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ENDEMIC INFECTION OF STRANDED SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*) WITH NOVEL PARVOVIRUS, POLYOMAVIRUS, AND ADENOVIRUS

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ABSTRACT: Over the past century, the southern sea otter (SSO; *Enhydra lutris nereis*) population has been slowly recovering from near extinction due to overharvest. The SSO is a threatened subspecies under federal law and a fully protected species under California law, US. Through a multiagency collaborative program, stranded animals are rehabilitated and released, while deceased animals are necropsied and tissues are cryopreserved to facilitate scientific study. Here, we processed archival tissues to enrich particle-associated viral nucleic acids, which we randomly amplified and deeply sequenced to identify viral genomes through sequence similarities. Anelloviruses and endogenous retroviral sequences made up over 50% of observed viral sequences. Polyomavirus, parvovirus, and adenovirus sequences made up most of the remaining reads. We characterized and phylogenetically analyzed the full genome of sea otter polyomavirus 1 and the complete coding sequence of sea otter parvovirus 1 and found that the closest known viruses infect primates and domestic pigs (*Sus scrofa domesticus*), respectively. We tested archived tissues from 69 stranded SSO necropsied over 14 yr (2000–13) by PCR. Polyomavirus, parvovirus, and adenovirus infections were detected in 51, 61, and 29% of examined animals, respectively, with no significant increase in frequency over time, suggesting endemic infection. We found that 80% of tested SSO were infected with at least one of the three DNA viruses, whose tissue distribution we determined in 261 tissue samples. Parvovirus DNA was most frequently detected in mesenteric lymph node, polyomavirus DNA in spleen, and adenovirus DNA in multiple tissues (spleen, retropharyngeal and mesenteric lymph node, lung, and liver). This study describes the virome in tissues of a threatened species and shows that stranded SSO are frequently infected with multiple viruses, warranting future research to investigate associations between these infections and observed lesions.

Key words: Adenovirus, *Enhydra lutris nereis*, metagenomics, parvovirus, polyomavirus, southern sea otter, virome.

INTRODUCTION

Genetic sequence from only four viruses has been reported in sea otters (SO; *Enhydra lutris*). A gammaherpesvirus (mustelid herpesvirus 2) genome was partially characterized by a 219 base pair (bp) PCR fragment amplified from ulcerated oral lesions, which was also detected in nasal secretions of healthy northern SO (NSO; *Enhydra lutris kenyoni*; Tseng et al. 2012). The partial sequence of a SO poxvirus DNA–dependent DNA polymerase gene (2,233 bp) was sequenced from ulcerated skin lesions from both NSO and southern SO (SSO; *Enhydra lutris nereis*; Tuomi et al. 2014). The partial sequence of phocine distemper virus, a morbillivirus, was amplified from nasal swabs obtained from live NSO and lung, lymph node, and brain from dead NSO (Goldstein et al. 2009). We recently reported the first complete viral genome from SSO, a papillo-

mavirus associated with oral lesions (Ng et al. 2015b).

Here, we present an initial characterization of the SO virome by using metagenomics screening of archival frozen tissues from necropsied SSO collected by the California Department of Fish and Wildlife and collaborating agencies from 2000–13. Using PCR, we estimated the prevalence and tissue distribution in available tissues of three novel DNA viruses, SO parvovirus 1, SO polyomavirus 1, and SO adenovirus-like sequence 1.

MATERIALS AND METHODS

Tissue samples

We necropsied SSO that were found dead with minimal autolysis or died under care. Postmortem examinations included detailed gross examination, histopathology, and storage at –80 C of representative tissues from each case. To assess infection patterns over time, we randomly selected a subsample of three to seven necropsied SSO for each year from 2000–13, for a total of 69 animals. For each case, spleen, retropharyngeal lymph node, axillary or hilar lymph node, inguinal, perigastric or mesenteric lymph node, liver, and lung, where available, were analyzed.

