DOI: 10.7589/2016-09-208 *Journal of Wildlife Diseases*, 54(1), 2018, pp. 000–000


# *STREPTOCOCCUS PHOCAE* IN MARINE MAMMALS OF NORTHEASTERN PACIFIC AND ARCTIC CANADA: A RETROSPECTIVE ANALYSIS OF 85 POSTMORTEM INVESTIGATIONS

**Nicole D. Taurisano,[1,7] Brian P. Butler,[1] Diana Stone,[1] Harry Hariharan,[1] Paul J. Fields,[2] Hugh W. Ferguson,[1] Martin Haulena,[3] Paul Cotrell,[4] Ole Nielsen,[5] and Stephen Raverty[6]**

[1] Department of Pathobiology, St. George's University, School of Veterinary Medicine, True Blue, St. George, Grenada, West Indies
[2] Windward Islands Research and Education Foundation, True Blue, St. George, Grenada, West Indies
[3] Vancouver Aquarium Marine Science Centre, PO Box 3232, Vancouver, British Columbia V6B 3X8, Canada
[4] Department of Fisheries and Oceans Canada, Suite 200-401 Burrard, Vancouver, British Columbia V6C 3S4, Canada
[5] Department of Fisheries and Oceans Canada, 501 University Cr., Winnipeg, Manitoba R3T 2N6, Canada
[6] Animal Health Center, 1767 Angus Campbell Road, Abbotsford, British Columbia V3G 2M3, Canada
[7] Corresponding author (email: ntaurisa@sgu.edu)

ABSTRACT: *Streptococcus phocae* is a pathogen of marine mammals, although its pathogenicity remains poorly understood. Recovery of this bacterium from asymptomatic carriers suggests that it is an opportunistic pathogen. We investigated the role of *S. phocae* in naturally occurring disease and its significance as a pathogen based on postmortem investigations. Between 2007 and 2012, 1,696 whole carcasses, tissue samples, or both were submitted from the northeastern Pacific and Arctic Canada for diagnostic testing. *Streptococcus phocae* was cultured from phocids ($n$=66), otariids ($n$=12), harbor porpoises (*Phocoena phocoena*; $n$=5), and sea otters (*Enhydra lutris*; $n$=2). Pathologic manifestations of *S. phocae*–associated disease included localized, as well as systemic, inflammatory lesions with common findings of suppurative bronchopneumonia ($n$=17) and bacteremia ($n$=27). Lung lesions were frequently culture-positive for *S. phocae*, suggesting commensal colonization of the oropharynx with subsequent opportunistic infection of the respiratory tract during tissue injury, coinfection, immunosuppression, or other debilitating conditions. The presence of a positive spleen culture, and interpretations at necropsy and histopathology, were used to determine the presence of *S. phocae* bacteremia. Less frequent lesions that were culture positive for *S. phocae* included abscesses ($n$=9), meningitis ($n$=7), and cellulitis ($n$=1). The majority of cases with *S. phocae* lesions featured pre-existing conditions that presumably contributed to some degree of debilitation or immunosuppression, including emaciation ($n$=29), liver mercury accumulation ($n$=29), trauma ($n$=22), severe pulmonary or cardiovascular nematodiasis ($n$=9), concurrent bacterial or viral infections ($n$=8), or sarcocystosis ($n$=6). These findings suggest that *S. phocae* could be characterized as an opportunistic pathogen, associated with debilitating conditions in stranded and rehabilitating marine mammals. Wildlife investigators can use these results to draw more definitive conclusions regarding positive *S. phocae* cultures during postmortem studies in marine mammals.

*Key words:* Bronchopneumonia, harbor porpoise, harbor seal, infection, marine mammals, sea otter, sepsis, *Streptococcus phocae*

## INTRODUCTION

*Streptococcus phocae*, named following its isolation during an epidemic in harbor seals (*Phoca vitulina*), is associated with streptococcal disease in marine mammals (Skaar et al. 1994). It has been recovered from clinically healthy and diseased animals and from postmortem lesions. Based on the epidemiology and clinical presentations of *S. phocae*, the bacterium is considered a commensal organism with a tropism for the oropharynx and upper respiratory tract, although there is no published evidence directly supporting these sites as being preferred.

