

# Animal Whodunit, Medical Mystery

## Scientists cross species barriers to diagnose West Nile encephalitis

By SUSAN MILIUS

**P**uzzles of pathology were abundant in New York last summer. The New York City Department of Health was in overdrive by late August, tracing people stricken with an odd viral encephalitis. A private veterinarian in Laurel was worrying about an equally odd cluster of sick horses on Long Island. In Albany, the sole wildlife pathologist employed by New York State was striving to find out what was suddenly killing hundreds of crows.

Then at the Bronx Zoo, the pathologists came back from Labor Day weekend to find a range of dying and strangely ill birds, such as a cormorant that spent its last hours swimming in circles and a bald eagle with tremors.

By the end of September, after much sampling and testing, researchers had finally figured out that their mysteries shared a common villain: the West Nile virus, a mosquito-borne pathogen found for the first time in the Western Hemisphere. It killed 7 of the 60 people eventually diagnosed with the virus, 9 of 23 horses, a variety of zoo birds including flamingos and a snowy owl, and several thousand wild crows.

As the outbreak waned, or possibly went into a winter lull, the Centers for Disease Control and Prevention (CDC) drew an unusual mix of investigators—specialists in human diseases, veterinary pathologists, and even an ornithologist—to a November workshop in Fort Collins, Colo., to sort out the lessons of the outbreak. New York City's assistant commissioner for communicable diseases, Marcelle Layton, argued that knowing the particulars of the animal cases sooner might have eased the way for physicians trying to identify the cause of the human outbreak.

The doings of nonhuman New Yorkers are now shedding light on questions of how the virus spreads and whether it will settle down to stay.

For the future, Layton urges public health officers to look out for "unusual events in animals." Layton had never met the Bronx Zoo pathologist before the West Nile outbreak, but now, she assured

the workshop assembly, they make it a point to do lunch.

**T**he horse had collapsed in the field and was thrashing, unable to get up, by the time John Andresen reached it on Aug. 26. "It was in bad shape," he remembers. Before he even managed to contact its owner, the horse died.

Looking back, Andresen recognizes this as an encounter with West Nile virus. At the time, though, he didn't suspect anything outside the usual realm of neurological miseries he'd diagnosed over

possums. As they potter around the countryside, they shed the parasite in their droppings, and horses pick up the disease as they graze outdoors or eat hay from a field visited by a possum. The parasite doesn't seem to bother many of the horses that carry it, but in stressed or older animals, it can work its way into the brain. These animals start staggering, dragging their hind legs, showing attitude changes, or displaying a host of other neurological symptoms.

Andresen took spinal fluid from the tripping horse, and the lab detected the EPM parasite. That result could mean that the parasite was indeed causing the disease or that a horse that tolerated EPM had coincidentally developed another neurological disease.

In all, Andresen did spinal taps on five horses, and all tests came back positive for EPM. "We were led a little astray," he says.

Even if EPM was knocking out Long Island's horses, why the sudden increase in cases? Typically Andresen sees four or five a year, not three in a week, clustered within about 5 miles of each other.

In early September, he called the state veterinarian to report the troubling mystery. "I can't remember the last time I did that," he says.

Like the doctors studying the human outbreak in the parallel universe in New York City, Andresen worked long hours, sometimes visiting patients at midnight. He saw horses with the illness in racing barns, in backyards. He saw 4-year-olds go down as well as elderly animals. Some died as quickly as his first patient, some lingered, and some, amazingly, bounced back.

He attended an Arabian mare that a woman had cherished for 19 years. One of his own horses, a Percheron, collapsed. Except for two horses at Belmont race track, close to the city, the disease stayed within Andresen's rural area, 60 to 80 miles east of the city on the North Fork of Long Island.

On Oct. 8, the U.S. Department of Agriculture dispatched one of its early-response teams, including a veterinary



Chilean flamingos at the Bronx Zoo proved vulnerable to West Nile virus.

33 years of practice. The equine specialist in a group practice, Andresen cares for a couple thousand horses in the rural part of Long Island.

