Avian Borna Virus

Almost certainly the cause of Proventricular Dilatation Disease (PDD)

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Introduction

Proventricular Dilatation Disease (PDD) is a horrible disease of psittacines, especially the large species such as macaws, African Greys and cockatoos. First recognized in the early 1980s, the disease was originally called Macaw Wasting Disease. In its classical form, the disease results from paralysis of the gastrointestinal tract, especially the ventriculus. Because of this paralysis, the flow of food through the ventriculus is blocked and so begins to accumulate in the proventriculus. The proventriculus enlarges as it fills with food, while the bird slowly starves to death. Subsequent studies traced the cause of the paralysis to a brain infection (encephalitis). It was soon recognized that proventricular dilatation, while common, was not the only symptom of this disease. Some birds, especially African Greys showed other signs of brain damage such as behavioral changes, loss of balance, blindness and paralysis.

From the earliest days, the disease was assumed to be infectious and likely of viral origin. The disease clearly occurred in outbreaks and numerous reports testified to its transmissibility. Unfortunately, it proved very difficult or impossible to isolate the virus responsible. Large psittacines may carry several viruses not responsible for PDD. Thus Dr. Christian Grund in Germany suggested that a paramyxovirus related to Newcastle disease virus was the cause. Unfortunately, although present in some PDD birds, it was also absent in many cases and is almost certainly not the cause. Likewise a coronavirus was isolated by Dr Gough and his colleagues in the United Kingdom from a PDD case but was not a consistent finding. Investigators were frustrated with our inability to determine the cause of the disease.

Discovery of the virus

The impasse was broken in the late summer of 2008 when two groups of investigators used sophisticated modern screening techniques to identify a hitherto unknown virus while a third group succeeded in isolating this virus from the brains of birds with PDD. These investigators, Dr Kistler and her colleagues at UCSF in San Francisco and Dr Honkavuori and her colleagues at Columbia University in New York, had been developing methods for the very rapid diagnosis of new and unknown viral infections. Thus they isolated viral nucleic acids from infected tissues, sequenced them and then ran the results through large computers to determine if

there was any match with known viruses. Using PDD-derived material, both groups found unique viral genes that were related to a virus that causes encephalitis in mammals. It is called Borna Virus after the town in Germany where the disease was first identified. Additional analysis of this new virus from PDD cases showed that it was distinctly different from mammalian Borna Virus so it was called Avian Borna Virus (ABV).

Simply identifying the presence of a virus in the tissues of a bird with PDD does not, of course, prove that that virus actually causes PDD. Nevertheless, testing numbers of birds for the virus can provide suggestive evidence. Thus the UCSF group tested eight birds with confirmed PDD and detected the virus in five of them. They also tested 14 birds not known to have PDD and all were negative for ABV. The Columbia University group tested tissues of three PDD birds and all were positive for PDD while none of four unaffected birds were positive,

Meanwhile, a third research group at the Schubot Center at Texas A&M University approached the problem from a different direction. We reasoned that sick birds would respond by mounting an immune response and making antibodies against the virus. We took blood from affected birds and investigated whether it would react with anything in the tissues of other PDD affected birds by using a technique called a Western Blot assay. Indeed we found that PDD-affected birds all made antibodies against one or more proteins in infected bird brain. These proteins were absent in normal birds brains and appeared to belong to a virus. Initially, we had no idea what virus we were detecting but we found it in 22 of 25 PDD cases and two of 25 apparently healthy birds. We seemed to have a diagnostic test!

Viral growth and isolation

As we studied PDD cases, we also made attempts to isolate and grow the virus in tissue culture. We took fresh brain,

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put it in tissue culture for several weeks and then tested the cultures for the presence of the viral protein–it was present in large amounts–we were growing the virus! Our first two viral isolates were from an African Grey and from a Yellow-collared Macaw. We sent these cultured viruses to Thomas Briese and his colleagues at Columbia University who confirmed indeed that we were indeed growing ABV. Since then we have succeeded in growing ABV from the brains of seven additional PDD cases. It is beginning to look like ABV is the cause of PDD.

