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SHORT COMMUNICATIONS

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Seroprevalence of Selected Disease Agents from Free-ranging Black Bears in Florida

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ABSTRACT: Sera obtained from 66 free-ranging Florida black bears (*Ursus americanus floridanus*) from three geographic areas of Florida (USA) between November 1993 and August 1995 were tested for antibodies to 13 disease agents. Antibody prevalences were 3 positive of 37 tested (8%) *Coxiella burnetii*, 37 of 66 (56%) *Toxoplasma gondii*, 3 of 61 (5%) blue-tongue virus/epizootic hemorrhagic disease virus (BTV/EHDV), 4 of 66 (6%) canine adenovirus-type 1, 5 of 66 (8%) canine distemper virus (CDV), 10 of 62 (16%) canine parvovirus (CPV), 7 of 66 (11%) eastern equine encephalitis virus, 4 of 66 (6%) western equine encephalitis virus, 2 of 66 (3%) Venezuelan equine encephalitis virus, and 11 of 66 (17%) St. Louis encephalitis virus. No samples had serologic evidence of exposure to *Brucella* spp. ($n = 37$), *Francisella tularensis* ($n = 40$), or pseudorabies virus ($n = 37$). This is the first known published report of antibodies to BTV/EHDV, CDV, and CPV in black bears.

Key words: Black bear, disease, serologic survey, *Ursus americanus floridanus*.

The Florida black bear (*Ursus americanus floridanus*) population, which is listed as threatened by the state of Florida (USA), is becoming increasingly fragmented by urban and agricultural development. Small, isolated populations may be more vulnerable to inbreeding, infectious disease, and other stressors. As predators and scavengers, bears may contact a variety of disease agents. Further, the propensity of black bears to frequent human habitations may increase exposure to domestic animals. Causes of natural mortality in black bears and the role of bears as potential sources of exposure for other wild and domestic animals is largely unknown (Binninger et al., 1980). There is limited published data concerning diseases of the

Florida black bear (Pirtle et al., 1986; Forrester, 1992). This study was conducted in order to determine the serum antibody prevalence of selected disease agents in bears from three geographic areas in Florida.

Bears were captured in three separate geographic areas of Florida (Fig. 1). Twenty-one bears consisting of seven females (F) and 14 males (M) were captured in the Apalachicola region (AR) of the Florida panhandle (29°50' to 30°20'N, 83°40' to 85°30'W) during June to August 1995. Eight (2F, 6M) bears were captured in the Osceola National Forest/Pinhook Swamp region (OR) of north peninsular Florida (30°10' to 31°10'N, 82°10' to 82°40'W) during July and August 1994. Thirty-seven (16F, 21M) bears were captured in the Wekiva River region (WR) of central Florida (28°40' to 28°50'N, 81°30' to 81°40'W) during November 1993 to June 1995.

Bears were captured with Aldrich spring-activated foot-snares (Johnson and Pelton, 1980) or culvert traps. Bears in WR were immobilized with ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) at a dosage of 11.0 mg/kg and xylazine hydrochloride (Rompun®, Mobay Corporation Animal Health Division, Shawnee, Kansas, USA) at a dosage of 0.5 to 4.0 mg/kg. Bears in OR and AR were immobilized with tiletamine hydrochloride and zolazepam hydrochloride (Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) at a dosage of 3.0 mg/kg. Age was estimated by examination of premolar cementum annuli

