UiT
THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Bioscience, Fisheries and Economics
Department of Arctic and Marine Biology

Pathogen exposure surveillance in Southern Ocean pinnipeds

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Sandra Núñez Egido

BIO-3950 Master thesis in Biology
May 2019

UNIVERSITY OF TROMSØ

Cover photo retrieved from *https://oceanwide-expeditions.com/to-do/wildlife/antarctic-fur-seal*

Photo of a female Antarctic fur seal (*Arctocephalus gazella*)

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Supervisors

Morten Tryland, UiT-The Arctic University of Norway

Andrew Lowther, Norwegian Polar Institute

Ingebjørg Helena Nymo, Norwegian Veterinary Institute

Preface

This thesis has been written as an article for submission to the «Journal of Wildlife Diseases». The manuscript has thus been prepared according to the editor guidelines for this journal. Co-authors Eva M. Breines and Jörn Klein performed DNA extractions and phylogenetic trees, respectively.

I would like to thank my supervisors Morten Tryland, Andrew Lowther and Ingebjørg Helena Nymo for giving me the opportunity to work in such interesting and enriching study. I have gained so much knowledge due to their constant input and support. I feel really privileged I had the chance to work with such experienced researchers and so lucky they developed in me the motivation in keeping on the research path.

The study would not have been completed without the assistance of Eva M. Breines during laboratory analyses, it is really rewarding I could develop new skills under her supervision, and also I would like to recognize Jörn Klein's work for his input in bioinformatics and phylogenetics. Special thanks to Javier Sánchez Romano and the whole research group of Arctic Infection Biology, who are always willing to help and contribute with a nice working atmosphere. Also, I feel grateful for the guidance provided by Siv Andreassen.

I find this part the hardest to write and I could not skip it without acknowledge my family and friends back home for all the sacrifices they made for my future and all the trust they always had on me. Thanks to Stakkevollan squad for being my family during my stay in Tromsø and to my office mates for all the bounding after numerous shared complaints. Finally, Chiara grazie per aver condiviso questa avventura insieme and Greg, my biggest support this whole time.

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ABSTRACT

An increased pressure of human activities that can introduce new infectious agents and therefore alter host-pathogen interactions, resulting in epizootics and biodiversity loss, challenges the Southern Ocean. Health status information and the potential effect of disease outbreaks in the Southern Ocean fauna is scare and evaluating the susceptibility to new infectious agents is decisive for its conservation. Herein we assess the exposure of Antarctic fur seals (AFS, *Arctocephalus gazella*) and Southern elephant seals (SES, *Mirounga leonina*) to pathogens known to be present in pinnipeds elsewhere. AFS (n = 65) serum and swab samples and SES (n = 13) serum samples from the sub-Antarctic island of Bouvet (54º41'S, 03º29'E) were collected in two austral summers (2014-2015, 2017-2018). Three PCR protocols for parapoxvirus DNA detection amplifying the DNA polymerase, *B2L* and *GIF* genomic regions were performed. The genomic region encoding for glycoprotein B (gB) was targeted to detect Phocine herpes virus-1 (PhHV-1) viral DNA. Sera were assayed for *Toxoplasma gondii* and smooth *Brucella* spp. antibodies with indirect enzyme linked immunosorbent assays (iELISAs). PCR amplicons of the expected size were generated for parapoxvirus in two of the 29 AFS pups (nasal swabs, 2014-2015), for the *B2L* (n=2) and DNA polymerase (n=1) genes, whereas the *GIF* PCR did not amplify relevant sequences. Sequencing of the PCR amplicons was carried out and blasted in GenBank finding most homology with a seal parapoxvirus, confirming the presence of the virus in AFS for the first time. Antibodies against *T. gondii* or smooth *Brucella* spp. were not detected, and no PhHV-1 amplicons were generated. Overall, our data indicate that these seals are not threatened by being exposed to the pathogens tested, although parapoxvirus infections may cause severe disease in young and stressed individuals. These data contribute to the baseline for the evaluation and management of these seal populations.

Key words: Antarctic fur seals, parapoxvirus, Southern elephant seal, Southern Ocean, wildlife disease, zoonotic.

INTRODUCTION

An increased monitoring of infectious diseases has been reported in the marine environment, with factors such as climate change and human activities contributing to the impact of diseases in the ocean (Harvell et al., 1999; Burek et al., 2008). Marine mammals are shown to have potential as sentinel species to evaluate ocean ecosystem health by providing early warning about current or future threats, which enable a better management of potential negative impacts (Bossart, 2011). Little is known about the stressors these marine species are exposed to, such as persistent organic pollutants (POPs), which has been shown to impair immune responses in pinnipeds (De Swart et al., 1996), or the presence and prevalence of potential pathogens. Such factors could affect population dynamics with major biological implications; disease outbreaks can trigger loss of biodiversity by mass mortalities, increasing the risk of local extinctions (Daszak et al., 2000). Additionally, some of these infectious agents might be transmissible between animals and humans and thus present a risk to public health (Tryland, 2017).

The Southern Ocean is a pristine wilderness hotspot for biodiversity, but the recent increased development in anthropogenic activities and the occurrence of introduced animal species into this environment coupled with global warming effects, may lead to the exposure of immunologically naïve animals to new infectious agents (Edwards et al., 1998; Frenot et al., 2005). It is still not clear whether infectious agents present in pinniped species elsewhere are present in the Southern Ocean, with few studies having addressed the exposure of Southern Ocean seals to various microorganisms, including human pathogens. Four *Salmonella* serotypes, enteropathogenic *Echerichia coli* and *Campylobacter* spp. have been isolated from Antarctic fur seals (AFS, *Arctocephalus gazella*) (Palmgren et al., 2000; Hernández et al., 2007; García-Peña et al., 2010). Antibodies against morbillivirus have also been found in serum samples from Weddell seals (*Leptonychotes weddellii*; 6.6 %), crabeater seals (*Lobodon carcinophagus;* 33.3 %) and leopard seals (*Hydrurga leptonyx;* 66.7 %) (Bengtson et al., 1991; McFarlane, 2009).

Poxviruses in genus *Parapoxvirus,* are large, enveloped and double-stranded DNA viruses that cause cutaneous lesions in wildlife and domestic fauna, mainly sheep, goat and cattle (Tryland et al., 2018). Evidence of exposure to parapoxvirus have been found in members of the families *Phocidae*, *Otariidae* and *Odobenidae* (Table 1), suggesting sealpox virus and California sea lion (*Zalophus californianus*) poxvirus as tentative members of the genus *Parapoxvirus* (Becher et al., 2002; Nollens et al., 2006_b). Parapoxvirus infection in pinnipeds

causes skin lesions characterized by firm, raised, single or multiple, proliferative nodules from 0.5 – 3.0 cm in diameter on the head, neck, flippers and mucosa that may ulcerate (Müller et al., 2003). Transmission takes place through contact with dermal abrasions or contaminated crusts (Tryland, 2011). Studies have shown that the virus can be transmitted from pinnipeds to humans after contact or following a physical trauma, developing classic nodular abrasions as the ones reported with other parapoxviruses infections (Hicks and Worthy, 1987; Clark et al., 2005). The isolation of a parapoxvirus from a Weddell seal in Dronning Maud Land (Tryland et al. 2005_a) is the only evidence to date confirming the presence of the virus among Southern Ocean pinnipeds.

