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Outbreaks of Disease Possibly Due to a Natural Avian Herpesvirus Infection in a Colony of Young Magnificent Frigatebirds (*Fregata magnificens*) in French Guiana

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Abstract:

The Ile du Grand Connétable nature reserve is a rocky island off the Northern Atlantic coast of South America that hosts a unique population of Magnificent Frigatebirds (*Fregata magnificens*, Pelecaniformes). A high chick mortality, associated with nodular proliferative lesions, involving featherless areas, such as legs, neck, eyelids, and beak, was recorded during a consecutive 2 yr and affected almost half of the generation. Investigations were, therefore, conducted to determine the cause of these epidemics. Although histopathologic investigations suggested that malnutrition, because of fewer resources in the Frigates' fishing area, could be the cause of the epidemic, a novel alphaherpesvirus, tentatively called *Fregata magnificens* herpesvirus, was detected in cutaneous crusts on the diseased birds. Although in this study, we do not prove the causal link of this new virus to the symptoms observed, it can nevertheless be suggested that in debilitated hosts, a productive herpesvirus infection might accelerate, and/or be accelerated by, population declines. These results emphasize the need to take into consideration the possible role of herpesviruses in weakened populations of wild birds in conservation management plans.

Keywords: Alphaherpesvirus, chick mortality, *Fregata magnificens*, Frigatebird

Mortality outbreaks in wild bird populations are mainly related to acute, chronic pollution and infectious diseases. These epidemic events have increasing impacts on population conservation (Daszak et al., 2001; Wellehan et al., 2003). Among viruses, herpesvirus infection has been widely reported in wild bird populations for decades; the induced diseases are among the most common health problems in aquatic birds, parrots and passerines (Converse and Kidd, 2001; Newman et al., 2007; Tomaszewski et al. 2001; Johnson and Tyack, 1995). Herpesvirus infections have also been reported in vultures, falcons, wild turkeys, penguins and ducks (Cardoso et al., 2005; Forbes et al. 2000; Grant et al. 1975; Kincaid et al. 1988; Wojcinski et al., 1991).

The Ile du Grand Connétable nature reserve (4°49'30N ; 51°56'00W) is a rocky island located off the Northern Atlantic coast of South America that hosts a unique population of Magnificent frigate birds (*Fregata magnificens*, Pelecaniformes). With about 5% of the Caribbean population, this Frigate bird colony is one of the most important of the region and the sole nesting site between the islands of Tobago and Fernando do Noronha (Dujardin and Tostain, 1990). In July 2005, after the annual hatching period, 25 chick cadavers were found while 30 live chicks among the 250 nests on the island had clinical cutaneous signs. They presented nodular proliferative lesions involving featherless areas such as legs, neck, eyelids and beak, and keratitis and conjunctivitis were also recorded (Figure 1). The cadavers and affected birds were not geographically restricted but widespread over the entire island, in proximity to healthy animals. No adult or other species present on the island (*Sterna maxima*, *Sterna eurygnatha*, *Sterna fuscata*, *Anous stolidus* and *Larus atricilla*) had detectable symptoms. Two weeks later, 33 more cadavers were recorded, and 27 chicks presented clinical signs. One month later, 41 other cadavers were recorded. Through intense monitoring of nests, it was confirmed that symptoms were always lethal: no animal with clinical signs recovered.

