



the detectives

An outbreak of equine influenza among racing greyhounds in Jacksonville, Fla., last January has turned out to be far from a fluke, as the virus struck again and again from June through August, extending to every state that has greyhound race tracks and possibly turning up in other breeds of dog as well. The cross-species nature of the illness worries some researchers, as they consider the implications of viruses that jump from animal to animal and, potentially, to humans.

Investigators were initially baffled when a severe respiratory illness struck greyhound kennels in Florida in 2003–2004. In January 2004, eight of the 24 affected dogs died. Ultimately, Cornell virologists working with researchers in Florida and the Centers for Disease Control (CDC) determined that the dogs were stricken by a type of influenza that is ordinarily found only in

Careful investigative work in a Cornell lab turns up an influenza virus that made an interspecies leap

by Claudia Wheatley

horses. This was the first scientific report of an equine influenza virus that jumped the species barrier.

Finding the culprit took careful detective work that included the combined talents and resources of three former colleagues from the College of Veterinary Medicine. There have been reports of a flu-like illness among dogs at racetracks from Florida to New England since at least the early 1990s, but they were sporadic. The increasing

severity of the 2003–2004 outbreaks prompted the University of Florida (UF) College of Veterinary Medicine Racing Laboratory and the Florida Division of Pari-Mutuel Waging, which regulates greyhound racing in that state, to fund an investigation. Cynda Crawford, a UF immunologist, collected and analyzed tissue samples from five of the dogs that had died in January 2004. She also collected blood and nasal fluid from both affected and unaffected dogs at the kennel. The pathology results suggested a viral disease, but did not identify the offending microorganism. But Crawford's lab lacked the resources required to solve the puzzle. So she turned to Cornell's Animal Health Diagnostic Lab, a full-service operation that has been conducting standard antibody screening related to this problem for several years.

"We anticipated that we were looking for something out of the ordinary," says Ed Dubovi, director of the virology center at the Cornell lab, "but we weren't necessarily thinking about influenza." However, Dubovi's group was already collaborating with a colleague in England who had come across a new respiratory ailment in dogs that seemed closely related to bovine coronavirus. With that in mind, they set up a whole

range of tissue culture cells capable of detecting the new coronavirus, if it was present. "Fortunately, with the first dog we put up in culture, something started happening on one of the cell culture systems," Dubovi says. "We eventually worked it up enough to prove that it was real. Then we started doing our in-house testing and concluded that we did indeed have an influenza virus."

But which influenza virus did they have? There are 15 different hemagglutinin types (H-types), and the Cornell lab only had the necessary reagents to rule out two: the H5s and H7s, which are associated with avian flu. Fortunately one of Dubovi's former graduate students, Ruben Donis, had just been hired at the CDC in December 2003. Dubovi arranged to have samples sent to Donis at the CDC's Influenza



Branch, which routinely monitors influenza outbreaks that appear to be zoonotic (involving interspecies transmission). The CDC staff scientists concluded that the Florida greyhounds were infected with H3, a strain of influenza that is ordinarily found in horses.

The finding was so unexpected that Dubovi was suspicious. The Cornell lab routinely handles equine influenza cases, raising the possibility that the samples had been cross-contaminated. "So CDC did a nucleotide sequence of the hemagglutinin gene of a laboratory strain of equine influenza and the new isolate. Comparisons with these sequences and those in databases clearly showed that the Cornell isolate was different and not a lab contaminant," Dubovi says. In the meantime, Dubovi's lab analyzed serum samples on dogs from the affected kennel. "We started doing some antibody testing on those serum samples, and 13 out of the 26 animals sero-converted; that is, they had no or low antibody titers at the beginning of the process, and after the outbreak they were positive for antibodies to equine influenza. So we had evidence from the serology that what we did from the isolation testing wasn't a fluke, these dogs actually had it."

Former Cornell veterinary college professor William Castleman, a pulmonary pathologist specializing in respiratory viral diseases at the University of Florida, provided further evidence by setting up immunohistochemistry staining on lung tissues taken from affected dogs. Several of the samples indicated flu antigen in the lung tissue. "So that adds even more weight to the argument that this was not just a who-cares finding, that in fact the virus was capable of replicating and causing disease in the dogs," Dubovi points out.

"It's possible that dogs have been catching equine influenza all along," Dubovi adds. The virus causes relatively mild illness in horses, and dog breeds other than greyhounds may be similarly hardy. "The greyhound is a finely tuned athlete," Dubovi says. "It may have been the canary in the mineshaft that alerted us to this cross-over; it's so sensitive, unusual things happened to it. As we

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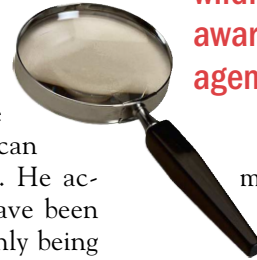
look at this, we may find that what people thought was kennel cough is actually equine influenza.”

Epidemiological studies are now underway to find out how the greyhounds could have been exposed to the virus. There is little mystery to how it spread so quickly through racing dogs this summer; owners tend to keep greyhounds in close quarters, and the dogs are constantly moving from track to track, giving the virus plenty of opportunities to travel across state lines.

The whole experience underlines Dubovi’s longstanding concern over the extent to which public health officials monitor the transmission of disease from one species to another. In addition to greyhounds with equine influenza, there are reports of equine herpes virus in llamas, and a virus that affects fruit bats has been found in pigs in

Southeast Asia. In October, about 30 tigers died at a zoo in Thailand after they were fed poultry infected with avian influenza—a virus that has also claimed the lives of more than two dozen humans in Thailand and Vietnam. “As populations get denser and domestic animals mix with each other and with wildlife, we have to be aware that disease-causing agents can jump species,” Dubovi says. He acknowledges that this may have been happening all along and is only being picked up now because of the increased number and sensitivity of contemporary testing methods. But Dubovi strongly feels that the system may be missing some important opportunities.

The current approach is one of targeted surveillance: for example, if a flock of chickens dies, the U.S. Depart-



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ment of Agriculture will pay to test it for avian influenza and exotic Newcastle disease. If those two diseases are ruled out, that may be the end of the investigation. “In New York State, the public health people take the approach that, ‘If we find something interesting in an animal, we’ll send it your way and we’ll pay you for doing it,’ but that’s a backwards situation,” Dubovi says. “You



Alexis Wenski-Roberts/Image Lab

Dubovi

want to have a system in place so if you have a sick dog or a sick chicken, you come up with the answer to the problem. Ruling out a single disease doesn't tell you anything. There's a big chance of missing something significant in the very animal you have in the system, because there's not enough funding behind it to actually [find out] why this animal has the disease."

Dubovi would like to set up a surveillance system for companion animals, possibly associated with organizations like the SPCA and based in high-population areas like New York City, "so that if there is a new and emerging disease coming out there, we

can spot it," he says. New York State's Department of Agriculture and Markets already contracts with Cornell to perform similar services for the food and fiber industry, subsidizing testing as a way of encouraging farmers to come forward with health problems among their livestock. State law gives the agriculture commissioner responsibility for the health and welfare of all animals in New York, including wildlife. But current concern over the potential for bioterrorist attacks has drawn much of the state's attention—and money—away from more mundane risks like influenza. "The funding base, particularly here in New York, just isn't there for zoonotic disease surveillance," Dubovi says, "and I'm not sure we'll ever see it until or unless we have a major outbreak of disease that makes it politically unacceptable to ignore the situation."