The Gram- negative bacteria *Brucella* spp. is known to infect a wide range of domestic and wild, endothermic and ectodermic animals, including humans (Godfroid, 2002; Eisenberg et al., 2012). Even though the zoonotic potential of the marine *Brucella* spp. is unclear (Larsen et al., 2013; Nymo et al., 2016_a), it has been related with spinal osteomyelitis and neurobrucellosis in humans that had been in contact with raw products from the sea (Sohn et al., 2003, McDonald et al., 2006). Since it was first isolated in 1994 from four harbor seals (*Phoca vitulina*), two harbor porpoises (*Phocoena phocoena*) and a common dolphin (*Delphinus delphis*), *Brucella* spp. has been isolated from various organs in apparently healthy marine mammal species in the Northern hemisphere (Table 1). However, observations of *Brucella*-associated pathology in pinnipeds have exclusively been observed in eared seals (Duncan et al., 2014). Retamal et al., 2000 showed the first indication of *Brucella* spp. in the Southern Ocean when detecting *Brucella* antibodies in AFS (31.3 %) and in a Weddell seal. Subsequent studies have detected antibodies in AFS (7.7 %) (Abalos et al., 2009; Table 1), Weddell seals (36. 8 %), a Ross seal (*Ommatophoca rossii*, 5.0 %), crabeater seals (11.1 %) (Tryland et al., 2012; Table 1) and Southern elephant seals (SES, *Mirounga leonina*, 4.2 %) (Jensen et al., 2013; Table 1).

A neurotropic alpha-herpesvirus, Phocine herpesvirus-1 (PhHV-1), was first isolated and characterized after a disease outbreak among harbor seals in a rehabilitation center in the Netherlands (Osterhaus et al., 1985). Fatal generalized infections are mostly seen in neonates and weanlings causing hepatitis and necrosis of the adrenal cortex (Borst et al., 1986; Gulland et al., 1997). Isolation of the virus and antibody detection against PhHV-1 has been numerously reported in pinnipeds in the Northern hemisphere (Table 1). Indication of PhHV-1 exposure in the Southern Ocean was first published in 1991 when antibodies were detected in all the 25 investigated Weddell seals and in three crabeater seals. This was further supported by the detection of antibodies against the virus in Ross seals (15.0 %) and AFS (56.7 %) (Tryland et

al., 2012; Table 1). Even though there is no clear evidence that PhHV-1 is zoonotic (Tischer and Osterrieder, 2010), it is considered as an important widespread seal pathogen (Table 1) that could affect population recruitment.

Toxoplasma gondii is an obligate intracellular protozoan suspected to act as an opportunistic parasite in immunocompromised individuals (Mazzariol et al., 2012). In marine mammals toxoplasmosis is associated with encephalitis (Dubey et al., 2009), adrenalitis (Cruickshank et al., 1990) and lymphadenitis (Domingo et al., 1992). *T. gondii* oocysts, which are shown to remain viable in seawater for extended periods of time (Lindsay and Dubey, 2009), can infect marine mammals through surface runoff and sewage discharge (Miller et al., 2002). Investigations carried out in the Southern Ocean have demonstrated seroprevalence of 18.2 % and 6.2 % in Weddell seals and SES, respectively (Jensen et al., 2012; Table 1) and in SES (76.9 %), Weddell seals (41.9 %), AFS (2.4 %) and in one out of two crabeater seals (Rengifo-Herrera et al., 2012; Table 1).

Sub-Antarctic islands provide an ideal setting for the development of disease epizootics, since animals haul out in huge numbers in a relatively small space during breeding and molting (Norman, 2008). Apart from its geographical isolation, the Norwegian sub-Antarctic island of Bouvet (Bouvetøya, 54º41'S, 03º29'E) represents a crucial contact point between the Antarctic ecosystem and the rest of the world, in the Atlantic Ocean south of the Antarctic Circumpolar Current (Figure 1). This small land of 49 km^2 is home to a number of marine species, including the true seal SES, the eared seal AFS, brown skuas (*Catharacta antarctica lonnbergi*), Southern giant petrels (*Macronectes giganteus*) and macaroni (*Eudyptes chrysolophus*) and chinstrap penguins (*Pygoscelis antarcticus*) (Huyser, 2001). Some of these animals conduct long range seasonal migrations (Bester, 1989; Arthur et al., 2017), being in contact with different waters, food chains and ecosystems and are therefore potential hosts and vectors for infectious agents affecting the ecology and pristine environment of the Island. AFS and SES have a Southern circumpolar distribution, inhabiting areas north and south of the Antarctic Polar Front (Bonner, 1968; Laws, 1994). The second largest breeding colony of AFS is found at Bouvet Island, while SES breed and molt in small numbers (Kirkman et al., 2001; Hofmeyr et al., 2005). Lactating females are constrained to forage close to the breeding colony travelling up to 100 km to feed (Biuw et al., 2009). However, once their respective lactation periods are over, both SES and adult female AFS adopt a pelagic behavior dispersing over wider areas of known high productivity (Boyd et al., 2002; Hindell et al., 2016). AFS gather in dense concentrations at Bouvet Island and share a terrestrial breeding ground at Nyrøysa with different animal species (Figure 1), increasing the potential of cross-species transmission of infectious agents. A

previous study conducted at the island did not detect *T. gondii* neither *Brucella* antibodies in 68 and 64 AFS serum samples, respectively, but found 42 (56.8 %) seropositive individuals against PhHV-1 (Tryland et al., 2012).

The aim of this study was to evaluate the exposure of AFS and SES which breed on Bouvet Island to selected pathogens, known to cause significant mortality and/or morbidity in pinnipeds. Pathogens were selected on the basis of its presence in other investigated seal populations, of their zoonotic potential and their possible implications for population demography. Results from the project are regarded as valuable in the future evaluation and management of these seals by helping to characterize infectious disease risks in these species.

MATERIALS AND METHODS

Sampling

Sampling was carried out under permits from the Norwegian Food Safety Authorities (Permit ID: 12411). AFS (n = 65) serum and swab samples and SES (n = 13) serum samples from Bouvet Island were collected by the Norwegian Polar Institute over two austral summers (2014-2015 and 2017-2018, henceforth referred to as 2015 and 2018, respectively) (Table 2). The study was conducted at Nyrøysa, a platform of land approximately 2 km long and 500 m wide on the west side of the Island (Figure 1).

Adult (>1 year) SES and male AFS were immobilized with a combination of tiletamine and zolazepam (Zoletil vet®) (1 mg/kg), administrated remotely using a dart gun (PAXARMS, Domett, New Zeland). Adult female AFS were captured using a fuhrman flex net and sedated with isoflurane derived through a portable anaesthetic system (Advanced Anaesthesia Specialists, Gladesville, Australia), initially inducted at 5 % and maintained between 0.5 – 1.5 %. Offspring of the year were captured by hand and manually restrained.

For SES, blood was collected from the extradural intravertebral vein in blood tubes with sodium-heparin (BD Vacutainer®; BD, Plymouth, UK) using a 72 mm spinal tap needle. For AFS, blood samples were taken from the interdigital vein from the hind flippers using a hypodermic 18 gauge 45 mm needle mounted on a 5 ml syringe into blood tubes with sodium-heparin. Due to technical challenges, centrifugation of the sera was not conducted in the field and full blood was stored at -20 °C. Before analysis, the blood tubes were thawed and

centrifuged for 5 minutes at 3000 rpm. One ml aliquots of the supernatant were taken and stored at -20 ºC until analysis.

Swab samples were taken from the AFS nose and eye. Cotton swabs for virology (Applimed SA, Châtel-St-Denis, Switzerland) were rubbed against the mucosa of the nostrils and conjunctival fornix and transferred into sterile cryotubes with 800 µl of Eagle's Minimum Essential Medium (EMEM) with antibiotics (final concentrations of 100 IU/ml of penicillin, 100 µg/ml of streptomycin, 50 µg/ml of gentamicin and 2.5 µg/ml amphotericin B). Samples were stored at -20 ºC until analysis.

