Detection and Phylogenetic Characterization of a Novel Herpesvirus in Sooty Terns *Onychoprion fuscatus*

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Since 2005, we have recorded annual episodes of alphaherpesvirus outbreaks in chicks of magnificent frigatebird *Fregata magnificens* on the Ile du Grand Connétable Nature Reserve in French Guiana. In 2009, we found sooty terns, *Onychoprion fuscatus*, that live sympatrically with frigatebirds, with visible clinical signs of a potential viral infection. To determine if the symptoms observed in sooty terns could be associated with an alphaherpesvirus previously identified in frigatebirds, we carried out molecular screening of samples collected from seven individuals. We identified and characterized a novel viral sequence from five birds. BLAST searches, pairwise nucleotide, and amino acid sequence comparisons, as well as phylogenetic analyses confirmed that the sequence belonged to the *Herpesviridae* family, of the *Alphaherpesvirinae* subfamily. We observed that it clustered with strains isolated from Podargidae (Caprimulgiformes), Columbiformes, and Falconiformes, but was distinct from the frigatebird herpesvirus. We have tentatively named it *Onychoprion fuscatus* alphaherpesvirus 1, (OfusAHV1). These two sequences, although found syntopic on the Ile du Grand Connétable, belong to two distinct alphaherpesvirus strains. Thus, the clinical symptoms showed by sooty terns do not likely result from a cross-species transmission event. Future work is needed to better characterize the virus and to investigate herpesvirus prevalence in healthy, free-ranging sooty terns, and to assess the impact of the virus on population viability.

**Keywords:** alphaherpesvirus, French Guiana, *Onychoprion fuscatus*, seabirds, disease

**INTRODUCTION**

Herpesviruses are DNA viruses found in many animal species, from invertebrates to mammals (1). Herpesviruses are thought to have evolved in association with their hosts. However, some studies reported cases of cross-species transmission, indicating that such events could occur more frequently than previously thought (2–4). These “spillover” infections in alternative hosts can result in dramatic outbreaks of disease (5–7). Because of their ability to establish a latent infection, herpesviruses do not generally pose a threat to their host species. However, some viruses can cause
severe diseases and induce high mortality rates in their natural 
hosts (8, 9). This is the case for avian herpesviruses that remain 
one of the major causes of fatal infectious diseases in many bird 
species (10–12).

In 2005, we found several chicks of magnificent frigatebird 
_Fregata magnificens_ on the Ile du Grand Connétable Nature 
Reserve (4°49’ 36” N, 51°56’ 38” W), a rocky island located 
off the coast of French Guiana, that showed clinical cutaneous 
signs or were found dead (13). In particular, chicks showed 
nodular proliferative skin lesions in legs and in the neck, and 
hyperkeratosis (13). A few years later, we characterized 
a novel alphaherpesvirus sequence from those chicks (13). In 
the following years, we have started a monitoring program of 
the population of frigatebirds, and have found that the disease 
is widespread in chicks, causing a number of physiological 
alterations associated with a high mortality rate (14–16). Since 
the first appearance of clinical signs in frigatebirds, we have 
also started annual monitoring programs for the other species 
that breed sympatrically in the natural reserve. On the 30th 
of April 2009, we found several dead or dying adult sooty 
terns _Onychoprion fuscatus_ showing similar clinical signs of 
frigatebirds (bone frailty, hyperkeratosis) as described previously 
(13, 16, 17). Our goal was to determine if the observed 
symptoms could be due to a cross-species transmission of 
the alphaherpesvirus that affect magnificent frigatebirds or the 
results of an infection with an unknown herpesvirus.

