Soon after introduction of the antiparasitic drug ivermectin in 1981, individual idiosyncratic toxicoses were recognized in a proportion of Collies after extra- label use of a product formulated for cattle. Subsequently, it was found that dogs within some herding breeds, including Old English Sheepdogs, Shetland Sheepdogs, Australian Shepherds, and sighthound-type breeds (including Whippets), share this sensitivity to ivermectin. This phenomenon has subsequently been found to apply to the general antiparasitic classes of AVMs (including selamectin and ivermectin) and the closely related MBs (including MBO and moxidectin), which have a similar structure and chemical actions. Therefore, it would be appropriate to describe the condition as AVM-MB sensitivity and AVM-MB toxicosis.

Attributions
AVM Avermectin
GABA γ-Aminobutyric acid
MB Milbemycin
MBO Milbemycin 5-oxime
PGP P-glycoprotein

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bile and urine. In the endothelial cells of the blood-brain barrier, PGP functions to actively transport many drugs out of the CNS and back into the blood. Thus, inhibition or a lack of the PGP system can lead to increases in drug bioavailability and greater systemic drug concentrations, with accumulation of drug in the CNS.

Dogs that are homozygous for the MDR1 gene mutation readily exhibit sensitivity to AVMs and MBs at dosages that, although much greater than the label dose rates for dogs, cause no adverse effects in dogs that do not have the mutation. Dogs heterozygous for the MDR1 mutation have been suggested to be less sensitive to toxic effects of AVMs and MBs than are homozygous dogs, but heterozygous dogs are more susceptible than are homozygous normal dogs to the toxic effects of drugs affected by PGP. In addition to its sensitizing effect, the MDR1 gene mutation may also render dogs more susceptible to the toxic effects of other drugs that are affected by the PGP system. Such drugs include gastrointestinal drugs (eg, loperamide), antimicrobials (eg, erythromycin, tetracycline, and ketoconazole), antiscabetic chemotherapeutics (eg, doxorubicin and paclitaxel), hormones (eg, estradiol), corticosteroids (eg, dexamethasone), and immunosuppressants (eg, cyclosporine and tacrolimus). Spinosad is an antiparasitic product for oral administration to dogs that can provide a 1-month duration of effectiveness against Ctenocephalides felis. Primarily consisting of 2 major members of the spinosyn class (spinosyn A and spinosyn D), spinosad has a structure that consists of a tetracyclic ring system to which an amino sugar (O-forosamine) and a neutral sugar (tri-O-methylrhamnose) are attached. Although they have no effect on mammalian nervous systems, the spinosyns have a novel mode of action on insect nervous systems that is modulated primarily through binding at nicotinic acetylcholine receptor sites distinct from those at which other insecticides bind and secondarily through binding at GABA receptors. Exposure to spinosyns causes hyperexcitation in insects, which is manifested as involuntary muscle contractions and tremors that result in prostration, paralysis, and rapid death. Experiments that involved use of increased dose rates were conducted to achieve registration with the FDA; they revealed that at the label dose rate, spinosad had a wide safety margin in dogs. Additional data generated in field trials allowed approval of a label without contraindications, although with a precautionary statement on use in breeding females.

As a tetracyclic macrolide, spinosad is in a different macrocyclic lactone category than are AVMs and MBs, and its effect on the PGP system is undetermined. Therefore, the study reported here was conducted to achieve 2 primary objectives. The first objective was to determine whether dogs with the MDR1 gene mutation would have adverse effects as a result of exposure to spinosad doses greater than the recommended monthly dose of 30 to 60 mg of spinosad/kg. The second objective, as an investigational step in product development, was to determine whether administration of a combination of spinosad and MBO would precipitate AVM-MBO toxicosis in these dogs.