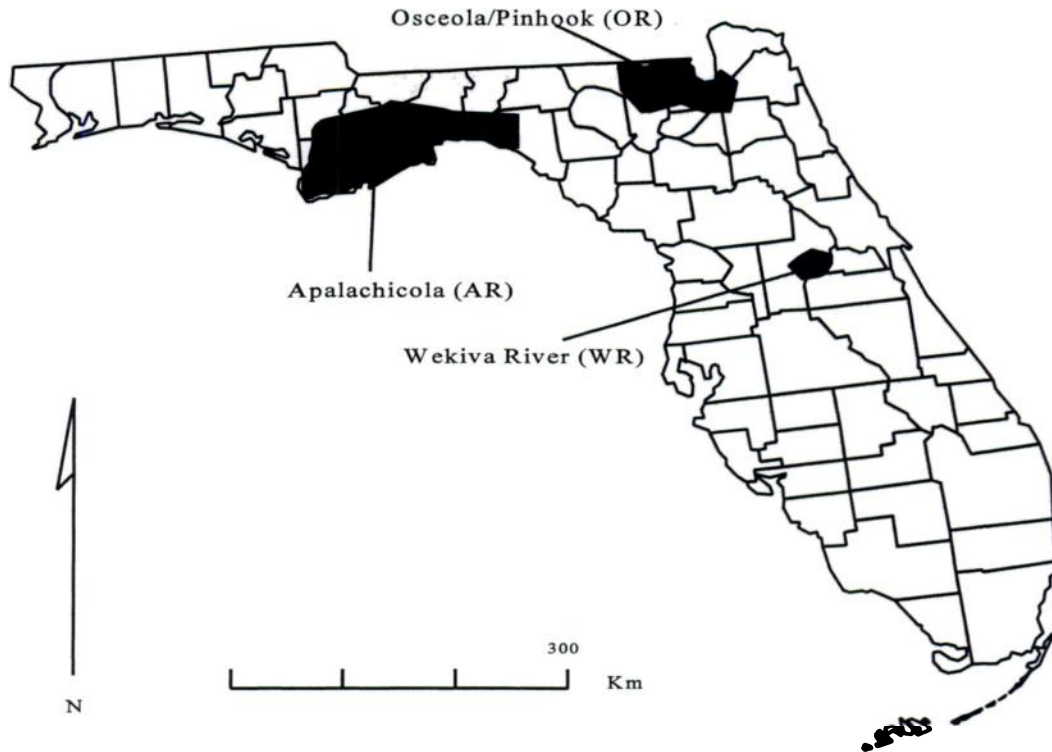


FIGURE 1. Map of Florida depicting three black bear study areas investigated during November 1993 to August 1995.

($n = 38$) (Willey, 1974), or from tooth development and wear, physical condition, and pelage characteristics ($n = 28$) (Marks and Erickson, 1966). Bears not aged by cementum annuli were classified as cubs, yearlings, juveniles, or adults. Mean age of bears aged by cementum annuli was 6.0 yr (range 1–14 yr) and those aged by other techniques included 1 cub, 2 yearlings, and 25 adults.

Blood from 25 F and 41 M black bears was collected in serum separator tubes by venipuncture of the femoral vein. Whole blood was refrigerated for 1 to 12 hr before centrifugation at approximately 2,000 rpm for 10 to 15 min. Serum was decanted and stored at -20 C until analyzed. Serum samples were tested for antibodies to *Brucella* spp., *Francisella tularensis*, *Coxiella burnetii*, *Toxoplasma gondii*, bluetongue virus/epizootic hemorrhagic disease virus (BTV/EHDV), canine adenovirus-type 1

(CAV-1), canine distemper virus (CDV), canine parvovirus (CPV), pseudorabies virus (PRV), eastern equine encephalitis virus (EEE), western equine encephalitis virus (WEE), Venezuelan equine encephalitis virus (VEE), and St. Louis equine encephalitis virus (SLE). Bears in OR were not tested for *Brucella* spp., *F. tularensis*, *C. burnetii*, and PRV. The serologic test methods, antigens used, threshold titers, and references for standard test procedures are summarized in Table 1. Sera with titers greater than or equal to threshold titers are referred to as positive. Modifications to the described tests included a 2-fold serial dilution for CAV-1 serum neutralization and kaolin adsorption to remove nonspecific inhibitors for arbovirus hemagglutination inhibition. Antibodies to bluetongue virus and EHDV could not be differentiated with the agar gel precipitation test used in this study. All statistical

TABLE 1. Antigens, threshold titers, references, and tests used to detect serum antibodies against selected disease agents in free-ranging black bears from Florida, 1993–1995.

Antigen	Test	Threshold	
		Titer	Reference
<i>Brucella</i> spp.	card ^a	Qual. ^b	U.S. Department of Agriculture (undated)
<i>Francisella tularensis</i>	slide agglutination ^{c,d}	Qual. ^b	Pezzlo (1994)
<i>Toxoplasma gondii</i>	microtiter latex agglutination ^{a,e}	≥64	Tsubota et al. (1977)
<i>Coxiella burnetii</i>	complement fixation ^c	≥20	Powell and Stallman (1982)
Bluetongue virus/epizootic hemorrhagic disease virus	agar gel precipitation ^a	Qual. ^b	Pearson and Jochim (1979)
Canine adenovirus-type I	serum neutralization ^f	≥4	Appel et al. (1973)
Canine distemper virus	serum neutralization ^f	≥8	Appel and Robson (1973)
Canine parvovirus	hemagglutination inhibition ^f	≥10	Carmichael et al. (1980)
Pseudorabies virus	latex agglutination ^{a,g}	>4	Pirtle et al. (1986)
Eastern equine encephalitis	hemagglutination inhibition ^h	>10	Beaty et al. (1989)
Western equine encephalitis	hemagglutination inhibition ^h	>10	Beaty et al. (1989)
Venezuelan equine encephalitis	hemagglutination inhibition ^h	>10	Beaty et al. (1989)
St. Louis encephalitis	hemagglutination inhibition ^h	>10	Beaty et al. (1989)

^a Performed at the University of Florida, College of Veterinary Medicine, Gainesville, Florida 32611, USA.