Opportunistic streptococcal infections in humans and domestic animals result from underlying disease or immunodeficiency. Information regarding the natural history of *S. phocae* in marine mammals in the northeastern Pacific is sparse. Case reports in pinnipeds describe suppurative lesions typical of streptococcus infections (Skaar et al. 1994; Henton et al. 1999; Bartlett et al. 2016). The first clinical signs associated with *S. phocae* infec-

tion during a harbor seal morbillivirus outbreak in northwestern Europe were pneumonia and sinusitis (Skaar et al. 1994). Respiratory infection was again reported during a later outbreak of emaciation, vulvovaginitis, and abortion in Cape fur seals (*Arctocephalus pusillus*) in South Africa (Henton et al. 1999). Pyometra and lymphadenopathy were documented in a spotted seal (*Phoca largha*) in Alaska (Hueffer et al. 2011). *Streptococcus phocae* has also been associated with urogenital neoplasia in Steller sea lions (*Eumatopias jubatus*) and skin abscesses in southern sea otters (*Enhydra lutris nereis*; Johnson et al. 2006; Bartlett et al. 2016).

Despite these case reports, there is insufficient understanding of the role of *S. phocae* in marine mammal disease. We investigated the presence of *S. phocae*–associated lesions identified at postmortem examination of marine mammals of the northeastern Pacific and Arctic Canada and considered the most frequently associated risk factors.

## MATERIALS AND METHODS

As part of an ongoing health assessment, harbor seals from local rehabilitation facilities and dead beachcast marine mammals in British Columbia, Canada, are submitted for necropsy to the Animal Health Centre in Abbotsford, British Columbia. This study retrospectively analyzed postmortem reports of marine mammals in the Northeastern Pacific and Arctic Canada between 2007 and 2012. In this period, 1,696 marine mammals were either reported stranded alive and presented to rehabilitation, or they were recovered dead and necropsied. At postmortem, a systematic suite of tissues was cultured and included lung, spleen, brain, lymph node, and small intestine. Additional tissues were cultured at the discretion of the attending pathologist. Initial inoculation was made onto Columbia Blood agar with 5% sheep blood and MacConkey agar (Oxoid, Nepean, Ontario, Canada). *Streptococcus phocae* was suspected based on the β-hemolytic pattern and confirmed with 16s-RNA (Vossen et al. 2004). The Biolog identification system (Biolog Inc., Hayward, California, USA) and gene sequencing provided speciation (Truu et al. 1999). For this case series, only animals that were culture positive for *S. phocae* were evaluated. Between 2007 and 2012, 85 marine mammals fit this criterion and were included in our study; mammals in the study were those stranded within the Northeastern Pacific region and western Canadian arctic.

*Streptococcus*-associated lesions were defined as tissue lesions yielding *S. phocae* in pure culture. Pure culture from the spleen, lymph nodes, or a combination, in addition to determinations made by the attending veterinary pathologist, indicated likely cases of sepsis. We calculated the frequency of *S. phocae* isolation from each tissue and any accompanying lesions.

Segments of intestinal tracts from all pinnipeds, cetaceans, and sea otter specimens were cultured for *Salmonella* spp. *Brucella* sp. was ruled out with PCR for all cetaceans, otters, gravid pinnipeds, and aborted pups (Casanas et al. 2001). Pooled tissues were submitted for *Leptospira* spp. PCR for every otariid and sea otter (Cameron et al. 2008). Histologic tubulointerstitial nephritis with intratubular agyrophilic spirochetes supported a diagnosis of leptospirosis. Pooled tissues including lung, brain, lymph node, and spleen were tested with a PCR for canine distemper virus in all marine mammal species (Stanton et al. 2004). Tissue for testing using a PCR for influenza virus PCR was only submitted from pinniped species (Spackman et al. 2002). Microscopic detection of necrotizing adrenocortical adenitis, hepatitis, or interstitial pneumonia with intranuclear inclusion bodies was considered typical of phocine herpesvirus-1 (PHV-1). We evaluated cases reported as having heavy parasite loads and severe associated lesions for potential predisposition to bacterial translocation. When intact parasites could be identified, morphology determined metazoan parasite speciation. For protozoa, immunohistochemistry was done for *Sarcocystis neurona* and *Toxoplasma gondii*, and PCR for Apicomplexa (Gibson et al. 2011).

We determined liver concentrations of mercury (Hg) to be potentially harmful if its ratio with liver-selenium (Se), the Se:Hg ratio, was $<1$. In this study, only those cases where the ratio was below 1, and where the liver Hg measured above 2 ppm were considered for potential immune suppression and predisposition to opportunistic infection (De Guise et al. 1996). A boarded pathologist (S.R.) evaluated carcasses for evidence of antemortem trauma that contributed to significant morbidity or subacute mortality. Cases were assigned to the antemortem trauma category when a physical wound appeared to be directly related to mortality, or when pre-existing wounds included intralesional coccobacilli and pure cultures of *S. phocae*. Animals with no conclusive or significant primary predisposing conditions were assigned to an "inconclusive" category, in which predisposition to *S. phocae*–associated disease was speculative based on signalment, or else it was unidentified. Carcasses were deemed emaciated

based on blubber thickness, muscle mass, recorded weights, or gross observations during necropsy.

