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VIRAL ANTIBODIES IN COYOTES FROM CALIFORNIA

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ABSTRACT: Prevalence of antibodies against canine parvovirus (CPV), canine distemper virus (CDV), and canine adenovirus type 1 (CAV) were determined among 152 coyotes (*Canis latrans*) at the Naval Petroleum Reserves (NPRC; California, USA) from 1985 to 1990. Overall prevalence of antibodies to CPV, CDV, and CAV was 66%, 37%, and 68%, respectively. Prevalence of CPV and CDV varied significantly among years. Antibody prevalence did not differ between sexes for any disease, but did vary significantly among age classes and was lowest for pups (<1-yr-old). Among pups, antibody prevalence increased with age for all three diseases. Coyotes are a potential source of viral exposure for endangered San Joaquin kit foxes (*Vulpes macrotis mutica*), but variation in coyote abundance did not appear to influence antibody prevalence among kit foxes.

Key words: Canine adenovirus type 1, canine distemper, canine parvovirus, *Canis latrans*, coyote, endangered species, San Joaquin kit fox, *Vulpes macrotis mutica*.

INTRODUCTION

Coyotes (*Canis latrans*) frequently have antibodies to a variety of infectious canine diseases (Pence and Custer, 1981). Serologic surveys for evidence of viral infection among coyotes have been conducted in numerous locations in the United States, including Texas (Trainer and Knowlton, 1968), New York (Monson and Stone, 1976), Utah and Idaho (Thomas et al., 1984), Colorado (Gese et al., 1991), Georgia (Holzman et al., 1992), and Wyoming (Gese et al., 1997), but incidence and effects of viral diseases on populations are difficult to determine from such data.

In the San Joaquin Valley of California (USA), coyotes are sympatric with endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) and constitute a potential source of viral exposure for foxes. To assess the potential for transmission of viruses from coyotes to kit foxes, we conducted a serological survey for viral antibodies among coyotes at the Naval Petroluem Reserves in California (NPRC). Our objectives were to determine antibody prevalences for canine parvovirus (CPV), canine distemper virus (CDV), and canine adenovirus type 1 (CAV) among coyotes, and to determine the prevalences of these antibodies among sex and age classes within the coyote population.

MATERIALS AND METHODS

Naval Petroleum Reserves in California is an area of active petroleum production encompassing 31,293 ha and located 42 km southwest of Bakersfield, California (Kern County; 35°17′N, 119°28′W). McCue and O'Farrell (1988) provide a description of the study area.

Blood samples were collected from coyotes killed during a control program conducted at NPRC from 1985 to 1990 (Cypher and Scrivner, 1992). All coyotes were killed by gunshot. Blood samples were collected by cardiac puncture whenever possible; otherwise, samples were collected from pooled blood in the thoracic or abdominal cavities. All samples were centrifuged to obtain serum, which was stored at −20 C until analysis.

Analyses of sera for the presence of viral antibodies were conducted by the Immunology/Virology Diagnostic Laboratory (Veterinary Medical Teaching Hospital, University of California, Davis, California, USA). Antibodies against CPV were detected using an indirect fluorescent antibody (IFA) test (Rose et al., 1992). Serum dilutions (1:10 to 1:520) were incubated on commercially prepared substrate slides (Veterinary Medical Research Diagnostics, Pullman, Washington, USA) for 30 min and then rinsed for 10 min in phosphate buffered saline (PBS). The slides then were flooded with rabbit anticanine immunoglubulin G (IgG) (The Binding Site, San Diego, California, USA) for 30 min. Slides then were washed for

TABLE 1. Annual prevalence of antibodies to canine parvovirus (CPV), canine distemper (CDV), and canine adenovirus type 1 (CAV) among coyotes at the Naval Petroleum Reserves in California, Kern County, from 1985 to 1990.

	CPV		CDV		CAV	
Year	Samples	Percent positive	Samples	Percent positive	Samples	Percent positive
1985	26	77	26	65	26	85
1986	32	47	32	38	32	69
1987	7	86	7	29	7	71
1988	31	48	31	26	31	68
1989	51	80	50	34	52	58
1990	4	75	4	0	4	75
Total	151	66	150	37	152	68

10 min in PBS, counter-stained for 5 min in Evans Blue, and rinsed again for 10 min in PBS. A coverslip was affixed using a glycerol-based mounting media, and the slides were examined under a fluorescent microscope. Titer was determined from the highest dilution giving positive fluorescence and titer levels were considered seropositive at levels ≥1:20.

Antibodies to CAV were determined by a serum neutralization test (Appel et al., 1975). Increasing dilutions (1:2 to 1:1024) of test sera were mixed with a constant concentration of CAV 100 $TCID_{50}$. Serum-virus mixtures then were incubated on Madin-Darby canine kidney tissue culture cells (American Type Culture Collection, Rockville, Maryland, USA) for 3 days at 35 C in 5% CO_2. Titer was determined from the highest serum dilution that prevented 50% of cultures from exhibiting cytopathic effects after incubation. Samples were considered seropositive at titer levels ≥1:8.