^b Qualitative tests, no titers determined.

^c Performed at National Veterinary Services Laboratory, 1800 Dayton Road, Ames, Iowa 50010, USA.

^d Bacto[®] Francisella Tularensis Antigen (Slide) (2240), Bacto Francisella Tularensis Antigen (Tube) (2251), Bacto Francisella Tularensis Antiserum (2241), Bacto Febrile Negative Control (3239), DIFCO Laboratories, Detroit, Michigan 48232, USA.

^e Toxotest-MT-latex agglutination microtiter, Eiken Chemical Company, LTD, Tokyo, Japan.

^f Performed at Cornell University, New York State College of Veterinary Medicine, Diagnostic Laboratory, Ithaca, New York 14852, USA.

^g Pseudorabies virus antibody test kit - latex agglutination, Viral Antigens, Inc., Memphis, Tennessee, USA.

^h Performed at Kissimmee Diagnostic Veterinary Laboratory, 2700 North Bermuda Avenue, Kissimmee, Florida 34741.

tests were conducted using Chi-square (χ^2) analysis and/or Fisher's Exact Test.

Results are presented in Table 2. No clinical signs of disease were observed in any bears. *Toxoplasma gondii* is an enteric sporozoan of felids. There have been no reports of *T. gondii* causing disease in black bears; however, deaths in 4- to 7-month old captive Kodiak bears (*U. arctos middendorffi*) were attributed to septicemic toxoplasmosis (Kiupel et al., 1987). Antibody prevalence of *T. gondii* in our study (56%) was apparently higher than reported in black bears from Idaho (8%) (Binninger et al., 1980), Alaska (15%) (Chomel et al., 1995), and California (27%) (Ruppanner et al., 1982). Conversely, prevalence was apparently lower than reported from Pennsylvania (80%) (Briscoe et al., 1993). Our results were similar to findings in Ontario, Canada (44%) (Quinn et al., 1976), and Florida, (45%) (Forrester, 1992). There was not a significant difference (χ^2

= 3.66, 2 df, $P = 0.16$) in prevalence (AR, 67%; OR, 75%; and WR, 46%) among the three geographic areas. Prevalence and titers to *T. gondii* appeared to be similar among age classes, although only two of seven (29%) yearlings were positive. Antibody prevalence was marginally higher ($\chi^2 = 3.04$, 1 df, $P = 0.81$) among males (27 of 41, 66%) than females (11 of 25, 44%). One bear sampled three times over a 1.5-yr period remained positive and one positive bear was negative at initial capture 1.5 yr earlier. Because of the potential for disease, especially in young bears, further studies are needed on the effects of *T. gondii* in black bears.

Antibodies to BTV/EHDV were detected in two adult males and one adult female from WR. No antibodies were detected in sera from bears in either AR or OR. Bluetongue virus and EHDV usually infect wild and domestic ruminants in tropical, subtropical, and some temperate regions

TABLE 2. Prevalences and reciprocal antibody titers to selected disease agents in three combined study areas of black bears from Florida, 1993 to 1995.

Agent	No.	Positive	% Positive	Reciprocal titer min-max
<i>Brucella</i> spp.	37	0	0	ND ^a
<i>Francisella tularensis</i>	40	0	0	ND
<i>Coxiella burnetii</i>	37	3	8	10–20 ^b
<i>Toxoplasma gondii</i>	66	37	56	64–1024
Bluetongue virus/epizootic hemorrhagic disease virus	61	3	5	Qual. ^b
Canine adenovirus (type 1)	66	4	6	16–48
Canine distemper virus	66	5	8	153–1288
Canine parvovirus	62	10	16	10–80
Pseudorabies virus	37	0	0	ND
Eastern equine encephalitis virus	66	7	11	20–160
Western equine encephalitis virus	66	4	6	20–80
Venezuelan equine encephalitis virus	66	2	3	20–40
St. Louis equine encephalitis virus	66	11	17	20–40

^a ND = not determined.

