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Does Human Proximity Affect Antibody Prevalence in Marine-Foraging River Otters (*Lontra canadensis*)?

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ABSTRACT: The investigation of diseases of free-ranging river otters (*Lontra canadensis*) is a primary conservation priority for this species; however, very little is known about diseases of river otters that forage in marine environments. To identify and better understand pathogens that could be important to marine-foraging river otters, other wildlife species, domestic animals, and humans and to determine if proximity to human population could be a factor in disease exposure, serum samples from 55 free-ranging marine-foraging river otters were tested for antibodies to selected pathogens. Thirty-five animals were captured in Prince William Sound, Alaska (USA), an area of low human density, and 20 were captured in the San Juan Islands, Washington State (USA), an area characterized by higher human density. Of 40 river otters tested by indirect immunofluorescent antibody test, 17.5% were seropositive (titer ≥320) for *Toxoplasma gondii*. All positive animals came from Washington. Of 35 river otters tested for antibodies to *Leptospira interrogans* using the microscopic agglutination test, 10 of 20 (50%) from Washington were seropositive (titer ≥200). None of the 15 tested animals from Alaska were positive. Antibodies to *Neospora caninum* ($n=40$), *Sarcocystis neurona* ($n=40$), *Brucella abortus* ($n=55$), avian influenza ($n=40$), canine distemper virus ($n=55$), phocine distemper virus ($n=55$), dolphin morbillivirus ($n=55$), porpoise morbillivirus ($n=55$), and Aleutian disease parvovirus ($n=46$) were not detected. Identifying exposure to *T. gondii* and *L. interrogans* in otters from Washington State but not in otters from Alaska suggests that living proximal to higher human density and its associated agricultural activities, domestic animals, and rodent populations could enhance river otter exposure to these pathogens.

Key words: Disease, human population, *Leptospirosa*, *Lontra canadensis*, marine, river otter, *Toxoplasma gondii*.

The river otter (*Lontra canadensis*) is a semiaquatic mammal that ranges over much of North America. In Washington (WA; Jones, 2000), British Columbia (Stenson et al., 1984), and Alaska (AK; Bowyer et al., 1994, 2003), river otters inhabit coastal marine waters where they feed on a variety of marine fish and invertebrates and play an important role in the transfer of nitrogen and phosphorus from marine to terrestrial ecosystems (Ben-David et al., 1998, 2005). The World Conservation Union Species Survival Commission identified the investigation of diseases of free-ranging river otters as a primary conservation priority for this species (Foster-Turley et al., 1990). Diseases of river otters that live in inland freshwater systems have been reviewed by Kimber and Kollias (2000), but information on diseases of river otters that inhabit marine ecosystems is not available. Marine-foraging river otters hunt in marine waters to depths of at least 18 m (Scheffer, 1953) and move great distances over land, exposing them to both marine and terrestrial pathogens. Furthermore, human population expansion is most pronounced in coastal areas (Small and Cohen, 2004) so marine-foraging river otters could be at a higher risk for exposure to domestic animal diseases than are conspecifics in freshwater systems. We surveyed marine-foraging river otters in WA and AK for exposure to a suite of pathogens. Our goal was to better understand marine-foraging river otter exposure to pathogens that could be important to otters, other wildlife species, domestic animals, and humans and to determine if proximity to human population could be a factor in disease exposure.

Forty river otters were live-captured

and sampled in Prince William Sound, AK, USA (60°30′N, 147°40′W; $n=20$), an area with little human activity (0.8 people per square kilometer and a 2.8% population growth between 1990 and 2000; US Census Bureau, 2000), and in the San Juan Islands of WA, USA (48°35′N, 122°53′W; $n=20$), an area characterized by recent human development and higher human density (208.2 people per square kilometer in 2000 and a 40.3% population growth between 1990 and 2000; US Census Bureau, 2000). Animals were trapped along marine shorelines of five island and mainland sites in an area of 4,800 km^2 between June 1996 and May 1998 in AK, and at four island sites in an area of 1,609 km^2 between August 2002 and August 2003 in WA. As previously described by Blundell et al. (1999) and Bowyer et al. (2003), animals were trapped using #11 double-jaw leg-hold traps (Sleepy Creek®, Berkeley Springs, West Virginia, USA) or box traps (Tomahawk®, Tomahawk, Wisconsin, USA) and anesthetized using tiletamine-zolazepam (Telazol, Fort Dodge Laboratories, Fort Dodge, Iowa, USA) or a combination of medetomidine (Pfizer Animal Health, LaJolla, California, USA) and ketamine (Fort Dodge Laboratories) with or without atropine (Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, USA). Anesthetized river otters were weighed, measured, sexed, and given a complete physical examination; age was estimated using animal size, weight, and tooth wear (Blundell et al., 2002a, b). Otters were classified as juveniles (<1 yr), young adults (1–4 yr) or adults (>4 yr), as described by Baitchman and Kollias (2000). Such classification is highly correlated with age as determined by cementum annuli (Ben-David et al., 2001). Whole blood was collected via jugular venipuncture and allowed to clot; serum was separated from cells by centrifugation and stored at −40 C until tested. Otters were tagged and, after they recovered from anesthesia, released at the site of capture. Using similar trapping and sampling techniques, an additional 15 river otters were trapped in Price William Sound in May 1998 and taken into captivity as part of a companion study (Ben-David et al., 2000). Serum was collected from all animals at the time of capture.