**MATERIALS AND METHODS**

**Sample Collection**

To determine if the observed symptoms could be due to a 
cross-species transmission we collected biological material (i.e., 
tracheal swabs and blood) from sick birds, while small tissue 
samples (i.e., trachea, brain, lungs, liver, and heart) from dead 
birds were additionally collected. Trachea (3 samples), brain (6 
samples), lung (1 sample), liver (4 samples) heart (1 sample), and 
whole blood (4 samples) for a total of 19 samples were collected 
and placed in 2 mL Eppendorf tubes. Blood was centrifuged in 
whole blood (4 samples) for a total of 19 samples were collected 
samples), lung (1 sample), liver (4 samples) heart (1 sample), and 
whole blood (4 samples) from dead and dying adult sooty terns 
_Onychoprion fuscatus_ showing similar clinical signs of 
frigatebirds (bone frailty, hyperkeratosis) as described previously 
(13, 16, 17). Our goal was to determine if the observed 
symptoms could be due to a cross-species transmission of 
the alphaherpesvirus that affect magnificent frigatebirds or the 
results of an infection with an unknown herpesvirus.

**Virus Identification**

We extracted DNA by a classical phenol, phenol-chloroform 
(1:1 vol/vol), and chloroform technique and precipitated it by 
isopropanol. Then, we washed the DNA with 70% ethanol and 
resuspended it in TE buffer containing 10 mM Tris (pH 8.0) 
and 1 mM EDTA. We carried out molecular screening by semi-
nested PCR amplifications with degenerate consensus primers 
targeting highly conserved amino acid motifs of the herpesvirus 
_DNA polymerase_ gene. To this end, we used two sets of primers [First set: Freg1F: GTGTTGATTATTTGCGACGCTGTA 
TCC, Freg1R: ATGTTGCTTCCATGGTCTTACC, Freg2R: 
ACGTGCAGACGCAGCAGAAG; Second set: as explained in 
(18)] targeting the same region of the gene, with different 
levels of degeneracy. This was done for each DNA sample in 
separate reactions for the first-round PCR (Freg1F/Freg1R or 
DFASA/GDTD1B) and second-round PCR (Freg1F/Freg2R or 
VYGA/GDTD1B). The initial round of PCR contained 500 ng of 
genomic DNA, 30 pmols of degenerate primers, 2 mM MgCl2, 
0.2 mM each dNTP, 5 µL of 10 × PCR buffer, and 0.5 µL of 
AmpliTaq Gold DNA polymerase in a volume of 50 µL. We used 
2 µL of this reaction in the semi-nested reaction. The PCR cycling 
conditions were as follows: after the DNAs were denatured at 
94°C for 10 min, the reaction mixtures were cycled five times 
at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by 
30 cycles at 94°C for 30 s, 46°C for 30 s, and 72°C for 30 s. We 
made an extension of 10 min at 72°C on the last cycle (GeneAmp 
PCR system 9600 thermal cycler; Perkin-Elmer). Amplification 
products of the expected size (about 250 and 350 base pair, 
respectively) were cloned into pCR4-TOPO vectors using a TA 
cloning kit from Invitrogen and sent them for sequencing to 
Genewiz (https://www.genewiz.com/). For each PCR product, 
three clones of the “screening amplicons” were sequenced on 
both strands.

**Phylogenetic Analysis**

Raw sequences were analyzed and edited in MEGA 5.05 (19). 
The nucleotide sequence was 307 bp in size, excluding primers. 
We then carried out sequence homology analyses using the 
BLAST program at the National Center of Biotechnology 
Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast 
cgi). Then, a multiple sequence alignment was constructed 
using ClustalW with previously published avian herpesvirus 
sequences and representative sequences for each genus or 
subfamily retrieved from GenBank (http://www.ncbi.nlm.nih. 
gov/nucleotide) (13, 20). The alignment was checked manually.

We analyzed the phylogenetic relationships among 
herpesviruses using the Bayesian inference (BI) approach 
implemented in Beast 1.8.4 (21), based on a final alignment 
including 37 unique sequences of 104 amino acid positions. 
We assessed the best-fit model of amino acid evolution for 
the dataset using the smart model selection (SMS) approach 
implemented in the PhyML environment (23). We ran 
BI analyses with LG+G+I model of aminoacidic substitution 
(24), an uncorrelated relaxed molecular clock model with a 
log-normal distribution (25), and a Yule tree prior. Then, we 
analyzed the results from two independent runs of 10 million 
generations, sampled every 1,000 generations, using Tracer 1.7.1 
to check that the effective sample sizes for all parameters that 
exceeded 200 (26) and to assess the appropriate number of initial 
trees to discard as burn-in. Then, we combined the two runs 
using Logcombiner 1.8.4 [BEAST package, (27)]. We computed 
the Maximum Clade Credibility (MCC) tree summarizing the 
post-burn-in trees using TreeAnnotator 1.8.4, and we visualized 
the tree using FigTree 1.4.4 (26).

