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DISEASES, PARASITES AND SURVIVAL OF COYOTES IN SOUTH-CENTRAL GEORGIA

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ABSTRACT: Serologic testing, radio-telemetry and post-mortem diagnostic evaluations were used to investigate survival and causes of mortality among 17 coyotes (*Canis latrans*) in south-central Georgia (USA). Prevalence of canine heartworm (*Dirofilaria immitis*) microfilariae was lower ($P = 0.057$) among fall-captured (22%) than among winter-captured (75%) coyotes. Prevalence of heartworm was higher among adults than juveniles in the fall, but no significant difference was detected between animals captured in winter. Antibodies were found against canine parvovirus (65%), canine parainfluenza virus (59%), infectious canine hepatitis virus (41%), and *Toxoplasma gondii* (18%). Antibodies were not found to *Brucella canis*, canine coronavirus, five serovars of *Leptospira interrogans*, or canine distemper virus. Seroprevalence of canine parvovirus was lower ($P = 0.009$) among fall-captured animals (33%) than winter-captured animals (100%). The Kaplan-Meier estimate of annual survival was 0.500 for all animals. Juvenile survival did not differ ($P = 0.79$) from adult survival, but male survival ($\hat{S} = 0.217$) was lower ($P = 0.11$) than female survival ($\hat{S} = 0.804$). Two of nine (22%) mortalities were human-caused, one was due to concurrent canine parvovirus and canine distemper virus infections, one animal died of trauma, two were considered natural mortalities of unknown cause, and no cause of death could be determined for the remaining three animals. Natural mortality may be significant for coyotes in south-central Georgia, although there was no apparent link between exposure to pathogens and the animals' subsequent fate in our small sample.

Key words: Canine heartworm, canine parvovirus, *Canis latrans*, coyotes, disease, Georgia, parasites, survival, mortality factors.

INTRODUCTION

Studies of diseases and mortality factors of coyotes (*Canis latrans*) have included the effects of specific pathogens on captive animals (Green et al., 1934; Lundgren et al., 1957; Gier et al., 1978); serologic surveys of wild populations (Cook et al., 1965; Trainer and Knowlton, 1968; Riemann et al., 1975; Jorgenson et al., 1977; Drewek et al., 1981; Thomas et al., 1984; Davis et al., 1979); surveys for internal and external parasites (Bishopp and Trembley, 1945; Butler and Grundman, 1954; Gier, 1968; Mitchell and Beasom, 1974; Custer and Pence, 1981; Pence and Custer, 1981; Pence and Eason, 1980; Pence et al., 1981; Pence and Meinzer, 1979); and survival of ear-tagged and radio-collared animals (Nellis and Keith, 1976; Tzilkowski, 1980; Pence et al., 1983; Pyrah, 1984; Roy and Dorrance, 1985; Windberg et al., 1985; Harrison, 1986). Advances in radio-telemetry such as mortality sensors enable researchers to find dead animals more quickly, thus improving diagnostic opportunities and allowing better inferences regarding the effects of disease on survival. In one of the few studies to relate occurrence of pathogens to mortality, Pence et al. (1983) found significantly higher mortality among animals infected with sarcoptic mange than among uninfected animals.

Coyotes have occurred in Georgia (USA) since the 1950's (Hill et al., 1987); with the present population derived from both introduced animals and expanding populations from western North America. The coyote population in Georgia apparently has not increased as rapidly as it has in other southeastern states (Georgia Department of Natural Resources, unpublished data). We speculated that population growth may have been suppressed by either increased mortality or decreased na-

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tality. Our purpose was to investigate potential limiting factors on coyotes in south-central Georgia by determining the prevalence of pathogens known or suspected as capable of producing mortality in adult animals (Gier et al., 1978); estimating survival rates; and identifying sources of mortality.

