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Avian Pox in Shearwaters on Lord Howe Island

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Abstract

Avipoxvirus infections occur in a wide range of bird species worldwide. In Australia pox is common in the Australian magpie (*Cracticus tibicen*), Currawongs (*Strepera* spp.) and Silvereyes (*Zosterops lateralis*) but very little is known about the evolution of this family of viruses or the disease ecology of avian poxviruses in seabirds. Pox lesions have been seen in colonies of Shy Albatross (*Thalassarche cauta*) in Bass Strait but the epidemiology of pox in pelagic birds is not very well elucidated. Two novel avipoxvirus species demonstrated in Flesh-footed Shearwater (*A. carneipes*) and Wedge-tailed Shearwater (*A. pacificus)* (SWPV-2) recently discovered in birds from Lord Howe Island had relatively close relationships with *Canarypox virus,* particularly within the highly conserved polymerase gene of these viruses. This raised some concern regarding the potential for cosmopolitan pathogens to spill over into wildlife. However, the results highlight how mistakes in interpretation could occur if only highly conserved genes are used to detect and or characterise viral infections. The results also contribute to a deeper understanding of the genetic relationships and likely complex epidemiology among avipoxvirus species in wildlife species.

Introduction

The *Avipoxvirus* genus includes a divergent group of pox viruses that cause skin disease in wild and domestic birds (Bolte et al., 1999; van Riper and Forrester, 2007) where it typically results in so-called 'dry' pox which are encrusted, proliferative growths that are most commonly restricted to the eyes, beak or unfeathered skin of the body. However, internal infections can also develop in the upper alimentary and respiratory tracts as 'wet' or 'diptheritic' pox (van Riper and Forrester, 2007). Relatively little is known about the origins, worldwide host distribution and genetic diversity of avipoxviruses (Offerman et al., 2014). Skin lesions are rarely fatal and the incubation period of avipoxvirus infection is variable but secondary bacterial or fungal infections are common and contribute to mortality (van Riper and Forrester, 2007). Until recently six avipoxvirus genomes were published; a pathogenic American strain of *Fowlpox virus* (Afonso et al., 2000), an attenuated European strain of *Fowlpox virus* (Laidlaw and Skinner , 2004), a virulent *Canarypox virus* (Tulman et al., 2004), a pathogenic South African strain of *Pigeonpox virus*, a *Penguinpox virus* (Offerman et al., 2014) and a pathogenic Hungarian strain of *Turkeypox virus* (Banyai et al., 2015).

On Lord Howe Island in eastern Australia, the Flesh-footed Shearwater *Ardenna carneipes* is listed as Vulnerable in the state of New South Wales (Reid et al., 2013) due to the threat of plastic pollution and other threats (Bond and Lavers, 2011) so the species may be more susceptible to the detrimental effects of introduced pathogens. Avipoxviruses have been identified as threat for island avifauna of various archipelagos (van Riper et al., 2002) and the emergence of distinctive avipoxvirus with a high prevalence (88%) in Hawaiian Laysan Albatross (*Phoebastria immutabilis*) enabled one of the first detailed studies of the epidemiology and population-level impact of the disease in the seabirds (Young and VanderWerf, 2008). Overall, relatively little is known about the epidemiology or effects of poxviruses in seabird populations, including for shearwaters (*Ardenna* or *Puffinus* spp.) and the purpose of this presentation is to discuss the broader implication of pox in breeding colonies of Flesh-footed Shearwater and Wedge-tailed Shearwater recently detected in juvenile birds from Lord Howe Island (Sarker et al., 2017).

