**Novel alphaherpesvirus in a wild South American sea lion (*Otaria byronia*) with pulmonary tuberculosis**

**Running title: Alphaherpesvirus and tuberculosis in a sea lion**

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**SUMMARY**

In 2017, an adult male South American sea lion (*Otaria byronia*) presenting emaciation and a cervical abscess stranded alive in Florianópolis, southern Brazil. The animal was directed to a rehabilitation center, where leukocytosis and hyperproteinemia - indicative of systemic infection, were diagnosed, dying a few days later. On necropsy, the main gross findings were necrotizing lymphadenitis of the right prescapular lymph node and nodular bronchopneumonia. A novel alphaherpesvirus - tentatively named *Otariid alphaherpesvirus 1*, was amplified in brain, lung, liver, spleen, adrenal, esophagus and prescapular lymph node samples. No histopathologic findings associated with viral infection were observed. Additionally, pulmonary tuberculosis by *Mycobacterium pinnipedii* was diagnosed by histopathological, immunohistochemical and molecular techniques. Several bacteria were cultured from antemortem and postmortem samples, including *Proteus mirabilis* from the cervical abscess and cardiac blood, and *Escherichia coli* from the cervical abscess and pericardial effusion. Flavivirus, morbillivirus and Apicomplexa were not detected by molecular techniques. Herein we report a novel alphaherpesvirus in a pinniped species of the family Otariidae. Although previously described in Southern Hemisphere pinniped species, including South American sea lions, there is limited information regarding *M. pinnipedii* impact over this group. Further research is required to determine the associated pathogenesis of this novel herpesvirus, and prevalence of *Otariid alphaherpesvirus 1* and *M. pinnipedii* in the reproductive colonies.

**Keywords:** herpesvirus, *Mycobacterium*, pinniped, South America, stranding, virology.

**INTRODUCTION**

The South American sea lion (*Otaria byronia*, family Otariidae) is a generalist gregarious pinniped species that breeds in Uruguay, Argentina, the Falklands/Malvinas islands, Chile and Peru (Cárdenas-Alayza et al., 2016). Even though considered nonmigratory, this species performs post-reproductive seasonal movements, especially during the austral winter and early spring. Such movements are possibly favored by the Malvinas current northward flow (Vaz-Ferreira, 1982), when individuals from Uruguay, and in a small scale from Argentina and the Falklands/Malvinas, visit Brazilian waters (Rosas et al., 1994; Hoffman et al., 2016). Nevertheless, there are non-breeding colonies in southern Brazil (Pavanato et al., 2013).

Infectious diseases, including those of viral etiology, are recognized as threats to pinniped species (sea lions and fur seals [family Otariidae], seals [family Phocidae], and walruses [family Odobenidae]) (Anthony et al., 2012; Van Wormer et al., 2019). Nevertheless, little is known about the occurrence of viral agents in South American sea lions, which is limited to the report of cutaneous lesions by poxvirus and adenovirus-associated fatal hepatitis in captive animals from Canada and Japan, respectively, and low antibody titers against parvovirus and morbillivirus in free-ranging animals from Chile (Wilson & Poglayen-Neuwal, 1971; Inoshima et al., 2013; Sepúlveda et al., 2015). To this date, to the best of our knowledge, there are no studies available regarding neither herpesvirus infection nor exposure in South American sea lions.

Herpesviruses are enveloped, large, linear double-stranded DNA viruses able to establish latency in their natural hosts (ICTV, 2020). Some herpesviruses have co-evolved with their natural hosts, generally causing mild disease (Kaján et al., 2019; Pellet & Roizman, 2013). To date, only herpesviruses of the subfamilies *Alphaherpesvirinae* and *Gammaherpesvinae* have been described in pinnipeds, both of family *Herpesviridae* (Maness et al., 2011; Reisfeld et al., 2019a). In Otariidae, seven gammaherpesvirus species presenting different clinicopathological presentations - from asymptomatic infections to urogenital carcinomas, have been described (Lipscomb et al., 2000; Cortés-Hinojosa et al., 2016; Sacristán et al., 2018; Reisfeld et al., 2019a). In Odobenidae, there is a report of gammaherpesvirus infection not linked to specific lesions (Melero et al., 2014). Finally, in Phocidae, an alphaherpesvirus (*Phocid alphaherpesvirus 1*) associated with pneumonia, adrenal and hepatic necrosis (Gulland et al., 1997), and at least eight gammaherpesvirus species have been proposed (initially described as phocine herpesvirus 2 to phocine herpesvirus 7, harp seal herpesvirus, and hooded seal herpesvirus), associated with a variety of clinical manifestations and lesions (Bodewes et al., 2015; Bellehumeur et al., 2016). A sequence named ‘ringed seal herpesvirus’, highly similar to ‘harp seal herpesvirus’, was also reported (Bellehumeur et al., 2016). In spite of that, to date, the only pinniped herpesviruses recognized by the International Committee on Taxonomy of Viruses (ICTV) are the alphaherpesvirus *Phocid alphaherpesvirus 1,* and the gammaherpesviruses *Phocid gammaherpesvirus 2* and *Phocid gammaherpesvirus 3* - the last two corresponding to herpesvirus species initially described as phocine herpesvirus 2 and harp seal herpesvirus, respectively (ICTV, 2020).

Aside from viral diseases, another relevant disease in pinnipeds is tuberculosis. Tuberculosis is caused by acid-alcohol-fast bacilli (AAFB) from the zoonotic *Mycobacterium* *tuberculosis* Complex (MTBC) – comprising the validated species *M. tuberculosis*, *M. africanum, M. bovis*, *M. caprae*, *M. microti*, and *M. pinnipedii* (among others), sharing average 99% nucleotide genome identities (Brites et al., 2018; Chiner-Oms et al., 2019). In pinnipeds, tuberculosis was initially described in recently captured captured hooded seals (*Cystophora cristata*) from the Arctic (Blair, 1912). Ever since, cases of tuberculosis have been reported in captive and free-ranging pinnipeds, mainly in otariids from the Southern Hemisphere (Forshaw & Phelps, 1991; Kriz et al., 2011; de Amorim et al., 2014; Roe et al., 2019).

The current knowledge regarding herpesvirus species infecting free-ranging pinnipeds from the Southern Hemisphere is limited to the description of three gammaherpesvirus species in a South American fur seal (*Arctocephalus australis*) and a subantarctic fur seal (*Arctocephalus tropicalis*) (Sacristán et al., 2018; Reisfeld et al., 2019a). The records of *Mycobacterium* infection in pinnipeds from Brazil are also scarce (De Amorin et al., 2014; Martins Melo et al., 2019). Herein we report the coinfection by alphaherpesvirus and *Mycobacterium pinnipedii* in a South American sea lion that stranded alive in Brazil.

**MATERIALS AND METHODS**

**Monitoring and Clinical history**

An adult male South American sea lion was initially sighted resting in Laranjeiras beach (26°59'50" S, 48°35'27" W, Balneário Camboriú, northern coast of Santa Catarina state, southern Brazil) in June 19th 2017, by the Santos Basin Beach Monitoring Project (Projeto de Monitoramento de Praias da Bacia de Santos, PMP-BS). Because the animal was in good body condition, and apparently healthy and alert, the veterinary team decided not to intervene at that moment, but marked its dorsum with yellow dye for remote monitoring. In the following days, the same individual appeared in several beaches along the state coast and spent approximately one month in the beaches of Florianópolis city. After that, the animal was not observed by the field team by two weeks, and when re-sighted on August 12th 2017, was in poor body condition and presenting marked swelling on the right lateral neck area. Due to the individual’s poor clinical condition, it was immediately rescued at Pântano do Sul beach (27°46'59” S, 48°30'23” W, Florianópolis) and directed to the nearest rehabilitation center (Centro de Pesquisa, Reabilitação e Despetrolização de Animais Marinhos – CEPRAM), located in the same city.

