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## SEROLOGIC RESPONSE OF CAPTIVE COYOTES (*CANIS LATRANS* SAY) TO CANINE PARVOVIRUS AND ACCOMPANYING PROFILES OF CANINE CORONAVIRUS TITERS

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**ABSTRACT:** Fifty-five of 66 (83%) coyote pups from bitches vaccinated against canine parvovirus (CPV) were seropositive for CPV antibodies at birth. The CPV antibody titer in the pups declined with a half-life of 6.7 days until by the 8th week, only two of 41 (5%) pups were seropositive for CPV antibodies. At 8 wk, 41 of the pups were vaccinated against CPV (killed feline origin vaccine), but only one of 37 (3%) was positive for CPV antibodies at 11 wk. The 8-wk-old pups were either too young to respond to the CPV vaccine; they had sufficient undetectable, maternally-derived CPV antibodies to block active immunization; 3 wk was not a sufficient time for an immunological response from the pups; or the vaccine was poorly antigenic. Twenty of the 66 pups (30%) were seropositive for canine coronavirus (CCV) antibodies at birth, and all but three of the 20 were whelped from bitches that were also seropositive for CCV antibodies. Vaccination of females prior to whelping appeared to provide protection to their pups from CPV-induced mortality.

### INTRODUCTION

Canine parvovirus (CPV) is the most recently identified of three parvoviruses known to infect dogs (Carmichael and Binn, 1981). CPV has also been identified in wild canids (Fletcher et al., 1979; Evermann et al., 1980a; Mann et al., 1980). Dual infection with CPV and canine coronavirus (CCV) was reported in dogs (Appel et al., 1979) and in coyotes (Evermann et al., 1980b), and a more severe enteritis was observed when both viruses were present.

The response of dogs to inactivated CPV and feline panleukopenia virus vaccines was recently reported (Pollock and Carmichael, 1982a). Passive immunity to CPV in dog pups is derived from an immune bitch through the placenta and colostrum, and such passive immunity interferes with active immunization by a variety of CPV vaccines (Pollock and Carmichael, 1982b).

Although such features of the immune response may be similar in other canids, they have not yet been documented. The purpose of this study was to document the level of CPV and CCV antibody titers in captive, adult female coyotes that had been previously vaccinated against CPV and to determine the subsequent CPV antibody titer in their whelps over time. The prevalence of CPV-induced mortality in the whelps was also noted.

### MATERIALS AND METHODS

#### Animals

Forty-six male-female pairs of coyotes were used in the study. Thirty-one pairs were housed in individual pens (6 × 1.8 × 1.8 m), and 15 pairs were housed in kennel runs (3.7 × 0.9 × 2.1 m). Twenty-three pairs (all in individual pens) were maintained in relative isolation in scattered field locations, and the remaining 23 pairs were housed in a central kennel area that contained up to 100 additional coyotes.

All coyotes received commercial dry dog chow and water ad lib. and had been in captivity from 1 to 5 yr. The coyotes received canine distemper, hepatitis, leptospirosis, parainfluenza, and rabies vaccines. Anthelmintics were routinely administered. All of the coyotes had been vaccinated against CPV with a killed feline parvovirus vaccine of feline cell origin (Parvocine, Dellen Laboratories, Inc., Omaha, Nebraska 68134, USA) 9 mo previous to the

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study. The initial CPV vaccination was followed by a booster given 4 wk later.

### Sampling

The study began in early March 1981 by administration of a killed feline origin CPV booster vaccine (Parvocine) to all 46 pairs of coyotes. Previously collected data (Green, unpubl. data) indicated that conception was completed by early March in females that whelped during that particular reproductive season. At the time of vaccination a 5-ml blood sample was collected from the jugular vein of each female coyote, and a second 5-ml blood sample was collected from each female 2 wk later.

Attempts were made to obtain a 0.5-ml sample of colostrum from each female as soon after parturition as possible. Following whelping, 1-ml jugular blood samples were obtained from up to three pups from each litter at 1 to 3, 7, 14, and 21 days of age. The pups were removed from the adults at approximately 4 wk of age and were vaccinated with a killed feline origin CPV vaccine (Parvocine) at 8 and 11 wk of age. A 1-ml blood sample was obtained at the time of each vaccination. Serum was obtained from each blood sample by centrifugation within several hours of collection and frozen ( $-23^{\circ}\text{C}$ ) until analyzed for CPV and CCV antibody titers.