^b Qual. = Qualitative test, no titer determined.

of the world. However, abortion and death due to BTV serotype 11 infection were recently reported in domestic dogs following vaccination with a contaminated modified live combination canine vaccine (Evermann et al., 1994). Epizootic hemorrhagic disease has been reported in white-tailed deer (*Odocoileus virginianus*) throughout Florida (Forrester, 1992) and Stallknecht et al. (1991) reported serum antibody prevalence of 48% from the coastal plain of the southeastern United States. Ingestion of ruminants infected with BTV is believed to have caused natural infection in a variety of carnivores in Africa (Alexander et al., 1994). Except for a finding of five of 45 (11%) Florida panthers (*Felis concolor coryi*) seropositive for BTV/EHDV (M. Dunbar, unpubl. data), this is the first known published report of antibodies to BTV/EHDV in wild carnivores, including black bears, in North America. The only other report of antibodies to BTV/EHDV in carnivores in North America was in a study by Howerth et al. (1995) where 1 of 130 domestic dogs from Georgia (USA) was positive. The effect BTV/EHDV may have on bears is unknown, but warrants further investigation.

Antibody titers to CAV-1, also known as

infectious canine hepatitis virus, in our study were limited to bears from WR. All positive bears ($n = 4$) were ≥ 5 -yr old. One positive bear was negative upon recapture 1 yr later. Foreyt et al. (1986) found only one positive antibody titer in 33 black bears tested in Washington. However, Zarnke and Evans (1989) reported a CAV-1 serum antibody prevalence of 12% in adult grizzly bears (*Ursus arctos*) and 0% in grizzly bears <2 yr of age in Alaska. They hypothesize that mortality occurs in young bears exposed to the virus, and further suggest that CAV-1 may limit grizzly bear populations in Alaska. Canine adenovirus-type 1 was isolated from two captive black bear cubs in Georgia that had clinical and postmortem signs of canine infectious hepatitis (Pursell et al., 1983). An epizootic of CAV-1 infection occurred in captive black bears in South Dakota (USA) with similar results (Collins et al., 1984). In both instances, the adenovirus was believed to have been transmitted directly or indirectly from canids. The virus also infects red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), wolves (*Canis lupus*), striped skunks (*Mephitis mephitis*) (Cabasso, 1981) as well as domestic dogs. The coexistence of canids and black bears in

the wild, and the known susceptibility of captive black bears to a CAV-1 suggest that this disease may be a cause of mortality in free-ranging black bears.

Schultze et al. (1986) reported that PRV contributed to the death of a captive black bear housed near domestic swine. Zanin et al. (1997) reported the deaths of four captive European brown bears (*U. arctos*) from pseudorabies was linked to ingestion of raw pork. Florida black bears utilize feral swine as food (Maehr, 1984). Van der Leek et al. (1993) reported a 35% prevalence of antibodies to PRV in Florida's large feral swine population. Pirtle et al. (1986) found antibodies to PRV in one of 13 black bears from central Florida, however, we found no seropositive bears. Failure to detect antibodies to PRV in black bears in our study may be due to a low probability of exposure and/or to a high mortality rate following exposure.

Antibody prevalence of CDV was low (8%) in black bears in our study. Four positive bears were from WR and one from AR. The positive bears were three females, ages 5, 12, and 14 yr and one male, age 12 yr. However, this is the first report of titers to CDV found in bears of North America. Canine distemper is a common, highly infectious viral disease of domestic and wild, free-ranging and captive Canidae, Procyonidae, and Mustelidae (Budd, 1981), but has not been reported in free-ranging Ursidae in North America. Canine distemper has been reported as the cause of death in captive polar bears (*U. maritimus*) and one spectacled bear (*Tremarctos ornatus*) in Austria (Schonbauer, 1984). Antibody titers to CDV were found in both captive and free-ranging Marsican brown bears (*U. arctos marsicanus*) from Italy (Marsilio et al., 1997). Cook and Pelton (1978) found no titers using serum neutralization tests to CDV in 47 black bears in Tennessee. Forrester (1992) reported several epizootics in gray fox (*Urocyon cinereoargenteus*), and raccoons (*Procyon lotor*) in Florida due to CDV. Bears in our study may be exposed to in-

fecting wildlife such as gray fox and raccoon. The effect of CDV on black bear populations in Florida or elsewhere is unknown, but due to known susceptibility of members of the family Ursidae, effects could be significant when epizootics due to CDV are occurring in wildlife to which bears may be exposed.

