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## Serologic Survey for Selected Infectious Disease Agents in Swift and Kit Foxes from the Western United States

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**ABSTRACT:** A serologic survey of swift fox (*Vulpes velox*) and kit fox (*V. macrotis*) from the western USA was conducted for 12 infectious diseases. Samples from swift fox were collected between 1987 and 1992 from Colorado ( $n = 44$ ), Kansas ( $n = 10$ ), and Wyoming ( $n = 9$ ). Samples from kit fox were collected in California ( $n = 86$ ), New Mexico ( $n = 18$ ), Utah ( $n = 9$ ), and Arizona ( $n = 6$ ). Overall antibody prevalence rates were 33 of 110 (30%) for canine parvovirus (CPV), 9 of 72 (13%) for canine distemper virus (CDV), 23 of 117 (20%) for vesicular stomatitis New Jersey, 16 of 117 (14%) for vesicular stomatitis Indiana, six of 117 (5%) for Cache Valley virus, five of 117 (4%) for Jamestown Canyon virus, one of 97 (1%) for rabies virus, one of 117 (1%) for Colorado tick fever virus, and one of 117 (1%) for western equine encephalitis virus. In addition, antibodies were not found to *Yersinia pestis*, *Francisella tularensis*, and *Borrelia burgdorferi* in serum from 25 Colorado swift fox. Adult swift fox from Colorado had serologic evidence of exposure to CPV more often than juveniles. No juvenile swift fox from Colorado had serum antibodies to CDV. There were season-specific differences in serum antibody prevalence for CPV for swift fox from Colorado. No viruses were isolated from ectoparasites or fox from Colorado.

**Key words:** Swift fox, kit fox, canine distemper virus, canine parvovirus, rabies virus, arbovirus, bacteria, rickettsia.

Diseases can threaten the long-term viability of small populations of host species (Thorne and Williams, 1988). Some swift fox (*Vulpes velox*) and kit fox (*V. macrotis*) populations in the western USA are considered threatened or recovering. Exposure to pathogens could threaten population recovery efforts.

Swift fox and kit fox are 1.4 to 3.0 kg

arid-land foxes of western North America (O'Farrell, 1987; Scott-Brown et al., 1987). These fox species are closely related. They compose a taxonomic group (clade) with arctic fox (*Alopex lagopus*) which is distinct from other members of the genus *Vulpes* (Wayne and O'Brien, 1987; Mercure et al., 1993). Swift fox inhabit the Great Plains from Texas to Canada. Kit fox inhabit arid regions west of the Rocky Mountains. A limited area of hybridization between the two species exists in New Mexico and western Texas (Mercure et al., 1993). Both species of fox feed primarily on small vertebrates and insects, and occupy ground dens.

Swift fox numbers plummeted in the early 1900's due to efforts to poison other carnivores (Scott-Brown et al., 1987; Herrera et al., 1991), and this species is a candidate species for listing under the U.S. Endangered Species Act (U.S. Department of the Interior, 1996). Kit fox populations were similarly affected by predator control efforts (O'Farrell, 1987). The San Joaquin kit fox (*V. macrotis mutica*) is listed as threatened by the state of California, and endangered under the U.S. Endangered Species Act (Disney and Spiegel, 1992).

We report the results of a broad geographic serologic survey to identify exposure to selected pathogens in swift and kit fox. We also report serology results for samples from Colorado with respect to demography.

Colorado swift fox were trapped in box

(National Live Trap Corporation, Tomahawk, Wisconsin, USA) and enclosure traps (Covell, 1992) at the U.S. Army's Piñon Canyon Maneuver Site (PCMS; Las Animas County, USA; 37°20'N, 103°40'W) as part of a study on swift fox dispersal and energetics (Covell, 1992). Behavioral data was collected using radio telemetry and visual observations at dens (Covell, 1992). Trapping was done from August, 1989 through January, 1991. Fox were manually restrained for collection of samples, tagged, radio-collared (Telonics, 932 East Impala Avenue, Mesa, Arizona, USA) and released. One to five ml blood samples were collected by venipuncture with 0.1 ml liquid heparin, promptly chilled on ice packs, centrifuged within 6 hr, plasma separated, and stored at -20 C. Forty-four fox were sampled on at least one date and multiple collections were made from 11 fox which were recaptured. Additional serum samples collected during 1987-1992 were provided by the University of California (Los Angeles, California, USA) (Table 1). These samples had been collected as part of a genetics study of swift and kit fox (Mercure et al., 1993). Due to limited volumes of sera, it was not possible to assay each sample for each pathogen.

