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Prevalence of Selected Vector-borne Organisms and Identification of *Bartonella* Species DNA in North American River Otters (*Lontra canadensis*)

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ABSTRACT: Trapper-killed North American river otters (*Lontra canadensis*) in North Carolina, USA, were screened for multiple vector-borne bacteria known to be pathogenic to mammals. Blood was collected from 30 carcasses in 2006, from 35 in 2007, and from one live otter in 2008. Samples were screened using conventional polymerase chain reaction (PCR) tests for DNA from *Bartonella* spp., *Ehrlichia* spp., and spotted fever group *Rickettsia* spp. All samples were negative for *Rickettsia* spp. Twelve of 30 samples from 2006 produced amplicons using the assay designed to detect *Ehrlichia* spp., but sequencing revealed that the amplified DNA fragment was from a novel *Wolbachia* sp., thought to be an endosymbiote of a *Dirofilaria* sp. Between 2006 and 2007, DNA from a novel *Bartonella* sp. was detected in 19 of 65 animals (29%). Blood from one live otter captured in 2008 was found positive for this *Bartonella* sp. by both PCR and culture. The pathogenicity of this *Bartonella* species in river otters or other mammals is unknown.

Key words: *Bartonella*, disease, *Ehrlichia*, *Lontra canadensis*, *Rickettsia*, river otter.

North American river otters (*Lontra canadensis*) have been successfully translocated from eastern North Carolina, USA, to reestablish breeding populations in the western portion of the state (Spelman et al., 1997). Intensive health assessment of the animals used for translocation did not identify tick infestation (Tocidlowski et al., 2000). However, in a separate study, several tick species, including *Ixodes banksi*, *I. cookei*, *Amblyomma americanum*, and *Dermacentor variabilis*, were rarely documented feeding on river otters in the eastern USA (Kimber and Kollias, 2000). *Amblyomma americanum* and *D. variabilis* are integral in the transmission of *Ehrlichia chaffeensis* and *Ehrlichia ewingii*, and of *Rickettsia rickettsii*, respectively, to dogs (*Canis familiaris*) and humans (Cohn, 2003). In both previous river otter studies, no evidence of vector-borne diseases was found on blood smears; however, more sensitive molecular-based diagnostics were not used. In a previous study from our laboratory, a novel *Babesia* sp. was identified in river otters from North Carolina (Birkenheuer et al., 2007). The presence of at least one vector-borne organism in river otters led us to speculate that they could be susceptible to other vector-borne infections. Otters in eastern North Carolina share habitat with numerous wild and domestic carnivores that are susceptible to a spectrum of vector-borne pathogens and the potential for disease transmission between river otters and other species exists. This study was performed to screen a convenience population of otters for selected vector-borne organisms.

Otters from six counties in eastern North Carolina were legally trapped for fur collection during January and February of 2006 and 2007. The carcasses were stored frozen by a licensed fur dealer. Carcasses were thawed at room temperature and blood was collected by cardiac puncture. Thirty animals were sampled in 2006 and 35 were sampled in 2007. One additional live-caught otter was sampled by jugular venipuncture during a training exercise conducted by the North Carolina Wildlife Resources Commission. The animal was released after collection of blood.

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All blood samples were collected into tubes containing EDTA and frozen at −80 C until being processed. Blood samples were initially processed by mixing 100 μl of whole blood with 100 μl of phosphate-buffered saline. DNA was extracted from all samples for polymerase chain reaction (PCR)-based testing using a QIAmp tissue mini kit (Qiagen, Valencia, California, USA). DNA concentration and quality were determined using an absorbance ratio of 260:280 nm. A 1-ml aliquot of whole blood from the single antemortem sample was added to 8 ml of liquid *Bartonella* alpha proteobacteria growth medium (BAPGM) for culture. The BAPGM liquid enrichment culture was subcultured onto blood agar plates (Fisher Scientific, Pittsburgh, Pennsylvania, USA). DNA was extracted from an enrichment liquid BAPGM culture following inoculation and incubation of the single antemortem sample.

Samples were screened for *Ehrlichia* spp., *Bartonella* spp., and *Rickettsia* spp. using conventional PCR of DNA extracted directly from blood. The DNA extracted from the enrichment liquid BAPGM culture following inoculation and incubation of the single antemortem sample was only tested for *Bartonella* spp. by PCR. Detection of *Ehrlichia* DNA was performed using the GEPs forward and GEPr reverse primers (Billeter et al., 2007) and all suspected positive samples were rerun with truncated GEPs and GEPr primers (Table 1). Screening for the *Bartonella* 16S–23S internal transcribed spacer region using the 325s forward and 1100 reverse primers (Table 1) was performed using the same thermocycling conditions as the *Ehrlichia* PCR. Screening for *Rickettsia* spp. was performed using the ompA 107 forward and ompA 299 reverse primers (Table 1; Kidd 2006). Amplicons from positive samples were sequenced directly (MWG Biotech, Inc., Huntsville, Alabama, USA) and compared to known sequences using a GenBank® BLAST homology search (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov).