### Materials and Methods

#### Animals

Twenty-four purpose-bred adult Collies, all of which had the MDR1 gene mutation, were available for use in the study. Of these, 1 was ineligible for enrollment because of severe bilateral conjunctivitis. The remaining 23 Collies were subjected to an initial screening for ivermectin sensitivity; 20 dogs were selected for the study. The study dogs weighed between 16.3 and 34.4 kg and were provided by a commercial kennel; all dogs were returned to the kennel after the study. The study protocol was modeled in accordance with Veterinary International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products to explore the safety of the combination of spinosad and MBO and was approved by a university institutional animal care and use committee.

All dogs enrolled in the study were considered healthy on the basis of results of a physical examination and complete hematologic analysis, serum biochemical analysis, and urinalysis (all variables were within the respective reference ranges). The only dog that did not have a unique ear tattoo for identification was easily recognizable by its distinct coat color and body size. Dogs were housed in 1.83 X 1.37-m, raised stainless-steel pens with plastic-coated metal floors and a resting board, in compliance with established National Research Council guidelines. Temperature and humidity were monitored. A regimented light cycle (12 hours of light and 12 hours of darkness) was maintained. Dogs were fed daily by use of separate feed hoppers, and water was provided ad libitum via automatic waterers.

**Prestudy screening for AVM-MB sensitivity**—Brush samples of cheek cells were collected from each of the 23 dogs and submitted to a university laboratory for testing to detect the MDR1 gene mutation to assess AVM-MB sensitivity. Within 1 day after collection of the cheek cells, the AVM-MB sensitivity of each dog was further assessed by oral administration of ivermectin (120 µg/kg) and observation of the dogs for signs of toxicosis at 2, 4, 6, 8, 12, 16, 20, and 24 hours (+ 30 minutes) after administration. Evidence of AVM-MB toxicosis, including signs of depression, ataxia, mydriasis, and salivation-drooling, was assessed in accordance with the reaction scoring system developed in another study. Severity of clinical signs was ranked on a scale of 0 to 3 (0, no reaction; 1, mild reaction; 2, moderate reaction; and 3, severe reaction). A washout period of 28 days was allowed between screening for AVM-MB toxicosis and the first day of the study (day 0).

**Procedure**—Body weight of each dog was obtained on days –14, –2, 26, and 35. Body weights recorded on days –2 and 26 were used to calculate drug doses for the 5-day dosing sequence that began 2 days following weight measurement (day 0 and 28, respectively). Accuracy of the scale was verified by use of certified weights before and after each weighing period. Blood samples and urine samples (midstream catch) for serum biochemical analysis and urinalysis, respectively, were obtained on days –2 and 35.
Treatments—Combined scores for each of the clinical signs of AVM-MB toxicosis were used to calculate a mean value for each dog, and blocks were formed (n = 5 dogs/block) on the basis of mean ivermectin sensitivity scores. Randomization was performed by use of a random-number generator, and dogs within a block were assigned to 1 of 5 treatments (placebo [control group], spinosad\(^4\) at 300 mg/kg [5 times the upper limit of the labeled monthly dose of 30 to 60 mg/kg; 5X/0X group], spinosad at 180 mg/kg combined with MBO\(^7\) at 3 mg/kg [3 times the labeled monthly dose; 3X/3X group], spinosad at 300 mg/kg combined with MBO at 5 mg/kg [5X/5X group], and spinosad at 300 mg/kg combined with MBO at 10 mg/kg [5X/10X group]).

Treatments were administered orally once daily for 5 consecutive days (first day of treatment was designated as day 0) to achieve the allocated multiples of the monthly dose rate. Daily administration of doses for 5 days, rather than a single bolus dose, was used to maximize total exposure. The sequence of treatment doses was repeated starting on day 28; thus, each dog allocated to a treatment group was exposed 2 separate times to a maximum cumulative dose equivalent to 3 or 5 times the upper limit of the recommended monthly dose of spinosad and concurrent treatment with 3, 5, or 10 times the labeled monthly dose of MBO. Control dogs received placebo capsules on each of the 5 treatment days.