Background

In 2015 pox lesions, liver, and blood samples were examined from Flesh-footed Shearwater (*A. carneipes*) and Wedge-tailed Shearwater (*A. pacificus*) of breeding colonies on Lord Howe Island located approximately 500 km from the coast of eastern Australia (Sarker et al., 2017). After histopathological confirmation of the disease, PCR screening was conducted for avipoxvirus, (Huw Lee and Hwa Lee, 1997), novel circoviruses (Sarker et al., 2015,

2016), reticuloendotheliosis virus (Biswas et al., 2011) and the internal transcribed spacer (ITS) region for detecting fungal pathogens (Kumar and Shukla, 2005). Tests for circovirus and reticuloendotheliosis virus were negative but Sanger sequencing of positive avipoxvirus PCR gel bands resulted in a 578 bp sequence after trimming with the best match (99%) to *Canarypox virus* 4b core protein gene. Two samples that were PCR positive for poxvirus were further assessed by NextGen sequencing performed on a HiSeq4000 sequencing platform (Illumina) by Novogene, China. This yielded complete *Shearwaterpox virus* (SWPV) genomes from *A. pacificus* and *A. carneipes,* both showing the highest degree of similarity with *Canarypox virus* (98% and 67%, respectively). The assembled complete genomes of SWPV-1 and -2 were 326,929 and 351,108 nt, respectively. The novel SWPV-1 complete genome from *A. carneipes* was missing 43 genes compared to *Canarypox virus* and contained 4 predicted genes which are not found in any other poxvirus, whilst, SWPV-2 complete genome was missing 18 genes compared to *Canarypox virus* and a further 15 genes significantly fragmented as to probably cause them to be non-functional. There were several relatively short syntenic regions where 1) SWPV-1 matched *Canarypox virus* significantly better than the majority of the genome, and 2) SWPV-2 matched *Canarypox virus* significantly less than the majority of its genome. BLASTN searches of all poxvirus sequences for these regions of interest provided best matches to *Canarypox virus* suggesting that these sequences originated from avipoxvirus genomes not represented in the public databases and yet to be discovered. As shown in Table 1 a further 31 recombination events were detected amongst the newly sequenced SWPV-1 and SWPV-2 genomes with other avipoxviruses.

Discussion

The value of complete genome characterization and analysis is highlighted in the discovery of SWPV-1 and SWPV-2 since a high sequence identity (98%) and preliminary phylogenetic relationships based on a single gene, in this case the highly conserved polymerase gene, may have falsely implicated *Canarypox virus* as a potential emerging disease from domesticated birds. Although it was not possible to trace the primary source of SWPV-1 and SWPV-2 infection in the shearwater fledglings, it is likely that pox lesions in the affected birds resulted from parental feeding and or arthropod mediated transmission from other bird species residential to Lord Howe Island (Shearn-Bochsler et al., 2008). While, the reservoir host of these novel *Shearwaterpox viruses* is unknown, mosquitoes and other ectoparasite vectors likely play a significant part in transmission within the island. Avipoxvirus infections appear to be relatively rare in seabirds, but pox has been reported in several species when they occur on human-inhabited islands that harbor mosquito vectors (VanderWerf and Young, 2016). Tritrophic relationships have been implicated in some studies of prey-predator poxvirus epidemiological studies in eastern imperial eagles, which acquire pox from their dove prey, and northern harriers from passerine prey (Gyuranecz et al., 2013). So poxvirus in shearwater species may well be spill over infections transmitted from other island bird species including other migratory seabirds such as the Black-winged petrel (*Pterodroma nigripennis*) which is also suspected to be susceptible to pox (Lavers unpublished). Nevertheless, *Shearwaterpox virus 1* in Flesh-footed Shearwaters and *Shearwaterpox virus 2* in Wedge-tailed Shearwaters are the first avipoxvirus species that have been fully characterized in marine seabirds (Sarker et al., 2017).

