Bartonella spp. Exposure in Northern and Southern Sea Otters in Alaska and California

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Abstract

Since 2002, an increased number of northern sea otters (Enhydra lutris kenyoni) from southcentral Alaska have been reported to be dying due to endocarditis and/or septicemia with infection by Streptococcus infantarius subsp. coli. Bartonella spp. DNA was also detected in northern sea otters as part of mortality investigations during this unusual mortality event (UME) in Kachemak Bay, Alaska. To evaluate the extent of exposure to Bartonella spp. in sea otters, sera collected from necropsied and live-captured northern sea otters, as well as necropsied southern sea otters (Enhydra lutris nereis) unaffected by the UME, were analyzed using an immunofluorescent antibody assay. Antibodies against Bartonella spp. were detected in sera from 50% of necropsied and 34% of presumed healthy, live-captured northern sea otters and in 16% of necropsied southern sea otters. The majority of sea otters with reactive sera were seropositive for B. washoensis, with antibody titers ranging from 1:64 to 1:256. Bartonella spp. antibodies were especially common in adult northern sea otters, both free-living (49%) and necropsied (62%). Adult stranded northern sea otters that died from infectious causes, such as opportunistic bacterial infections, were 27 times more likely to be Bartonella seropositive than adult stranded northern sea otters that died from noninfectious causes ($p<0.001$; 95% confidence interval 2.62–269.4). Because Bartonella spp. antibodies were detected in necropsied northern sea otters from southcentral (44%) and southwestern (86%) stocks of Alaska, as well as in necropsied southern sea otters (16%) in southcentral California, we concluded that Bartonella spp. exposure is widely distributed among sea otter populations in the Eastern Pacific, providing context for investigating future disease outbreaks and monitoring of Bartonella infections for sea otter management and conservation.

Key Words: Northern sea otter—Southern sea otter—Bartonella spp.—Antibodies—Bartonella washoensis—Alaska.

Introduction

Northern and southern sea otters (Enhydra lutris kenyoni and E. l. nereis) are keystone predators that inhabit the near-shore coastal waters of Alaska and California, respectively (Riedman and Estes 1990). Because of a decline in the southwestern stock of northern sea otters in Alaska and slow population recovery of southern sea otters in California, these populations are currently listed as “threatened” under the United States Endangered Species Act (US Fish and Wildlife Department 2008; US Geological Survey 2010). Causes of the decline and slow recovery are multifactorial and mediated through complex interactions among sea otter populations and numerous biotic and environmental factors, including infectious diseases (Kreuder et al. 2003, Goldstein et al. 2009, Miller et al. 2010b, Counihan-Edgar et al. 2012). Epidemiological studies have demonstrated that southern sea otters are at high risk for exposure to gastrointestinal protozoal pathogens and opportunistic bacteria in the coastal waters of central California (Miller et al. 2008, 2010b).
Miller et al. 2010a). Recent data also suggest that northern sea otters in Alaska have suffered increased exposure to and infection with pathogenic microorganisms that could make them more susceptible to predation and disease-associated mortality (Goldstein et al. 2009, Goldstein et al. 2011, Brownstein et al. 2011).

Because a large number of northern sea otters were found dead in southcentral Alaska beginning in 2006, the US Working Group on Marine Mammal Unusual Mortality Events declared an unusual mortality event (UME; Gill 2006). Since that time, diagnostic efforts have been directed toward understanding the causes of the sea otter UME. *Streptococcus infantarius* subsp. *coli* (a member of *Streptococcus bovis*/equinus complex, SB/E) infection was identified to be highly associated with the mortality in Kachemak Bay and surrounding areas. This bacterium was isolated from heart valves of northern sea otters with vegetative valvular endocarditis (VVE), with or without septicemia (Gill 2006). Recently, we reported that up to 45% of examined northern sea otters had detectable *Bartonella* spp. DNA, and 33% of the heart valves from animals that died with VVE were co-infected with *Bartonella* spp. and *S. infantarius* subsp. *coli* (Carrasco et al. 2014).

