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SEROLOGICAL SURVEY FOR DISEASES IN FREE-RANGING COYOTES (*CANIS LATRANS*) IN YELLOWSTONE NATIONAL PARK, WYOMING

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ABSTRACT: From October 1989 to June 1993, we captured and sampled 110 coyotes (*Canis latrans*) for various diseases in Yellowstone National Park, Wyoming (USA). Prevalence of antibodies against canine parvovirus (CPV) was 100% for adults (>24 months old), 100% for yearlings (12 to 24 months old), and 100% for old pups (4 to 12 months old); 0% of the young pups (<3 months old) had antibodies against CPV. Presence of antibodies against canine distemper virus (CDV) was associated with the age of the coyote, with 88%, 54%, 23%, and 0% prevalence among adults, yearlings, old pups, and young pups, respectively. Prevalence of CDV antibodies declined over time from 100% in 1989 to 33% in 1992. The prevalence of canine infectious hepatitis (ICH) virus antibodies was 97%, 82%, 54%, and 33%, for adults, yearlings, old pups, and young pups, respectively. The percentage of coyotes with ICH virus antibodies also declined over time from a high of 100% in 1989 to 31% in 1992, and 42% in 1993. Prevalence of antibodies against *Yersinia pestis* was 86%, 33%, 80%, and 7%, for adults, yearlings, old pups, and young pups, respectively, and changed over time from 57% in 1991 to 0% in 1993. The prevalence of antibodies against *Francisella tularensis* was 21%, 17%, 10%, and 20%, for adults, yearlings, old pups, and young pups, respectively. No coyotes had serologic evidence of exposure to brucellosis, either *Brucella abortus* or *Brucella canis*. No coyotes were seropositive to *Leptospira interrogans* (serovars *canicola*, *hardjo*, and *icterohemorrhagiae*). Prevalence of antibodies against *L. interrogans* serovar *pomona* was 7%, 0%, 0%, and 9%, for adults, yearlings, old pups, and young pups, respectively. Antibodies against *L. interrogans* serovar *grippotyphosa* were present in 17% of adults and 0% of yearlings, old pups, and young pups. Many infectious canine pathogens (CPV, CDV, ICH virus) are prevalent in coyotes in Yellowstone National Park, with CPV influencing coyote pup survival during the first 3 months of life; eight of 21 transmitted pups died of CPV infection in 1992. The potential impact of these canine pathogens on wolves (*C. lupus*) reintroduced to Yellowstone National Park remains to be documented.

Key words: Coyote, *Canis latrans*, canine parvovirus, canine distemper virus, infectious canine hepatitis, plague, tularemia, brucellosis, leptospirosis, *Yersinia pestis*, *Francisella tularensis*, *Brucella* spp., *Leptospira* spp.

INTRODUCTION

The prevalence of antibodies against various diseases has been reported for many species in the family Canidae. Antibodies against viral and bacterial diseases have been reported for populations of wolves (*Canis lupus*) in Alaska (USA) (Zarnke and Ballard, 1987), Minnesota (USA) (Goyal et al., 1986), Montana (USA) (Johnson et al., 1994), and Canada (Choquette and Kuyt, 1974). Coyote (*Canis latrans*) populations in Kansas (USA) (Gier and Ameel, 1959), Texas (USA) (Thomas et al., 1984), Colorado (USA) (Gese et al., 1991), Utah (USA) (Thomas et al., 1984), Idaho (USA) (Thomas et al., 1984), California (USA) (Thomas and Hughes, 1992), Georgia (USA) (Holzman et al., 1992), Wyoming (USA) (Williams et al., 1988), and Ontario, Canada (Barker et

al., 1983) also had evidence of exposure to various diseases.

Current plans to reintroduce wolves to Yellowstone National Park, Wyoming (U.S. Fish and Wildlife Service, 1987), or their recovery through natural recolonization, have warranted an examination of the role of the coyote as a possible reservoir for infectious diseases, as well as an indicator species of possible diseases that wolves may encounter upon returning to the park. Conversely, coyotes could be susceptible to diseases brought in by reintroduced wolves. Baseline data prior to wolf reintroduction or recovery is needed to document the prevalence of certain diseases in the coyote population. We report the results of a serological survey for evidence of canine parvovirus (CPV), canine distemper virus (CDV), infectious canine hepatitis (ICH) virus, leptospirosis, plague, tularemia, and brucellosis in free-ranging coyotes in Yellowstone National Park, Wyoming.