During the week after the thrashing horse died, he received calls from two alarmed owners whose horses were acting strangely. One horse walked with its head tilted, and it leaned against a fence as if trying to keep its balance. The other had begun to stagger and trip over its own hind legs. Then it started tripping over its front legs, too.

Andresen worked through a list of possible causes for the illness. Was an ear infection disorienting the horses? Rabies?

Or was it EPM, short for equine protozoal myelitis? This disease comes from infection by the parasite *Sarcocystis neuromona*, which spends part of its life cycle in

pathologist and an epidemiologist, to scrutinize each place where a horse had gotten sick, and they collected samples for analysis.

**A**lso starting in August, pathologist Ward Stone faced another animal-disease mystery. People around New York City and Long Island reported unusual numbers of dead crows. Looking back at his records, Stone notes that a dead crow collected sometime between Aug. 2 and Aug. 9 was sent to him from Nassau County. At the time, Stone couldn't tell much. "It was warm weather," he says, and the carcasses had started to decompose fast.

As more crows arrived, he spent hours trying to figure out the cause of death. He logged more than 100 necropsies, some taking 15 minutes and others taking hours. Toxicology tests ruled out poisoning by lead or a range of pesticides. "We did a lot of toxicology," Stone remembers glumly.

"I like crows—I've raised them," he says. "I know they didn't normally die in these numbers." Yet he couldn't figure out a disease diagnosis that made sense.

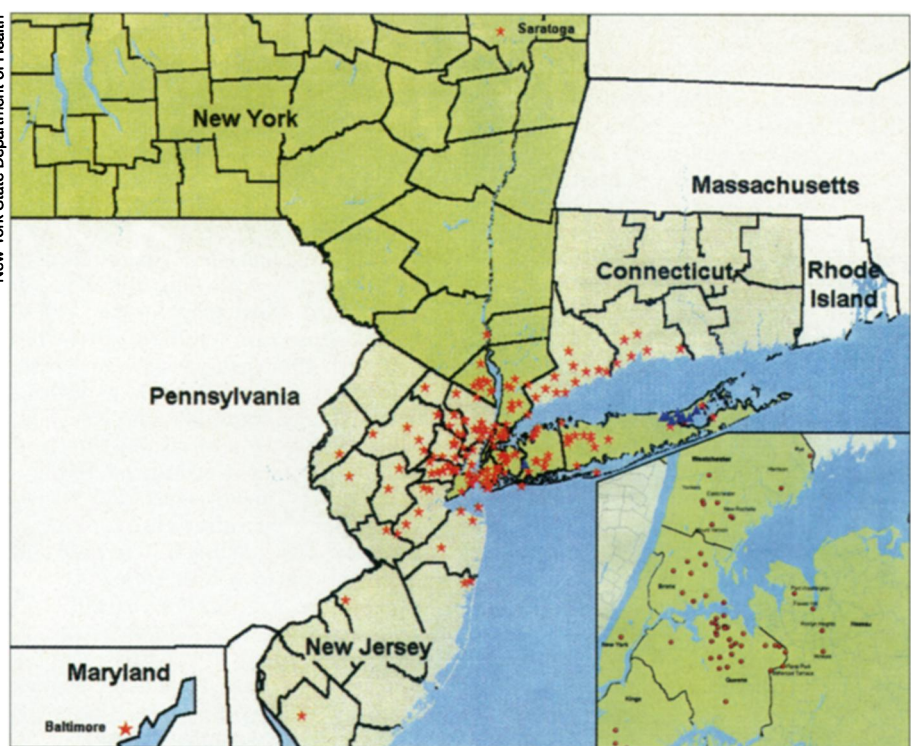
"I thought it could be something new," he recalls. The boom in fast travel and trade, jettisoning people and their goods from continent to continent in just hours, spreads wildlife pestilences just as it does human diseases. Stone notes, for example, the arrival of duck plague, a European disease that has killed 10,000 ducks at a time in the United States. Drawing on his 3 decades as a wildlife pathologist, he predicts that invading diseases will appear with increasing frequency. "That's what's been happening," he adds grimly.

