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Adenovirus Diversity in Fur Seal and Penguin Colonies of South America

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ABSTRACT: Adenoviruses are medium size nonenveloped viruses with a trend of coevolution with their hosts. We surveyed South American fur seals (*Arctocephalus australis*) and Humboldt penguins (*Spheniscus humboldti*) for adenoviruses at two sites from 2009 to 2012. Despite the common pattern of host specificity, some of the adenoviruses in our study were present in samples from unexpected host species. We identified mastadenoviruses, aviadenoviruses, and siadenoviruses in *A. australis* from Peru and Chile and in *S. humboldti* from Peru. The El Niño Southern Oscillation (ENSO) significantly reduces the productivity of the Humboldt upwelling system, which can change trophic and other ecological interactions, facilitating exposure to new pathogens. One aviadenovirus was detected in both the penguins and the fur seals, an interclass distance. This finding occurred only during the 2009 ENSO and not in 2010 or 2012. Further studies of viral diversity in sites with high-density mixed species populations are necessary to better understand viral evolution and the effect of environmental change on viral evolution and host specificity.

Key words: Adenovirus, *Arctocephalus australis*, Chile, El Niño, Humboldt penguin, Peru, Peruvian American fur seal.

The Humboldt Current upwelling system is one of the most productive marine ecosystems in the world, bringing cold, nutrient-rich water from the Antarctic to the west coast of South America (Gutierrez et al. 2016). This system supports diverse marine fauna in Peru and northern-central Chile (Alheit and Niquen 2004). In this region, the El Niño Southern Oscillation (ENSO) cycle results in changes in sea surface temperature, reducing upwelling and primary productivity in the ocean. These changes have been associated with significant mortalities of apex predators, especially among juveniles (Soto et al. 2006).

The viruses of the family *Adenoviridae* are nonenveloped DNA viruses with high host specificity. Members of the genus *Mastadenovirus* are found in mammalian hosts, while the genus *Aviadenovirus* are found in avian hosts. Host jumping is rarely reported, with exceptions including canine adenovirus 1 (Park et al. 2007), Titi monkey adenovirus (Chen et al. 2011), and California sea lion adenovirus 1 (CSLAdV1; Cortés-Hinojosa et al. 2016). A study from Kohl and colleagues (2012) infers that canine adenoviruses originated from an ancient jump from bats, which could explain the aggressive clinical presentation in multiple carnivore hosts, including Eurasian river otters (*Lutra lutra*; Park et al. 2007). Hepatitis associated with CSLAdV1 has been reported in multiple pinniped species (Cortés-Hinojosa et al. 2016). A recent report of an aviadenovirus in pine martens (*Martes martes*), a terrestrial carnivore, using Next Generation Sequencing further expands the complexity of host jumping of adenoviruses (Walker et al. 2017).

South American fur seals (SAFSs; *Arctocephalus australis*) have an extensive range of distribution along both coasts of South America. A gap of more than 2000 km isolates the Peruvian subspecies found in Peru and northern Chile from the southern subspecies, which ranges from Guafo Island in northern Chilean Patagonia to Rio Grande do Sul in

Brazil (de Oliveira and Brownell 2014; Paves et al. 2016). Humboldt penguins (HPs; *Spheniscus humboldti*) have an overlapping range of distribution from Isla Foca in Peru to Guafo Island. Punta San Juan is one of the most critical breeding rookeries for both species (Mattern et al. 2004) and has been studied for years. Disease surveillance in SAFSs has detected several pathogens (Jankowski et al. 2015), but serology for adenoviruses has not been reported. Serologic testing for an atadenovirus in HPs at the Peruvian site found 4/61 positive animals (Smith et al. 2008).

Adenoviruses are nonenveloped and thus more resistant to environmental conditions. Human adenoviruses can persist up to 384 days in groundwater and 77–85 days in seawater at 15 C (Enriquez et al. 1995; Ogorzaly et al. 2010). This environmental persistence results in high risk of exposure for animals, particularly in colonial species like SAFSs and HPs, which cohabit in high population densities. With high resource variability and dense reproductive populations of different species, the Peruvian site represents an ideal situation to evaluate adenoviral diversity and host jumping in a natural system.