MATERIALS AND METHODS

Coyotes from 17 packs were captured in the Lamar River Valley (44°55′N, 110°15′E) and Blacktail Plateau region (44°55′N, 110°35′E) in Yellowstone National Park. Nine packs in the Lamar Valley and eight packs in the Blacktail region were sampled. The two study areas were separated by about 15 km. Elevations in the park ranged from 1,500 to 3,400 m with the study areas at about 2,000 m above sea level. Winters are long and cold with most of the annual precipitation falling as snow (Despain, 1990). Coyotes ≥4 mo of age were captured with padded, offset-jaw, leg-hold traps with attached tranquilizer tabs (Balser, 1965). Coyotes were immobilized with ketamine hydrochloride and xylazine hydrochloride (Cornely, 1979) for removal from the trap and processing. Animals were weighed, had their sex determined, were aged by tooth wear (Gier, 1968), were eartagged, and radiocollared (Advanced Telemetry Systems, Inc., Isanti, Minnesota); their first vestigial premolar from the lower jaw was extracted for aging by cementum annuli analysis (Linhart and Knowlton, 1967). A 20-ml blood sample was extracted from the cephalic or saphenous vein of trapped coyotes. Coyote pups were captured when 8 to 12 wk old and surgically implanted with an intraperitoneal transmitter (Telonics, Inc., Mesa, Arizona, USA). A 4 to 5 ml blood sample was collected from the pups from the jugular vein.

Each blood sample was placed into a glass serum tube (Vacutainer, Becton Dickinson, Rutherford, New Jersey, USA) and centrifuged for 30 min; the serum was harvested and stored at −20 C. Coyotes were classed as young pups (≤3 mo old), old pups (4 to 11 mo old), yearlings (12 to 24 mo old), and adults (>24 mo old). The distinction between young and old pups was made because young pups were captured at the den and may have had maternal antibodies, whereas old pups were trapped in the fall and maternal antibodies would have declined by 5 to 6 mo of age (Gorham, 1966; Green et al., 1984).

The serum samples were analyzed for antibodies against CPV, CDV, and ICH virus; five serovars of *Leptospira interrogans*; as well as *Yersinia pestis*, *Francisella tularensis*, and *Brucella* spp. at the School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin (USA) and the Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, Wyoming. We used the hemagglutination inhibition (HI) test following the procedures outlined by Carmichael et al. (1980) to detect antibodies against CPV. A titer of ≥1:100 was considered positive for CPV antibodies. A CPV titer level of ≥1:1,280 was evidence for a recent infection (Carmichael et al., 1980). Canine distemper virus antibody was determined by the serum virus neutralization test described by Appel and Robson (1973). A titer ≥1:16 was considered positive for antibodies against CDV. Antibodies against infectious canine hepatitis virus were determined by the virus neutralization test (Appel et al., 1975). A titer level of ≥1:10 was considered positive.

To determine the prevalence of antibodies against *Y. pestis*, we used passive hemagglutination against fraction 1 antigen (Centers for Disease Control, undated a); a titer of ≥1:8 was considered positive. Briefly, serum was heated to 60 C for 20 min and 0.1 ml dispensed into microcentrifuge vials. Two Pasteur pipette drops of packed sheep red blood cells were added to the serum and incubated for 3 hr or at 4 C overnight. Vials were centrifuged at 1,500 × *G* for 5 min, serum removed, and twofold serial dilutions were made in 0.01% normal rabbit serum in 0.85% NaCl the hemagglutination (HA) diluent, and in HA diluent containing 0.2 mg/ml *Y. pestis* fraction 1 antigen (Centers for Disease Control, Fort Collins, Colorado). Twenty-five μl of 10% sensitized sheep red blood cells (Centers for Disease Control, Fort Collins) were added to 50 μl of diluted serum in round (u)-bottomed 96-well microtiter plates, mixed and incubated at 22 C

TABLE 1. Prevalence of antibodies against canine parvovirus (CPV), canine distemper virus (CDV), infectious canine hepatitis (ICH) virus, *Yersinia pestis*, *Francisella tularensis*, and *Brucella* spp. in coyotes, Yellowstone National Park, Wyoming, 1989 to 1993.

Age class	CPV	CDV	ICH	*Y. pestis*	*F. tularensis*	*Brucella* spp.
Adult	100 (33)[a]	88 (33)	97 (33)	86 (14)	21 (14)	0 (14)
Yearling	100 (11)	54 (11)	82 (11)	33 (6)	17 (6)	0 (6)
Old pup	100 (13)	23 (13)	54 (13)	80 (10)	10 (10)	0 (10)
Young pup	0 (53)	0 (53)	33 (51)	7 (43)	20 (40)	0 (40)

[a] Prevalence (sample size tested).

for 4 hr. Controls on each plate were serum with high titers of *Y. pestis* antibody and normal rabbit serum.

We used the microscopic agglutination test for detecting antibodies against *F. tularensis* (Centers for Disease Control, undated b); a titer of ≥1:128 was considered positive. Two-fold serial dilutions of serum were made in 0.01% normal rabbit serum in phosphate buffered saline in u-bottomed microtiter plates. Twenty-five μl of *F. tularensis* antigen (Difco Laboratories, Detroit, Michigan, USA) were added to 50 μl of diluted serum, agitated gently, and incubated overnight at room temperature (22 C). Controls on each plate included serum with high titers of *F. tularensis* antibody and normal rabbit serum.

