Neurotoxic effects of ivermectin administration in genetically engineered mice with targeted insertion of the mutated canine ABCB1 gene

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Objective—To develop in genetically engineered mice an alternative screening method for evaluation of P-glycoprotein substrate toxicosis in ivermectin-sensitive Collies.

Animals—14 wild-type C57BL/6J mice (controls) and 21 genetically engineered mice in which the abcb1a and abcb1b genes were disrupted and the mutated canine ABCB1 gene was inserted.

Procedure—Mice were allocated to receive 10 mg of ivermectin/kg via SC injection (n = 30) or a vehicle-only formulation of propylene glycol and glycerol formal (5). Each was observed for clinical signs of toxic effects from 0 to 7 hours following drug administration.

Results—After ivermectin administration, considerable differences were observed in drug sensitivity between the 2 types of mice. The genetically engineered mice with the mutated canine ABCB1 gene had signs of severe sensitivity to ivermectin, characterized by progressive lethargy, ataxia, and tremors, whereas the wild-type control mice developed no remarkable effects related to the ivermectin.

Conclusions and Clinical Relevance—The ivermectin sensitivity modeled in the transgenic mice closely resembled the lethargy, stupor, disorientation, and loss of coordination observed in ivermectin-sensitive Collies with the ABCB1-1∆ mutation. As such, the model has the potential to facilitate toxicity assessments of certain drugs for dogs that are P-glycoprotein substrates, and it may serve to reduce the use of dogs in avermectin derivative safety studies that are part of the new animal drug approval process. (Am J Vet Res 2012;73:1477–1484)
ening neurotoxic effects because of an accumulation of ivermectin in brain tissue.17,11,12,18

Having broad substrate specificity, P-gp has the potential to affect the pharmacokinetics, tissue distribution, and toxicity profile of any new avermectin drug, which is a class of drugs observed to yield toxic effects in dogs with ABCB1-1A, as well as new nonavermectin drugs that are P-gp substrates. Because of these adverse events, new avermectin drugs undergo an additional safety study in Collies with defective P-gp transporter activity as part of the US FDA drug approval process. However, the number of available ivermectin-sensitive Collie colonies for drug approval studies is diminishing and drug sponsors are finding it increasingly difficult to acquire these dogs to test the safety of avermectins. The scarcity of Collies available for safety studies has also precluded use of these dogs to investigate potential safety issues that might be posed by other nonavermectin P-gp substrates that could have clinical consequences in dogs with defective P-gp activity. Thus, an alternative means of assessing the potential toxicity of P-gp substrates is needed.

In contrast to the single ABCB1 gene found in dogs and most vertebrate species, 2 homologous isoforms have been identified in mice: abcb1a and abcb1b.9 The murine abcb1a gene is highly expressed in the intestinal epithelium, liver, and blood-brain barrier, whereas the murine abcb1b gene is expressed predominantly in the adrenal glands, gravid uterus, and ovaries.10-22 In developing a model of ivermectin sensitivity in genetically engineered mice to mimic phenotypic responses in ivermectin-sensitive Collies, both abcb1a and abcb1b genes must first be targeted to eliminate the P-gp produced by these genes and remove any possible artifacts that could be attributed to their presence. Next, the mutated form of the canine ABCB1 gene must be inserted into the mouse genome so that the mouse solely expresses the truncated form of the protein. Following successful generation of the transgenic mouse, ivermectin would be administered to assess whether the transgenic mouse response to a P-gp substrate mirrors the adverse neurotoxic effects observed in dogs without functionally intact P-gp.

Transgenic animals, particularly those created through elimination of drug transporters (also referred to as knock-out models), are potentially valuable for use in preclinical studies and for gaining insight into the pharmacokinetics of certain drugs.23 The purpose of the study reported here was to establish an alternative screening method for drugs that are P-gp substrates and that potentially could have altered toxicity profiles in dogs with the ABCB1-1A mutation. Determining whether the adverse effects observed in dogs could be reproduced in these transgenic mice following ivermectin administration would allow for validation of the model of ivermectin sensitivity in genetically engineered mice and could facilitate the identification of new P-gp substrates potentially toxic to ivermectin-sensitive Collies.