We collected dry nasal swabs (2009–10) and paired nasal and fecal swabs (2011–12) placed in RNAlater (Thermo Fisher, Gaithersburg, Maryland, USA) from SAFSs. We collected 83 samples at Punta San Juan, Peru (15°22′S, 75°12′W) and 35 at Guafo Island, Chile (43°35′34.9″S, 74°42′48.53″W; Fig. 1 and Table 1). Additionally, dry swabs were collected from conjunctiva, choana, and cloaca of 81 HPs at the Peruvian site in 2011–12 (Table 1) and then stored at –80 C until extractions were carried out in a Maxwell 16 automated extractor (Promega, Madison, Wisconsin, USA). Adenoviruses were detected using a previously described PCR protocol for the DNA-dependent DNA polymerase gene (Wellehan et al. 2004). Host verification was carried out using PCR for the *cyclooxygenase 1* gene (Ward et al. 2005). Fragments of the expected size were sequenced using ABI 3130 DNA sequencers (Applied Biosystems, Foster City, California, USA). Bayesian and maximum likelihood phylogenetic analyses were carried out as previously described (Cortés-Hinojosa et al. 2015, 2016).

Several novel adenoviruses were identified, including five mastadenoviruses, four aviadenoviruses, and one siadenovirus in SAFSs, and three mastadenoviruses, two aviadenoviruses, and three siadenoviruses in HPs. One aviadenovirus and one siadenovirus were detected in both HPs and SAFSs in Peru (Table 1 and Supplementary Material Table S1). There are no previous reports of mastadenoviruses in birds or aviadenoviruses in marine mammals. The host *cyclooxygenase 1* gene sequence results confirmed that the adenovirus positive samples corresponded to the host species from which the sample had been taken.

Chi-square analyses revealed statistically significant differences in the prevalence of aviadenoviruses (P=0.002), but not mastadenoviruses (P=0.67), in Peruvian SAFS pups between 2009 and 2010. Increased adenoviral pathogenicity occurs with high host densities and immunosuppressed hosts and when stressful conditions exist (Sanchez et al. 2015). The Humboldt Current along the Pacific coast of Peru and Chile supports an extremely high density of marine wildlife, but it is periodically disrupted by naturally occurring ENSO, creating stressful conditions for marine predators (Trillmich and Limberger 1985).

We detected four aviadenoviruses in SAFS pups (SAFS-Avia1 to 4) in 2009 during an ENSO event. One of these novel aviadenoviruses (SAFS-Avia2) was detected in three SAFS nasal swabs and 22 HP samples. Given that aviadenoviruses are usually found in avian hosts, and this virus was found in HPs with greater prevalence than any other adenovirus, the HP is the most likely endemic host for this virus (Table 1). The other three SAFS aviadenoviruses were detected solely in SAFSs and, given that we did not find them in HPs, are most likely endemic in other sympatric avian hosts, such as *Sula variegata*, *Pelecanus thagus*, or *Phalacrocorax bougainvillii*, which were not surveyed. Lack of primary prey species caused by ENSO may cause shifts toward avian predation or co-