Polymerase Chain Reaction (PCR)

DNA was extracted from the swab samples using the Maxwell® 16 Buccal Swab LEV DNA Purification Kit (Promega, Wisconsin, USA) according to the guidelines provided by the manufacturer. The quality and content of the DNA was assessed by spectrophotometry using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific™, Massachusetts, USA). Samples were considered acceptable if the A260/280 ratio was higher than 1.7. The PCR reactions were run in a Perkin Elmer GeneAmp@ PCR System 9700 (Perkin Elmer Corp., Norwalk, Connecticut, USA).

Parapoxvirus

Three different PCR protocols were conducted. Part of the genomic region *B2L* encoding the putative virion envelope antigen p42K of parapoxviruses (Sullivan et al., 1994) and the *GIF* gene, which encodes for a protein inhibiting of granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-2 (IL-2) (Deane et al., 2000), were targeted using specific primers (Table 3). DNA extracted from mucosal tissues (nose and conjunctiva) was amplified as previously reported (Klein and Tryland, 2005), but with an annealing temperature of 68 ºC (*B2L*) and an incubation period of five minutes (*B2L, GIF*). Water was used as a negative control while DNA extracted from a goat kid (*Capra hircus*) with contagious ecthyma and verified Orf virus (ORFV) infection (M Tryland, Norwegian Veterinary Institute, Tromsø, Norway) was used as a positive control.

Primers PPV/DNApol-F and PPV/DNApol-R (Table 3) were used to target the DNA polymerase gene for those samples that yielded amplicons with the PCR protocols described above. The PCR was carried out as previously reported (Bracht et al., 2006), but increasing the annealing temperature to 65 ºC. Water was used as negative control. DNA extracted from an Atlantic harbor seal with a verified parapoxvirus infection (M Garron, National Oceanic and Atmospheric Administration, Washington DC, USA) was used as positive control.

Phocine Herpesvirus-1

The genomic region encoding for glycoprotein B (gB), a highly conserved component of herpesvirus cell-fusion machinery (Spear and Longnecker, 2003) was targeted to detect PhHV-1 viral DNA (Table 3). An initial incubation at 94 ºC for 4 minutes was followed by 25 cycles of denaturation (94 ºC, 30 sec), annealing (54 ºC, 30 sec) and extension (72 ºC, 50 sec). Water was used as a negative control and PhHV-1 DNA from an isolate obtained from a harbor seal (Frey et al., 1989) was used as a positive control.

Gel electrophoresis and sequencing

Ten microliters of the PCR products were separated by electrophoresis in a 1 % agarose gel (Ultra-pure agarose gel, Life Technologies, California, USA) in a 1 x TBE buffer (0.04 M Trisborate, 1.0 mM EDTA) containing 0.0005 % GelRed, with a separation time of 1.5 h at 110V. The bands were visualized under ultraviolet light and photographed with a gel documentation system (Bio-Rad Laboratories, California, USA). ExoSAP-IT™ (Amersham Pharmacia Biotech, Sweden) was used to clean the relevant amplicons by removing unused dNTPs and primers. Following the clean-up, The Big Dye® Terminator v3.1 Cycle Sequencing protocol (Applied Biosystems, Norway) was performed. Three µl of the purified PCR products were mixed with 3 µl "Ready Reaction Premix", 2 µl sequencing buffer, 1 µl of each primer and 11 µl dH_20, the mixture was subsequently thermal cycled as described by Klein and Tryland*.,* 2005. Sequencing of the gene amplicons was carried out by the genetics laboratory at UiT The Arctic University of Norway, Tromsø, Norway. Sequences were submitted to GenBank (NCBI, Bethesda, MD, USA) under Accession Numbers MK908011, MK908012 and MK910261.

Phylogenetic analysis

The evolutionary history for the *B2L* and DNA polymerase gene was inferred by using the Tamura 3-parameter model and Maximum Likelihood method (Tamura, 1992). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 59.69 % sites for polymerase gene). Trees are

drawn to scale, with branch lengths measured in the number of substitutions per site. These analyses involved 14 (*B2L*) and 17 (polymerase gene) nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 529 (*B2L*) and 355 (polymerase gene) positions in the final datasets. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Serology

A protein A/G indirect enzyme-linked immunosorbent assay (iELISA), validated to detect smooth *Brucella* spp. antibodies in seals, was performed as previously described (Nymo et al., 2013).

The ID Screen Toxoplasmosis Indirect Multi-species ELISA kit (ID.vet, Montpellier, France) was used to test the presence of anti-*T. gondii* antibodies as reported in the manufacturer instructions.

RESULTS

From the nose swab samples, products of the expected size were generated for the *B2L* gene in two AFS pups from the 2015 sampling period (Table 4). Within different parapoxvirus gene sequences compared, the sequences generated from the two pups demonstrated 99 % (pup 15; MK908011) and 88 % (pup 5; MK908012) sequence identity with a California sea lion parapoxvirus-2 (DQ273137.1). The PCR targeting the DNA polymerase gene did only amplify DNA from pup 5 (MK910261), having the highest nucleotide homology (97.5 %) with a pinniped parapoxvirus isolated from a spotted seal (*Phoca largha*; AY780678.1). We were not able to amplify parapoxvirus-specific DNA from any of the samples using the *GIF* PCR protocol. None of the three parapox PCRs were able to generate amplicons of expected size in any of the eye swab samples.

The trees with the highest log likelihood (-1658.62 for *B2L* and -1334.58 for polymerase gene) are presented (Figure 2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The phylogenetic analysis of the sequences generated from the *B2L* genomic region (Figure 2a) of the two cases revealed that they were seal parapoxvirus, grouping them among other seal and sea lion isolates and differentiating them from other parapoxviruses.

The phylogenetic analysis based on the PCR amplicon from the DNA polymerase gene sequence showed that the AFS parapoxvirus resembled seal parapoxvirus more than other

parapoxviruses, such as Bovine popular stomatitis virus (BPSV), Pseudocowpox virus (PCPV) and ORFV (Figure 2b).

No PhHV-1 amplicons were generated in any of the swab samples. All serum samples were below the cut-off of the iELISA detecting *Brucella* spp. (Table 4). The mean %P in SES was 16.2 %P (SD: 19.1) and the mean %P in AFS was 3.6 %P (SD: 5.3). Similarly, no antibodies against *T. gondii* were detected in any of the serum samples.

DISCUSSION

Parapoxvirus

The phylogenetic analysis of the PCR amplicons generated by the primers targeting the *B2L* gene showed that two individuals included in this study had parapoxvirus-specific DNA on their nasal mucosa. We thus report the first verified case of parapoxvirus infection in AFS and the second case in Southern Ocean pinnipeds. The samples of AFS clustered well together with other pinniped isolates for the two genes that generated amplicons, the *B2L* and the DNA polymerase gene. This was evident for both genes since the node branch likelihood had a value of 100 (*B2L*, Figure 2a) and the branch likelihood at the separation point among pinniped parapoxvirus and other parapoxvirus was 99 (polymerase gene, Figure 2b), suggesting a well-supported node and separation point for these specific genomic regions, and that the AFS virus belongs to the tentative seal parapoxvirus species (Nollens et al., 2006$_b$). Even though the phylogeny of the DNA polymerase gene indicates that AFS virus is closely related with a parapoxvirus isolated from a Spotted seal (AY780678.1), in general and for both genes, parapoxviruses from *Phocidae* were more closely related to each other than to parapoxvirus in *Otariidae,* which might indicate differences in host susceptibility. For the *B2L* phylogeny, AFS parapoxvirus were most closely related to a California sea lion parapoxvirus-2 (DQ273137.1) with a branch likelihood of 100, thus even though the distribution area of both species does not overlap, indirect contact and occasionally transmission of the pathogen might occur. It has already been hypothesized that pinniped parapoxvirus can infect multiple pinniped host species belonging to the same phylogenetic family (Nollens et al., 2006$_b$).

