Original Article

PCR detection of multidrug resistance 1 (MDR1) gene mutation in dogs presented at the University of the Philippines Veterinary Teaching Hospital

Keighley Shayne Y. Wee-Ebol[1] Listya Purnamasari[2]

Jorge Michael D. Dominguez[3] Joseph F. dela Cruz[1*]

Abstract

The multidrug resistance 1 (MDR1) gene codes for the p-glycoprotein (P-gp) efflux transporter function to limit the entry of certain xenobiotics, P-gp substrate drugs, in various physiological barriers of the body but most importantly in the blood-brain barrier. Mutation of the MDR1 gene results in a severely truncated P-gp that can lead to adverse drug reactions in patients treated with P-gp substrate drugs. This study aims to determine the presence of the mutation in the dogs presented to. Results revealed that many dogs possess the MDR1 gene mutation, with many possessing at least one copy of the mutation. The study discovers the mutation in two breeds and further proves the presence of the mutation in the German Shepherd dog. Furthermore, a large percentage of mixed breeds and Aspins carry at least one copy of the mutation. Although no adverse drug reactions have been reported, caution is still advised when treating patients with P-gp substrate drugs.

Keywords: ABCB1, aspin, dog, MDR1, P-glycoprotein

[1]*Department of Basic Veterinary Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, the Philippines, 4031*

[2]*Department of Animal Husbandry, Faculty of Agriculture, University of Jember, Jember, Indonesia, 68121*

[3]*Animal Breeding Division, Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, the Philippines, 4031*

****Correspondence**: jfdelacruz@up.edu.ph* (J.F.D. Cruz)

Received September 21, 2022

Accepted December 7, 2022

https://doi.org/10.14456/tjvm.2022.88

Introduction

In small animal medicine, patients are treated on an individual basis and each individual may have different responses to treatment. Generally, these differences are negligible. However, some patients have increased sensitivity, even at therapeutic doses, that can lead to adverse drug reactions (Archer, *et al*., 2014). Numerous cases have shown that some dogs experience toxicity upon administration of certain xenobiotics, the most infamous of these is ivermectin (Hopper *et al*., 2002). Symptoms of neurologic toxicity include ataxia, depression, salivation, tremor, apparent blindness and mydriasis (Nürnberger *et al*., 2021).

The major factor behind these adverse reactions is a four-base-pair deletion in the multidrug resistance 1 (MDR1) gene. The MDR1 gene is also known as the ABCB1 gene because it codes for a membrane-bound transporter in the superfamily ATP-binding cassette (ABC), subfamily B, member 1. This transporter is known as the permeability glycoprotein (P-gp) efflux transporter (Seelig, 2020; Merola and Eubig, 2012). P-gp is located in the plasma membrane and many organs with secretory or excretory functions, including the small intestine, liver and kidneys (Lee, *et al*., 2019). The transporter serves to decrease drug absorption and intracellular drug bioavailability in the small intestines whilst increasing drug elimination in the liver and kidneys. Moreover, P-gp is found in physiological barriers such as the placenta, Sertoli cell barrier and, most especially, the blood-brain barrier (Roulet, *et al*., 2003; Monobe, *et al*., 2015). It functions to limit the entry of P-gp substrates into these organs. The MDR1 gene mutation causes a frameshift mutation that creates premature stop codons which result in the loss of function of the P-gp (Geyer and Janko, 2012).

Dogs with a mutation in the MDR1 gene experience a myriad of side effects upon administration of the P-gp substrate drugs. Side effects can vary depending on the drug administered. Several herding breeds and some sighthounds commonly exhibit this mutation (Marelli *et al*., 2020). Mixed breeds can also be affected (Gramer *et al.* 2011). Recent discoveries have also found the mutation in breeds previously not known to possess it (Donner *et al*., 2016; Parker *et al*., 2017). With many breeds at risk of adverse drug reactions due to MDR1 gene mutation, it is essential to detect the mutation before drug administration.

No study has been conducted on the frequency, or even presence, of the mutant MDR1 in dogs in the Philippines. This study is the first to report on this as well as to provide an overview of the status of the mutation in dogs in the Philippines.