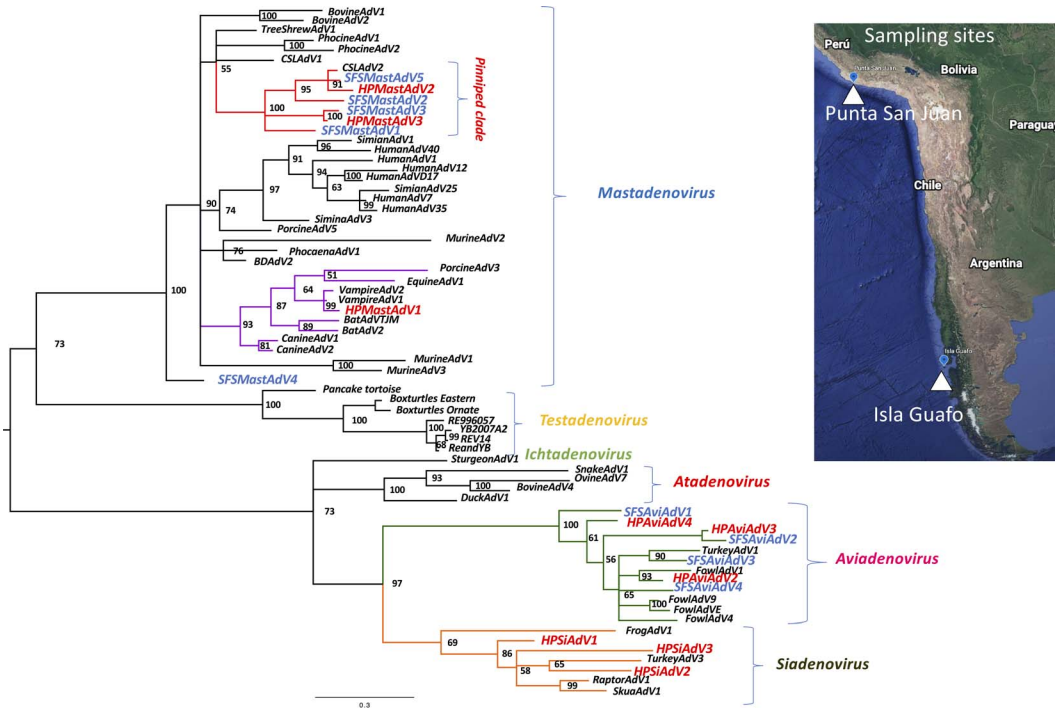

FIGURE 1. Bayesian analysis phylogram depicting the relationship of the novel adenoviruses from Humbolt penguins (*Spheniscus humboldti*) and South American fur seals (*Arctocephalus australis*) sampled at two sites in Peru, 2009–12, to representatives from each of the genera in the family *Adenoviridae*, based on partial amino acid sequences for the adenoviral DNA-dependent DNA polymerase gene (93 AA characters including gaps and poorly aligned sequence) using the LG+G model of evolution. Numbers at each node represent the posterior probability. Branch lengths are based on the number of inferred substitutions, as indicated by the scale (accession numbers for adenoviruses used in this figure are located in Supplementary Material Table S1). Each adenovirus genus is indicated by brackets; novel penguin adenoviruses start with "HP" in the label, and novel SAFS adenoviruses start with "SFS" in the label. Figure shows South America with each sample site indicated with a white triangle.

prophagy in SAFSs. Though SAFSs do not generally prey on birds, avian predation is reported in other pinniped species (Rogers and Bryden 1995). Direct transmission through contact with seabird guano is also possible. No aviadenoviruses were detected in samples of SAFSs from the Chilean site, where several bird species interact closely with fur seal pups (Seguel et al. 2017). This finding may be related to sample collection occurring during non-ENSO years, and because this colony is located at a southern latitude where ENSO impact on the environment is milder, further study is needed to investigate this (Gutierrez et al. 2016).

The most common mastadenovirus, SAFS-Masta2, was sequenced in both Peruvian and Chilean SAFS populations. However, SAFS-Masta1, 3, and 5 were present only at the Peruvian site, and SAFS-Masta4 only at in the Chilean site (Table 1). This difference in adenovirus distribution could indicate limited pathogen flow between SAFS populations, which may be due to the 2000 km gap in the distribution range between subspecies, unlike HPs, which have a continuous population across this range. Future analyses of more variable regions of SAFS-Masta2 may help clarify the evolutionary history of SAFSs.