Upon clinical examination, emaciation, mild bilateral pulmonary crepitations on auscultation and a 15 cm in diameter purulent ulcerated abscess located on the right lateral neck area were observed. The animal weighed approximately 150 kg. At first, the animal was treated with penicillin (Shotapen®, Virbac Laboratories, Carros, France, 0.1 mg/kg, IM SID EOD for three days), ketoprofen (Venco Laboratory, Londrina, Brazil, 2 mg/Kg, IM SID for two days), tramadol (Teuto, Anápolis, Brazil, 2 mg/Kg, IM BID for five days), and a single dose of an energetic and electrolytic supplement (Polijet HD, Vetoquinol, Mairiporã, Brazil, 20 mg/kg, SC). In the following 24 hours, because the animal was eating on its own, all medications were transferred to oral administration, to decrease the stress associated with manual restraining. Additionally, two different multivitamin complexes were administered (Aminomix Pet, Vetnil Produtos Veterinários Ltda, São Paulo, Brazil, and a vitamin complex based on Mazuri). The ulcerated cervical mass was thoroughly cleaned. On the fifth day of treatment, the animal presented hyporexia, and received subcutaneous omeprazole (40 mg/kg, Cristália, Itapira, Brazil), ranitidine (Farmace, Barbalha, Brazil, 25 mg/kg), and Ringer’s lactate (10 ml/kg). Because on physical examination the oral mucosa was pale, the animal also received a multivitamin (Hemolitan, Vetnil, Brazil, 1ml/10 kg, PO BID). Based on the results from the antimicrobial susceptibility test (see Table 1), penicillin was replaced by enrofloxacin (Dechra Brasil, Londrina, Brazil, 5 mg/kg, IM BID for 2 days) after four days of treatment. On August 19th 2017, the animal presented anorexia and hypotension, dying a few hours later.

**Gross and histopathologic examination**

The South American sea lion was necropsied within a few hours after its death, following a standard protocol (Geraci & Loundsbury, 2005). Representative tissue samples of brain, thyroid and parathyroid glands, lung, stomach, small and large intestines, liver, spleen, kidney, bladder, adrenal glands, skeletal muscle, heart, large vessels, and prescapular, mediastinal and mesenteric lymph nodes were fixed in 10% buffered formalin and subsequently embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin (HE). The histochemical stain Ziehl-Neelsen was selected to further characterize histopathologic findings. Fresh tissue samples of adrenal gland, brain, diaphragm, esophagus, heart, kidney, liver, lung, the enlarged right prescapular lymph node, submandibular, mesenteric and pulmonary lymph nodes, pancreas, pericardium, salivary gland, skeletal muscle, stomach, spleen, trachea, urinary bladder, and small and large intestines were also collected during necropsy and stored at -20 ºC.

**Microbiological study**

Bacterial cultures were performed from Stuart swabs sampled from the cervical abscess upon the initial clinical examination (August 12th 2017), and from the cervical abscess, and nasal, oral, urine and rectal swabs sampled at necropsy (August 19th 2017). Samples were plated onto blood agar medium for up to 72 h. Intracardiac blood and pericardial exudates, and blood from the jugular vein were withdrawn, placed in blood culture media and incubated overnight at 35 ± 2 °C. All isolated colonies were submitted to Gram staining and identified by API20 E system (Biomeriex®, France). Antimicrobial susceptibility was tested through the disc diffusion method using human and veterinary antibiotics, including amoxicillin/clavulanic acid, ampicillin, cefalexin, imipenem, meropenem, ertapenem, tetracycline, amikacin, gentamicin, enrofloxacin, ciprofloxacin and norfloxacin.

**Hematological and biochemical analyzes**

On August 14th 2017, blood was withdrawn by venipuncture from the interdigital vein of a posterior limb using a 21 gauge 3.8 cm needle. Approximately 2 ml were aliquoted in EDTA tubes and in tubes without additive for hematology and biochemistry evaluation. Analyzes were performed in a commercial veterinary laboratory. The hematological and biochemical values previously described for South American sea lions by Ruoppolo & Loureiro, (2014) were used as reference values.

**Immunohistochemistry**

Formalin fixed paraffin-embedded (FFPE) lung and lymph node samples presenting microscopic granulomatous lesions were selected for *Mycobacterium* spp. immunohistochemistry (IHC). Alphaherpesvirus IHC was performed in all available FFPE tissue samples.

Briefly, deparaffinized 3 μm sections of FFPE tissues silanized slides were submitted to antigen retrieval (citrate buffer citric acid solution 10mM pH 6.0 in a pressure cooker, for 3 min, at 120º C. Samples were submitted to blockage of endogenous peroxidase (H2O2, 30 min) and incubated overnight (4 °C) with (1) antibody anti-bacillus Calmette−Guérin (BCG) diluted in bovine serum albumin (BCG, rabbit, Dako, Santa Clara, CA, USA), followed by amplification with micropolymers conjugated with peroxidase (Reveal - Spring Bioscience -HRP Polymer Detection System, Pleasanton, USA) and visualization with 3,3'-Diaminobenzine chromogen (DAB) for 3 min (3’3-diaminobenzidine, Sigma D5637, St. Louis, USA) or (2) monoclonal antibody against Varicella zoster (*Human alphaherpesvirus 3*,clone c90.2.8 – Abcam,Cambridge, United Kingdom) at a concentration of 1/50, using signal amplification by Novolink polymer detection system (Leica Biosystems, Newcastle, UK) for 60 min, and visualization by DAB. The samples were counterstained with Harris Hematoxylin for 20 seconds followed by dehydration and slide mounting with synthetic resin.

For both immunohistochemistries, tissue sections in which the primary antibodies were replaced by non‐immune serum of those species where antibodies were raised served as negative controls. Human cases of *M. tuberculosis* and *Human alphaherpesvirus 3* infections were used as positive controls.

**Molecular study**

Total RNA was extracted from brain, lung, liver, spleen, esophagus, enlarged right prescapular lymph node and kidney using the RNEasy Mini kit (Qiagen, Hilden, Germany). Total DNA was extracted from the above mentioned tissues and also from the adrenal gland, small and large intestine frozen samples using the DNeasy Blood & Tissue kit (Qiagen).

A one-step real-time RT-PCR using universal primers (Moureau et al., 2008) was performed to partially amplify the NS5 gene of flaviviruses in brain, kidney, liver, lung, spleen and prescapular lymph node. The same samples were tested by RT-PCR to partially amplify the phosphoprotein gene of several species within the genus *Morbillivirus*, including *Phocine morbillivirus*, *Canine morbillivirus* and *Cetacean morbillivirus* (Barret et al., 1993).

The nested PCR protocols described by Vandevanter et al. (1996) and Ehlers et al. (2008) were selected to partially amplify the DNA polymerase (DPOL) and glycoprotein B (gB) genes of herpesviruses, respectively. The former amplifies a broad diversity of alpha-, beta- and gammaherpesviruses, while the latter is restricted to gammaherpesviruses. In order to detect and differentiate species of *M. tuberculosis* complex, we selected a multiplex PCR using nine primers to determine the absence or presence of the regions of difference 2, 9 and 12 of *M. tuberculosis* complex, as described before (Warren et al., 2006). This PCR protocol allows differentiating *M. pinnipedii* to the remaining species into de *M. tuberculosis* complex according to amplicon size (168, 108 and 369 bp, respectively, for RD2, RD9 and RD12 of *M. pinnipedii*). A nested PCR to amplify Apicomplexa of the family Toxoplasmatinae and *Sarcocystis* spp. was also performed in brain, enlarged prescapular lymph node, esophagus, lung, liver and spleen samples, as previously described (Soares et al., 2011; Reisfeld et al., 2019a).