Twelve samples collected in 2006 produced amplicons using the assay designed to screen for the presence of *Ehrlichia* spp. DNA. A BLAST search revealed that the amplified sequence shared 99% homology with a species of *Wolbachia*, thought to be an endosymbiote of *Dirofilaria immitis*. All positive samples were rerun with truncated primers designed to amplify *E. canis*, *E. chaffeensis*, and *E. ewingii* and not *Wolbachia* spp. and no *Ehrlichia* spp. DNA was detected. Samples collected in 2007 were screened using the truncated primers and one sample was positive for *Ehrlichia* spp. DNA, which was determined by sequence analysis to be most similar to *Ehrlichia ewingii*. From the 2006 samples, 13/30 animals (43%) were PCR-positive for *Bartonella* spp. DNA and in 2007 6/35 (17%) were positive. The antemortem sample collected in 2008 was positive for *Bartonella* spp. by conventional PCR and by real-time PCR after culture in BAPGM, but *Bartonella* was not isolated following subculture on an agar plate. The DNA sequences obtained from positive samples from 2006, 2007, and 2008 were identical and had the highest degree of sequence identity (338/450 bp or 86%) with *Bartonella volans* (GenBank accession number EU294521). There was also 92% homology (423/459) with a *Bartonella volans*–like sequence detected in heart samples obtained from sea otters that died of vegetative valvular endocarditis in south-central Alaska (Chomel et al., 2009). All samples screened for *Rickettsia* from 2006 and 2007 were PCR negative.

The detection of a *Wolbachia* species thought to be an endosymbiote of *Dirofilaria immitis* from the blood of these otters is suggestive of *Dirofilaria* spp. infection. Both *D. immitis* and *D. lutrae* have been identified in the heart (Snyder et al., 1989) and subcutaneous tissues (Orihel, 1965), respectively, of river otters. Complete postmortem examinations, cy-

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TABLE 1. Genes used as targets for identification of *Rickettisa*, *Bartonella*, and *Ehrlichia* DNA in North American river otters (*Lontra canadensis*) from North Carolina, USA, 2006–2008.

Organism and gene	PCR primer
Rickettsia ompA 107	GCT TTA TTCACC ACC TCA AC
Rickettsia ompA 299	TRA TCA CCA CCG TAA GTA AAT
Bartonella ITS 325s	CCT CAG ATG ATG ATC CCA AGC CTT TT
Bartonella ITS 1100	GAA CCG ACG ACC CCC TGC TTG CAA
Ehrlichia GEPs	CTG GCG GCA AGC YTA ACA CAT GCA AGT CGA ACG GA
Ehrlichia GEPr	CTT CTR TRG GTA CCG TCA TTA TCT TCC CYA YTG
Ehrlichia GEPs truncated	GCA AGC YTA ACA CAT GCA AGT CGA
Ehrlichia GEPr truncated	TAT AGG TAC CGT CAT TAT CTT CCC TAT TG

tologic examinations of blood smears, or Knott's tests were not performed, so other evidence of *Dirofilaria* spp. infections was not specifically investigated or detected.

Bartonella spp. are gram-negative, aerobic intracellular bacteria known to infect erythrocytes in wild and domestic mammals, as well as humans. An increasing number of *Bartonella* species have been determined to be pathogenic to humans and domestic animals, including *B. henselae* and *B. quintana* (Billeter et al., 2008). Recently *Bartonella* species have been identified in porpoises (*Phocoena phocoena*; Maggi et al., 2005) and loggerhead sea turtles (*Caretta caretta*; Valentine et al., 2007), suggesting that the host range of the organism is much broader than previously suspected and that infection of animals in an aquatic environment is possible. It is unknown whether *Bartonella* spp. isolated from aquatic animals pose a zoonotic risk. The *Bartonella* sp. DNA sequences identified from the otters in this study were identical among all positive samples. The pathogenicity of this *Bartonella* sp. in the North American river otter is unknown, as is the transmissibility to domestic animals, wildlife, and humans. Transmission of *Bartonella* spp. between animals is typically carried out by insect vectors, including fleas, sandflies, lice, and potentially biting flies and ticks (Billeter et al., 2008). While tick infestation in otters has only been detected rarely, the apparent prevalence of three vector-borne organisms, *Babesia*, *E. ewingii*, and *Bartonella*, suggests that the potential for vector-associated transmission of disease is greater than previously appreciated.

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