Viral nucleic acid extraction and metagenomics library preparation

Prior to testing, tissue samples were grouped in 14 pools containing tissues from three to seven animals each, according to the year of collection. Each tissue pool was homogenized in 600 µL of phosphate-buffered saline with a tissue homogenizer (Omni International, Inc., Kennesaw, Georgia, USA) and frozen thawed thrice on dry ice. The homogenate was then filtered through a 0.45-µm filter (Merck Millipore, Billerica, Massachusetts, USA) to remove large cellular debris, and 200 µL of the filtrate was treated with DNase and RNase enzymes (Turbo DNase, Thermo Fisher Scientific, Waltham, Massachusetts, USA; Baseline Zero DNase Epicentre, Madison, Wisconsin, USA; and RNase A, Thermo Fisher Scientific) at 37 C for 90 min to degrade unprotected nucleic acid. The DNA and RNA protected from degradation within viral particles were then extracted with a QIAamp viral RNA Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. We added RiboLock RNase Inhibitor (Thermo Fisher Scientific) to prevent RNA degradation. Viral RNA and DNA were stored at –20 C.

Viral metagenomics were performed according to a previously described protocol (Ng et al. 2012, 2015a). Briefly, reverse transcription was performed using a 28-base oligonucleotide whose 3′ end consisted of a random hexamer (Primer N1_8N, CCTTGAAGGCGGACTGTGAGNNN NNNNN). After incubation at 85 C for 2 min, the reactions were cooled on ice for 2 min and SuperScript III (Thermo Fisher Scientific), deoxynucleoside triphosphate (dNTP; Thermo Fisher Scientific), DTT (Thermo Fisher Scientific), and 1× First-Strand Buffer (Thermo Fisher Scientific) were added and incubated at 25 C for 10 min, 50 C for 60 min, 70 C for 15 min and then at 95 C for 2 min. Subsequently, the second strand of DNA was synthetized with Klenow Fragment (New England Biolabs, Ipswich, Massachusetts, USA) at 37 C for 1 hr. The resulting double-stranded cDNA and DNA was then PCR-amplified by using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific) using Primer N1, CCTTGAAGGCGGACTGTGAG.

Libraries were prepared for each pool with Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, California, USA) according to the manufacturer's protocol, with 12 cycles of PCR amplification, and purified with QIAquick PCR Purification Kit (Qiagen). Fragments ranging in size from 400 to 800 bp were selected by using Pippin Prep (Sage Science, Inc., Beverly, Massachusetts, USA) and quantified by qPCR with the KAPA library quantification kit (Kapa Biosystems, Wilmington, Massachusetts, USA). Purified libraries were sequenced on a Miseq Illumina platform (Illumina) using 2×250 paired ends.

Data analysis

Sequence reads (reads) were grouped based on the dual barcodes used. Bacterial reads were filtered by using Bowtie 2 (Langmead and Salzberg 2012) with RefSeq genomes database (Brister et al. 2015). Identical reads were identified, and only one retained. Remaining reads were trimmed to remove nucleotide positions with Phred quality score lower than 10 and were then used for de novo assembly by using Ensemble Assembler 1.0 (Deng et al. 2015). The contiguous sets of overlapping sequences (contigs) generated by de novo assembly and the single reads (singlets) were compared with the viral proteome database in RefSeq by using the Basic Local Alignment Tool translated nucleotide versus protein (BLASTx; Camacho et al. 2009). Sequences showing an expectation (E value) <0.01 were then compared with an in-house, nonviral proteome database derived from GenBank's NR (nonredundant) database by using BLASTx, and only sequences more closely related to viral than non-viral sequences (with lower E scores) were retained. Sequences showing an E value of $<10^{-5}$

against viral proteins were sorted by viral taxonomy.

Generation of genome sequences

Potential new viral sequences were aligned with the most similar GenBank viral genome by using Geneious Version 6.1.8 (Biomatters, Auckland, New Zealand), and PCR primers were designed to bridge gaps and complete the genome sequences by PCR and Sanger sequencing. Protein sequences (NS1 for the parvovirus and large T for polyomavirus) were translated and aligned in Geneious Version 6.1.8 (Biomatters). Resulting alignments were assessed via MEGA6 (Tamura et al. 2013) to create maximum likelihood phylogenetic trees.