We used the standard plate test (U.S. Department of Agriculture, undated) to detect antibodies against *Brucella* spp. Briefly, serum was diluted at 1:25, 1:50, 1:100, and 1:200 in *B. abortus* plate agglutination test antigen (National Animal Disease Laboratory, Ames, Iowa, USA) and mixed on a scored plate. The plate was rotated four times, incubated for 4 min at 22 C, rotated four times, and incubated again for a total of 8 min. Agglutination reactions were read using illumination; a titer ≥1:50 was considered positive. Antibodies against five serovars of *L. interrogans* (serovars *canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona*) were detected using the microscopic agglutination test (National Veterinary Services Laboratory, 1987). A titer ≥1:100 was considered evidence for exposure to leptospires.

For all statistical tests, the sampling unit was each individual coyote. All coyotes were represented by only one sample; there were no repeated samples from the same coyote. The chi-square test was used to statistically analyze the prevalence of antibodies among age classes, between the two study areas, and among years (Sokal and Rohlf, 1981). We used the Fisher exact test when the contingency table contained an expected frequency of less than 1.0 in any cell (Zar, 1984).

RESULTS

Blood samples were collected from 110 coyotes (60 males and 50 females) captured from 17 different resident packs from October 1989 to June 1993. Age classes of the captured coyotes were 33 adults (17 males: 16 females), 11 yearlings (5 males: 6 females), 13 old pups (11 males: 2 females), and 53 young pups (27 males: 26 females). Thirteen coyotes were sampled in 1989, 14 in 1990, 36 in 1991, 38 in 1992, and nine in 1993.

Laboratory analysis for CPV antibodies was completed on serum samples from all 110 coyotes. Young pups had significantly lower antibody prevalence to CPV compared to adults, yearlings, and old pups (Table 1) (Chi-square = 110.00, 3 df, $P < 0.001$). Positive antibody titers ranged from 1:320 to 1:81,920 for adults, 1:1,280 to 1:10,240 for yearlings, and 1:1,280 to 1:10,240 for old pups; young pups had no positive titers. For coyotes >4 mo of age, the prevalence of recent infections was influenced by age (Chi-square = 8.46, 2 df, $P = 0.014$). Twenty-one (64%) adults, 10 (91%) yearlings, and 13 (100%) old pups had titers ≥1:1,280, evidence for a recent infection. No young pups had evidence of recent infection. The prevalence of recent infection was not different among years (Chi-square = 1.37, 3 df, $P = 0.71$), or between the two study areas (Chi-square = 0.23, 1 df, $P = 0.62$).

Serology for CDV antibodies was completed on 110 coyotes. Prevalence of CDV antibodies increased with coyote age (Table 1) (Chi-square = 72.18, 3 df, $P <$

0.001). Positive titers ranged from 1:32 to 1:1,600 for adults, 1:16 to 1:1,600 for yearlings, and 1:16 to 1:400 for old pups; no young pups were positive. Prevalence of antibodies against CDV declined through the study period (Chi-square = 25.75, 3 df, $P < 0.001$). Seroprevalence was 100% in 1989, 100% in 1990, 39% in 1991, and 33% in 1992, for adult, yearling, and old pups combined. None of the young pups had CDV antibodies in any year. Prevalence of CDV antibodies also varied between the two study areas. For coyotes >4 mo old, prevalence of CDV antibodies in both study areas was 100% in 1989 and 1990. In 1991, prevalence of CDV antibodies was 62% and 20% in Blacktail and Lamar Valley, respectively (Chi-square = 3.37, 1 df, $P = 0.066$). In 1992, prevalence of antibodies against CDV was 57% in Blacktail and 0% in Lamar Valley (Chi-square = 4.28, 1 df, $P = 0.038$).

Prevalence of antibodies against ICH virus was determined using samples from 108 coyotes. Based on the ICH virus titers, canine adenovirus (CAV) was present. However, distinguishing between CAV-type 1 and CAV-type 2 was not possible based on serology. Age of the coyote was a factor influencing prevalence of ICH virus antibodies (Table 1) (Chi-square = 36.34, 3 df, $P < 0.001$). Positive antibody titers ranged from 1:10 to 1:1,600 for adults and yearlings, 1:10 to 1:800 for old pups, and 1:10 to 1:200 for young pups. For all coyotes combined, the prevalence of antibodies against ICH virus changed over time (Chi-square = 32.11, 4 df, $P < 0.001$). Prevalence of ICH virus antibodies was 100% in both 1989 and 1990, declining to 64% in 1991, and then to 31% and 43% in 1992 and 1993, respectively. This decline did not occur among adult coyotes (Fisher exact test, $P = 0.42$), yearlings (Fisher exact test, $P = 0.27$), or old pups (Fisher exact test, $P = 0.12$). The change in ICH virus prevalence over time occurred only among young pups (Chi-square = 8.05, 2 df, $P = 0.018$). The prevalence of seropositive antibodies against ICH virus was not different between the two study areas (Chi-square = 2.42, 1 df, $P = 0.12$).