Appropriate positive and negative controls were included in all PCR reactions except for *Mycobacterium*, i.e., RNA samples from a non-human primate infected with yellow fever virus for flavivirus, RNA from a cetacean morbillivirus case for morbillivirus, DNA from a herpesvirus-positive Magellanic penguin for herpesviral DPOL PCR, DNA from a herpesvirus-positive South American fur seal skin sample for herpesviral gB, and a *T. gondii* isolate for Apicomplexan PCRs. Diethylpyrocarbonate-treated water was selected as no template control.

Amplicons of the expected size were purified with Exo-SAP-IT (Atria Genetics, San Francisco, USA), and directly sequenced by Sanger technology. The consensus deduced amino acid herpesviral sequence was aligned in Mega7 by MUSCLE with alphaherpesvirus sequences of species recognized by the ICTV, and the gammaherpesvirus *Human gammaherpesvirus 4* was selected as outgroup. The program ProtTest (version 3.4.2, Darriba et al., 2011) was used to select the best model of evolution prior to the construction of the deduced amino acid maximum likelihood DPOL phylogenetic tree.

**RESULTS**

**Hematological and biochemical findings**

The main hematological and biochemical findings were macrocytic anemia, leukocytosis, azotemia, hypoalbuminemia and hyperproteinemia (Table 2).

*Gross findings*

Emaciation, moderate mucopurulent oral and bilateral ocular secretion, and a focal abscess of approximately 15 cm in diameter at the right cervical region were observed during external examination. Upon necropsy, it was verified that the fistulated abscess originated from the right prescapular lymph node. Nodular pneumonia was also observed (Figure 1.1). Live anisakid nematode larvae were found in the esophagus and stomach. Other significant findings were depletion of subcutaneous and cardiac fat, marked muscular atrophy, thick mucous secretion in the distal trachea, pericardial thickening and effusion with yellowish exudates, moderate cardiac congestion and gastric ulcers.

**Histopathologic findings**

The main histopathologic findings were marked multifocal granulomatous necrotizing pneumonia associated with dystrophic mineralization and presence of intralesional AAFB (Figure 1.6), marked focally expansive necrotizing lymphadenitis associated with dystrophic mineralization (without any AAFB detectable by Ziehl Neelsen stain), marked hepatocelullar atrophy, moderate to marked sinusoidal dilatation and congestion, moderate to marked multifocal cardiomyolysis, moderate to marked multifocal rhabdomyolysis, moderate to marked multifocal necrotizing splenitis, mild multifocal mononuclear interstitial nephritis and mild gliosis and satellitosis. Additionally, adult lungworms were observed in alveolar spaces, morphologically consistent with nematodes of the order Strongylida, superfamily Metastrongyloidea (Figure 1.4). Their main characteristics were lateral chords, heavily pigmented intestine, artifacts on cuticle, celomyarian musculature and the presence of eggs and developing larvae in uteri (Figure 1.4.1, 1.4.2, 1.4.3). The complete microscopic findings are summarized in Table 3.

**Immunohistochemistry**

The AAFB found in lungs were positive in anti-BCG immunohistochemistry. No antigen was detected in the necrotizing lesion in lymph node. Additionally, there was multifocal positive immunolabeling on the reticular cells of the adrenal cortex by anti-Varicella IHC (Figure 1.3).

**Microbiological results**

The bacteria *Shewanella putrefaciens* and *Proteus mirabilis* were isolated in antemortem swabs from the cervical abscess. Different bacteria were isolated from the samples collected during necropsy: *P. mirabilis* from the cervical abscess and cardiac blood, *Proteus* sp. from the cervical abscess, pericardial effusion, nasal and urine swabs, *Escherichia coli* from the cervical abscess, pericardial effusion, oral and anal swabs, *Salmonella* sp. from the oral cavity, and *Serratia liquefaciens* from the anal swab. Multiresistant profiles (defined as those bacteria resistant to three of more antimicrobial classes [Magiorakos et al., 2012; Sacristán et al., 2014]) were identified in all of the isolated bacterial species, except in *Shewanella putrefaciens* (Table 1).

**Molecular results**

Appropriate DPOL sequences were amplified in brain, lung, liver, spleen, esophagus, prescapular lymph node and adrenal gland. No DPOL amplification was observed in kidney, small intestine and large intestine samples. No gammaherpesviral sequences were recovered using the glycoprotein B protocol. All obtained DPOL sequences were identical; thus, a representative sequence was submitted to GenBank under accession number MW264409. Sequences presented 74.9% nucleotide similarity with *Equid alphaherpesvirus 3* (AF141885) (96% query coverage). The highest similarities (73.7%) for predicted amino acid were with sequences found in carnivores, i.e., *Phocid alphaherpesvirus 1* (QBN85151) sequence of a non-specified species from the Netherlands, and with a mustelid alphaherpesvirus 1 (ASE05858) found in a badger (*Meles meles*) from France. The South American sea lion herpesvirus sequence do not clustered with other viral species of the genus *Varicellovirus* (Figure 2). Amplicon sizes of 168, 108 and 369 bp were obtained from the tested lung and enlarged prescapular lymph node samples for the regions of difference RD2, RD9 and RD12, respectively. According to this result, the regions of difference 2 and 9 were absent, while the region of difference 12 was present. This pattern is consistent with *M. pinnipedii* (Warren et al., 2006). All analyzed samples tested negative for flavivirus, morbillivirus, Toxoplasmatinae and *Sarcocystis* spp.

**DISCUSSION**

In the present work we detected an alphaherpesvirus in several tissue samples of a South American sea lion stranded in southern Brazil. There is only one previous alphaherpesvirus species described in pinnipeds – *Phocid alphaherpesvirus 1* (previously known as phocid herpesvirus 1 or seal herpesvirus 1, genus *Varicellovirus*) in harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*) (Osterhaus et al., 1985; Gulland et al., 1997; Baily et al., 2019). *Phocid alphaherpesvirus 1* was initially reported in European harbor seal (*P. v. vitulina*) pups that died in the Netherlands, several of them presenting focal hepatitis, acute pneumonia and acute gingivostomatitis (Osterhaus et al., 1985). In Pacific harbor seals (*P. v. richardsi*) from North America, the virus was mainly associated with adrenal necrosis, followed by hepatic necrosis (Gulland et al., 1997). Most recently, Baily et al. (2019) suggested that the virus caused hepatic necrosis, thymic atrophy and oral ulceration in grey seal (*Halichoerus grypus*) pups from Europe. *Phocid alphaherpesvirus 1* is highly prevalent in harbor and grey seals and considered a significant cause of morbidity and mortality in these species (Osterhaus et al., 1985; Gulland et al., 1997; Baily et al., 2019). The draft genome of the virus obtained from the brain of a neonatal harbor seal from the Pacific coast of the United States was recently published (Rosales & Thurber, 2019).

Exposure to alphaherpesvirus(es) has been previously described in pinnipeds from the Southern Hemisphere. In the Antarctic, seroneutralization against alphaherpesviruses was described in all Weddell seals (*Leptonychotes weddellii*, n=25) and crabeater seals (*Lobodon carcinophagus*, n=3) tested by Harder et al. (1991), and in 72.2% (13/18) of the Weddell seals analyzed by Stenvers et al. (1992). Two decades later, antibodies against alphaherpesvirus(es) were detected by indirect ELISA in Weddell seals (100%, 20/20), crabeater seals (44%, 4/9), Antarctic fur seals (*Arctocephalus gazella*,58%, 42/74), and Ross seals (*Ommatophoca rossii*, 3/20, 15%), some of them also positive by seroneutralization (Tryland et al., 2012). In South America, neutralizing antibodies against *Phocid alphaherpesvirus 1* or other related alphaherpesviruses were identified in 85.7% (24/28) of the tested South American fur seals (*Arctocephalus australis*) (Jankowski et al., 2015). Unfortunately, none of these studies molecularly identified the etiological agent.