The prevalence of antibodies against CPV has been reported for populations of many species in the family Canidae. However, titers to CPV were detected for the first time in the family Ursidae in European brown bears in Croatia in 1993 (Madic et al., 1993). They found 7 of 22 (32%) bears were positive with titers ranging from 20 to 1,280. Marsileo et al. (1997) also found the presence of antibodies to CPV in both free-ranging (4 of 11, 36%) and captive (5 of 12, 42%) Marsican brown bears in Italy with titers ranging from 20 to 640 using hemagglutination-inhibition tests. We found a 16% prevalence in bears in our study with titers ranging from 10 to 80. The positive bears were six males and four females. Ages of positive bears ranged from cub to 12 yr. All three study areas had bears that were positive. Although the WR area had the highest prevalence of 21%, there were no significant differences ($\chi^2 = 1.385$, 2 df, $P = 0.50$) among the three study areas. We are not aware of other published reports on serologic testing of black bears in North America for antibodies to CPV. To the best of our knowledge, this is the first published report of black bears showing serologic evidence of exposure to CPV. The significance of CPV to free-ranging black bears in Florida is unknown.

Exposure to the encephalitis viruses appeared to be related to location. There were significant differences ($\chi^2 = 10.54$, 2 df, $P = 0.005$) in prevalence of EEE in bears among the three study areas. And, AR had a higher prevalence ($P = 0.003$) than the OR and WR study areas combined. There were also significant differences ($\chi^2 = 9.12$, 2 df, $P = 0.010$) in prevalence of WEE in bears among the three

areas and AR had a higher ($P = 0.008$) prevalence than the OR and WR areas combined. However, there was no differences ($\chi^2 = 4.42$, 2 df, $P = 0.110$) in prevalence of VEE in bears among the three areas. There was a significant difference ($\chi^2 = 16.31$, 2 df, $P = 0.001$) in prevalence of SLE in bears among the three areas. Bears from OR had the highest prevalence (63%) of SLE among the three areas. The reasons for differences in prevalences of titers to encephalitis viruses among the three areas are unknown. One bear positive for EEE and SLE was negative upon recapture 1.5 yr later, and one positive for SLE was negative upon recapture 7 mo later. Binninger et al. (1980) reported low prevalence of WEE and SLE antibody titers in black bears in Idaho. The significance of these encephalitis viruses in black bear populations is unknown.

Coxiella burnetii causes Q fever in humans and is infectious to ruminants. We found only three adult (2M, 1F) bears seropositive to *C. burnetii*, all from AR (bears from OR were not tested). A low prevalence of antibodies to *C. burnetii* has been reported in black bears from Idaho (Binninger et al., 1980) and California (Ruppanner et al., 1982). Although prevalence of antibodies to *Brucella* spp. has been reported in black bears from California (Drew et al., 1992) and Idaho (Binninger et al., 1980), we found no evidence of exposure of black bears to *Brucella* spp. in Florida. Forrester (1992) also did not find antibodies to *Brucella* spp. in 34 bears tested in north central Florida.

Francisella tularensis causes tularemia and is a disease of wild lagomorphs and rodents and is also an important zoonotic disease. The disease is present in Florida (Forrester, 1992). However, we found no antibody titers to *F. tularensis*. Binninger et al. (1980) reported a 19% prevalence of *F. tularensis* in black bears in Idaho. The effects of this disease upon black bear populations are unknown.

Although interpretation of serology should be done cautiously (Gardner et al.,

1996), periodic serologic surveys in free-ranging wildlife populations may assist wildlife managers in determining prevalence of exposure to pathogens and identification of risk factors. Serologic evidence of exposure to disease agents combined with the known effects of the disease on captive or free-ranging wildlife can give clues as to the effect of infectious disease on free-ranging populations. Knowledge of exposure status of specific populations also can assist in decisions regarding translocation programs.

Although sample sizes were small, we did find significant differences in prevalence of titers to some disease agents among the three geographic areas. Bears in the WR area were exposed more to disease agents that were atypical for bears such as BTV/EHDV, CAV-1, and CDV. Bears in AR appeared to be exposed more to the encephalitis viruses than bears from the two other areas. The reasons for these observations are not known. We did find that bears in this study had evidence of exposure to a variety of infectious disease agents, including zoonotic diseases and diseases affecting other wildlife and domestic animals. And, we did find that bears in this study had exposure to disease agents including BTV/EHDV, CDV, and CPV that have not been reported before in black bears. Ruppanner et al. (1982) suggested that black bears, because of their scavenging and omnivorous food habits, may serve as sentinels for a variety of infectious diseases. This study supports that concept. The effects these disease agents may have on the population dynamics of black bears in Florida are unknown, but due to known susceptibility of bears to some of these agents, the possible effects on bear populations should be cause for concern and warrants further investigation.

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