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BY MICHAL MEYER



CYNDA CRAWFORD WAS BORN TO LOVE ANIMALS, ESPECIALLY DOGS. The family Collie had puppies the day of her birth; baby photos are full of gamboling pups, and Crawford. She went to vet school with two dogs, and gained two more while training. The number is up to eight now, mostly greyhounds picked up during the course of her work, dogs unable to race or past their running days.

These days Crawford runs as fast as she can, chasing the birth of a virus. In 2004, the University of Florida veterinary researcher discovered an unknown virus ravaging the greyhound population of a Florida racetrack. Identified as influenza A subtype H3N8, the virus likely represents an equine influenza virus that jumped species, subsequently spreading into the nation's dog population.

Like the H5N1 avian flu virus, H3N8 equine flu virus leaped from its original host into a new species. But while H3N8 now spreads easily from dog to dog, H5N1 virus can only leap from bird to human, and, to the world's collective relief, so far is not known to have spread from human to human.

Says Crawford: "I don't know how many times the H3N8 virus had to go horse to dog, horse to dog before it changed enough to go from dog to dog. That's what we worry about in H5N1."



RACING



FLU

UF VETERINARY RESEARCHERS
DISCOVER A NEW DISEASE
IN DOGS



Kristen Bartlett



William Castleman

Paul Gibbs, a virologist with a background in emerging diseases, helped set up the initial study.

SARS, West Nile virus, avian flu — all dangerous emerging diseases in a world where jet planes shrink time and space, where growing populations intersect with wild-life and their diseases, and mass tourism reaches into cities and jungles alike. It's a world in which vets play a vital role as guardians of human health, says John Dame, chair of the Department of Infectious Diseases and Pathology at UF's College of Veterinary Medicine.

Crawford cannot think of an emerging disease without an animal component to it. "Behind every good disease is an animal," she says, only half jokingly.

And behind every sick animal stands a vet. Crawford discovered greyhounds in 1988, her senior year of veterinary school. Her first patient, a greyhound with a broken leg, would never race, and Crawford adopted it. A second greyhound soon followed. Word got out, and the third and fourth greyhounds turned up. Crawford also fosters ex-racers looking for new homes, and touts their value as pets.

"They're very calm and quiet," she says, "and curl up into little balls like cats." Crawford took her greyhound interests with her when she turned to research in 1998. Or, rather, returned to research. With a Ph.D. in immunology and infectious diseases from UF prior to her vet training, Crawford soon brushed up her research skills.

In mid 2003, greyhounds stopped running. Recurrent outbreaks of kennel cough, a catch-all phrase for a number of contagious ailments, that, as Crawford puts it, describes

"a dog with a cough that lives in a kennel," turned threatening. Quarantine, and the sheer number of sick dogs, shut down the racing industry. And dogs died.

A nationwide outbreak of kennel cough in 1999 originally caught Crawford's interest and provided a puzzle.

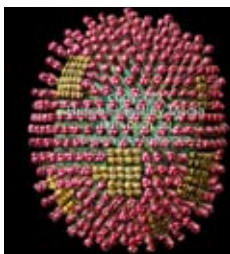
"We have a vaccine to provide protection against kennel cough," Crawford says. "It did not make sense that racing greyhounds were involved in these tremendous outbreaks of kennel cough nationwide — thousands of dogs simultaneously. It did not make sense to me that many dogs were dying from an unusual form of pneumonia."

The mid-2003 outbreak turned puzzlement into action. It began with Richard Hill's trip to the Sanford-Orlando racetrack and its sick dogs. Hill, a UF internist who specializes in greyhounds, took blood samples and looked for pathogens known to cause respiratory illnesses in dogs. He looked for *Bordetella bronchiseptica* and parainfluenza, common culprits in kennel cough, and found them nowhere. He sent samples to a government lab, which came back negative. He talked to Crawford, an infectious diseases specialist. "She grabbed the ball and started to run with it," says Hill.

