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A NOVEL GAMMAHERPESVIRUS IN NORTHERN FUR SEALS (*CALLORHINUS URSINUS*) IS CLOSELY RELATED TO THE CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS*) CARCINOMA-ASSOCIATED OTARINE HERPESVIRUS-1

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ABSTRACT: *Otarine herpesvirus 1* (OtHV1) is strongly associated with California sea lion (CSL, *Zalophus californianus*) urogenital carcinoma, the most common cancer documented in marine mammals. In addition to CSL, OtHV1 has also been found in association with carcinoma in South American fur seals (*Arctocephalus australis*), demonstrating it can infect related species. Northern fur seals (NFS, *Callorhinus ursinus*) are sympatric with CSL, and copulation between these species has been observed; yet, there are no reports of urogenital carcinoma in NFS. We describe a new Otarine herpesvirus found in vaginal swabs from NFS, herein called OtHV4. Partial sequencing of the polymerase gene and the glycoprotein B gene revealed OtHV4 is closely related to OtHV1, with 95% homology in the region of polymerase sequenced, and phylogenetic analyses demonstrate that they are sister taxa. An OtHV4-specific hydrolysis probe quantitative PCR was developed and validated, and its use on vaginal swabs revealed 16 of 50 (32%) wild adult female NFS were positive for OtHV4. The identification of a virus highly similar to the carcinoma-associated OtHV1 in a sympatric species without carcinoma suggests that comparative genomics of OtHV1 and OtHV4 may identify candidate viral oncogenes.

Key words: Herpesvirus, northern fur seal, *Otarine herpesvirus 1*, *Otarine herpesvirus 4*, phylogeny.

INTRODUCTION

Herpesviruses are enveloped, large, double-stranded DNA viruses with high host fidelity (Pellett et al. 2011). The order *Herpesvirales* has three families: *Alloherpesviridae*, *Malacoherpesviridae*, and *Herpesviridae*. There are three subfamilies of *Herpesviridae*: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Only alphaherpesviruses and gammaherpesviruses have been reported in marine mammals, with at least 19 of these characterized using partial polymerase sequences (Maness et al. 2011). Three gammaherpesviruses have been reported from otariids: *Otarine herpesvirus 1* (OtHV1) is strongly associated with urogenital carcinoma in California sea lions (CSL, *Zalophus californianus*; Lipscomb et al. 2000; King et al. 2002) with a prevalence of 22% in adult females and 46% in adult males (Buckles et al. 2007); OtHV2 was identified from an ocular swab of a CSL with conjunctivitis, and OtHV3, first identified in an animal with lymphoma, was found in 30.7% of CSL samples surveyed (Maness et al. 2011; Venn-Watson et al. 2012).

Otarine herpesvirus 1 is primarily detected in the CSL urogenital tract, and sexual transmission, similar to *Human herpesvirus 2* (HHV2) transmission, has been hypothesized because OtHV1 has a higher prevalence in adult males than in adult females or juveniles (Buckles et al. 2007). Urogenital carcinomatosis has been commonly reported in stranded CSL in California since 1979 (Gulland et al. 1996) and is a major cause of mortality in CSL; some studies report OtHV1 in 100% of these

urogenital carcinomas (Lipscomb et al. 2000; King et al. 2002; Colegrove et al. 2009). Dagleish et al. (2012) found OtHV1 in a captive female South American fur seal (*Arctocephalus australis*) in association with urogenital carcinoma, consistent with the hypothesis that OtHV1 has a significant role in the etiology of urogenital carcinoma, and indicating other otarid species may be susceptible to OtHV1 infection.

Herpesviruses are considered to have high host fidelity (Pellett et al. 2011), but host jumps have been described among closely related host species. For example, human herpesvirus 1 (HHV1) has been detected in white-handed gibbons (*Hylobates lar*), causing a multifocal lymphoplasmacytic meningoencephalitis (Landolfi et al. 2005), and in a confiscated eastern lowland gorilla (*Gorilla beringei graueri*) with vesicular stomatitis (Gilardi et al. 2014). Human herpesvirus 4 (HHV4) causes lymphoma in infected cotton-top tamarins (*Saguinus oedipus*; Young et al. 1989). Northern fur seals (NFS, *Callorhinus ursinus*) are sympatric with CSL along the west coast of the US, and copulation between these two species in the wild has been observed.

