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Prevalence of *Bartonella henselae* Antibody in Florida Panthers

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ABSTRACT: Serum samples from 28 free-ranging Florida panthers (*Puma concolor coryi*) and seven mountain lions from Texas (*P. concolor stanleyana*) living in south Florida (USA) between 1997 to 1998 were tested for antibodies to *Bartonella henselae.* Twenty percent (7/35) of the samples were reactive to *B. henselae* antisera with a subspecies prevalence of 18% (5/28) for Florida panthers and 28% (2/7) for cougars from Texas (USA). There was not a significant sex related difference in infection rates among the Florida panthers. Antibody prevalence was higher in panthers <2-yr of age (40%) compared to panthers >2-yr (13%). Compared to studies of antibody prevalence in mountain lions (*P. concolor*) from California (USA), overall seroprevalence was lower as was prevalence in panthers >2-yr-old. However, the seroprevalence in animals <2-yr from southern Florida was similar to prevalences reported in mountain lions or domestic felids in California.

Key words: Bartonellosis, *Bartonella henselae,* Florida panther, mountain lion, *Puma concolor coryi, Puma concolor stanleyana,* serology.

Bartonella henselae is a gram-negative bacterium, that causes the zoonotic disease bartonellosis or cat scratch disease. Several clinical syndromes in humans may occur following infection including lymphadenopathy, malaise, fever, and in immunocompromised individuals, bacillary angiomatosis (Breitschwerdt and Kordick, 1995). Cases in humans arise from transmission of the agent from domestic cats by either a scratch or bite (Chomel et al., 1996). Transmission between domestic cats has been associated with the cat flea (*Ctenocephalides felis*) (Chomel et al., 1996). Younger cats (<1-yr-old) are more likely to be bacteremic than older cats (Chomel et al., 1995) and other than a transient febrile state and transient lymphadnopathy, there are no long term effects known to occur (Breitshwerdt and Kordick, 1995; Greene et al., 1996).

Recent studies by Yamomoto et al. (1998), have demonstrated the presence of antibodies in a variety of free-ranging and exotic felids from California (USA). The prevalence of infections in bobcats (*Lynx rufus*), mountain lions (*Puma concolor*) and captive felids (genera *Acinonyx, Panthera* and *Felis*) was 53%, 35%, and 30%, respectively. Similarily, Jameson et al. (1995) found high prevalence rates in domestic cats from the southeast (55%) and coastal California (40%). Specifically, the seroprevalence was 35% for domestic cats from Florida (USA; Jameson et al., 1995).

The purpose of this study was to determine the presence of *B. henselae* antibodies in free-ranging panthers (*P. concolor coryi*) in southern Florida based on age, sex, or subspecies. (*P. concolor stanleyana*) from Texas mountain lions (USA) introduced for genetic restoration also were evaluated. In addition, preliminary recommendations for capture personnel who are exposed to potential bites and scratches may be made from this data.

Serum from 28 free-ranging Florida panthers (13 males; 15 females) ranging from 8-mo-old to 16-yr-old and mountain lions translocated from Texas (7 females) in 1995 ranging from 6- to 8-yr-old were collected from October to April 1997 to 1998 in southern Florida (south of 27°00′N). The animals were handled for routine radiotelemetry collar replacement, physical examination, and biomedical sample collection. The capture event involved treeing the panthers by a houndsmen (Maehr et al., 1991), and a 3 cc intramuscular dart with a 1.5 × 20 mm uncollared needle was devlivered via a CO_2 powered rifle (Telinject, Saugus, California, USA).

Many combinations of anesthetic drugs have been utilized for anesthesia including ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA), xylazine hydrochloride (Rompun®, Mobay Corporation Animal Health Division, Shawnee, Kansas, USA), and tiletamine hydrochloride/zolazepam hydrochloride (Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA). Blood was collected via a 21 gauge butterfly catheter into a 7 ml tube without anticoagulant, placed on cold packs, transferred back to the laboratory, and spun down. Approximately 1 ml of serum was sent overnite on cold packs to the laboratory for analysis (College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA). Blood was kept frozen at −70 C for a period of 3 to 5 days before the analysis procedure.