To the authors’ knowledge, this is the first molecular description of an alphaherpesvirus in a pinniped of the family Otariidae. The low amino acid similarity (73.7%) in comparison with the closest alphaherpesviruses for a fragment of a well-conserved gene (DPOL) and its identification in a novel host, are in accordance with the definition criteria of the ICTV for a novel herpesviral species ([Davison et al., 2005](#B005)), herein proposed as *Otariid alphaherpesvirus* *1*. Based on postmortem, microbiological and molecular findings, and in the species biology, we hypothesize that the alphaherpesvirus was likely reactivated from latency due to the individual’s impaired immune system function. Such condition was probably triggered by undernourishment (Bourke et al., 2016) and bacteremia (see below), as well as by pulmonary tuberculosis. Hypoalbuminemia possibly resulted from malnutrition. The observed white pulp depletion could be due to chronic stress or a response to infectious agents. Infection by immunosuppressive viruses of the genus *Morbillivirus* was not identified. Alphaherpesviruses are known to cause lytic cell infections, necrotizing lesions, syncytia formation, eosinophilic intranuclear inclusions, and latency (Caswell & Williams, 2015). Nevertheless, in our case the necrotizing lesions (i.e., necrotizing pneumonia, necrotizing lymphadenitis, and necrotizing splenitis) likely have a bacterial etiology; neither intranuclear inclusion bodies nor syncytia were observed. The pulmonary lesions were consistent with tuberculosis. It is not possible to establish if South American sea lions are the natural hosts of *Otariid alphaherpesvirus* *1* based solely in one case. Due to the presence of different pinniped species (South American sea lions and South American fur seals) in the same breeding colonies, cross-species transmission of infectious agents such as herpesviruses should be considered. Based on the impact of *Phocid alphaherpesvirus 1* in pinnipeds from the Northern Hemisphere, we highlight the importance of exploring the epidemiology of the novel *Otariid alphaherpesvirus* *1* in pinnipeds from the Southern Hemisphere.

Intralesional AAFB consistent with *Mycobacterium* were observed in the pulmonary granuloma of this South American sea lion and positively marked by immunohistochemistry, and *M. pinnipedii* was identified by multiplex PCR in prescapular lymph node and lung samples. Our findings mirror those reported by de Amorim et al. (2014), which found *M. pinnipedii* in the pulmonary granuloma and enlarged lymph nodes of a starved free-ranging South American sea lion stranded in southern Brazil (de Amorim et al., 2014), presenting a superinfection by two different *M.* *pinnipedii* strains, possibly reflecting high population prevalence (Silva-Pereira et al., 2019). Tuberculosis by the same agent was also identified in another South American sea lion presenting poor body condition, pulmonary granulomas and enlarged lymph nodes found in southern Brazil (Martins Melo et al., 2019). Moreover, tuberculosis cases have also been diagnosed in South American sea lions, South American and subantarctic fur seals and a Southern elephant seal (*Mirounga leonine*) sampled in Argentina and Uruguay (Romano et al., 1995; Bernardelli et al., 1996; Bastida et al., 1999; Arbiza et al., 2012). Of note, multifocal granulomas with central necrosis are one of the main microscopical features of pinniped tuberculosis cases, whereas mineralization and giant cell formation are not consistently observed (Forshaw & Phelps, 1991). MTBC complex species, including *M. pinnipedii,* have zoonotic potential (Zmak et al., 2019); thus, appropriate biosecurity measures should be adopted by personnel dealing with rescue, rehabilitation and necropsy of pinnipeds.

Aside from the alphaherpesvirus and *M. pinnipedii*, several bacteria from the cervical abscess were identified antemortem and postmortem: *Shewanella putrefaciens*, *P. mirabilis*, *Proteus* sp., and *E. coli*. These Gram-negative bacteria were likely associated with the observed right prescapular lymphadenitis that leads to the fistulated abscess. Due to its anatomical location, the abscess could have resulted from a bite during intra- or interspecific interactions (e.g., dogs). *S. putrefaciens* is found in aquatic environments (Janda, 2014) and was previously reported as part of the genital microbiota of another pinniped species, the California sea lion (*Zalophus californianus*) (Johnson et al., 2006). Additionally, this bacterium has been identified in humans with ulcers and traumatic injuries (Holt et al., 2005), as reported in a fisherman with a leg ulcerative skin lesion following contact with riverine environment in northeastern Brazil (Da Silva et al., 2011). Bacteria of the genus *Proteus* were previously described in wounds in pinnipeds, as reported by Reisfeld et al. (2019b) in *Fusarium*-associated cutaneous lesions in a captive South American sea lion and by Dailey et al. (2002) in northern elephant seal (*Mirounga angustirostris*) skin lesions caused by the copepod *Pennella balaenopterae*. Moreover, the hematological and biochemical results, along with the widespread isolation of *P. mirabilis*, *Proteus* sp. and *E. coli* suggest that these Enterobacterales caused the bacteremia and were involved in lymph node lesions. In humans, *P. mirabilis* and *E coli* are known agents present in bloodstream infections (O'Hara et al., 2000; Micenková et al., 2017). Most of the isolated strains were multiresistant or presented intrinsic resistance to the prescribed antimicrobials (i.e., *S. putrefaciens* to penicillin) (Holt et al., 2005), which likely hampered therapeutic success. Bacteremia and pulmonary tuberculosis possibly contributed to the animal’s death.

Finally, nematodes of the superfamily Metastrongyloidea were found in the lung of the animal. These endoparasites are widely known to infest the respiratory system of several species of terrestrial and marine carnivores, and generally require an intermediate host (mollusks or small fish). The main genera reported in pinnipeds are *Otostrongylus* and *Parafilaroides* (Gardiner & Poynton, 1999; Measures, 2001).

To the authors’ knowledge, this is the first description of herpesvirus infection in South American sea lion, herein proposed as *Otariid alphaherpesvirus* *1*, widening the host range of herpesvirus in pinnipeds. It is also the first alphaherpesvirus detected in Otariidae, and the second in pinnipeds worldwide. This herpesvirus was likely reactivated from latency due to immunosuppression caused by the individual’s poor body condition and septicemia. *Mycobacterium* infections seem to be endemic in reproductive colonies; thus, all pinnipeds admitted for rehabilitation should be considered as potential hosts. Future surveillance studies on the presence and prevalence of this novel alphaherpesvirus in South American sea lions (e.g., in reproductive and wintering colonies and in rehabilitation centers) are necessary to establish if these pinnipeds are the natural host of this novel alphaherpesvirus and its potential impact over the species. Furthermore, surveillance studies aiming on the prevalence and impact of *Mycobacterium* in this population are also needed, due to this agent’s clinical relevance, zoonotic potential and ability to further overwhelm the host’s immune system.

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**Ethics Statement:** all procedures were performed in accordance with the Ethical Committee in Animal Research of the School of Veterinary Medicine and Animal Sciences, University of São Paulo (Process number 7151291019).

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**REFERENCES**

Anthony, S. J., St Leger, J. A., Pugliares, K., Ip, H. S., Chan, J. M., Carpenter, Z. W., Navarrete-Macias, I., Sanchez-Leon, M., Saliki, J. T., Pedersen, J., Karesh, W., Daszak, P., Rabadan, R., Rowles, T., & Lipkin, W. I. (2012). Emergence of fatal avian influenza in New England harbor seals. *mBio*, *3*, e00166-12. <https://doi.org/10.1128/mBio.00166-12>

Arbiza, J., Blanc, A., Castro-Ramos, M., Katz, H., Ponce de León, A., & Clara, M. (2012). Uruguayan Pinnipeds (*Arctocephalus australis* and *Otaria flavescens*): Evidence of influenza virus and *Mycobacterium pinnipedii* infections. In A. Romero, & O. E. Keith (Eds.), *New approaches to the study of marine mammals.* (pp. 151-182) Rijeka, Croatia: InTech.