The results also indicated that there are likely to be many more unique avipoxvirus species in wild birds. The DNA sequences of SWPV-1 and SWPV-2 are significantly different to each other but nevertheless had closest similarity with *Canarypox virus* (67% and 98%, respectively). The phylogeny of SWPV-1 also indicates that shearwaters and perhaps other long-lived, vagile marine birds could be important hosts for avipoxvirus dispersal around the globe. The natural hosts of SWPV-1 and SWVP-2 may be the Lord Howe population of shearwaters, other migratory birds that use Lord Howe Island for breeding or resident forest avian host reservoir species. Species such as the Lord Howe White-eye (*Zosterops tephropleura*) and Lord Howe Golden Whistler (*Pachcephala petoralis contempta*) are candidate passerine birds that might provide such a function. It is also evident from the phylogeny that SWPV-2 is the most closely related to *Canarypox virus* with both SWPV-1 and SWPV-2 containing several genes that are more closely related to *Canarypox virus* throughout their respective genomes (Sarker et al., 2017). It is reasonable to postulate that these viruses originated from a common ancestor that diverged from a *Canarypox virus*-like progenitor related to *Fowlpox*, *Penguinpox* and *Pigeonpox* viruses. Finer resolution of the phylogenetic relationship between avipoxviruses in this clade will likely be dependent on discovering more avipoxvirus detection and sequencing. Given their genetic diversity, it is perhaps not surprising that Shearwater species can be exposed to multiple pox virus infections and this is supported by the recombination analysis shown in Table 1. Studies (Barnett et al., 2015) suggest that the species specificity of poxviruses can be variable. Some genera, such as *Suipoxvirus* are highly restricted to individual vertebrate hosts, swinepox for instance, whereas others, such as avipoxviruses demonstrate some evidence of cross-species infections within a predator-prey system (Gyuranecz et al., 2013). This suggests that the avipoxviruses can infect a diverse range of bird species if they are within a close enough proximity to each other (Haller et al., 2014). Thus far, there were no clear patterns regarding species-specificity in the *Shearwaterpox viruses* described here.

Avian pox tends to be a self-limiting localized infection of apterial skin of the face, feet and legs with full recovery possible. Studies in chickens and other birds have shown that most birds develop a life-long immunity following the resolution of lesion if the birds do not succumb

to secondary infections or are not infected by different strains (Winterfield and Reed, 1985, Lierz, 2002). As shown in affected shearwaters, secondary infections can occur and these may contribute to morbidity and mortality (Johnson and Castro, 1986; Shivaprasad, 2009; Reza et al., 2013; Sarker et al., 2017). Stressful conditions, poor nutrition, overt environmental contamination and other underlying causes of immunosuppression and ill health may contribute to the pathogenesis of such lesions. This was the primary reason for testing for avian circovirus and other potential pathogens. These new *Shearwaterpox virus* complete genomes also provide evidence that recombination may play an important role in the evolution of avipoxviruses. A number of genes in SWPV-1 appear to have been rearranged compared to *Canarypox virus* and blocks of unusual similarity scores were seen in both SWPVs. Such relatively small exchanges of DNA may exert important influences on virus evolution, and has been predicted to have been a driver in the evolution of smallpox (Smithson et al., 2014).

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References

Afonso, C. L., E. R. Tulman, Z. Lu, L. Zsak, G. F. Kutish and D. L. Rock (2000). The genome of fowlpox virus. *Journal of Virology* **74**(8): 3815-3831.

Banyai, K., V. Palya, B. Denes, R. Glavits, E. Ivanics, B. Horvath, S. L. Farkas, S. Marton, A. Balint, M. Gyuranecz, K. Erdelyi and A. Dan (2015). Unique genomic organization of a novel Avipoxvirus detected in turkey (*Meleagris gallopavo*). Infection, Genetics and Evolution **35**: 221-229.

Barnett, J., A. Dastjerdi, N. Davison, R. Deaville, D. Everest, J. Peake, C. Finnegan, P. Jepson and F. Steinbach (2015). Identification of Novel Cetacean Poxviruses in Cetaceans Stranded in South West England. PLoS ONE **10**(6): e0124315.

Biswas, S. K., C. Jana, K. Chand, W. Rehman and B. Mondal (2011). Detection of fowl poxvirus integrated with reticuloendotheliosis virus sequences from an outbreak in backyard chickens in India. *Veterinaria Italiana* **47**(2): 147-153.

Bolte, A. L., J. Meurer and E. F. Kaleta (1999). Avian host spectrum of avipoxviruses. *Avian Pathology* **28**(5): 415-432.

Bond, A. L. and J. L. Lavers (2011). Trace Element Concentrations in Feathers of Flesh-footed Shearwaters (*Puffinus carneipes*) from Across Their Breeding Range. *Archives of Environmental Contamination and Toxicology* **61**(2): 318-326.

