Heartworm Resistance: Should Californians Worry?
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In the last 24 months, there have been significant revelations relative to the presence of resistant isolates of heartworm in the field that are capable of developing in dogs on the preventive products. The research shows that this issue now a reality rather than a supposition and will display the underlying research that has brought the situation to light and discuss how we might be able to minimize its further development and spread.

In the area extending along the Mississippi River from Tennessee through Louisiana, there were many practitioners who contended in the early 2000s that heartworm preventives were no longer protecting dogs from infection (Hampshire, 2005). These concerns were vocally raised by veterinarians practicing throughout this area of the United States, and were vociferously raised by practitioners in 2010 at the meeting of the American Heartworm Society in Memphis, TN. Microfilariae from dogs that had purportedly been infected while on prevention when examined for their susceptibility to macrocyclic lactones ultimately were shown to survive in the presence of increased concentrations of these drugs (Blagburn et al., 2011). In addition, when some of the isolates were grown to third-stage larvae in mosquitoes that were used to infect other dogs, it was shown in the same assay that they were as unsusceptible to the macrocyclic lactones as their parents, indicating that these phenotypes were inheritable.

Also, in 2011, there was a report that persistent microfilariae in a Katrina rescue dog taken to Canada that had been treated repeatedly for its adult heartworms had persistent microfilariae long-after the adults were cleared by treatment and after receiving repeated high doses of ivermectin or milbemycin oxime (Bourguinat et al, 2011).

As part of the development of the new product, Trifexis®, a new product containing milbemycin oxime at the same dosage as had been used in Interceptor and Sentinel, using an isolate of heartworms identified as MP3 - named after the naturally infected dog from Georgia, USA from which it was isolated, “Miss Piggy,” it was found that 3 of the 10 infected dogs treated with Trifexis® had 3 worms at necropsy, 1 had 1 worm and 2 had 2 worms (Snyder et al., 2011a&b). Additional work showed that MP3 could also develop in dogs treated with Heartgard Plus Chewables for Dogs® and Interceptor Flavor Tabs for Dogs & Cats® (As marketed: dose band of 0.5-1.0 mg milbemycin oxime/kg). Additional work with Trifexis® showed that of 10 dogs treated twice with Trifexis® 30 and 60 days after infection, there was a single worm present at necropsy at 150 days postinfection in 1 dog, but no worms present in dogs treated three times. Thus, the Trifexis® label has the statement "For heartworm prevention, give once monthly for at least 3 months after exposure to mosquitoes." Continuing treatment for three months following the end of mosquito season should protect dogs from infection by killing any worms acquired during the last month of transmission.

Bayer Animal Health, as part of product development, had been working on a new macrocyclic lactone-containing preventive (two formulations of ivermectin-containing products with target minimum doses of 6 mcg/kg and 9 mcg/kg; personal communication as to dose bands used) (Blagburn et al., 2011). In this work using the same dose of ivermectin as an already marketed product or one that was at a dose 1.5 times higher, the MP3 laboratory strain was less susceptible to traditionally effective doses of an ivermectin-based preventive in a limited number of dogs. Bayer then decided to examine the efficacy of four of the five commercial monthly products against this problematic strain. In this study, MP3 developed to adulthood in dogs treated with single doses of Heartgard Plus®, Interceptor®, and Revolution™, but not in the Advantage Multi®-treated dogs. Thus, with the MP3 isolate overall, there have been eight reported instances in which dogs became infected in spite of being given a single heartworm preventive treatment 30 days after infection: 1 study with Trifexis®, 2 studies with Heartgard Plus®, 2 studies with Interceptor®, 1 study with Revolution™, and 2 studies with ivermectin in a product undergoing development. There was also one study where one dog had one adult heartworm at necropsy after having received two Trifexis® treatments 30 and 60 days after infection. In a new product, approved but not yet marketed in the US, Sentinel® Spectrum® Tasty Chews (a combination product of 0.5-1.0 mg milbemycin oxime/kg, lufenuron, and praziquantel), one or two monthly treatments did not protect all dogs against MP3, but 6 consecutive monthly treatments were 100% effective (NADA 141-333 Sentinel® Spectrum®).

At the 58th Annual Meeting of the American Association of Veterinary Parasitologists Chicago July, 2013, a number of studies representing the culmination of a decade of work by Novartis on the issue of resistance were presented. The work dealt with a number of experiments performed using the isolates that Dr. Blagburn at Auburn University, Auburn, AL, had been collecting from dogs that had undergone treatment failures in the Mississippi River valley over the last 10 or so years and which he had passed from dog to dog to isolate different characterized strains (Blagburn et al., 2013a). The microfilariae, third-stage larvae, and adults of these worms were used in various trials to identify potential molecular markers of resistance, to examine the trends of these markers in microfilariae from dogs given repeated treatments with ivermectin, and in studies as to the efficacy of different preventives to block the development of adult worms.
In one such study (Blagburn et al., 2013b), it was shown that using certain markers that as dogs were repeatedly treated with escalating doses of weekly ivermectin that the markers did indeed increase within the populations of microfilariae remaining in circulation despite the weekly treatments. These same markers were shown to be dramatically correlated with worms collected from dogs in which adult worms had developed in spite of the worms being on preventive therapy (Prichard et al., 2013).