*Bartonella* spp. are fastidious Gram-negative bacteria that infect erythrocytes and vascular endothelial cells of hosts and are usually transmitted by blood-sucking arthropods (Chomel et al. 2009). This facultative intracellular bacterium causes persistent asymptomatic bacteremia and has been associated with debilitating and possibly life-threatening illnesses, including encephalopathies, bacillary angiomatosis, myocarditis, and valvular endocarditis in domestic animals and humans (Breitschwerdt et al. 2010). Clinical manifestations vary on the basis of the immune status of the host, the infecting *Bartonella* strain, and the co-evolutionary history of a *Bartonella* spp. with its animal host (Chomel et al. 2009). In their mammalian reservoir hosts, *Bartonella* spp. often cause asymptomatic chronic bacteremia, whereas in the nonreservoir hosts this pathogen is typically associated with multiple disease processes (Chomel et al. 2009). *Bartonella* spp. have been increasingly isolated or detected in blood or lesions from live-captured or moribund marine animals (Maggi et al. 2008, Morick et al. 2009); however, little is known about the epidemiological significance, as hosts and transmission dynamics of this organism in the marine environment are relatively unstudied.

Because *Bartonella* DNA was recently detected in northern and southern sea otter carcasses from Alaska and California (Carrasco et al. 2014), our main objective was to assess exposure in northern and southern sea otters to this usually vector-borne pathogen. The goals of this study were two-fold: (1) To determine the frequency and distribution of serum antibodies against selected *Bartonella* species in northern and southern sea otters and (2) to evaluate risk factors associated with *Bartonella* exposure in both populations. Our findings provided baseline serological data and insight into the epidemiology of bartonellosis in sea otters to contribute to conservation efforts.

**Materials and Methods**

**Sampling**

Serum samples were collected from 44 apparently healthy, live-captured northern sea otters sampled in Kachemak Bay, Alaska, in 2007 by the United States Fish and Wildlife Service (USFWS), Anchorage, Alaska. Blood was collected from anesthetized sea otters using well-established protocols for immobilization, sampling, and release (Doroff and Badajos 2010). Samples were also obtained from 48 northern sea otters that stranded and were necropsied between 2004 and 2009. Postmortem examinations of northern sea otters were performed at the Anchorage USFWS office by a veterinary pathologist or by veterinarians with marine mammal necropsy experience.

At necropsy, northern sea otters were categorized into either the infectious or noninfectious group on the basis of the diagnosed cause of death, such as opportunistic bacterial infections. Thirty-three individuals were categorized into an “infectious” group, whereas those animals dying due to trauma and/or other causes and with no evidence of concurrent infectious disease were included in a “noninfectious” group (n = 15). Bacterial pathogens isolated or detected in lesions of necropsied northern sea otters included *S. infantarius* subsp. *coli* or SB/E organisms, *Bartonella* spp., and *Streptococcus phocae*, as previously described (Carrasco et al. 2014). Stranded sea otters were found dead or moribund in south-central Alaska, Kodiak Archipelago, and the western side of Cook Inlet, including Ninilchik, Homer and Kachemak Bay, Whittier, Kodiak Island, Hallo Bay, and Kamishak Bay (Fig. 1). Sea otters from southcentral stock that had stranded in the Ninilchik, Whittier, Homer, and Kachemak Bay regions were further subcategorized into a “Kachemak Bay area” group, whereas animals from southwest stock that had stranded in Kamishak Bay, Hallo Bay, and Kodiak Island were pooled into a “southwest Alaska” group. These categories were selected because mortalities caused by SB/E infection were common in northern sea otters from southcentral stock in Kachemak Bay and surrounding areas (adjacent to the threatened southwestern stock).

In addition, 148 serum samples were obtained from necropsied southern sea otters that had stranded during 2001–2009 in California. Postmortem examinations of southern sea otters were performed at the Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife (CDFW), Santa Cruz, California, by a veterinary pathologist to establish the cause of death. As with the northern sea otters, southern sea otters that were classified into the “infectious” group generally were diagnosed with a cause of death due to either bacterial or parasitic pathogens or had bacterial or parasitic infections as a contributing cause of death (n = 72 animals), whereas animals that were diagnosed with other noninfectious causes of death or had contributing factors that were not associated with infectious disease were included in a “noninfectious” group (n = 76). Acknowledged pathogens of southern sea otters included a range of bacterial, protozoal, and acanthocephalan parasites, as previously described (Kreuder et al. 2003, Miller et al. 2010a). These sea otters were found dead or moribund on beaches in various geographic areas along the central California coast, including Monterey Bay, Spanish Bay, Carmel Bay, Julia Pfeiffer State Beach, San Simeon Bay and surroundings, Morro Bay and surroundings, Diablo Canyon, and San Luis Obispo Bay and Pismo Beach (Fig. 1.).