Serum was tested for the presence of antibodies to *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis neurona* with indirect immunofluorescent antibody tests (IFAT) as described (Miller et al., 2002b) at the University of California, Davis, School of Veterinary Medicine (Davis, California, USA). The IFAT for *T. gondii* had not been validated in river otters so we used a cutoff titer of 320, which was established by Miller et al. (2002b) for sea otters (*Enhydra lutris*); a closely related mustelid.

Serum was tested for antibodies to *Leptospira interrogans* at the California Animal Health and Food Safety Laboratory (Davis, California, USA) by microscopic agglutination test (MAT; Colagross-Schouten et al., 2002). Specific antisera were used to test samples for exposure to six *L. interrogans* serovars (bratislava, canicola, gryppotyphosa, hardjo, icterohemorrhagiae, and pomona). The MAT had not been validated for river otters; however, titers were considered to be positive if ≥200, as recommended by the laboratory performing the tests.

For the 40 river otters live-trapped in WA and AK, serum samples were tested for antibodies to *Brucella abortus* at the Washington Department of Agriculture (Olympia, Washington, USA) using the *Brucella* buffered plate agglutination test antigen (BAPA), the brucellosis card test using buffered *Brucella* antigen (BBA), the Rivanol precipitation test, and the complement fixation (CF) test using official protocols (Office International Des Epizooties, 1996). Official guidelines are not available for interpreting brucellosis serology test results from river otters. Therefore we applied standards used for

interpreting marine mammal sera tested for *Brucella abortus* antibodies (Garner et al., 1997). Only samples that were positive for antibodies to *B. abortus* on either the BAPA or BBA tests were then tested using the CF test and the Rivanol precipitation test. Samples that were positive on either or both the CF or Rivanol tests were considered positive for antibodies to *Brucella* spp.

The avian influenza agar gel immunodiffusion (AGID) test as described by Beard (1970) was used to test samples for group-specific antibodies to type A influenza at the Animal Health Monitoring Laboratory (Abbotsford Agriculture Centre, Abbotsford, British Columbia, Canada). Samples were tested at the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma, USA) for antibodies to canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV) using a differential serum neutralization assay as previously described by Garner et al. (2000). Serum samples from 31 of the 40 river otters were tested for antibodies to Aleutian disease parvovirus (ADP) at United Vaccines (Madison, Wisconsin, USA) using counterimmunoelectrophoresis (Kenyon et al., 1978). Adequate serum was not available for the evaluating ADP exposure in nine samples.

Sera from the additional 15 river otters trapped in AK in May 1998 were tested at the Oklahoma Animal Disease Diagnostic Laboratory for antibodies to *L. interrogans* serovars (bratislava, canicola, gryppotyphosa, hardjo, icterohemorrhagiae, and pomona), *B. abortus*, and morbilliviruses (CDV, PDV, DMV, and PMV). Sera from these animals also were tested for antibodies to ADP at United Vaccines.

To explore whether animals from the two areas differed morphologically, lengths and weights (key components of aging) were compared by sex with multivariate analysis of variance (MANOVA; Zar, 1998). The distribution of ages among the samples in the two locations was compared using a *t*-test (Zar, 1998). In these analyses, all 55 animals were included. The χ^2 goodness-of-fit test (Zar, 1998) was used to compare the prevalence of disease exposure in otters in WA and AK. Differences in antibody prevalence between sexes was evaluated because male and female river otters that inhabit marine environments exhibit differences in sociality and movements (Blundell et al., 2002a, b), which could result in different probabilities of pathogen exposure. The effects of age also were evaluated because younger animals have less time to be exposed to pathogens.

