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AN HHMI TEAM ENTERS THE MYSTERIOUS DOMAIN OF  
PARROT BREEDING TO DECIPHER THE VIRUS  
THAT'S KILLING EXOTIC BIRDS.

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## THE TINY PATIENT

was feathered and failing. The prognosis: grim. On a hot Friday before Labor Day 2008, Amy Kistler fought holiday traffic to make a house call. She was greeted by a high cyclone fence and snapping rottweilers. When the owner opened the gate and dismissed the guard dogs, Kistler stepped into the world of a parrot breeder.

Behind the house, dozens of cages—some as big as 6 feet square and 8 feet tall, with wooden nesting boxes inside—littered the yard. Each held a pair of parrots. Majestic macaws, big cream-colored crested cockatoos, African grays with blood-red tails.

As the owner showed Kistler around, the birds screamed and shrieked—a cawing cacophony. “There were more than 100 birds, and they absolutely flipped out,” Kistler says. “I had never seen anything like it before.”

Ignoring the riot, the owner explained the situation: A young macaw was sick. It had stopped eating and was losing weight. During the past six weeks, other parrots in his collection, mostly chicks that lived inside the house, had wasted to the bone and died. Kistler collected the red bird, put its carrier in the backseat of her car, and drove to a local bird veterinarian, Jeanne Smith.

At Smith’s office, the pair euthanized the sick bird and opened it up. Smith pointed to one of the digestive organs, the proventriculus. Situated in the upper gastrointestinal tract of birds, the proventriculus normally passes partially digested food farther down the line. In this macaw, though, the organ was grotesquely swollen and clogged with food. This young bird had PDD, proventricular dilatation disease. This and the other dead birds meant an outbreak was spreading.

PDD is the AIDS of the parrot world, devastating captive flocks and stigmatizing breeders whose birds carry it. How Kistler, a virology postdoctoral fellow at the University of California, San Francisco (UCSF), got involved is a story of DNA chips, perseverance, and good timing.

## THE VIRUS HUNTERS

When she arrived at UCSF in 2003, Kistler had never heard of PDD, let alone touched a bird. She had signed on for a post-

doctoral position with two HHMI investigators, Joseph DeRisi and Don Ganem, attracted to the operation they had built to link viruses to human diseases. (See “Modern Day Virus Hunters,” *HHMI Bulletin*, August 2006.) Ganem, the physician of the operation, had found in the late 1990s that human herpesvirus 8 causes Kaposi’s sarcoma, a tumor found in many AIDS patients. DeRisi, the lab polymath, is a pioneer in DNA microarrays—glass slides spotted with thousands of genetic fragments.

In 2002, DeRisi and David Wang, a postdoctoral fellow in his lab at the time, invented the Virochip, a DNA array for detecting every virus found in plants, animals, insects, and humans. The Virochip can also identify unknown viruses if they share a smattering of genetic code with a known virus.

In 2003, the Virochip pinned severe acute respiratory syndrome (SARS) on a previously unknown coronavirus, proving the worth of the chip in a pressure-cooker international outbreak that killed hundreds of people.

Since then, Ganem and DeRisi had received stacks of entreaties from researchers wanting to tap their technology. In 2006, two unusual requests arrived. Both asked the researchers to help unravel the mystery of a parrot disease, PDD.

“Parrots seemed kind of off the subject, so at first we just filed the letters,” Ganem recalls.

## ELUSIVE BIRD KILLER

At the time, Kistler was studying upper respiratory illnesses with the Virochip, but she had a growing interest in animal viruses. When she got wind of the letters, she read up on PDD and discovered the disease first appeared in macaws imported to the United States from Bolivia in the 1970s. Since then, veterinarians had recorded it in more than 50 species of Psittaciformes, the avian order that includes many pet species, such as parrots, macaws, cockatoos, cockatiels, lorikeets, parakeets, budgerigars, conures, parrotlets, and lovebirds. The disease jumps between birds in close contact, suggesting an infectious agent.

