Further Investigation into the Etiology of Internal Papillomatosis of Parrots and Detection of Birds Unapparently Infected with Pacheco's Disease Virus

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Internal papillomatosis (IP) is a disease primarily of macaws, conures, Amazon and hawk-headed parrots.[1,2,3,4,5] An IP-like disease has also been described in cockatoos and African grey parrots, but is extremely rare (Phalen, unpublished observation). IP appears to be more common in imported parrots but occurs in domestically raised parrots as well.[2,5,6] IP causes the lining of the digestive tract to develop into a localized or more generalized papillomas. Papillomas are growths that, like a mushroom, have a narrow base and a broad head. These growths can be found in the mouth at the base of the tongue, on the roof of the mouth, and around the opening of the trachea. If oral papillomas become large they can obstruct the birds airways. In certain species of birds, particularly green-winged macaws and hawk-headed parrots, papillomas will also develop in the crop, esophagus, and in the glandular stomach. Birds with severe changes to their digestive tract will gradually lose weight and eventually die. Another common place to find papillomas is just inside the vent. Papillomas that form on the edges of the vent will usually reach a size that will cause them to prolapse. When they do, they appear as a large round mass that generally will ooze blood. Papillomas associated with the vent are painful and birds will often strain and even grunt when they make a dropping.[3,4,7]

If left alone, papillomas will generally become smaller over a period of several months, but will then return. Untreated birds have lived with IP for many (more than 10) years (Phalen, unpublished oberservation). However, when the disease is extensive, some birds may only live for a year or less.[3,4] For some, as yet, unexplained reason many birds with IP will ultimately develop cancer of the liver or pancreas and sometimes both. These types of cancer do not metastasize, but as they grow they destroy the normal liver and pancreatic tissue eventually resulting in the bird's death.[4,6,8,9] The progression of these cancers is very slow and birds with this type of liver cancer have been known to live a quality life for more than two years after the initial diagnosis. These liver tumors are readily recognized with ultrasonography and have been associated with elevations in serum gamma glutamyl transferase concentrations.[6]

There are many treatments for IP, but there is no cure. When papillomas cause pain or interfere with breathing they can be removed. Many different methods can be used to remove them, including freezing them, applying silver nitrate to them, using electrocautery, or simply cutting them off with a scalpel blade. Generally, the surgery necessary to remove the papilloma is not risky.[10] Occasionally, however, following surgery of the vent, scar tissue will form narrowing the vent so that the bird cannot produce a dropping.[11] Autogenous vaccines made from macerated lesions have been tried, but long term follow up reports on the treated animals have not been done.

Because the cause of IP is not known there are no specific diagnostic tests that can be used to screen birds for this disease. The only way that is even moderately effective of keeping IP out of a bird collection is to have all new birds, especially macaws, Amazons, conures, and hawk-heads, examined carefully by a veterinarian before they are released from quarantine. Often, the early stages of IP are very subtle and so a very careful examination must be made. Early changes in the mouth include a thickening of the choanal slit, a loss of the normal projections around the choana, and a discoloration of the choana. Changes around the vent may also be subtle. In order to see them, a moistened cotton swab must be inserted into the vent and the lining of the vent as it joins the skin examined. This surface should be pink and smooth. In early IP, the tissue becomes roughened (cobblestone-like) and may bleed. Dilute vinegar applied to early IP lesions will cause them to become white.[2,5]

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Increasingly persuasive evidence suggests that IP is caused by an infectious agent. If a bird with IP is introduced into an aviary, other lesions in other birds often develop. Also, IP is especially common in birds that are in direct contact with birds having this disease. Finally, at least some offspring of birds raised by parents with IP will develop IP (Phalen, unpublished).

Both papillomaviruses and herpesviruses are potential etiologic agents of this disease. Papillomaviruses have been purified from cutaneous papillomatous lesions of European finches,[12] canaries,[13] and African grey parrots.[7,14] One or more herpesviruses have also been described in papillomatous lesions of the feet of macaws and cockatoos,[15] and herpsevirus virions were identified in a cloacal papilloma from a conure.[16] Recently, in my laboratory, we have shown that a high percentage of birds with IP have antibodies in their blood that will neutralize an avian herpesvirus known as the Pacheco's disease virus (PDV). PDV causes an acute and rapidly fatal disease in some birds, but unapparently infects others. Unapparently infected birds shed virus intermittently and are the source of infection for others. Until now, it has been impossible to detect birds that were unapparently infected with PDV.[10]