Even though PCR targeting *GIF* is a robust technique to discriminate among species within genus *Parapoxvirus* (Deane et al., 2009), it did not produce any amplicons in the present study. *GIF* is not considered an essential gene for virus replication, being located in the right terminal of the *Parapoxvirus* genome (Haig et al., 2002). Thus, it can go thorough re-adjustments in order to adapt to changes in the host immune response (Klein and Tryland,

2005), and such changes might explain why no amplicons were detected in our samples. The DNA polymerase PCR protocol has been used in previous studies to detect parapoxvirus in seal samples (Burek et al., 2005; Bracht et al., 2006). In our study, this PCR only generated relevant amplicons in one of the two samples positive on the *B2L* PCR. This supports the view that the use of multiple PCRs targeting a combination of different genes may be helpful in order to detect parapoxviruses (Klein and Tyland, 2005).

Parapoxvirus infection has been suggested to be prevalent in pinnipeds in the Northern hemisphere after the detection of virus specific antibodies in several seal species (seroprevalence 10.0 – 40.0 %) from North West Europe, North America and Siberia (Osterhaus et al., 1994) and in 693 (91.1 %) free ranging California sea lions (Nollens et al., 2006$_a$). This contrast with the apparently low presence of the virus agent in the Southern Ocean (Tryland et al., 2005$_a$). There is limited knowledge regarding parapox transmission patterns among wild seals and whether this pathogen is endemic in Southern Ocean pinnipeds. Bouvet Island is a protected nature reserve, where activities, sewage disposal and traffic are strictly regulated. The island is sporadically visited for a few months during scientific expeditions, making it quite unlikely that human disturbances would be facilitating the emergence of infectious diseases in this area. AFS disperse widely in the Southern Ocean, travelling long distances beyond their normal range and interacting with different wildlife, oceans and ecosystems (Boyd et al., 2002; Hofmeyr et al., 2006). Consequently, AFS vagrants could have acquired the virus when in contact with different environments and introduced it to Bouvet Island.

Evidence of seal parapoxvirus in this study was documented in two pups whilst parapox specific DNA was not detected in their respective mothers, indicating that weanlings might be exposed to the virus within their first weeks of life (Nollens et al., 2006$_a$). Parapoxviruses have an opportunistic behavior, infecting and causing disease in immunosuppressed or stressed individuals. Nutritional stress in combination with insufficient protection by maternal antibodies could have influenced the susceptibility of the pups to parapoxvirus infection (Müller et al., 2003; Tryland et al., 2005a). Pups were not weaned at the moment of sampling and factors such as starvation and poor milk quality can lower their immune status, favoring infections. The main prey of AFS, the Antarctic krill *Euphasia superba* (Kirkman et al., 2000), relies on ice for predator avoidance (Marschall, 1988) and ice-algae for food (Quetin and Rodd, 1991). Models predict that the influence of global warming in the South Pole will yield a striking decline in krill stocks (Flores et al., 2012), contributing significantly to nutritional stress in AFS (Siniff et al., 2008) and therefore predisposing this specie to infections and diseases.

Even though our findings indicate that the animals were harboring the infection at the moment of sampling, epidemiological data indicates that parapoxvirus infection in wild pinnipeds does not cause a high mortality rate. Thus, at population level, infection with the virus does not seem to represent a major threat, since it is considered common in the wild and lesions usually heal spontaneously (Nollens et al., 2006_a). In captive facilities, however, parapoxvirus infections may pose a major risk, with a high morbidity rate, where captured-induced stress and change of environment may initiate disease outbreaks and facilitate transmission (Hicks and Worthy, 1987; Müller et al., 2003). Feeding and respiratory functions can be impaired when the nodules develop in the oral cavity or nostrils, which together with secondary bacterial infections, can be fatal (Tryland, 2011). Animal handlers should be aware of the zoonotic potential of this pathogen (Tryland, 2017).

Brucella spp.

Anti-*Brucella* antibodies were not detected in the present study, which is in agreement with previous studies (Tryland et al., 2012). The detection of antibodies is, however, dependent on the time post-infection. Microorganisms sharing epitopes with *Brucella* may also cross-react with the bacteria, yielding false positives (Kittelberger et al., 1998; Godfroid et al., 2002). Thus, the gold standard for *Brucella* spp. diagnostics remains isolation of the bacteria. Attempts to isolate *Brucella* spp. has so far not succeeded in Southern Ocean pinnipeds (Abalos et al., 2009; McFarlane, 2009).

Even though all the samples tested in our study were below the cut-off, the %P values for SES were higher than those found in AFS (Supplementary material A, B). The same pattern has previously been described in Alaskan true and eared seals (Nymo et al., 2018). This difference between the seal families has been suggested to be due to greater resistance to infection in eared seals or different diets (Nymo et al., 2018). The survival of *Brucella pinnipedialis* in Atlantic cod (*Gadus morhua*) suggests that the infection might be transmissible through the food web (Nymo et al., 2016_b). AFS from Bouvet Island mainly feed on *E. superba*, while small myctophid fishes and squid are taken opportunistically (Kirkman et al., 2000). Even if myctophids and squid dominate SES food intake (Bradshaw et al., 2003), recent investigations emphasize the contribution of crustaceans in their diet (Lübcker et al., 2017). Further studies are warranted in order to clarify if differences in prey item consumption are linked to differences in *Brucella*-exposure in *Phocidae* and *Otariidae.*

Although terrestrial *Brucella* infection has been recognized as a cause of abortion and infertility, the few evidences of *Brucella*-associated reproductive failures in pinnipeds have been reported in otariids (Goldstein et al., 2009; Duncan et al., 2014). Despite the high *Brucella* seroprevalence in phocids from the Northern hemisphere (Table 1) and the abortion in cattle experimentally infected with a harbor seal *Brucella* isolate (Rhyan et al., 2001), *Brucella*-associated pathology linked to a reduced reproductive success in *Phocidae* remains to be documented (Nymo et al., 2011). These differences in pathology among seals together with the lack of *in vitro B. pinnipedialis* multiplication in hooded seal (*Cystophora cristata*) epithelial cells have led to the hypothesis that true seals may not be the definitive host (Larsen et al., 2015). Whether true and eared seals are infected by different *Brucella* strains is currently unknown.

The seroprevalence detected in previous studies in the Southern Ocean (Table 1) suggest that *Brucella* spp. might be present in this environment. The lack of anti-*Brucella* antibodies in the AFS and SES populations in Bouvet Island could be due to a lack of exposure to infection in these seals or, alternatively, animals with high susceptibility to *Brucella* spp. could have been infected and removed from the population due to mortality. The latter seems unlikely taking into account the lack of *Brucella*-associated pathology in true seals (Nymo et al., 2011). Additional work aiming at the isolation of the bacteria would be required to determine the *Brucella* spp. infection dynamics in Southern Ocean seals.