Materials and Methods

The Study Population: The study population included canine patients, regardless of health status, sex and age, encountered at the University of the Philippines Veterinary Teaching Hospitals - Diliman (VTH-DL) and Los Banos stations (VTH-LB). This study was conducted in compliance with the requirements of the Institutional Animal Care and Use Committee (IACUC) at the University of the Philippines Los Banos (Number: 2020-015). The blood was drawn and used for the study and consent was also taken from the pet owners.

Purposive sampling was implemented due to the limited number of samples that could be tested. A priority list of samples was selected which went from (1) herding breeds, (2) Aspins, and (3) mixed breeds. The herding class of dog breeds was selected because it was of interest to study whether breeds related to the predisposed breeds also possessed the mutation. For all dogs, it was asked explicitly whether xenobiotics such as ivermectin had been used in the past and whether any signs of neurological toxicity subsequently appeared. Moreover, analysis of the MDR1 gene mutation in other pure breeds already established to not possess it was not necessary. A total of 95 dogs were sampled with 18 herding breeds, 40 Aspins, and 36 mixed breeds. Among the herding breeds, five were German Shepherd dogs, six Belgian Malinois, six corgis, and one Shetland sheepdog. The duration of the sample collection was three months.

Blood Collection: Whole blood (1 ml) was placed in EDTA-containing tubes from the dogs. In VTH-DL, the tubes were labelled with the patient number for the day and stored in a polyethylene plastic bag each day; whereas, in VTH-LB, the tubes were labelled with the patient's name and stored in small boxes. The patient information was taken from the Animal Disease Diagnostic Laboratory record book and the digital records of VTH-DL and VTH-LB, respectively. The patient data provided included species, breed, sex and age. The samples were stored at -12℃ until transport to the Pharmacology Laboratory of the Department of Basic Veterinary Sciences of the College of Veterinary Medicine in the University of the Philippines Los Baños where they were stored at the same temperature.

Genomic DNA Extraction: Genomic DNA was extracted using the NucleoSpin® Tissue protocol (Machery-Nagel, Germany) albeit with a few modifications. Sample incubation at 70℃ was lengthened from 10-15 mins to one hour. The 100% ethanol was pre-cooled to 0℃ before addition. Centrifugation times alternated between 1 min and 2 mins. The elution buffer was preheated to 70℃ and was separated into two additions. After the first 50 µl elution buffer was added, the samples were incubated at 70℃ for 5 mins and then centrifuged at 11000 xg for 1 min. This was followed by the addition of the second 50 µl elution buffer then the samples were incubated at room temperature for 1 min and centrifuged at 11000 xg for 2 mins. The result was a 100 µl genomic DNA sample eluted in the microcentrifuge tube. To determine the concentration and purity of the extracted genomic DNA, spectrophotometry and gel electrophoresis using 1% agarose were performed.

Polymerase Chain Reaction: To differentiate between the mutant MDR1 gene and the normal, or wild-type, MDR1 gene, the protocol developed by Asawakarn *et al.* (2012), was utilized. Two PCR amplifications were performed; one using the forward and reverse primers for the mutant (forward and reverse mut P') and the other using forward and reverse primers for the wild type (forward and reverse wt P'). The PCR products

were separated using 2% agarose gel electrophoresis for 30 mins and viewed using Bio-Rad GelDoc XR (Bio-Rad Laboratories, Inc., USA).

DNA Sequencing: DNA sequencing was conducted to verify whether the amplicons were from the target region. Two samples that (1) have A260/280 ratio between 1.8 to 1.9, (2) have DNA concentrations greater than 100 ng/uL, and (3) heterozygous for both wild-type and mutant MDR1 gene were sent out to AsiaGel Corporation for DNA sequencing. After receiving the DNA sequences, a similarity index was conducted using the BLAST option of ncbi.nlm.nih.gov.

Data Analysis: For each breed, the number of samples with a given genotype was divided by the total number of samples for that breed resulting in its genotypic frequency. This was computed for the homozygous wild-type, heterozygous mutant and homozygous mutant genotypes. The allelic frequency is the number of times an allele appears in a population. Thus, the allelic frequency of the wild-type allele was computed by taking the genotypic frequency of the homozygous wild-type and half of the genotypic frequency of the heterozygous. The same was done in the determination of the allelic frequency of the mutant allele.