Baily, J. L., Willoughby, K., Maley, M., Chapman, J., Pizzi, R., Hall, A. J., & Dagleish, M. P. (2019). Widespread neonatal infection with phocid herpesvirus 1 in free-ranging and stranded grey seals *Halichoerus grypus*. *Diseases of Aquatic Organisms*, *133*, 181-187. https://doi.org/10.3354/dao03345

Barrett, T., Visser, I. K. G., Mamaev, L., Goatley, L., Van Bressem, M. F., & Osterhaus, A. D. M. E. (1993). Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. *Virology*, *193*, https://doi.org/1010-1012. 10.1006/viro.1993.1217

Bastida, R., Loureiro, J., Quse, V., Bernardelli, A., Rodríguez, D., & Costa, E. (1999). Tuberculosis in a wild Subantarctic fur seal from Argentina. *Journal of Wildlife Diseases*, *35*, 796–798. https://doi.org/10.7589/0090-3558-35.4.796

Bellehumeur, C., Nielsen, O., Measures, L., Harwood, L., Goldstein, T., Boyle, B., & Gagnon, C. A. (2016). Herpesviruses including novel gammaherpesviruses are widespread among phocid seal species in Canada. *Journal of Wildlife Diseases*, *52*, 70-81. https://doi.org/10.7589/2015-01-020

Bernardelli, A., Bastida, R., Loureiro, J., Michelis, H., Romano, M. I., Cataldi, A., & Costa, E. (1996). Tuberculosis in sea lions and fur seals from the southwestern Atlantic coast. *Revue Scientifique et Technique (International Office of Epizootics)*, *15*, 985–1005. https://doi.org/10.20506/rst.15.3.963

Blair, W. R. (1912). Report of the Veterinarian on the mammals. In New York Zoological Society (Eds.), *17th Annual Report of the New York Zoological Society*. (pp.74-77) New York, NY: New York Zoological Society.

Bodewes, R., Contreras, G. J. S., García, A. R., Hapsari, R., van de Bildt, M. W., Kuiken, T., & Osterhaus, A. D. (2015). Identification of DNA sequences that imply a novel gammaherpesvirus in seals. *Journal of General Virology*, *96*, 1109-1114. https://doi.org/10.1099/vir.0.000029

Bourke, C. D., Berkley, J. A., & Prendergast, A. J. (2016). Immune Dysfunction as a Cause and Consequence of Malnutrition. *Trends in Immunology*, *37*, 386-398. <https://doi.org/10.1016/j.it.2016.04.003>

Brites, D., Loiseau, C., Menardo, F., Borrell, S., Boniotti, M. B., Warren, R., Dippenaar, A., Parsons, S., Beisel, C., Behr, M. A., Fyfe, J. A., Coscolla, M., & Gagneux, S. (2018). A New Phylogenetic Framework for the Animal-Adapted *Mycobacterium tuberculosis* Complex. *Frontiers in Microbiology*, *9*, 2820. https://doi.org/10.3389/fmicb.2018.02820

Cárdenas-Alayza, S., Crespo, E. & Oliveira, L. (2016). *Otaria byronia*. The IUCN Red List of Threatened Species 2016. Retrieved from <https://www.iucnredlist.org/species/41665/61948292>

Caswell, J. L. & Williams, K. J. (2015). Respiratory System. In M. G. Maxie (Ed.), *Jubb, Kennedy and Palmer’s pathology of domestic animals*, 6th ed. vol 2 (pp. 465-590), Toronto, Canada: Saunders Ltd. https://doi.org/10.1016/B978-0-7020-5318-4.00011-5

Chiner-Oms, Á., Sánchez-Busó, L., Corander, J., Gagneux, S., Harris, S. R., Young, D., González-Candelas, F.,& Comas, I. (2019). Genomic determinants of speciation and spread of the *Mycobacterium tuberculosis* complex. *Science Advances*, *5*, eaaw3307. https://doi.org/10.1126/sciadv.aaw3307

Cortés-Hinojosa, G., Gulland, F. M. D., DeLong, R., Gelatt, T., Archer, L., & Wellehan, J. F.X. Jr. (2016). A novel gammaherpesvirus in northern fur seals (*Callorhinus ursinus*) is closely related to the California sea lion (*Zalophus californianus*) carcinoma associated Otarine herpesvirus-1. *Journal of Wildlife Diseases*, *52*, 88-95. http://dx.doi.org/[10.7589/2015-03-060](https://doi.org/10.7589/2015-03-060)

Dailey, M. D., Haulena, M., & Lawrence, J. (2002). First report of a parasitic copepod (*Pennella balaenopterae*) infestation in a pinniped. *Journal of Zoo and Wildlife Medicine*, *33*, 62-65. http://dx.doi.org/10.1638/1042-7260(2002)033[0062:FROAPC]2.0.CO;2

Da Silva, A. R., Batista de Matos, W., Lima, J. F., Barbosa, A. V., Hofer, E., & Gonçalves, E. D. G. D. R. (2011). *Shewanella putrefaciens* em lesão cutânea traumática. *Revista Pan-Amazônica de Saúde*, *1*, 125-128. http://dx.doi.org/10.5123/S2176-62232010000300016

Davison, A. J., Eberle, R., Hayward, G. S., McGeoch, D. J., Minson, A. C., Pellett, P. E., Roizman, B., Studdert, M. J., & Thiry, E. (2005). Family Herpesviridae. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*. (pp. 193–212) New York, NY: Elsevier.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2011). ProtTest 3: fast selection of best-fit models of protein evolution. *Bioinformatics*, *27*, 1164-1165. https://doi.org/10.1093/bioinformatics/btr088

de Amorim, D. B., Casagrande, R. A., Alievi, M. M., Wouters, F., De Oliveira, L. G., Driemeier, D., Tavares, M., Ikuta, C. Y., Telles, E. O., & Ferreira-Neto, J. S. (2014). *Mycobacterium pinnipedii* in a stranded South American sea lion (*Otaria byronia*) in Brazil. *Journal of Wildlife Diseases*, *50*, 419–422. https://doi.org/10.7589/2013-05-124

Ehlers, B., Dural, G., Yasmum, N., Lembo, T., Lembo, T., de Thoisy, B., Ryser-Degiorgis, M. P., Ulrich, R. G., & McGeoch, D. J. (2008). Novel mammalian herpesviruses and lineages within the Gammaherpesvirinae: cospeciation and interspecies transfer. *Journal of Virology*, *82*, 3509–3516.

Forshaw, D., & Phelps, G. R. (1991). Tuberculosis in a captive colony of pinnipeds. *Journal of Wildlife Diseases*, *27*, 288–295. <https://doi.org/10.7589/0090-3558-27.2.288>

Gardiner, C. H., & Poynton, S. L. (1999). An Atlas of Metazoan Parasites in Animal Tissues. Armed Forces Institute of Pathology, Washington, DC: American Registry of Pathology. Geraci, J. R., & Lounsbury, V. J. (2005). Marine mammals ashore: a field guide for strandings, 2nd ed. Baltimore, MD: National Aquarium in Baltimore.

Gulland, F. M., Lowenstine, L. J., Lapointe, J. M., Spraker, T., & King, D.P. (1997). Herpesvirus infection in stranded Pacific harbor seals of coastal California. *Journal of Wildlife Diseases*, *33*, 450−458. https://doi.org/10.7589/0090-3558-33.3.450

Gulland, F. M., Dierauf, L. A., & Whitman, K. L. (2018). CRC handbook of marine mammal medicine. CRC Press.