To ensure that each dog received the correct dose of spinosad, gelatin capsules containing the calculated dose were prepared for each dog on the basis of the body weight recorded 2 days before the initial day of each 5-day administration sequence. Spinosad and MBO were combined in hard gelatin capsules <48 hours before administration. No active ingredients were placed in the capsules administered to placebo-treated dogs. Capsules for all dogs were individually packaged, labeled with the animal identification, and shipped via overnight courier to the study site. Capsules containing spinosad, spinosad and MBO, and placebo were identical in appearance. Food was withheld overnight from all dogs prior to capsule administration on all treatment days. Because the administration of spinosad with food can enhance absorption,\(^7\) each dog was required to consume approximately 25% of the recommended daily amount of diet in the form of a canned food before capsule administration (dietary amounts were based on each dog’s body weight). Two hours after capsule administration, the remaining 75% of the ration for each dog for that day was provided in the form of a dry diet.

Observations and AVM-MB toxicosis scores—Study personnel responsible for data collection were trained to score signs of AVM-MB toxicosis and were not aware of the treatment group to which each dog was assigned. Dogs were monitored continuously for any abnormalities in the results of CBCs, serum biochemical analyses, or urinalyses performed on days 0, 7, 14, 21, and 28. No abnormalities were detected during physical examinations for any dog, except for a hyperexcitable dog in the M/M group that had a rectal temperature >39.4°C during examinations conducted before and after treatment administration and a dog in the 5X/5X group that had diarrhea, lethargy, and urinary tract problems prior to treatment administration. The latter received amoxicillin with clavulanic acid and lactated Ringer’s solution before the first dosing sequence began and enrofloxacin and physiologic saline (0.9% NaCl) solution during the first dosing sequence. The dog responded well to treatment. There were no significant abnormalities in the results of CBCs, serum biochemical analyses, or urinalyses performed on days 0, 7, 14, 21, and 28.

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Observations and AVM-MB toxicosis scores—Study personnel responsible for data collection were trained to score signs of AVM-MB toxicosis and were not aware of the treatment group to which each dog was assigned. Dogs were monitored continuously for approximately the first 2 hours and then at 4, 6, 8, 12, 16, 20, and 24 hours (± 1 hour) after treatment; thus, there were 320 scoring times for each clinical sign for each treatment. Dogs were observed for any abnormal clinical signs in addition to ataxia, signs of depression, mydriasis, and salivation that were scored specifically to detect AVM-MB toxicosis. Any signs of AVM-MB toxicosis were scored as for the prestudy screening for ivermectin sensitivity. Daily observations were also made for any other abnormal clinical signs on days on which dogs were administered treatments.

Statistical analysis—For each variable (ie, signs of depression, ataxia, mydriasis, and salivation), mean and maximum values for each dog for each day, each period, and among periods were summarized by treatment group. Mean and maximum of each variable for each treatment group were also summarized within and between dose administration periods. Daily number of dogs vomiting and vomiting rates were summarized for treatment group and dose administration period and between dose administration periods. The Fisher exact test was used to test the rate of vomiting among treatment groups for each dose administration period and between dose administration periods.

Results

All 23 dogs screened for study enrollment had positive results when tested for the MDR1 mutation. Three dogs (2 were heterozygous for MDR1 and 1 was homozygous for MDR1) were not included because they had a score of 0 when tested for AVM-MB sensitivity. Of the 20 dogs enrolled in the study, 17 were homozygous for the MDR1 mutation, with mean AVM sensitivity scores ranging from 0.25 to 1.25 (Table 1). The 3 heterozygous dogs had mean AVM sensitivity scores of 0.25, 0.25, and 0.5, respectively.