Results

Genotype Analysis: The resulting gel was documented after PCR was examined to determine the genotype of the samples. Bands at 341 bp present on the wild-type primer set reveal the presence of at least one copy of the wild-type MDR1 gene as shown in Figure 1.

Whereas, bands at 577 bp present on the mutant primer set reveal the presence of at least one copy of the mutant MDR1 gene as shown in Figure 2.

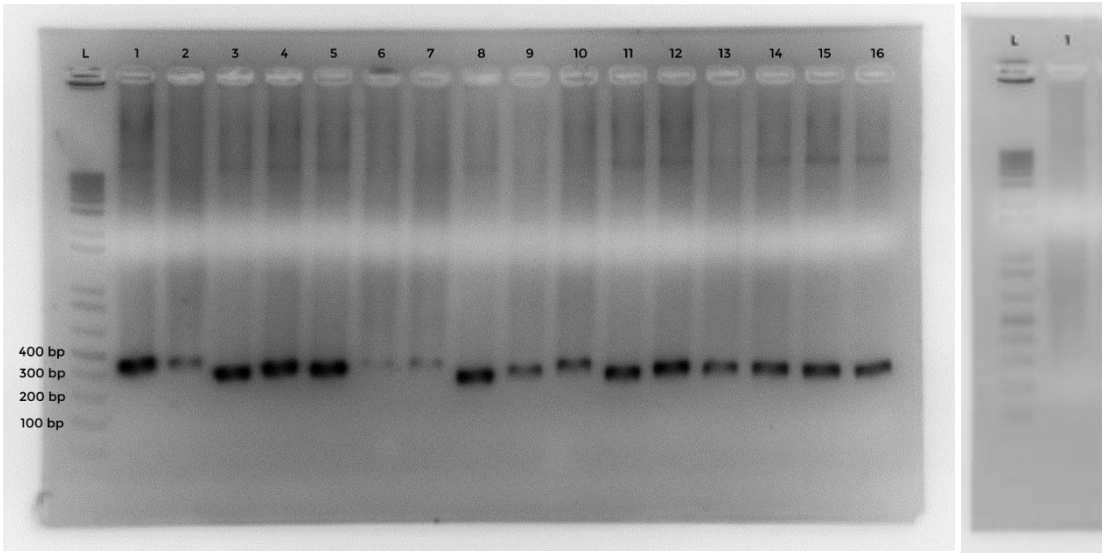
Samples that present bands only on the wild-type primer set are homozygous for the wild-type MDR1 gene. In the same way, samples that present bands only on the mutant primer set are homozygous for the mutant MDR1 gene. Samples that present bands on both sets are heterozygous. Negative control of a documented gel in Figures 1 and 2 was run on a separate gel due to lack of space (Figure 3).

BLAST: The amplicons were verified using BLAST. The canine MDR1 sequence was obtained from the GenBank database (accession no. AF045016). Percent identity is the percentage of the nucleotide sequence that have the same residues and positions as the reference sequence (Fassler and Cooper, 2011). With percent identities ranging from 95.65% to 97.53% (Table 1), we are confident that the target region was amplified.

Genotypic and Allelic Frequency Analysis: The data for herding breeds was pooled due to the small sample size. Table 2 enumerates the breeds in this category.

