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STREPTOCOCCUS PHOCAE ISOLATED FROM A SPOTTED SEAL (*PHOCA LARGHA*) WITH PYOMETRA IN ALASKA

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Abstract

A spotted seal harvested by subsistence hunters in Kotzebue Sound, Alaska (USA), showed a grossly enlarged uterus and associated lymph nodes. *Streptococcus phocae* was isolated from the purulent uterine discharge. Histopathologic examination revealed inflammation that was limited to the uterine mucosa. Lymph nodes draining the affected organ were reactive but no evidence of active infection was found in the lymph nodes. This report is the first *Streptococcus phocae* isolated from spotted seals as well as the first report of pyometra as the main pathologic finding associated with this pathogen. Isolation of this pathogen from Alaska expands the reported range to arctic pinnipeds. Zoonotic potential remains unknown.

Keywords

Alaska; pyometra; spotted seal; *Streptococcus phocae*

INTRODUCTION

Spotted seals (*Phoca largha*) are distributed from the East Siberian Sea and the Chukchi Sea to the Yellow Sea.¹ In the United States, they are managed under the Marine Mammal Protection Act. Currently, the spotted seal in Alaska (USA) is not listed as endangered; however, the reduction in sea ice has been discussed as a potential threat to this and other northern pinnipeds (e.g., ice seals). Investigating cases of apparent infectious disease in these ice seals is required in the context of rapid environmental change (e.g., loss of sea ice, increasing air and water temperatures, changes in prey abundance and distribution) as outlined in Burek et al.² Typically, samples for pathologic, histopathologic, and microbiologic studies are limited to captive or stranded animals. Therefore, subclinical to mild clinical cases in noncaptive animals are unnoticed and not incorporated into management decisions. Subsistence harvests in Alaska offer a unique but limited opportunity for surveillance of wild animals and assessment of possible health threats to the population outside of stranding events.

Streptococcus phocae was first described in 1994 in seals from Norway.⁹ In that study, *S. phocae* was isolated from lungs and other organs such as liver and kidney, indicating

septicemia. Since this first description, it has been implicated in disease in multiple marine mammal species including harbor seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in Germany¹¹ and sea otters (*Enhydra lutris nereis*) stranded in central California.⁵ In addition to its presence in marine mammals, *S. phocae* has been identified as an emerging pathogen in Atlantic salmon (*Salmo salar*).^{3,8,10} In farmed salmon, this pathogen can cause up to 25% mortality.⁸

CASE REPORT

An adult female spotted seal was harvested by subsistence hunters in Kotzebue Sound (66°54'N, 162°35'W) in November 2009. The animal measured 144 cm (tip of nose to tip of tail), weighed 71.9 kg, and was determined to be in good body condition (umbilical blubber thickness 5.5 cm, axillary blubber thickness 5 cm). During butchering, subsamples of tissues were collected for various research projects. The uterus and associated lymph nodes in the mesometrium were enlarged bilaterally. The uterus contained about 20 ml green to yellow purulent discharge. Gram stain of the discharge revealed gram-positive cocci that formed string-like arrangements (Fig. 1). Plating of the discharge on tryptic soy blood agar (TSB agar) resulted in colonies of uniform morphology that showed β -hemolysis.

Polymerase chain reaction on DNA isolated directly from the discharge and from the isolated bacteria grown on TSB agar, using primers specific for the 16S rRNA gene,¹¹ resulted in clear bands of the expected size (primer sequences shown in Table 1). Sequencing of the products and search of relevant databases revealed similarities to *Streptococcus phocae* strain CCUG 35103 (99% identity) for the 16S rRNA gene. The sequence was submitted to GenBank (accession number: HM032023). The 16S rRNA sequence obtained from this case was compared with type strains of all *Streptococcus* species using phylogenetic trees estimated with the maximum likelihood method implemented in PAUP* 4.0b10.10 (Sinauer and Associates, Sunderland, MA, USA). The models selected by ModelTest 3.7 were generalized time reversible with invariable sites and gamma-distributed rate variation.⁷ The resulting phylogenetic trees distinctly grouped the isolate with *S. phocae*, resulting in bootstrap support of 100 (Fig. 2A). Bootstrap supports of branches were obtained from 1,000 replications. In addition, DNA sequence of the chaperonin 60 gene⁴ (*cpn60*; Fig. 2B) and superoxide dismutase A (*sodA*; Fig. 2C) were amplified and submitted to GenBank (accession numbers: *cpn60*, HM067702; *sodA*, HM067703). Phylogenetic trees constructed using the Tamura-Nei variable base frequencies with invariable sites and γ distribution confirmed the grouping of the Alaska isolate with *S. phocae* with a bootstrap support of 100 for both genes.

Sections of the formalin-fixed uterus and lymph nodes were submitted to the University of Georgia Veterinary Diagnostic and Investigational Laboratory for histopathologic evaluation. The formalin-fixed tissues were routinely processed using a commercial tissue processor. The processed tissues were embedded in paraffin, sectioned at 4 μ m onto glass slides, stained with hematoxylin and eosin, and viewed by light microscopy. Additionally, Gram and Grocott-Gomori methenamine-silver stains were performed to attempt to visualize bacteria and fungi.