At the same time, Florida's Division of Pari-Mutuel Wagering, which regulates the racing industry, took action. Worried by the number of sick dogs, the economic impact on owners, racing tracks, and state government coffers, it approached UF's College of Veterinary Medicine for help and offered money for research. Crawford, along with Hill,



Reg Carron



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— Cynda Crawford

jumped aboard. Paul Gibbs, a virologist with a background in emerging diseases, helped set up the initial study. Pathologist William Castleman offered to perform postmortem examinations for clues to the disease. By the end of 2003 the group put together a plan of attack.

Jacksonville greyhounds took sick in January 2004. Trainers might leave a healthy-looking dog at the midnight bed check, only to return to a corpse in the morning. Crawford headed out to the track. She saw the deaths first hand — out of 22 sick dogs, eight died.

“It was devastating to me,” she says, “not only as a greyhound lover, but as a vet.” But it gave the team a chance to put their plan into action. Crawford collected blood samples and nose swabs, and dead dogs for Castleman.

“We looked real hard for the common causes of kennel cough and found no evidence,” Crawford says. Instead of frothy, light-pink lungs, postmortems showed lungs looking like hunks of liver, due to massive bleeding. A blood infection was a candidate. But tests came back negative. Crawford returned to Jacksonville three weeks later for more blood samples from convalescing dogs. Already thinking in terms of an unknown virus, she believed blood from recovering dogs might show a recent viral infection.

“The outbreaks were explosive and highly contagious, that smacks of a virus,” she says, “and there is nothing more contagious than a respiratory virus. We thought it was a new agent because of the severity of the disease in the dogs

that died so quickly. We could not think of another virus that infected dogs like that.”

Crawford needed a viral lineup of suspects to identify the culprit. She sent lung samples to Edward Dubovi, director of Cornell’s Animal Health Diagnostic Center’s virology section. Dubovi found an influenza-like virus, which he sent on to Ruben Donis at the Centers for Disease Control’s Influenza Branch. Donis, a molecular virologist, sequenced the unknown virus’ genes. He compared its nucleic acids, the building blocks of genes, to the nucleic acids of other known influenza viruses. The results stunned Crawford. The unknown virus was extraordinarily similar to a horse influenza virus subtype, known as H3N8.

“Influenza never crossed my mind,” Crawford says. “It’s not been associated with clinical disease in dogs.” Doubt struck the team. Were they barking up the wrong tree?

Says Castleman: “Everybody worries every time you get something new; is this virus really associated with the disease?” A mistake, thought Crawford, or contamination of the samples. She went back to the blood taken from convalescing dogs. They showed high levels of antibodies to the horse virus. A fluke, Crawford concluded; a one-off event unrelated to the respiratory infections that regularly raced around the country’s dog tracks.

In June 2004, another outbreak roared through Florida’s racing kennels. Crawford crisscrossed Florida again and again, collecting blood samples and nose swabs, and return-



William Castleman

ing three weeks later for another round. The culprit's identity? The H3N8 influenza virus. Within two months, dogs at racing tracks in other states were falling ill.

"The story was starting to come together," says Crawford. "By August we had evidence from dogs at different tracks in different states."

In January 2005, the virus again struck the nation's racing greyhounds.

As spring melted into summer Crawford began fielding calls from vets with a heartbreakingly familiar story. It was time to hunt further afield.

"The more we looked at non-greyhound dogs, starting in Florida and then in other states — a puppy store in New Jersey, a vet clinic and boarding kennel in New York — the more we realized that this was not unique to greyhounds."

The UF team began calling it the canine influenza virus. One piece of good news: the virus proved less lethal than originally thought, killing between one and five percent of infected dogs.

By August 2005, with the virus erupting in dog shelters, boarding kennels, pet stores and even dog spas, Crawford thought it time to officially alert the State Veterinarian. An information release intended for vets rocketed through the Internet, sparking panic among dog owners and making headlines in national newspapers.

The Washington Post's headline: "Dog Flu Jumped from Horses: No Evidence that Illness can Spread to Humans, CDC Says."

No one thinks the H3N8 virus is likely to jump to humans.

"Remember the route of this virus," says Crawford. "It

"Influenza, being the great masquerader that it is, with its ability to change and infect different hosts, means you don't turn your back on it. You stay diligent."