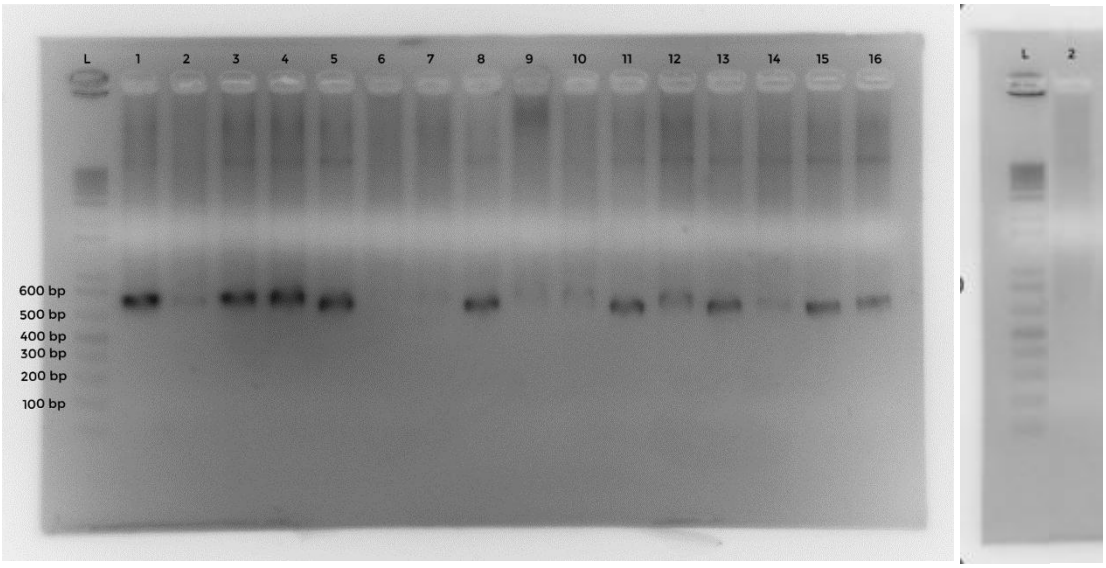
All the sampled Belgian Malinois were found to have the heterozygous MDR1 genotype as opposed to half of the corgis and 80% of German Shepherd dogs. The one Shetland sheepdog sampled was homozygous wild-type. None of the herding breeds were found to be homozygous mutants (Table 2).

All in all, the herding breeds had allelic frequencies of 63.89% for the wild-type MDR1 and 36.11% for the mutant. Most were heterozygous (72.22%) whilst the remaining were homozygous wild-type (27.87%) (Table 3). The Aspins sampled presented with 45% allelic frequency for the MDR1 gene mutation with 85% being heterozygous and 2.5% being a homozygous mutant. Only 12.5% were homozygous wild-type (Table 3). The mixed breeds had a similar allelic frequency of 43.06% for the mutation with 81.08% heterozygous and 2.7% homozygous mutant. 16.22% were homozygous wild-type (Table 3).



(a) (b)

Figure 1 (a) Detection of wild-type MDR1. The presence of bands at 341 bp in lanes 1 to 16 indicates that respective samples possess at least one copy of wild-type MDR1. Lane L represents a 100 bp DNA ladder. (b) Negative control for the detection of wild-type MDR1.



(a) (b)

Figure 2 (a) Detection of mutant MDR1. The presence of bands at 577 bp in lanes 1 to 16 indicates that respective samples possess at least one copy of mutant MDR1. Lane L represents a 100 bp DNA ladder. (b) Negative control for the detection of mutant MDR1.

Table 1 Average percentage identities of sequenced sample with reference sequence Accession No. AF045016

Sample	Allele	Average Percent Identity (%)
Belgian Malinois	Wt	95.04
Belgian Malinois	Mut	95.65
German Shepherd dog	Wt	95.04
German Shepherd dog	Mut	97.53

Legend: Wt = wild-type; Mut = mutant

Table 2 Genotype and allele frequencies for the herding breeds with reference sequence Accession No. AF045016

		Genotypic Frequency			Allelic Frequency	
Breed	**n**	**MDR1 Wt/Wt**	**MDR1 Wt/Mut**	**MDR1 Mut/Mut**	**Wt**	**Mut**
German Shepherd	5	0.20	0.80	0.00	0.60	0.40
Belgian Malinois	6	0.00	1.00	0.00	0.50	0.50
Corgi	6	0.50	0.50	0.00	0.75	0.25
Shetland sheepdog	1	1.00	0.00	0.00	1.00	0.00

Legend: Wt = wild-type; Mut = mutant

Table 3 Genotype and allele frequencies of MDR1 gene in different dog breeds with reference sequence Accession No. AF045016

		Genotypic Frequency			Allelic Frequency	
Breed	**n**	**MDR1 Wt/Wt**	**MDR1 Wt/Mut**	**MDR1 Mut/Mut**	**Wt**	**Mut**
Herding Breeds	18	0.2778	0.7222	0.0000	0.6389	0.3611
Aspin	40	0.1250	0.8500	0.0250	0.5500	0.4500
Mixed	36	0.1622	0.8108	0.0270	0.5694	0.4306
TOTAL	**95**	0.1684	0.8105	0.0211	0.5745	0.4255

Legend: Wt = wild-type; Mut = mutant

As shown in Table 3, the most frequently occurring genotype was the heterozygous MDR1 (81.05%), followed by the homozygous wild-type MDR1 (16.84%), and the lowest observed genotype was homozygous mutant MDR1 (2.11%). In total, the wild-type allele has a frequency of 57.45% whilst the mutant allele has a frequency of 42.55%.