The age of each sea otter was estimated at the time of necropsy based on body mass, weight, dentition, pelage characteristics, and reproductive status (Riedman and Estes 1990, Von Biela et al. 2011). Pups, juveniles, and subadults
were categorized as "immature" otters, and adults and aged adults were categorized as "adult" otters. Nutritional condition was assessed by the amount of subcutaneous fat present and by palpation of the ribs, backbone, and other bony protuberances (Doroff and Mulcahy 1997). Otters were categorized as being in "poor" nutritional condition if they were thin or emaciated with scant subcutaneous fat and evidence of muscle loss, or in "good" nutritional condition if they had moderate to abundant subcutaneous fat.

**Serology**

Antibodies against *Bartonella henselae* type II (also known as Marseille; isolate U4, University of California–Davis), *Baronella clarridgeiae* (ATCC 51734), and *Bartonella washoensis* (isolate UCD-dog2, University of California–Davis) in serum and/or whole blood samples from northern and southern sea otters were detected using an indirect immunofluorescent antibody (IFA) assay. The IFA procedure was similar to the procedure described by Henn et al. (2007) with a slight modification. Fluorescein isothiocyanate (FITC)-conjugated goat anti-ferret immunoglobulin G (IgG) (Bethyl Laboratories, Montgomery, TX) was used for detection of primary antibodies. *B. henselae*, *B. clarridgeiae*, and *B. washoensis* strains were used as surrogate bacteria for this assay due to the lack of success obtaining a *Bartonella* isolate from sea otter tissues. Samples were examined at dilutions of 1:64 and 1:128 using a fluorescence microscope at 400× magnification. The intensity of bacillus-specific fluorescence was scored subjectively from 1 to 4, as previously described (Chang et al. 2000). Samples with a fluorescence score of ≥2 at a dilution of 1:64 were considered positive because this antibody titer has proven to indicate prior exposure and current infection by *Bartonella* spp. in dogs and cats (Chomel et al. 1995, Pappalardo et al. 1997). Samples testing positive were titrated in serial two-fold dilutions until the fluorescence score was below the limit of detection (<2). Each slide was examined in a blinded fashion by three readers, and fluorescence scores were averaged.

**Statistical analysis**

Data collected on each necropsied northern and southern sea otter included animal identification number, age class, sex,
stranding date and location, date of sampling, weight, nutritional condition, and cause of death from necropsy findings, including pathological lesions and reported laboratory results for any postmortem bacterial culture or parasitology result. Additional SBE/E or *S. infantarius* subsp. *coli* and *S. phocae* culture results were provided by the USFWS from microbiological reports from the Microbiology Laboratory (Veterinary Medical Teaching Hospital, University of California–Davis). Chi-squared analyses were used to evaluate associations between *Bartonella* IFA results and age, sex, nutritional condition, stranding area, and animal grouping as “infectious” or “non-infectious” cause of death using JMPv8.0 software (SAS Institute Inc, www.jmp.com). Associations between *Bartonella* IFA results and live-captured otters and necropsied northern sea otters were also analyzed using chi-squared analyses. Stratified analyses were considered where appropriate after univariate analyses were completed. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of association of *Bartonella* IFA results with each of the variables that were significant in the stratified analysis. A Fisher exact test was performed to analyze variables with limited sample sizes (Fisher 1935). For all tests, *p* values ≤0.05 were considered statistically significant. Sampling sites were mapped using ArcGIS 10.2 software (ESRI, Redlands, CA) for all necropsied northern and southern sea otters tested by serology.

Results

Serological findings

Antibodies reactive to *Bartonella* spp. were detected in 34% (15/44) and 50% (24/48) of serum samples from live-captured and necropsied northern sea otters, respectively, with antibody titers ranging from 1:64 to 1:256 (Tables 1 and 2). By comparison, *Bartonella* spp. seroprevalence was lower (16%, 24/148) for southern sea otters (Table 1). The majority of positive sea otter sera were reactive for *B. washoensis*, as antibodies reactive to *B. washoensis* (≥1:64 IFA titers) were detected in 27% (12/44) of sera from live-captured northern sea otters (Table 1). Two (4.5%, 2/44) were seropositive for *B. clarridgeiae* and *B. washoensis*, and one sample was seropositive only to *B. clarridgeiae* (≥1:128). Of 48 necropsied northern sea otters sampled, seven (14%, 7/48) reacted only to *B. washoensis* and 12 (25%, 12/48) were reactive to *B. henselae* and *B. washoensis* (Table 1). Antibodies reactive to *B. washoensis* (≥1:64) were detected in 13% (19/148) of sera from necropsied southern sea otters (Table 1). Seven samples (4.7%, 7/148) were seropositive only to *B. washoensis*, and eight samples (5.4%, 8/148) tested positive for *B. henselae* and *B. washoensis*.

