Do Two Current Canine Parvovirus Type 2 and 2b Vaccines Provide Protection Against the New Type 2c Variant?*

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CLINICAL RELEVANCE

Three groups (n = 9 or 10) of 12-week-old canine parvovirus type 2 (CPV-2) antibody–negative puppies were vaccinated: one group with a product containing modified-live CPV-2b (Galaxy DA2PPv; Schering-Plough Animal Health), one group with a product containing modified-live CPV-2 (Continuum DAP, Intervet), and one group (controls) with sterile saline. All puppies receiving CPV-2 and CPV-2b vaccines developed antibody as determined by the hemagglutination inhibition assay. All groups of puppies were challenged with a combination of virulent CPV-2b and CPV-2c 5 weeks after vaccination. All puppies in the CPV-2 and CPV-2b vaccinated groups were protected from disease, whereas all control group puppies developed disease and 50% died or were euthanized. This study demonstrated that the CPV-2 and CPV-2b vaccine components of the Continuum DAP and Galaxy DA2PPv products, respectively, provided protection against the CPV-2b virus and also provided complete protection against the new CPV-2c variant.

INTRODUCTION

Canine parvovirus type 2 (CPV-2) first appeared in 1978 and rapidly spread worldwide, causing severe enteric disease in the canine population.[1–4] When it first appeared in the United States, CPV-2 was also associated with myocardial disease,[2–4] but that is rarely, if ever, seen in the United States today. The development of myocarditis requires viral infection of the fetus or pup before or shortly after parturition. Because most adult dogs now have antibody, which was not the case in the late 1970s and 1980s, the virus rarely infects the fetus or puppy during the periparturient period.[2–4]

Genotypic changes leading to antigenic and biotypic differences in CPV-2 began to occur very early after the virus was first isolated and characterized.[5] The first change was recognized

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in the early 1980s and consisted of several amino acid changes as well as a host range change.[5,6] The new variant was termed CPV-2a. Although CPV-2 presumably originated as a mutant of feline panleukopenia virus (FPV), a very closely related parvovirus that was probably in the feline species for hundreds of years, the original CPV-2 virus failed to replicate in feline cells and could not infect domestic cats.[5,6] In contrast, the CPV-2a variant could replicate in feline cells, and it was possible to experimentally infect cats susceptible to FPV, but the virus did not cause disease. Within 2 to 4 years after CPV-2a appeared, another variant, designated CPV-2b, emerged.[5–7] This variant had only one amino acid change. The CPV-2b variant replicated as well, actually better, in feline cells as in canine cells in vitro.[7–9] CPV-2b has been reported to cause a "panleukopenia-like" syndrome in a small percentage of cats.[5,6] Cats that are immunized against FPV are protected from disease caused by CPV-2b as well as FPV.[10]

About 15 to 16 years after the appearance of CPV-2b (i.e., in 2000 to 2001), a new variant, CPV-2c, was reported in Europe[8] but was not detected in the United States until approximately 2005 or 2006.[8,9] The CPV-2c variant had an amino acid change in the 426th amino acid residue, but instead of it being Asn 426 Asp, as in CPV-2b, it was Glu 426. These minor mutations should not be unexpected because CPV-2 is a single-stranded DNA virus, and mutations would be more likely in a single-stranded DNA virus than in a double-stranded DNA virus; however, the mutation rate in CPV-2 would be less likely than for single-stranded RNA viruses.[7–9] Although these mutations have occurred at various intervals since CPV-2 first infected the canine species in the late 1970s, the important question is whether any of the mutations have significance with regard to vaccine-induced or natural immunity.[11] Some researchers suggest that the recent mutations raise concerns about the efficacy of current parvovirus vaccines against the mutant viruses, especially the new CPV-2c variant.[11,12]

The important question is whether any of the mutations that have occurred in canine parvovirus have significance with regard to vaccine-induced or natural immunity.

The present study was designed to determine if a vaccine containing modified-live (ML) CPV-2b or one containing ML CPV-2 provides protection against experimental challenge with a combination of CPV-2b and CPV-2c.