MATERIALS AND METHODS

Coyotes were trapped in two locations in south-central Georgia. Site 1 encompassed approximately 100 km² of primarily forested habitat in Telfair county, Georgia (31°50′N, 82°50′W), 25 km south of McRae. Sixteen percent of the area was hardwood forest, predominately blackgum (*Nyssa sylvatica*), sweetgum (*Liquidambar styraciflua*), and oak (*Quercus* spp.) along the Ocmulgee River and its tributaries. Mixed pine-hardwood stands of loblolly pine (*Pinus taeda*), slash pine (*P. elliottii*), oaks, and sweetgum comprised 31% of the area and plantations of loblolly and slash pine of various ages comprised 23% of the area. Twelve percent was agricultural land, mainly in peanut cultivation. The remaining 18% included clearcuts, brushy areas, old fields, pecan orchards, pasture, ponds, and swamps.

Site 2 encompassed approximately 230 km² of Irwin County, Georgia (31°35′N, 83°25′W) in a region with many farms and small towns. Forty-one percent of the study area was agricultural land. Common crops included peanuts, cotton, winter wheat, and hay. Twenty-three percent was bottomland hardwood forest along the Alapaha River and associated creeks. The area included 10% mixed pine-hardwood forest and 9% pine-plantations. The remaining 17% was comprised of old fields, oak scrub, brushy areas, orchards, ponds, homes, and industry.

Climate for both study sites is characterized by hot summers and mild winters. Average annual rainfall is 1,164 mm, and daily temperatures range from a mean minimum of 4.6 C in December to a mean maximum of 33.6 C in July (Soil Conservation Service, 1969).

Coyotes were captured September 1987 through February 1988 using #3 rubber-jawed leghold traps (Woodstream Corp., Lititz Penn., USA) or snares set along trails and field edges. We checked sets daily and initially anesthetized captured animals with a 5:1 ketamine hydrochloride (Ketaset) Aveco Company, Inc., Fort Dodge, Iowa, USA) : xylazine hydrochloride (Rompun, Mobay Corp., Shawnee, Kansas, USA) mixture at 7 mg/kg estimated body mass (Anonymous, 1986). This dosage was later increased to 13.2 mg/kg to provide a minimum of 1 hr of immobilization. After weighing animals on a spring balance, we delivered more anesthetic appropriate to the animal's true weight, if necessary. Coyotes were aged by tooth wear (Gier, 1968) and, when possible, cementum annuli analysis of the first lower premolar (Linhart and Knowlton, 1967). We used the ratio of molar row length to palatal width to detect and eliminate any coyote-dog hybrids or domestic dogs from the sample (Howard, 1949).

Live anesthetized coyotes were examined for ticks, fleas, and evidence of mange mite infestations by brief visual inspection of pelage, particularly including the ears, axillae, and ventral portions of the trunk. Ticks and fleas were removed and preserved in 70% alcohol for later identification. We took 10 to 15 ml of blood from the jugular vein of each coyote. One ml of whole blood was mixed with 10 ml of 2% formalin for a subsequent Knott's test for microfilariae of the canine heartworm (*Dirofilaria immitis*) (Soulsby, 1971). Two thin blood smears were prepared from fresh blood, air dried, fixed in 100% methanol, stained with Giesma stain, and examined microscopically at 40 to 1,000× for blood parasites. We allowed the remaining blood to clot, centrifuged it, and removed and froze the serum (−10 C for 9 mo). Serum neutralization tests (Appel and Robson, 1973) were used to test for antibodies to canine coronavirus (CCV), canine distemper virus (CDV), infectious canine hepatitis (ICH), and canine parainfluenza (SV-5) virus. A hemagglutination inhibition test (Carmichael et al., 1980) was used to detect antibodies to canine parvovirus type 2 (CPV-2). Microscopic agglutination tests (Shotts, 1976) were used to test for antibodies to the *pomona*, *hardjo*, *icterohemorrhagiae*, *grippotyphosa*, and *canicola* serovars of *Leptospira interrogans*. A tube test (U.S. Department of Agriculture, no date) was used to test for antibodies to *Brucella canis*, and a commercial latex agglutination test (Toxotest-MT "Eiken," Tanabe U.S.A., Inc., San Diego, California USA) was used to detect antibodies to *Toxoplasma gondii*.