We investigated the possibility of interspecies transmission of OtHV1 between CSL and NFS. We identified a novel otarid herpesvirus, herein called OtHV4, that is the sister taxon of OtHV1, and developed a hydrolysis probe quantitative PCR (qPCR) assay specific for OtHV4 to screen additional samples.

MATERIALS AND METHODS

Samples

Vaginal swabs from 20 apparently healthy adult female NFS were collected in the Pribilof Islands, Alaska, US, in 2011 (Buckles et al. 2007) under Marine Mammal Protection Act permit 932-1905iMA-009526. We used 51 NFS samples for the qPCR assay, including the first set of 20 samples and a second set of 30 samples collected from adult females at San Miguel Island, California, US, in 2012. One additional vaginal swab from an animal in rehabilitation at The Marine Mammal Center (Sausalito, California, USA) was used. Additionally, 13 pinniped samples positive for other gammaherpesviruses were used for assay validation. We extracted DNA from the first set of samples using a commercial kit (DNeasy Blood and Tissue, Qiagen Inc., Valencia, California, USA). We extracted DNA from the additional samples with a Maxwell 16 Buccal Swab Purification kit using a Maxwell automated extractor (Promega, Madison, Wisconsin, USA).

PCR and phylogenetic analysis

For the first set of 20 samples, amplification was done using a previously described, consensus, nested PCR protocol targeting the DNA-depended DNA polymerase (pol) gene (VanDevanter et al. 1996). The same PCR conditions were used in both rounds using Platinum Taq® DNA Polymerase (Platinum *Taq* DNA polymerase, Invitrogen, Carlsbad, California, USA). The PCR products from the second round were electrophoresed in 1% agarose gels, and bands of the expected size were cut and extracted using QIAquick gel extraction kits (Cat No. 28706; Qiagen). The PCR products were then sequenced using ABI 3130 DNA sequencers (Life Technologies, Carlsbad, California, USA). Extension of the pol sequence was carried out using a seminested protocol with the same amplification protocol mentioned above. We used a degenerate forward primer, SIIQ, and two OtHV4-specific reverse primers designed from the sequence already obtained; OtHV4R1 was used in the first round and OtHVR2 was used in the second round (Table 1). To obtain a second gene, published, nested protocols targeting a portion of the glycoprotein B gene (glyB) were used (Ehlers et al. 2008; Table 1). To compare it with OtHV4, the OtHV1 glyB was amplified and sequenced following the same protocol.

Partial polymerase amino acid sequences of 39 *Herpesviridae* viruses, including all recognized genera, were selected from GenBank and aligned using MAFFT with default settings (Katoh and Toh 2008). To determine the best amino acid substitution model, a corrected Akaike information criterion (AIC_c) was used in ProtTest 3.2.2 (Darriba et al. 2011).

Bayesian analyses of the amino acid alignments were performed using MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003) on the CIPRES server (Miller et al. 2015); HHV1 (GenBank accession CAA26941) was designated as the outgroup. The Bayesian analyses were run for 1,000,000 generations. Convergence among runs was evaluated by calculating the average split deviation using a threshold of

TABLE 1. Primers used for detection of Otarine herpesviruses and quantitative PCR (qPCR) assays from Northern fur seals (*Callorhinus ursinus*) from Alaska and California, USA, 2011–12.

