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Dogs left homeless after Hurricane Katrina have been found to carry treatment-resistant parasites.

DOGS

The riddle of resistance

In the southern United States, heartworm parasites are acquiring resistance to preventives that once offered complete protection, raising concerns for dog owners.

BY MICHAEL EISENSTEIN

Perfection is rare in medicine. But with heartworm, veterinarians have grown accustomed to ironclad protection for their canine clients. There are multiple preventives on the market, all of which use active ingredients based on macrocyclic lactones — antiparasitic drugs derived from a compound that earned its discoverers a share of the 2015 Nobel Prize in Physiology or Medicine. These drugs are so effective against heartworm that the US Food and Drug Administration (FDA) will only consider approval for agents that match their track record of 100% protection. "It's a hard standard to beat," says Tim Geary, director of McGill University's Institute of Parasitology in Ste Anne de Bellevue, Canada.

But this protection may be slipping. Over the past decade, macrocyclic-lactone-resistant parasites have emerged in a region of the Mississippi Delta spanning parts of four states — Arkansas, Tennessee, Mississippi and Louisiana — that has long been a hotbed for heartworm. Heartworm can be fatal to dogs, so this resistance has raised deep concerns among veterinarians and dog owners. The nature and magnitude of the problem are poorly understood and are the subject of heated debate. The situation is further compounded by a limited toolbox for studying resistance and a poor understanding of how the preventive treatment works. The consensus is that resistance is real, but questions remain as to whether it represents an immediate threat that requires urgent attention.

The heartworm parasite (*Dirofilaria immitis*) is transmitted to canines as third-stage larva through mosquito bites. Within a week or so, these L3 larvae enter the L4 stage, after which they transition to an adult state and set up shop in the host's pulmonary artery, which carries blood from the heart to the lungs. Once ensconced there, the adult worms release early-stage larvae known as microfilariae into circulation. These microfilariae are subsequently taken up by mosquitoes during a blood meal to continue the cycle of transmission. Untreated, the adult worms can block blood flow and cause fatal heart failure. Macrocyclic lactones, if given every 30 days, will eradicate L3 or L4 larvae before they become adult worms, which are less vulnerable to the treatment. If dog owners miss a dose, the protection lapses.

RUMOUR VERSUS REALITY

Sporadic reports of lack of efficacy for macrocyclic lactones emerged in the late 1990s. An FDA evaluation[1] of this issue was published in 2005; it focused on case reports submitted to the agency by heartworm-preventive manufacturers in response to complaints by customers. "The preventatives are labelled as 100% effective," says Adrian Wolstenholme, a parasitologist at the University of Georgia in Athens. "And if a compliant owner has an animal that gets an infection, the drug company may pay for treatment of the infection and will report the treatment failure to the FDA." The 2005 report also offered guidelines to help distinguish cases caused by treatment failure from those arising because of poor compliance. But there was still a lack of robust proof of resistance.

Hard evidence arrived years later, after researchers had studied parasites isolated from lack-of-efficiency cases. In 2011, it was revealed that single doses of some macrocyclic lactones delivered little protection against a strain called MP3 (ref. 2). But the strain, which was isolated from a dog in Georgia, would subsequently prove vulnerable to more persistent treatment. This was not the case for a dog rescued from Louisiana in the aftermath of 2005's Hurricane Katrina[3]. The dog's heartworm exhibited robust resistance, with infection persisting after multiple rounds of aggressive treatment.

These findings have since been replicated with several other strains. In dogs experimentally infected with two different heartworm isolates taken from suspected lack-of-efficacy cases from the Delta region, the parasites survived repeated treatment with a macrocyclic lactone. The dogs received double the recommended dose, monthly for six months, under the watchful eye of veterinarians[4]. "There's no question that these are resistant parasites," says Wolstenholme, who has had similar findings in his own research into resistant strains.

The Delta is an unsurprising place for resistance to emerge — conditions there are ideal for the spread of heartworm. "It's among the wettest and warmest areas of the US, with the longest mosquito season," says Clarke Atkins, a veterinary cardiologist at North Carolina State

University in Raleigh. But lack-of-efficacy reports are also popping up elsewhere, says Dwight Bowman, a veterinary parasitologist at Cornell University in Ithaca, New York. He cites the example of JYD-34, a strain found in Illinois by a drug company looking to re-test the efficacy of its heartworm product — as is routinely required by the FDA. "My belief is they got an isolate from Illinois because they thought it would not be resistant — companies want to kill the parasites after all," says Bowman. "They ran into JYD-34, and everything that has been tested against it has failed."

SEEKING SURVIVORS

Geary and his colleague at McGill, Roger Prichard, monitor reports of resistant strains, but getting a firm handle on the numbers is tough. "There have certainly been reports of as many as 20 isolates," says Geary, "but you can't take that number too seriously because of the difficulty of proving resistance."

In many cases, the problem lies not with the drug, but with the dog owners who are responsible for administering it. Several years ago, Atkins partnered with animal-health company Merial in Duluth, Georgia, to investigate the situation. His team combed through hundreds of records pertaining to putative lack-of-efficacy cases from 19 veterinary clinics throughout the Delta region, using software that allowed them to determine whether pet owners had purchased enough preventives to protect their dogs[5]. "It's a way to look at the validity of the claims that the drug failed," says Atkins. Strikingly, around 80% of the cases could be linked to inadequate compliance, whereas only 1.7% — just five cases — represented possible instances of true lack of efficacy, in which there was no other explanation for treatment failure. "We know that compliance for preventives is bad, and it's probably worse in the Mississippi Delta because it's more socioeconomically deprived," says Atkins. He acknowledges that resistance exists, but adds: "I do not believe that is the explanation for what is going on in the Delta."