As Stone worried about the dying crows, he sent samples of their tissue for analysis to specialized labs, including the National Veterinary Services Laboratory in Ames, Iowa.

**A**t the Wildlife Conservation Society's Bronx Zoo, Tracey McNamara also got her first hint of trouble from crows. Calls came in from New Yorkers puzzled by unusual numbers of dead birds, and fallen crows began turning up on the zoo grounds. She sent some of them to Stone, but starting Sept. 7, the crisis intensified.

Birds in several of the zoo exhibits began to develop neurological oddities and, not infrequently, die soon afterward. "We had a trumpeter swan doing the backstroke," she remembers. The swan recovered, but a Chilean flamingo, guanay cormorant, bald eagle, and snowy owl were not so lucky.

At first, McNamara wondered whether one of two dreaded bird diseases—Newcastle disease and highly pathogenic avian influenza—had slipped through the zoo's tight quarantine and hygiene procedures. Chicken and other poultry farmers



People with West Nile encephalitis (locations marked with circles in inset) were clustered around New York City, as were birds (stars) but not horses (blue triangles).

fear huge losses from these infections, so potential outbreaks shoot tremors through the veterinary community.

"I'm probably the only pathologist in New York City who's seen both," she says, and her intuition told her she was dealing with something else among the zoo birds. "Plus," she adds, "our chickens were fine."

Examining the deceased birds, McNamara found signs of hemorrhage in their brains. Could they have died from eastern equine encephalitis, which despite its name can attack birds, especially emus, and produce these symptoms? The pattern of species affected didn't make sense in that case. "Our emus were fine," she said. "Luckily, we had emus."

As she talked to other pathologists about the zoo deaths, she encountered skepticism for her idea that the disease was novel. "If I had a nickel for every person who told me I was nuts, I'd retire," she says.

In early September, McNamara rushed tissue samples of the birds to the National Veterinary Services Laboratory and the CDC laboratory in Fort Collins, Colo.

**B**y this time, New York was feverish with the news of the viral encephalitis outbreak in people. On Sept. 3, CDC had reported a probable outbreak of St. Louis encephalitis, which is spread by mosquitoes. The agency's preliminary tests had screened the encephalitis patients for only viruses known to strike in North America. No one had reported the St. Louis encephalitis so far north before, but New York City didn't

wait for confirmatory tests. The mayor ordered spraying to kill mosquitoes in Queens, which had come to be called the hot zone of the outbreak.

Medical entomologists in helicopters roared over neighborhoods looking for possible mosquito breeding havens, like half-filled swimming pools or mounds of abandoned tires. The city bought and distributed more than 300,000 containers of DEET bug repellent.

As the city widened its mosquito-control spraying, debate flared about the safety of the insecticides being used in the operation. Police escorted the truck carrying the pesticide into the city.

Could the CDC results apply to the animals' illnesses? With a wry chuckle at the power of hindsight, Andresen recalls that two of the horse owners insisted that their animals had the same disease as the people in New York. However, he discounted that idea because there had been no reports of such problems in city horses.

Amid all the furor, McNamara immediately wondered if the disease that was striking people could be killing her zoo's birds. However, previous U.S. outbreaks of St. Louis encephalitis hadn't affected birds. As she puts it: "Why were we losing North American and South American species if it's something that's always been here?"

Results from the veterinary lab in Ames started confirming McNamara's hunches. The lab ruled out the dread poultry diseases as well as eastern equine encephalitis. Working with tissue from a dead zoo bird, they isolated the virus and took vital measurements under a transmission electron microscope. This turned out to be

the first isolation of the new virus, which the Ames lab sent to the CDC facility in Fort Collins for identification.

Meanwhile, CDC researchers there were also working on samples from people in New York, performing more sensitive tests to distinguish closely related viruses. These analyses showed a slight indication of St. Louis encephalitis but a vastly stronger reaction for West Nile virus.

Named for the province of Uganda where it was first described in the 1930s, the West Nile virus spreads via mosquitoes and causes encephalitis in people and horses in Africa, Australia, the Middle East, and parts of Europe.