Primer name	Sequence	Gene	Source
SIIQ	AGY ATH ATH CAR GCN CAY	Polymerase	Maness et al. 2011
OtHV4R1	CGATTTTTCAAGCATTGTGC	Polymerase	This study
OtHV4R2	CGATGAATTGCCTCGATTTT	Polymerase	This study
2759s	CCTCCCAGGTTCARTWYGCMTAYGA	Glycoprotein B	Ehlers et al. 2008
2762as	CCGTTGAGGTTCTGAGTGTARTARTTRTAYTC	Glycoprotein B	Ehlers et al. 2008
2760s	AAGATCAACCCCACNAGIGTIATG	Glycoprotein B	Ehlers et al. 2008
2761as	GTGTAGTAGTTGTACTCCCTRAACATIGTYTC	Glycoprotein B	Ehlers et al. 2008
OtHV4F1	GGCGATAATGCCTTCGCTTAAA	Polymerase	This study
OtHV4F2	TCCACAATGATACTCGGATGAAGA	Polymerase	This study
OtHV4R4	CTAGAATTGCACGACGCTGT	Polymerase	This study
QPCR_OtHV4F2	CTTCAACATTAGCTCCGGATT	Polymerase	This study
QPCR_OtHV4R2	CTTTACGCTTTGTTAGCCATGT	Polymerase	This study
QPCR_OtHV4probe2	AAAAAGCCATATATGTCAATCGCTACTATCAAA	Polymerase	This study

0.02%. Chains were sampled every 100 generations, and the first 20% were discarded as a burn-in. To determine whether this number of generations was adequate and to calculate whether we had effective sample sizes we used TRACER 1.5 (Rambaut and Drummond 2013). Maximum likelihood (ML) analyses were run in PhyML 3.0 (Guindon et al. 2010) with the best model of evolution according to AIC_c, using 100 bootstrap replicates to test the strength of the tree topology (Felsenstein 1985). Trees were edited using FigTree v1.3.1. (Rambaut 2010).

Quantitative PCR

Primers QPCR_OtHV4F2, QPCR_OtHV4R2, and QPCR_OtHV4probe2, were designed using Primer express version 3.0 (Applied Biosystems, Foster City, California, USA) to target a conserved area of the DNA-dependent DNA polymerase gene (Table 1). The probe was designed with Black Hole Quencher® and FAM as a reporter dye. To obtain a template for standard curves, the OtHV4 DNA-dependent DNA polymerase was amplified from positive samples using specific primers, with OtHV4F2 as the forward primer and OtHV4R4 as the reverse primer, which generated a fragment of 217 base pairs (bp), including primers (Table 1). The product was run in a 1% agarose gel and extracted using a QIAquick Gel extraction kit. The product was then quantified using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA), and dilutions were made with Tris-EDTA buffer ranging from 1 to 10^7 copies per well.

Each sample was run in duplicate, with one well of universal eukaryote 18S ribosomal RNA primer/probe (VIC Probe, Applied Biosystems) as an internal control. Each 20-μL reaction consisted of 4 μL DNA extract, 10 μL universal qPCR mix (TaqMan® Fast Universal PCR Master Mix 2×, Applied Biosystems), 3 μL water, and the concentrations of primers and probe found to be optimal. All reactions were run in a 7500 Fast Real-Time PCR System (Applied Biosystems) under a standard fast protocol. The amplification conditions were standard; 20 s of initial denaturation at 95 C, followed by 45 cycles of 95 C for 3 s and 62 C for 30 s. The slope and R^2 were calculated with the 7500 Fast Real-Time PCR System software. Results were calculated as copies detected per nanogram of DNA in the reaction. To test for nonspecific reactivity of the assay, samples containing other herpesviruses, including OtHV1 and OtHV3, were tested.

For confirmation of positive samples, we used the consensus, nested PCR protocol

targeting the pol gene (VanDevanter et al. 1996) and a combination of OtHV4 primers; using OtHV4F1 as the forward primer and OtHV4R2 as the reverse primer or OtHV4F2 as the forward primer and OtHV4R2 as the reverse primer (Table 1). For this assay, we used Platinum Taq DNA polymerase (Platinum *Taq* DNA polymerase). The initial denaturation was for 5 min at 95 C, followed by 45 cycles of denaturation at 95 C for 1 min, primer annealing at 6 C below the manufacturer-predicted melting temperature of the set of primers for 1 min, and extension at 72 C for 1 min, followed by a final elongation step at 72 C for 7 min. The PCR products were run in a 1% agarose gel, and fragments of the expected size were cut, extracted, and sequenced in both directions as noted earlier.