Discussion

Homozygous wild-type dogs possess two copies of the wild-type, or normal, MDR1 gene. Conversely, homozygous mutant dogs have two copies of the mutated MDR1 gene, rendering them sensitive to P-gp substrate drugs. Heterozygous dogs possess one copy of the normal gene and one copy of the mutant gene. These dogs are also at risk of P-gp substrate drug

toxicity although a higher dose is generally required compared to homozygous mutants (Mealey, 2016).

Among the dogs included in this study, eighteen were herding breeds with the Shetland sheepdog and German Shepherd being breeds previously reported to possess the MDR1 gene mutation (Monobe *et al*., 2015; Lerdkrai and Phungphosop, 2021). One out of the five German shepherd dogs sampled were homozygous wild-type whilst the rest were heterozygous. None was found to be a homozygous mutant. This is contrary to similar studies done in Germany, Thailand, the United Kingdom and Brazil where none of their sampled German Shepherd dogs carried the mutant gene (Gramer *et al*., 2011; Asawakarn *et al*., 2012; Tappin *et al*., 2012; Monobe *et al*., 2015). It is, however, comparable with a study in North America, where they discovered the mutant gene in this breed and a more recent study in Israel (Mealey and Meurs, 2008; Dekel *et al*., 2017). Mealey and Meurs (2008) had a sample size of 166 German Shepherd dogs with genotypic frequencies of 90% homozygous wild-type, 8% heterozygous, and 2% homozygous mutant. Dekel *et al*. (2017), sampled 21 German Shepherd dogs with frequencies of 95.2% homozygous wild-type, 4.8% heterozygous and 0% homozygous mutant. Although the current study resulted in 20% homozygous wild-type, 80% heterozygous and 0% homozygous, the sample size was smaller with only five dogs of this breed. Further research by Firdova *et al*., (2016) was necessary to rule out the presence of ABCB1 mutation in American Akitas which were potentially more influenced by crossing with German Shepherds. This explains the skew toward heterozygous German Shepherd dogs but provides more evidence that the MDR1 mutation is indeed present in this breed.

The MDR1 gene mutation is thought to be associated with white-factored German Shepherds wherein the dog expresses white coat color or has a white-colored ancestor. The MDR1 mutation is related to an allele for the white color coat (Mealey and Meurs, 2008; Pires *et al*., 2021). Although an investigation of the individual dog's pedigree was not done in this study, it is a possibility that the dogs sampled were white-factored German Shepherd dogs.

Only one Shetland sheepdog was genotyped and did not have the MDR1 mutation. The allelic frequency of the mutation in this breed has been reported to be 8% in the United States, 30% in Germany, 1% in Japan, 36% in the United Kingdom and 26.9% in Israel (Neff *et al*., 2004; Geyer *et al*., 2005; Kawabata *et al*., 2005; Tappin *et al*., 2012; Dekel *et al*., 2017).

Interestingly, all Belgian Malinois and half of the corgis sampled, breeds previously unreported to have the mutation, were heterozygous (Neff *et al*., 2004; Mealey and Meurs, 2008; Gramer *et al*., 2011). Nevertheless, the DNA sequencing results revealed percent identities ranging from 95.65% to 97.53%. Percent identity is the percentage of the nucleotide sequence that have the same residues and positions as the reference sequence (Fassler and Cooper, 2011). Thus, we are confident that the target region was detected. Moreover, one of the sequenced samples was from a heterozygous Belgian Malinois which further shows that the breed indeed bears the mutation.