Three mastadenoviruses were detected in HPs (HP-Masta1 to 3). Both HP-Masta2 and HP-Masta3 cluster in a pinniped host clade, consistent with crossovers from a pinniped to HPs; however, we did not detect either HP-

TABLE 1. Findings of adenoviruses from Humbolt penguins (*Spheniscus humboldti*) and South American fur seals (*Arctocephalus australis*) sampled at two sites in Peru, 2009–12, showing locations and years of sampling. Adenoviruses were detected with PCR.[a]

Virus (sample type)	Peru 2009 (SAFS n=30)	Peru 2010 (SAFS n=30)	Peru 2011 (HP n=48)	Chile 2011 (SAFS n=35)	Peru 2012 (SAFS n=23, HP n=33)
SAFS-Masta1 (resp.)	1	0	0	0	1 SAFS
SAFS-Masta2 (resp. and fecal)	0	2	0	9	0
SAFS-Masta3 (resp.)	1	2	0	0	1 SAFS
SAFS-Masta4 (resp.)	0	0	0	1	0
SAFS-Masta5 (fecal)	0	0	0	0	1 SAFS
SAFS-Avia1 (resp.)	5	0	0	0	0
SAFS Avia2 (resp.)–HP-Avia1 (mix)	3	0	13	0	9 HPs
SAFS-Avia3 (resp.)	1	0	0	0	0
SAFS-Avia4 (resp.)	1	0	0	0	0
HP-Masta1 (mix)	—	—	1	—	2 HPs
HP-Masta2 (mix)	—	—	1	—	0
HP-Masta3 (mix)	—	—	2	—	0
HP-Avia2 (mix)	—	—	1	—	2 HPs
HP-Avia3 (fecal)	—	—	0	—	3 HPs
HP-Avia4 (fecal)	—	—	0	—	1 HP
HP-Sia1 (mix)	—	—	1	—	2 HPs
HP-Sia2 (mix)	—	—	3	—	6 HPs, 1 SAFS
HP-Sia3 (mix)	—	—	1	—	0

[a] SAFS = South America fur seal; HP = Humbolt penguin; resp. = choanal (HP) or nasal swab (SAFS); fecal = cloacal (HP) or rectal swab (SAFS); mix = conjunctival-choanal-cloacal swab; — = no data.

Masta2 or HP-Masta3 in SAFSs. Interestingly, HP-Masta1 clusters in a canine-horse-bat host clade. Vampire bat (*Desmodus rotundus*) feeding on HP chicks has been reported (Luna-Jorquera and Culik 1995). *Desmodus rotundus* adenovirus 1 and 2 (Lacoste et al. 2017) are the closest relatives of HP-Masta1, with sequence homologies of 96% and 94%, respectively, and strong phylogenetic support (Fig. 1 and Supplementary Material Fig. S1) suggests that HP Mastadenovirus-1 may represent a host jump from *D. rotundus*. Exposure of HPs to *D. rotundus* feces is also plausible, particularly by HPs nesting in burrows frequented by bats.

Birds are common hosts for siadenoviruses and aviadenoviruses; we detected two siadenoviruses and three aviadenoviruses in HPs. In addition, we detected a siadenovirus in one SAFS fecal sample, supporting the hypothesis of HP predation by SAFSs (Table 1).

Our data also provide insight into the evolution of adenoviruses in Carnivora. Our Bayesian analysis shows four possible independent lineages of pinniped adenoviruses. The first lineage includes only CSLAdV1, clustering outside of a canine-bat-equine clade. A second pinniped clade includes most otariid adenoviruses, herein called the "Pinniped clade." A third clade includes the two known phocid adenoviruses (Phocine adenovirus 1 and 2). SAFS-Masta4 represents a possible fourth clade at the root of the genus *Mastadenovirus* (Fig. 1). Our maximum likelihood analysis does not resolve this pattern clearly, most likely due to poor resolution (Supplementary Material Fig. S1). We detected a low GC% content in most pinniped adenoviruses, which has been hypothesized to correlate with host jumping events in other virus/host systems (Wellehan et al. 2004). Further analyses, including sequence data from Brazil (Chiappetta et al. 2017), more genes, and molecular clock calibration, may help to clarify the history of pinniped adenoviruses. Future studies should include

disease correlations and histologic examination, as well as long-term data sets that include several sample seasons with data from multiple ENSO.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-20-00118.

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