Detection of viral DNA in tissues

Tissue samples were pooled by animal, homogenized in 600 µL of phosphate-buffered saline with tissue homogenizer (Omni International), and the nucleic acid was extracted directly with a QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer's protocol. Subsequent nested PCR reactions with Taq DNA Polymerase (New England Biolabs) were performed for SO adenovirus-like sequence 1, SO parvovirus 1, and SO polyomavirus 1 in all samples and for SO retrovirus-related sequence in 10 random samples with the primers described in the following. Each PCR reaction contained 2 µL of the nucleic acid extracted or first-round product, 0.75 µL (3.75 units) of Taq DNA Polymerase (New England Biolabs), 1× PCR buffer, 1.25 µL of dNTP (10 mM), and 2.5 µL of each primer (10 µM) in a 50-µL reaction volume. Individual tissue samples from virus-positive animals were tested by using a direct extraction PCR kit (MyTaq Extract-PCR, Bioline, London, UK). The first round was performed with 12.5 µL of MyTaq HS Red Mix, 1 µL of each primer (10 µM), and 1 µL of the extraction product in 25-µL reaction volume. The second round was performed as described previously with 1 µL of the first-round PCR product and Taq DNA Polymerase (New England Biolabs). The conditions for all PCR reactions were 95 C for 2 min, 5 cycles of 95 C for 1 min, 55 C for 1 min and 72 C for 1 min, followed by 35 cycles of 95 C for 30 s, 55 C for 30 s, and 72 C for 30 s, and a final extension at 72 C for 10 min. PCR products of the correct size were detected by 1.5% agarose gel electrophoresis. The primers designed for nested adenoviral PCR amplified a 450-bp fragment (SOadenoF1 5′-GTCGCCGTCTATGTCAGTG, SOadenoR1 5′-TCCGTCTGAGGGAAGGAACA, SOadenoF2 5′-GGGTAATGTTTGGAAGGTTTG, and SOadenoR2 5′-GCCTCTCTATGACACAACTAA). Nested primer pairs for parvoviral detection amplified a product of 319 bp (SOParvoF16 5′-TTTGGAGGGAATCCTACACATA, SOParvoR16 5′-TCCATTTTCCAGAGGCTTGTGGT, SOParvoF26 5′-AAACACAAAAATAACTGCTCACTCT, and SOParvoR26 5′-CTGCTGTTAGTATAAGTCCTTG). Nested primer pairs designed for polyomaviral detection amplified a 335-bp fragment (SOPolyoF14 5′-TGATGAGGGGAGAGCATTCC, SOPolyoR14 5′-TAAAAAACCCCTACCCAGTGAATG, SOPolyoF24 5′-CAGAAGACACAGATGATGGATT, and SOPolyoR24 5′-GGCTGTTTTCTTCAAGTATCCCA). Finally, retroviral primer pairs were designed for seminested PCR with a final product of 320 bp (SORetroF1 5′-TTTTGGTCCTCCCTAATGCCC, SORetroR1 5′-GGAGGTTCTGTACTCACGCAA, and SORetroF2 5′-GGGAAGCAAAGGAAGGGATCA). All primers were based on sequences obtained from the metagenomics analysis and deposited in GenBank accession nos.: KU561553 (SO adenovirus-like sequence 1), KU561552 (SO parvovirus 1), NC_025259 (SO polyomavirus 1), and KX018258 (endogenous SO retrovirus). Chi-square statistic was used to test whether the prevalence of infections changed over the 14 yr of sampling.

RESULTS

Tissues from 69 stranded SSO were analyzed by using viral metagenomics. Tissues were pooled by year of collection from 2000–13, and viral sequences were characterized by deep sequencing after random amplification of nuclease-resistant RNA and DNA from homogenized and filtered samples. Liver was included from all 69 animals, followed by lung and spleen (n=67 each), mesenteric lymph node and retropharyngeal lymph node (n=55 each), inguinal lymph node (n=5), hilar lymph node (n=3), axillary lymph node (n=2), and perigastric lymph node (n=1).