We attached ear-tags and radio-collars with mortality sensors (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA) to 17 coyotes before release. Daily (September 1987 to June 1988) or weekly (June to December 1988) attempts were made to locate each coyote and determine its status (Cochran, 1980). Time of death was estimated as the midpoint between an animal's last recorded location and date of discovery, unless carcass condition provided a basis for a more exact date.

Carcasses were placed on ice within 1 hr of retrieval and delivered to the Southeastern Cooperative Wildlife Disease Study, University of

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Georgia, Athens, Georgia within 5 hr for necropsy. Necropsies were oriented to determine cause of death; thus, our procedures and diagnostic tests differed among cases depending on diagnostic circumstances and findings. However, we carefully examined all animals for external parasites. Carcasses were then skinned and examined for traumatic injuries. Samples from all major organ systems and lesions were preserved in 10% neutral buffered formalin for histopathologic study. External parasites were preserved in 70% alcohol, and helminth parasites were preserved in 5% formalin for later identification. Unless parasitism was suspected to be related to the cause of death, no effort was made to quantify parasitic infections. Because necropsy procedures were not designed to detect and enumerate internal parasites, generally only the more obvious helminth parasites were noted in the five animals for which we retrieved carcasses.

Fisher's exact test (Steele and Torrie, 1980) was used to test for age, sex, and seasonal differences in the prevalence of canine heartworm microfilariae and antibodies to *T. gondii*, CPV-2, ICH, and canine parainfluenza virus. We estimated overall survival and obtained separate estimates for adults, juveniles, males, and females by Pollock et al.'s (1989) modification of Kaplan and Meier's (1958) non-parametric technique. This method allows the staggered entry of animals into a study, censoring animals that can no longer be located or have left the study site, and testing for differential survival between ages or sexes. Kaplan-Meier estimates (S(t)) of survival over 6 September 1987 to 1 January 1989 (482 days) were converted to annual survival rates ($\hat{S}$) to enable comparisons with other studies, by the formula:

$$\hat{S} = S(t)^{365/482}$$

with approximate standard error by the delta method (Seber, 1982):

$$SE(\hat{S}) = SE[S(t)] \cdot \{365/482 \cdot S(t)^{[365/482-1]}\}$$

where SE[S(t)] was obtained from Cox and Oakes (1984). Logrank tests (Pollock et al., 1989) were used to compare survival rates between ages and sexes.

RESULTS

We captured six adult (four male, two female) and 12 juvenile (six male, six female) coyotes, obtained 17 blood samples, and radio-collared 17 animals. Four coyotes were captured at site 1, 14 at site 2. One juvenile female at site 2 died during handling, and we were unable to collect blood from a juvenile male at site 1. Juvenile females had the lowest mean body mass ($\bar{x}$ = 7.95 kg, SE = 0.89) and adult males the highest ($\bar{x}$ = 13.8 kg, SE = 0.09). The mean (±SE) body masses for adult females and juvenile males were 12.15 kg ± 0.55 and 11.1 kg ± 1.10, respectively. We did not perform statistical tests of body mass between age and sex classes because of differences in capture probabilities by season. All animals appeared healthy, and in most cases the trapped foot had only minor swelling.

We collected ticks from 13 animals: American dog ticks (*Dermacentor variabilis*) from eight animals, Gulf coast ticks (*Amblyomma maculatum*) from six animals, and black-legged ticks (*Ixodes scapularis*) from four animals. Two species of fleas, *Pulex simulans* and *Cediopsylla simplex*, were recovered from one animal. Unidentified hookworms were found in two animals, *Physaloptera* sp. was discovered in two animals, and *Ancylostoma caninum* eggs or adults were discovered in two animals. Unidentified tapeworms were found in two animals, and *Taenia* sp. was recovered from two animals. We observed cysts of *Sarcocystis* sp. in the skeletal muscle of a juvenile female. Hematotrophic protozoans were not found in any of 17 blood smears. Eight of 17 animals had microfilariae of *Dirofilaria immitis* when examined by the Knott's test; fall prevalence (two of nine) was lower (P = 0.06) than winter prevalence (six of eight). One juvenile female negative for microfilariae when captured in the fall had adult heartworms at necropsy 2 wk later. A winter-caught juvenile male positive for microfilariae when captured also had adult heartworms at necropsy 4 mo later.