RESULTS

PCR and phylogenetic analysis

The nested PCR amplified a region of herpesvirus polymerase from one of the initial 20 samples, resulting in a product of 166 bp after primers were edited out. The protocol to obtain longer sequence using specific reverse the primers amplified a product of 450 bp after the primers were edited out. The sequence was submitted to GenBank under accession KP861868. For glyB, we obtained sequences of 453 bp after the primers were edited out (OtHV4 GenBank KP861869; OtHV1 GenBank KP861870).

Nucleotide sequence comparison revealed that this virus is 95% similar to OtHV1 using partial polymerase or partial glyB. At the amino acid level, partial polymerase of this virus is 95% similar to OtHV1, and the partial glyB is 97% similar to OtHV1.

Phylogenetic analysis was carried out using the best model of evolution available for MrBayes (RtREV substitution matrix with a gamma distribution, a proportion of invariant sites, and differing base frequencies) and ML (LG substitution matrix with a gamma distribution, a proportion of invariant sites, and differing base frequencies). Both analyses supported this herpesvirus as a novel gammaherpesvirus and as a sister taxon of OtHV1 with 100% posterior probability and 100% ML bootstrap support (ML tree, Fig. 1).

Quantitative PCR

The standard curve was represented by a linear relationship with an average slope of $-3.63 \pm 0.18\%$, an average efficiency of $88.93 \pm 5.37\%$, and an average R^2 of $98.58 \pm 0.69\%$. The dynamic range was from 10^7 to 10^1, and samples with amplification below 10 copies/well were considered below the limit of detection. In the first set of samples, the only consensus PCR-positive sample was also positive in the qPCR assay. Four additional samples were qPCR positive, and two other samples showed curves below the limit of detection. In the second set of samples, 11 samples were qPCR positive, with two of those samples only qPCR positive. Samples positive for OtHV1 and OtHV3 did not show amplification in the OtHV4 qPCR assay, indicating good analytical specificity.

Of the 51 samples analyzed by qPCR, 17 were positive (Table 2), five from the first set of samples and 11 from the second set, representing 25% and 37% prevalence for OtHV4 in each group, respectively. The additional stranded animal sample was also positive (CU13027).

DISCUSSION

Few viruses have been reported in samples from NFS; San Miguel Sea lion virus was reported by Sawyer et al. (1978), poxvirus infection was reported by Hadlow et al. (1980), and a polyomavirus was reported in placental tissue by Duncan et al. (2013). This is a low number, considering the diversity of viruses that can infect a species. There are eight known endemic human herpesviruses, and multiple herpesviruses reported in other marine mammals (Maness et al. 2011). This lack of known NFS viral diversity is likely due to a lack of investigation, and more research is needed to understand the virome of NFS.