Vitamin A concentrations in the liver were measured routinely in all species. We used previously developed reference values (Puls 1994): 200–3,500 μg/g for the harbor seal and 150–3,500 μg/g for the harbor porpoise (*Phocoena phocoena*), extrapolating these ranges for use in investigating sea otter and otariid vitamin A status. We used the Mantel-Haenszel chi-square test to determine associations between animals' ages and underlying primary disease, further identifying populations at risk.

## RESULTS

Male and female animals each represented 48.2% of the sample population, with sex not determined for 3.5% (Table 1). Eight species of marine mammals of various ages were represented (Table 1). Lung tissue was culture-positive for *S. phocae* most frequently, comprising 25% (47 of 186) of the total positive tissue cultures. Of all cases where lung tissue was cultured, 58% yielded pure (17%, 14 of 81), or mixed (41%, 33 of 47), growth of *S. phocae*. Of all positive splenic and lymph node cultures, *S. phocae* was isolated in pure culture from 47% (17 of 36) and 60% (17 of 28), respectively. Twenty-seven cases had pure culture from one or both of the spleen and lymph nodes. The cases with pure culture from the spleen and lymph nodes were considered most likely to have *S. phocae*–associated sepsis. The most common isolate found in mixed culture with *S. phocae* was a nonhemolytic *Escherichia coli*.

Bronchopneumonia, meningoencephalitis, and cellulitis were the findings most consistent with an *S. phocae*–associated lesion, because coccobacilli could be visualized within lesions that yielded *S. phocae* in pure culture (Fig. 1). Bronchopneumonia was the most consistent lesion ($n$=17) reported in *S. phocae* culture-positive lung tissue, identified in 64% (9 of 14) of cases where *S. phocae* grew in pure culture from the lung (Table 2). Meningitis, encephalitis, or both were present in 40% (4 of 10) of the cases where *S. phocae* was the only bacterial isolate from brain tissue, although less frequently present ($n$=7) overall. Abscesses that cultured positive for *S. phocae* were reported from the integument ($n$=2), lung ($n$=4), lymph node ($n$=1), brain ($n$=1), and umbilical vein ($n$=1).

Despite past reports of abortion and pyometra, lesions in the reproductive tract were not consistent findings in our case series (Henton et al. 1999; Hueffer et al. 2011). Our sample population did include two pregnant females; four neonates with injuries suggestive of dystocia; and four cases of omphalitis, from which *S. phocae* grew in pure or mixed culture. *Streptococcus phocae* grew in pure or mixed culture from reproductive tissues, including umbilicus, placenta, uterus, and ovary, with no observed pathologic changes.

Animals with mixed microbial infection ($n$=8) had the largest percentage of cases with *S. phocae*–associated sepsis (63%, 5 of 8) most of which ($n$=6) were California sea lions (CSLs) with leptospirosis (Table 3). Most CSLs with leptospirosis had bronchopneumonia ($n$=5), four of which grew *S. phocae* in mixed culture. The remaining animals with mixed microbial infection were harbor seals with active PHV-1 infections ($n$=2). One animal exhibited *S. phocae* brain abscesses, and the other animal had encephalitic sarcocystosis with mixed *S. phocae* culture from the brain.

In animals with liver Se:Hg ratios $<1$ ($n$=29), the ratio ranged from 0.02 to 0.9, with Hg concentrations ranging from 15.8 to 69.4 mg/L. Coinciding pathologic changes suspected as factors contributing to individual mortality were present in all but two animals and included leptospirosis ($n$=6), emaciation ($n$=5), sarcocystosis ($n$=2), severe verminous pneumonia ($n$=2), physical trauma ($n$=2), and intestinal acanthocephalans ($n$=1). Pure culture from the spleen, lymph nodes, or a combination was obtained in 41% (12 of 29) cases. The finding of increased liver-Hg was significantly associated with increasing age of an animal ($P<0.001$).