Due to a limited quantity of serum, we analyzed only 73 coyote serum samples for antibodies against *Y. pestis*. The prevalence of antibodies varied significantly among the four age classes (Table 1) (Chi-square = 39.96, 3 df, $P < 0.001$). Positive titers of *Y. pestis* ranged from 1:8 to >1:512 for adults and yearlings, 1:8 to 1:256 for old pups, and 1:8 to 1:512 for young pups. The prevalence of antibodies changed over time (Chi-square = 12.04, 2 df, $P = 0.002$). During the 3 yr in which we had samples, titers declined significantly from 57% in 1991, to 29% in 1992, then to 0% in 1993. This decline over the 3 yr occurred in both study areas, but at different prevalences. In 1991, seroprevalences differed between Blacktail (80%) and Lamar Valley (44%) (Chi-square = 3.31, 1 df, $P = 0.068$). In 1992, seroprevalences differed in Blacktail (45%) and Lamar Valley (0%) (Chi-square = 10.61, 1 df, $P = 0.001$). Both study areas had 0% prevalence of antibodies against *Y. pestis* in 1993.

Serum samples from 70 coyotes were analyzed for antibodies against *F. tularensis*. Seroprevalence did not vary significantly among age classes (Table 1) (Chi-square = 0.62, 3 df, $P = 0.88$). Positive titers ranged from 1:128 to 1:256 for adults and old pups, 1:128 to 1:512 for yearlings, and 1:128 to 1:1,024 for young pups. Due to limited serum, we tested for tularemia during only 3 yr of the study; the seroprevalence was 7% in 1991, 30% in 1992, and 0% in 1993, and this annual difference was significant (Fisher exact test, $P = 0.043$). The study areas were not significantly different in the prevalence of antibodies against *F. tularensis* (Chi-square = 0.70, 1 df, $P = 0.40$).

We tested 70 coyotes for serum antibodies against *Brucella* spp. None of the adults, yearlings, old pups, or young pups were seropositive (Table 1).

We analyzed serum samples from 73

coyotes (14 adults, six yearlings, 10 old pups, 43 young pups) for antibodies against five serovars of *L. interrogans*. No coyotes had antibodies to serovars *canicola*, *hardjo*, and *icterohemorrhagiae* of *L. interrogans*. One adult, no yearlings or old pups, and four young pups were seropositive for serovar *pomona* (Fisher exact test, $P > 0.90$) Prevalence of antibodies against serovar *pomona* was 10%, 6%, and 0%, for the years of 1991, 1992, and 1993, respectively (Fisher exact test, $P = 0.82$). The two study areas had a similar prevalence of antibodies (Chi-square = 0.05, 1 df, $P = 0.80$). Two adults, and no yearlings, old pups, or young pups had evidence of exposure to serovar *grippotyphosa* (Fisher exact test, $P = 0.112$). The prevalence of antibodies against serovar *grippotyphosa* did not differ significantly among years (Fisher exact test, $P > 0.90$) or between the two study areas (Fisher exact test, $P > 0.90$).

DISCUSSION

Coyotes in Yellowstone National Park were last studied in the 1930's and 1940's (Murie, 1940; Robinson and Cummings, 1951; Robinson, 1952). No serological survey for diseases in the coyote population has been conducted in the park. Based on our results, the prevalence of antibodies against CPV in Yellowstone coyotes was high when compared to other coyote populations. We found 100% exposure to CPV in adults, yearlings, and old pups, and no exposure among young pups. In Texas, Utah, Idaho, and Colorado, >70% of the coyotes had antibodies against CPV (Thomas et al., 1984; Gese et al., 1991). In Georgia, 65% of the coyotes sampled had antibodies against CPV (Holzman et al., 1992). High prevalence of antibodies are often associated with a highly contagious, but nonfatal infection, because prevalence is measured among the survivors (Thomas et al., 1984). However, active infections in the Yellowstone coyote population may have contributed to high pup mortality. Of 21 coyote pups implanted with radio transmitters in 1992, 14 pups died; eight of these were known to have died from CPV infection based on gross and histologic lesions and detection of a parvovirus by electron microscopy. More pups may have died from CPV, but carcasses were recovered too late for accurate diagnosis. In 1991 and 1992, nine pups died of unknown causes, but at the same age as the parvoviral deaths in 1992.

Maternally derived antibodies to CPV in pups are passed from the breeding female through the placenta and colostrum (Pollock and Carmichael, 1982). None of the young pups had detectable antibodies to CPV. Green et al. (1984) found that females with antibodies usually produce pups with antibodies. However, the level of maternally-derived CPV antibody rapidly declines (half-life of 6.7 days) through the third week after birth (Green et al., 1984). Green et al. (1984) found only two (5%) of 41 pups at 8 wk of age had detectable antibody titers to CPV. In our sample of young pups (8 to 12 wk old), maternal antibodies were not detected, possibly making these young pups susceptible to viral infection; hence the high mortality due to CPV among the implanted pups. All the pups 5 to 6 mo old captured in the fall had antibodies to CPV, and all indicated recent infection based on the level of antibody detected at the time of sampling. None of these pups died and they apparently survived the CPV infection. The prevalence of recent infections (titers ≥1:1,280) declined with age; most of these older animals had likely been exposed to CPV at a younger age and survived.