MATERIALS AND METHODS

Twenty-eight 12-week-old antibody-negative beagle pups of both sexes were used. Nine pups were vaccinated once with Galaxy DA2PPv (Schering-Plough Animal Health, serial number 212274B; canine distemper–adenovirus type 2–parainfluenza–parvovirus vaccine), a combination product containing ML CPV-2b. Nine pups were vaccinated once with Continuum DAP (Intervet, serial number 90066003A; canine distemper–adenovirus type 2–parvovirus vaccine), which contains ML CPV-2 patented strain 154. Ten pups were vaccinated once with sterile saline. Pups were segregated by vaccine group and housed in groups of four or five in the Isolation Unit of the Charmany Instructional Facility of the University of Wisconsin-Madison School of Veterinary Medicine, an

TABLE 1. Additive Clinical Scoring System used for CPV-2

Symptom	*Score*
Lethargy	1
Vomiting	1
Diarrhea	1
Dehydration	3
Bloody diarrhea	5
Anorexia	
1st day	1
2nd day	6
3rd day	12
Moribund	12

Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility.

Five weeks after vaccination, control pups were distributed to rooms containing vaccinated pups, and all pups were challenged intranasally and/or orally with a combination of virulent CPV-2b and CPV-2c. Control pups were housed with vaccinates to increase the severity of challenge, as the vaccinates would be exposed to further virulent CPV-2b and CPV-2c shed in feces of control pups over a period of days.

The challenge virus consisted of field isolates from puppies with clinical parvovirosis. Viruses were genetically typed using polymerase chain reaction analysis by Dr. J. Saliki of the University of Georgia. Each challenge dose contained approximately 1×10^6 $TCID_{50}$ (median tissue culture infective dose) each of CPV-2b and CPV-2c.

Clinical signs of disease were recorded daily, fecal samples were collected for detection of virus on specific days, and blood was collected weekly for viral serology. Clinical scores were assigned and totaled according to the rubric outlined in Table 1. Pups developing dehydration were treated with fluids as previously described.[13] Pups with clinical scores greater than 6 for 2 days or greater than 12 at any time were euthanized, as per the approved Animal Care and Use Protocol. The hemagglutination inhibition (HI) assay was used to measure antibody to CPV-2 and was performed as previously described.[14] Fecal samples were tested for CPV-2 as described for the SNAP Parvo Antigen Test (IDEXX Laboratories). All positive fecal antigen tests were confirmed by replicate testing. Laboratory personnel conducting both serology and antigen testing were blinded to study groups. Statistical analysis was not needed for this study.

RESULTS

The results of clinical scores for disease and mortality can be seen in Table 2. Table 3 shows antibody titers as determined by HI assay. Table 4 shows viral shedding as detected by the SNAP Parvo Antigen Test. Clinical signs were seen in all saline-vaccinated puppies, virus was detected in their feces, and antibody developed only after challenge. In contrast, there were no signs of disease in any of the vaccinated puppies. HI antibody titers remained the same after challenge, as the level of HI antibody at postchallenge (PC) day 14 did not differ from PC day 0 by more than a four-fold difference. This suggests that the virus was neutralized at the time of challenge, as would be expected in a CPV-2–vaccinated, antibody-positive puppy.[15–17] Virus was not detected in feces of the Galaxy-vaccinated group but was present in two of the nine puppies in the Continuum-vaccinated group. These puppies did not show any clinical signs, nor did their antibody titers increase.

DISCUSSION

The present study demonstrated that two of the current combination vaccines, Galaxy DA2PPv (CPV-2b) and Continuum DAP (CPV-2), provide complete protection against