Sarcocystosis manifested as encephalitis in most (83%, 5 of 6) cases. Sarcocystis-associated necrotizing lymphadenitis affected one harbor seal. Harbor seals with evidence of sarcocystis-associated disease had concurrent evidence of *S. phocae* bacteremia ($n$=3),

TABLE 1. Demographics of stranded marine mammals that were culture positive for *Streptococcus phocae* postmortem. All animals were from the northeastern Pacific Ocean, except for three ringed seals (*Pusa hispida*) that originated in Arctic Canada. Two ringed seals and one harbor seal (*Phoca vitulina*) had unrecorded ages and are excluded from the age classification.

| Species | No. | Sex (M/F/U[a]) | Age classification | | | | |
|---|---|---|---|---|---|---|---|
| | | | Fetus | Neonate | Weaned pup | Subadult | Adult |
| Harbor seal (*Phoca vitulina*) | 61 | 28/31/2 | 2 | 19 | 11 | 10 | 18 |
| Ringed seal (*Pusa hispida*) | 5 | 1/2/2 | | 1 | | 2 | |
| Harbor porpoise (*Phocoena phocoena*) | 5 | 3/2/0 | | 1 | 1 | 1 | 2 |
| California sea lion (*Zalophus californianus*) | 7 | 3/4/0 | | | | 5 | 2 |
| Steller sea lion (*Eumatopias jubatus*) | 3 | 2/1/0 | 1 | | | 1 | 1 |
| Sea otter (*Enhydra lutris*) | 2 | 1/1/0 | | | | | 2 |
| Guadalupe fur seal (*Arctocephalans twonsendii*) | 1 | 0/1/0 | | | | 1 | |
| Elephant seal (*Mirounga angustirostris*) | 1 | 1/0/0 | | | | | 1 |

[a] M=male; F=female; U=unknown.

meningitis (*n*=2), bronchopneumonia (*n*=1), and otitis externa (*n*=1).

Emaciated animals (*n*=29) often presented with overlapping conditions, including Hg accumulation (*n*=11), trauma (*n*=6), verminous pneumonia with ectopic vascular migration (*n*=2), and encephalitic sarcocystosis (*n*=2). Emaciation was the only evidence for underlying debilitation in 28% (8 of 29) animals. In these cases, manifestations of *S. phocae*–associated disease included bronchopneumonia (*n*=4), lung abscesses (*n*=2), meningoencephalomyelitis (*n*=3), and abscessing oropharyngitis (*n*=1). Some (*n*=7) of these animals were of nursing age, suggestive of failure to nurse (Table 4).

Three harbor seals had evidence of severe intestinal parasitism that may have predisposed to intestinal perforation (acanthocephalans) and mucosal disruption (coccidiosis) with subsequent seeding of the peritoneal cavity with bacteria or hematogenous dissemination. *Streptococcus phocae* grew in mixed culture with *E. coli* in each case and was isolated in pure culture in the spleen in one case.

Sixteen animals lacked evidence of an underlying primary disease. This category contains harbor seals and harbor porpoises with bronchopneumonia (*n*=6), sepsis (*n*=5), omphalitis (*n*=1), and lung abscesses (*n*=1) associated with *S. phocae*. Most (62%, 10 of 16) were of nursing age and exhibited depleted liver stores of vitamin A (*n*=14, 7–70 μg/g [normal range 150–200 to 3,500 μg/g]) and/or lymphoid depletion of the thymus and/or spleen (*n*=8). In our sample, 43% animals had decreased vitamin A in the liver, of which only 10 had *S. phocae* isolated in pure culture from the spleen, lymph nodes, or a combination.

Physical trauma accounted for mortality in 22 animals. Ten individuals had wounds suggestive of anthropogenic events, such as gunshot (*n*=4), propeller strike (*n*=2), net entanglement (*n*=2), and blunt force trauma to the head (*n*=2). Seven had injuries consistent with predation or conspecific aggression. Four neonatal harbor seal pups presented with apparent dystocia or crush injuries. Other findings that may have predisposed these animals to traumatic interactions, or exacerbated debilitation afterward, included emaciation (*n*=4), encephalitic sarcocystosis (*n*=1), and increased liver Hg levels with low Se:Hg ratio (*n*=1).

Nine animals had pulmonary or cardiac nematodiasis potentially severe enough to contribute to secondary, opportunistic bacterial infections. *Streptococcus phocae* was