An average of 256,000 sequence reads were generated from each of the 14 tissue pools. After trimming to remove primer sequence, removal of duplicates and low quality reads, and de novo assembly, an average of 148,048 (range: 1,588–268,660) sequences were compared with a viral database using BLASTx. Contigs and single reads were analyzed to detect translated sequence similarity to proteins encoded by all eukaryotic viruses in GenBank's viral genome reference database.

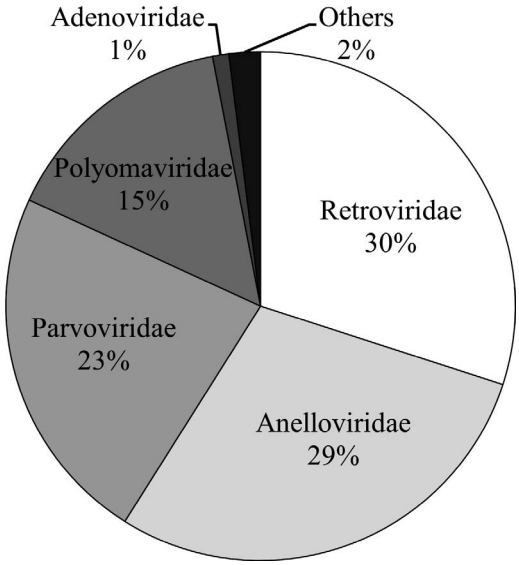

FIGURE 1. Distribution of sequence reads generated by viral metagenomics with translated protein sequence similarity to different viral families for samples from necropsied southern sea otters (*Enhydra lutris nereis*) sampled from 2000–13.

Sequences from the viral families *Retroviridae*, *Anelloviridae*, *Parvoviridae*, *Polyomaviridae*, and *Adenoviridae* were the most prevalent in these tissue pools. A smaller number of sequences showing similarities to those of the families *Circoviridae*, *Papillomaviridae*, and *Herpesviridae* were also found (Fig. 1). We focused further sequencing on two of the four viruses generating the most sequence reads from the *Parvoviridae* and *Polyomaviridae* families. Anelloviruses were not further analyzed as this highly diverse and common family of viruses is known to infect many mammals resulting in chronic viremia and is generally considered to be a commensal infection. Only some anelloviruses in pigs have been associated with disease during coinfections with porcine circovirus 2 (Okamoto 2009; Kekarainen and Segales 2012; Spandole et al. 2015).

Retrovirus-related sequences were also detected, including a 4,350 base long contig (submitted to GenBank, accession no. KX018258), to which 25% of the retrovirus-like sequences aligned. The closest match in GenBank was an endogenous retrovirus from the domestic ferret (*Mustela putorius furo*), with 92% nucleotide identity over 59% of the query. Sea otters and ferrets belong to the same family (Mustelidae), in the suborder Caniformia. The genome of ferrets is currently the closest available genome to that of SO. We designed primers to this retrovirus-related sequence, and all 10 random SSO sample pools tested were PCR positive for this sequence in the absence of reverse transcription. The retroviral contig, therefore, likely reflects the presence an endogenous (germline) SO retrovirus. Other retroviral sequences could be assigned to the reverse transcriptase enzyme used in the generation of the metagenomic library (Li et al. 2015).

Novel SO parvovirus and polyomavirus

Parvovirus-related contigs were detected, which were most closely related to the partial genome from domestic pig protoparvovirus Zsana/2013/HUN (GenBank accession no. KT965075), described by Hargitai et al. (2016). These contigs were joined by PCR and sequenced by Sanger to generate a nearly complete genome from SO parvovirus 1 with 4,639 bases. The NS1 shared 67% amino acid identity to the partial NS1 available from the domestic pig protoparvovirus Zsana/2013/HUN and 59% to the complete NS1 of the rhesus macaque (*Macaca mulatta*) WUHARV parvovirus (GenBank accession no. JX627576) described by Handley et al. (2012). The SO parvovirus 1 VP1 capsid protein shared 64% identity to the partial protein from the protoparvovirus Zsana/2013/HUN, and 61% to that of the rhesus macaque WUHARV parvovirus. These results, together with phylogenetic analysis, indicate that the SO parvovirus 1 belongs to a new species in the *Protoparvovirus* genus (Cotmore et al. 2014), whose closest known relative infects pigs (Fig. 2).