In a more recent study we looked into the archives. For 10 years between 1987 and 1998, our colleague, Dr. David Graham offered a diagnostic service for exotic birds and identified many cases of PDD. And, he didn't throw anything away! So we looked at 24 avian brain samples obtained in 1992. Thirteen had been diagnosed with PDD while the other 11 were diagnosed with other diseases. We tested each brain sample for the presence of ABV. All 13 cases diagnosed as having PDD had ABV in their brains. One bird diagnosed as not having PDD but which had brain inflammation and a proventricular ulcer also had ABV in its brain. Once again, the evidence is mounting to support the claim that ABV causes PDD.

While identifying the causal agent of PDD as a unique new virus is immensely satisfying, that is, of course, only the beginning. Our role is to save birds' lives and prevent their suffering. We want to eliminate PDD. In order to achieve this we need several things. One is a diagnostic test that will enable us to identify infected birds. Once we have such a test that we can keep infected and non-infected birds apart. We need to characterize the virus and find out how it spreads so that we know how to stop it. We also need to know just how this virus causes disease so that we can treat and ideally, cure infected birds. Finally, it would be ideal if we could prevent birds from acquiring this infection. For this we need to develop an effective vaccine.

Diagnostic tests

As described earlier we have developed a western blot assay that appears to detect ABV-infected birds; however, blood tests are rarely 100% accurate. Occasional samples will give false positive results while others will be falsely negative and miss infected birds. When we develop a test we need to know how often do false results occur. For example, out of about 25 healthy birds we tested, two gave positive blood tests for Avian Borna Virus. In other words, while healthy, they appeared to be infected. This is unsurprising. These birds likely are infected but have yet to get sick or they may simply be healthy carriers. We need to find out just what is happening. Likewise, two birds were diagnosed as having PDD had a negative blood test out of 25. This suggests that they had somehow failed to respond to the virus or perhaps their

disease was misdiagnosed. If some birds fail to respond to the virus we need to know how many and how often.

The new blood test thus needs to be validated before we can offer it to bird owners with confidence. We believe that we need to test blood from about 100 birds (50 positive and 50 negative) before we can be reasonably confident about its accuracy. Samples are coming in slowly since we really want to test birds of known PDD status. If you have a bird known to have PDD or conversely, have birds that you are sure don't have PDD, please consider asking your veterinarian to send us a small blood sample.

While the blood test measures a bird's response to Avian Borna Virus, a more direct test for infected birds is to look for the virus in their droppings. Using a PCR test, we have found that many of the PDD birds in our own collection are shedding the virus in their feces. We would like to know how common this is and how this test compares with the blood test. Ideally, we would like to receive paired feces and blood samples for comparison.

A vaccine or a cure?

Only when we can identify infected birds with confidence can we seek an effective treatment and monitor its effectiveness. To help us plan this we can look at the experiences with Mammalian Borna Virus. In mammals, the brain damage that occurs in Borna disease is due not only to the direct effects of the virus on brain cells but also to the body's own immune response. As the bird tries to fight the virus it causes local tissue damage. This means that the outlook for an effective vaccine is not encouraging. Indeed it is possible that vaccination may make the disease worse. The news is not all bad, however. Published papers indicate that Mammalian Borna Virus is susceptible to some common antiviral drugs. This clearly needs to be investigated.

Possible control procedures

What should you do in the meantime if you have known PDD birds in your collection? We already know that PDD birds spread the virus in their droppings. Prudent control procedures would therefore include isolating infected birds as much as practical. Only handle PDD birds after you have handled healthy birds. Do not breed PDD birds. Mammalian Borna Virus is rapidly killed by chlorine-containing disinfectants. Wash your hands with an alcohol wash after handling infected birds and exercise normal prudent hygienic measures.

The future

It is clear that we have lots of work ahead if we are to control and eliminate this dreadful disease. The events of the past few months have been very encouraging and bode well for the future. Rapid progress is being made. As in so many areas; however, the rate of progress depends on the resources allocated to the problem. Resources in turn are determined by available finances. I would encourage all of you who love birds to consider donating to some of the laboratories engaged in this exciting research.