No abnormalities were detected during physical examinations for any dog, except for a hyperexcitable dog in the 5X/10X group that had a rectal temperature >39.4°C during examinations conducted before and after treatment administration and a dog in the 5X/5X group that had diarrhea, lethargy, and urinary tract problems prior to treatment administration. The latter received amoxicillin with clavulanic acid and lactated Ringer’s solution before the first dosing sequence began and enrofloxacin and physiologic saline (0.9% NaCl) solution during the first dosing sequence. The dog responded well to treatment. There were no significant abnormalities in the results of CBCs, serum biochemical analyses, or urinalyses performed on days 0, 7, 14, 21, and 28.

Table 1—Results of prescreening testing of 20 Collies with sensitivity to AVM-MB that were enrolled in the study.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>MDR1 status</th>
<th>Mean AVM score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>M/M (n = 4)</td>
<td>0.25–0.75</td>
</tr>
<tr>
<td>5X/0X</td>
<td>M/M (n = 4)</td>
<td>0.25–0.75</td>
</tr>
<tr>
<td>3X/3X</td>
<td>M/M (n = 3)</td>
<td>0.50–0.75</td>
</tr>
<tr>
<td>5X/5X</td>
<td>M/M (n = 2)</td>
<td>0.50–1.25</td>
</tr>
<tr>
<td>5X/10X</td>
<td>M/M (n = 4)</td>
<td>0.25–0.75</td>
</tr>
<tr>
<td></td>
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</table>

The AVM-MB sensitivity of each dog was assessed by oral administration of ivermectin (120 µg/kg) and observation of the dogs for signs of toxicosis at 2, 4, 6, 8, 12, 16, 20, and 24 hours (± 30 minutes) after administration.

* Treatments were as follows: control = placebo, 5X/0X group = spinosad at 300 mg/kg, 3X/3X = spinosad at 180 mg/kg combined with MBO at 3 mg/kg, 5X/5X = spinosad at 300 mg/kg combined with MBO at 5 mg/kg, and 5X/10X = spinosad at 300 mg/kg combined with MBO at 10 mg/kg. † Value reported is the range of mean AVM scores for all dogs in that category.

M/M = Homozygous, M/N = Heterozygous, n = Number of dogs.
samples collected on days –2 (before administration) and 35 (after administration).

All adverse events detected subsequent to the first treatment period were transient and mild, and none of the dogs required medical treatment. Of the events that were detected, vomiting was the most frequently recorded. None of the dogs vomited after capsule administration on day 0, and during the subsequent 4 days of capsule administration, isolated vomiting events were observed in 3 dogs in the 5X/0X group, 2 in the 3X/3X group, and 3 in the 5X/5X group. None of the dogs in the control or 5X/10X groups vomited. There was no evidence of administered drug in the vomitus, and none of the dogs was administered a second dose after vomiting. None of the dogs vomited during the second 5-day administration period. There were no significant (values ranged from P = 0.143 to P = 1.000) differences among groups for the number of dogs that vomited or for the overall rate of vomiting. Diarrhea was observed in 1 dog in the 5X/5X group for 1 day after the first dose in the first 5-day administration period and again 3 days later (after the third dose in this sequence). Other than vomiting, no adverse events were observed in > 1 dog in any treatment group; therefore, statistical comparisons of these events were not performed.

One dog in the control group and 1 dog in the 3X/3X group salivated after administration during the first 5-day administration period. During prestudy screening for AVM-MB sensitivity, both of these dogs had salivated and were ataxic and the dog in the 3X/3X group also had signs of depression. For the assessments of AVM-MB toxicosis during the study, salivation was scored as mild at 5 scheduled observation times for the control dog and as mild during 1 scheduled observation time for the 3X/3X dog. No other signs associated with AVM-MB toxicosis were observed during the study.

**Discussion**

The only potential sign of AVM-MB toxicosis observed in any dog during the study was mild salivation. The fact that this 1 sign was observed in a dog of the control group and a dog of the 3X/3X group but not observed in any dogs receiving the highest dose of spinosad-MBO suggested that it was not caused by exposure to the test compounds.