We did not detect antibodies to *B. canis*, CCV, or CDV in coyote serum samples. No animals were positive for antibodies to *L. interrogans* serovars. Three animals had antibodies to *Toxoplasma gondii* (Table 1), but there were no significant differences in prevalence between ages, sexes, or capture seasons (all comparisons, $P >$

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TABLE 1. Serological titers to selected pathogens among coyotes captured in south-central Georgia, September 1987 through February 1988.

	Dirofilaria immitis[a]	*Toxoplasma gondii*[b]	CPV-2[c]	ICH[d]	SV-5[e]
Captured September–October					
Adult males					
C11	N[f]	1:32	1:1,280	1:2,512	N
Adult females					
C24	P	N	1:2,560	1:158	N
Juvenile males					
C88	N	N	1:160	N	N
C64	N	N	N	N	1:20
Juvenile females					
C05	N	N	N	1:10	N
C66	P	N	N	N	1:10
C96	N	N	N	N	N
C28	N	N	N	N	1:10
C01	N	N	N	N	1:20
Captured December–February					
Adult males					
C15	P	N	>1:10,240	N	N
C13	P	N	>1:10,240	N	1:20
C91	N	N	>1:10,240	1:32	1:316
Adult females					
C94	P	N	>1:10,240	1:794	1:10
Juvenile males					
C69	N	1:16	1:2,560	N	N
C02	P	N	>1:10,240	N	1:10
C61	P	N	>1:10,240	1:20	1:316
Juvenile females					
C04	P	1:16	1:10,240	1:3,981	1:32

[a] Presence of *Dirofilaria immitis* microfilariae determined by Knott's Test.
[b] Antibody titer to *Toxoplasma gondii*.
[c] Antibody titer to canine parvovirus type 2.
[d] Antibody titer to infectious canine hepatitis (canine adenovirus type 2).
[e] Antibody titer to canine parainfluenza virus.
[f] P, positive; N, negative.

TABLE 2. Kaplan-Meier annual survival probabilities for coyotes in south-central Georgia, September 1987 through Dec 1988.

Group	n[a]	$\hat{S}$[b]	95% confidence interval
All animals	17	0.500	0.239 to 0.761
Males[c]	10	0.217	−0.045 to 0.480
Females	7	0.804	0.460 to 1.149
Adults[d]	6	0.499	0.093 to 0.906
Juveniles	11	0.525	0.191 to 0.858

[a] n = Sample size of coyotes.
[b] $\hat{S}$ = Estimated survival rate of coyotes from 6 September 1987 to 1 January 1989, transformed to a 12 mo survival estimate (see text).
[c] Logrank test of males vs. females ($\chi^2 = 2.51$, df = 1, $P = 0.11$).
[d] Logrank test of adults vs. juveniles ($\chi^2 = 0.867$, df = 1, $P = 0.35$).

0.58). Eleven of 17 animals had antibodies to CPV-2, and there was a greater prevalence of CPV-2 among males than females ($P = 0.05$); among adults than juveniles ($P = 0.04$); and among animals captured in winter than those captured in fall ($P = 0.009$). In fall, both adults, and one of two juvenile males had antibodies, whereas none of five juvenile females had antibodies. In contrast, all animals captured in winter had antibodies. Seven of 17 animals had antibodies to ICH virus, and 10 of 17 coyotes had antibodies to canine parainfluenza virus. There were no significant differences (all comparisons, $P > 0.16$) in the prevalence of antibodies to these two viruses between ages, sexes, or capture seasons.