The result in the Aspins and mixed breeds is in stark contrast with similar studies that included mixed breed dogs in Israel, Germany and North America (Dekel *et al*., 2017; Gramer *et al*., 2011; Mealey and Meurs, 2008). The MDR1 gene mutation in Dekel *et al*. (2017), had an allelic frequency of 0.9% with no homozygous mutants and only 1.9% heterozygous mixed breeds out of the 594 sampled. Although Gramer *et al*. (2011), expected to find heterozygous MDR1 and homozygous MDR1 mutants, the allelic frequency was only 2% for mixed breeds and 8% for herding breed mixes. None of their mixed breeds was a homozygous mutant and 3% were heterozygous. Whereas, for their herding breed mixes, 2% were homozygous mutant and 12% were heterozygous. The rest were normal. Even though Mealey and Meurs (2008) report relatively higher frequencies of the mutation in mixed breed dogs with an allelic frequency of 7%, 3% homozygous mutant and 8% heterozygous, they are still comparatively lower than the current study.

These results are surprising since many patients are being treated with P-gp substrate drugs in Veterinary Teaching Hospitals with no reported adverse effects. Ivermectin is known to cause neurologic effects in homozygous mutant dogs at doses as low as 100 µg/kg (Nelson *et al*., 2003). This is much lower than the dose for deworming used in the hospital at 250 µg/kg and even more compared to the dose for the treatment of mange at 300 µg/kg. Mealey (2016b), however, has been able to administer this drug in heterozygous mutant dogs for the treatment of sarcoptic mange and demodicosis (300-600 µg/kg) successfully. Moreover, in a study by Bissonnette *et al*. (2009), a heterozygous mixed breed treated with 670 µg/kg ivermectin daily did not experience neurotoxicity until seven weeks into treatment concluding, therefore, that increased ivermectin-sensitivities of heterozygous dogs remains to be formally proven through further clinical studies. Having one wildtype MDR1 allele may provide protection even in high doses of ivermectin (Geyer and Janko, 2012). P-gp substrates such as ivermectin, technetium 99-m, loperamide, acepromazine and others have been documented in dogs homozygous for MDR1-1Δ resulting in increased susceptibility to adverse effects compared to dogs with normal P-gp function (Deshpande *et al*., 2016).

Interbreed variation may also play a role in the lack of reported adverse effects. This is demonstrated in a study by Han *et al*. (2010), where they compared wild-type Beagles' sequences to wild-type Border collies. Keep in mind that these are wild-type Border collies; they do not experience adverse reactions to P-gp substrate drugs. Eight single nucleotide polymorphisms are found in all the wild-type Border collies in comparison to the wild-type Beagles. P-gp is expressed by enterocytes within the gastrointestinal tract and by renal tubular cells, it is also possible that oral bioavailability and renal excretion of P-gp substrates could differ in dogs homozygous for MDR1-1Δ, as compared to wild-type dogs (Heit *et al*., 2021).

Non-recognition of clinical signs by pet owners also contributes to the lack of reported adverse drug reactions. In the case of ivermectin neurotoxicity, clinical signs can range from excessive drooling,

lethargy and weakness to ataxia, seizures and coma (Bissonnette *et al*., 2009; Merola and Eubig, 2012). They may take several hours to days or even weeks to develop (Bissonnette *et al*., 2009). Multiple factors may also prevent or dissuade owners from seeking veterinary care even when their pet's condition is dire (Lue *et al*., 2008). It can take multiple occurrences of unusual signs for pet owners to communicate these to their veterinarians (Monobe *et al*., 2013). Furthermore, genetic testing is unavailable at the Veterinary Teaching Hospital, nor is it available in most veterinary clinics and hospitals in the Philippines. As a result, without sufficient evidence for the diagnosis of adverse drug reaction due to MDR1 gene mutation, the condition may be under or misdiagnosed by veterinarians.

In conclusion, The MDR1 gene mutation was found in many breeds of dogs presented at the University of the Philippines Veterinary Teaching Hospital. These include herding breeds not previously noted to have the mutation, Belgian Malinois and corgis, although they were heterozygous. Surprisingly, a large percentage of Aspins were found to possess at least one copy of the mutation. Even though there have been no reports of adverse drug reactions, caution is advised in the treatment of these breeds with P-gp substrate drugs because of the high percentage of patients with the MDR1 gene mutation. The presence of the MDR1 gene mutation in other breeds in the Philippines should be explored given the high percentage of mixed breeds carrying it. Further investigation should be done to demystify the relationship between dogs heterozygous for the MDR1 mutation and the occurrence of adverse drug reactions. A larger sample size may also be of benefit for future studies.