Of the 40 river otters trapped and released, 10 females and 10 males were trapped in WA and 8 females and 12 males were trapped in AK. All animals, including the 15 males trapped for the companion study, appeared to be healthy on physical examination. Animals from AK were larger than those captured in WA (AK males, length=128.9±1.3 cm (mean± SE), weight=9.98±0.22 kg; AK females, length=126.3±2.2 cm, weight=7.94±0.28 kg; WA males, length=113.5±5.6 cm, weight=8.34±0.85 kg; WA females, length=107.3±4.4 cm, weight=7.09±0.68 kg; MANOVA, overall $P<0.001$), and males were significantly larger than females in both study areas, although males from WA and females from AK overlapped in size (MANOVA, sex effect length $P=0.184$, weight $P=0.002$). There was no difference in age composition of animals captured in AK ($n=35$; average estimated age=2.0± 0.12 yr) and those captured in WA ($n=20$; 2.2±0.13 yr; $P=0.283$).

Fifteen of 40 otters tested had *T. gondii* IFAT titers ≥40. Observed titers were 40 ($n=2$), 80 ($n=4$), 160 ($n=2$), 320 ($n=2$), 640 ($n=3$), 1280 ($n=1$), and 10,240 ($n=1$). Using the cutoff titer of 320 for sea otters, 17.5% (7/40) of the river otters were seropositive for *T. gondii*. All seven of these animals came from WA ($\chi^2=10.8$, $P=0.001$). Among the WA animals, age

(χ^2=2.49, P=0.29) and sex (χ^2=0.49, P=0.85) were not related to exposure.

Using the cutoff titer of 200, 10 of 20 river otters from WA were seropositive to *L. interrogans*. No animals from AK were positive. In four animals, titers to more than one serovar were present. Assuming the predominate serovar was the one with the highest titer, one animal was exposed to the gryppotyphosa serovar (3,200) and eight were exposed to the pomona serovar. Observed titers of the pomona serovar were 400 (n=3), 800 (n=2), and 1,600 (n=3). The 10th animal had a titer of 200 to the bratislava and pomona serovars. Of the 35 river otters sampled from AK, serum contamination with an unknown crystalline substance interfered with reading the MAT assay for the original 20 otters trapped. Antibodies to the six *L. interrogans* serovars were not detected in the additional 15 animals sampled from AK (χ^2=14.9, P=0.001). Among the WA animals, sex (χ^2=0.59, P=0.89) was not related to *Leptospira* exposure, but younger animals had a lower probability of being exposed (χ^2=8.2, P=0.015; Fig. 1).

Using the tests described, antibodies to *N. caninum*, *S. neurona*, or *B. abortus* were not detected in any of the river otters tested. Similarly, we did not detect antibodies to avian influenza, morbilliviruses (CDV, PDV, DMV, PMV), or ADP.

These data suggest that proximity to higher human density and its associated agricultural activities, domestic animals, and rodent populations could be a factor influencing marine-foraging river otter exposure to *T. gondii* and *L. interrogans*. Despite their spatial separation, the nearshore marine ecosystems of Prince William Sound and the San Juan Islands are very similar, as are the biology and natural history of marine-foraging river otters in both locations (Bowyer et al., 1994, 2003; Jones, 2000). These regions do differ significantly, however, in their human density, recent rates of human population expansion, and human-associated activities, and it is noteworthy that domestic

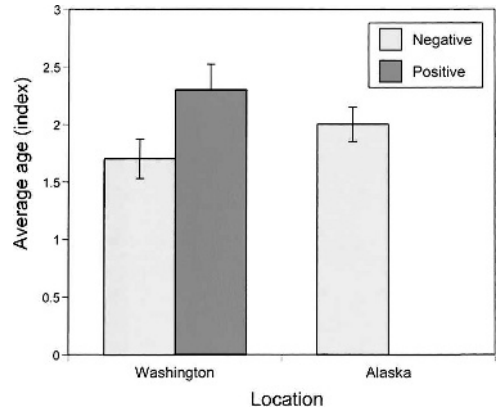

FIGURE 1. Average age (±SE) of otters exhibiting antibodies to *Leptospira interrogans* in Washington as compared with averaged ages of nonexposed animals in Washington and Alaska.

felids (Tenter et al., 2000) and domestic animals and human-associated rodents (Faine, 1994) have been associated with the transmission of *T. gondii* and *L. interrogans*, respectively.

Two issues, however, should be considered. First, our sampling effort included only one area of high and one of low human density (Hurlbert, 1984). Nonetheless, the large spatial extent of our sampling in the two areas suggests that our data are representative. Future work should evaluate exposure in river otters to these pathogens in additional locations. Second, based on morphometric data (Wozencraft, 1993) and DNA analyses (Koepfli and Wayne, 1998), river otters tested in AK and WA represent two distinct subspecies (*Lontra canadensis* subsp. *kodiacensis* and *Lontra canadensis* subsp. *pacifica*, respectively). It has been suggested that for some animals innate resistance to diseases can vary between subspecies (Gaydos et al., 2002). Nonetheless, it is unlikely that differences in subspecies would account for significant differences in exposure as indicated by antibody prevalence.