Antibodies to CDV were detected using an IFA test (Rose et al., 1992). Substrate slides were made from mink kidney cells infected with the virus (American BioResearch Inc., Seymour, Tennessee, USA). Serial dilutions (1:2 to 1:512) of serum were incubated on the substrate slides for 30 min, and then washed for 10 min in PBS. Slides then were flooded with rabbit anticanine IgG and incubated for another 30 min. After counter-staining for 5 min in Evans Blue, the slides were rinsed for 10 min in PBS and a coverslip was affixed using a glycerol-based mounting media. The slides then were examined under a fluorescent microscope and the titer was determined from the highest dilution giving positive fluorescence. Samples were considered seropositive at titer levels of ≥1:8.

Prevalence of antibodies to CPV, CDV, and CAV was determined for all coyotes combined, and by year, sex, and age class. Ages of coyotes were determined by cementum annuli analysis of a lower canine tooth (Matson's Laboratory, Milltown, Montana, USA). Age classes were defined as pup (<1-yr-old), yearling (1-yr-old), and adult (≥2-yr-old). Contingency-table analyses using a G statistic (Zar, 1984) were used to test for differences in antibody prevalence among years, sexes, and age classes. To determine the chronology of antibody acquisition among pups, contingency-table analyses were used to test for differences in prevalence among pups taken during three time periods: April to June, July to October, and February to March. P-values ≤0.05 were considered statistically significant.

RESULTS

Among coyotes sampled, antibody prevalence was 66% for CPV, 37% for CDV, and 68% for CAV (Table 1). Prevalence of CPV antibodies varied significantly among years (G = 17.15, 5 df, $P < 0.01$), as did prevalence of CDV antibodies (G = 14.45, 5 df, P = 0.01) (Table 1). Prevalence of CAV antibodies did not vary among years (G = 6.32, 5 df, P = 0.28). Also, antibody prevalence did not differ between sexes for CPV (G = 1.31, 1 df, P = 0.25), CDV (G = 0.87, 1 df, P = 0.35), or CAV (G = 2.36, 1 df, P = 0.13).

Antibody prevalence varied among age classes (Table 2) for all three viruses (CPV: G = 27.26, 2 df, $P < 0.01$; CDV: G = 19.05, 2 df, $P < 0.01$; and CAV: G = 28.73, 2 df, $P < 0.01$). Prevalence of CDV and CAV antibodies was highest among adults and lowest among pups, but prevalence of CPV antibodies was highest among yearlings and lowest among pups. Among

TABLE 2. Prevalence of antibodies to canine parvovirus (CPV), canine distemper (CDV), and canine adenovirus type 1 (CAV) among coyote age classes at the Naval Petroleum Reserves in California, Kern County, from 1985 to 1990.

	CPV		CDV		CAV	
Age class	Samples	Percent positive	Samples	Percent positive	Samples	Percent positive
Pup (<1 year)	47	38	47	15	48	48
Yearling (1–2 years)	37	89	36	36	37	54
Adult (≥2 years)	67	73	67	54	67	90

pups, antibody prevalence varied among time periods for CPV ($G = 17.66$, 2 df, $P < 0.01$), but not for CDV ($G = 0.27$, 2 df, $P = 0.87$) or CAV ($G = 1.22$, 2 df, $P = 0.55$) (Table 3). Prevalence of CPV antibodies was highest among older pups taken during February to March and lowest among younger pups taken during April to June.

DISCUSSION

Based on the prevalence of CPV antibodies among coyotes sampled at NPRC (66%), a large proportion of the population had been exposed to this virus. Thomas et al. (1984) considered CPV antibody prevalence >50% to be "high" and indicative of a highly contagious infection. This virus is persistent in the environment (Thomas et al., 1984) which may contribute to the high exposure rates observed at NPRC and elsewhere. Gese et al. (1997) reported that CPV antibody prevalence was 100% among coyotes ≥4-mo-old in Wyoming. Antibody prevalence was 71% among coyotes in southeastern Colorado (Gese et al, 1991) and 65% among coyotes in Georgia (Holzman et al., 1992). Thomas et al. (1984) conducted serological surveys for CPV antibodies among coyotes in Utah, Idaho, and Texas from 1972 to 1983, and found that prevalence exceeded 90% in all three locations by the end of the study.

Thomas et al. (1984) and Gese et al. (1991) both reported that CPV antibody prevalence in coyotes did not differ between sexes, which is consistent with results from NPRC. However, prevalence was higher among male coyotes in Georgia (Holzman et al., 1992). In Colorado, antibody prevalence did not differ between adults (≥1-yr-old) and juveniles (<1 yr old) (Gese et al., 1991). But similar to results from NPRC, CPV antibody prevalence was significantly higher among adults in Georgia (Holzman et al., 1992). In Wyoming, no CPV antibodies were found in pups ≤3-mo-old, but prevalence was 100% in older individuals (Gese et al., 1997). Higher CPV antibody prevalence among older age classes may be a function of greater mortality among pups resulting in a lower proportion of seropositive survivors in younger age classes (Gese et al., 1991).

