactivated feline panleukopenia vaccine intended for cats was not effective at stimulating an antibody response after a single inoculation. Even after two doses titers were low in three dogs and nondetectable in the fourth. This confirms our field experience with this type of vaccine, although Carmichael, Pollock and Appel (8) reported higher, but nonpersistent titers, following two doses of the seven commercial killed feline panleukopenia vaccines they tested. Those authors have reported also that 42 percent of dogs vaccinated with modified live feline panleukopenia virus vaccine failed to develop protective titers following a single dose. Even two doses still left 10 to 20% of dogs unprotected.

The killed feline panleukopenia virus vaccine, licensed for use for preventing CPV in dogs, did produce regular antibody responses after one dose. However mean serum antibody titers after one or two doses, in this experiment, were some tenfold lower than with the double adjuvanted CPV-2 vaccine. Also prevention of viral shedding after challenge was less complete than with the CPV-2 vaccine.

The safety of the inactivated CPV-2 vaccine lots, demonstrated initially by standard *in vitro* laboratory testing, was confirmed by the continued health of the dogs following vaccination. The subcutaneous nodules noted at the inoculation site in some dogs receiving the adjuvanted CPV vaccines in trial 2 were painless and transient. Unreported histological studies have shown these nodules to be a mild granulomatous response almost certainly due to the adjuvant.

The subcutaneous and intramuscular routes of administration for the inactivated CPV-2 vaccine appear equally effective in stimulating serum antibody and in preventing disease. At least with the representative live modified canine distemper, adenovirus and parainfluenza vaccine studied, the CPV-2 vaccine could be used to reconstitute that freeze-dried vaccine without interfering with the antibody response to either distemper or adenovirus. Responses to the parainfluenza fraction were not measured.

A delayed onset of protection can be a disadvantage for some killed virus vaccines. However in the course of the compatibility trial it was demonstrated that the double adjuvanted vaccine had stimulated antibody titers of 1:48 to 1:96 within five days of the first injection. The duration of protection with killed vaccines is also sometimes questioned, particularly when detectable antibody can disappear within three months as demonstrated for some killed feline panleukopenia and CPV vaccines in dogs (2). Duration of immunity studies with this vaccine are in progress. The preliminary data from trial 2 indicates an excellent persistence of antibody for at least nine months with the double adjuvanted CPV-2 vaccine. Although the dog which had received only single adjuvanted vaccine had lost detectable antibody after six months, this dog was able to resist challenge at nine months.

Johnson and Spradbrow (11) suggested that the possible source of the "new" canine parvovirus might have been a veterinary biological product and advised that any attempt to control the syndrome in dogs should be made using inactivated rather than attenuated vaccines. The development of the inactivated CPV-2 vaccine reported here, with the efficacy, safety and good duration of immunity associated with the double adjuvanted vaccine, improves upon the results reported by Appel *et al* (2, 4) and extends the results reported by Eugster (9) who has also used formalin inactivated CPV-2 as a vaccine. Although in general, results obtained with the non-adjuvanted, single adjuvanted and double adjuvanted vaccine appears to be the preparation of choice in terms of its consistently strong induction of good serum titers, solid protection against challenge, and steady persistence of antibody.

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ABSTRACT

Pedersen, N.C., Pool, R.R. and O'Brien, T. Feline chronic progressive polyarthritis. American Journal of Veterinary Research (1980) 41: 522-535. (Dep. Med., Sch. Vet. Med., Univ., Davis, California 96161, USA).

Twenty cats with a chronic progressive polyarthritis were studied. The disorder occurred exclusively in male cats, and all but six were between 1.5 and 5 years of age. There were two forms of the disease as determined by radiographic changes, joint instability and deformity, and clinical course. The most prevalent form was characterized by osteopenia and periosteal new bone formation surrounding affected joints. Marginal periarticular erosions and collapse of the joint spaces with fibrous ankylosis occurred with time, but joint instability and deformities were not seen. The second form of the disease was characterized by severe subchondral marginal erosions, joint instability, and deformities. The periosteal proliferative form resembled Reiter's arthritis of man, and the deforming type resembled human rheumatoid arthritis. The disease began as tenosynovitis and synovitis, with subsequent changes in the articular cartilage and periosteal bone. Histopathological changes in these cats were similar to those in both chronic Reiter's and rheumatoid arthritis of man. Chronic progressive polyarthritis of cats was not caused by identifiable bacteria or mycoplasma, but was aetiologically linked to feline leukaemia virus (FELV) and feline syncytia-forming virus (FeSFV) infections. The FeSEV was isolated from the blood or was detected by a serological test in all of the cats with the disease, whereas FeLV was isolated or identified by immunofluorescence technique in 60% of the cats. The arthritis could not be reproduced by inoculation of cell-free synovial tissue from diseased cats or with tissue culture fluid containing FeSFV and FeLV isolates. It was postulated that arthritis was an uncommon manifestation of FeSFV infection that occurred in predisposed male cats. FeLV virus may not have been directly involved in the disease, but may have acted in some way to potentiate the pathogenic effects of FeSFV.

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