Histologic examination revealed mild to moderate freeze and crush artifact but cellular detail was otherwise well preserved. The lamina propria of the uterine mucosa was infiltrated by inflammatory cells that often obscured glandular architecture. The inflammatory cell infiltrate was predominated by plasma cells and admixed with fewer macrophages, neutrophils, and lymphocytes. These infiltrates extended through the lamina propria but did not infiltrate the muscular wall in the examined sections (Fig. 3A). Glands were often plugged with cellular debris (glandular abscesses; Fig. 3B). Occasional cellular

debris and inflammatory cells were noted along the luminal epithelium but infectious agents were not discernible and special stains for Gram organisms or fungi were negative. The sections of lymph node revealed proliferation of lymphoid follicles but inflammatory cells were not noted.

CONCLUSIONS

This report of *S. phocae*-associated pyometra in a spotted seal in Kotzebue Sound (Alaska, USA) indicates a wider geographic and species distribution of this potentially pathogenic agent. Pyometra as the primary finding has not been described for *S. phocae*. Because this animal was sampled during subsistence harvesting nothing is known about the route of infection and if the pyometra was preceded by an abortion. Johnson et al. found *S. phocae* in preputial swabs of California sea lions, and suggested potential sexual transmission.⁶ That study further found an association between *S. phocae* colonization in female sea lions and urogenital carcinoma. The pathologic findings described in this case study suggest that *S. phocae* can influence populations without obvious signs such as mass strandings or abortions. The inevitable reduced fertility in affected animals will lead to reduced reproductive success, potentially resulting in negative consequences for population dynamics. However, more knowledge about this pathogen and its prevalence is needed to assess this possibility. The effects may be potentially dire for very small populations, especially when considering the possible morbidity and mortality experienced by reproductively active females. Although no human cases involving *S. phocae* have been described in the literature, the potential for this bacterium to cause infections in humans handling infected pinnipeds cannot be excluded. Given that *S. phocae* is not restricted to pinnipeds but also is a major pathogen in salmon mariculture, this bacterium may be of greater importance in the marine ecosystem than previously thought.

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Figure 1. Gram stain of uterine contents. Gram-positive cocci in chain-like arrangement are indicated by arrows.

Figure 2.

Phylogenetic trees based on genomic sequences from isolated bacteria obtained from the uterus of a spotted seal in Alaska. **A.** 16S ribosomal RNA gene. *Streptococcus phocae* sequences as well as the most closely related *Streptococcus* type strain sequences with at least 800 base pairs aligning with our isolates were downloaded from the 16S rRNA database project and a tree constructed using the most likelihood method and the program PAUP*. Individual *S. phocae* sequences are indicated by their GenBank accession numbers. The grouping of the isolate with *S. phocae* sequences has a bootstrap support of 100. **B.** Phylogenetic tree using partial sequences of the *cpn60* gene also yielded bootstrap support

of 100 for the grouping of the isolate with *S. phocae*. C. Phylogenetic tree based on partial sequence of the *sodA* gene also yielded a bootstrap support of 100 for the grouping of the isolate with *S. phocae*.

Figure 3.

Photomicrographs of the uterus of a spotted seal (*Phoca largha*) with pyometra. Hematoxylin and eosin stain. **A.** The distribution of the inflammatory infiltrate was generally limited to the mucosal surface (arrows). **B.** The inflammation was characterized by glandular abscesses (arrow) and an inflammatory cell infiltrate composed of plasma cells, macrophages, neutrophils, and lymphocytes (arrow-heads). Double arrows demonstrate freeze artifact.

Table 1

Primers used for amplification and sequencing of *S. phocae* genes.

Target gene	Primer name	Sequence 5'–3'
16S rRNA	F1	GAG TTT GAT CCT GGC TCA G
16S rRNA	R1	GWA TTA CCG CGG CKG CTG
16S rRNA	16s_StrpGenome_F	CTG TCA GCG ACA GAA AGA ACG AGT
16S rRNA	16s_StrpGenome_B	CCA GCC GCA CCT TCC GAT ACG
16S rRNA	16s_StrpGenome_FM	GAT ACC CTG GTA GTC CAC GC
<i>cpn60</i>	H729	CGC CAG GGT TTT CCC AGT CAC GAC GAI III GCI GGI GAY GGI ACI ACI AC
<i>cpn60</i>	H730	AGC GGA TAA CAA TTT CAC ACA GGA YKI YKI TCI CCR AAI CCI GGI GCY TT
<i>cpn60</i>	M13F	CGC CAG GGT TTT CCC AGT CAC GAC
<i>cpn60</i>	M14B	AGC GGA TAA CAA TTT CAC ACA GGA
<i>sodA</i>	sodAd1_F	CCN TAY NCN TAY GAY GCN YTN GAR CC
<i>sodA</i>	sodAd2_R	ARR TAR TAN GCR TGY TCC CAN ACR TC