Confirming resistance is labour intensive, requiring cultivation of isolates in mosquitoes, infection of treated and control cohorts of dogs, and months of waiting to monitor infection. "It's a hideously long and expensive process, and no use at all to veterinarians," says Wolstenholme. Unfortunately, there are no good options for *in vitro* laboratory testing. Some groups have employed an assay that tests how macrocyclic lactones affect the movement of microfilariae in a culture dish, but many are sceptical about the results. "The drug concentrations required to affect the motility of microfilariae are way higher than you would ever see in a blood sample, so they're not pharmacologically relevant," says Geary. His team is exploring an alternative strategy in which it monitors the extent to which treatment clears microfilariae from the bloodstream of infected dogs. Although the microfilarial stage is not the main target of preventive treatment, these immature worms are highly vulnerable and their response could offer a useful surrogate marker.

Geary and Prichard are also exploring the molecular roots of resistance, and have assembled genetic data from various resistant isolates[6]. Based on these data, Geary thinks that his team has identified possible signatures of adaptation to macrocyclic lactones. "We've found very strong evidence for a selection event that has led to a genomically distinct population of heartworms," he says, "but we haven't identified the resistance gene." Efforts to home in on candidate genes have been further confounded by a poor understanding of how macrocyclic lactones kill the parasite at the concentrations used in the medicines. One possibility is that the drugs prevent heartworms from secreting peptides that mask the parasite from the host's immune system, a defence mechanism that Wolstenholme likens to the technology that turns spacecraft invisible in *Star Trek*. "It could be that the drug is actually switching off this cloaking device so the parasite reappears," he says.

DEFINING THE DANGER

The biggest question is how great a threat does resistance pose. From Atkins' perspective, the risk of it becoming a nationwide problem is low. Most of the areas that report lack of efficacy, he says, are routinely hammered with tropical storms, which create conditions for mosquitoes to thrive and spread parasites to the Delta's numerous feral dogs and wild canids, as well as the many pet dogs that live largely outdoors. The resulting explosion in heartworm incidence, which Atkins says peaked around 2009, made it inevitable that even a small number of resistant strains would emerge among the large number of cases being reported. Atkins points to data from multiple sites in the Delta to support his hypothesis. "I can show a statistical relationship where there's roughly a three-year lag between storm activity and bursts of heartworm infection," he says. "This underlying heartworm epidemic caused us to find resistance."

The good news is that resistant strains that do arise will probably have difficulty propagating in the region. With many untreated dogs — as well as foxes, wolves and coyotes — running free, there is little selective pressure to promote the expansion of a drug-resistant parasite population. Indeed, the number of lack-of-efficacy reports has not shown the steady climb that would be seen with a resistant bacterium or virus. "Things peaked in 2009, and then started to fall," says Atkins. Since, he says, the number of claims of lack of efficacy has risen and fallen with no obvious pattern to indicate the emergence of widespread resistance.

Nevertheless, some researchers think it's a mistake to think that resistance is confined to the Delta — especially because many dogs from the region are adopted elsewhere in the United States. "People in other parts of the country don't want to recognize that they may have resistance," says Bowman. "I honestly don't know if this is the calm before the storm." He also contends that poor preventive practices, such as the sloppy application of macrocyclic lactones to animals that already have a full-blown infection — which is normally treated with a combination of antibiotics and a worm-killing drug called melarsomine — could fuel the emergence of further resistance. "Some of these dogs continue to have microfilariae swimming around being bathed in the drug, and those microfilariae can live for years," he explains.

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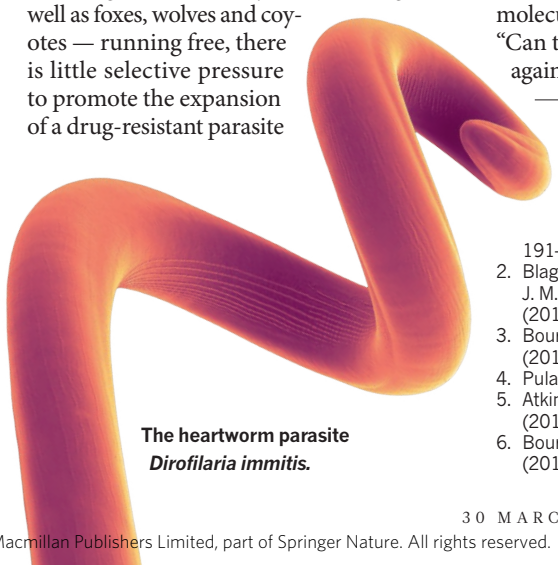
However great or small the threat is now, being more diligent with the application of preventive treatments should keep the situation from getting worse. "These drugs are amazing, but we as a society haven't used them adequately," says Atkins. "If we lose them, we will have lost a big piece of our armamentarium for prevention — and that's a big deal." Established infections of resistant strains can still be killed with an aggressive therapeutic regimen that uses a non-macrocyclic-lactone-based treatment. However, this is expensive and labour intensive — it can cost up to US$1,000 and animals require close monitoring. Although drug companies are investigating alternatives for prevention, the success of macrocyclic lactones makes them a hard act to follow. "New molecules are hard to come by," says Bowman. "Can they find something that monumental again? I just don't know." ■



DENNIS KUNKEL MICROSCOPY/SPL

The heartworm parasite *Dirofilaria immitis*.

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