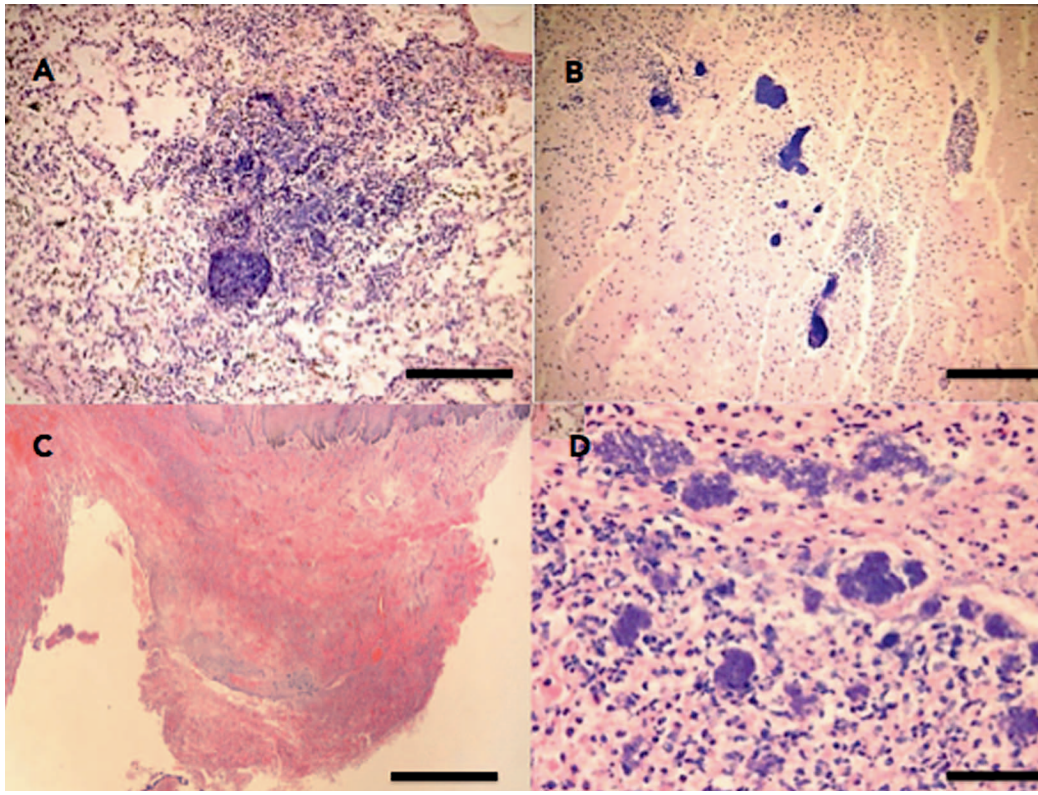


FIGURE 1. Postmortem lesions with intralesional coccobacilli from stranded marine mammals that cultured positive for *Streptococcus phocae*. All sections were stained with H&E. (A) Lung tissue from an adult emaciated harbor seal (*Phoca vitulina*). Fibrinosuppurative and necrotizing bronchopneumonia. Bar=500 µm. (B) Brain tissue from the same animal as (A). Necrotizing meningoencephalitis. Bar=500 µm. (C) Skin from a female harbor porpoise (*Phocoena phocoena*) calf with evidence of net entanglement. Necrosuppurative cellulitis with intravascular and intralesional cocci. Bar=500 µm. (D) Same tissue as (C). Bar=50 µm.

isolated from bronchopneumonia in two cases, and it was isolated in pure culture from the spleen, lymph nodes, or a combination in three cases.

Young animals were more likely ($P<0.001$) to exhibit decreased vitamin A (Table 4). Older animals were significantly more affected by cardiopulmonary nematodiasis and liver Hg/Se imbalance than younger animals ($P=0.03$ and $P<0.001$, respectively). Emaciation, sarcocystosis, physical trauma, and with comicrobial infection all did not demonstrate a significant association with age. The small number of individuals with severe intestinal parasitism and nontraumatic skin pathology precluded statistical analysis.

## DISCUSSION

*Streptococcus phocae* is historically associated with respiratory, reproductive, and integumentary infections in marine mammals (Skaar et al. 1994; Henton et al. 1999; Bartlett et al. 2016). In this retrospective study, we reported bronchopneumonia, sepsis, meningitis and encephalitis, omphalitis, cellulitis, and abscesses. Documented in seven cases, suppurative meningoencephalitis was the second most commonly observed pathologic manifestation of infection, likely the result of sepsis and hematogenous dissemination to the central nervous system.

The majority of positive *S. phocae* cultures 25% (47 of 186) were from lung tissue, which

TABLE 2. Tissue lesions described from 81 marine mammals found stranded in Northeastern Pacific and arctic Canada waters. Only lesions yielding pure or mixed *Streptococcus phocae* culture are listed. The Guadalupe fur seal (*Arctocephalans townsendii*), elephant seal (*Mirounga angustirostris*), and southern sea otters (*Enhydra lutris nereis*) are excluded from this table due to lack of observed suppurative lesions, despite pure culture from several tissues.