**Materials and Methods**

### Construction of targeting vectors

To disrupt abcb1b gene function, a targeting vector4 that incorporated the mutated coding sequence for the canine ABCB1 gene (accession No. AJ419568) was inserted in frame with the abcb1a gene initiation codon (ATG) in a process referred to as knocking out. The targeting vector also had the following attributes: homology regions isogenic with the embryonic stem cell line that was used (129Sv/Pas), a positive selection neomycin gene flanked by loxP-sites inserted downstream of the mutated canine gene to enable the selection of the transplanted embryonic stem cell clones, a negative selection marker (diphtheria toxin A) located upstream of the mutated canine cDNA cassette, and the exogenous human growth hormone polyadenylation signal (hGH pA).

To disrupt abcb1b gene function (a process referred to as knocking out), another targeting vector was designed and constructed containing homologous genomic abcb1b sequences with a deletion of the abcb1b region encompassing exons 3 and 4 (resulting in the deletion of 3.4 kbp of genomic sequences) and subsequent replacement with a flippase recognition target—flanked hygromycin-positive selection cassette. Similar to the abcb1a targeting vector, the abcb1b targeting vector contained an upstream negative selection marker (diphtheria toxin A), which, in both vectors, served to reduce the isolation of nonhomologous recombined embryonic stem cell clones and enhance the chance of isolating embryonic stem cell clones with correct integration events.

### Electroporation and screening of embryonic stem cells and model generation

—On the basis of findings from the bioinformatic analysis of the organization of the 2 murine abcb1 genes, a strategy was designed in which 2 successive electroporations were performed on the same targeted embryonic stem cell clone to eliminate any residual production of murine P-gp. For the first homologous recombination, the abcb1b targeting vector was linearized by restriction digest and 40 μg of the resulting 14-kbp fragment was purified and transfected into 129Sv/Pas embryonic stem cells4 by electroporation. Hygromycin-resistant clones were analyzed by PCR assay, and Southern blotting was used to isolate embryonic stem cell clones that had undergone correct homologous recombination at both the 5′ and 3′ junction and contained no additional random integrations or recombinations of the targeting vector. Two of these embryonic stem cell clones harboring the disrupted abcb1b allele (knock-out allele) were selected for the second electroporation phase, in which they were transfected with 40 μg of linear abcb1a vector (a 16.2-kbp targeting fragment). Positive selection was performed with G418 to isolate neomycin-resistant clones. Polymerase chain reaction screening and Southern blotting of the clones allowed characterization of recombined clones with confirmed 5′ and 3′ targeting events.

Seven correctly targeted embryonic stem cell clones were expanded and selected for microinjection into host blastocysts originating from C57BL/6j mice.6 Mouse coat color markers were used to assess whether the embryonic stem cells contributed to the germ layer of the chimeras, given that the agouti coat color of the 129Sv/Pas embryonic stem cells is dominants over the black coat color of the C57BL/6j mice. Highly chime-
ric males, selected on the basis of coat color markers, were consequently generated and mated with female C57BL/6J Cre-recombinase-expressing mice to induce excision of the neomycin selection cassette through recombination between the 2 loxP sites. This step was performed to avoid any dysregulation of the mutant canine ABBC1 attributable to the strong phosphoglycerate kinase promoter driving the neomycin resistance gene. Polymerase chain reaction screening and Southern blot analyses were used to identify double-targeted heterozygous mice carrying the neomycin-excised ABBC1-1Δ knock-in allele and the abcb1b knock-out allele. Finally, interbreeding of first filial heterozygous mice led to the generation of 17 homozygous mice, of which 6 females and 3 males were selected for proliferation. Mice were housed and reared in a specific and opportunistic pathogen-free environment and were then transferred to a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Animals—Thirty-five to 42-week-old mice were used: 21 transgenic mice (body weight range, 22.8 to 51.4 g) and 14 wild-type C57BL/6J control mice (26.5 to 55.6 g). All mice were individually housed in standard polycarbonate microisolator filtered cages with cellulose bedding and had unrestricted access to deionized water and a standard certified pellet rodent diet, in compliance with all relevant guidelines and institutional policies. A lighting cycle of 12 hours of light and 12 hours of dark was used, and the environmental temperature was maintained within a range of 18° to 26°C and humidity between 40% and 70%.

Sentinel mice were used to monitor the health status of the study mice as part of the Center for Food Safety and Applied Nutrition animal program. All serum and fecal samples from sentinels in rooms housing the study mice yielded negative results when tested for at least 18 common disease agents of mice. All experimental procedures involving mice were reviewed, approved, and monitored by the Center for Food Safety and Applied Nutrition Institutional Animal Care and Use Committee.