### Antibody determinations

Antibodies to CPV and CCV were detected by the indirect fluorescent antibody (IFA) method as described by Helfer-Baker et al. (1980). Briefly, samples to be analyzed for CPV- and CCV-specific immunoglobulin type G (IgG) were initially diluted 1:25 in phosphate buffered saline followed by fourfold serial dilutions to 1:1,600. Rabbit-origin anti-canine IgG conjugated to a fluorescent marker, FITC (Antibodies Inc., Davis, California 95616, USA), was used to detect CPV and CCV antibodies. Crandell feline kidney cells infected with either the Cornell strain of CPV (78-0929) or the 1-71 strain of CCV were grown in eight-well chamber slides (Miles Laboratories, Inc., Naperville, Illinois 60540, USA) and served as the substrate for the IFA test. Serum antibody titers were expressed as the reciprocal of the highest dilution of serum showing specific fluorescence.

With the IFA test, dilutions  $<25$  are not valid due to nonspecific fluorescent reactions. However, the IFA test is more specific than the hemagglutination inhibition (HI) test where HI titers  $<80$  are not considered valid due to nonspecific serum inhibitors (Carmichael et al., 1980).

Data were analyzed using chi-square, Student's *t*-test, and simple linear regression. *P* values  $<0.05$  were considered statistically significant.

## RESULTS

### Canine parvovirus

A significantly greater number ( $P < 0.01$ ) of kennel-area females were seropositive for CPV (21/23) antibodies than the field females (9/23) prior to the March CPV vaccine booster. Two weeks following the CPV booster, all of the kennel-area females and 19 of the 23 field females had antibody titers to CPV (Table 1).

There was a significant difference ( $P < 0.01$ ) in the change of the CPV antibody level following the booster between the field and the kennel coyotes. In the field, the CPV antibody titers of 15 females in-

TABLE 1. Pre- and post-CPV-booster CPV titers for field and kennel-area adult coyotes. (Titers are highest reactive IgG titers by IFA.)

Field coyotes		Kennel coyotes	
Pre-booster CPV titer	Post-booster CPV titer	Pre-booster CPV titer	Post-booster CPV titer
$<25$	400	25	400
$<25$	100	100	400
$<25$	100	25	25
100	400	$<25$	400
$<25$	25	100	400
$<25$	$<25$	400	400
$<25$	100	1,600	100
100	400	400	100
100	400	100	100
$<25$	$<25$	100	100
400	400	400	100
$<25$	25	100	100
$<25$	$<25$	$<25$	25
25	400	100	100
25	400	400	400
$<25$	25	100	100
$<25$	$<25$	100	100
$<25$	25	400	100
$<25$	100	100	100
1,600	1,600	400	100
$<25$	100	100	25
100	100	100	100
400	100	100	25

TABLE 2. CPV serum antibody titers of pups born to bitches seropositive for CPV antibodies. (Titers are highest reactive IgG titers by IFA.)

Bitch	Prewhelping titer to CPV		Pup	Serum CPV titer of whelps				
	Serum	Colostrum		1-3 day	1 wk	2 wk	3 wk	8 wk
319	25	100	1	25	25	<25	—*	—
			2	<25	<25	<25	—	—
			3	<25	—	—	—	—
312	25	no sample	1	25	<25	<25	<25	<25
			2	<25	<25	<25	<25	<25
			3	<25	<25	<25	<25	<25

\* Dash indicates that pup died.

creased, one decreased (still seropositive), and seven were unchanged. In the kennel area, the CPV antibody titers of five females increased, seven decreased (still seropositive), and 11 were unchanged following the March CPV booster (Table 1).

Twenty-nine of the 46 (63%) females whelped including 20 of 23 (87%) field females and nine of 23 (39%) kennel females. Twenty-three females (17 field and 6 kennel) kept their study pups ( $n = 66$ ) for at least 1 wk, and 15 females (11 field and 4 kennel) had study pups ( $n = 37$ ) that survived for the 11 wk of the project.

Colostrum was collected from 13 females. Ten (77%) samples were positive for CPV antibodies. There was no significant difference between the serum and colostrum CPV antibody level in any of the females from which both serum and colostrum were sampled.