| | | | Isolation of *S. phocae* | |
|---|---|---|---|---|
| Species | No. tested | Lesion | Mixed culture | Pure culture |
| Harbor seal (*Phoca vitulina*) | 61 | Bronchopneumonia | 4 | 5 |
| | | Meningoencephalitis | 3 | 4 |
| | | Brain abscess | 1 | 0 |
| | | Lung abscess | 1 | 2 |
| | | Lymph node abscess | 0 | 1 |
| | | Necrotizing lymphadenitis | 0 | 1 |
| | | Embolic pneumonia | 0 | 1 |
| | | Pleuropneumonia | 1 | 1 |
| | | Peritonitis | 1 | 0 |
| | | Abscessing oropharyngitis | 1 | 0 |
| | | Otitis externa | 1 | 0 |
| | | Omphalitis | 1 | 0 |
| | | Abscessing omphalophlebitis | 0 | 1 |
| Harbor porpoise (*Phocoena phocoena*) | 5 | Bronchopneumonia | 1 | 3 |
| | | Cellulitis | 0 | 1 |
| California sea lion (*Zalophus californianus*) | 7 | Bronchopneumonia | 3 | 0 |
| | | Lung abscess | 1 | 0 |
| Ringed seal (*Pusa hispida*) | 5 | Dermatitis | 2 | 0 |
| | | Hyperkeratosis | 2 | 0 |
| Steller sea lion (*Eumatopias jubatus*) | 3 | Bronchopneumonia | 1 | 0 |

TABLE 3. Risk factors for *Steptococcus phocae*–associated disease 81 marine mammals found stranded in Northeastern Pacific and arctic Canada waters. Categories of underlying disorders are listed in descending order of how often each yielded pure *S. phocae* culture from the spleen and lymph nodes.

| | | Isolation of *S. phocae* in pure culture from spleen/lymph node tissue | |
|---|---|---|---|
| Risk factor | Total | No. | % |
| Comicrobial infection | 8 | 5 | 63 |
| Sarcocystosis | 6 | 3 | 50 |
| Emaciation | 29 | 13 | 45 |
| Liver Se:Hg <1 | 29 | 12 | 41 |
| Cardiopulmonary nematodiasis | 9 | 3 | 33 |
| Severe intestinal parasitism | 3 | 1 | 33 |
| Nontraumatic skin pathology | 3 | 1 | 33 |
| Inconclusive | 16 | 5 | 31 |
| Decreased liver stores of vitamin A | 37 | 10 | 27 |
| Physical trauma | 22 | 5 | 23 |

TABLE 4. Age association with predisposition to *Streptococcus phocae*–associated disease. Young animals were more likely ($P$=0.0001) to exhibit decreased vitamin A (range <5 to 186 μg/g). Conversely, older animals were significantly associated with low Se:Hg ratio and cardiopulmonary nematodiasis ($P$<0.001 and $P$=0.03, respectively). Two ringed seals and one harbor seal had unrecorded ages and are excluded from the age classification.

| | | Age classification | | | | |
|---|---|---|---|---|---|---|
| Co-morbidity | Number | Fetus | Neonate | Weaned pup | Subadult | Adult |
| Co-microbial infection | 8 | | 1 | | 6 | 1 |
| Sarcocystosis | 6 | | | | 2 | 4 |
| Emaciation | 29 | | 7 | 4 | 9 | 9 |
| Liver Se:Hg <1 | 29 | | | 1 | 9 | 18 |
| Cardiopulmonary nematodiasis | 9 | | | | 6 | 3 |
| Severe intestinal parasitism | 3 | | | 2 | | 1 |
| Non-traumatic skin pathology | 3 | | | | | 1 |
| Inconclusive | 16 | 1 | 9 | 3 | 1 | 2 |
| Decreased liver stores of vitamin A | 37 | 2 | 16 | 9 | 2 | 7 |
| Physical trauma | 22 | | 8 | 4 | 2 | 8 |

along with findings of *S. phocae*–associated bronchopneumonia, supported the concept of opportunistic colonization of the respiratory tract. It did not, however, rule out underlying disorders that predispose animals to respiratory infection. The increased prevalence of mixed culture versus pure culture was confounding, because mixed culture from lung tissue was not a reliable indicator of true infection associated with any of the isolated bacterial species. Pure cultures were isolated more often than mixed from the spleen and lymph nodes. Furthermore, when *S. phocae* grew in mixed culture from lung tissue, it was isolated in pure culture from the spleen and lymph nodes 30% of the time, suggesting greater pathogenic potential than the other bacteria that grew in mixed culture from lung tissue. Although postmortem interval and microbial invasion from the gastrointestinal tract may have also been considerations, the spleen and lymph nodes were much more reliable sources for a positive culture associated with true infection (Tsokos and Puschel 2001; Morris et al. 2006). Case-control studies demonstrate that splenic culture can be negative in 65–82% or proven *Streptococcus*-associated pneumonia (Fredette 1916; Kurtin and Maike 1958). The actual incidence of true *S. phocae*–associated disease in our study was likely best reflected in, and potentially was greater than, the frequency of pure culture from the spleen and lymph nodes.