Genotyping of mice—Blood samples from mice were obtained from the submandibular vein for subsequent genotyping. Sterile 5.0-mm lancets were used to control the depth of skin puncture, and 2 to 4 drops of blood were collected and spotted on blood stain cards. Genomic DNA was extracted from two 2-mm disks punched from the whole blood dried on the blood stain cards by use of a PCR kit in accordance with the manufacturer’s instructions.

Polymerase chain reaction amplification was performed with a ready-to-use mixture and 4 primer sets for specific allele detection (Appendix). The following thermocycling conditions were used: 1 cycle at 94°C for 2 minutes; 35 cycles of denaturing at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 68°C for 5 minutes (1 minute for abcb1a wild-type primer pair); and final extension at 68°C for 8 minutes. The abcb1a knock-in primer set was used to detect the abcb1a knock-in allele with the sense primer hybridizing within the transgene upstream of the remaining loxP site and the antisense primer hybridizing downstream of the targeting vector sequence. The abcb1a wild-type primer pair was used to detect the murine abcb1a wild-type allele, with the forward primer hybridizing upstream of abcb1a exon 1 and the reverse primer hybridizing within abcb1a intron 2. The abcb1b knock-out primers hybridized within the hygromycin selection cassette, downstream of the targeting vector homology sequence, allowing for specific detection of the abcb1b knock-out allele. Finally, the abcb1b wild-type primer set was used to detect the murine abcb1b wild-type allele by hybridizing within abcb1b intron 4 (region deleted by knock-out locus) and downstream of the targeting vector sequence (Figure 1). Polymerase chain reaction products were separated by electrophoresis on a 1.2% agarose precast gel and visualized under UV illumination.

Ivermectin administration—Injectable ivermectin (10 mg/mL) consisted of solubilized 1% ivermectin formulated in 40% glycerol formal and 59% propylene glycol. Dilutions of the stock were prepared in propylene glycol to generate concentrations that would allow for doses of 10 mg/kg administered in a total injection volume of 100 µL.
Results from a preliminary trial demonstrated that a dose of 0.2 mg/kg, which is the standard dose administered to dogs, was not sufficient to yield any observable clinical signs of toxic effects within 6 hours after administration in either group of mice. Therefore, a dose of 10 mg/kg was selected on the basis of the LD₅₀ of ivermectin in wild-type mice (40 to 60 mg/kg). The selected dose was sufficient to rapidly affect transgenic mice with the mutated canine ABCB1 gene but did not result in any observable toxic effects in wild-type mice.

Ivermectin solution was administered to 18 transgenic and 12 wild-type mice in the dorsal region of the neck via SC injection with a 1-mL tuberculin syringe and a 26-gauge, 3/8-inch needle. A subset of wild-type (n = 2) and transgenic (3) mice received 100 µL of vehicle only, which was a mixture of glycerol formal and propylene glycol formulated by weight. Mice were observed for signs of toxic effects from just prior to drug administration (time 0) to 7 hours after drug administration, with observations recorded at 1-hour intervals at a minimum. At each observation point, mice were assessed for the following behavioral and physiologic conditions: hyperactivity and startle reflex, locomotor activity, gait, body position and posture, depth and frequency of respiration, eye closure, salivation, lacrimation, lethargy, recumbency, righting reflex, uncontrolled body tremors, and onset of comatose state, as described elsewhere.11,12,23,28

Although all observed changes from a mouse’s pre-injection condition were recorded, ataxia, lethargy, and tremors were monitored most closely because of their high prevalence in preliminary trials and were ranked on a 4-point scale. The scale used for assessment of motor coordination (ataxia) was as follows: 0 = no change in body and muscle tone, with coordination unaffected; 1 = slight gait irregularity and mild motor uncoordination; 2 = moderately impaired staggering gait and uncoordination evidenced as a wobble, with a slightly slowed righting reflex; and 3 = heavily impaired motor coordination with righting reflex severely slowed or absent. Degree of lethargy was evaluated as follows: 0 = no change in energy and exploratory activity, with typical movement away from touch; 1 = slightly dulled locomotor activity, more time spent huddling, and slowed response to touch; 2 = moderate lethargy and sluggish movements and significant prompting required for the mouse to move but otherwise no spontaneous efforts made to move; and 3 = severe lethargy and sluggishness, with refusal and to move even with considerable prodding. Lastly, tremors were characterized by the following scale: 0 = no tremor and no change in behavior; 1 = first observation of infrequent, single-burst, low-intensity twitches; 2 = more frequent moderate-intensity whole body tremor bursts; and 3 = severe-intensity spontaneous tremors and involuntary spasms occurring repeatedly and progressing to convulsive episodes.