Author's contribution: KSYW, JMDD, and JFC conceived and designed the experiments. KSYW performed the experiments. All authors analysed the data. KSYW and LP writing of the manuscript. All authors critically revised the manuscript and approved the final version.

Acknowledgements

The authors thank the animal owners for providing blood samples and/or buccal swabs from their dogs and for their helpful cooperation.

References

Archer TM, Boothe DM, Langston VC, Fellman CL, Lunsford KV and Mackin AJ 2014. Oral Cyclosporine Treatment in Dogs: A Review of the Literature. J Vet Intern Med 28:1-20.

Asawakarn S, Ruangchaiprakarn V, Srisowanna N, Wongwan L, Kanuengthong A and Suriyaphol G 2012. Determination of multidrug resistance (MDR1) gene and its mutations in dogs by using polymerase chain reaction. Thai J Vet Med. 42 (1): 37-42.

Bissonnette S, Paradis M, Daneau I and Silversides DW 2009. The ABCB1-1Δ mutation is not responsible for subchronic neurotoxicity seen in dogs of non-collie breeds following macrocyclic lactone treatment for generalized demodicosis. Vet Derm. 20 (1): 60-66.

Dekel Y, Machluf Y, Stoler A, Aderet A, Baumel D, Kellerman E, Plotsky Y, Partouche ON, Elhalal G, Ben-Shlomo I and Bercovich D 2017. Frequency of canine nt230(del4) MDR1 mutation in prone pure breeds, their crosses, and mongrels in Israel - insights from a worldwide comparative perspective. BMC Vet Res. 13: 333.

Deshpande D, Hill KE, Mealey KL, Chambers JP and Giesag MA 2016. The effect of the canine ABCB1-1D mutation on sedation after intravenous administration of acepromazine. J Vet Int Med., 30 (2): 636–641.

Donner J, Kaukonen M, Anderson H, Möller F, Kyöstilä K, Sankari S, Hytönen M, Giger U and Lohi H 2016. Genetic panel screening of nearly 100 mutations reveals new insights into the breed distribution of risk variants for canine hereditary disorders. PLoS ONE 11(8):e0161005. doi:10.1371/journal.pone.0161005

Fassler J and Cooper P 2011. BLAST Glossary. https://www.ncbi.nlm.nih.gov/books/NBK62051/. Accessed on 23 March 2022.

Firdova Z, Turnova E, Bielikova M, Turna J and Dudas A 2016. The prevalence of ABCB1:c.227_230delATAG mutation in affected dog breeds from European countries. Res Vet Sci. 106:89-92 https://doi.org/10.1016/j.rvsc.2016.03.016

Geyer J, Döring B, Godoy JR, Leidolf R, Moritz A and Petzinger E 2005. Frequency of the nt230(del4) MDR1 mutation in Collies and related dog breeds in Germany. J Vet Pharm Ther. 28: 545–551.

Geyer J and Janko C. 2012. Treatment of MDR1 Mutant Dogs with Macrocyclic Lactones. Curr Pharm Biotech. 13: 969-986. https://doi.org/10.2174%2F138920 1128 00399301

Gramer I, Leidolf R, Doring B, Klintzsch S, Kramer E, Yalcin E, Petzinger E and Geyer J 2011. Breed distribution of the nt230(del4) MDR1 mutation in dogs. The Vet J. 189: 67-71.

Han J, Son H, Park S and Na K 2010. A novel insertion mutation of ABCB1 gene in an ivermectin-sensitive Border Collie. J Vet Sci. 11 (4): 341-344.

Heit MC, Mealey KL and King SB 2021. Tolerance and Pharmacokinetics of Galliprant™ Administered Orally to Collies Homozygous for MDR1-1Δ. J Vet Pharmacol Ther. 44(5):705–713. https://doi.org/10.1111%2Fjvp.12984

Kawabata A, Momoi Y, Inoue-Murayama M and Iwasaki T 2005. Canine mdr1 gene mutation in Japan. J Vet Med Sci. 67: 1103–1107.