Evidence of CPV has been reported in many wolf populations. In Minnesota, over 50% of the wolves had been exposed to CPV (Goyal et al., 1986; Mech et al., 1986). In Alaska, 31% of the wolves had antibodies against CPV (Zarnke and Ballard, 1987). The high prevalence of CPV in the Yellowstone coyote population is evidence that CPV is persistent in the population, and that coyotes will be a potential

source of viral infection to introduced or recolonizing wolves. Canine parvoviral infection has been implicated as a major cause of wolf pup mortality (Mech and Goyal, 1993; Johnson et al., 1994). In Minnesota, Mech and Goyal (1993) found that, over a 12-yr period, both the annual percent population increase and the proportion of pups in the population were inversely related to the percentage of wolves seropositive for CPV. They concluded that the prevalence of CPV did not impede population growth in the large Minnesota wolf population, but could limit population growth during times when CPV prevalence exceeded 76% in adults (Mech and Goyal, 1995) or in a small, recolonizing population of wolves (Mech and Goyal, 1993). For a recolonizing population of wolves in northwestern Montana, high CPV prevalence in the adults may have contributed to higher pup mortality in certain packs (Johnson et al., 1994).

We found an overall CDV seroprevalence of 76% among adult, yearling, and old pup coyotes combined. Young pups had no antibodies to CDV. In Texas, 37% (Trainer and Knowlton, 1968) and 56% (Guo et al., 1986) of the coyotes had antibodies to CDV in two separate studies, respectively. Williams et al. (1988) reported that 50% of coyotes in Wyoming tested positive for CDV antibodies. In Georgia, no coyotes were found to have been exposed in CDV (Holzman et al., 1992). Our result of 76% prevalence is one of the highest reported. We found an increase in prevalence as age increased; Guo et al. (1986) found similar results in Texas. The higher prevalence in adults and yearlings may be due to animals surviving infection, adults being more likely to survive exposure, adults having a longer time period to be exposed to the virus and develop a long-persisting titer, or declining maternal antibodies as pups grow older (Gorham, 1966; Green et al., 1984).

Similar to coyotes, wolf populations in North America have also been exposed to CDV. In two studies in Alaska, 7% and 12%, respectively, of the wolves were seropositive to CDV (Stephenson et al., 1982; Zarnke and Ballard, 1987). In Canada, Choquette and Kuyt (1974) found only two of 86 wolves had antibodies for CDV. In a recolonizing wolf population in Montana, 29% of the wolves had positive titers for CDV. Effects of CDV on a coyote or wolf population are not fully understood, but CDV could play a role in pup survival, especially in a recolonizing population (Johnson et al., 1994). However, because both canids have been exposed to CDV there is little chance of widespread losses from CDV with either species.

Prevalence of antibodies against ICH virus was high (>80%) in coyotes >1-yr old in both our study areas; thus many coyotes may have been exposed to ICH virus and survived. Coyotes in Texas and Georgia had a lower prevalence (41 to 51%) of ICH virus exposure (Trainer and Knowlton, 1968; Holzman et al., 1992). In wolf populations, seroprevalence was high (>80%) in Alaska (Stephenson et al., 1982; Zarnke and Ballard, 1987), and lower prevalences (<40%) were observed in Canada and Montana (Choquette and Kuyt, 1974; Johnson et al., 1994). The degree to which ICH virus affects canid populations is unknown. The coyote population in Yellowstone was healthy and robust with normal annual recruitment of pups into the population (Gese, 1995). In Alaska, 95% of the wolves had antibodies to ICH virus and the researchers concluded that the population had normal recruitment (Stephenson et al., 1982). Infectious canine hepatitis virus can be transmitted via urine from infected animals, and is relatively resistant to chemical and physical agents (Stephenson et al., 1982), allowing for a high exposure to ICH virus in most canid populations. The finding that ICH virus antibody prevalence was steadily declining during our study is evidence that ICH may be enzootic with a previous epizootic in the coyote population in Yellowstone.

The high prevalence of antibodies

against *Y. pestis* in the coyote population in Yellowstone was similar to results in Colorado and Idaho (Barnes, 1982). In contrast, coyotes sampled in California had very low antibody prevalence (<6%) to plague (Thomas and Hughes, 1992). Coyotes may become infected with *Y. pestis* by being bitten by fleas or by ingesting infected rodents (Thomas et al., 1989). When coyotes are infected, they usually do not develop clinical signs, but develop antibody titers which last about 6 mo (Barnes, 1982). This makes coyotes an indicator species for plague. Thus, changes in prevalence of plague in the coyote population are likely related to changes in the prevalence of plague in the coyotes prey base, such as small mammals. We did see a decline in seroprevalence in the coyote population over the 3 yr sampled, possibly indicative of the epizootic nature of plague in rodent populations. The impact of plague on canid populations is unknown.