Seven of 17 animals died between September 1987 and December 1988. Radio contact was lost for an adult male one week after capture. This loss may have been due to radio failure or dispersal; an aerial search of approximately 300 km^2 was unsuccessful. An adult male (C13) was found dead in February 1989, and a juvenile male (C69) was lost 77 days after capture and was found dead 1 yr later approximately 67 km from its last known location. All other animals were located at intervals of 7 days or less over September 1987 to December 1988 and constitute the sample used to estimate survival.

Juvenile survival was not significantly different ($P = 0.35$) from adult survival (Table 2). Most juvenile mortality (four of six) occurred during the first 6 mo. of the study. In contrast, no adults died until 10 mo after the study began. We determined the cause of death for three of nine mortalities, and classified the remaining six deaths as natural or human-caused mor-

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TABLE 3. Causes of mortality of radio-collared coyotes in south-central Georgia, September 1987 through May 1989.

Coyote	Date of capture mo/day/yr	Date of discovery of remains mo/day/yr	Major diagnostic findings, comments	Cause of death
Adult males				
C11	09/10/87	07/15/88	Only bones recovered.	Unknown
C15	12/27/87	07/15/88	Only bones recovered.	Unknown
C13	01/30/88	02/25/89	Only bones recoverd, automotive fan-belt tied around neck	Unknown, human-caused
Juvenile males				
C88	09/06/87	11/18/87	Carcass autolytic, corneal smear FA positive for CDV antigen	Unknown, natural
C12	12/2/87	12/14/87	Dehydration and emaciation, diarrhea and severe gastroenteritis; canine parvovirus infection based on virus isolation, histopathology and EM; canine distemper infection based on FA and histopathology; severe *Sarcocystis* infection in intestinal mucosa	Concurrent canine parvovirus and canine distemper virus infections
C64	09/15/87	01/11/88	Carcass not recovered	Shot during deer hunting season
C02	01/26/88	05/25/88	Luxation with hemorrhage and edema in the region of the first thoracic vertebra caused by fallen tree	Accidental trauma
C69	12/23/88	1/10/89	Only normal skull recovered	Unknown
Juvenile females				
C05	09/08/87	09/25/87	Carcass autolytic, minimal fat reserves, *Dirofilaria immitis* in right ventricle, possible canine distemper viral inclusion bodies in brain lesions	Unknown, natural

talities (Table 3). Although we had evidence that canine distemper may have caused the deaths of C05 and C88 (Table 3), the cause of death in both animals could not be confirmed because of autolysis. However, both deaths were considered natural mortalities because no evidence of human-caused injuries (trauma, broken bones, or embedded shot) was observed. The carcass of C12 was in good condition, and we were able to confirm concurrent CPV and CDV infection as the cause of death of this juvenile male. The skeletons of C11 and C15 were discovered during very hot weather in July 1988, and these animals had been dead for several days. Both were found in woods near fields, and there were no indications of human-caused mortality such as embedded shot or broken bones. The skeleton of C69 also was found in woods near an open field, 67 km from its original capture location. It had been missing for 10 mo. Although the death of C13 probably was human-caused, it was unclear whether the animal was suffocated or whether an automotive fan-belt found around the animal's neck had been placed there after death.

DISCUSSION

Coyotes sampled were not heavily parasitized by arthropods. The endoparasites discovered at necropsy are common throughout the range of the coyote (Mitchell and Beasom, 1974; Fayer and Johnson, 1975; Gier et al., 1978; Pence and Meinzer, 1979; Foreyt and Foreyt, 1982). Because heartworms were the only helminths

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sought during necropsy, little can be said about the effects of other helminths on coyote mortality.