Sampling of animals led us to observe thickening of the skin, hyperkeratosis, bone frailty and severe emaciation. Blood samples were collected from 11 chicks, 5 from healthy animals and 6 from animals with clinical signs. In addition, skin samples were collected from 3 of the 6 sick animals and preserved in Hanks medium. One dead animal was brought back for anatomopathological investigations. Biochemical investigations showed a strong hypophosphatemia (1.78 mmol/l, n=4, *vs*. 4.03 mmol/l in healthy chicks, Works 1996), while calcemia and hematocrit and hemoglobin measurements were within normal range values (Work 1996). Histopathological investigations revealed that the esophagus, intestine and trachea had no inflammatory lesions. Lungs were moderately congested, and muscles showed a slight interstitial edema. The liver had minor perivascular inflammatory infiltrates, and the kidneys presented with two foci of interstitial inflammatory cells. Bone mineralization was deficient, with a thin primary ossification area and a persistent cartilage. Finally, dermal tissues presented hyperkeratosis, and epidermal keratinocytes had ballooning degeneration, but no inflammatory process or intranuclear viral inclusions were noticed. All these data are typical of a poor nutritional status. Bacterial cultures and microscopic investigations on skin samples excluded the possibility of a bacterial infection and the presence of ectoparasites. A search for avian poxvirus DNA was carried out by PCR using previously published procedures (Kim et al., 2003). This yielded negative results. We then attempted to amplify herpesviral sequences using a nested PCR approach with degenerate consensus primers targeted to highly conserved amino acid motifs within the herpesvirus DNA polymerase gene (Rose et al., 1997). Amplification products of the expected size (about 250-bp) were identified in one skin sample and sequenced after cloning. Database searches using the BLAST web server revealed the presence of a new herpesvirus sequence. To extend the nucleotide sequence upstream, a specific nondegenerate oligonucleotide was designed from the complementary sequence of the small fragment and was used in a nPCR amplification with the DFASA primer pool (Lacoste et al. 2000). The PCR products from the initial PCR were used as template DNA in the subsequent amplification reactions. The upstream nPCR products were subsequently cloned and sequenced. The resulting sequences were

assembled to give a total of 476-bp (excluding primers). The obtained consensus was deposited in GenBank under accession number EU867220. BLAST searches showed that this novel sequence was most similar to the DNA polymerases of the *Alphaherpesvirinae* subfamily. Comparison of amino acid identities among alphaherpesviruses indicated that the Frigate bird herpesvirus, tentatively named FmagHV for *Fregata magnificens Herpesvirus*, was most closely related to the Vulture herpesvirus VHV, exhibiting 83.5% identity with it (Cardoso et al. 2005). Furthermore, within the *Alphaherpesvirinae* subfamily, our frigate bird herpesvirus sequence was more closely related to the human herpes simplex virus types 1 and 2 (HSV1 and HSV2) of the simplex genus (81.4 and 79.7% amino acid identity, respectively) than to the other bird alphaherpesviruses (73% of amino acid identity with Marek's disease virus type 2 and 65% with Psittacid herpesvirus 1 and Passerine herpesvirus 1). Phylogenetic analyses were performed on 124 amino acids. The amino acid sequence was aligned using ClustalW (Thompson et al. 1994) with other previously published sequences, and alignments were checked manually. The ProtTest program (Abascal et al. 2005) was used to determine the optimal model of amini acid evolution for the data set. A Bayesian approach was performed with the program BEAST version 1.4.7 (Drummond and Rambaut, 2007) to infer phylogenetic relationships. Analysis was performed using a WAG model of amino acid substitutions with a gamma distributed rate of variation among sites and six rate categories. We ran the analysis assuming a constant population size and a relaxed molecular clock (uncorrelated lognormal). Results from the run (10,000,000 generations with the first 1,000,000 discarded as burn-in and parameter values sampled every 100 generations) were analyzed using the program Tracer version 1.4 (Rambaut and Drummond, 2003). The phylogenetic analysis placed the frigate bird herpesvirus (FmagHV) close to the VHV with a posterior probability value of 1. This cluster is associated with the *simplex* genus encompassing HSV-1, HSV-2, CeHV-1, CeHV-2, as well as BHV-2 with less support (0.76) (Figure 2).