**RESULTS AND DISCUSSION**

This study aimed at assessing the presence of a herpesvirus from 
dead and dying adult sooty terns and to determine its relationship 
with other members of the _Herpesviridae_ family. Out of the 19 
tissue samples, 7 samples (4 blood samples and 3 brain samples, 
collected from both dead and dying birds) tested positive for
herpesvirus. A unique and novel viral sequence was obtained from five out of the seven individuals with PCR positive results. This sequence is tentatively designated as *Onychoprion fuscatus* alphaherpesvirus 1 (*OfusAHV1*) in the Alphaherpesvirinae subfamily. From a phylogenetic perspective, *OfusAHV1* clustered with strains detected from Podargidae (Caprimulgiformes), Columbiformes, and Falconiformes (posterior probability = 0.86; Figure 1). We also found that this novel alphaherpesvirus sequence of sooty terns was distinct from the Frigatebird herpesvirus (nucleotide p-distance: 0.28), which belonged to a different well-supported monophyletic lineage. These results seem to indicate that no cross-species transmission has occurred between frigatebirds and sooty terns. This is not surprising given that herpesviruses are usually associated with a single host species (28), and co-evolve with their host over long periods of time (28).

Because herpesviruses establish latent infections and have a high prevalence in natural hosts (11), symptoms of herpesvirus infection and the associated appearance of clinical signs may only occur when birds undergo a stressful situation (29). This raises the question of whether sooty terns were undergoing any form of stress and/or immune suppression. The severe clinical signs found in dying birds with positive PCR results may also suggest that sooty terns had no prior contact with this specific virus. Although very little is known about this population, recent work showed that sympatrically breeding frigatebirds have high blood concentrations of mercury while sooty terns showed very low mercury concentrations (30, 31). Mercury exposure can therefore likely be ruled out as a plausible candidate stressor for this population. However, suppression of the immune system in these birds might also be due to malnutrition, as has previously been suggested and recently corroborated in frigatebirds (13, 17).

This study does not conclusively prove the causal link between this herpesvirus and the occurrence of clinical signs and mortality in this population of sooty terns. The number of tissue samples and birds included in the present study was limited, and additional work would prove beneficial for a more solid interpretation of the results. However, the viral sequence here reported is novel and may be well-specific to this species, further supporting the fact that distinct avian populations are naturally infected with distinct herpesviruses. Seabirds aggregate
at high densities during the breeding season, which may favor viral spread among conspecific and may lead to severe outbreaks in wild populations. We do not know the effect that this virus may have on this and other populations of sooty terns in terms of reproductive success and survival. However, given that herpesviruses in wild animals are usually only detected when they cause disease outbreaks, future works are needed to investigate herpesvirus prevalence in healthy, free-ranging seabirds. Seabirds are currently facing a strong decline in food resources (32, 33) and an increasing exposure to environmental contaminants and plastic pollution (34–36) which may increase their susceptibility to viral outbreaks.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/ (MT364351).

**REFERENCES**

1. Davison AJ. Herpesvirus systematics. Vet Microbiol. (2010) 143:52–69. doi: 10.1016/j.vetmic.2010.02.014

2. Ehlers B, Dural G, Yasum N, Lembo T, De Thoisy B, Ryser-Degorgis MP, et al. Novel mammalian herpesviruses and lineages within the Gammaherpesvirinae: cospeciation and interspecies transfer. J Virol. (2008) 82:3509–16. doi: 10.1128/JVI.02646-07

3. Leendertz FH, Deckers M, Scheep W, Lankester F, Boesch C, Mugisha L, et al. Novel cytomegaloviruses in free-ranging and captive great apes: phylogenetic evidence for bidirectional horizontal transmission. J General Virol. (2009) 90:2386–94. doi: 10.1099/vir.0.011866-0