Serum samples were tested for antibodies to canine parvovirus (CPV) and canine distemper virus (CDV) by the Colorado State University Diagnostic Laboratory (Fort Collins, Colorado, USA), utilizing serum neutralization (Collins et al., 1988) and hemagglutination inhibition (Joo et al., 1976; Lennette and Schmidt, 1979), respectively. Samples were tested for virus neutralizing antibodies (VNA) to rabies virus (RV) at the CDC, Division of Viral and Rickettsial Diseases (Atlanta, Georgia, USA), utilizing a modification of the rapid fluorescent focus inhibition test (Reagan et al., 1983). Samples were tested for antibodies to vesicular stomatitis virus New Jersey (VSNJV, Hazelhurst M15284A strain), vesicular stomatitis virus, Indiana (VSINV, M1977cc strain), Cache Valley virus (CVV, 6V633 strain), Jamestown Can-

yon virus (JCV, 61V-2235 strain), Colorado tick fever virus (CTFV, Florio strain), and western equine encephalitis (WEEV, Fleming M12959C strain) by the CDC Division of Vector-Borne Infectious Diseases (DVBID, Fort Collins, Colorado, USA) using serum-dilution, constant virus, plaque-reduction neutralization tests in Vero cell culture (McLean et al., 1987, 1993). Serum samples were originally tested at a 1:10 final dilution and then retested at increasing dilutions to confirm positive results. End-point titers were determined for VSNJV and VSINV. These serotypes were distinguished by a monotypic reaction to only one virus, or by the presence of an antibody titer against one virus which was fourfold or greater than that against the other virus, or were considered positive to both. The DVBID also conducted tests on PCMS samples for antibodies to *Yersinia pestis*, the causative agent of plague, using a passive hemagglutination technique (Wolff and Hudson, 1974), and *Francisella tularensis*, the causative agent of tularemia, using a microagglutination technique (Stewart, 1988). The School of Veterinary Medicine, University of Wisconsin (Madison, Wisconsin, USA) conducted tests on Colorado swift fox samples for serum antibodies to *Borrelia burgdorferi*, the causative agent of Lyme borreliosis, using the indirect fluorescent antibody test (Burgess et al., 1986). The fluorescent antibody used was a fluorescein isothiocyanate conjugated rabbit antidog IgG. Homology between dog and fox sera was established using rabbit antidog sera in a gel diffusion precipitation reaction.

Positive threshold antibody titers were determined by the laboratory in which the analyses was performed: CPV, >8; CDV,  $\geq 2$ ; RV, >5; VSNJV, VSINV, CVV, JCV, CTFV, and WEEV,  $\geq 10$ ; *Y. pestis* >64; *F. tularensis*  $\geq 128$ ; *B. burgdorferi*  $\geq 128$ . Sera with titers above established thresholds were considered indicative of previous natural exposure to a given pathogen. These sera will be referred to as positive.

Nasal swab ( $n = 28$ ), fecal ( $n = 22$ ), and

TABLE 1. Antibody prevalences to nine infectious disease agents in swift and kit foxes from seven western states of the USA.

	CPV (%) <sup>a</sup>	CDV (%) <sup>a</sup>	RV (%) <sup>a</sup>	VSNJV (%) <sup>a</sup>	VSINV (%) <sup>a</sup>	CVV (%) <sup>a</sup>	JCV (%) <sup>a</sup>	CTFV (%) <sup>a</sup>	WEEV (%) <sup>a</sup>
Colorado	17/44 (39) <sup>b</sup>	4/22 (18)	0/40 (0)	10/39 (26)	2/39 (5)	1/39 (3)	2/39 (5)	0/39 (0)	0/39 (0)
Kansas <sup>c</sup>	4/8 (50)	1/5 (20)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)
Wyoming	4/4 (100)	2/2 (100)	1/2 (50)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
California									
SLO	1/14 (7)	0/13 (0)	0/9 (0)	2/13 (15)	3/13 (23)	1/13 (8)	0/13 (0)	0/13 (0)	0/13 (0)
Kern A	1/13 (8)	0/7 (0)	0/9 (0)	4/13 (31)	4/13 (31)	1/13 (8)	0/13 (0)	1/13 (8)	0/13 (0)
Kern B	2/12 (17)	0/10 (0)	0/13 (0)	2/10 (20)	1/10 (10)	1/10 (10)	0/10 (0)	0/10 (0)	0/10 (0)
Death Valley	0/1 (0)	—	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)
New Mexico	1/1 (100)	0/1 (0)	0/1 (0)	1/18 (6)	2/18 (11)	1/18 (6)	1/18 (6)	0/18 (0)	0/18 (0)
Utah	2/9 (22)	1/8 (13)	0/9 (0)	2/8 (25)	1/8 (13)	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Arizona	1/4 (25)	1/4 (25)	0/6 (0)	2/5 (40)	3/5 (60)	1/5 (20)	2/5 (40)	0/5 (0)	0/5 (0)
Total	33/110 (30)	9/72 (13)	1/97 (1)	23/117 (20)	16/117 (14)	6/117 (5)	5/117 (4)	1/117 (1)	1/117 (1)

<sup>a</sup> CPV = canine parvovirus, CDV = canine distemper virus, RV = rabies virus, VSNJV = vesicular stomatitis, New Jersey, VSINV = vesicular stomatitis, Indiana, CVV = Cache Valley Virus, JCV = Jamestown Canyon virus, CTFV = Colorado tick fever, WEEV = Western equine encephalitis.