Harder, T. C., Plötz, J., & Liess, B. (1991). Antibodies against European phocine herpesvirus isolates detected in sera of Antarctic seals. *Polar Biology*, *11*, 509-512. <https://doi.org/10.1007/BF00233087>

Hoffman, J. I., Kowalski, G. J., Klimova, A., Eberhart-Phillips, L. J., Staniland, I. J., & Baylis, A. M. (2016). Population structure and historical demography of South American sea lions provide insights into the catastrophic decline of a marine mammal population. *Royal Society Open Science*, *3*, 160291. https://doi.org/10.1098/rsos.160291

Holt, H. M., Gahrn-Hansen, B., & Bruun, B. (2005). *Shewanella algae* and *Shewanella putrefaciens*: clinical and microbiological characteristics. *Clinical Microbiology and Infection*, *11*, 347-352. https://doi.org/10.1111/j.1469-0691.2005.01108.x

ICTV (International Committee on Taxonomy of Viruses). (2020). Virus Taxonomy: 2019 Release. EC 51, Berlin, Germany, July 2019. Email ratification March 2020 (MSL #35) Retrieved from <https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsdna-viruses-2011/w/dsdna_viruses/91/herpesviridae>

Inoshima, Y., Murakami, T., Ishiguro, N., Hasegawa, K., & Kasamatsu, M. (2013). An outbreak of lethal 323 adenovirus infection among different otariid species. Veterinary *Microbiology*, *165*, 455-459. https://doi.org/10.1016/j.vetmic.2013.04.013

Janda, J. M., & Abbott, S. L. (2014) The genus *Shewanella*: from the briny depths below to human pathogen. *Critical Reviews in Microbiology*, *40*, 293−312. https://doi.org/10.3109/1040841X.2012.726209

Jankowski, G., Adkesson, M. J., Saliki, J. T., Cárdenas-Alayza, S., & Majluf, P. (2015). Survey for infectious disease in the South American fur seal (*Arctocephalus australis*) population at Punta San Juan, Peru. *Journal of Zoo and Wildlife Medicine*, *46*, 246-254. https://doi.org/10.1638/2014-0120.1

Johnson, S., Lowenstine, L., Gulland, F., Jang, S., Imai, D., Almy, F., Delong, R., & Gardner, I. (2006). Aerobic bacterial flora of the vagina and prepuce of California sea lions (*Zalophus californianus*) and investigation of associations with urogenital carcinoma. *Veterinary Microbiology*, *114*, 94–103. https://doi.org/10.1016/j.vetmic.2005.11.045

Kaján, G. L., Doszpoly, A., Tarján, Z. L., Vidovszky, M. Z., & Papp, T. (2019). Virus–host coevolution with a focus on animal and human DNA viruses*. Journal of Molecular Evolution*, *88*, 41-56. https://doi.org/10.1007/s00239-019-09913-4

Kriz, P., Kralik, P., Slany, M., Slana, I., Svobodova, J., Parmova, I., Barnet, V., Jurek, V., & Pavlik, I. (2011). *Mycobacterium pinnipedii* in a captive Southern sea lion (*Otaria flavescens*): a case report. *Veterinární Medicína*, *6*, 307-313. https://doi.org/10.17221/1549-VETMED

Lipscomb, T. P., Scott, D. P., Garber, R. L., Krafft, A. E., Tsai, M. M., Lichy, J. H., Taubenberger, J. K., Schulman, F. Y., & Gulland, F. M. (2000). Common metastatic carcinoma of California sea lions (*Zalophus californianus*): evidence of genital origin and association with novel gammaherpesvirus. *Veterinary Pathology*, *37*, 609-617. https://doi.org/10.1354/vp.37-6-609

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Maness, H. T., Nollens, H. H., Jensen, E. D., Goldstein, T., LaMere, S., Childress, A., Sykes, J., St Leger, J., Lacave, G., Latson, F. E., & Wellehan, J. F., Jr. (2011). Phylogenetic analysis of marine mammal herpesviruses. *Veterinary Microbiology*, *149*, 23-29. <https://doi.org/10.1016/j.vetmic.2010.09.035>

Measures, L. N. (2001). Lungworms of marine mammals. In W. M. Samuel, M. J. Pybus, & A. A. Kocan (Eds.), *Parasitic diseases of wild mammals* (pp. 279-300) Ames, IO: The Iowa State University Press.

Melero, M., García-Párraga, D., Corpa, J. M., Ortega, J., Rubio-Guerri, C., Crespo, J. L., Rivera-Arroyo, B., & Sánchez-Vizcaíno, J. M. (2014) First molecular detection and characterization of herpesvirus and poxvirus in a Pacific walrus (*Odobenus rosmarus divergens*). *BMC Veterinary Research, 10*,968. https://doi.org/10.1186/s12917-014-0308-2

Micenková, L., Beňová, A., Frankovičová, L., Bosák, J., Vrba, M., Ševčíková, A., Kmeťová, M., & Šmajs, D. (2017). Human *Escherichia coli* isolates from hemocultures: septicemia linked to urogenital tract infections is caused by isolates harboring more virulence genes than bacteraemia linked to other conditions. *International Journal of Medical Microbiology*, *307*, 182–189. https://doi.org/10.1016/j.ijmm.2017.02.003

Martins Melo, A., Silva Filho, R., von Groll, A., Reis, A. J., Diniz, J., Perdigão, J., Portugal, I., da Silva, P., Borelli Grecco, F., Orzechowski Xavier, M. (2019). Tuberculosis caused by *Mycobacterium pinnipedii* in a wild South American sea lion *Otaria flavescens* stranded in southern Brazil. *Diseases of Aquatic Organisms*, *133*, 189–194. https://doi.org/10.3354/dao03342

Moureau, G., Temmam, S., Gonzalez, J.P., Charrel, R.N., Grard, G., & de Lamballerie, X. (2008). A real time RT-PCR method for universal detection and identification of flaviviruses. *Vector-Borne and Zoonotic Diseases*, *7*, 467-478. http://dx.doi.org/10.1089/vbz.2007.0206

O'Hara, C. M., Brenner, F. W., & Miller, J. M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews*, *13*, 534-534. http://dx.doi.org/10.1128/cmr.13.4.534-546.2000

Osterhaus, A. D. M. E., Yang, H., Spijkers, H. E. M., Groen, J., Teppema, J. S., & Van Steenis, G. (1985). The isolation and partial characterization of a highly pathogenic herpesvirus from the harbor seal (*Phoca vitulina*). *Archives of Virology*, *86*, 239-251.