In birds, either in poultry or in captive species, symptoms of herpesviral infections are diverse. They are associated with different diseases such as Marek's disease, duck viral enteritis, infectious laryngotracheitis and Pacheco's disease. Pacheco's disease has no evident clinical signs in parrots, and some animals may recover (Tomaszewski et al 2001). In passerines, clinical signs are mainly respiratory, with or without conjunctivitis (Wellehan et al. 2003). In wild bird populations, it has been suggested that herpesvirus outbreaks are often facilitated by immune suppression of animals resulting from polluted environments (Goldberg et al., 1990). Nevertheless, despite an impressive number of clinical cases recorded, molecular investigations have rarely been conducted during herpesvirus outbreaks. The particular outbreaks as well as the virus we described in this frigate bird colony thus raised two major questions. Firstly, concerning the immune status of the target population, no hypothesis to explain a possible immune suppression of these animals has been highlighted. No marine pollution record has been evidenced in the nature reserve, but Frigate birds cover an average of 223 km per foraging trip (Weimerskirch et al., 2003), therefore certain individuals may have been contaminated. far from the control area. Nevertheless, since clinical signs only pertain to juveniles, deficiency due to malnutrition could also be advocated. Frigate birds often feed on refuse from fishing ships (more than 120 frigate birds frequently observed on a single trawler) (Calixto-Albarran and Osorno, 2000) but in French Guiana, the activity of the industrial shrimp trawling fisheries had decreased for economic reasons one month before the appearance of the first chick symptoms. The observed clinical signs, such as hypocalcemia, hypophosphatemia and hyperkeratosis support this model. Secondly, the origin of the virus is unknown. It has been suggested that migratory birds would facilitate herpesvirus outbreaks and dissemination (Hubalek, 2004, Newman et al., 2007). This assumption means that outbreaks could be caused by cross-species infections. The herpesvirus sequence we isolated in Frigate bird tissues could therefore belong to a virus of one of the island's other bird species. Nevertheless, though occasional transfer to other species can occur *in natura*, as a general rule, the natural host range of individual viruses is highly restricted, and most herpesviruses are thought to have evolved in association with

their host species (Davison, 2002). In addition, as herpesviruses are highly adapted to their hosts, and severe infections are usually observed only in the very young, the fetus or the immunosuppressed, we believe that the virus we characterized here is very likely indigenous to frigate birds. The fact that the reported sequence has never been identified in any other bird species supports this hypothesis. One could therefore suggest that this virus, indigenous to the frigate birds and latent in healthy animals, could be reactivated in immunosuppressed individuals. Though in this study we do not prove the causal link between this virus and the outbreaks (the classical herpetic viral inclusion bodies and associated dermatitis were not observed and histological signs were more likely related to malnutrition), the virus was only identified in skin lesions, suggesting that it might be reactivated and that it might therefore interact with the host's fitness. Our results thus suggest that, in a context of debilitated hosts, productive herpesvirus infection might accelerate and/or be accelerated by population declines. The possible role of herpesviruses in weakened populations of wild birds will therefore have to be taken into consideration in conservation plans (Cardoso et al., 2005).

Acknowledgments

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Figures

Figure 1. Skin lesions and cornea alteration in a Frigate bird chick.