Fifty-five of 66 (83%) pups were seropositive for CPV antibodies within 3 days

of birth. There was a significant relationship ( $P < 0.01$ , by chi-square) between the presence of CPV antibodies in bitches and in their pups. Generally, bitches with antibodies produced pups with antibodies, and bitches without antibodies produced pups without antibodies. Several exceptions were observed, however. Two bitches (319 and 312) that were seropositive for CPV antibodies produced pups that were seronegative for CPV antibodies (Table 2). Colostrum was obtained from one of the bitches (319), and it was also seropositive for CPV antibodies. Two bitches (200 and 261) that were seronegative for CPV antibodies produced pups that were seropositive for CPV antibodies (Table 3). Colostrum from one bitch (200) was positive for CPV antibodies, and colostrum from the other (261) was negative for CPV antibodies.

Despite the positive relationship between the presence of antibodies in bitch-

TABLE 3. CPV serum antibody titers of pups born to two bitches that were seronegative for CPV antibodies. (Titers are highest reactive IgG titers by IFA.)

Bitch	Prewhelping titer to CPV		Pup	Serum CPV titer of whelps				
	Serum	Colostrum		1-3 day	1 wk	2 wk	3 wk	8 wk
200	<25	100	1	25	25	<25	<25	<25
			2	25	25	25	25	<25
			3	<25	<25	<25	<25	<25
261	<25	<25	1	25	25	25	<25	<25
			2	25	25	<25	<25	<25
			3	25	25	<25	<25	<25

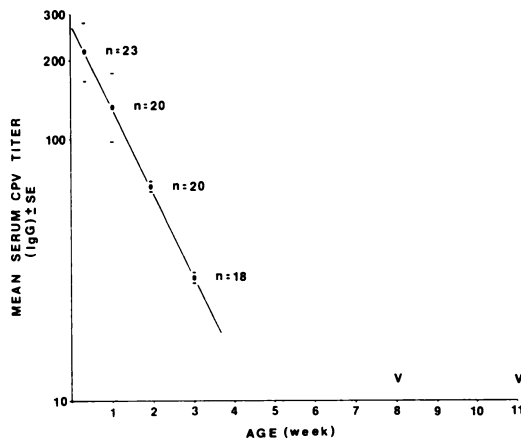


FIGURE 1. Mean serum CPV titer per litter ( $n$ ) of coyote pups from birth through 11 wk (V = vaccination against CPV). Each point was obtained by first averaging the titers within each litter and then averaging the litter means. The regression line is a least squares fit with  $r = -0.9997$ .

es and their pups, there was no significant correlation between the levels of the CPV antibody titer in the bitches and their pups. That is, bitches with high CPV titers did not necessarily whelp pups with high titers. There was no consistent difference in the level of antibody titer to CPV between male and female pups.

The level of CPV antibody titer in the pups steadily declined through the 3rd wk of life (Fig. 1). By the 8th wk only two of 41 (5%) of the pups had detectable antibody titers to CPV. The half-life of the maternally-derived CPV antibody was 6.7 days (calculated from the regression equation used to construct Fig. 1). Despite the fact that all pups were vaccinated against CPV at 8 wk of age, only one of 37 (3%) pups had a titer to CPV at 11 wk.

#### Canine coronavirus

As was noted for CPV, a significantly greater number ( $P < 0.01$ ) of kennel-area females were seropositive for CCV (21/23) antibodies than the field females (7/23) prior to the March CPV vaccine booster. Nineteen kennel-area females and four

field females had antibody titers to both CPV and CCV. Six of the kennel-area females and two of the field females that previously had CCV antibody titers showed no detectable CCV antibody titer following the March CPV booster. One field female seroconverted (seronegative to seropositive) to CCV following the March CPV booster.

The level of CCV antibody titer increased in one female, decreased in 15 females, and was unchanged in 30 females following the March CPV booster. There was no significant difference in the change in CCV antibody level between the field and kennel-area females.

Samples of colostrum from six of the 13 (46%) females were positive for CCV antibodies. There was no significant difference between the serum and colostrum CCV antibody level in any of the females from which both serum and colostrum were sampled.

Pups were tested for antibody to CCV at the first (1–3 day) and last (11 wk) bleeding only. Twenty of 66 (30%) pups were seropositive to CCV antibodies within 3 days of birth, and generally, pups that were positive for CCV antibodies were whelped from bitches that were positive for CCV antibodies. All of the 37 pups remaining at 11 wk of age were seronegative to CCV antibodies.