Phocine Herpesvirus -1

Tryland et al*.,* 2012 found antibodies against PhHV-1 in 42 (56.8 %) AFS from Bouvet Island. This high seroprevalence indicates that the Bouvet Island AFS breeding population has been exposed to PhHV-1. We were unable to detect any active virus shedding at the moment of sampling, most likely due to the small fraction of the population tested.

Seropositive Antarctic pinnipeds have also been reported in Dronning Maud Land and the Weddell Sea (Table 1). Even though the finding of seropositive Weddell seals against PhHV-1 (100.0 %), feline herpes virus -1 (FHV-1; 12.0 %) and canine herpes virus (CHV; 64.0 %) has led to speculate that terrestrial carnivores introduced into Antarctica and sub-Antarctic Islands might have worked as vectors of the virus (Harder et al., 1991), herpesvirus transmission from dogs to seals in the Southern Ocean is not well documented. PHV-1 is suspected to be an endemic seal pathogen in Antarctic pinnipeds, although vagrant movements as the ones

reported in crabeater seals could facilitate the spread of the virus over a wider geographical spectrum (Harder et al., 1991).

Whether PhHV-1 has been introduced into the Southern Ocean or remains as an endemic virus in Antarctic seals, requires further investigation. Future studies with a larger sample size from seals at Bouvet Island will be needed to clarify if PhHV-1 is circulating in these seal populations.

Toxoplasma gondii

Our results and the lack of previous indications of *T. gondii* infection or antibodies in seals from Bouvet Island suggest that the parasite is absent or present in very low prevalence in these populations (Tryland et al., 2012). However, our findings differ from earlier investigations in which *T. gondii* antibodies were detected in Southern Ocean seals when the direct agglutination test (DAT) was applied (Jensen et al., 2012; Rengifo-Herrera et al., 2012). DAT and modified agglutination tests (MAT) have extensively been used in serological detections of *T. gondii* antibodies in various animals (Huong et al., 1998; Zarnke et al., 2000), including marine mammals (Table 1). Nonetheless, the accuracy of the agglutination tests has been questioned suggesting that they might yield false positives in lipid rich samples, such as the ones from marine mammals, and therefore ELISA is considered to be a more reliable test for *Toxoplasma* serological screening (Blanchet *et al*., 2014) and was hence used in the present study.

Based on our results, Southern Ocean pinnipeds are not facing serious infectious disease risks caused by the investigated pathogens. However, surveillances such as the one presented here illustrate data for only a small fraction of the population, and more systematic monitoring with larger sample sizes and time series are required in order to draw solid conclusions on the health status of these pinnipeds and the impact of the seal pathogens that are present in the Southern Ocean. Numerous indications of infectious diseases and outbreaks in Arctic pinnipeds have been recorded (Table 1) as a result of the human encroachment this region is experiencing. Similar patterns are expected to happen in the Southern hemisphere if the human-wildlife interface increases (Daszak et al., 2001). Additionally, global warming and bioaccumulative immunotoxic compounds may facilitate the vulnerability of wildlife populations to infectious diseases. These stressful ecological changes might increase pathogen survival and distribution, as well as the host susceptibility and disease-transmission rate (Ross et al., 2002; Burek et al., 2008). Infectious diseases may impact both the health of the seal populations as well as humans, since many of the seal pathogens are zoonotic.

Seals are apex predators and prime sentinel species in the marine ecosystems. The current health status of AFS and SES at our study location indicate that these populations and their environment are not seriously compromised by the seal pathogens studied. Whether this can be applied to other Southern Ocean regions warrants further investigation. The finding for the first time of parapoxvirus infection in AFS shows that these animals can host the virus. Future work aiming to isolate parapoxvirus at Bouvet Island will help to better understand its epidemiology in these seals. Further investigations integrating climate change, immunotoxic contaminants and human activities will help to clarify the contribution of these factors to emerging infectious diseases in the Southern Ocean.

ACKNOWLEDGMENTS

We thank the logistic support from the South African Department of Environmental Affairs and the Captain of the SA Agulhas II and biologist/collaborators from the Norwegian Polar Institute and University of Pretoria for sample collection. Further thanks to Javier Sánchez Romano for his assistance in sequence submission. Financial support was provided by the Norwegian Antarctic Research Expedition (NARE).

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Table 1. Summary of marine mammal-associated pathogens evaluated in this study. References are listed in chronological order for each pathogen.

Patoghen Taxonomy				**Notes**				
Specie	**Order**	**Family**	**Host**	**Method***	**Sample**	**Location**	**Year**	**Reference**
Parapoxvirus spp.	-	Poxviridae	Bottlenose dolphin (*T. truncatus*) White -sided dolphin (*L. acutus)*	LM, EM	Tissue	Marathon, Florida Wellfeet and captive facility, Massachusetts	-	Geraci et al., 1979
			Dusky dolphin (*L. obscurus*) Burmeister's porpoise (*P. spinipinnis*)	TEM	Tissue	Peru	August, September and December 1990	Van Bressem et al., 1993
			Harbor seal (*P. vitulina)*	LM, EM, in situ hybridization, PCR	Swab, tissue	Wadden sea, Germany	Summer 2000	Müller et al., 2003
			Steller sea lion (*E. jubatus*)	LM, negative staining EM, PCR	Biopsy	Prince William Sound, Alaska	2000, 2001	Burek et al., 2005
			Weddell seal (*L. weddellii)*	EM, PCR	Tissue	Queen Maud Land, Antarctica	2001	Tryland et al., 2005_a
			California sea lion (*Z. californianus*)	iELISA	Sera	Rehabilitation facility and central coast of California	January 2000-August 2004	Nollens et al., 2006_a

			Spotted seal (*P. largha*)	PCR	Swab, sera, tissue, nodules biopsy	Nagoya, Japan	March 2010	Ohno et al., 2011
			Southern right whale (*E. australis*)	TEM, PCR	Tissue	Península Valdés, Argentina	2012-2013	Fiorito et al., 2015
			Atlantic walrus (*O. rosmarus rosmarus*)	PCR	Swab	Svalbard, Norway	July 2014 August 2015	Scotter et al., 2019
Brucella spp.	Rhizobiales	Brucellaceae	Harbor seal (*P. vitulina*) Harbor porpoise (*P. phocoena*) Common dolphin *(D. delphis*)	Isolation	Tissue	Scottish coast and North East England	1991-1994	Ross et al., 1994
			Ringed seal (*P. hispida*) Atlantic walrus (*O. rosmarus rosmarus)*	cELISA, tube agglutination test	Sera	Canadian Arctic	1987-1994	Nielsen et al., 1996
			Common bottlenose dolphin (*T. truncatus)*	Tissue culture, SAT, CT, RIV, PCR, hitopathology and immunoperoxidase technique	Dead fetus, placenta, sera, swab	Captive facility, USA	1992, 1997	Miller et al., 1999
			Hooded seal (*C. cristata*) Harp seals (*P. groenlandica*)	iELISA, SAT, EDTA modified SAT, RBT, CFT, anti-complement ELISA, isolation	Sera, tissue	Iceland, Jan Mayen, Northern coast of Norway, Kola Peninsula,	1983-1996	Tryland et al., 1999

Ringed seals (*P.hispida*) Minke whales (*B. acutorostrata*) Fin whales (*B. physalus*) Sei whales (*B. borealis*)			West Svalbard and Barents Sea		
Ringed seal (*P. hispida*) Harp seal (*P. groenlandica*)	cELISA, Isolation	Sera, lymph node tissue	Canadian Arctic	1995, 1996	Forbes et al., 2000
Antarctic fur seals *(A. gazella)* Weddell seal (*L. weddellii)*	RBT, CFT, AGID, cELISA	Sera	Livingston Island, Antartica	December 1998-January 1999	Retamal et al., 2000
Dusky Dolphin (*L.obscurus)* Long-beaked common dolphin (*D. capensis)* Common bottle-nose dolphin (*T. truncatus*)	cELISA, iELISA	Sera samples	Peru and Spanish Mediterranean coast.	1993-1995, 1997-1999	Van Bressem et al., 2001