Evidence of tularemia was found in the coyote population in Yellowstone, but at relatively low levels (<25%). In Texas, Trainer and Knowlton (1968) found no serologic evidence of tularemia in 33 coyotes. In contrast, 88% of the coyotes sampled in Idaho were seropositive (Gier et al., 1978). In Alaska, 25% of the wolves tested had antibodies to tularemia (Zarnke and Ballard, 1987). The impact of tularemia on canids is unknown. Canids may contract the disease, but appear to be relatively unsusceptible and most healthy animals will probably recover (Gier and Ameel, 1959; Zarnke and Ballard, 1987).

We found no serologic evidence of brucellosis (*B. canis* or *B. abortus*) among the coyote population in Yellowstone. Similarly, coyotes sampled in Texas and Georgia had not been exposed to brucellosis (Trainer and Knowlton, 1968; Holzman et al., 1992). Among wolves, seroprevalence of brucellosis was 1% in southcentral Alaska (Zarnke and Ballard, 1987), but 30% in northern Alaska (Neiland, 1970, 1975). For wolves, caribou (*Rangifer tarandus*) are the primary reservoir of brucellosis, and wolves are likely infected by preying or scavenging on infected caribou (Neiland, 1970). In Yellowstone, bison (*Bison bison*) act as primary hosts of *B. abortus* (Johnson, 1992). Coyotes in the northern range of the park rely upon ungulate carrion throughout the winter (Murie, 1940; Houston, 1978; Gese, 1995). Serological tests cannot distinguish among the six species of *Brucella*. However, the absence of antibodies for any of the *Brucella* species is evidence that coyotes were not involved as significant hosts for brucellosis. Should brucellosis become more prevalent among the different canid species in Yellowstone, impacts on an individual animal could include reproductive failure as a result of infection (Carmichael and Kennedy, 1970; Neiland and Miller, 1981). However, since coyotes and wolves usually do not develop clinical signs from brucellosis, the likelihood of reproductive failure is minimal for both canids.

We found a low seroprevalence of leptospirosis in the coyote population in Yellowstone, similar to results for coyotes sampled in Texas (Trainer and Knowlton, 1968) and Georgia (Holzman et al., 1992). In contrast, four of nine coyotes tested in Arizona were seropositive for leptospirosis (Drewek et al., 1981). For wolves, Zarnke and Ballard (1987) found a low (1%) antibody prevalence of leptospirosis in Alaska. In contrast, wolves near farming areas in Minnesota had a higher prevalence (20%) of antibodies against *L. interrogans* than wolves sampled in a wilderness areas (8%) (Khan et al., 1991). The impact of leptospirosis on a canid population is unknown, but infected canids may survive and remain carriers for a short time (Drewek et al., 1981).

Routes of exposure and transmission of various canine diseases may occur from within the wild canid populations themselves, or from domestic dogs coming into the park. Over three million people from all over the world visit Yellowstone each year. Both study areas were traversed by a paved road along which there were many

pullouts, trailheads, picnic areas, two campgrounds, and a lodge. The National Park Service had restrictions on pets, including no pets permitted 30 m beyond established roads and parking areas, pets must be on a leash, pet owners must properly remove fecal material, and no pets were allowed in the backcountry. Unfortunately, many of these regulations are ignored by visitors and infected dogs likely deposit urine and feces along the road, permitting the opportunity for exposure to the wild canid population.

In summary, wolf populations are exposed to various viral and bacterial diseases, especially CPV and CDV (Stephenson et al., 1982; Goyal et al., 1986; Mech et al., 1986; Zarnke and Ballard, 1987). Current plans to reintroduce wolves to Yellowstone National Park (U.S. Fish and Wildlife Service, 1987), or their recovery through natural recolonization, warranted an examination of the role of the coyote as a possible reservoir for infection, as well as an indicator species for diseases to which wolves will be exposed in the park. Similarly, reintroduced wolves could introduce potential diseases to the coyote population. However, the seroprevalence of CPV, CDV, and ICH is evidence that the coyote population as a whole is immunologically protected via previous exposure; and thus coyotes are not at risk to diseases from the reintroduced wolves because these canine diseases are already present in the park. The occurrence of CPV and CDV in the Yellowstone coyote population may increase the chances of exposure and infection to a reestablishing wolf population, which may decrease wolf pup survival (Mech and Goyal, 1993; Johnson et al., 1994). Also, CPV is extremely resistant to heat and dessication (Thomas et al., 1984) allowing for exposure to be maintained through environmental contamination, as when wolves excavate and utilize old coyote dens. Management decisions and handling procedures of reintroduced wolves to the park should include an awareness of the canine diseases currently present in the coyote population in Yellowstone National Park.

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LITERATURE CITED

APPEL, M., AND D. S. ROBSON. 1973. A microneutralization test for canine distemper virus. American Journal of Veterinary Research 34: 1459–1463.