#### Pup mortality

At least five of the pups that died during the study exhibited gross external signs similar to those associated with parvovirus (bloody diarrhea, emaciation, dehydration). However, parvovirus was not identified in three of the five pups that were clinically examined. *Coccidia* (*Isospora canis* and *I. ohioensis*) were thought to be a contributory cause of the deaths.

#### DISCUSSION

Significantly more kennel-area females had pre-booster antibody titers to CPV

than did the field females despite the fact that all coyotes had previously received CPV vaccinations. It follows, therefore, that fewer kennel females responded to the booster with an increase in CPV antibody titer. Kennel females also had higher pre-booster antibody titers to CCV than did field females. Perhaps the closer proximity of the coyotes in the kennel area was more favorable for the perpetuation of a continual challenge by field strains of CPV and CCV, thus maintaining positive CPV and CCV antibody titers in the coyotes.

Since 61% of the adult coyotes had antibody to CCV without CCV vaccination, it is obvious that CCV had been present already in the population. The extent to which CPV was present naturally could not be determined because of vaccination against CPV.

Canine parvovirus and CCV have been identified as causative agents of canine enteritis, and both viruses have been found simultaneously in dogs (Appel et al., 1979) and coyotes (Evermann et al., 1980a). Therefore, enteritis can be viewed as a disease complex potentially involving several viruses. However, no consistent relationship between CPV and CCV in coyotes could be determined from this study. In addition, an explanation for the decreases in CPV titers and the changes in the CCV titers of some of the bitches following the March CPV booster was not readily apparent.

The half-life of maternally-derived antibody to CPV in the coyote pups (6.7 days) was shorter than that reported in dogs (9.7 days) (Pollock and Carmichael, 1982b). This difference, however, may not be significant and may be due to sampling and other procedural differences between the studies.

Colostrum transfer accounted for 90% of the maternally-derived antibody to CPV in dogs (Pollock and Carmichael, 1982b), and similarly high levels of colostrum antibody transfer in dogs were found for ca-

nine distemper (Gillespie et al., 1958). We were unable to sample pre-suckling coyote pups for CPV antibody titer, but we speculate that colostrum transfer of antibodies is also high in coyotes. However, in one of the 13 females from which a colostrum sample was obtained, the colostrum was negative for CPV antibodies while the serum sample was positive. Three of her pups were sampled near birth, and all were seropositive for CPV antibodies.

Maternally-derived antibody to CPV prevented a response to vaccination with inactivated CPV vaccines in dog pups until they had been seronegative for 4 wk, or until they were 14 to 16 wk old (Pollock and Carmichael, 1982b). An experimental attenuated canine origin CPV vaccine was effective in controlling CPV-induced diarrheal disease in pups from a closed beagle colony when administered in a single subcutaneous vaccination at 7.5 wk of age or in two vaccinations at 6.5 and 8 wk of age (Glickman and Appel, 1982). Because serological data on pups in this study were collected only until 11 wk, we were not able to conclude firmly that there was a maternally caused post-vaccination suppression of antibody development in coyote pups. However, eight pups (from 4 bitches that were seronegative for CPV antibodies) that survived through the study were seronegative for CPV antibodies from birth, and despite vaccination against CPV at 8 wk, they all failed to develop a detectable positive response by 11 wk of age. Only one of 37 pups was positive for CPV antibodies at the final 11 wk sampling. Several possible explanations exist. Either the pups were too young to respond to the parvovirus antigens in the vaccine; greater than 3 wk were required for the immunological response; undetectable low levels of maternal CPV antibody in the pups interfered with a serologic response to the CPV vaccine; or the vaccine was poorly antigenic.

Females that were seropositive for CPV antibodies did not consistently whelp pups

that had detectable antibody titers for CPV, and three pups that were seropositive for CPV antibodies at birth were whelped from a bitch that had no detectable colostral or serum antibody titer for CPV. It is possible that the IFA method lacked the sensitivity to detect low levels of CPV antibodies in bitches and pups, however, a more sensitive yet practical test was not available.

Parvovirus infection was not identified as a cause of mortality of pups during the study. In the year previous to this study, 26 of 52 (50%) coyote pups (approximately 6 to 9 wk of age) at the kennel area died of enteritis with CPV identified as one of the causative agents (Evermann et al., 1980b). None of the bitches had been vaccinated against CPV.

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