Under the microscope, veterinarians see tiny round specks in tissue from sick birds, specks that resemble virus particles. Veterinarian Susan Clubb had written one of the letters pleading for a viral investigation of PDD. “For a very long time, researchers all over the world have been trying to find the agent that causes the disease,” says Clubb, who has a bird clinic in Loxahatchee, Florida. “It’s been very elusive.”

Clubb calls PDD an obsession. Over her 30-year career, she’s treated hundreds of birds with it. She’s watched PDD wipe out whole indoor aviaries in Canada and the northern United States. “It’s devastating,” says Clubb, who counts 280 parrots in her own breeding flock.

Like AIDS, PDD is a wasting disease. Inflammation attacks the nerves at the base of the proventriculus, paralyzing the organ. As the organ swells, birds stop eating, regurgitate, and pass undigested food in their feces. Affected parrots often become clumsy and fall off their perches. Seizures sometimes strike. But

symptoms can wax and wane, and birds might rally for a time after treatment for secondary bacterial and yeast infections.

Most cases are diagnosed symptomatically. Owners sometimes spring for an arduous and costly crop biopsy, in which veterinarians search for tell-tale inflammation of the gastrointestinal nerves. Even then, biopsies miss about half of positive cases, says Clubb. A definitive diagnosis—as confirmed by the inflamed nerves—can be made only via necropsy.

There are cultural parallels to AIDS, too. PDD is freighted with stigma. No breeder wants a reputation for keeping birds with the disease. Organizations of parrot lovers have sprung up to raise awareness, some launching a campaign called Stop PDD, which made a PDD quilt, each square hand-sewn to recall a parrot that had passed.

As Kistler discovered, parrot breeding is a big backyard business, exploding after a 1992 law banned the importation of most wild parrots. Now, virtually all the estimated 12 million pet parrots in the United States are captive bred, providing income for several thousand breeders, according to the American Federation of Aviculture. Rare, hard-to-breed, “super-exotics,” such as the magnificent hyacinth macaw, can fetch \$10,000 to \$15,000. African grays, known for their intelligence and longevity, typically cost \$1,200 retail.

#### NEW TERRITORY

Ganem delved into the pathology of the parrot disease to determine whether it resembled any human ailment. He made a

connection with a condition called achalasia. In this disease, the esophagus—analogueous in form and function to the proventriculus of birds—is affected, impairing the peristalsis that normally propels food along the digestive tract. The cause is unknown, but esophageal biopsies of achalasia patients show “active inflammation of the nerves and ganglia,” Ganem says, “which in fact is the exact pathology of PDD. I realized this wasn’t just important for parrot owners, it actually had relevance to human disease.”

Buttressed by Ganem’s findings and the enthusiasm of Kistler and Alexander Greninger, an M.D./Ph.D. student who joined the project, the team decided to make PDD the first veterinary disease they would study with the Virochip. Clubb sent tissue from birds that died of PDD and birds that died of natural causes. The writer of the second letter, an Israeli veterinarian named Ady Gancz, sent his own samples.

In January 2007, Kistler selected one of the samples Clubb had sent from Florida, extracted the genetic material, added fluorescent tags, and then washed the mixture over the Virochip. She slipped the chip into a scanner, which reads each of its 20,000 spots, and watched bright dots scroll up the screen: green spots are hits, white spots are strong hits.

Kistler noticed a white spot on the right edge, one she’d never seen in the hundreds of human disease Virochips she had processed. The computer identified it as “bornavirus.” She’d never heard of it. An Internet search revealed that “Borna disease virus,” named for the town in Germany where a peculiar outbreak



AMY KISTLER KNOWS MORE ABOUT BIRDS THAN SHE EVER INTENDED WHEN SHE JOINED THE GANEM AND DERISI LABS. HER RESEARCH ON A DEADLY AVIAN VIRUS HELPED SOLVE A 30-YEAR PUZZLE AND OPENED HER EYES TO VETERINARY RESEARCH.