TABLE 3. Prevalence of antibodies to canine parvovirus (CPV), canine distemper (CDV), and canine adenovirus type 1 (CAV) among coyote pups collected during three time periods at the Naval Petroleum Reserves in California, Kern County, from 1985 to 1990.

	CPV		CDV		CAV	
Time period	Samples	Percent positive	Samples	Percent positive	Samples	Percent positive
April to June	21	14	21	14	20	50
July to October	16	38	16	13	18	39
February to March	10	90	10	20	10	60

The prevalence of CDV antibodies among coyotes at NPRC varied annually. CDV prevalence may be positively correlated with host density (Budd, 1981; Pence and Custer, 1981), and coyote abundance at NPRC declined about 85% between 1985 and 1991 (Cypher and Scrivner, 1992), which may have contributed to the observed variation in antibody prevalence. In Texas, antibody prevalence among coyotes increased from 30% in 1975–76 to 86% in 1984, and CDV was considered to have become enzootic because prevalence had increased to ≥60% (Guo et al., 1986). In Wyoming, prevalence of CDV antibodies declined from 100% in 1989 and 1990 to 31% in 1992 (Gese et al., 1997). Conversely, prevalence among coyotes in Colorado (57%) did not differ among years (Gese et al., 1991). Prevalence of CDV antibodies reported from other locations included 0% in Georgia (Holzman et al., 1992), 37% in Texas (Trainer and Knowlton, 1968), and 50% in Wyoming (Williams et al., 1988).

Consistent with results from NPRC, CDV antibody prevalence was similar between sexes for coyotes in Texas (Guo et al., 1986) and Colorado (Gese et al., 1991). Also, consistent with results from NPRC, prevalence differed significantly among coyote age classes in Texas where prevalence for pups, yearlings, and adults was 25%, 67%, and 91%, respectively (Guo et al., 1986). Similarly, CDV antibody prevalence among adults (62%) was significantly higher than among juveniles (33%) in Colorado (Gese et al., 1991). In Wyoming, CDV antibody prevalence increased with age and was 0%, 23%, 54%, and 88% among young pups (≤3-mo-old), older pups (4- to 11-mo-old), yearlings, and adults, respectively (Gese et al., 1997).

Relatively little information is available regarding the prevalence of CAV antibodies among free-ranging coyotes. The 68% prevalence observed at NPRC is somewhat higher than the 57% prevalence reported from Texas (Trainer and Knowlton, 1968) and 41% prevalence reported from Georgia (Holzman et al., 1992). In Wyoming, overall antibody prevalence declined from 100% in 1989 and 1990 to 31% in 1992, and was attributed to declining prevalence among pups ≤3-mo-old (Gese et al., 1997). Similarly, prevalence of CAV antibodies increased with age at NPRC. However, prevalence did not differ among coyote age classes in Georgia (Holzman et al., 1992), but consistent with results from NPRC, prevalence of antibodies to CAV also did not differ between sexes in Georgia.

The effects of CPV, CDV, and CAV on the coyote population at NPRC are unknown. Coyote population indices at NPRC varied considerably from 1985 to 1995, but these indices were strongly correlated with prey availability (Cypher and Spencer, 1998). The most likely population effect from these viruses is reduced survival among young pups. Gese et al. (1997) reported that at least eight of 21 transmittered pups died of CPV at Yellowstone National Park in Wyoming, although recruitment rates were considered to be "normal". Also, CPV was implicated as a significant cause of mortality for wolf (*Canis lupus*) pups (Mech and Goyal, 1993; Johnson et al., 1994), and was considered to be a potential limiting factor for wolf population growth (Mech and Goyal, 1995). Others have suggested that CPV, CDV, and CAV have the capacity to exist within coyote populations in an enzootic state (Thomas et al., 1984; Guo et al., 1986), and may only cause significant mortality during stressful conditions such as high density, food scarcity, or parasitism (Trainer and Knowlton, 1968; Pence and Custer, 1981).

Coyotes constitute a potential source of exposure to CPV, CDV, and CAV for endangered kit foxes at NPRC. The opportunity for interspecific transmission of viral pathogens is high because coyotes are present in all areas of NPRC occupied by kit foxes. Although transmission can occur through direct contact, coyotes commonly kill the kit foxes they encounter (Cypher

and Spencer, 1998). Therefore, transmission is more likely to occur through environmental contamination (e.g., feces or urine). Prevalence of viral antibodies among kit foxes at NPRC was determined from serum samples collected in 1981, 1982, and 1984, and was 81% for CPV, 10% for CDV, and 16% for CAV (McCue and O'Farrell, 1988). Additional samples (n = 140) were collected from 1988 to 1991, and antibody prevalence among kit foxes was 76% for CPV, 6% for CDV, and 12% for CAV (B. Cypher, unpubl. data). Antibody prevalence among kit foxes was similar between the two sampling periods despite the fact that coyote abundance declined significantly between these periods (Cypher and Scrivner, 1992). Thus, variation in coyote abundance did not appear to influence the prevalence of viral antibodies among kit foxes.

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