For SO polyomavirus 1, the large T protein sequence clustered closest to that of some primate polyomaviruses (Fig. 2). The complete genome was 5,145 bases and was 60% identical to both *Chlorocebus pygerythrus*

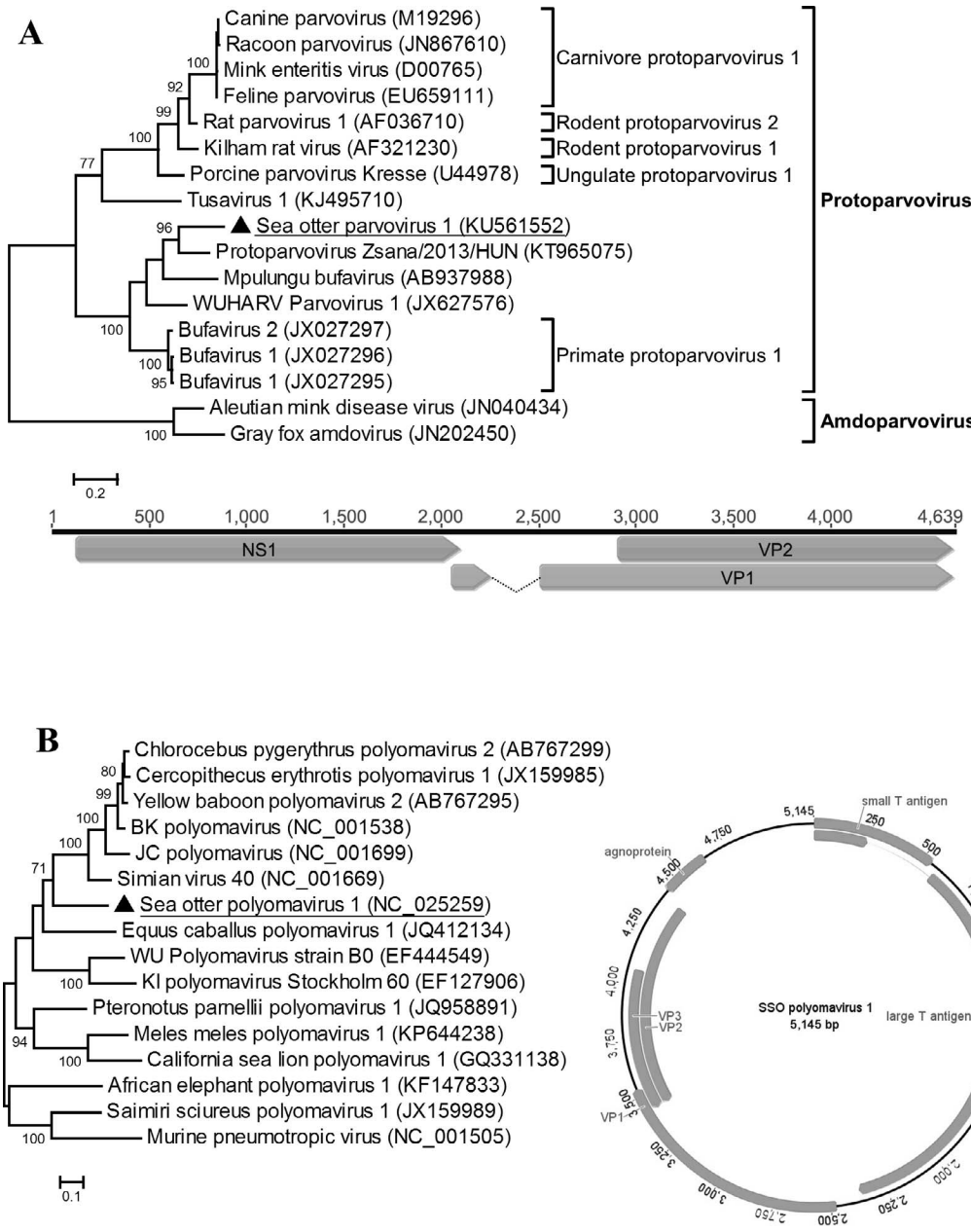

FIGURE 2. Phylogenetic analyses and genome organization of sea otter (*Enhydra lutris*) parvovirus 1 and sea otter polyomavirus 1. Phylogenetic analysis of parvovirus NS1 (A) and polyomavirus large T protein (B) using the maximum likelihood method in MEGA6 (Tamura et al. 2013). SSO=southern sea otter.

