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SEROLOGICAL SURVEY FOR SELECTED DISEASES IN THE ENDANGERED SAN JOAQUIN KIT FOX (*VULPES MACROTIS MUTICA*)

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ABSTRACT: Blood from endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) inhabiting the Elk Hills Naval Petroleum Reserve, Kern County, and the Elkhorn Plain, San Luis Obispo County, California, was collected in 1981, 1982 and 1984 and sera were tested for antibodies against 10 selected pathogens. Proportions of kit fox sera containing antibodies against pathogens were: canine parvovirus, 100% in 1981–1982 and 67% in 1984; infectious canine hepatitis virus, 6% in 1981–1982 and 21% in 1984; canine distemper virus, none in 1981–1982 and 14% in 1984; *Francisella tularensis*, 8% in 1981–1982 and 31% in 1984; *Brucella abortus*, 8% in 1981–1982 and 3% in 1984; *Brucella canis*, 14% in 1981–1982 and none in 1984; *Toxoplasma gondii*, 6% in 1981–1982; *Coccidioides immitis*, 3% in 1981–1982; and *Yersinia pestis* and *Leptospira interrogans* serotypes *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, and *pomona*, none in 1981–1982. Although antibodies against selected pathogens were present, no clinical indications of disease were observed in these fox populations.

Key words: *Vulpes macrotis mutica*, San Joaquin kit fox, serology, survey, viral diseases, bacterial diseases, coccidioidomycosis, toxoplasmosis.

INTRODUCTION

The San Joaquin kit fox is a small nocturnal canid endemic to arid regions of the San Joaquin Valley of south-central California. It is protected under the Endangered Species Act of 1973 (U.S. Public Law 93-205) because significant losses of habitat threaten the continued existence of the subspecies. A detailed review of the life history and status of this fox is provided in its Recovery Plan (O'Farrell, 1983).

To obtain a better understanding of the role of disease in the life history of this animal, a serological survey was conducted to determine whether antibodies against selected pathogens were present. Tests were conducted for diseases that had occurred previously in the kit fox or other canids, were known to impact wildlife populations, or were endemic to the southern San Joaquin Valley of California.

MATERIALS AND METHODS

Kit foxes were collected on the Elk Hills Naval Petroleum Reserve which is located approximately 48 km west-southwest of Bakersfield, California (Kern County; 35°17′N, 119°28′W) and on the Elkhorn Plain located 65 km southwest of Bakersfield (San Luis Obispo County; 35°03′N, 119°31′W). The Elk Hills consist of a long ridge of alluvium projecting eastward from the Temblor Range into the southwestern corner of the San Joaquin Valley. Elevations range between 88 and 470 m above sea level. The major vegetation association is Lower Sonoran Grassland (Twisselmann, 1967) that has a ground cover of red brome (*Bromus rubens*) and red-stemmed filaree (*Erodium cicutarium*), and a shrub cover of common saltbush (*Atriplex polycarpa*), spiny saltbush (*A. spinifera*), cheese-bush (*Hymenoclea salsola*) and matchweed (*Gutierrezia bracteata*). The Reserve is being extensively developed for petroleum production; livestock grazing is proscribed.

The Elkhorn Plain is a high Coast Range valley that is separated from the San Joaquin Valley by the Temblor Range. Elevations of the fox capture sites ranged between 705 and 717 m above sea level. Vegetation of the valley floor was dominated by a grazing disclimax of Upper and Lower Sonoran Grassland species (Twisselmann, 1967) that included red brome, Arabian grass (*Schismus arabicus*), red-stemmed filaree, matchweed and Mormon tea (*Ephedra californica*) (O'Farrell and McCue, 1981). It is heavily grazed by cattle and sheep, but petroleum development is limited to a few widely scattered exploratory wells.

Kit foxes were trapped in National® live traps (National Live Trap Corporation, Tomahawk, Wisconsin 54487, USA) and handled using methods published previously (McCue and O'Farrell, 1987). Blood samples were taken from

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the jugular vein in 10-ml syringes and transferred into sterile Vacutainers® (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey 07070, USA). After field collection, all blood samples were refrigerated, centrifuged and serum was frozen (−20 C) until analyzed.