TABLE 2. Clinical Scores and Mortality after Challenge

		Clinical Scores				
Vaccine Group	***Animal ID***	*PC Day 5*	*PC Day 6*	*PC Day 7*	*PC Day 8*	*PC Day 9*
Control	C A	2	21	Dead	—	—
	C B	0	1	5	0	0
	C C	2	10	26	Dead	—
	C D	1	3	11	1	0
	C E	0	0	6	1	0
	C F	2	20	Dead	—	—
	C G	1	1	1	0	0
	C H	2	17	Dead	—	—
	C I	1	21	Dead	—	—
	C J	0	0	1	0	0
Galaxy	SP 1	0	0	0	0	0
	SP 2	0	0	0	0	0
	SP 3	0	0	0	0	0
	SP 4	0	0	0	0	0
	SP 5	0	0	0	0	0
	SP 6	0	0	0	0	0
	SP 7	0	0	0	0	0
	SP 8	0	0	0	0	0
	SP 9	0	0	0	0	0
Continuum	IC 1	0	0	0	0	0
	IC 2	0	0	0	0	0
	IC 3	0	0	0	0	0
	IC 4	0	0	0	0	0
	IC 5	0	0	0	0	0
	IC 6	0	0	0	0	0
	IC 7	0	0	0	0	0
	IC 8	0	0	0	0	0
	IC 9	0	0	0	0	0

C = control; IC = Intervet Continuum; PC = postchallenge; SP = Schering-Plough.

the new CPV-2c as well as the CPV-2b variant. Because control puppies were present in the rooms with vaccinated puppies, we suspect virus detected in the two pups vaccinated with Continuum was the result of coprophagy of virus-laden feces from control pups in the room. Although dogs were not directly observed ingesting stool, diarrheic feces were found only in protected areas beneath resting areas, with stained areas remaining where diarrhea had been elsewhere in the room. The pups used in this study were free of maternally derived antibody at the time of vaccination. Therefore, one dose of vaccine was adequate to immunize all pups in the viral-vacci-

TABLE 3. CPV-2 Hemagglutination Inhibition Assay Titers before and after Challenge

		Clinical Scores		
Vaccine Group	***Animal ID***	*Day 0*	*PC Day 0*	*PC Day 14*
Control	C A	<20	<20	Dead
	C B	<20	<20	20,480
	C C	<20	<20	Dead
	C D	<20	<20	20,480
	C E	<20	<20	20,480
	C F	<20	<20	Dead
	C G	<20	<20	10,240
	C H	<20	<20	Dead
	C I	<20	<20	Dead
	C J	<20	<20	10,240
Galaxy	SP 1	<20	5,120	2,560
	SP 2	<20	10,240	5,120
	SP 3	<20	2,560	10,240
	SP 4	<20	10,240	5,120
	SP 5	<20	5,120	5,120
	SP 6	<20	5,120	5,120
	SP 7	<20	10,240	20,480
	SP 8	<20	5,120	2,560
	SP 9	<20	10,240	5,120
Continuum	IC 1	<20	2,560	2,560
	IC 2	<20	2,560	1,280
	IC 3	<20	2,560	2,560
	IC 4	<20	5,120	2,560
	IC 5	<20	2,560	1,280
	IC 6	<20	2,560	2,560
	IC 7	<20	10,240	5,120
	IC 8	<20	5,120	5,120
	IC 9	<20	2,560	1,280

C = control; IC = Intervet Continuum; PC = postchallenge; SP = Schering-Plough.

nated groups. Challenge studies showed that the vaccinated pups that developed antibody as detected by the HI test were protected from infection and clinical disease.[13,16–19]

At the present time, CPV-2c represents only a small percentage of parvovirus isolated in the United States. Although large-scale studies determining the prevalence of variants isolated from puppies in the United States have not been reported recently, ongoing research in our laboratory of approximately 100 isolates obtained from dogs nationwide would suggest that (1) CPV-2 is no longer present, (2) the CPV-2a variant constitutes a small percentage (less than