Toxoplasma gondii is a major mortality factor and contributor to the slow rate of population recovery in southern sea otters (Conrad et al., 2005) and could be a cause

of morbidity or mortality in closely related river otters that forage in the marine environment. River otters are susceptible to infection as demonstrated in a serologic survey where 47% of adult and 39% of juvenile freshwater-foraging river otters from North Carolina had antibodies to *T. gondii* (Tocidlowski et al., 1997). We found similar levels of exposure (35% prevalence) in marine-foraging river otters from WA.

Marine-foraging river otters in WA and AK are primarily piscivorous but also prey upon marine invertebrates such as crustaceans and mollusks (Bowyer et al., 1994; Jones, 2000), none of which are known *T. gondii* intermediate hosts. Rarely, marine-foraging river otters have been observed eating marine birds (Speich and Pitman, 1984), which could be intermediate hosts of *T. gondii*. Because of their dietary preference for marine fish and invertebrates, we hypothesize that most marine-foraging river otters are being exposed to *T. gondii* via oocysts that are shed in the feces of felids and transported via freshwater runoff into freshwater ponds and streams and the marine ecosystem, as has been purported for the route of transmission to sea otters (Miller et al., 2002a). In the San Juan Islands, domestic felids would be the most likely source of *T. gondii* oocysts because wild felids do not occur in the archipelago. Arkush et al. (2003) demonstrated that mussels can serve as paratenic hosts for *T. gondii* by concentrating infective oocysts; otter exposure to *T. gondii* oocysts could come through ingestion of infected bivalves, such as mussels (*Mytilus* spp.), which river otters are known to eat in WA and AK. Alternately, infection could come through direct ingestion of contaminated freshwater runoff into marine waters. Unlike true marine mammals, marine-foraging river otters are obligate freshwater drinkers (Bowyer et al., 2003). Near where otters were trapped in WA, one of the authors (J.K.G.) witnessed marine-foraging river otters drinking freshwater directly from storm culverts that drain into marine waters.

It is interesting to note that as reported here, a study comparing *T. gondii* exposure in sea otters from areas of high and low human density, Southern California and AK, respectively (Hanni et al., 2003), found high seroprevalence in southern sea otters but no exposure in Alaskan sea otters. If proximity to high human density is a factor influencing coastal river otter exposure to *T. gondii*, it is likely related to the increased presence of feral and domestic cats associated with increasing human populations.

River otters in WA are exposed to *Leptospira* species, most likely *L. interrogans*, serovars pomona and gryppotyphosa. Otters could be maintenance hosts or accidental hosts exposed to this bacterium from terrestrial or marine hosts and more work is needed to understand the epidemiology of leptospirosis in marine-foraging river otters. River otter exposure to this bacterium in the San Juan Islands could have come from marine or terrestrial wild animals such as sea lions (*Zalophus californianus*; Gulland et al., 1996) or raccoons (*Procyon lotor*; Mitchell et al., 1999), domestic animals such as cattle or dogs (few pigs are present in the San Juan Islands), or increased rodent populations associated with human habitation. Similar to the work reported here on river otters, Hanni et al. (2003) found low seroprevalence to *L. interrogans* in sea otters from California but no exposure in Alaskan sea otters. It is interesting that age, but not sex, influenced exposure to *L. interrogans* in WA otters. We expected that males would have a higher likelihood for disease exposure because in coastal environments they tend to forage in large social groups whereas females are usually solitary (Blundell et al., 2002a).

The fact that antibodies to *N. caninum*, *S. neurona*, marine *Brucella* spp., influenza A viruses, ACP, CDV, PDV, DMV, and PMV were not detected in river otters could be because of limitations in sample

size relative to disease prevalence, inability of the tests used to detect antibodies to these pathogens in river otters, or true lack of exposure to these pathogens in the populations sampled.

This study raises the question about the potential effects of human density and population growth on the prevalence of *T. gondii and L. interrogans* exposure in marine-foraging river otters. Clearly more work is needed to elucidate the causal connections between exposure of wildlife to diseases and proximity to human development. To determine which diseases impact marine-foraging river otter populations, and to better understand the role of river otters in transmitting pathogens to other species, efforts should be made to increase serological surveys, obtain fresh otter carcasses for complete postmortem necropsies, and monitor disease prevalence in sympatric terrestrial and marine carnivores and otter prey species. These combined efforts have the potential to better elucidate the impact of pathogens for which we identified antibodies in river otters, identify those diseases that cause mortality, and uncover novel pathogens and transmission modes as well as highlight the potential effects of human density on disease dynamics in this species.

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