<sup>b</sup> Number of foxes with antibody/number of foxes examined (%).

<sup>c</sup> Kansas, Wallace County (38°50'N, 101°45'W; *n* = 10), Wyoming, Laramie County, (41°20'N, 104°40'W; *n* = 9), California, San Luis Obispo County (SLO) (35°15'N, 119°52'W; *n* = 24) (White and Ralls, 1993), California, Kern County, (Kern A) (35°18'N, 119°37'N; *n* = 31) (Disney and Spiegel, 1992), California, Kern County, Naval Petroleum Reserves (Kern B) (35°17'N, 119°28'W; *n* = 33), California, Inyo County, Death Valley, California (36°40'N, 116°55'W; *n* = 1), New Mexico, Chaves County, (33°30'N, 104°20'W; *n* = 18), Utah, Juab and Millard Counties, (39°25'N, 112°20'W; *n* = 9), Arizona, Maricopa County, (33°5'N, 112°8'W; *n* = 6).

urine ( $n = 11$ ) samples were collected for virus isolation from Colorado swift fox. Nasal swabs were collected with cotton tipped swabs with wooden shafts, broken, and placed in minimal essential media with 50  $\mu\text{g/ml}$  gentamicin for storage (Lennette and Schmidt, 1979). Fresh fecal samples were opportunistically collected from traps. Urine samples were collected from disinfected pans placed under traps or were midstream collections. These samples were promptly stored and transported on dry ice until virus isolation was attempted at the School of Veterinary Medicine, University of Wisconsin. Fleas and ticks were collected for species identification during August 1989 (Miller et al., 1998), placed in labeled screw-capped glass vials, and stored on dry ice for virus isolation at the DVBID.

Ticks and fleas were identified to the species level, when possible (Miller et al., 1998). Ticks were tested individually for virus isolation in Vero cell culture (McLean et al., 1985). Ticks were tested for the presence of spirochetes by dark-field microscopy and by culturing in BSK II media (McLean et al., 1993). No attempts were made to isolate agents from fleas. Virus isolation was attempted on nasal swabs, fecal, and urine samples using minimal essential media and Vero tissue culture at the School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA (Lennette and Schmidt, 1979). Virus isolation was conducted with cells grown in minimal essential media with 10% fetal bovine serum (Sigma Chemicals, St. Louis, Missouri) and 1% penicillin-streptomycin.

Colorado swift fox <1-yr-old were classified as juveniles. Those  $\geq 1$ -yr-old were classified as adults. Colorado fox trapped in June, July, and August were grouped as summer fox, whereas those trapped in January were classified as winter fox. Although sampling size and sampling procedures do not permit meaningful statistical comparison, serologic data for swift fox from Colorado are reported with respect to age, sex, and season.

Prevalence rates for serum antibodies to CPV varied by sample (Table 1). More Colorado adults [14 of 20 (60%)] than juveniles [five of 24 (21%)] had serum antibodies to CPV, and no sex-based differences in the data were observed. Fewer Colorado samples had antibodies to CPV during summer sampling [eight of 27 (30%)] than winter [11 of 17 (55%)]. Summer samples were composed primarily of juveniles [19 of 27 (70%)]. Eight fox were sampled more than once for CPV. No fox had a significant change in titer during the course of the study.

Prevalences for fox with antibodies to CDV varied by sample, with most samples showing low rates of exposure (Table 1). None of nine Colorado juveniles had antibodies to CDV. One fox from Wyoming was classified as positive for serum antibody to rabies virus based on a titer of 135 (to 2 IU/ml). No other fox had antibodies to rabies virus. Titers ranged from 1:20–1:320 for both VSNJV and VSINV. One Utah fox had identical titers to VSNJV and VSINV (1:20) and was considered to have serum antibodies to both viruses. Five fox had serum antibodies to VSNJV or VSINV and to one other arbovirus.

Antibodies to *Y. pestis*, *F. tularensis*, and *B. burgdorferi* were not detected in 25 swift fox from Colorado. *Ixodes sculptus* ( $n = 2$ ), *I. kingi* ( $n = 19$ ), *I. sp.* ( $n = 1$ ), and *Pulex irritans* ( $n = 69$ ) were collected from 22 swift fox from Colorado (Miller et al., 1998). Tick, nasal swab, fecal, and urine samples in swift fox from Colorado did not yield virus or spirochete isolates.