— Cynda Crawford

has existed in horses in this country for 43 years, without any evidence of people acquiring influenza from infected horses." Crawford adds a caveat. "Influenza, being the great masquerader that it is, with its ability to change and infect different hosts, means you don't turn your back on it. You stay diligent."

Wildlife biologists and vets, research vets and even vets in practice often are the first to notice emerging diseases in animals — the pool from which new human diseases emerge.

"We are the first people to look at animal diseases with the background to know what is common and what is uncommon," says Crawford. Gibbs, the emerging diseases specialist, emphasizes the importance of wildlife as early warning signals. America, so far untouched by the 2003 emergence of avian influenza, has stepped up surveillance of birds migrating from Europe and Asia. In Florida, says Crawford, testing of wild birds has increased 400 percent.

While vets may be the Homeland Security for the animal world, the university as a whole is responding to new health threats. UF's Emerging Pathogens Initiative (EPI) is a campuswide effort to marshal expertise from medicine (animal and human), food and agricultural sciences, engineering, public health, sciences, and even dentistry to investigate diseases threatening humans, animals, and even plants.

"It is," says Dame, "a unique opportunity for the study of emerging pathogens."

The state legislature agrees, earmarking more than \$26 million dollars this year for the project. Including construction to house the researchers, the project should swing into

UF PARTNERS WITH CYNTEGRA TO DEVELOP DOG FLU DIAGNOSTIC TEST

Just months after Cynda Crawford confirmed the existence of canine influenza, the University of Florida licensed a diagnostic test she developed for the disease to CYNTEGRA, a San Diego, Cal.-based company.

In April, CYNTEGRA announced the launch of VIOGENIX DUO, a rapid test for cats and dogs that detects both H5N1 avian influenza and H3N8 canine influenza. CYNTEGRA's diagnostic technology is similar to that used to detect influenza and SARS in humans.

"Viruses survive by adapting to both existing and new hosts," said CYNTEGRA CEO Simon Brodie. "We should not be surprised by the ability of these viruses to infect cats and dogs. There is even concern that our pets might contract avian flu and transmit it to humans. CYNTEGRA's decision to launch a combined H5N1/H3N8 test immediately was twofold: first, VIOGENIX DUO provides an accurate bird flu test for both dogs and cats. Second, VIOGENIX DUO permits further differentiation between the avian and dog flu in dogs, given the similarity of symptoms."

Brodie noted that in trials, VIOGENIX DUO detected dog flu within 12 hours of infection, days before the animals started to show any clinical signs of illness.

"With results provided in just a few hours, and a processing capacity of thousands per day, VIOGENIX DUO ensures that infected animals can be quickly isolated and further contain the infection," Brodie said.

CYNTEGRA specializes in low-cost, high-throughput molecular diagnostics for multiple animal pathogens, with an online data reporting system for tracking disease occurrences. CYNTEGRA allows state and private veterinarians to screen for multiple pathogens at an affordable cost to the pet owner.

gear around 2009. For Dame, Crawford's work showcases the value of vets in surveillance and in making the connections when diseases jump species. Adds Gibbs: "We've been working on emerging diseases and foreign animal diseases since this college was founded in 1976. We could be one of the bedrock programs of the EPI, from basic science right through to the control and prevention of disease."

To date, canine influenza has infected pet dogs in 21 states and the District of Columbia. While a private company works on a vaccine under license, Crawford patiently works back in time to find that moment when H3N8 first leaped into greyhounds. Stored blood samples show it as early as 2000, and she is testing samples going back to the mid 1990s. Crawford still conducts syndromic surveillance — looking for dog populations showing signs of influenza infection.

"It doesn't tell us the true prevalence of the virus," she says, "but is useful in tracking the movement of the virus, in identifying hot spots of activity, and to define high-risk groups."

High-risk groups include shelter dogs. With few resources, shelter organizations face an agonizing choice in the face of an outbreak — quarantine and spend their limited cash on sick dogs or euthanize. Crawford, passionately

committed to keeping these dogs alive, is figuring out risk factors and ways of managing outbreaks.

"I've fulfilled one dream of a researcher," says Crawford, "helping unravel the story of a newly emerging respiratory pathogen in dogs, so we can move on to prevention and control. As a dog owner myself that is the payoff." ✕

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