polyomavirus 2 (GenBank accession no. AB767299) from the Vervet monkey (*C. pygerythrus*; Yamaguchi et al. 2013), and BK polyomavirus (GenBank accession no. NC_001538) from humans (Seif et al. 1979). The large T antigen protein also shared 60% amino acid identity to that of the *C. pygerythrus* polyomavirus 2 and BK polyomavirus.

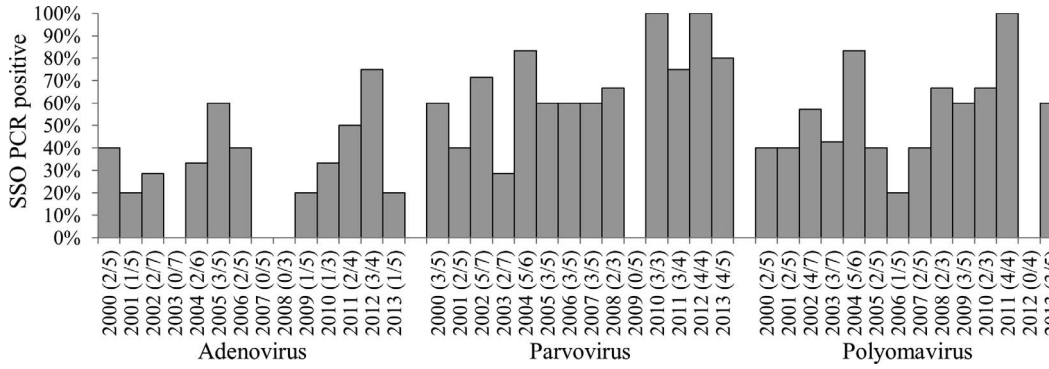

FIGURE 3. Prevalence of sea otter adenovirus-like, parvovirus, and polyomavirus DNA in a subset of tissue pools from 69 stranded southern sea otters (SSO; *Enhydra lutris nereis*) necropsied from 2000–13.

Sea otter adenovirus

We found 10 adenovirus sequence reads in tissue pools from six different years, showing between 40% to 85% identity to different *Mastadenovirus* proteins (penton, pIIIa, IVa2, 100K, DNA polymerase, and fiber). The longest sequence of 500 bases (hereafter designated as SO adenovirus-like sequence 1), was deposited in GenBank (accession no. KU561553) and showed 69% amino acid identity to penton base protein from equine adenovirus 2 (GenBank accession no. KT160425; Giles et al. 2015).

Prevalence of SO adenovirus, parvovirus, and polyomavirus DNA over time and in different tissues

Pooled tissue samples from each of 69 animals were created and screened by PCR with primers designed to detect SO adenovirus-like sequence 1, SO parvovirus 1, and SO polyomavirus 1. Overall 80% (55/69) of tested SO were infected with at least one of the three novel viruses. The SO parvovirus 1 infection was the most prevalent (61%), followed by SO polyomavirus 1 (51%), and SO adenovirus (29%). Coinfection by all three viruses was detected in eight animals (12%), and dual infections occurred in 26 otters (38%). Infections were present in almost all sample years from 2000–13 (Fig. 3). The proportion of SSO testing positive for any of these 3 viruses did not significantly increase or decrease over time.

Individual tissues from PCR-positive tissue pools were separately extracted, and PCR was performed to assess tissue-specific viral prevalence. Among 20 animals testing positive for SO adenovirus-like sequence 1, the virus was detected at least once in all tissues tested, except in a single inguinal lymph node. The prevalence of adenovirus in the different tissues varied from 35% to 90% (Table 1). Among 42 animals testing positive for SO parvovirus 1, detection was most common in mesenteric lymph nodes (78%), compared with all other examined tissues (<10%). For 35 animals infected with SO polyomavirus 1, nearly all spleens were PCR positive (34/35; 97%), while livers, lungs, and lymph nodes were less commonly positive (Table 1).