**Table 1. Prevalence of Antibodies against *B. henselae* (*Bh*), *B. clarridgeiae* (*Bc*), and *B. washoensis* (*Bw*) in Apparently Healthy, Live-Captured Northern Sea Otters and Necropsied Northern and Southern Sea Otters Using an Indirect Immunofluorescent Antibody (IFA) Test**

<table>
<thead>
<tr>
<th>Bartonella spp.</th>
<th>Live-captured northern sea otters (n = 44)</th>
<th>Necropsied northern sea otters (n = 48)</th>
<th>Necropsied southern sea otters (n = 148)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. washoensis</em></td>
<td>27% (12/44)</td>
<td>14% (7/48)</td>
<td>4.7% (7/148)</td>
</tr>
<tr>
<td><em>B. clarridgeiae</em></td>
<td>2.2% (1/44)</td>
<td>ND</td>
<td>1.3% (2/148)</td>
</tr>
<tr>
<td><em>B. henselae</em></td>
<td>ND</td>
<td>2.0% (3/148)</td>
<td></td>
</tr>
<tr>
<td><em>Bh</em> and <em>Bw</em></td>
<td>ND</td>
<td>25% (12/48)</td>
<td>5.4% (8/148)</td>
</tr>
<tr>
<td><em>Bh</em> and <em>Bc</em></td>
<td>2% (1/48)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td><em>Bc</em> and <em>Bw</em></td>
<td>4.5% (2/44)</td>
<td>2% (1/48)</td>
<td>1.3% (2/148)</td>
</tr>
<tr>
<td><em>Bh</em>, <em>Bc</em>, and <em>Bw</em></td>
<td>6.2% (3/48)</td>
<td>1.3% (2/148)</td>
<td></td>
</tr>
<tr>
<td>Total <em>Bartonella</em> positives</td>
<td>34% (15/44)</td>
<td>50% (24/48)</td>
<td>16% (24/148)</td>
</tr>
</tbody>
</table>

ND, Not detected.

**Table 2. The Distribution of *Bartonella* Indirect Immunofluorescent Antibody Titers in Live-Captured Northern Sea Otters and Necropsied Northern and Southern Sea Otters**

<table>
<thead>
<tr>
<th>Bartonella IFA titer</th>
<th>Live-captured northern sea otters (n = 44)</th>
<th>Necropsied northern sea otters (n = 48)</th>
<th>Necropsied southern sea otters (n = 148)</th>
<th>Total number of samples by titer level (n = 240)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1:256‡</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>≥1:128‡</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>≥1:64‡</td>
<td>7</td>
<td>9</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>&lt;1:64</td>
<td>29</td>
<td>24</td>
<td>124</td>
<td>177</td>
</tr>
</tbody>
</table>

‡Antibody titers ≥1:64 were classified as seropositive. IFA, indirect immunofluorescent assay.

Association of *Bartonella* seroprevalence with demographic and spatial factors

Significant associations were identified between *Bartonella* seropositivity in northern sea otter carcasses and adult age class, poor nutritional condition, and death due to infectious causes (Table 3). *Bartonella* seropositivity was not more common in necropsied northern sea otters when compared to apparently healthy live animals. A much higher proportion of *Bartonella*-seropositive stranded northern sea otters died with infectious disease as a primary or major contributing cause of death, when compared to stranded northern sea otters that died from noninfectious causes (Table 3). Adult and aged adult stranded northern sea otters that died with infectious disease were 27 times more likely (Fisher exact test, *p* < 0.001; 95% CI 2.62–269.4) to be seropositive to *Bartonella* spp. than northern sea otters that died from noninfectious causes. A similar age association was observed for *Bartonella*-seropositive, live-captured northern sea otters (*p* = 0.04). Of 28 adult, live-captured northern sea otters tested, 12 (49%) were seropositive, compared to two out of 16 immature sea otters (13%; *p* = 0.04).

In contrast to observed patterns for northern sea otters, no significant associations were observed between *Bartonella* seropositivity and age class, sex, nutritional condition, or cause of death for southern sea otter carcasses. Southern and northern sea otter carcasses that tested positive for *Bartonella* spp. were found on beaches in various geographic areas of California and Alaska (Fig. 1).