Lenny Gonzalez



DON GANEM AND JOE DERISI WEREN'T PLANNING ON STUDYING A BIRD DISEASE, BUT AN ENTERPRISING POSTDOC AND SIMILAR PATHOLOGY TO A HUMAN AILMENT MOVED THEM TO TAKE A CLOSER LOOK AND PINPOINT THE VIRUS THAT CAUSES PDD.

in horses was first described in 1885, causes poor balance, motor control problems, and abnormal behavior in horses, sheep, and other livestock. The virus latches onto nerves and can cause encephalitis.

The association to PDD was striking. Five of the 8 samples, but none of the controls, lit up with bornavirus.

“That was it,” says DeRisi, recalling when he and Kistler watched the first data roll in. “That was a thrilling moment. It gave us an answer that was pretty clear cut.” In two days, the team and their Virochip had solved a 30-year mystery.

Next, Kistler fished out segments of the virus’s genetic code. The segments confirmed that they had found a bornavirus unlike any seen before. The viruses recovered from horses and sheep all closely resembled each other at the genetic level, but the parrot viruses looked much different from those viruses and from each other. The team called them avian bornaviruses (ABVs). After analyzing samples from more dead birds, the team discovered five ABV subtypes across a range of parrot species. Kistler, Ganem, and DeRisi eventually spelled out the entire genome—all six genes—of one strain of the new virus. They had found their quarry.

#### THE OUTBREAK

The team published the discoveries in *Virology Journal* in July 2008. “We became rock stars of the parrot world,” Ganem says. Soon the calls began. Veterinarians asked if a diagnostic test was available. (“Nothing commercially available.”) Breeders hoped the discovery somehow meant a cure was at hand. (“Not yet.”)

That’s when bird doctor Jeanne Smith called. She suspected an active PDD outbreak. Did the researchers want to play disease detective? At this point, Kistler had worked only with tissue samples from dead parrots. She jumped at a chance to investigate live cases, and on that scorching Friday in August 2008, she drove three hours and met Smith at the breeder’s compound.

The facts of the case: Several months earlier, a female African gray named Kiwi had fallen ill, lost weight, and developed a nasty fungal skin infection. The breeder isolated Kiwi outside, but brought her inside to the kitchen to medicate her. Too close, it turned out, to where he kept the hatchlings, 50 chicks, stacked in cages in the erstwhile living room and dining room and in the pantry, next to the kitchen. In July, some of the chicks started showing symptoms; a few quickly died.

Kiwi also died, and the necropsy tissue was positive for PDD. “He introduced a sick bird with PDD into the nursery. He wasn’t washing his hands, washing the counters, taking precautions,” Smith says. “So it just spread like wildfire.”

The breeder was worried. He agreed to let Kistler take samples, but only if he could remain anonymous.

On Kistler’s first visit, she collected the young red macaw, and she and Smith sampled nearly every organ. At the UCSF lab, Kistler ran the tissue through a polymerase chain reaction (PCR) test she had developed with the ABV genome data. The test can quickly fish out bits of ABV. The brain and digestive tract were marinating in virus. Kistler returned to the compound for another dying bird. Same results.

The breeder panicked. He wanted all the indoor chicks tested. In mid-September, Kistler and Smith arrived armed with vials for blood and a batch of large Q-tips for swabbing the birds' cloaca, or backsides. They started with the African grays and spent three hours wrangling screeching parrots. When Kistler ran the samples at the lab, she found virus in 12 of 46 chicks. She e-mailed the results to the breeder. He never wrote back. The breeder also cut off contact with Smith, his veterinarian for 10 years. "He went into denial," Smith says.