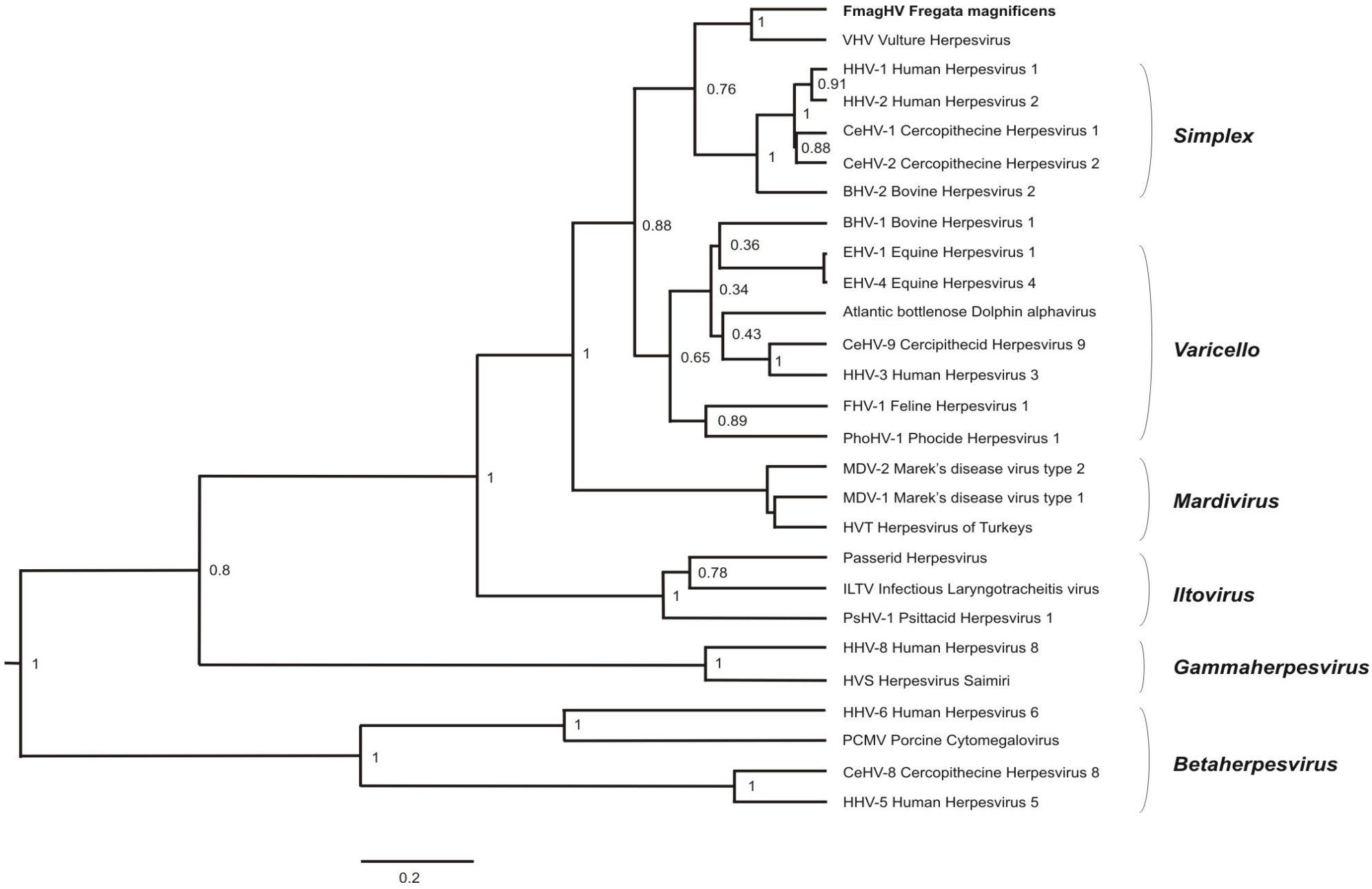
FmagHV Fregata magnificens
VHV Vulture Herpesvirus
HHV-1 Human Herpesvirus 1
HHV-2 Human Herpesvirus 2
CeHV-1 Cercopithecine Herpesvirus 1
CeHV-2 Cercopithecine Herpesvirus 2
BHV-2 Bovine Herpesvirus 2
BHV-1 Bovine Herpesvirus 1
EHV-1 Equine Herpesvirus 1
EHV-4 Equine Herpesvirus 4
Atlantic bottlenose Dolphin alphavirus
CeHV-9 Cercipithecid Herpesvirus 9
HHV-3 Human Herpesvirus 3
FHV-1 Feline Herpesvirus 1
PhoHV-1 Phocide Herpesvirus 1
MDV-2 Marek's disease virus type 2
MDV-1 Marek's disease virus type 1
HVT Herpesvirus of Turkeys
Passerid Herpesvirus
ILTV Infectious Laryngotracheitis virus
PsHV-1 Psittacid Herpesvirus 1
HHV-8 Human Herpesvirus 8
HVS Herpesvirus Saimiri
HHV-6 Human Herpesvirus 6
PCMV Porcine Cytomegalovirus
CeHV-8 Cercopithecine Herpesvirus 8
HHV-5 Human Herpesvirus 5
Simplex
Varicello
Mardivirus
Iltovirus
Gammaherpesvirus
Betaherpesvirus
0.2

Figure 2. Phylogenetic tree (based on 124 amino acid sequences of the DNA Polymerase gene, using a bayesian procedure) showing relationships between *Fregata magnificens Herpesvirus* and other selected herpesviruses.