4. Escalera-Zamudio M, Rojas-Anaya E, Kolokotronis S-O, Taboada B, Loza-Rubio E, Méndez-Ojeda ML, et al. Bats, primates, and the evolutionary origins and diversification of mammalian gammaherpesviruses. MBio. (2016) 7:e01425–16. doi: 10.1128/mBio.01425-16

5. Huff J, Barry P. B-Virus (Cercopithecine herpesvirus 1) infection in humans and macaques: potential for zoonotic disease. Emerg Infect Dis. (2003) 9:246–50. doi: 10.3202/eed092/020272

6. Parrish CR, Holmes EC, Morens DM, Park EC, Burke DS, Calisher CH, et al. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev. (2008) 72:457–70. doi: 10.1128/MMBR.00084-08

7. Tischer BK, Ostertiedner N. Herpesviruses—a zoonotic threat? Vet Microbiol. (2010) 140:266–70. doi: 10.1016/j.vetmic.2009.06.020

8. Longa CS, Bruno SF, Pires AR, Romijn PC, Kimura LS, Costa CHC. Human herpesvirus 1 in wild marmosets, Brazil, 2008. Emerg Infect Dis. (2011) 17:1308–10. doi: 10.3202/eed1707.100333

9. Long SY, Latimer EM, Hayward GS. Review of elephant endarteriopathic herpesviruses and acute hemorrhagic disease. J Ar. J. (2016) 56:283–96. doi: 10.1093/ilar/ilv041

10. Tomaszewski E, Wilson VG, Wigle WL, Phalen DN. Detection and heterogeneity of herpesviruses causing Pacheco’s disease in parrots. J Clin Microbiol. (2001) 39:533–8. doi: 10.1128/JCM.39.2.533-538.2001

11. Kaleta EE, Docherty DE. Avian Herpesviruses. In: Thomas NJ, Hunter DB, Atkinson CT, editors. Infectious Diseases of Wild Birds. New Jersey, NJ: Wiley (2008). p. 63–86. doi: 10.1007/9780470344668.ch3

12. Dhamma K, Kumar N, Saminathan M, Tiwari R, Karthik K, Kumar MA, et al. Duck virus enteritis (duck plague) – a comprehensive update. Veterinary Quart. (2017) 37:57–80. doi: 10.1007/s10621-017-12988-5

13. De Thoisy B, Lavergne A, Semelin J, Pouliquen JF, Blanchard F, Hansen E, et al. Outbreaks of disease possibly due to a natural avian herpesvirus infection in a colony of young Magnificent Frigatebirds (Fregata magnificens) in French Guiana. J Wildl Dis. (2009) 45:802–7. doi: 10.7589/0090-3558-45.3.802

14. Sebastiano M, Eens M, Abd Elgawad H, Thoisy V, Pineau K, et al. Oxidative stress biomarkers are associated with visible clinical signs of a disease in frigatebird nestlings. Sci Rep. (2017) 7:1599. doi: 10.1038/s41598-017-01417-9

15. Sebastiano M, Eens M, Thoisy V, Pineau K, Chastel O, Costantini D. Corticosterone, inflammation, immune status and telomere length in frigatebird nestlings facing a severe herpesvirus infection. Conserv Physiol. (2017) 5:cow073. doi: 10.1093/conphys/cow073

16. Sebastiano M, Eens M, Messina S, Abdelgawad H, Pineau K, Beemster GTS, et al. Resveratrol supplementation reduces oxidative stress and modulates the immune response in free-living animals during a viral infection. Punct Ecol. (2018) 32:2509–19. doi: 10.1111/1365-2435.13195

17. Sebastiano M, Eens M, Pineau K, Chastel O, Costantini D. Food supplementation protects Magnificent frigatebird chicks against a fatal viral disease. Conserv Lett. (2019) 12:e12630. doi: 10.1111/conl.12630

