

## The Multi Drug Resistance Gene

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A problem with the multi drug resistance gene (MDR-1 also known as ABCB1) was first noticed when a worming drug called ivermectin was given to Rough Collies and unexpectedly caused severe neurological signs and deaths in some dogs, where as other Rough Collies and dogs of other breeds appeared fine. Dr Mealey investigating at the Washington State University eventually found that the susceptible Rough Collies had abnormal copies of the multi drug resistance gene. The multi drug resistance gene stores the genetic information for a cellular pump called p-glycoprotein. This pump protects the brain and other organs in the body, by pumping drugs and toxins out of tissues and preventing them from accumulating in high concentrations. If this gene is abnormal then the pump will not be produced normally and drugs and toxins are not pumped out of the cells as they should be which can lead to toxicity. As a result, affected Rough Collies accumulated much more ivermectin in their brain cells than normal, leading to severe toxic effects such as seizures. Further investigations revealed that many other drugs were also transported by the p-glycoprotein transporter and these include some of the more commonly used veterinary drugs such as heart drugs, analgesics and chemotherapy agents, these can lead to unexpected side effects if the MDR mutation is present.

### **Drugs with documented problems in dogs with the MDR1 mutation:**

- **Acepromazine (ACP)** is a drug commonly used as a sedative in veterinary medicine. Dogs with the MDR1 mutation tend to have longer and more profound sedation. It is suggested to reduce the dose by 25% in heterozygotes and 50% in homozygotes.
- **Butorphanol** is another commonly used sedative and cough suppressant. In dogs with MDR1 mutations prolonged sedation is seen and similar dose reductions to ACP are suggested.
- **Erythromycin** is an antibiotic which is occasionally used for specific infections. There are case reports of this drug causing neurological signs in homozygote Rough collies, thus this drug is best avoided in dogs with the MDR1 mutation.
- **Ivermectin** is used in the USA to treat heartworm. This is not endemic in the UK, thus this drug is not needed for this purpose in the UK. It is sometimes used to treat mange but should not be used in dogs with the MDR mutation as it will lead to neurological signs.

- **Loperamide (Imodium)** is occasionally used to treat diarrhoea in dogs. It should not be used in dogs with the MDR1 mutation as it causes neurological signs.
- **Selamectin (Stronghold), Milbemycin (Milbemax) and Moxidectin (Advocate)** these are all antiparasitic agents and have been documented to cause neurological signs at high doses. These drugs are safe to use in dogs with the MDR mutation if applied following the manufacturers recommendations.
- **Vincristine, Vinblastine and Doxorubicin** are all chemotherapy agents, used in the treatment of a variety of tumours. Ongoing research suggests that normal tissues in dogs with the MDR1 mutation are more sensitive to these drugs, increasing the likelihood of an adverse reaction. This is most likely to manifest as gastrointestinal effects (vomiting, diarrhoea and inappetence) or bone marrow suppression (reduced white blood cell numbers). Dose reductions of 25-30% are typically made for dogs with the mutation.

**Drugs which use p-glycoprotein (the product of the MDR1 gene) but known to be safe in dogs with MDR1 mutations:**

- **Cyclosporin (Atopica)** this is an immunosuppressive agent used in the management of a wide range of conditions such as allergic skin disease (Atopy) and inflammatory bowel disease. No adverse reactions have been noted in dogs with the MDR1 mutation.
- **Digoxin** is a commonly used drug for the management of cardiac arrhythmias such as atrial fibrillation. Levels are usually carefully monitored to be within a therapeutic range. Dogs with MDR1 mutations appear to be able to tolerate this drug well, if levels are maintained within the normal therapeutic window.
- **Doxycycline** is an antibiotic used for treating specific infections (e.g. Borrelia which causes Lyme's disease). No adverse reactions have been recorded and dose reductions are not suggested.
- **Morphine, Buprenorphine and Fentanyl** are all opioid analgesic drugs. They have been reported to use p-glycoprotein pumps in people, but we are not yet sure if this is the case in dogs. No adverse reactions have been reported to these drugs in dogs with the MDR1 mutation, thus dose reductions are not suggested.