We documented a novel herpesvirus from NFS, which is a close relative to

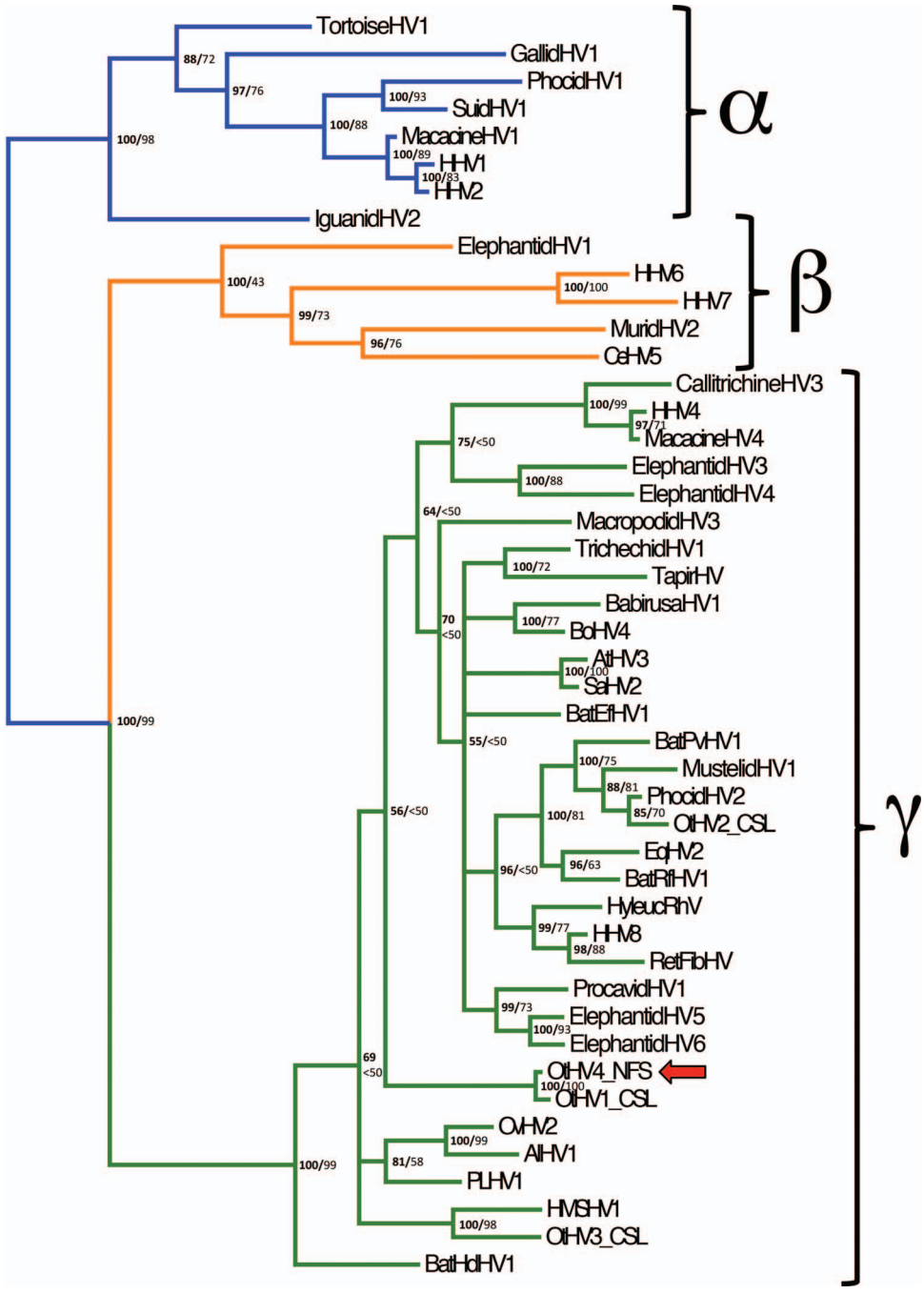

FIGURE 1. Bayesian phylogram depicting the relationship of the novel Otarine herpesvirus 4 (OtHV4, marked with an arrow) from a Northern fur seal (NFS; *Callorhinus ursinus*) with OtHV1 and other herpesviruses based on predicted amino acid sequences of herpesviral DNA-dependent-DNA polymerase sequences with MAFFT alignment. Bayesian posterior probabilities of branchings as percentages are in bold, and maximum likelihood bootstrap values for branchings based on 100 resamplings are given to the right or below. Numbers less than 50 are considered insignificant. Human herpesvirus 1 (HHV1) was used as an outgroup. Subfamilies are marked with brackets. Sequences retrieved from GenBank for phylogenetic analysis included AlHV1 (NP_065512), AtHV3 (NP_047983), BatRfHV1 (ABH05842),

OtHV1, with 95% amino acid sequence identity in the conserved region of the partial polymerase amplified. This similarity is comparable to that found in the same region between HHV1 and HHV2 (94%) and to that between HHV4 and *Macacine herpesvirus 4* (96%), supporting the hypothesis that these two viruses are independent, closely related species (Fig. 1). Although the genetic distance seen between OtHV1 and OtHV4 is slightly smaller than the distance between HHV1 and HHV2, which is often used as a benchmark for distinguishing species (Pellett et al. 2011), the ecologic distinction of host species indicates that OtHV1 and OtHV4 are separate species.