DISCUSSION

Reports of viral infection in SO have been published over the years, but little is known about the virome commonly infecting these marine mustelids. Herpesvirus (mustelid herpesvirus 2; Tseng et al. 2012), poxvirus (SO poxvirus; Tuomi et al. 2014), morbillivirus (phocine distemper virus; Goldstein et al. 2009), and papillomavirus (*Enhydra lutris* papillomavirus 1; Ng et al. 2015b) nucleic acid and positive serology for influenza (White et al. 2013) have been reported in

TABLE 1. Frequency of detection of DNA of three viruses in various tissues from stranded southern sea otters (SSO; *Enhydra lutris nereis*) necropsied from 2000–13.

Tissue	% Specific tissues from SSO PCR positive for viruses (no. sampled)		
	Adenovirus[a]	Parvovirus	Polyomavirus
Liver	35 (20)	7 (42)	17 (35)
Lung	60 (20)	5 (42)	21 (34)
Lymph node axillary	—	0 (1)	100 (1)
Lymph node hilar	—	0 (2)	0 (1)
Lymph node inguinal	0 (1)	0 (1)	100 (1)
Lymph node mesenteric	47 (17)	78 (37)	13 (31)
Lymph node retropharyngeal	61 (18)	9 (35)	33 (30)
Spleen	90 (20)	7 (41)	97 (35)

[a] — = not applicable.

SO. Among viruses previously described from SO, we also detected a few reads mapping to the family *Herpesviridae* that did not overlap with a 219-bp sequence from the DNA-dependent DNA polymerase gene of the previously reported gammaherpesvirus from NSO (Tseng et al. 2012), precluding direct comparison. Our metagenomics analysis detected nucleic acids from several viral families not previously reported in SO, including a high proportion of reads from the family *Anelloviridae* (29%), *Parvoviridae* (23%), and *Polyomaviridae* (15%). The discovery of sequences from viral families known to infect mammals, detection in internal tissues rather than nonsterile sites exposed to the environment, such as feces and respiratory samples, and common PCR amplification from a large fraction of tissues from animals collected over a number of years increases our confidence that these viral genomes indicated SSO infection, rather than environmental or other contamination. Because our testing was performed on a subsample of stranded, randomly selected SSO, the high frequency of virus detection is likely reflective of their overall prevalence in this population. The prevalence of these viruses in nonstranded animals is unknown.

Anelloviruses have been reported in a wide range of mammals, including in feces or tissue from the Caniformia suborder (e.g., Pacific harbor seal, *Phoca vitulina*; Ng et al. 2011), sea lion (*Zalophus californianus*; Ng et al. 2009; Fahsbender et al. 2015), American pine marten (*Martes americana*; van den Brand et al. 2012), and domestic ferret (Smits et al. 2013). We also detected retrovirus sequence, but based on universal PCR detection (without reverse transcription of RNA) in all SSO samples tested and closely related sequences in the domestic ferret genome, these sequences likely represent endogenous retroviral elements.

A large proportion of viral sequence reads from SSO tissues belonged to a parvovirus whose genome was nearly completely characterized, and was most closely related to protoparvovirus Zsana/2013/HUN (GenBank accession no. KT965075) from domestic pigs. The SO parvovirus 1 was present in 61% (42/69) of stranded animals tested, during all but one of the 14 yr analyzed, demonstrating persistence of this virus in this population. The most common site of detection was the mesenteric lymph node. Infection with SO parvovirus 1 appears to be common in California SSO, and detection was most common in lymphoid tissue associated with the intestinal tract. Protoparvoviruses have been detected in animal feces and from human patients with and without diarrhea (Handley et al. 2012; Phan et al. 2012; Smits et al. 2014; Väisänen et al. 2014; Yahiro et al. 2014; Sasaki et al. 2015).