**Discussion**

The high proportion of *B. washoensis*–seropositive sea otters was consistent with our recent detection of *Bartonella* DNA in sea otter heart valves from Alaska and California.
(Carrasco et al. 2014). The *Bartonella* DNA detected was closely related to *B. henselae*, *B. wasoensis*, and *Candidatus Bartonella volans*, suggesting that northern and southern sea otters are infected with more than one *Bartonella* species (Carrasco et al. 2014). Consistent with that finding, seroreactivity to greater than one *Bartonella* spp. was detected in 67% (16/24) and 50% (12/24) of *B. wasoensis*-positive samples from northern and southern sea otter carcasses, respectively. This result was not surprising given that serological cross-reactivity of these three *Bartonella* spp. and others has been previously reported in sera from *Bartonella*-infected humans and animals (Maurin et al. 2002, Chomel et al. 2003, Tsuneoka et al. 2004, Boulouis et al. 2005). In contrast to samples from sea otter carcasses, the majority of the sera from presumably healthy, live-captured northern sea otters showed exclusive reactivity against *B. wasoensis* (86%; 12/14) as described (Carrasco et al. 2014). Interestingly, five of these six seropositive animals had antibodies reactive to *B. wasoensis* and detectable *Bartonella* DNA that was closely related to *B. wasoensis* and *Candidatus B. volans* (Carrasco et al. 2014). Some *B. wasoensis*-seropositive northern sea otters that had died due to VVE yielded titers $\geq1:256$, as commonly reported in *Bartonella*-associated endocarditis in dogs and humans (Maurin et al. 2002, MacDonald et al. 2004). However, the clinical impact of disseminated bartonellosis in sea otters was not clear. The observed high mortality due to VVE for northern sea otters from this region and confirmation of co-infection by *Bartonella* and other pathogenic bacteria in prior studies (Brownstein et al. 2011, Carrasco et al. 2011, Carrasco et al. 2014) suggests that *Bartonella* co-infections may be a contributor to the development of debilitating disease in northern sea otters.

Previous studies in dogs and humans with infective endocarditis have shown evidence of serological cross-reactivity between *Bartonella* ssp. and intracellular bacteria (LaScola and Raoult 1996, Maurin et al. 2002, MacDonald et al. 2004). Although it is reasonable to speculate that SB/E-infected northern sea otters could have cross-reactive antibodies to *Bartonella* antigens using our IFA-based assay, data from our laboratory and others have shown that cross-reactivity between *Bartonella* ssp. and *Streptococcus* ssp. is rare when samples are evaluated with this serological assay (Sykes et al. 2006, Vermeulen et al. 2010). However, a potential limitation of our findings is that we cannot rule out the possibility of cross-reactive antibodies to other intracellular pathogens that have shown to interfere in the diagnosis of bartonellosis in dogs and humans (Maurin et al. 2002, MacDonald et al. 2004).

Exposure to *Bartonella* ssp. was widely distributed among sea otters from the southcentral stock of Alaska, because positive samples were detected in otters from Ninilchik, Whittier, and Kachemak Bay. In addition, *Bartonella* ssp. antibodies were detected in the southwestern stock of northern sea otters that inhabit areas adjacent to Kachemak...
Bay, including Kamishak Bay and Kodiak Island. This broad distribution of seropositivity suggested that this bacterium is endemic in northern sea otters. Due to shared haul-out sites and rafting behavior of sea otters in southcentral Alaska (Doroff and Badajos 2010) and the wide range and diversity of seroprevalence to Bartonella spp. documented here, it appears that Bartonella may be transmitted horizontally between sea otters, possibly by yet unidentified arthropod vectors. It is also possible that a common vector may be involved in the transmission of Bartonella between northern and southern sea otters since fecal analyses in haul-out sites in Kachemak Bay showed that otters likely used the same haul-out sites as coastal river otters at different times of day (Doroff et al. 2012).

In conclusion, this study together with our recently published molecular findings (Carrasco et al. 2014) confirms that Bartonella organisms have been present in sea otters over the past decade and provides evidence for widespread exposure to Bartonella spp. among northern and southern sea otters. This bacterium seems to be regionally endemic for northern sea otter subspecies in Kachemak Bay and surrounding areas. Our findings provide baseline data on the frequency and distribution of Bartonella exposure among northern and southern sea otters in Alaska and California, providing context for investigating future disease outbreaks and monitoring of Bartonella infections for sea otter management and conservation.

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Author Disclosure Statement

No competing financial interests exist.

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