Although OtHV1 was first detected in CSL, it was recently detected in a urogenital carcinoma from a wild-born South American fur seal that was kept under human care for 11 yr in a zoological facility (Dagleish et al. 2012). This clinical case demonstrates that OtHV1 can infect otariids other than CSL. Northern fur seals are sympatric with CSL, and copulation between the two species has been observed, but urogenital carcinoma has not been reported in NFS. We identified OtHV4, a novel virus closely related to OtHV1, with 95% nucleotide similarity in two conserved partial genes, in a species without documented carcinoma. Based on our study, the prevalence of OtHV4 in NFS is comparable to the prevalence of OtHV1 in healthy CSL females (22%) (Buckles et al. 2007). As urogenital carcinoma has not been reported in NFS, this may indicate that this sister virus to OtHV1 lacks carcinogenic features.

Vaccination with a virus of low virulence can protect against closely related virulent viruses, with perhaps the best-studied example being the use of *Meleagrid herpesvirus 1* from turkeys to vaccinate chickens against Marek's disease (Eidson et al. 1973). If OtHV1 has a significant etiologic role in urogenital carcinoma, one possible explanation for the lack of detection of urogenital carcinoma in NFS is that the acquired immune response to OtHV4 may cross-protect against OtHV1 infection. The comparison between pathogenicity, host specificity, and genomes of these viruses may provide clinically useful insights toward a better understanding OtHV1-associated urogenital carcinoma.

It is probable that assays that did not identify virus to the level of sequence would not have distinguished these viruses in surveillance. Serologic assays are likely to cross-react with genetically closely related organisms (Nollens et al. 2008). With 95–97% homology over the available sequences, primers designed without specifically targeting the differences between OtHV1 and OtHV4 would be likely to bind to sequences of both viruses, resulting in amplification of both viruses. This emphasizes the need for understanding the diversity of pathogens in understudied species before limited disease surveillance data can be reliably interpreted.

In conclusion, we identified OtHV4, a novel gammaherpesvirus in NFS, which is closely related to the urogenital carcinoma-associated OtHV1 of CSL and appears to be common in healthy wild NFS. The ecology and epidemiology of this virus merits

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BatEfHV1 (ABH05841), BatPvHV1 (AGK25934), BatHdHV1 (BAH57334), BabirusaHV1 (AAO46907), BoHV4 (AAC59454), CallitrichineHV3 (NP_733857), CeHV5 (AAM75149), ElephantidHV1 (ADG03758), ElephantidHV3 (ABB54684), ElephantidHV4 (ABB54685), ElephantidHV5 (ABK41480), ElephantidHV6 (ABP96774), EqHV2 (NP_042605), GallidHV1 (YP_182359), HMSHV1 (AAY90140), HHV2 (NP_044500), HHV1 (YP_009137105), HHV4 (YP_401712), HHV6 (NP_042931), HHV7 (YP_073778), HHV8 (AAB62593), HyleucRhV (AAS17748), IguanidHV2 (AAO84913), MacacineHV1 (AF533768), MacacineHV4 (YP_068007), MacropodidHV3 (ABO61861), MuridHV2 (AAW57296), MustelidHV1 (AAL55728), OtHV1_CSL (ACV86603), OtHV2_CSL (GQ429148), OtHV3_CSL (AFP23381), OtHV4_NFS (This study), OvHV2 (AAC59455), PhocidHV1 (AAB93518), PhocidHV2 (ACV86607), PLHV1 (AAF16520), ProcavidHV1 (ABK41481), RetFibHV (AAC57975), SaHV2 (CAC84304), SuidHV1 (YP_068333), TapirHV (AAD30142), TortoiseHV1 (BAB40430), and TrichechidHV1 (ABB54686).

TABLE 2. Northern fur seal (*Callorhinus ursinus*) vaginal swab samples tested for Otarine herpesviruses (OtHV4) and comparison between quantitative PCR (qPCR) and PCR results, Alaska and California, USA, 2011–12.