An outbreak of canine parvovirus 2 infection was described in Asian small-clawed otters (*Aonyx cinerea*; Gjeltema et al. 2015), and serology for this virus was positive in North American river otters (*Lontra canadensis*; Kimber et al. 2000). Another parvovirus known to infect mustelids is the *Amdoparvovirus* Aleutian mink disease virus, which can be pathogenic in American mink (*Neovison vison*), European mink (*Mustela lutreola*), and other mustelids (Bloom et al. 1994; Nituch et al. 2011; Nituch et al. 2012). Interestingly, Aleutian mink disease virus was not found in river otters sympatric with infected American Mink (Bowman et al. 2014). Although Aleutian disease appears to be an emerging issue of free-ranging striped skunks (*Mephitis mephitis*), including animals from coastal California (LaDouceur et al. 2015), this virus was not detected in the partial virome of sympatric SSO from the current study.

The SO polyomavirus 1 is the second polyomavirus described in hosts from the Mustelidae family (Hill et al. 2015). This polyomavirus genome showed 60% nucleotide identity with the closest known polyomavirus genomes: *C. pygerythrus* polyomavirus 2 from the Vervet monkey, and BK polyomavirus from humans (Johne et al. 2011). Phylogenetic analysis indicates that the SO polyomavirus 1 clusters with BK, JC, and nonhuman primate polyomaviruses, with greater genetic distance from the polyomavirus infecting European badgers (*Meles meles* polyomavirus 1; Hill et al. 2015). Polyomavirus DNA was detected in SSO tissue during all but one of the 14 yr analyzed, suggesting endemic infection. The tissue with the highest prevalence of polyomavirus DNA detection was spleen (97%; 34/35) disregarding the inguinal and axillary lymph nodes with only one available sample each. Analysis of polyomavirus infections of nonhuman primates also reported spleen as the most frequently infected tissue (Scuda et al. 2013). The *Meles meles* polyomavirus 1 was most prevalent in badger liver and mediastinal and bronchial lymph nodes (Hill et al. 2015), while human and nonhuman primate polyomavirus genomes closest to the SO polyomavirus 1 were detected mainly in kidney, spleen, intestine, and respiratory tract (Scuda et al. 2013; Yamaguchi et al. 2013).

The *Adenoviridae* family of viruses is genetically diverse. Several adenoviruses have been reported from aquatic mammals, including canine adenovirus 1 from a captive Eurasian river otter (*Lutra lutra*; Park et al. 2007) and California sea lion adenovirus 1 from California sea lions, northern fur seals (*Callorhinus ursinus*), and South American sea lions (*Otaria flavescens*; Goldstein et al. 2011; Inoshima et al. 2013). The SO adenovirus-like sequence 1 DNA was detected in 29% of tested SSO, and in most tissues analyzed, including liver, spleen, lung, and mesenteric and retropharyngeal lymph nodes. Canine adenovirus can also infect multiple tissues, including lung, liver, spleen, kidney, brain, lymph nodes, heart, and intestine, and the sea lion adenovirus 1 was associated with endothelial cell infection of multiple tissues (Benetka et al. 2006; Goldstein et al. 2011; Choi et al. 2014).

The PCR detection rates for SO parvovirus 1, polyomavirus 1, and adenovirus-like DNA did not change significantly during 14 yr of stranded SSO sampling. Given their high prevalence, it is possible that infection by these viruses is asymptomatic or of low pathogenicity. It is also conceivable that some specific pathologies may be induced by these viruses in SSO in poor health due to starvation, stress, or by other infections or factors that prevent an effective response to viral infection. The low genetic diversity of SO (Larson et al. 2012) due to historical overharvest could also enhance susceptibility to viral infection, when compared with more outbred species. The viruses described here and in prior studies are unlikely to reflect all viruses infecting SSO, as transient and rare infections may not have been sampled. Future screening of samples not tested as part of the current study, including brain, feces, and respiratory secretions, is likely to yield additional viruses with distinct tropisms. Our partial description of the SSO virome and PCR detection in a subset of

tissues from stranded SSO shows a high prevalence of these endemic viruses and warrants further studies to assess their genetic diversity, evolution, and pathophysiology.

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