Tests in government and academic labs soon confirmed that in New York, people, horses, crows, and zoo birds had succumbed to the West Nile virus.

**J**ust as epidemiologists predicted, the outbreak died down after mosquito spraying. Then, cold weather presumably curbed the adult mosquitoes that had been carrying the disease.

Andresen's own horse has recovered from encephalitis, as has the beloved 19-year-old Arabian. Crows seem again to be doing more flying than dying. During this merciful lull, as the public health community plots its strategy for next year, many of the big questions involve animals.

Adults of *Culex pipiens*, the mosquito suspected of most frequently spreading

the disease, can survive the winter, perhaps lurking in New York's sewers, says Nicholas Komar of CDC in Fort Collins. Once infected, a mosquito bears the virus for life. Yet the presence of virus-carrying mosquitoes doesn't guarantee another outbreak.

One important factor will be how the species that the mosquitoes bite react. West Nile virus has been isolated from at least 23 species, according to Komar. Although not all showed symptoms, a wide range of birds contracted the virus—the flamingos, the owl, domestic geese, chickens, a Cooper's hawk, a yellow-billed cuckoo, a sandhill crane, a pigeon, a house sparrow, and so on. The bird species stretched over at least 10 taxonomic orders. The virus has even turned up in tissues from a stray cat.

Labs are now starting to test bats and other mammals from New York.

An animal that hosts the virus doesn't necessarily spread it. The virus has to reach high levels in the bloodstream before a feeding mosquito is likely to pick up enough virus to pass the disease to the next animal it samples.

The reservoir issue arouses particular passion among horse owners, explains USDA veterinary epidemiologist Oliver Williams in Riverdale, Md. This fall, the European Union, Hong Kong, and several other racing destinations closed their borders to horses that had passed through New York, New Jersey, or Con-

necticut within the previous 2 weeks. This raised tensions over whether horses would be able to reach races with multimillion-dollar purses.

"There's no evidence to date that the horse can serve as a sufficient source of virus to infect mosquitoes," says Peter J. Timoney, director of the Maxwell H. Gluck Equine Research Center in Lexington, Ky. He says that a study in France and another in Egypt more than 30 years ago failed to find significant concentrations of West Nile virus in the blood of horses exposed to the microbe.

It's clear that the virus can reproduce and increase its numbers in birds, Komar says. Using data collected in studies in other parts of the world, he estimates that a bird can pass along West Nile infection to a mosquito for perhaps 1 to 4 days. If Komar had to guess which species play the most important role in New York's risk of outbreak, he says he'd pick starlings, house sparrows, and pigeons. Surveys suggest that fewer than 15 percent of them have been exposed to the virus, but their huge numbers give them significance.

Though a popular emblem of the West Nile outbreak, crows make an unlikely reservoir. For a rough estimate, Komar and his colleagues examined blood samples from 65 crows still flying around New York City. Only two showed evidence of exposure to the virus. Considering the number of dead crows previously reported and the probable population, thanks to several Christmas bird counts, Komar estimates that when West Nile virus swept through the region, it killed about 98 percent of birds it infected. Because reservoir animals must survive to incubate a disease, crows are more likely to serve as sentinels for the disease than as reservoirs, he concludes.

Just what creature brought the virus to the United States makes another great conservation starter. The latest clue, a slim one at best, comes from Robert Lanciotti at CDC's Fort Collins laboratory.

He's sequenced the virus' genes and compared them with those of the other strains in the three main geographical branches of West Nile virus. The virus from the New York outbreak shares more than 99 percent of the sequenced region with a strain collected in Israel from a goose in 1998, but it doesn't as closely resemble African or Australian strains.

All the talk of animals as reservoirs, disease carriers, or warning signs left Stone sounding a little testy by the end of the CDC workshop. He fretted about whether the disease will ravage New York's small band of ravens, relatives of the susceptible crows, or will threaten wild bald eagles.

Although studies in nonhuman species can be useful to understanding diseases in people, Stone wants to see concern for animals in their own right. "They're beautiful. They play an ecological role," he says. "I'm worried about the wildlife." □

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