Sample ID	OtHV4 qPCR (average viral counts/ nanogram DNA)[a]	Specific OtHV4 PCR/ sequencing	Consensus herpesvirus PCR/ sequencing
CU11001	1609.29	OtHV4	OtHV4
CU11002	0.00	Not done	Negative
CU11003	0.00	Not done	Negative
CU11004	0.00	Not done	Negative
CU11005	BLD	Not done	Negative
CU11006	0.00	Not done	Negative
CU11007	0.00	Not done	Negative
CU11008	0.00	Not done	Negative
CU11009	BLD	Not done	Negative
CU11010	0.00	Not done	Negative
CU11011	59.30	Not done	Negative
CU11012	0.00	Not done	Negative
CU11013	0.00	Not done	Negative
CU11014	0.11	Not done	Negative
CU11015	1.72	Not done	Negative
CU11016	0.25	Not done	Negative
CU11017	0.00	Not done	Negative
CU11018	0.00	Not done	Negative
CU11019	0.00	Not done	Negative
CU11020	0.00	Not done	Negative
CU12008	0.00	Negative	Not done
CU12009	0.00	Negative	Not done
CU12010	0.00	Negative	Not done
CU12011	0.00	Negative	Not done
CU12012	0.86	Negative	Negative
CU12013	2750.70	OtHV4	Not done
CU12014	2934.50	OtHV4	Not done
CU12015	0.00	Negative	Not done
CU12016	0.00	Negative	Not done
CU12017	0.00	Negative	Not done
CU12018	4425.36	OtHV4	Not done
CU12019	259.29	OtHV4	Not done
CU12020	0.00	Negative	Not done
CU12021	20328.84	OtHV4	Not done
CU12022	0.00	Negative	Not done
CU12023	0.00	Negative	Not done
CU12024	0.00	Negative	Not done
CU12025	0.00	Negative	Not done
CU12026	1.46	Negative	Not done
CU12027	0.00	Negative	Not done
CU12028	0.00	Negative	Not done
CU12029	0.00	Negative	Not done
CU12030	144245.99	OtHV4	Not done
CU12031	91.55	Negative	Not done
CU12032	0.00	Negative	Not done
CU12033	0.00	Negative	Not done
CU12034	175788.54	OtHV4	Not done
CU12035	0.00	Negative	Not done
CU12036	0.00	Negative	Not done
CU12037	5.09	Negative	OtHV4
CU13027	21610.61	Not done	OtHV4

[a] BLD = below limit of linear detection.

further study, and comparative genomic data of the two viruses may illuminate the origin of OtHV1 and its role in carcinogenesis in CSL.

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LITERATURE CITED

Buckles EL, Lowenstine LJ, DeLong RL, Melin SR, Vittore RK, Wong HN, Ross GL, St Leger JA, Greig DJ, Duerr RS, et al. 2007. Age-prevalence of otarine herpesvirus-1, a tumor-associated virus, and possibility of its sexual transmission in California sea lions. *Vet Microbiol* 120:1–8.

Colegrove KM, Gulland FM, Naydan DK, Lowenstine LJ. 2009. Tumor morphology and immunohistochemical expression of estrogen receptor, progesterone receptor, p53, and Ki67 in urogenital carcinomas of California sea lions (*Zalophus californianus*). *Vet Pathol* 46:642–655.

Dagleish MP, Barrows M, Maley M, Killick R, Finlayson J, Goodchild R, Valentine A, Saunders R, Willoughby K, Smith KC, et al. 2012. The first report of Otarine Herpesvirus-1-associated urogenital carcinoma in a South American fur seal (*Arctocephalus australis*). *J Comp Pathol* 149: 119–125.

Darriba D, Taboada GL, Doallo R, Posada D. 2011. ProtTest 3: Fast selection of best-fit models of protein evolution. *Bioinformatics* 27:1164–1165.

Duncan C, Goldstein T, Hearne C, Gelatt T, Spraker T. 2013. Novel polyomaviral infection in the placenta of a northern fur seal (*Callorhinus ursinus*) on the Pribilof Islands, Alaska, USA. *J Zoo Wildl Med* 49:163–167.

Ehlers B, Dural G, Yasmum N, Lembo T, De Thoisy B, Ryser-Degiorgis MP, Ulrich RG, McGeoch DJ. 2008. Novel mammalian herpesviruses and lineages within the Gammaherpesvirinae: Cospeciation and interspecies transfer. *J Virol* 82: 3509–3516.

Eidson CS, King DD, Connell HE, Anderson DP, Kleven SH. 1973. Efficacy of turkey herpesvirus vaccine against Marek's disease in broilers. *Poult Sci* 52:1482–1491.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.