Gyuranecz, M., J. T. Foster, Á. Dán, H. S. Ip, K. F. Egstad, P. G. Parker, J. M. Higashiguchi, M. A. Skinner, U. Höfle, Z. Kreizinger, G. M. Dorrestein, S. Solt, E. Sós, Y. J. Kim, M. Uhart, A. Pereda, G. González-Hein, H. Hidalgo, J.-M. Blanco and K. Erdélyi (2013). Worldwide Phylogenetic Relationship of Avian Poxviruses. *Journal of Virology* **87**(9): 4938-4951.

Haller, S. L., C. Peng, G. McFadden and S. Rothenburg (2014). Poxviruses and the Evolution of Host Range and Virulence. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases **21**: 15-40. doi: 10.1016/j.meegid.2013.10.014.

Huw Lee, L. and K. Hwa Lee (1997). Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. *Journal of Virological Methods* **63**(1-2): 113-119.

Johnson, B. J. and A. E. Castro (1986). Canary pox causing high mortality in an aviary. *Journal of the American Veterinary Medical Association* **189**(10): 1345-1347.

Kumar, M. and P. K. Shukla (2005). Use of PCR Targeting of Internal Transcribed Spacer Regions and Single-Stranded Conformation Polymorphism Analysis of Sequence Variation in Different Regions of rRNA Genes in Fungi for Rapid Diagnosis of Mycotic Keratitis. *Journal of Clinical Microbiology* **43**(2): 662-668.

Laidlaw, S. M. and M. A. Skinner (2004). Comparison of the genome sequence of FP9, an attenuated, tissue culture-adapted European strain of Fowlpox virus, with those of virulent American and European viruses. *Journal of General Virology* **85**(Pt 2): 305-322.

Lierz, M. (2002). **EAZWV Transmissible Disease Fact Sheet No. 6**. Select 'Fact Sheets 006 - Avian Pox'.

Offerman, K., O. Carulei, A. P. van der Walt, N. Douglass and A.-L. Williamson (2014). The complete genome sequences of poxviruses isolated from a penguin and a pigeon in South Africa and comparison to other sequenced avipoxviruses. *BMC Genomics* **15**(1): 1-17.

Reid, T., M. Hindell, J. L. Lavers and C. Wilcox (2013). Re-Examining Mortality Sources and Population Trends in a Declining Seabird: Using Bayesian Methods to Incorporate Existing Information and New Data. *PLoS ONE* **8**(4):

e58230.

Reza, K., A. Nasrin and S. Mahmoud (2013). Clinical and pathological findings of concurrent poxvirus lesions and aspergillosis infection in canaries. *Asian Pacific Journal of Tropical Biomedicine* **3**(3): 182-185.

Sarker, S., S. Das, J. L. Lavers, I. Hutton, K. Helbig, J. Imbery, C. Upton and S. R. Raidal (2017). Genomic characterization of two novel pathogenic avipoxviruses isolated from pacific shearwaters (*Ardenna* spp.). *BMC Genomics* **18**(1): 298.

Sarker, S., C. Lloyd, J. Forwood and S. R. Raidal (2016). Forensic genetic evidence of beak and feather disease virus infection in a Powerful Owl, *Ninox strenua*. *Emu* **116**(1): 71-74.

Sarker, S., K. G. Moylan, S. A. Ghorashi, J. K. Forwood, A. Peters and S. R. Raidal (2015). Evidence of a deep viral host switch event with beak and feather disease virus infection in rainbow bee-eaters (*Merops ornatus*). *Scientific Reports* **5**: 14511.

Shearn-Bochsler, V., D. E. Green, K. A. Converse, D. E. Docherty, T. Thiel, H. N. Geisz, W. R. Fraser and D. L. Patterson-Fraser (2008). Cutaneous and diphtheritic avian poxvirus infection in a nestling Southern Giant Petrel (*Macronectes giganteus*) from Antarctica. *Polar Biology* **31**(5): 569-573.

Shivaprasad, H. L., T. Kim, D. Tripathy, P. R. Woolcock and F. Uzal (2009). Unusual pathology of canary poxvirus infection associated with high mortality in young and adult breeder canaries (*Serinus canaria*). *Avian Pathology* **38**(4): 311-316.