Antibodies against canine parvovirus (CPV) were detected by JM Veterinary Laboratories (San Diego, California 92154, USA) using an IFA test. Substrate slides using an established canine kidney cell line (U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, Iowa 50010, USA) were used to determine titer of IgG and presence or absence of IgM. Antibodies to infectious canine hepatitis (ICH) virus were determined by a serum neutralization (SN) test that used a constant concentration of ICH virus 100 $TCID_{50}$ mixed with increasing dilutions of test sera. Serum-virus mixtures were incubated on Madin-Darby canine kidney tissue culture cells (American Type Culture Collection, Rockville, Maryland 20852, USA) for three days in 35 C in 5% CO_2. Titer was determined by the highest serum dilution that prevented 50% of cultures from showing cytopathic effects (CPE) after incubation. Antibodies to canine distemper (CD) virus were detected by indirect fluorescent antibody (IFA) tests. Serial dilutions of sera (1:2 to 1:256) were made in PBS and then transferred to substrate slides containing Vero-M cells infected with CD virus. Slides were incubated in a moist chamber at 37 C for 45–60 min, washed, and then flooded with fluorescein conjugated rabbit anticanine gamma globulin (U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, Iowa 50010, USA). After an additional incubation period (37 C, 45–60 min) slides were counterstained with Evans blue, and then rinsed with PBS and distilled water. Titers were read by recording the highest dilution where fluorescence occurred. IFA and SN tests for antibodies against CD and ICH viruses were conducted by the Immunology/Virology Diagnostic Laboratory (Veterinary Medical Teaching Hospital, University of California, Davis, California 95616, USA). Antibodies to *Francisella tularensis* were detected using slide agglutination tests. Tube agglutination tests were performed on positive samples to determine the titer. Test protocols, antigen and antiserum were prepared by DIFCO Laboratories (Detroit, Michigan 48232, USA). Complement fixation (CF) (Lennette and Schmidt, 1969) and buffered *Brucella* antigen card tests (Hynson, Westcott and Dunning, Inc., Baltimore, Maryland 21201, USA) were used to screen sera for antibodies against *Brucella abortus*. Titers of sera showing positive reactions on card tests were determined by standard agglutination tests (U.S. Department of Agriculture, no date). *Brucella canis* was detected using CF and 2-mercaptoethanol (ME) tube tests (Alton et al., 1975). Antibodies to *Toxoplasma gondii* were detected by indirect hemagglutination tests (Riemann et al., 1975) using commercially available antigens (International Biological Laboratories, Inc., Rockville, Maryland 20850, USA). Antibodies against *Coccidioides immitis* were detected by immunodiffusion tests (Pappagianis, 1980) at the Coccidioidal Serology Laboratory (University of California, Davis, California 95616, USA). Passive hemagglutination tests for antibodies against *Yersinia pestis* were conducted by the Plague Branch (Center for Disease Control, Fort Collins, Colorado 80522, USA) using procedures recommended by the World Health Organization (Hudson and Kartman, 1967). Live-antigen microagglutination (MA) tests for antibodies against *Leptospira interrogans* serotypes *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, and *pomona* (Cirone et al., 1978) were conducted by the Animal Health Division (Wisconsin Department of Agriculture, Central Animal Health Laboratory, Madison, Wisconsin 53705, USA).

RESULTS AND DISCUSSION

Blood samples from 71 adult (>10 mo old) kit foxes (57 from Elk Hills, 14 from the Elkhorn Plain) were collected between 7 August 1981 and 7 January 1982. An additional 29 samples were collected from Elk Hills between 26 June and 26 July 1984. All tests were not conducted on each serum sample (Table 1). Information on titers obtained is available in McCue and O'Farrell (1986).

Canine parvovirus (CPV)

All 12 serum samples tested in 1981 and 1982 (11 from Elk Hills; one from Elkhorn Plain) were positive for IgG antibodies against CPV. Five of these (four from Elk Hills; one from Elkhorn Plain) were also positive for IgM antibodies (Table 1). In 1984, 10 of 15 (67%) of the serum samples collected on Elk Hills were positive for IgG and two of 15 (13%) were positive for IgM antibodies against CPV.