Any mouse judged to have life-threatening signs, in the opinion of the study director or in consultation with the Center for Food Safety and Applied Nutrition veterinary staff, was euthanized prior to the study endpoint, whereas all remaining mice were euthanized at the 7-hour observation point via carbon dioxide asphyxiation. Once euthanized, brain tissue was collected and stored in tissue containers for genomic analysis.

qRT-PCR analysis—Total RNA from mouse brain tissue was isolated with an RNA kit in accordance with the manufacturer’s instructions. Quantitative real-time reverse transcription PCR was performed with primers custom designed with a desktop tool and synthesized by a vendor, for the detection of mutated canine ABCB1 gene expression in the double-targeted, homozygous, transgenic mice. The sense primer for the ABCB1 gene was 5’-AAGGAAGAAAAACCTGTCAGCAGC-3’ and the antisense primer was 5’-CATGAGAGGAGATGCAGCTC-3’.

Total RNA was reverse transcribed with a qRT-PCR kit. Briefly, 25 µL of reaction mix, 1 µL of polymerase, 20 µL of diethylpyrocarbonate-treated water, and 1 µL each of 10 µM concentration of sense and antisense primers were combined to create the master mix. Twenty-four microliters of master mix was added to a sealed, pressurized polypropylene tube system after which extracted RNA samples were added. Real-time detection was performed with a real-time PCR system with the following parameters: cDNA synthesis at 30°C for 15 minutes and 95°C for 5 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Melt curve analysis was performed from 72°C to 99°C in 1°C increments every 5 seconds. Cycle threshold values were recorded, and specificity of PCR amplification was verified on the basis of melting curve analysis.

Results

Generation and analysis of transgenic mice—A lineage of double-targeted abcb1a knock-in/abcb1b knock-out homozygous mice with targeted insertion of mutant canine ABCB1 was successfully generated. These mice, examined until 12 months of age, were free of adverse effects on fertility, physiology, anatomy, or mortality rate. Litter yields were of comparable size to those resulting from wild-type interbreeding. Additionally, breeding demonstrated germ line transmission of the knock-in and knock-out alleles.

Genotyping and qRT-PCR knock-out analysis—Correctly sized amplicons generated via PCR with primers abcb1a knock-in (2,993 bp) and abcb1b knock-out (2,929 bp) allocated a mouse to the abcb1a knock-in–abcb1b knock-out group, as long as no products were generated by PCR with primers abcb1a wild-type and abcb1b wild-type (Figure 2). Conversely, products generated via PCR with primers abcb1a wild-type (942 bp) and abcb1b wild-type (2,969 bp) with no amplicons generated by the other two primers resulted in a wild-type mouse designation. Genotyping of the mice confirmed 14 of the 35 mice used in this study as wild-type and the remaining 21 as transgenic with complete disruption of murine abcb1a and abcb1b.

qRT-PCR analysis—Gene expression of mutated canine ABCB1 cDNA was analyzed by performing qRT-PCR assays on total RNA extracted from brain tissue recovered from abcb1a knock-in–abcb1b knock-out and wild-type mice. Quantitative real-time reverse transcription PCR analysis revealed that the mutated canine...
ABCB1 transcript was expressed and detectable in brain tissue from all abcb1a knock-in/abcb1b knock-out mice (n = 21) and not detectable in any wild-type mice (14), validating the model of ivermectin sensitivity in genetically engineered mice by demonstrating disruption of murine abcb1a gene expression and replacement by the canine ABCB1 cDNA.

Sensitivity of mice to ivermectin—Physiologic and behavioral assessment of mice following ivermectin administration revealed considerable differences in drug sensitivity between the abcb1a knock-in/abcb1b knock-out mice and wild-type mice.

Immediately following SC injection of ivermectin or the vehicle control, 23 of the 35 mice were observed to have initial signs of discomfort, irritation, and scratching, with severity ranging from minor signs of discomfort to extensive self-mutilation. However, given that transgenic and wild-type mice were both affected, the initial irritation and scratching that occurred were independent of mouse genotype. The signs of discomfort were also unrelated to ivermectin itself, given that both ivermectin-treated and vehicle-treated mice were observed to be scratching. Results from the preliminary trial (not shown) demonstrated that when mice were injected SC with a higher volume of propylene glycol (200 µL) than was used in this evaluation, many had skin irritation with self-mutilation (raw lesions visible at the scruff region from severe scratching), early-onset lethargy, and uncoordination. These clinical signs resembling ethanol intoxication had a rapid onset, suggesting these effects might have been similar to those associated with the acute phase of ethylene glycol toxicosis.29 The scratching, mild ataxia, and lethargy typically subsided after 2 to 3 hours, and by the final observation 7 hours after injection, the mice affected by the propylene glycol had fully recovered or had noticeably improved. Clinical signs related to the propylene glycol were virtually eliminated with a 50% reduction in SC injection volume to 100 µL.