Smithson, C., A. Purdy, A. J. Verster and C. Upton (2014). Prediction of Steps in the Evolution of Variola Virus Host Range. *PLOS ONE* **9**(3): e91520.

Tulman, E. R., C. L. Afonso, Z. Lu, L. Zsak, G. F. Kutish and D. L. Rock (2004). The genome of canarypox virus. *Journal of Virology* **78**(1): 353-366.

van Riper, C. and D. J. Forrester (2007). Avian Pox. In: Thomas NJ, Hunter DB, Atkinson CT, editors. Infectious Diseases of Wild Birds, Oxford: Wiley Blackwell Publishing.

van Riper, C., S. G. van Riper and W. R. Hansen (2002). Epizootiology and Effect of Avian Pox on Hawaiian Forest Birds. *The Auk* **119**(4): 929-942.

VanderWerf, E. A. and L. C. Young (2016). Juvenile survival, recruitment, population size, and effects of avian pox virus in Laysan Albatross (*Phoebastria immutabilis*) on Oahu, Hawaii, USA. The Condor **118**(4): 804-814.

Winterfield, R. W. and W. Reed (1985). Avian pox: infection and immunity with quail, psittacine, fowl, and pigeon pox viruses. *Poultry Science* **64**(1): 65-70.

Young, L. C. and E. A. VanderWerf (2008). Prevalence of avian pox virus and effect on the fledging success of Laysan Albatross. *Journal of Field Ornithology* **79**(1): 93-98.

Figure 1. Well circumscribed, multifocal, crusting pox nodules in the medial canthus of the eye and commissure of the beak (Photograph J. Lavers).

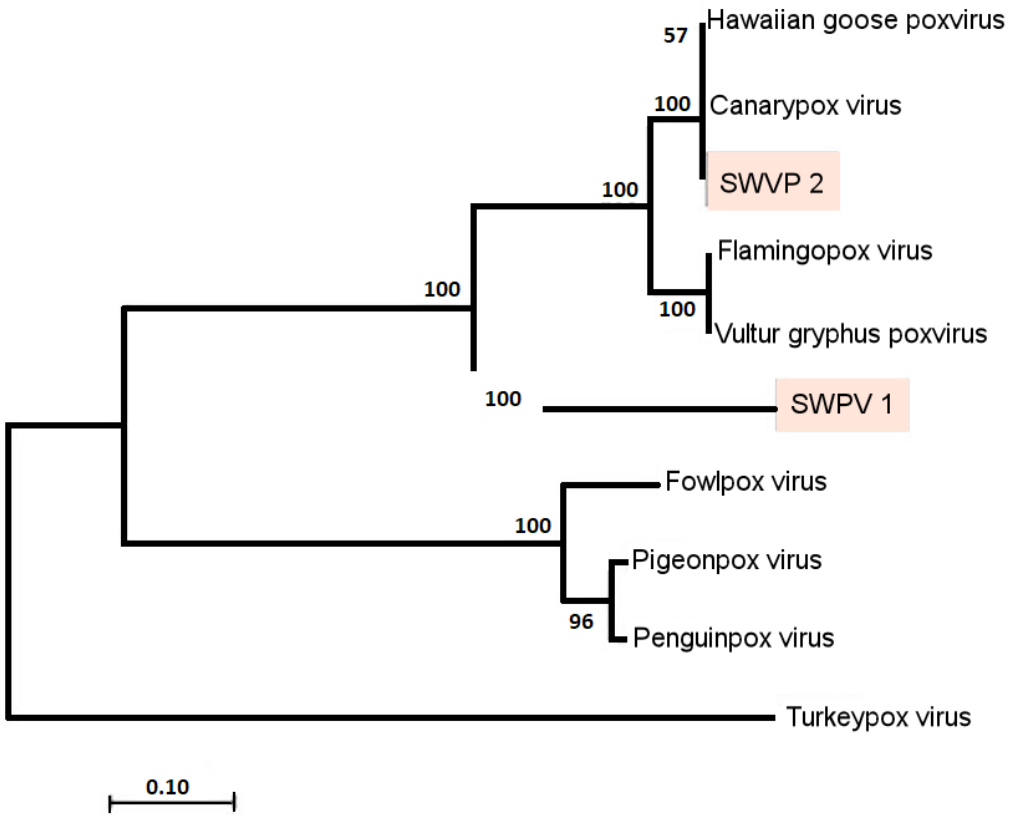

Figure 2. Phylogenetic relationship between Shearwaterpox viruses (SWPV-1 and 2) and other avipoxviruses based on the 173 kbp core region with large gaps removed.