Our results disagreed with current literature describing *S. phocae*–associated skin and reproductive lesions (Henton et al. 1999; Johnson et al. 2006; Hueffer et al. 2011). *Streptococcus phocae*–associated skin pathology is described in sea otters (Bartlett et al. 2016). Our study included three cases of phocids with bacterial overgrowth associated with nontraumatic skin lesions and one case of cellulitis and abscessation in a harbor porpoise with net entanglement wounds. Skin lesions may have been the result of direct invasion by skin commensals, or from environmental or hematogenous exposure. Routine cultures of the skin in marine mammals would be necessary to determine commensal flora and the potential for secondary *S. phocae* overgrowth in times of integumentary compromise.

We cultured *S. phocae* from the placenta, umbilicus, uterus, ovary, aborted fetuses, and deceased neonatal dystocia victims, usually unassociated with disease. This suggested that the bacterium was a commensal in the female urogenital tract, although the incidence of reproductive lesions did not seem to be as prevalent as respiratory lesions. It is possible

that the neonate and placenta are exposed to *S. phocae* during parturition or in cases of ascending infection to the uterus. Current literature documents *S. phocae* isolation from the reproductive tract in healthy CSL, but also in association with squamous cell carcinoma in CSL, pyometra in a spotted seal, and abortion in Cape fur seals (Henton et al. 1999; Johnson et al. 2006; Hueffer et al. 2011). Further studies are required to substantiate its status as a commensal in the species described in this study.

Cases with positive bacterial cultures from the spleen, lymph nodes, or a combination likely represented true infections as opposed to postmortem translocation or contamination (Tsokos and Puschel 2001; Morris et al. 2006). Association between certain underlying diseases and the increased frequency of positive cultures from the spleen or lymph nodes identified animals at risk for developing secondary *S. phocae*–associated disease (Table 3). Underlying microbial infections had the largest percentage of *S. phocae* bacteremia (63%, 5 of 8) and included leptospirosis in CSL ($n$=6) and PHV-1 in harbor seals ($n$=2).

It was interesting to note that all cases of leptospirosis in CSL had increased liver Hg with decreased Se:Hg ratios. A low Se:Hg ratio indicated that Se was not increased to the point of protecting against Hg, because Se binds with Hg in a 1:1 ratio to keep Hg from accumulating. It is not known what concentrations of liver Hg may be toxic and, when toxic, what the range of detrimental effects are (Bossart 2011). In cases where Hg accumulation in the liver was not accompanied by a protective Se increase, it is not possible to conclude whether Hg accumulation contributed to debilitation. The Se:Hg imbalance commonly overlapped with other debilitating conditions. However, concurrent pure culture of *S. phocae* from spleen and lymph nodes raised suspicion for a significant role of Hg in antemortem morbidity in these cases.

Opportunistic infection and underlying disease can exacerbate each other. It is then unclear which manifested first. In cases of sarcocystosis in harbor seals, although cause of death was attributed to severe primary protozoal infection, opportunistic *S. phocae* infection likely contributed to morbidity and mortality. This establishes a link between the two diseases, albeit a tenuous link. Preexisting debilitation is thought to predispose to both encephalitic sarcocystosis and *S. phocae* infection, independently (Saville et al. 2001; Dubey et al. 2003). Sarcocystosis is also known to depress lymphocyte function in horses (*Equus caballus*; Witonsky et al. 2008). Therefore, once present, the two may exacerbate each other regardless of which one is the first to infect.

Emaciated animals ($n$=29) presented with one or more other debilitating conditions. Although poor body condition can by itself predispose to opportunistic bacterial infections, it often results in a vicious cycle with other infections: poor nutrition predisposes to another disease, which in turn hinders foraging ability, exacerbating any negative energy balance. The primary cause is not clear in most cases. Emaciated harbor seal pups ($n$=7) likely had not sufficiently nursed. Harbor seals have a short suckling period of 3–4 wk, critical for immune system development (Ross et al. 1994; Marquez et al. 2003). If maternal colostrum is inadequate or insufficient, little transfer of passive immunity occurs, predisposing to opportunistic infection and sepsis due to both poor nutritional status and inability to mount a proper immune system. In 16 animals, postmortem examination did not reveal comicrobial infection, trauma, parasitism, integumentary disease, or emaciation. Based on signalment, as most of these animals were of nursing age, failure to nurse with subsequent immune incompetence was a prime consideration.