			Burmeister's porpoise (*P. spinipinnis*) Striped Dolphin (*S.coeruleoalba*)				
			Ringed seal (*P. hispida*) Hooded seal (*C. cristata*)	EDTA-modified SAW, RBT, CFT, iELISA, Isolation	Sera, tissue	Svalbard and Greenland	2002, 2003	Tryland et al., 2005$_b$
			Antarctic fur seals *(A. gazella)*	cELISA	Sera	Livingston Island, Antarctica	-	Abalos et al, 2009
			Weddell seal (*L. weddellii)* Ross seal (*O.rossii*) Crabeater seal (*L. carcinophaga*)	RBT, iELISA	Sera	Queen Maud Land, Antarctica	December 2000 January/February 2001	Tryland et al., 2012
			Weddell seal (*L. weddellii)* Southern elephant seal (*M. leonina*)	RBT, iELISA	Sera	Hutton Cliffs, East Antarctica	October-December 2003	Jensen et al., 2013
Phocine alpha-herpesvirus 1	Herpesvirales	Herpesviridae	Harbor seal (*P. vitulina*)	Isolation, indirect IF, EM, ether sensitivity test, buoyant density in sucrose, NT	Sera, tissue	Captive facility, The Netherlands	-	Osterhaus et al., 1985

California sea lion (*Z. californianus*)	Isolation	Sera, major tissues	Captive facility	-	Kennedy-Stoskopf et al., 1986
Harbor seal (*P. vitulina*)	Isolation, NT	Sera, major organ tissues	North and Wadden sea	1988	Frey *et al.,* 1989
Weddell seal (*L. weddellii*) Crabeater seal (*L. carcinophagus*)	NT	Sera	Weddell sea, Southern Ocean	January/ February 1990	Harder et al., 1991
Harp seal (*P. groenlandicus*) Hooded seal (*C.cristata*)	NT	Sera	Barents sea and Jan Mayen	April/May 1991-1992	Stuen et al., 1994
Harbor seal (*P. vitulina*)	EM	Sera, major organ tissues	Central and Northern California	1990-1991	Gulland et al., 1997
Walrus (*O. rosmarus*) Northern fur seal (*C. ursinus*) Harbor seal (*P. vitulina*) Spotted seal (*P. largha*) Ribbon seal (*H. fasciata*) Steller sea lion (*E. jubatus*)	Microneutralization assay	Sera	Alaska and Russian coast	1978-1994	Zarnke et al., 1997

			Bearded seal (*E. barbatus*) Ringed seal (*P. hispida*)					
			Antarctic fur seal *(A. gazella*) Ross seal (*O. rossii*)	iELISA	Sera	Bouvetøya and Queen Maud Land, Antarctica	December 2000 January/ February 2001	Tryland et al., 2012
Toxoplasma gondii	Eucoccidiorida	Sarcocystidae	Grey seal (*H. grypus*) Hooded seal (*C. cristata*) Harbour seal (*P. vitulina*)	MAT	Sera	East Coast of Canada	1995-97	Measures et al., 2004
			Bottlenose dolphin (*T. truncatus*) Pacific walrus (*O. rosmarus divergens)*	MAT, Isolation	Sera, major organ tissues	Captive facility, Canada	2007	Dubey et al., 2009
			Ringed seals *(P. hispida*) Adult bearded seals (*E. barbatus)* Polar bears *(U. maritimus)*	DAT	Sera	Svalbard, Norway	1992-2008	Jensen et al., 2010

Weddell seal (*L. weddellii*) Southern elephant seal (*M. leonina)*	DAT	Sera	Maquarie Island and Hutton cliffs, Antarctica	2003-2008	Jensen et al., 2012
Weddell seal (*L. weddellii*) Southern elephant seal (*M. leonina)* Antarctic fur seal *(A. gazella)* Crabeater seal (*L .carcinophaga)*	DAT	Sera	South Shetland Island and Antarctic Peninsula	2007, 2010-2011	Rengifo-Herrera et al., 2012

*AGID, Agar Gel Immunodiffusion Test
cELISA, Competitive Enzymed Linked Immunosorbent Assay
CFT, Complement Fixation Test
DAT, Direct Agglutination Test
EM, Electron Microscopy
iELISA, Indirect Enzymed Linked Immunosorbent Assay
IF, Immunofluorescence Technique
LM, Light Microscopy
MAT, Modified Agglutination Test
NT, Neutralization Test
PCR, Polymerase Chain Reaction
RBT, Rose Bengal Test
RIV, Rivanol Precipitation Plate Agglutination Test
SAT, Serum Agglutination Test
SAW, Slow Agglutination Wright
TEM, Transmission Electron Microscopy

Table 2. Number of samples taken from Antarctic fur seals (*Arctocephalus gazella)* and Southern elephant seals (*Mirounga leonina*) with the respective sampling period. Some animals were sampled for both, blood and swabs, while others were just sampled for either blood or nose/eye swabs.

	Blood samples						Nose and eye swab samples			
	2014-2015			**2017-2018**			**2014-2015**		**2017-2018**	
Seal species	Female[a]	Male	Pup	Female	Male[b]	Pup	Female	Pup	Female	Pup
Antarctic fur seal	17	-	17	11	8	4	36	39	28	17
Southern elephant seal	-	-	-	7	6	-	-	-	-	-

[a] Mothers of the pups are included within the "Female group".
[b] Blood samples from males were taken only during the 2017-2018 sampling period.

Table 3. Genes targeted and the size in base pair (bp) of each, with the respective primers used.

Virus	Gene target	Primers (5'→3')	Amplicon (bp)	Reference
PhHV-1	Glycoprotein B	For: acgtgatggagctcatacagaac Rev: gctagctcgttgctaatcattgg	450	Goldstein et al., 2004
Parapoxvirus	Major envelope protein gene	PPP1: gtcgtccacgatgagcagct PPP4: tacgtgggaagcgcctcgct	594	Inoshima et al., 2000
	GM-CSF/IL-2 Inhibition factor	GIF 5: gctctaggaaagatggcgtg GIF 6: gtactcctggctgaagagcg	408	Klein and Tryland, 2005
	Polymerase gene	PPV/DNApol-F: gcgagcacctgcatcaag PPV/DNApol-R: ctgtticggaagcccatgag	536	Bracht et al., 2006