Lee JJ, Lin HY, Chen CA, Lin CS and Wang LC 2019. Development of an oligonucleotide microarray for simultaneous detection of two canine MDR1 genotypes and association between genotypes and chemotherapy side effects. J Vet Sci. 20 (1): 27-33. https://doi.org/10.4142%2Fjvs.2019.20.1.27

Lerdkrai C and Phungphosop N 2021. Prevalence of the MDR1 gene mutation in herding dog breeds and Thai Ridgebacks in Thailand. Vet World 14 (11): 3015–3020 https://doi.org/10.14202%2Fvetworld.2021.3015-3020

Lue TW, Patenburg DP and Crawford PM 2008. Impact of the owner-pet and client-veterinarian bond on the care that pets receive. J American Vet Med Ass. 232: 531-540.

Marelli SP, Polli M, Frattini S, Cortellari M, Rizzi R and Crepaldi P 2020. Genotypic and allelic frequencies of MDR1 gene in dogs in Italy. Vet Rec Open 7 (1): 1-5. https://doi.org/10.1136%2Fvetreco-2019-000375

Mealey KL 2016. How Should I Treat Dogs & Cats with MDR1 Mutation. Plumb's Therapeutics Brief 12-23.

Mealey K and Meurs K 2008. Breed distribution of the ABCB1-1Δ (multidrug sensitivity) polymorphism among dogs undergoing ABCB1 genotyping. J American Vet Med Ass 233 (6): 921-924.

Merola VM and Eubig PA, 2012. Toxicology of avermectins and milbemycins (macrocyclic lactones) and the role of P-Glycoprotein in dogs and cats. Vet Clinics of North America: Small Anim Prac. 42 (2): 313–vii. doi:10.1016/j.cvsm.2011.12.005.

Monobe MM, Lunsford KV, Araujo JP and Bulla C 2013. Detection of heterozygous MDR1 nt230(del4) mutation in a mixed-breed dog: case report of possible doxorubicin toxicosis. Vet Med Res Rep. 4: 35-38.

Monobe MM, Araujo JP, Lunsford KV, Silva RC and Bulla C 2015. Frequency of the MDR1 mutant allele associated with multidrug sensitivity in dogs from Brazil. Vet Med Res Rep. 6: 111-117.

Neff MW, Robertson KR, Wong AK, Safra N, Broman KW, Slatkin M, Mealey KL and Pedersen NC 2004. Breed distribution and history of canine MDR1-1Δ, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. Proc. of the National Academy of Sciences of the United States of America 101 (32): 11725-11730.

Nürnberger D, Müller SF, Hamann M and Geyer J 2021. First Sequencing of Caprine Mdr1 (Abcb1) mRNA Due to Suspected Neurological Adverse Drug Reaction in a Thuringian Goat Following Extra-Label Use of Doramectin. Front Vet Sci. 8: 1-9. https://doi.org/10.3389/fvets.2021.682393

Parker HG, Dreger DL, Rimbault M, Davis BW, Mullen AB, Carpintero-Ramirez G and Ostrander EA 2017. Genomic analyses reveal the influence of geographic origin, migration, and hybridization on modern dog breed development. Cell Reports 19: 697-708.

Pires ADR, Gerardi D, Tisotti TDM, Serpa PBDS and Natalini CC 2021. First report of nt230(del4) mutation in the MDR1 gene in German Shepherds in Southern Brazil. Ciência Rural Santa Maria 51: 1-5 http://doi.org/10.1590/0103-8478cr20200690

Roulet A, Puel O, Gesta S, Lepage J, Drag M, Soll M, Alvinerie M and Pineau T 2003. MDR1-deficient genotype in collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. European J Pharm. 460: 85-91.

Seelig A 2020. P-Glycoprotein: One mechanism, many tasks, and the consequences for pharmacotherapy of cancers. Front Oncol. 1-16.

Tappin SW, Goodfellow MR, Peters IR, Day MJ, Hall EJ and Mealey KL 2012. Frequency of the mutant MDR1 allele in dogs in the UK. Short Communications 171: 72. doi: 10.1136/vr.100633.