Nearly half (43%, 37 of 85) of the animals in our sample exhibited decreased vitamin A stores, which can reflect nutritional deficiencies and serve as an indirect marker for decreased immune function (St. Leger et al. 2011). It can also be an indicator of poor carcass condition, because vitamin A is fat soluble and oxidizes in a decomposing body. The significant association between nursing pups and low vitamin A was highly suggestive of poor nutritional status and inadequate

colostrum consumption. However, the fact that decreased vitamin A stores had the lowest incidence of pure culture from the spleen and lymph nodes suggested that there was less of an association between decreased vitamin A and *S. phocae*–associated disease overall. Vitamin A is likely only a significant indicator of debilitation and a factor in *S. phocae*–associated disease in young animals of nursing age.

*Streptococcus phocae* was cultured from animals with underlying parasitic disease in both the intestinal tract ($n$=3) and cardiopulmonary vasculature ($n$=9). Overwhelming lungworm and heartworm infections can prevent proper foraging in marine mammals by hindering their ability to dive. This creates a negative energy balance that may exacerbate parasitism, potentially predisposing animals to secondary infections. In cases of intestinal coccidiosis/acanthocephalans, parasite load was severe enough to raise suspicion of mucosal disruption or perforation, facilitating bacterial invasion into adjacent tissues and blood stream. *Escherichia coli* normally inhabit the intestines, and so culture of this organism with *S. phocae* in each case supported the idea that infection began with a compromise in gastrointestinal tract integrity. It was impossible to determine the role of *S. phocae* in antemortem morbidity in these animals. *Streptococcus phocae* culture in these cases may have been incidental or may have synergistically caused disease with *E. coli*. Overall, *S. phocae* was isolated in mixed culture with nonhemolytic *E. coli* in more instances than any other species of bacteria. This may have been a significant finding and requires further research into possible synergistic pathogenicity between *S. phocae* and *E. coli*.

Physical trauma, identified in 22 cases, may have predisposed animals to bacterial infections by compromising integumentary defenses. Infection may remain localized, or be disseminated hematogenously. However, physical trauma may also be the result of preexisting debilitation that renders animals more susceptible to predation and anthropogenic encounters. Eight animals had wounds consistent with anthropogenic events. When injury accounted for acute death of an animal, one possible conclusion is that preexisting encephalitis or *S. phocae*–associated disease predisposed the individual to trauma, rather than the other way around. This was the case for five animals with pre-existing *S. phocae*–associated disease for which cause of death was attributed to gunshot ($n$=3), net entanglement ($n$=1), and propeller strike ($n$=1). Seven individuals presented with injuries suggestive of predation or intraspecies aggression. Animals with other overlapping evidence of underlying debilitation, such as emaciation and thymic depletion, may have been predisposed to both *S. phocae*–associated disease and physical injury. It was not always possible to determine which occurred first. It was not possible to determine the significance of positive culture of *S. phocae* in neonates with injuries suggestive of dystocia. Presence of any bacteria species in those animals was likely due to exposure from the birth canal and postmortem proliferation.

We also found mixed bacterial proliferation in the skin occurred secondary to nontraumatic skin lesions such as hyperkeratosis and dermatitis. Dermatitis was necroulcerative in one case and was accompanied by bronchopneumonia. A pure culture of *S. phocae* was isolated from the spleen of this animal, suggesting that localized *S. phocae*–associated disease progressed to systemic disease.

Our study isolated *S. phocae* most frequently from lung tissue and in association with bronchopneumonia, meningitis, cellulitis, omphalitis, and abscesses. Pure culture predominating from the spleen and lymph nodes was strong supporting evidence for its role in antemortem morbidity relative to the other bacteria with which it usually grew in mixed culture in other tissues. *Streptococcus phocae*–associated disease in marine mammals occurred secondary to debilitation, which in younger animals, was more likely associated with inadequate colostral transfer and possible malnutrition. In older animals, it accompanied severe parasitic disease and Hg/Se imbalance. Studies with antemortem blood cultures

before postmortem bacteriologic testing of lung, spleen, brain, and lymph nodes would greatly help to establish sensitivity and specificity of postmortem *S. phocae* cultures.

## ACKNOWLEDGMENTS

We thank the laboratory staff of the Animal Health Center and the biologists and technicians involved with stranding response, veterinary rehabilitation, and carcass recovery for dedicated efforts.

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*Submitted for publication 11 September 2016.*
*Accepted 17 April 2017.*