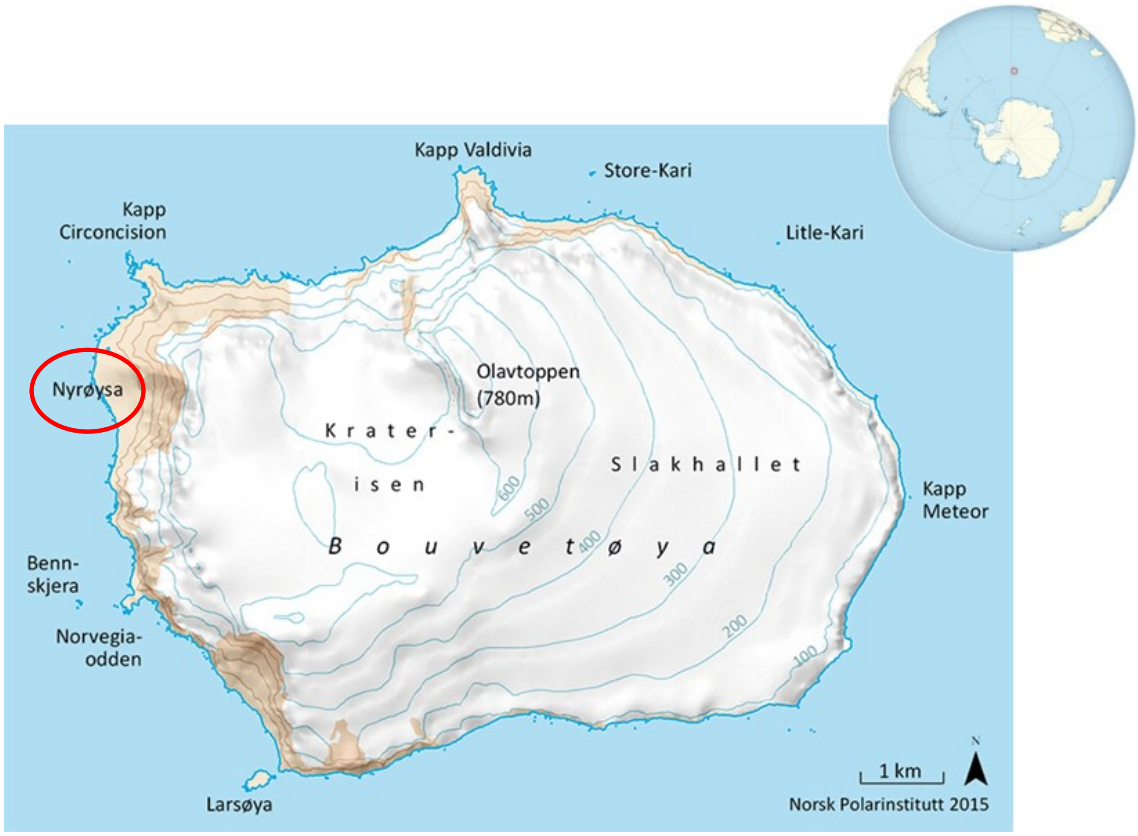
Table 4. Detection of virus specific DNA from the swab samples tested for parapoxvirus and phocine herpesvirus-1, together with the results from detection of specific antibodies against smooth *Brucella* spp and *Toxoplasma gondii* from Antarctic fur seals (*Arctocephalus gazella*) and Southern elephant seals (*Mirounga leonina*) serum samples, Results are presented as number positives/number tested (percentage prevalence).

Seal species	**Parapoxvirus**[a]	***Brucella spp.***	**PhHV-1**[b]	***Toxoplasma gondii***
Antarctic fur seals: adult females	0/34 (0)	0/28 (0)	0/64	0/28 (0)
Antarctic fur seals: adult males	NT	0/8 (0)	NT	0/8 (0)
Antarctic fur seals: pups	**2/29 (7)**	0/21 (0)	0/56	0/21 (0)
Antarctic fur seals (total)	**2/63 (3)**	0/57 (0)	0/120	0/57 (0)
Elephant seals: adult females	NT	0/6(0)	NT	0/6 (0)
Elephant seals: adult males	NT	0/7(0)	NT	0/7(0)
Elephant seals (total)	NT	0/13(0)	NT	0/13(0)

[a] Nose swabs.
[b] Eyes and nose swabs.

Figure 1. Map of Bouvet Island (54º25'S, 03º22'E) and its location in the Southern Ocean (globe). Bouvet Island is highlighted in the globe and the breeding ground Nyrøysa, where the animals were sampled, is highlighted in the map. Map: Norwegian Polar Institute (NPI).

Figure 2. a) Phylogenetic comparison of the parapoxvirus sequences generated from nasal mucosal tissue of two Antarctic fur seal pups (*Arctocephalus gazella*) from the putative viral envelope gene *B2L* with similar sequences from NCBI, including the type strain of ORFV and **b)** comparison of the sequences obtained from the DNA polymerase gene from one Antarctic fur seal with other parapoxvirus sequences (NCBI). Numbers (bootstrap values) describe the reliability for each clade in percent. Arrows point the studied sequences.


Kapp Valdivia
Store-Kari
Kapp
Circoncision
Litle-Kari
Nyrøysa
Olavtoppen
(780m)
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isen
Slakhallet
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600
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Norsk Polarinstitutt 2015

Figure 1.