Of the 5 mice (2 wild-type and 3 transgenic) that received 100 µL of vehicle only (glycerol formal and propylene glycol), 4 had no signs other than mild initial scratching during the 7-hour evaluation period. One of the vehicle-treated mice had slight ataxia 1 hour after injection but recovered completely and had no signs of adverse effects after 2 hours.

Similarly, there were no lasting effects of ivermectin administration in the 12 transgenic mice that received an SC injection of 10 mg of ivermectin/kg. Immediately following drug administration, 8 of 12 wild-type mice were observed to be scratching at the injection site. Nine of 12 mice had no other observable signs. The remaining 3 mice had slight gait impairment approximately 2 to 4 hours after drug administration, but all signs had completely subsided by 5 to 6 hours after injection and mice remained free of these signs for the remainder of the study.

Conversely, all 18 transgenic mice that received 10 mg of ivermectin/kg were judged to have extreme sensitivity to ivermectin when assessed for physiologic and behavioral changes following drug administration. The most ubiquitous signs of ivermectin sensitivity, in order of typical progression, were gait impairment and ataxia, followed by lethargy and uncontrolled body tremors comparable to the neurobiological aberrations that result from ethanol intoxication in mice.30–32 The first signs of neurotoxic effects became apparent anywhere from 1 to 6 hours after ivermectin injection. Of the 18 mice, 9 developed severe signs of ataxia, 7 had moderate ataxia, 1 had mild ataxia, and 1 had no signs of ataxia by the 7-hour endpoint of the observation period. Few mice had a complete loss of righting reflex after 7 hours, but it was apparent that the conditions of all mice were still worsening at the study endpoint. High severity of ataxia was occasionally obscured because this loss of motor coordination often coincided with increasing lethargy and unwillingness to move.

Intrinsic variations in the degree of energy among mice were noted prior to start of study to ensure that
only differences in degree of lethargy were recorded and that a mouse’s typical behavior was not confused with behavioral abnormalities resulting from drug administration. Prior to ivermectin administration, mouse behavior ranged from highly active and quick to explore to less inclined to move with a preference for huddling. Of the 18 ivermectin-treated transgenic mice, 6 had severe lethargy, 9 had moderate lethargy, 1 had mild lethargy, and 2 had no signs of lethargy at the study endpoint.

Following the development of signs of ataxia and lethargy, tremors began to manifest, typically beginning as barely noticeable low-intensity twitches localized to the head or back regions and rapidly progressing to spontaneous whole body tremors and convulsions. Of the 3 clinical signs evaluated, tremors were the most consistent and reliable indicator of progressing neurotoxic effects and rendered mice incapable of controlled movement. Of the 18 ivermectin-treated transgenic mice, 5 had severe tremors, 8 had moderate tremors, 4 had mild tremors, and 1 had no apparent tremors at the study endpoint. Crippling convulsions and loss of righting reflex with anticipated progression to a comatose state were the primary reasons for early euthanasia of 2 of the 18 ivermectin-treated transgenic mice.

Additional signs observed occasionally and inconsistently in the ivermectin-treated transgenic mice included shallow and rapid respiration, hyperactivity, hypersensitivity, heightened startle reflex, hunchback, head tilt, and choppy movements and hopping hind limbs. Additionally, several mice engaged in circling movements elicited by severe tremors. Abnormal vocalization, hypersalivation, and an increase in lacrimation were not observed in ivermectin-treated mice, although these signs develop in ivermectin-sensitive dogs.11,12

Discussion

The present study was conducted to develop and characterize a model of ivermectin sensitivity in genetically engineered mice with targeted insertion of mutant canine ABCB1. Both murine abcb1 isoforms were successfully disrupted and replaced by the mutant canine ABCB1 to address the need for alternative screening methods for identifying potential P-gp substrate toxicity in dogs. The rapidly progressing signs of toxic effects observed in the transgenic mice closely resembled the lethargy, stupor, disorientation, and loss of coordination observed in ivermectin-sensitive Collies with the ABCB1-Δ mutation when challenged with certain P-gp substrates.11,12