18. Rose TM, Strand KB, Schulz ER, Schaefer G, Rankin GW Jr, Thouless ME, et al. Identification of two homologs of the Kaposi’s sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. J Virol. (1997) 71:4138–44. doi: 10.1128/JVI.71.5.4138-414.1997

19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. (2011) 28:2731–9. doi: 10.1093/molbev/msr121

20. Cardoso M, Hyatt A, Selleck P, Lwowski, S, Prakash V, Pain D, et al. Phylogenetic analysis of the DNA polymerase gene of a novel alphaherpesvirus isolated from an Indian Gyps vulture. Virus Genes. (2005) 30:371–81. doi: 10.1007/s11262-005-6678-8

21. Drummond AJ, Suchard MA, Xie D, Rambaut A. BEAST 2.0. Mol Biol Evol. (2019) 36:371–9. doi: 10.1093/molbev/msz121

22. Lefort V, Longueville JE, Gascuel O. SMS: smart model selection in PhyML. Syst Biol. (2010) 59:307–21.

23. Le Q, Gascuel O. An improved general amino acid replacement matrix. Mol Biol Evol. (2008) 25:1307–20. doi: 10.1093/molbev/msn067

**ETHICS STATEMENT**

The animal study was reviewed and approved by Prefet de la region Guyane.

**AUTHOR CONTRIBUTIONS**

MS wrote the article. DCa, RB, and DCo performed phylogenetic analyses. AL and VL performed virus identification analyses. KP and OC contributed to data collection. All authors contributed to the article and approved the submitted version.

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25. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* (2006) 4:e88. doi: 10.1371/journal.pbio.0040088

26. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in bayesian phylogenetics using tracer 1.7. *Syst Biol.* (2018) 67:901–4. doi: 10.1093/sysbio/syy032

27. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* (2018) 4:vey016. doi: 10.1093/vey/vey016

28. Davison AJ. Evolution of the herpesviruses. *Vet Microbiol.* (2002) 86:69–88. doi: 10.1016/S0378-1135(01)00492-8

29. Goldberg DR, Yuill TM, Burgess EC. Mortality from duck plague virus in immunosuppressed adult mallard dUCKS. *J Wildl Dis.* (1990) 26:299–306. doi: 10.7589/0090-3558-26.3.299

30. Sebastiano M, Bustamante P, Costantini D, Eulaers I, Malarvannan G, Mendez-Fernandez P, et al. High levels of mercury and low levels of persistent organic pollutants in a tropical seabird in French Guiana, the Magnificent frigatebird, *Fregata magnificens*. *Environ Pollut.* (2016) 214:384–93. doi: 10.1016/j.envpol.2016.03.070

31. Sebastiano M, Bustamante P, Eulaers I, Malarvannan G, Mendez-Fernandez P, Churlaud C, et al. Trophic ecology drives contaminant concentrations within a tropical seabird community. *Environ Pollut.* (2017) 227:183–93. doi: 10.1016/j.envpol.2017.04.040

32. Wagner EL, Boersma PD. Effects of fisheries on seabird community ecology. *Rev Fisheries Sci.* (2011) 19:157–67. doi: 10.1080/10641262.2011.562568

33. Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, Hua X, Karanewsky CJ, Ryu HY, et al. How does climate change cause extinction? *Proc R Soc B Biol Sci.* (2013) 280:20121890. doi: 10.1098/rspb.2012.1890

34. Vo A-TE, Bank MS, Shine JP, Edwards SV. Temporal increase in organic mercury in an endangered pelagic seabird assessed by century-old museum specimens. *Proc Natl Acad Sci USA.* (2011) 108:7466–71. doi: 10.1073/pnas.1013865108

35. Bond AL, Hobson KA, Branfireun BA. Rapidly increasing methyl mercury in endangered ivory gull (*Pagophila eburnea*) feathers over a 130 year record. *Proc R Soc B Biol Sci.* (2015) 282:20150032. doi: 10.1098/rspb.2015.0032

36. Wilcox C, Van Sebille E, Hardesty BD. Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proc Natl Acad Sci USA.* (2015) 112:11899–904. doi: 10.1073/pnas.1502108112

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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