In Louisiana, Crowell et al. (1977) found only five of nine coyotes with adult heartworms also had microfilariae in the blood. In our study, adult heartworms found in one juvenile coyote that had been negative for microfilariae two weeks earlier may not have been reproductively mature at the time of capture. This phenomenon may explain the lower heartworm prevalence in our fall-caught sample as compared to the higher prevalence among winter-caught animals. Winter heartworm prevalence for our study was similar to that reported from coyotes sampled in other southeastern states (Custer and Pence, 1981; Crowell et al., 1977). Higher prevalences of heartworm among older coyotes have been reported in other studies (Custer and Pence, 1981; Crowell et al., 1977; Graham, 1975). Based on our study, we suggest that age-related differences in heartworm exposure may disappear by winter. Both Gier et al. (1978) and Custer and Pence (1981) suggested that heartworm disease may be an important factor in coyote morbidity and mortality. Future studies, involving larger sample sizes, could help to explain this relationship.

Although no animals had antibodies to CDV, one animal that died had concurrent CPV and CDV infections. Canine distemper may be lethal to young coyotes (Gier and Ameel, 1959; Gier et al., 1978), minimizing the chance of capturing a living animal with antibodies. A widespread serologic survey involving large samples would be needed to determine the prevalence of exposure to CDV in coyotes in south-central Georgia.

Exposure to CPV-2 was widespread. Sixty-five percent of all animals captured were seropositive for CPV-2, and all coyotes captured in the winter had titers to CPV-2. The remaining six animals, all caught in September to October, had titers between 1:20 and 1:40; these generally are not considered positive. During serologic surveys of coyotes conducted in Texas, Utah, and Idaho from 1972 to 1983, Thomas et al. (1984) found no evidence of parvoviruses until 1979, after which seroprevalence increased to >70% by 1982. Although CPV may be widespread throughout their range (Thomas et al., 1984; Gese et al., 1991), neither we nor Thomas et al. (1984) found reports of parvovirus-related enteritis in free-ranging coyotes. Our finding of a wild juvenile male coyote dying from CPV enteritis, complicated by a concurrent distemper infection, is the first description of such a case.

The Kaplan-Meier method entails the assumption that animals that disperse or whose radios fail survive at the same rate as the rest of the sample (Pollock et al., 1989). In our study, radio contact was lost for only 1 animal. Our annual survival rate (0.5) is in the range of that reported for coyotes in other parts of North America. Harrison (1986) reported an annual survival rate of 0.59 for 47 juvenile and 8 adult coyotes in Maine. An annual survival rate of 0.38 was reported for Alberta coyotes (Roy and Dorrance, 1985). Juvenile survival in our study was not significantly different from adult survival. Lower juvenile survival has been reported in other studies. Survival rates of 0.70 for adults and 0.42 for juveniles were estimated in a study of Texas coyotes (Windberg et al., 1985). Davison (1980) also reported lower annual survival for juveniles in Idaho.

In south-central Georgia, most human-related mortality appears incidental. We noted less human-related mortality (22%) than previous studies in other regions. In Maine, 60% of pup loss was due to human activities (Harrison, 1986). In Wyoming, human-caused mortality accounted for 93% of 41 recoveries (Tzilkowski, 1980). In Texas, shooting, trapping, and road fatalities accounted for 51% of mortalities (Windberg et al., 1985). Also in Texas, Andelt (1985) reported 38% human-caused mortality. Fourteen of 27 recoveries of radio-collared coyotes in Texas were associated with human activities while the

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cause of death in the remaining thirteen cases was undetermined (Pence et al., 1983). Roy and Dorrance (1985) reported that pups were more vulnerable to hunting than adults early in the winter but there was no difference in vulnerability after the first of January.

Four of nine (44%) mortalities were classified as natural either from direct or circumstantial evidence. Of these natural mortalities, we were able to definitively diagnose one disease-related mortality, and the circumstances surrounding two other deaths suggested disease (Table 3). Because observed survival was similar to other studies, we speculate that disease and other natural factors may have been important mortality factors for the coyote populations we studied. We recommend that future studies should be focused on larger-scale serologic surveys, combined with large samples of marked animals to enable more definitive conclusions regarding the role of pathogens in limiting coyote populations.

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