Gilardi KV, Oxford KL, Gardner-Roberts D, Kinani JF, Spelman L, Barry PA, Cranfield MR, Lowenstine LJ. 2014. Human Herpes Simplex Virus Type 1 in confiscated gorilla. *Emerg Infect Dis* 20:1883–1886.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Bio* 59:307–321.

Gulland FM, Trupkiewicz JG, Spraker TR, Lowenstine LJ. 1996. Metastatic carcinoma of probable transitional cell origin in 66 free-living California sea lions (*Zalophus californianus*), 1979 to 1994. *J Zoo Wildl Med* 32:250–258.

Hadlow WJ, Cheville NF, Jellison WL. 1980. Occurrence of pox in a northern fur seal on the Pribilof Islands in 1951. *J Zoo Wildl Med* 16:305–312.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* 9:286–298.

King DP, Hure MC, Goldstein T, Aldridge BM, Gulland FM, Saliki JT, Buckles EL, Lowenstine LJ, Stott JL. 2002. Otarine herpesvirus-1: A novel gammaherpesvirus associated with urogenital carcinoma in California sea lions (*Zalophus californianus*). *Vet Microbiol* 86:131–137.

Landolfi JA, Wellehan JF, Johnson AJ, Kinsel MJ. 2005. Fatal human herpesvirus type 1 infection in a white-handed gibbon (*Hylobates lar*). *J Vet Diagn Invest* 17:369–371.

Lipscomb TP, Scott DP, Garber RL, Krafft AE, Tsai MM, Lichy JH, Taubenberger JK, Schulman FY, Gulland FM. 2000. Common metastatic carcinoma of California sea lions (*Zalophus californianus*): Evidence of genital origin and association with novel gammaherpesvirus. *Vet Pathol* 37:609–617.

Maness HT, Nollens HH, Jensen ED, Goldstein T, LaMere S, Childress A, Sykes J, St Leger J, Lacave G, Latson FE, et al. 2011. Phylogenetic analysis of marine mammal herpesviruses. *Vet Microbiol* 149:23–29.

Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH, Passarotti M, Kaufman S, O'Leary MA. 2015. A RESTful API for Access to Phylogenetic Tools via the CIPRES Science Gateway. *Evol Bioinform Online* 11:43–48.

Nollens HH, Ruiz C, Walsh MT, Gulland FM, Bossart G, Jensen ED, McBain JF, Wellehan JF. 2008. Cross-reactivity between immunoglobulin G antibodies of whales and dolphins correlates with evolutionary distance. *Clin Vaccine Immunol* 15:1547–1554.

Pellett PE, Davison AJ, Eberle R, Ehlers B, Hayward GS, Lacoste V, Minson AC, Nicholas J, Roizman B, Studdert MJ, et al. 2011. Herpesvirales. In: *Virus taxonomy, 9th report of the International Committee on Taxonomy of Viruses*, King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Elsevier, San Diego, California, pp. 99–123.

Rambaut A. 2010. FigTree: Tree figure drawing tool, Version 1.3.2. http://tree.bio.ed.ac.uk/software/figtree/. Accessed April 2014.

Rambaut A, Drummond A. 2013. Tracer (version 1.5). http://beast.bio.ed.ac.uk/Tracer. Accessed April 2014.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.

Sawyer JC, Madin SH, Skilling DE. 1978. Isolation of San Miguel Sea Lion virus from samples of an animal food product produced from northern fur seal (*Callorhinus ursinus*) carcasses. *Am J Vet Res* 39:137–139.

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. *J Clin Microbiol* 34:1666–1671.

Venn-Watson S, Benham C, Gulland FM, Smith CR, St Leger J, Yochem P, Nollens H, Blas-Machado U, Saliki J, Colegrove K, et al. 2012. Clinical relevance of novel Otarine herpesvirus-3 in California sea lions (*Zalophus californianus*): Lymphoma, esophageal ulcers, and strandings. *Vet Res* 43:1–9.

Young LS, Finerty S, Brooks L, Scullion F, Rickinson AB, Morgan AJ. 1989. Epstein-Barr virus gene expression in malignant lymphomas induced by experimental virus infection of cottontop tamarins. *J Virol* 63:1967–1974.

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