These findings suggest that the complete disruption of murine abcb1a and abcb1b genes has no apparent deleterious effect on mice, unless the mice are challenged with P-gp substrates, despite the ubiquity of P-gp in mammals and the vast range of substrates that interact with the protein.9,10,19,33 The transgenic abcb1a knock-in/abcb1b knock-out mice with targeted insertion of mutant canine ABCB1 developed were also viable and fertile, with no physiologic abnormalities.34

Generally, ivermectin and other macrocyclic lactones have a high margin of safety in mammals because P-gp restricts the persistence of P-gp substrates in the CNS by actively pumping these compounds up their concentration gradient across the blood-brain barrier.34 Although the most commonly used standard therapeutic dose in dogs is 0.2 mg of ivermectin/kg, the oral LD50 for clinically normal dogs is approximately 80 mg/kg.22 In P-gp–deficient dogs, however, toxic effects have been observed in Collies treated with ivermectin at a dose as low as 0.1 mg/kg, PO.11,12 In clinically normal mice, the LD50 for ivermectin is reportedly 30 mg/kg via intraperitoneal injection36,37 and 40 to 60 mg/kg, PO.23 The higher SC dose of ivermectin used in the present study (10 mg/kg) was chosen to induce neurotoxic effects in mice with the mutated canine ABCB1 but not in wild-type mice, thus serving as a preliminary step in determining whether this model would mimic the responses observed in Collies possessing the ABCB1-Δ mutation. The 10 mg/kg dose was sufficient to consistently cause ataxia, lethargy, and tremors in the transgenic mice between 1 and 6 hours after administration, whereas no toxic effects related to ivermectin administration were observed in wild-type mice that received the same dose.

Different reactions to ivermectin administration are also observed in dogs known to be sensitive to the drug.11 Depending on the sensitivity of an individual Collie, the dog may react mildly or severely to an ivermectin dose of 0.2 mg/kg or not at all. Some dogs only react with considerably higher doses of ivermectin (0.6 to 2.5 mg/kg), even though they have the same ABCB1-Δ mutation. This suggests that factors other than the ABCB1-Δ mutation are likely to exist that impact a dog’s sensitivity toward P-gp substrates such as ivermectin. The model of ivermectin sensitivity developed in the present study may be useful in determining whether differences in ivermectin sensitivity among dogs are solely attributable to the known mutation or whether other mutations also contribute. Because P-gp has many substrates, each newly developed canine drug, including those not in the avermectin class, has the potential risk of causing adverse reactions when administered to dogs possessing the ABCB1-Δ mutation.

The model described in the present report has the potential to reduce the number of dogs used in avermectin safety studies as part of the drug approval process. It may also permit assessment of toxicity potential for other nonavermectin class P-gp substrates that are being developed for use in dogs. Ultimately, the model could influence how veterinary clinical data are generated and evaluated as well as the type of preclinical data requested by the US FDA to support drug approval by providing an alternative method to predict adverse reactions associated with avermectin administration in dogs.

a. genOway, Lyon, France
b. Charles River Laboratories, L’Arbresle Cedex, France.
c. US FDA Center for Food Safety and Applied Nutrition, Laurel, Md.
d. TEK-Fresh Laboratory Animal Bedding, Harlan Laboratories Inc, Indianapolis, Ind.
f. Extract-N-Amp Blood PCR Kit, Sigma-Aldrich, St Louis, Mo.
g. Platinum PCR SuperMix High Fidelity, Invitrogen, Carlsbad, Calif.
h. E-Gel, Invitrogen, Carlsbad, Calif.

Unauthenticated | Downloaded 10/09/23 02:20 AM UTC
i. Ivonec, Merial Ltd, Duluth, Ga.

j. PAXgene Tissue Containers, Qagen, Valencia, Calif.

k. PAXgene DNA RNA Kit, Qagen, Valencia, Calif.

l. PREMIER Biosoft International, Palo Alto, Calif.

m. AlleleID, Integrated DNA Technologies, Coralville, Iowa.

n. SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit, Invitrogen, Carlsbad, Calif.

o. 2X SYBR Green reaction mix, Invitrogen, Carlsbad, Calif.


q. SmartTubes, Cepheid, Sunnyvale, Calif.

r. SmartCycler, Cepheid, Sunnyvale, Calif.

s. Plus DNA Ladder, Invitrogen, Carlsbad, Calif.

References


Appendix appears on the next page
## Appendix

Oligonucleotide sequences of DNA primers used for genotyping of mice.

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F = Forward. R = Reverse.