# Testing for Psittacine Herpesviruses

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## Psittacid Herpesvirues and Pacheco's Disease

Pacheco's disease is an acute rapidly fatal disease of parrots. Outbreaks of this disease have resulted in massive die offs of captive parrots.[1,2,3,4] Disease in passerines is rare and there is only a single confirmed case of Pacheco's disease in a superb starling (*Lamprotornis superbus*) and 5 suspected cases of Pacheco's disease in 4 other species of passerine birds.[5] A disease with similar histological characteristics has uncommonly been seen in toucans.[6]

Pacheco's disease is caused by infection with the alphaherpesvirus, psittacid herpesvirus-1 (PsHV-1). PsHV-1 has four distinct genotypes. All four of the PsHV-1 genotypes have the potential to cause Pacheco's disease; however, the outcome of an infection will depend on the genotype of the virus, the species of parrot infected and other undefined factors. For example, all four PsHV-1 genotypes have been shown to cause Pacheco's disease in Amazon parrots, but only PsHV-1 genotypes 2, 3, and 4 have been identified in African grey parrots with Pacheco's disease, and only genotype 4 causes mortality in macaws and conures. Parrots from the Western Pacific distribution, e. g., Australia, Indonesia the Philippines and Southeast Asia, appear to be somewhat refractory to Pacheco's disease, but when they do succumb to PsHV 1 infection, it can be caused by any of the four PsHV-1 genotypes. Other unknown factors also play a role in the pathogenesis of Pacheco's disease; for example, PsHV-1 genotype 2 is the second leading cause of Pacheco's disease outbreaks in Europe, but has not been detected in parrots with Pacheco's disease in the USA.[7]

Recently, a second psittacid herpesvirus (PsHV-2) was discovered. PsHV-2 was identified in normal oral and cloacal tissues from African grey parrots and in a mucosal and a perimucosal papilloma from two African grey parrots. Genetically, it is closely related to PsHV-1. Aside from being associated with the rarely seen papillomas of African grey parrots, this virus has not been associated with other diseases.[8]

## Biology of Psittacid Herpesviruses

PsHV-1 and PsHV-2 are type 4 alpha herpesviruses that are genetically related to the finch herpesvirus and the herpesvirus that causes infectious laryngotracheitis in chickens. It has been postulated that the PsHV-1 variants have co-evolved with several Neotropical parrot species.[8,9] Which species these are is only beginning to be known, but it appears that PsHV-1 genotype 4 may have evolved with the Patagonian conure, genotype 3 may have evolved with the hyacinth macaw and possibly other closely related macaws, and genotype 1 and 2 may have evolved in some species of Aratinga conures.[9] Given that PsHV-2 has been identified in several African grey parrots and only one Neotropical species, it has been postulated that this virus has co-evolved with African parrots.[7]

Based on current observations, it appears that transmission of host adapted herpesvirus variants between an adapted host and another does not result in disease and initial infection may most likely occur in nestlings. This would be how the virus would be transmitted and maintained in wild birds. In captivity, virus transmission also occurs between parents and their offspring and there is also evidence that virus transmission can occur between naïve parrots and adult subclinically infected parrots or adult parrots that survived Pacheco's disease.9

Pacheco's disease is a disease of captivity and occurs when non-adapted parrots are exposed to PsHV-1 adapted species. Even under these circumstances, not all PsHV-1 infections result in Pacheco's disease and subclinical infections have been found in parrot species that would normally be considered to be highly susceptible to Pacheco's disease.[9]

The *Ara* species of macaws rarely develop Pacheco's disease when infected with PsHV-1 genotypes 1, 2, and 3. However, it has been shown that persistent infection with these viruses results in the development of papillomas of the mucous membranes of the upper digestive tract and the cloaca. It is also likely that bile duct carcinomas and pancreatic duct carcinomas, which develop in some birds with papillomas and contain PsHV-1 DNA, are also the result of the virus infection.6,10

It appears that all parrots latently infected with PsHV-1 will be infected for life. Low concentrations of viral DNA can be consistently found in the oral cavity and cloaca in these birds. It is not known, however, if the viral DNA is episomal or packaged in virions. Additionally, the factors that may cause an increase in virus shedding are not known. On more than one occasion, it has been suggested that reactivation of a PsHV-1 infection can result in Pacheco's disease in a latently infected bird. There is no evidence to support this assertion. However, there is excellent documentation to show that infection with one serotype does not protect from infection with another as dual infections have been found in several birds.[9] While infection with one serotype may not protect from infection with a second, it is not known if previous infection with another serotype will protect against disease from a second serotype.

**Screening for Psittacid Herpesvirus Infections**

A recent study examined multiple tissues from parrots latently infected with PsHV-1 that died spontaneously from other causes. In all but one of the 16 latently infected birds, viral DNA was found in the cloacal and/or the oral mucosa, suggesting that latently infected parrots can be readily detected by sampling these surfaces. Repeated sampling of latently infected live birds was also done in this study and all positive birds were positive again 5 years after the first sampling.[9] These data provide strong support for the conclusion that once infected, parrots will remain latently infected for life and that latently infected birds can be detected by testing samples of the oral and cloacal mucosa for PsHV-1 DNA.

Currently, the recommended testing method for parrots is to take a disposable plastic bacteriology loop and used it to scrape off epithelial cells from the surface of the oral cavity. The other end of the loop can be used to scrape the cloacal mucosa. Both ends are then put in a small plastic tube that can be sealed and are sent to the laboratory for delivery in 1 to 2 days. PsHV-1 DNA can be readily detected in these samples if a semi-nested or real time PCR assay is used with primers that detect all of the PsHV-1 variants. It should be noted that primer sets used in recent publications will also detect PsHV-2,[7,9] and the disease causing potential of this virus is not known.

PsHVs are considered to be exotic infections in Australia and New Zealand and studies have not found PsHVs in wild parrots in either of these countries (Phalen unpublished).[11] However, there are Neotropical parrots in both countries and both PsHV-1 PsHV-2 have been detected in African parrots and these species are also present in avicultural in both countries. It is possible, perhaps even likely, that PsHV-1 and 2 may be in some of these birds. Therefore, screening for these viruses in Neotropical and African species of parrots and Australian and New Zealand species of parrots exposed to them may be indicated.

## References


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