TABLE 4. CPV-2 Virus Shed in Feces after Challenge

		Fecal CPV Shed in Feces				
Vaccine Group	***Animal ID***	*PC Day 4*	*PC Day 5*	*PC Day 6*	*PC Day 7*	*PC Day 8*
Control	C A	Pos	Pos	Pos	Dead	—
	C B	Pos	Pos	Neg	Neg	Not done
	C C	Pos	Pos	Pos	Pos	Dead
	C D	Neg	Pos	Pos	Pos	Neg
	C E	Pos	Pos	Pos	Pos	Pos
	C F	Pos	Pos	Pos	Dead	—
	C G	Neg	Pos	Pos	Neg	Not done
	C H	Pos	Pos	Pos	Dead	—
	C I	Pos	Pos	Pos	Dead	—
	C J	Neg	Pos	Pos	Neg	Not done
Galaxy	SP 1	Neg	Neg	Neg	Neg	Neg
	SP 2	Neg	Neg	Neg	Neg	Neg
	SP 3	Neg	Neg	Neg	Neg	Neg
	SP 4	Neg	Neg	Neg	Neg	Neg
	SP 5	Neg	Neg	Neg	Neg	Neg
	SP 6	Neg	Neg	Neg	Neg	Neg
	SP 7	Neg	Neg	Neg	Neg	Neg
	SP 8	Neg	Neg	Neg	Neg	Neg
	SP 9	Neg	Neg	Neg	Neg	Neg
Continuum	IC 1	Neg	Pos	Pos	Neg	Neg
	IC 2	Neg	Neg	Neg	Neg	Neg
	IC 3	Neg	Neg	Neg	Neg	Neg
	IC 4	Neg	Neg	Neg	Neg	Neg
	IC 5	Neg	Neg	Neg	Neg	Neg
	IC 6	Neg	Neg	Neg	Neg	Neg
	IC 7	Neg	Neg	Neg	Neg	Neg
	IC 8	Neg	Neg	Neg	Neg	Neg
	IC 9	Neg	Pos	Neg	Neg	Neg

C = control; IC = Intervet Continuum; Pos = positive; Neg = negative; SP = Schering-Plough.

5%) of isolates, (3) the CPV-2b variant comprises about 85% to 90%, and thus remains the most prevalent, and (4) the CPV-2c variant represents approximately 10% of isolates. Similar to what has happened in the past, the new variant may likely become the predominant variant in the field after 2 to 4 years.[20]

The history of different variants of CPV used in vaccines since the early outbreak of CPV-2 in the late 1970s is varied. Vaccines used during the late 1970s and into the early 1980s were of FPV origin, as no CPV-2 vaccines were licensed until the early 1980s.[4] FPV remained in one canine vaccine until the mid-1990s, although

most vaccines developed in the 1980s and 1990s abandoned the FPV for an ML CPV-2 virus. The FPV vaccines were never very effective but were the only vaccines available for about 4 years after CPV-2 first appeared. All the initial ML CPV-2 vaccines were, as expected, made from the original CPV-2 isolates,[4,19,21] and some of those original CPV-2 isolates are still found in several of today's products. In the late 1980s, a vaccine with an ML CPV-2a variant was licensed[15,16] and remained on the market for about 10 years; it was never demonstrated to have any advantage over vaccines with CPV-2, even though CPV-2a was the most common variant in puppies at the time. The first of several vaccines containing ML CPV-2b appeared in the mid-1990s. At present, all the vaccines from the major US biologics manufacturers contain either ML CPV-2 or ML CPV-2b.[16–20] None contains both variants, and none knowingly contains either CPV-2a or CPV-2c.

Several groups have recently questioned whether the appearance of the CPV-2c variant raises concerns about the efficacy of current vaccines and, if so, whether new vaccines using the CPV-2c variant should be developed.[11,12] These are important questions that require answers, which can come from previous experimental studies and field experience with various vaccines as well as from new experimental studies and future field studies. We reviewed information based on previous studies and experience and presented the results of our present study, which included a CPV-2b and CPV-2c challenge in puppies vaccinated with products containing either ML CPV-2b or ML CPV-2.[13,17,18,22,23] Fortunately, to date, none of the CPV-2 genotypic mutations leading to new variants (e.g., 2a, 2b, 2c) appear to have led to changes that have significantly altered the antigenic properties of CPV-2. Therefore, at this time, it is not necessary to develop new vaccines. The current vaccines containing ML CPV-2 or ML CPV-2b protect against the variants CPV-2a, 2b, and 2c. However, that does not in any way guarantee that future mutations will also fail to significantly alter the antigenicity of this highly important pathogen of dogs; thus, we must ensure that current vaccines provide protection against all new future variants.

CONCLUSION

The present study, similar to our previous experience with other CPV-2 variants, shows that the ML CPV-2 and ML CPV-2b vaccines are effective in preventing infection and/or disease caused by all the variants known to be present in the United States at this time, including CPV-2c.[13,17–19] Furthermore, the current products can be expected to provide many years of immunity after vaccination.[13,17–19,22,23]

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