Pavanato, H., Silva, K. G., Estima, S. C., Monteiro, D. S., & Kinas, P. G. (2013). Occupancy dynamics of South American Sea-Lions in Brazilian Haul-outs. *Brazilian Journal of Biology*, *73*, 855–862. http://dx.doi.org/10.1590/S1519-69842013000400023

Pellett, P. E., & Roizman, B. (2012). *Herpesviridae*. In D. M. Knipe, P. M. Howley, J. I. Cohen, D. E. Griffin, R. A. Lamb, M. A. Martin, V. R. Racaniello, & B. Roizman, (Eds.), *Fields Virology*, 6th ed. (pp. 1802-1822) Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Reisfeld, L., Sacristán, C., Sánchez-Sarmiento, A. M., Costa-Silva,S., Díaz-Delgado,J., Groch,K. R., Marigo,J., Ewbank,A. C., Favero,C. M., Mariotti Guerra, J.,Réssio,R. A., Cremer, M. J., Esperón,F., & Catão-Dias, J. L. (2019a). Fatal pulmonary parafilaroidiasis in a free-ranging subantarctic fur seal (*Arctocephalus tropicalis*) coinfected with two gammaherpesviruses and *Sarcocystis* sp. *Revista Brasileira de Parasitologia Veterinária*, *28*, 499-503. <https://doi.org/10.1590/s1984-29612019029>

Reisfeld, L., Sacristán, C., Canedo, P., Schwarz, B., Ewbank, A. C., Esperón, F., & Catão-Dias, J. L. (2019b). Fusariosis in a captive South American Sea Lion (*Otaria flavescens*): A Case Report. *Mycopathologia*, *184*, 187-192. https://doi.org/10.1007/s11046-018-0270-9

Roe, W. D., Lenting, B., Kokosinska, A., Hunter, S., Duignan, P. J., Gartrell, B., Rogers, L., Collins, D. M., de Lisle, G. W., Gedye, K., & Price-Carter, M. (2019). Pathology and molecular epidemiology of *Mycobacterium pinnipedii* tuberculosis in native New Zealand marine mammals. *PloS One*, *14*, e0212363. https://doi.org/10.1371/journal.pone.0212363

Romano, M. I., Alito, A., Bigi, F., Fisanotti, J. C., & Cataldi, A. (1995). Genetic characterization of mycobacteria from South American wild seals. *Veterinary Microbiology*, *47*, 89–98. https://doi.org/10.1016/0378-1135(95)00103-h

Rosales, S. M., & Thurber, R. V. (2019). Draft Genome Sequence of Phocine Herpesvirus 1 Isolated from the Brain of a Harbor Seal. *Microbiology Resource Announcements*, *8*, e00210-19. https://doi.org/10.1128/MRA.00210-19

Rosas, F. C. W., Pinedo, M. C., Marmotel, M. & Haimovici, M. (1994). Seasonal movements of the South American sea lion (*Otaria flavescens*, Shaw) off the Rio Grande do Sul coast, Brazil. *Mammalia*, *58*, 51-59. https://doi.org/10.1515/mamm.1994.58.1.51

Ruoppolo, V., & Loureiro, J. D. (2014). Carnivora – Otariidae e Phocidae (Foca, Lobo-marinho e Elefante-marinho). In Z. S. Cubas, J. C. Ramos Silva, & J. L. Catão-Dias (Eds.), *Tratado de animais selvagens: medicina veterinária*. vol. 2 (pp: 893-916) São Paulo, Brazil: Roca.

Sacristán, C., Esperón, F., Herrera-León, S., Iglesias, I., Neves, E., Nogal, V., Muñoz, M. J., & de la Torre, A. (2014). Virulence genes, antibiotic resistance and integrons in *Escherichia coli* strains isolated from synanthropic birds from Spain. *Avian Pathology*, *43*, 172-175. https://doi.org/10.1080/03079457.2014.897683

Sacristán, C., Esperón, F., Ewbank, A. C., Costa-Silva, S., Marigo, J., Matushima, E. R., Kolesnikovas, C., & Catão-Dias, J. L. (2018). Identification of novel gammaherpesviruses in a South American fur seal (*Arctocephalus australis*) with ulcerative skin lesions. *Journal of Wildlife Diseases*, *54*, 592-596. https://doi.org/10.7589/2017-09-224

Sepúlveda, M. A., Seguel, M., Alvarado-Rybak, M., Verdugo, C., Muñoz-Zanzi, C., & Tamayo, R. (2015). Postmortem findings in four South American sea lions (*Otaria byronia*) from an urban colony in Valdivia, Chile. *Journal of Wildlife Diseases*, *51*, 279-282. https://doi.org/10.7589/2013-07-161

Silva-Pereira, T. T., Ikuta, C. Y., Zimpel, C. K., Camargo, N., de Souza Filho, A. F., Ferreira Neto, J. S., Heinemann, M. B., & Guimarães, A. (2019). Genome sequencing of *Mycobacterium pinnipedii* strains: genetic characterization and evidence of superinfection in a South American sea lion (*Otaria flavescens*). *BMC Genomics*, *20*, 1030. https://doi.org/10.1186/s12864-019-6407-5

Soares, R. M., Lopes, E. G., Keid, L. B., Sercundes, M. K., Martins, J., & Richtzenhain, L. J. (2011). Identification of *Hammondia heydorni* oocysts by a heminested-PCR (hnPCR-AP10) based on the *H. heydorni* RAPD fragment AP10. *Veterinary Parasitology*, *175*,168-172. https://doi.org/10.1016/j.vetpar.2010.09.022

Stenvers, O., Plötz, J., & Ludwig, H. (1992). Antarctic seals carry antibodies against seal herpesvirus. *Archives of Virology*, *123*, 421-424. https://doi.org/10.1007/BF01317275

Tryland, M., Nymo, I. H., Nielsen, O., Nordøy, E. S., Kovacs, K. M., Krafft, B. A., Thoresen, S. I., Åsbakk, K., Osterrieder, K., Roth, S. J., Lydersen, C., Godfroid, J., & Blix, A. S. (2012). Serum chemistry and antibodies against pathogens in antarctic fur seals, Weddell seals, crabeater seals, and Ross seals. *Journal of Wildlife Disea*ses, *48*, 632-645. https://doi.org/10.7589/0090-3558-48.3.632

VanDevanter, D. R., Warrener, P., Bennett, L., Schultz, E. R., Coulter, S., Garber, R. L., & Rose, T. M. (1996). Detection and analysis of diverse herpesviral species by consensus primer PCR. *Journal of Clinical Microbiology*, *34*, 1666–1671. https://doi.org/[10.1128/JCM.34.7.1666-1671.1996](https://doi.org/10.1128/jcm.34.7.1666-1671.1996)

VanWormer, E., Mazet, J., Hall, A., Gill, V. A., Boveng, P. L., London, J. M., Gelatt, T., Fadely, B. S., Lander, M. E., Sterling, J., Burkanov, V. N., Ream, R. R., Brock, P. M., Rea, L. D., Smith, B. R., Jeffers, A., Henstock, M., Rehberg, M. J., Burek-Huntington, K. A., Cosby, S. L., Hammond, J. A., & Goldstein, T. (2019). Viral emergence in marine mammals in the North Pacific may be linked to Arctic sea ice reduction. *Scientific Reports*, *9*, 15569. https://doi.org/10.1038/s41598-019-51699-4

Vaz-Ferreira, R. (1982). *Otaria flavescens* (Shaw). South American sea lion. In FAO Fisheries series, UNEP (Eds.), *Mammals in the seas, Small cetaceans, seals, sirenians and otters* (pp. 477-495). Rome, Italy: FAO Fisheries.

Warren, R. M., Gey van Pittius, N. C., Barnard, M., Hesseling, A., Engelke, E., de Kock, M., Gutierrez, M. C., Chege, G. K., Victor, T. C., Hoal, E. G., & van Helden, P. D. (2006). Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *The International Journal of Tuberculosis and Lung Disease*, *10*, 818–822.

Wilson, T. M., & Poglayen-Neuwall, I. (1971). Pox in South American sea lions (*Otaria byronia*). *Canadian Journal of Comparative Medicine*, *35*, 174-177.