There were still more casualties. Another client of Smith's, a second breeder, had boarded three hatchlings at the first breeder's house earlier that summer. After the second breeder brought the chicks home, one of them displayed symptoms and died. Kistler and Smith found ABV in 8 of 10 other birds exposed to the dead chick, even though some were asymptomatic. It was the same strain found in the first breeder's birds. The case was a slam dunk. "We linked the strains," says Ganem. "We proved the birds in the second aviary caught the strain we saw at the first place."

The second breeder wanted the virus gone, the birds out; she needed to protect her flock. She asked Smith to quarantine the exposed birds. Kistler and Smith watched the birds for a few months, but Smith eventually euthanized all of them to search for virus in their tissues.

Though they were long-time friends, the two breeders no longer talk. The second breeder, who eliminated the virus from her flock, also doesn't want to be identified. "It's one of those things that can severely damage my reputation," she says. "I'd never sell another bird again."

#### ANSWERS BREED QUESTIONS

DeRisi acknowledges that it is a complex situation. It's a small world and breeders are a close-knit community. "Maybe someday when birds have certificates of health, and you get a test result

from an independent testing service before you buy a bird, it will help clear that up."

DeRisi, Ganem, and Kistler are convinced that ABV causes PDD. In late 2008, Gancz, the Israeli veterinarian collaborating with the HHMI team, injected three cockatiels with an extract from ground-up ABV-infected parrot brain. One rapidly died from severe PDD, and the other two got sick. The group expects to publish its findings in an upcoming issue of the journal *Virology*.

A host of other questions now come into play. DeRisi wonders where the parrot outbreaks originate. Do wild ducks or geese carry it? "Maybe parrots are just the dead end," he says. "What about poultry?"

Kistler discovered that some asymptomatic birds test positive for the virus. Does that mean they're carriers, spreading the infection? And is ABV like HIV, infecting the host for a long period before causing symptoms? How accurate is the PCR test that Kistler produced? Can a vaccine be developed?

Already, the veterinary research community is jumping in, with teams in Canada and Germany also finding ABV in dead parrots. Any company can now license the technology that UCSF patented and commercialize a PCR diagnostic test. An accurate test could help eradicate PDD by identifying birds that need to be quarantined.

Ganem and DeRisi plan to back off PDD to focus on their work on achalasia and other human diseases. Ganem wants to know if a virus like ABV triggers achalasia, the human disease eerily similar to PDD. To that end, he has obtained esophagus biopsies from 10 patients, which he has begun scanning for viruses.

Kistler, though, might just make a career of it. She's looking for a faculty position at a university with a strong veterinary research program. "I'm going to work on it for a while," she says. "There are a lot of interesting questions left unanswered." ■

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#### SAVING THE RAREST OF BIRDS

colleagues have a chance to save a bird that exists only in captivity. ¶ On a lush island of vegetation in Qatar's desert lies an ark of sorts, Al Wabra Wildlife Preservation. Owned by a member of Qatar's royal family, the park shelters 2,000 animals from 100 rare species—including 50 Spix's macaws, the rarest bird

With the cause of a deadly viral disease in hand, HHMI researchers at UCSF and

in the world. The last wild Spix's, spotted in Brazil in 2000, is presumed dead. ¶ Veterinarians at Al Wabra breed the powder blue birds, and in 2008 the compound's owner bought a tract of land in Brazil with plans to reintroduce the Spix's there. But proventricular dilatation disease (PDD) is stalling his plans. Veterinarian Susan Clubb, a PDD expert, flew to Qatar and found the disease in several birds (she won't say how many). Ganem and DeRisi's research

into the viral cause of PDD might just save the species. After months of red tape wrangling, DeRisi won approval to import samples from the Spix's. He plans to run them through a battery of tests to search for signs of avian bornavirus. If the tests identify birds carrying the disease, the animals can be quarantined, reducing the risk to their flock-mates. "This is a case where finding PDD could make a difference in whether a species survives," Clubb says.

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