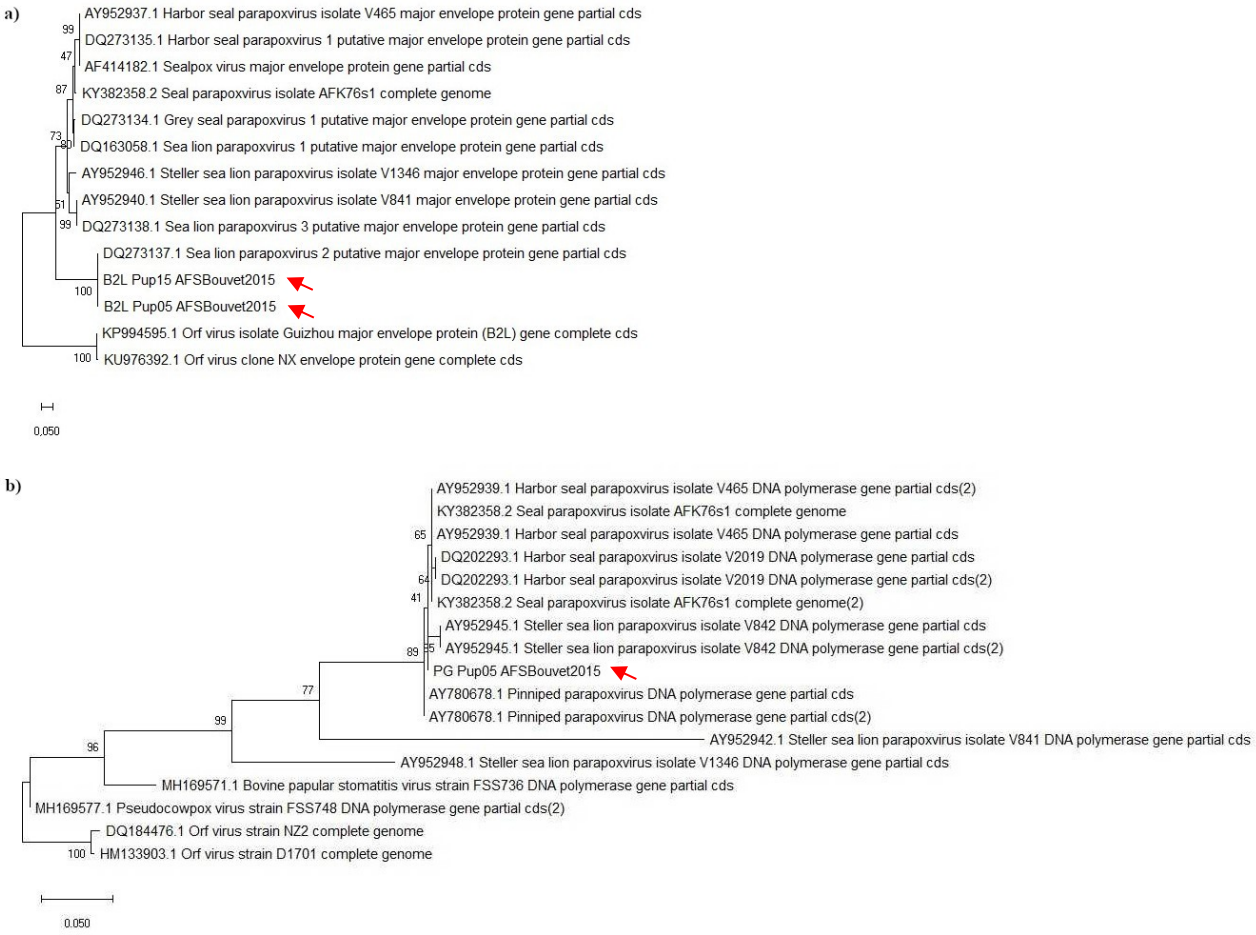
a)
AY952937.1 Harbor seal parapoxvirus isolate V465 major envelope protein gene partial cds
DQ273135.1 Harbor seal parapoxvirus 1 putative major envelope protein gene partial cds
AF414182.1 Sealpox virus major envelope protein gene partial cds
KY382358.2 Seal parapoxvirus isolate AFK76s1 complete genome
DQ273134.1 Grey seal parapoxvirus 1 putative major envelope protein gene partial cds
DQ163058.1 Sea lion parapoxvirus 1 putative major envelope protein gene partial cds
AY952946.1 Steller sea lion parapoxvirus isolate V1346 major envelope protein gene partial cds
AY952940.1 Steller sea lion parapoxvirus isolate V841 major envelope protein gene partial cds
DQ273138.1 Sea lion parapoxvirus 3 putative major envelope protein gene partial cds
DQ273137.1 Sea lion parapoxvirus 2 putative major envelope protein gene partial cds
B2L Pup15 AFSBouvet2015
B2L Pup05 AFSBouvet2015
KP994595.1 Orf virus isolate Guizhou major envelope protein (B2L) gene complete cds
KU976392.1 Orf virus clone NX envelope protein gene complete cds
0,050
b)
AY952939.1 Harbor seal parapoxvirus isolate V465 DNA polymerase gene partial cds(2)
KY382358.2 Seal parapoxvirus isolate AFK76s1 complete genome
AY952939.1 Harbor seal parapoxvirus isolate V465 DNA polymerase gene partial cds
DQ202293.1 Harbor seal parapoxvirus isolate V2019 DNA polymerase gene partial cds
DQ202293.1 Harbor seal parapoxvirus isolate V2019 DNA polymerase gene partial cds(2)
KY382358.2 Seal parapoxvirus isolate AFK76s1 complete genome(2)
AY952945.1 Steller sea lion parapoxvirus isolate V842 DNA polymerase gene partial cds
AY952945.1 Steller sea lion parapoxvirus isolate V842 DNA polymerase gene partial cds(2)
PG Pup05 AFSBouvet2015
AY780678.1 Pinniped parapoxvirus DNA polymerase gene partial cds
AY780678.1 Pinniped parapoxvirus DNA polymerase gene partial cds(2)
AY952942.1 Steller sea lion parapoxvirus isolate V841 DNA polymerase gene partial cds
AY952948.1 Steller sea lion parapoxvirus isolate V1346 DNA polymerase gene partial cds
MH169571.1 Bovine papular stomatitis virus strain FSS736 DNA polymerase gene partial cds
MH169577.1 Pseudocowpox virus strain FSS748 DNA polymerase gene partial cds(2)
DQ184476.1 Orf virus strain NZ2 complete genome
HM133903.1 Orf virus strain D1701 complete genome
0.050

Figure 2.

Supplementary material A. Mean of duplicates optical density (OD) and percent positivity (%P) values for the iELISA detecting smooth *Brucella* antibodies for the animals sampled in 2015. Cutoff for the iELISA is 73.6 % (Nymo et al., 2013).

Sample ID	Mean of duplicates OD 492-OD 600	%P	Sample ID	Mean of duplicates OD 492-OD 600	%P	Sample ID	Mean of duplicates OD 492-OD 600	%P
Pup[a] 05	0,0355	1,33684805	Mum 14	0,0775	2,918471098	Mum 21	0,0315	1,186217285
Pup 06	0,0275	1,03558652	Mum 15	0,11	4,142346074	Mum 23	0,053	1,995857654
Pup 07	0,034	1,28036151	Pup 17	0,0545	2,052344191	Mum 22	0,0625	2,353605724
Pup 08	0,1185	4,46243645	Pup 18	0,0645	2,428921107	Mum 25	0,0645	2,428921107
Pup 12	0,051	1,92054227	Pup 20	0,036	1,355676897	Pup 16	0,071	2,673696102
Pup 14	0,0505	1,90171342	Pup 21	0,0425	1,600451892	Pup 19	0,057	2,14648842
Pup 15	0,0505	1,90171342	Pup 23	0,071	2,673696102	Pup 24	0,0365	1,374505743
Mum[b] 05	0,046	1,73225381	Pup 22	0,0485	1,826398042	Mum 16	0,0655	2,466578799
Mum 06	0,0575	2,16531727	Pup 25	0,0465	1,751082659	Mum 19	0,0805	3,031444172
Mum 07	0,185	6,96667294	Mum 17	0,125	4,707211448	Mum 24	0,0695	2,617209565
Mum 08	0,119	4,4812653	Mum 18	0,0925	3,483336471			
Mum 12	0,05	1,88288458	Mum 20	0,0335	1,261532668			

[a] Offspring of the year
[b] Female Antarctic fur seals

Supplementary material B. Mean of duplicates optical density (OD) and percent positivity (%P) values for the iELISA detecting smooth *Brucella* antibodies for the animals sampled in 2017. Cutoff for the iELISA is 73.6 % (Nymo et al., 2013). The highest values for OD and %P (>10 %P) are highlighted.

Sample ID	Mean of duplicates OD 492-OD 600	%P	Sample ID	Mean of duplicates OD 492-OD 600	%P	Sample ID	Mean of duplicates OD 492-OD 600	%P
Mum[b] 07	0,0775	2,95519542	Mum 19	0,052	1,982840801	SES[d] 14671	0,0315	1,20114395
Mum 08	1,0155	38,7225929	Pup 4147	0,0455	1,734985701	SES 14647	0,314	11,9733079
Mum 09	0,033	1,25834128	Pup 4104	0,115	4,385128694	SES 14640	0,85	32,4118208
Mum 10	0,1065	4,06101049	Male[c] 673 A	0,141	5,376549094	SES 14641	0,3275	12,4880839
Mum 12	0,0435	1,65872259	Male 655 A	0,091	3,469971401	SES 14664	0,711	27,1115348
Mum 14	0,047	1,79218303	Male 676 A	0,0405	1,544327931	SES 14668	0,075	2,85986654
Mum15	0,0375	1,42993327	Male 673A	0,093	3,546234509	SES 14642	0,1035	3,94661582
Mum 16	0,064	2,44041945	Male 66 A	0,092	3,508102955	SES 14646	1,8045	68,8083889
Mum 17	0,0915	3,48903718	Male 651 A	0,307	11,70638704	SES 14672	0,474	18,0743565
Pup[a] 12	0,0305	1,16301239	Male 798 A	0,2085	1,163012393	SES 14643	0,0955	3,64156339
Pup 17	0,362	13,8036225	Male 659 A	0,058	2,211630124	SES 14645	0,0605	2,30695901
Mum 18	0,2205	8,40800763	Male 671 A	0,095	3,622497617	SES 14665	0,647	24,6711153
						SES 17673	0,041	1,56339371

[a] Offspring of the year
[b] Female Antarctic fur seals
[c] Male Antarctic fur seals
[d] Southern elephant seals