Zmak, L., Obrovac, M., Makek, M. J., Perko, G., & Trkanjec, J. T. (2019). From Peruvian mummies to living humans: first case of pulmonary tuberculosis caused by *Mycobacterium pinnipedii*. *The International Journal of Tuberculosis and Lung Disease*, *23*, 1283–1285. https://doi.org/10.5588/ijtld.19.0159

**TABLES**

**Table 1.** Antimicrobial resistance profile of the different bacterial species isolated from the South American sea Lion (*Otaria byronia*), according with collection date and sampled tissue. AMC = Amoxicillin/Clavulanic acid, AMP = Ampicillin, CFX = Cephalexin, MER = Meropenem, IMI = Imipenem, ERT = Ertapenem, TET = Tetracycline, AMK = Amikacin, GEN = Gentamicin, ENR = Enrofloxacin, CIP = Ciprofloxacin, Nor = Norfloxacin, S = susceptible, I = intermediate, R = resistant.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Bacterial species** | **Sampled tissue and collection date** | **Resistance profile** | | | | | | | | | | | |
|  |  | AMC (30μg) | AMP (10µg) | CFX (30μg) | MER (10µg) | IMI (10µg) | ERT (10µg) | TET (30µg) | AMK (30 μg) | GEN (10μg) | ENR (5 μg) | CIP (5 μg) | NOR (10μg) |
| *Shewanella putrefaciens* | Cervical mass 12/08/2017 | S | S | **R** | NA | NA | NA | **R** |  | S | S | S | NA |
| *Proteus mirabilis* | Cervical mass 12/08/2017 | S | S | S | NA | NA | NA | **R** | NA | S | S | S | NA |
| *Proteus mirabilis* | Cervical mass 19/08/2017 | **R** | **R** | **R** | NA | NA | NA | **R** | S | **R** | **R** | **R** | S |
| *Proteus* sp. | Cervical mass 19/08/2017 | **R** | **R** | **R** | **S** | **S** | **S** | S | S | **R** | **R** | **R** | **R** |
| *Proteus* sp. | Nasal swab 19/08/2017 | **R** | **R** | **R** | NA | NA | NA | **R** | **R** | **R** | **R** | S | **R** |
| *E. coli* | Cervical mass19/08/2017 | I | **R** | **R** | S | **R** | **S** | I | S | **R** | **R** | I | S |
| *E. coli* | Oral swab 19/08/2017 | S | **R** | **R** | NA | NA | NA | I | S | **R** | **R** | S | S |
| *E. coli* | Anal swab 19/08/2017 | **S** | **R** | S | NA | NA | NA | **R** | S | S | **R** | **R** | **R** |
| *Salmonella* sp. | Oral swab 19/08/2017 | S | **R** | **R** | NA | NA | NA | **R** | S | S | I | I | S |
| *Serratia liquefaciens* | Anal swab 19/08/2017 | **R** | **R** | **R** | NA | NA | NA | **R** | S | S | **R** | **R** | **R** |

**Table 2.** Hematological and biochemical results of the South American sea lion (*Otaria byronia*). The abnormal values are marked in bold.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **South American sea lion** | **South American sea lion reference values**† |
| **Hematology** | Red blood cell count (x10⁶/µL) | **0.98** | 3.99 – 5.72 |
| Hemoglobin (g/dL) | **10.00** | 12.8 – 19.2 |
| Hematocrit (%) | **23.00** | 37 – 52 |
| MCV (fL) | **234.60** | 83.92 – 114.56 |
| MCH (pg) | **102.04** | 29.93 – 36.90 |
| MCHC (g/dL) | **43.48** | 31.88 – 36.92 |
| Platelets (mammals)/µL | **34,300.00** | 267,000-299,000‡ |
| Leukocytes (x103/µL) | **29.05** | 7.9 – 11.3 |
| Segmented neutrophils (%) | **83.00** | 41 – 80 |
| Eosinophils (%) | 0.00 | 0 - 4 |
| Basophils (%) | 0.00 | 0 – 0 |
| Lymphocytes (%) | 14.00 | 13 – 52 |
| Monocytes (%) | 3.00 | 0 – 7 |
| **Biochemistry** | Uric Acid (mg/dL) | **3.00** | 0.42 – 1.6 |
| Albumin (g/dL) | **1.70** | 2.9 – 4.21 |
| Calcium (mg/dL) | 8.10 | 4.8 – 11.8 |
| Creatinine (mg/dL) | **2326.10** | 0.39 – 1.2 |
| Alkaline phosphatase (U/L) | 39.80 | 74 – 428 |
| Phosforus (mg/dL) | 4.80 | 4.36 – 7.2 |
| Glucose (mg/dL) | 83.00 | 60 – 112 |
| Total Proteins (g/dL) | **9.80** | 6.03 – 7.5 |
| Triglycerides (mg/dL) | 88.80 | 10 – 142 |

†According to Ruoppolo & Loureiro (2014).

‡The obtained platelets value was compared to that described in California sea lion (*Zalophus californianus*) by Gulland et al. (2018).

**Table 3.** Microscopic findings observed in the South American sea lion (*Otaria byronia*).

|  |  |
| --- | --- |
| **Organ** | **Morphologic diagnosis** |
| Lungs | Marked multifocal granulomatous necrotizing pneumonia associated with dystrophic mineralization. Presence of acid-alcohol-fast bacilli within granulomas. Moderate multifocal metastatic mineralization in alveolar wall. Free adult metastrongyloid nematodes in the alveolar lumen |
| Prescapular lymph node | Marked focally expansive necrotizing lymphadenitis associated with dystrophic mineralization |
| Liver | Marked hepatocellular atrophy. Moderate to marked sinusoidal dilatation and congestion |
| Myocardium | Moderate to marked multifocal cardiomyolysis. Mild multifocal hypereosinophilia and loss of striation |
| Skeletal muscle | Moderate to marked multifocal rhabdomyolysis |
| Spleen | Moderate to marked multifocal necrotizing splenitis. Moderate lymphoid depletion. Mild multifocal hemosiderosis. Mild reactive mesothelium. Mild arterial mineralization of tunica intima |
| Small intestine | Mild to moderate mineralization of tunica intima. Nematode larvae associated with mild eosinophilic enteritis |
| Thyroid | Mild to moderate colloid depletion associated with mild multifocal fibrosis. |
| Kidney | Mild multifocal mononuclear interstitial nephritis associated with minimal fibroplasia |
| Cerebrum | Mild gliosis and satellitosis |
| Adrenal glands, cerebellum, lymph node, pancreas | No significant findings |

**FIGURES**

**Figure 1.** **(1)** Macroscopic view of the South American sea lion (*Otaria byronia*) with severe emaciation. Note the concavities of the intercostal spaces. **(2)** Lung. Multifocal nodules in the pulmonary surface. **(3)** Adrenal gland. Nuclear immunostaining (brownish) of the reticular cells of the adrenal cortex on anti-Varicella immunohistochemistry (IHC, 400x). **(4)** Lung. Cross section of intra-alveolar metazoan parasites (40x) showing: **(4.1)** celomyarian musculature, ovary, heavily pigmented strongyloid intestine and developing larvae in uteri (400x), **(4.2)** lateral chords and eggs in uteri (400x), and **(4.3)** artifacts on cuticle (400x). H&E. (**5**) Lung. Granuloma with central necrosis (H&E, 40x), and **(5.1)** a population of epithelioid macrophages in the periphery (H&E, 400x) **(6)** Lung. Intralesional acid-alcohol--fast bacilli (Ziehl Neelsen, 200x). **(6.1)** Inset of the intralesional acid-alcohol fast bacilli (Ziehl Neelsen, 1000x). **(7)** Lung. Mycobacterial antigens detected by anti- bacillus Calmette−Guérin IHC (IHC, 100x), present mainly in the surrounding epithelioid macrophages (inset **7.1**, 400x).

**Figure 2.** DNA polymerase maximum likelihood phylogenetic tree of the alignment of the deduced amino acid alphaherpesvirus sequence obtained in the study (marked with a red dot) and other alphaherpesvirus sequences of the genera *Varicellovirus* and *Simplexvirus* retrieved from GenBank. *Human gammaherpesvirus 4* was selected as outgroup. The reliability of the tree was tested by bootstrap analysis with 1,000 replicates, and those bootstrap values lower than 70 were omitted.