Sera were analyzed by a microimmunofluorescence assay for immunoglobulin G (IgG) reactive for *B. henselae* Houston-1. Organisms were cultivated in Vero cells and were harvested when the cells were >80% infected (3 to 5 days postinoculation). Antigen for IFA testing was prepared by pelleting and resuspending the microorganisms in 0.5% bovine serum albumin in phosphate-buffered saline (PBS). Five microliter aliquots of crude antigen were applied to 30 well Teflon-coated slides (Celline, Erie Scientific, Portsmouth, New Hampshire, USA), air dried for 30 minutes, acetone fixed for 10 min and frozen at −70 C.

Twofold dilutions of serum in a diluent (PBS with 0.5% Tween 20, 0.5% nonfat dry milk and 1% normal goat serum) ranging from 1:16 to 1:8,192 were applied to slides in 10 μl aliquots; this was followed by a 30 min incubation (37 C) and wash for 30 min with gentle agitation. Fluorescein isothiocyanate (FITC)-conjugated goat anti-cat IgG (heavy and light chains) (Cappel, ICN Biomedicals, Costa Mesa, California, USA) was diluted 1:200 in PBS and was applied to each well containing feline serum in order to detect IgG-reactive antibodies in the samples. The slides were incubated for an additional 30 min, washed in PBS with 1.65% Eriochrome black as a counterstain and examined at 40× magnification with a fluorescent microscope. A sample was considered positive if fluorescence occurred at dilutions ≥1:64 (Childs et al., 1994).

Animals were placed into two age categories, <2-yr-old or >2-yr-old, to correspond to subadult and adult age classes. Twenty eight percent of the panthers (10/35) were <2-yr-old with an even sex distribution. In the >2-yr-old age group, approximately 50% (18/35) of the animals were females. A comparison of antibody prevalence between age classes was done for panthers within Florida. This was then compared to cougars from California (USA). A Fisher's exact test ($P \leq 0.05$) and relative risk was analyzed using EpiInfo version 6.04b (Dean et al., 1994; Center for Disease Control, Atlanta, Georgia, USA).

The seroprevalence among the Florida panthers was 18% (5/28) and 28% (2/7) for mountain lions from Texas. Within the Florida panther subspecies, 40% (4/10) <2-yr-old and 6% (1/18) >2-yr-old. In the >2-yr-old category, the lone seropositive panther was female and in the <2-yr-old, three of the positive panthers were male and one was female. Reciprocal titers were either 64 or 128 with one panther from each age category having a reciprocal titer of 128.

The results of this study indicate a lower, but not significant, prevalence of *B. henselae* infection in Florida panthers (20%) when compared to mountain lions (35%) in California or to domestic cats (28%) in the southeastern USA (Childs et al., 1995; Yamamoto et al., 1998). Unlike the Yamamoto et al. (1998) study where males were more likely to be seropositive than females, this was not observed (relative risk [RR] = 1.44, 95% confidence interval [CI] = 0.51, 4.06). Panthers <2-yr-old had a higher antibody prevalence which is similar to the findings of the Cal-

ifornia and domestic cat studies (Chomel et al., 1995; Yamamoto et al., 1998). Infection status was significantly lower ($P \leq 0.05$) for free-ranging Florida panthers >2-yr-old than for mountain lions (13% versus 39%) in California.

Seroprevalence was higher for mountain lions from Texas with 28% (2/7) having detectable antibodies. However, the prevalence of *B. henselae* infection was not known prior to their arrival; thus, these animals may have been infected in Texas.

This study provides serologic evidence that Florida panthers and translocated cougars from Texas can be infected with *Bartonella* sp. or a cross-reacting agent. The role of wild cats as a potential reservoir for *B. henselae* awaits further studies including speciation determined by blood culture isolation or molecular detection by PCR. Speciation is important, because several *Bartonella* sp. have been identified in wild rodents (Kosoy et al., 1997). Detrimental effects of bartonellosis have not been observed in seropositive panthers. *Bartonella henselae* may contribute to chronic disease manifestations in domestic cats with multiorgan infiltration by lymphocytes and plasma cells (Kordick et al., 1999). Evaluation of tissues of seroreactive panthers submitted for necropsy may provide evidence of the potential clinical importance.

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