Using one of the strains maintained by Dr. Blagburn, it was shown that Proheart® 6 was not protective to 4 dogs that had received their preventive 180 days prior to infection; in this same regimen, this same treatment had been 100% effective in the original pre-approval work with this product using locally obtained isolates (Bowman et al., 2013).

Also, 15 of 16 dogs treated with monthly ivermectin/pyrantel had worms at necropsy when infected with one of Dr. Blagburn’s strains, and it was also shown that worms of one of Dr. Blagburn’s strains developed in dogs treated with existing monthly heartworm prophylactics containing selamectin, milbemycin oxime, or moxidectin (Kaminsky et al., 2013).

Corroborating the field isolation work of Dr. Blagburn, two isolates from dogs in Louisiana with a history of efficacy failure with full monetary compensation by a commercial pharmaceutical firm, location in an area “hotspot,” microfilarial persistence 7 days after a typical microfilaricidal dose of macrocyclic lactone, and the presence of a high frequency of the molecular markers discussed above, developed to patent infections when each was transferred as third-stage larvae to a dog given monthly prophylactic treatments with ivermectin at 12 μg/kg from a month after infection until months after patency (Pulaski et al., 2013).

Thus, after these reports, it is fairly obvious that now there are dogs in the field with infections that are persisting in spite of the dog having been on monthly preventive that when transferred by mosquitoes to other dogs are capable of developing to adulthood even if these dogs are on monthly prophylaxis.

At the Triennial Symposium of the American Heartworm Society—Heartworms Today, the Search for Solutions, New Orleans, September, 2013 meeting there were two late breaking reports presented by Drs. Blagburn and McCall. Both of these groups used an isolate form Keytesville, MO.

In the first study by Blagburn, there were 5 groups of 8 dogs each: untreated controls, dogs treated three times with Ivermectin and pyrantel, dogs treated three times with milbemycin oxime and spinosad, dogs treated three times with selamectin, and dogs treated once with topically applied moxidectin with imidacloprid. All the dogs were given infective larvae a month before the first of their treatments. In this study, all dogs had adult worms at necropsy 5 months after infection, except the dogs that had received the topical moxidectin and imidacloprid treatment.

In the second study reported by McCall, there were again 5 groups of 8 dogs each: untreated controls, dogs treated three times with milbemycin oxime and spinosad, dogs treated three times with a 2X dose of milbemycin oxime, dogs treated one time with 2X milbemycin oxime, and dogs treated one time with topical moxidectin with imidacloprid. Again there were worms in all dogs except for the group receiving the moxidectin imidacloprid combination, except this time, one of the dogs in this group also had heartworms at necropsy.

The New Animal Drug Application supplement of Advantage Multi® for Dogs (NADA- 141-251) as a treatment for microfilariae in dogs infected with heartworms (Approved October 2013). As part of this research that provided the data that produced approval by the FDA, there were six clinical field sites: Zachary, LA; Seguin, TX; Oklahoma City, OK; Pell City, AL; Springfield, MO; and Collierville, TN. The results were excellent, however, as the report states: “The number of circulating microfilariae was reduced in the study population overall; however, not all dogs treated with 10% imidacloprid + 2.5% moxidectin topical solution had microfilariae counts reduced to zero. The microfilarial count in some heartworm-positive dogs increased or remained unchanged following treatment with 10% imidacloprid + 2.5% moxidectin topical solution alone or with melarsomine dihydrochloride. Effectiveness against circulating *D. immitis* microfilariae was > 90% at five of six sites; however, one site had effectiveness of 73.3%.” This suggests that there may be places where resistant microfilariae are circulating in higher prevalences in dogs than in other areas.

Very sadly, these reports combine to show that we have moved from the specter of potential resistance in the United States to one where resistance is present in the field. The current range of the resistant forms is not known, but isolates have been made now in Arkansas, Georgia, Louisiana, and Missouri. There are also rumors of additional clinical trials where isolates from other states in the southeastern United States have been involved with failures in products being tested as part of the FDA approval process.

The work at this time needs to focus on control to minimize the spread of these isolates, preventing the utilization of practices that promote further damage such as long-term preventive therapy to dogs with existing infections, verification that dogs on preventive have not become infected through increased diagnostic testing, and insuring that the veterinary community remains fully engaged in the ongoing developments and the control of this dreadful infection in dogs.

Californians do need to worry. Shelters in California, as in much of the nation, in efforts to save dogs from being euthanized, will transport them from other shelters in the nation. These source shelters are often in areas where these isolates have appeared—areas with too many dogs receiving inadequate care. Often these dogs are verified to be heartworm free before shipping, but we now know that methods for microfilarial suppression may cause antigen detection methods to be less than perfect (Little et al., 2014).
Therefore dogs that appear microfilarial and antigen negative coming from these areas may still have heartworms – and potentially resistant heartworms.

References