Vomiting was the most frequent adverse event in the study. This is consistent with results of a field study on the use of spinosad in dogs. In that report, the rate of vomiting during the month after the initial treatment was 12.7%, slightly higher than the 12.2% vomiting rate that was reported for the comparison group that was treated with topicaly applied selamectin. In the study reported here, the vomiting episodes decreased in frequency or there were no episodes of vomiting after the second treatment, which was consistent with results for the field study. Vomiting and other adverse events were mild and transient, and dogs did not require veterinary intervention or treatment.

The MDR1 gene deletion has been reported in 10 breeds, including Collies and Shetland Sheepdogs, and it may be present (but as yet not detected) in other breeds. It is widespread, with a frequency of > 50% in Collies but a lower frequency in other breeds, such as the Old English Sheepdog (< 4%). Therefore, it is helpful for veterinarians to be aware of whether any drug they administer or prescribe that can interact with the PGP system has the potential to precipitate signs of AVM-MB toxicosis in AVM-MB–sensitive dogs.

However, our results raise questions as to the interpretation of tests for dogs with the MDR1 gene deletion. It has been suggested that dogs heterozygous for the deletion are less sensitive to AVM than are homozygous dogs. However, results of the screening process in our study suggested that this may not reliably be the case. For instance, 1 dog homozygous for the MDR1 gene deletion did not have signs of toxicosis when exposed to an ivermectin dose of 120 µg/kg, whereas 3 dogs heterozygous for the MDR1 gene deletion had signs of toxicosis. Additionally, 1 heterozygous dog in the study had an AVM sensitivity score of 0.5, whereas 3 homozygous dogs each had a score of 0.25. Although this study was not designed to investigate the relative susceptibility of dogs with the MDR1 gene deletion to AVM, the screening results raise the questions as to whether all dogs homozygous for the mutation will have an increased sensitivity to AVM-MB and whether homozygous MDR1 dogs are necessarily more sensitive to AVM-MB than are heterozygous MDR1 dogs.

An alternative explanation for the results of the ivermectin screening procedure may be that it is not a reliable means of determining AVM-MB sensitivity. However, this would not appear to be true because in this study, the tests were performed by trained observers, and the scoring and screening process for AVM-MB toxicosis is reported to be repeatable and consistent. It may be possible that although the dose used for the screening test in the study reported here and other studies is adequate to assess ivermectin sensitivity, it is too low to accurately determine those dogs that will manifest overt clinical signs of neurologic toxicosis when higher doses are administered.

The randomized allocation procedure resulted in 2 of the 3 MDR1 heterozygous dogs in the same treatment group (ie, 5X/5X group). As mentioned previously, the heterozygotes in our study did not appear to be less sensitive than the homozygotes to AVM-MB toxicosis. However, even if this were not the case, the fact that no signs of toxicosis were evident in homozygous dogs receiving the highest doses of each product confirms the conclusion that the combination of products did not precipitate signs of AVM-MB toxicosis. The direct relevance of this study should be clear to clinicians. The FDA has issued a safety warning describing reports of AVM-MBO toxicosis in dogs treated concurrently with spinosad and high, extralabel doses of ivermectin. The results reported here revealed that doses up to 5 times the recommended monthly dose for spinosad, alone or in combination with up to 10 times the recommended monthly dose for MBO, administered during two 5-day periods with an interval of approximately 1 month between administration periods, did not induce signs of AVM-MB toxicosis in AVM-MB–sensitive Collies, regardless of whether those dogs were homozygous or heterozygous for the MDR1 gene deletion. Therefore, this report provides assurance to clinicians that when used concur-
rently with members of the AVM-MB class at recommended dose rates, spinosad has a wide therapeutic index in dogs with the MDRI mutation that are sensitive to AVM-MB. The results also indicated that a combination of spinosad and MBO, in an appropriate formulation, should not induce signs of AVM toxicity.

References