———, L. E. CARMICHAEL, AND D. S. ROBSON. 1975. Canine adenovirus type-2-induced immunity to two canine adenoviruses in pups with maternal antibody. American Journal of Veterinary Research 36: 1199–1202.

BALSER, D. S. 1965. Tranquilizer tabs for capturing wild carnivores. The Journal of Wildlife Management 29: 438–442.

BARKER, I. K., R. C. POVEY, AND D. R. VOIGT. 1983. Response of mink, skunk, red fox and raccoon to inoculation with mink virus enteritis, feline panleukopenia and canine parvovirus and prevalence of antibody to parvovirus in wild carnivores in Ontario. Canadian Journal of Comparative Medicine 47: 188–197.

BARNES, A. M. 1982. Surveillance and control of bubonic plague in the United States. Symposia of the Zoological Society of London 50: 237–270.

CARMICHAEL, L. E., AND R. M. KENNEDY. 1970. Canine brucellosis: The clinical disease, pathogenesis and immune response. Journal of the American Veterinary Medical Association 156: 1726–1736.

———, J. C. JOUBERT, AND R. V. H. POLLOCK. 1980. Hemagglutination by canine parvovirus: Serologic studies and diagnostic applications. American Journal of Veterinary Research 41: 784–791.

CENTERS FOR DISEASE CONTROL. Undated a. Passive hemagglutination test for Nobuto strips. Unpublished mimeo, Centers for Disease Control, Fort Collins, Colorado, 1 pp.

———. Undated b. Protocol for *Francisella tular-*

ensis microagglutination (MA). Unpublished mimeo, Centers for Disease Control, Fort Collins, Colorado, 1 pp.

CHOQUETTE, L. P. E., AND E. KUYT. 1974. Serological indication of canine distemper and of infectious canine hepatitis in wolves (*Canis lupus* L.) in northern Canada. Journal of Wildlife Diseases 10: 321–324.

CORNELY, J. E. 1979. Anesthesia of coyotes with ketamine hydrochloride and xylazine. The Journal of Wildlife Management 43: 577–579.

DESPAIN, D. G. 1990. Yellowstone vegetation: Consequences of environment and history in a natural setting. Roberts Rinehart Publishers, Boulder, Colorado, 239 pp.

DREWEK, J., JR., T. H. NOON, R. J. TRAUTMAN, AND E. J. BICKNELL. 1981. Serologic evidence of leptospirosis in a southern Arizona coyote population. Journal of Wildlife Diseases 17: 33–37.

GESE, E. M. 1995. Foraging ecology of coyotes in Yellowstone National Park. Ph.D. Thesis. University of Wisconsin, Madison, Wisconsin, 134 pp.

———, R. D. SCHULTZ, O. J. RONGSTAD, AND D. E. ANDERSEN. 1991. Prevalence of antibodies against canine parvovirus and canine distemper virus in wild coyotes in southeastern Colorado. Journal of Wildlife Diseases 27: 320–323.

GIER, H. T. 1968. Coyotes in Kansas (revised). Agricultural Experiment Station Bulletin 393, Kansas State University, Manhattan, Kansas, 118 pp.

———, AND D. J. AMEEL. 1959. Parasites and diseases of Kansas coyotes. Technical Bulletin 91, Agricultural Experiment Station, Kansas State University, Manhattan, Kansas, 34 pp.

———, S. M. KRUCKENBURG, AND R. J. MARLER. 1978. Parasites and diseases of coyotes. *In* Coyotes: Biology, behavior, and management, M. Bekoff (editor). Academic Press, New York, New York, pp. 37–71.

GORHAM, J. R. 1966. The epizootiology of distemper. Journal of the American Veterinary Medical Association 149: 610–618.

GOYAL, S. M., L. D. MECH, R. A. RADEMACHER, M. A. KHAN, AND U. S. SEAL. 1986. Antibodies against canine parvovirus in wolves of Minnesota: A serologic study from 1975 through 1985. Journal of the American Veterinary Medical Association 189: 1092–1094.

GREEN, J. S., M. L. BRUSS, J. F. EVERMANN, AND P. K. BERGSTROM. 1984. Serologic response of captive coyotes (*Canis latrans* Say) to canine parvovirus and accompanying profiles of canine coronavirus titers. Journal of Wildlife Diseases 20: 6–11.

GUO, W., J. F. EVERMANN, W. J. FOREYT, F. F. KNOWLTON, AND L. A. WINDBERG. 1986. Canine distemper virus in coyotes: A serologic survey. Journal of the American Veterinary Medical Association 189: 1099–1100.

HOLZMAN, S., M. J. CONROY, AND W. R. DAVIDSON. 1992. Diseases, parasites and survival of coyotes in south-central Georgia. Journal of Wildlife Diseases 28: 572–580.

HOUSTON, D. B. 1978. Elk as winter-spring food for carnivores in northern Yellowstone National Park. Journal of Applied Ecology 15: 653–661.