Canine parvovirus was first recognized in 1978 (Appel et al., 1980). The virus spread rapidly and was found on four continents within 1 yr (Appel et al., 1980). Serologic evidence of exposure to CPV has

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TABLE 1. Prevalence of serum antibodies against selected pathogens in San Joaquin kit foxes inhabiting the Elk Hills and Elkhorn Plain, California.

Disease agent	Elk Hills 1981–1982	Elk Hills 1984	Elkhorn Plain 1981
Canine distemper virus	0/13[a]	4/29	0/2
Infectious canine hepatitis virus	1/14	6/29	0/2
Canine parvovirus			
Immunoglobulin G	11/11	10/15	1/1
Immunoglobulin M	4/11	2/15	1/1
Brucella abortus			
Complement fixation test	3/23	ND[b]	0/13
Card test	1/24	1/29	ND
Brucella canis			
Complement fixation test	5/23	ND	0/13
Tube test	ND	0/20	ND
Francisella tularensis	5/51	9/29	0/13
Yersinia pestis	0/8	ND	0/3
Leptospira interrogans serotypes *canicola, hardjo, pomona, grippotyphosa, icterohaemorrhagiae*	0/23	ND	ND
Toxoplasma gondii	0/25	ND	2/10[c]
Coccidioides immitis	0/27	ND	1/9

[a] Number positive over number tested; information on titers may be found in McCue and O'Farrell (1986).
[b] ND = not determined.
[c] Partial hemagglutination at 1:64 dilution.

been found in coyotes (*Canis latrans*) (Barker et al., 1983; Thomas et al., 1984), red fox (*Vulpes vulpes*) (Barker et al., 1983), and wolves (*Canis lupus*) (Zarnke and Ballard, 1987). The prevalence of antibodies against CPV in wild coyotes captured in three western states between 1972 and 1983 coincided with the epizootic of CPV in domestic dogs (*Canis familiaris*) in North America (Thomas et al., 1984). The propensity for CPV to cause enteritis and myocarditis in dogs (Appel et al., 1980) makes this virus a significant potential pathogen for wild canids. The high prevalence of antibodies against CPV in the San Joaquin kit fox indicated that CPV was probably enzootic in this kit fox population. Presence of IgM antibodies in sera suggests a recent exposure to CPV.

Infectious canine hepatitis (ICH)

One of 14 (7%) samples collected in 1981 and 1982 and six of 29 (21%) collected in 1984 from Elk Hills were positive for antibodies against ICH virus (Table 1). Two samples from the Elkhorn Plain gave negative results.

Infectious canine hepatitis, also termed fox encephalitis, is distributed worldwide in members of the family Canidae (Cabasso, 1970). Foxes ≤6 mo old are very susceptible to ICH, and mortality rates as high as 80% are reported (Cabasso, 1970). In a natural epizootic the overall death rate may be 10–20% of the population (Cabasso, 1970). Evidence of ICH virus has been reported in wolves (Stephenson, et al., 1982; Zarnke and Ballard, 1987), coyotes (Trainer and Knowlton, 1968), gray fox (*Urocyon cinereoargenteus*) (Amundson and Yuill, 1981), and red fox (Amundson and Yuill, 1981).

Canine distemper (CD)

Four of 29 (14%) serum samples collected at Elk Hills in 1984 were positive for antibodies against CD virus (Table 1). Negative results were obtained on sera collected at Elk Hills (zero of 13) and the Elkhorn Plain (zero of two) in 1981 and 1982.

Canine distemper is a disease of the Canidae, Mustelidae and Procyonidae (Gillespie, 1962), and is distributed worldwide (Gillespie, 1962; Budd, 1970). It has been reported in wolves (Stephenson et al., 1982; Zarnke and Ballard, 1987), coyotes, (Trainer and Knowlton, 1968), gray fox (Armstrong and Anthony, 1942; Hoff et al., 1974), red foxes (Armstrong and Anthony, 1942; Amundson and Yuill, 1981) and kit foxes (Armstrong and Anthony, 1942).