Serum samples from several swift and kit fox populations was analyzed to provide information on the geographic distribution of selected pathogens in these species. The prevalence of infection for these pathogens, and test sensitivity and specificity have not been established in these species. Therefore, it is not possible to estimate the number of false-positive and false-negative results which occurred.

The population with the largest number of samples was the Colorado swift fox sam-



ple ( $n = 44$ ). Additional data for this population is available (Covell, 1992) which permits a preliminary assessment of the relationship between exposure to the pathogens tested for in this study and demographic characteristics. No mortality or morbidity was observed in the Colorado sample which could be associated with these pathogens, based on radiotelemetry data and necropsies on five fox which died during the study.

The higher prevalence of serum antibodies to CPV in adult Colorado swift fox was probably due to an increased probability of exposure to CPV with increasing age. The significance of fewer summer Colorado swift fox samples with antibodies to CPV is uncertain, but may be due to the greater number of juveniles in the summer sample. It is not surprising that fox in each state were found with antibody to CPV, given the widespread distribution of CPV in domestic dogs and wild canids, high rates of viral shedding in infected animals, and the stability of this virus in the environment (Appel and Parrish, 1987). From 1985–1988 the prevalence of CPV antibodies in coyotes at the Colorado study site was higher (71%) than the prevalence which we observed in swift fox (Table 1). The Kern B sample of kit fox (Table 1) was collected during December, 1987 and April, 1988. Samples from this site collected an earlier time were previously reported to have a higher prevalence of CPV (>65%) than we observed (McCue and O'Farrell, 1988). The significance of these comparisons is uncertain. However, mortality or decreased fecundity due to CPV in swift and kit fox populations has not been documented. Nevertheless, as mortality due to CPV may occur in free-ranging wolf pups (Mech and Goyal, 1993; Johnson et al., 1994), CPV should be evaluated for possible population level effects.

The relatively low prevalence of serum antibodies to CDV may be due to CDV's short survival time in the environment, and the need for close contact for transmission to occur (Gorham, 1966). The

prevalence of serum antibodies to CDV averaged 57% for coyotes at the Colorado study site (Gese et al., 1991), in contrast to the 18% for Colorado swift fox in our study. The absence of juveniles in the Colorado sample with antibodies to CDV may be due to the small sample size, the absence of exposure to CDV, or high mortality due to infection. The absence of antibodies to CDV in the California kit fox sample is in contrast to a prevalence of 14% for antibodies to CDV in a previous report on the Kern B California kit fox population. Empirical evidence for the source of CDV in wild canid populations is inconclusive. However, mortalities in free-ranging grey (*Urocyon cinereoargenteus*) and red fox (*V. vulpes*) (Monson and Stone, 1976; Nicholson and Hill, 1984; Davidson et al., 1992) suggest that CDV could be a source of mortality in free-ranging swift and kit fox.

Antibody to rabies virus was detected in one swift fox from Wyoming. Although no rabies virus was isolated to confirm this result, there were no indications of a cytopathic effect and rabies antibody has been found in a variety of free-ranging wildlife (Carey, 1985). Swift and kit fox are not known to be reservoirs for rabies virus. Therefore, rabies virus in swift and kit fox is likely due to sporadic exposure or spillover from reservoir species.

The prevalence of antibody to VSNJV (18%) and VSINV (17%) is relatively high compared to the prevalence in most other wildlife species (Trainer and Knowlton, 1968; Webb et al., 1987). The prevalence of antibodies to CVV, JCV, and WEE in wild canids generally appears to be low (Trainer and Knowlton, 1968; Zarnke et al., 1983; Buescher et al., 1970). Although the fox from California with antibody to CTFV was outside of the range of the Rocky Mountain wood tick (*Dermacentor andersoni*), the primary vector of CTFV, it was within the range of *D. occidentalis*, a known CTFV vector in California (Bowen, 1988). The significance of swift and kit fox in the transmission cycles of these viruses,

and the pathogenicity of these viruses for swift and kit fox, has not been documented.

Sera samples were screened for antibodies to *Y. pestis*, *F. tularensis*, and *B. burgdorferi* because these agents are of zoonotic interest, are of uncertain pathogenicity in swift and kit fox, and may influence fox prey numbers and distribution. A population of kit fox in Utah had serologic evidence of antibodies to *F. tularensis* (Vest et al., 1965). Previous studies of kit fox have not shown serologic evidence of exposure to *Y. pestis* (Vest et al., 1965; McCue and O'Farrell, 1988). It is unclear whether ectoparasites found on swift fox in Colorado (Miller et al., 1998) could be effective vectors for transmission of *B. burgdorferi*.

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