JOHNSON, M. R. 1992. The disease ecology of brucellosis and tuberculosis in potential relationship to Yellowstone wolf populations. *In* Wolves for Yellowstone? A report to the United States Congress, Vol. IV, J. D. Varley and W. G. Brewster (editors). National Park Service, Yellowstone National Park, Wyoming, pp. 671–694.

———, D. K. BOYD, AND D. H. PLETSCHER. 1994. Serologic investigations of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortalities. Journal of Wildlife Diseases 30: 270–273.

KHAN, M. A., S. M. GOYAL, S. L. DIESCH, L. D. MECH, AND S. H. FRITTS. 1991. Seroepidemiology of leptospirosis in Minnesota wolves. Journal of Wildlife Diseases 27: 248–253.

LINHART, S. B., AND F. F. KNOWLTON. 1967. Determining age of coyotes by tooth cementum layers. The Journal of Wildlife Management 31: 362–365.

MECH, L. D., AND S. M. GOYAL. 1993. Canine parvovirus effect on wolf population change and pup survival. Journal of Wildlife Diseases 29: 330–333.

———, AND ———. 1995. Effects of canine parvovirus on gray wolves in Minnesota. The Journal of Wildlife Management 59: 565–570.

———, ———, C. N. BOTA, AND U. S. SEAL. 1986. Canine parvovirus infection in wolves (*Canis lupus*) from Minnesota. Journal of Wildlife Diseases 22: 104–106.

MURIE, A. 1940. Ecology of the coyote in Yellowstone. Fauna Series 4, U.S. National Park Service, Washington D.C., 206 pp.

NATIONAL VETERINARY SERVICES LABORATORY. 1987. Microtiter technique for detection of *Leptospira* antibodies. Proceedings of the United States Animal Health Association 91: 65–73.

NEILAND, K. A. 1970. Rangiferine brucellosis in Alaskan canids. Journal of Wildlife Diseases 6: 136–139.

———. 1975. Further observations on rangiferine brucellosis in Alaskan carnivores. Journal of Wildlife Diseases 11: 45–53.

———, AND L. G. MILLER. 1981. Experimental *Brucella suis* type 4 infections in domestic and wild Alaskan carnivores. Journal of Wildlife Diseases 17: 183–189.

POLLOCK, R. V. H., AND L. E. CARMICHAEL. 1982. Maternally derived immunity to canine parvovirus infection: Transfer, decline, and interference with vaccination. Journal of the American Veterinary Medical Association 180: 37–42.

ROBINSON, W. B. 1952. Some observations on coyote predation in Yellowstone National Park. Journal of Mammalogy 33: 470–476.

———, AND M. W. CUMMINGS. 1951. Movements of coyotes from and to Yellowstone National Park. Special Science Report Wildlife No. 11, U.S. Fish and Wildlife Service, Washington, D.C., 17 pp.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed., W. H. Freeman and Co., San Francisco, California, 859 pp.

STEPHENSON, R. O., D. G. RITTER, AND C. A. NIELSEN. 1982. Serologic survey for canine distemper and infectious canine hepatitis in wolves in Alaska. Journal of Wildlife Diseases 18: 419–424.

THOMAS, C. U., AND P. E. HUGHES. 1992. Plague surveillance by serological testing of coyotes (*Canis latrans*) in Los Angeles County, California. Journal of Wildlife Diseases 28: 610–613.

THOMAS, N. J., W. J. FOREYT, J. F. EVERMANN, L. A. WINDBERG, AND F. F. KNOWLTON. 1984. Seroprevalence of canine parvovirus in wild coyotes from Texas, Utah, and Idaho (1972–1983). Journal of the American Veterinary Medical Association 185: 1283–1287.

THOMAS, R. E., M. L. BEARD, T. J. QUAN, L. G. CARTER, A. M. BARNES, AND C. E. HOPLA. 1989. Experimentally induced plague infection in the northern grasshopper mouse (*Onychomys leucoguster*) acquired by consumption of infected prey. Journal of Wildlife Diseases 25: 477–480.

TRAINER, D. O., AND F. F. KNOWLTON. 1968. Serologic evidence of diseases in Texas coyotes. The Journal of Wildlife Management 32: 981–983.

U.S. DEPARTMENT OF AGRICULTURE. Undated. National Animal Disease Laboratory Diagnostics Reagents Manual. National Animal Disease Laboratory, Ames, Iowa, 57 pp.

U.S. FISH AND WILDLIFE SERVICE. 1987. Northern Rocky Mountain wolf recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado, 119 pp.

WILLIAMS, E. S., E. T. THORNE, M. J. G. APPEL, AND D. W. BELITSKY. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24: 385–398.

ZAR, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, 718 pp.

ZARNKE, R. L., AND W. B. BALLARD. 1987. Serologic survey for selected microbial pathogens of wolves in Alaska, 1975–82. Journal of Wildlife Diseases 23: 77–85.

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