Like ICH, epizootics of CD occur when the population of susceptible animals becomes dense enough to ensure easy animal

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to animal transmission of the virus. Mortality rates due to these pathogens may be high for susceptible animals and populations (Gorham, 1966; Budd, 1970). Serologic evidence of antibodies against CD and ICH indicates that these pathogens are enzootic also. Therefore, it is speculated that the kit fox population on Elk Hills is not susceptible to epizootics of these diseases with their concomitant high rates of mortality (Gorham, 1966).

Tularemia

Five of 51 (10%) kit foxes sampled on Elk Hills in 1981 and 1982, and nine of 29 (31%) tested there in 1984 were positive for antibodies against *F. tularensis* (Table 1). The highest titer (1:320) was from a kit fox captured on Elk Hills in 1984. Negative results were obtained for 13 samples collected on the Elkhorn Plain.

Francisella tularensis causes tularemia, a disease that occurs primarily in the Northern Hemisphere. In North America it is associated with lagomorphs (*Lepus* spp. and *Sylvilagus* spp.) and rodents (Hopla, 1974). These mammals and their tick ectoparasites are the primary reservoirs of *F. tularensis* in nature. Serologic evidence of exposure to *F. tularensis* has been found in coyotes (Thorpe et al., 1965; Vest et al., 1965), gray foxes (McKeever et al., 1958) and red foxes (McKeever et al., 1958).

The proportion of seropositive kit foxes observed during the present study (15%) was similar to the 14% prevalence reported for kit foxes inhabiting the Great Salt Lake Desert region of western Utah from 1951 to 1964 (Thorpe et al., 1965). Kit foxes were probably exposed to *F. tularensis* after they ate infected prey or their ectoparasites. The reason for the difference in prevalence of antibodies against *F. tularensis* in foxes captured on Elk Hills and the Elkhorn Plain is unknown.

Brucellosis

Three of 23 (13%) kit foxes from Elk Hills captured in 1981 and 1982 were positive for *B. abortus* CF antibodies (Table 1); one fox had a titer of 1:128. Serum from that animal also showed a positive reaction on a *B. abortus* card test. One of 29 (3%) serum samples collected in 1984 was positive for antibodies to *B. abortus* on a card test. The antibody titer for that serum was determined by standard agglutination test to be 1:400. Thirteen serum samples collected on the Elkhorn Plain gave negative results. Five of 23 (22%) kit foxes from Elk Hills and none of 13 from the Elkhorn Plain captured in 1981 and 1982 had CF antibodies against *B. canis* (Table 1). All 20 animals tested from Elk Hills in 1984 by ME tube tests were negative.

Brucellosis is a highly contagious infectious disease of animals caused by *Brucella* spp. (Witter and O'Meara, 1970). Wild canids have been shown to host *B. abortus* (Davis et al., 1979), *B. canis* (Randhawa et al., 1977) and *B. suis* biotype 4 (Neiland, 1970). Antibodies againt *Brucella* spp. have been found in wolves (Neiland, 1970; Zarnke and Ballard, 1987), coyotes (Hoff et al., 1974; Randhawa et al., 1977; Hoq, 1978; Davis et al., 1979) and red foxes (Hoff et al., 1974). A previous serologic survey in California, that included animals collected in Kern County, revealed that coyotes, bobcats (*Felis rufus*), striped skunks (*Mephitis mephitis*) and spotted skunks (*Spilogale putorius*) trapped in livestock areas had titers $\geq$1:100 against *B. abortus* (Hoq, 1978). However, all kit foxes seropositive for *B. abortus* and *B. canis* were from Elk Hills where there were no livestock; positive samples were not obtained from the Elkhorn Plain, an area intensively grazed by livestock. This was the opposite of what was anticipated, since brucellosis has long been considered a disease associated with domestic animals (Bruner and Gillespie, 1973). The source(s) of exposure for Elk Hills kit foxes is not known. Moore and Schnurrenberger (1981) reviewed the natural occurrence of *Brucella* spp. infections, but the role of wildlife as reservoirs of *Brucella* spp. is still unclear.