Table 1. Recombination Events Detected Among Avipoxviruses.

Recombination Event Number	Begin	End	Recombinant Sequence(s)	Major Parent*	Minor Parent*	P value	Method†
1	74848	75223	CNPV	SWPV-2	SWPV-1	1.27E-47	RBT
2	74272	74831	CNPV	SWPV-2	SWPV-1	8.75E-42	RBMCT
3	75638	75858	SWPV-2	CNPV	SWPV-1	4.72E-31	RBMT
4	76297	76436	CNPV	SWPV-2	TKPV	1.17E-20	RBT
5	73361	73792	FWPV	PEPV, FeP2	SWPV-1	4.58E-20	RBMCT
6	66732	66833	SWPV-2	CNPV	SWPV-1	1.85E-18	RGT
7	148267	148690	SWPV-2	SWPV-1	PEPV	1.75E-15	RGBMCT
8	66400	66494	CNPV	SWPV-2	SWPV-1	4.23E-14	RGBM
9	75355	75495	SWPV-2	CNPV	Unknown	5.50E-19	RBMT
10	43019	43115	CNPV	SWPV-2	Unknown	1.79E-13	RBM
11	66849	66903	SWPV-2	CNPV	PEPV	1.27E-10	RBM
12	48258	48501	PEPV	FWPV	SWPV-1, SWPV-2	3.11E-10	RBMC
13	147907	148246	SWPV-1	SWPV-2	Unknown	4.76E-10	RGBMCT
14	93206	93746	PEPV	FeP2	FWPV	4.54E-09	RBMCT
15	66530	66612	SWPV-2	CNPV	Unknown	4.70E-08	RBT
16	1618	1786	SWPV-1	CNPV	PEPV, FeP2, FWPV	7.20E-07	RBMCT
17	176	358	FWPV	PEPV, FeP2	SWPV-1	1.85E-06	RBT
18	147442	147854	SWPV-2	SWPV-1	PEPV, FeP2	6.17E-06	RBMT
19	164076	164370	SWPV-1	PEPV, FeP2	TKPV	0.00014	RBMC
20	129440	129998	TKPV	SWPV-1	PEPV, FeP2, FWPV	1.04E-05	RBMC
21	76267	76293	SWPV-2	CNPV	Unknown	6.88E-07	RBC
22	492	646	SWPV-1	SWPV-2, CNPV	PEPV	0.001047	RBT
23	58825	59084	TKPV	Unknown	SWPV-2	0.006501	RBM
24	163198	163292	FWPV	FeP2	SWPV-1	0.007434	RGB
25	104074	104203	SWPV-1	Unknown	PEPV	2.17E-110	RBS
26	93843	93995	TKPV	Unknown	SWPV-1	0.011493	RMC
27	160678	160742	FWPV	PEPV	SWPV-1	0.020590	RBMC
28	185	405	SWPV-1	SWPV-2	Unknown	0.002067	RMC
29	97548	97704	SWPV-1	SWPV-2, CNPV	Unknown	0.025940	RBM
30	119182	119452	PEPV, FeP2, FWPV	SWPV-1	TKPV	0.013503	RGC
31	145646	145768	TKPV	SWPV-1	FeP2, FWPV	0.042229	RBC

* *Shearwaterpox virus* 1 (SWPV-1; KX857216), *Shearwaterpox virus* 2 (SWPV-2; KX857215), *Canarypox virus* (CNPV; NC_005309), *Pigeonpox virus* (FeP2; KJ801920), *Penguinpox virus* (PEPV; KJ859677) *Fowlpox virus* (FWPV; NC_002188), *Turkeypox virus* (TKPV; NC_028238).

† RDP4: Detection method coding R, G, B, M, C, S, and T represents methods RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan and 3Seq, respectively.