Preliminary Data

To determine if IP is associated with PDV infections, we have cloned a PDV isolate and developed six DNA primer sets (9F, 9F', 9R, 11F, 11R and 23F) that when used with the polymerase chain reaction (PCR) amplify PDV DNA. The primer sets were all found to amplify DNA from the original virus and did not amplify DNA nonspecifically. The ability of the primer sets to detect herpesvirus DNA in birds thought to have Pacheco's disease or another herpesvirus infection was then tested. Tissues from 15 psittacine birds, two pigeons, and a peafowl were probed with all six sets of primers (Table 1). Each of these birds was known to be infected with a herpesvirus based on immunofluorescent antibody staining with a PDV specific antibody, direct electron microscopic observation of a herpesvirus, the presence of characteristic histologic lesions, or isolation of a herpesvirus from the bird. One or more primer sets detected herpesvirus DNA in all of the tissues except for those of the second pigeon. Distinct patterns of DNA amplification were seen (Table 1). The most common pattern (75% of the psittacine herpesviruses) was pattern A where all 6 primer sets detected viral DNA. Two other patterns were also identified in the psittacine birds. Unexpectedly, five primer sets detected the herpesviruses in the peafowl and in one of the pigeons. Similar herpesvirus DNA sequences were not detected in the second pigeon. Primer set 11F detected viral DNA in all of the herpesviruses. Herpesvirus DNA has also been detected in cloacal biopsies from two Patagonian conures that were seropositive for PDV.

Twenty eight cloacal samples were probed with 5 of the PDV PCR primer sets (9F, 9R, 11F, 11R, 23F). All 28 either had cloacal papillomas at the time, or had them previously. Gross lesions were confirmed to be papillomas by histopathology. Herpesvirus DNA was detected in 22 of 28 samples (78.5%). Five different patterns of DNA amplification were identified. The predominate one was pattern B (68%). Pattern A, the predominate PDV pattern was only found in one papilloma. The 23F primer detected all the herpesviruses found in the papillomas, 9R detected none (Table 2). To verify that the amplification product was herpesvirus DNA, the 9F amplification product from a single papilloma was sequenced. The DNA sequence from this virus varied from the original PDV sequence by 4%.

Discussion

The initial data using the PDV primers with other suspected PDVs suggests that there is one predominate PDV and as many as two other avian herpesviruses capable of causing similar disease in the United States. This data also suggest that avian herpesviruses infecting nonpsittacine species have highly conserved regions of DNA and that primers made against PDV can be used to detect herpesvirus infection in some other birds as well. An alternative explanation for the nonpsittacine data is that the PDV may have crossed over into other species and is capable of causing disease in pigeons and gallinaceous birds.

Detecting PDV DNA pattern A in the cloaca of 2 patagonian conures, suggests that PCR of cloacal swabs may be a practical way to detect birds unapparently infected with PDV. This is an extremely important finding, as detection of latently infected birds will be essential to the control of PDV.

Detection of herpesvirus DNA in cloacal papillomas is an exciting first step in our work. The presence of herpesvirus DNA with a specific pattern suggests that a unique herpesvirus is present in these tissues. Given that herpesviruses are associated with cutaneous papillomatous lesions in fish,[17] macaws, cockatoos,[16] and

mallard ducks[18] we are optimistic that one element in the internal papilloma puzzle may have been identified. We are, however, acutely aware that this issue may be far more complex than our data suggests at this time. In humans, chickens, and many other animals, herpesvirus infections are often ubiquitous and are not necessarily associated with disease. It may be that we have found several non-pathogenic viruses that coincidentally grow in papillomas.

Another complicating issue is that preliminary evidence suggests that a papillomavirus-specific protein may also be present in these tissues.[19] This observations are particularly interesting, because in humans, there has always been the question of why so many people are infected with papillomaviruses, yet only certain people develop mucosal papillomas. Given that mucosal herpesvirus infections and papillomavirus infections are both extremely common in people, we speculate that co-infection may be necessary for papillomatous lesions to develop.

Table 1. Detection of avian Herpesviruses using 6 sets of PCR primers.

Species	9F	23F	9F'	9R	11F	11R	Pattern
Cockatoo	+	+	+	+	+	+	A
Cockatoo	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Amazon	+	+	+	+	+	+	A
Cockatiel	+	+	+	+	+	+	A
Cockatiel	+	+	+	+	+	+	A
Scarlet Macaw	+	+	+	+	+	+	A
African Grey	+	+	+	+	+	+	A
Amazon	+	+	+	-	+	+	B
African Grey	-	-	+	+	+	-	C
Amazon	-	-	+	+	+	-	C
Peafowl	+	+	wk[a]	+	+	+	D
Pigeon 1	+	+	wk	+	+	+	D
Pigeon 2	-	-	-	-	-	-	negative

[a] wk = amplifies weakly.

Table 2. Herpesvirus DNA amplification from papillomas

Species	9F	23F	9R	11F	11R	Pattern
Blue-front Amazon (4.5%)	+	+	+	+	+	A
Amazons and Macaws (68%)	+	+	-	+	+	B
B&G Macaw (4.5%)	+	+	-	+	_	E
GW Macaw (4.5%)	+	+	-	-	+	F
GW Macaw (4.5%)	-	+	-	-	-	G

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