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Toxoplasmosis

Two of 10 (20%) kit foxes from the Elkhorn Plain were suspected of having antibodies against *T. gondii* (Table 1) because partial hemagglutination occurred at a 1:64 dilution. No evidence of antibodies against *T. gondii* was found in 25 samples from Elk Hills.

Riemann et al. (1975) reported that antibodies against *T. gondii* were found in 55% of wild felids and 28% of wild canids tested from the Central Valley of California. Bobcats and feral cats are suitable definitive hosts and were present on Elk Hills, but no seropositive kit foxes were captured there. Antibodies against *T. gondii* were found in 20% of the serum collected from the Elkhorn Plain where bobcats and feral cats were probably present. Serologic evidence of *T. gondii* exposure has been previously reported in coyotes (Riemann et al., 1975, 1978; Marchiondo et al., 1976; Tizard et al., 1976), gray foxes (Marchiondo et al., 1976; Riemann et al., 1978), red foxes (Tizard et al., 1976), and kit foxes (Marchiondo et al., 1976).

Coccidioidomycosis

All 27 kit foxes from Elk Hills tested negative for antibodies against *C. immitis*. Serum from one of nine (11%) kit foxes from the Elkhorn Plain showed a positive reaction with the immunodiffusion test (Table 1).

Coccidioides immitis, the causative organism of coccidioidomycosis, is endemic to the San Joaquin Valley of California. Egeberg and Ely (1956) isolated *C. immitis* from soil samples collected from Elk Hills and noted that the microclimate of animal burrows may be conducive to the growth and/or concentrations of this fungus. Because individual kit foxes may use as many as 24 dens distributed over an area of 3 km^2 or more (Morrell, 1971), it was assumed that a large proportion of foxes would have been exposed to the fungus. However, serum from only one of 36 (3%) kit foxes provided a positive reaction.

Plague

Eleven kit foxes (eight from Elk Hills; three from Elkhorn Plain) tested negative for antibodies against *Y. pestis* (Table 1) which has been found to be widely distributed in California (Willeberg et al., 1979). Evidence of plague in Kern County, California, includes periodic epizootics in California ground squirrels (*Spermophilus beecheyi*) (Evans et al., 1943), a seropositive coyote (Willeberg et al., 1979) and two cases of human plague, one of which was fatal (California Morbidity, 1976, 1977). Antibodies against *Y. pestis* were found previously in coyotes (Willeberg et al., 1979), red foxes (Poland et al., 1973), and gray foxes (Poland et al., 1973). Carnivores are recognized as valuable sentinels in the detection of *Y. pestis* in wildlife populations (Willeberg et al., 1979).

Leptospirosis

Twenty-three kit foxes from Elk Hills tested negative for antibodies against *L. interrogans* serotypes *canicola*, *hardjo*, *grippotyphosa*, *icterohaemorrhagiae*, and *pomona* (Table 1). This result was unexpected because previous studies have indicated a high prevalence of leptospiral antibodies among wildlife, especially carnivores, in the Central Valley of California (Cirone et al., 1978). Antibodies against or isolations of *L. interrogans* serotypes have been documented in coyotes (Hanson, 1961; Trainer and Knowlton, 1968; Cirone, 1976), gray foxes (Cirone, 1976; Amundson and Yuill, 1981), red foxes (Amundson and Yuill, 1981) and wolves (Zarnke and Ballard, 1987).

CONCLUSIONS

The presence of serum antibodies against a pathogen suggests prior exposure to that pathogen (Paul and White, 1973), but seropositive samples do not necessarily indicate the presence of clinical disease. Clinical indications of diseases were not observed in any of the kit foxes we sampled and diseases were not identified as a major

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source of mortality in radiocollared kit foxes between 1980 and 1986 (Berry et al., 1987). However, it must be emphasized that long-term effects of the diseases for which serologic evidence was detected have not been determined. Knowledge of infectious diseases within the kit fox population is important when assessing the overall spectrum of ecological factors affecting the species, and it is essential before qualitative and comprehensive wildlife management guidelines can be proposed or implemented. It will be helpful also in assessing whether foxes inhabiting portions of Elk Hills being developed for petroleum production are exposed more frequently to infectious diseases than foxes living nearby in undeveloped habitats.

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