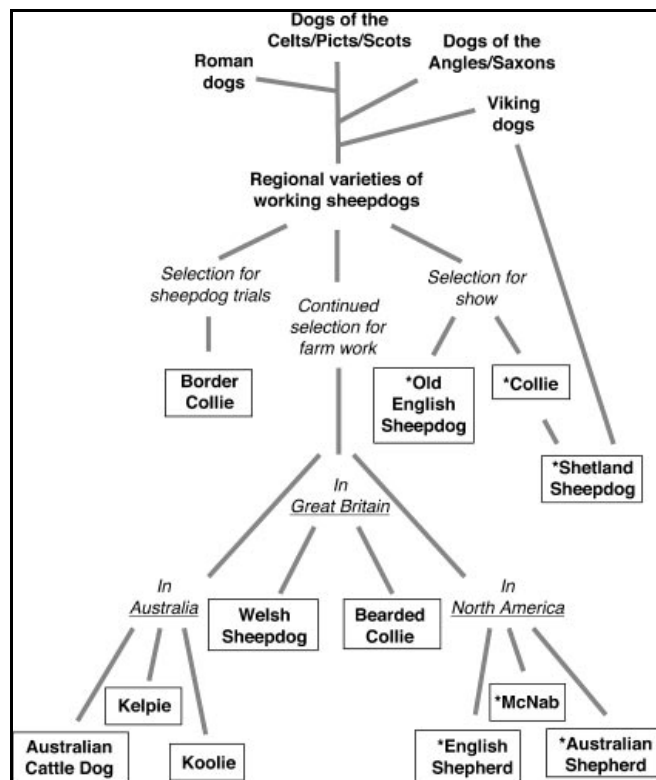
**Drugs which use P-glycoprotein in people and problems have been reported, but where there is no data in veterinary medicine:**

- Ondansetron
- Ketoconazole
- Mitoxantrone
- Verapamil
- Rifampicin

Since this discovery several other breeds, these include:

- Australian Shepherd
- Border Collie
- Cross-breeds (esp. of herding origin)
- English Shepherd
- German Shepherd Dogs (Possible linkage to white hair coat?)
- Long-haired Whippet
- McNab
- Old English Sheepdog
- Rough Collie
- Shetland Sheepdog
- Sliken Windhound

Almost all the breeds found to carry the MDR1 mutation are of herding origin and using linkage DNA studies it is possible to theorise that the mutation arose in the UK in the late 1800's.



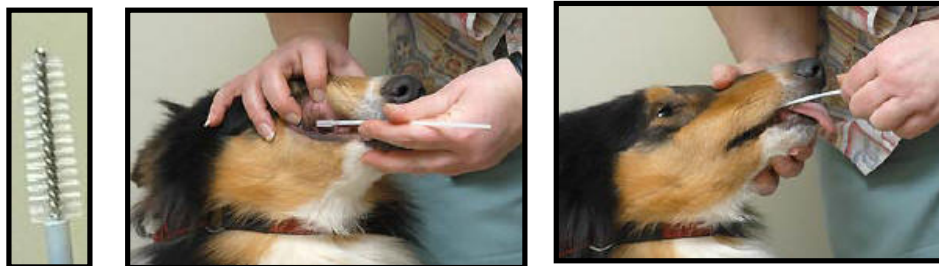
**Figure 1:** Breed heritage and selection, \* documents breeds with known MDR1 mutations. Taken from: Neff, M.W., Robertson, K.R., Wong, A.K., Safra, N., Broman, K.W., Slatkin, M., Mealey, K.L. & Pedersen, N.C. (2004) Breed distribution and history of canine *mdr1-1A* a pharmacogenetics mutation that marks the emergence of breeds from the collie lineage. *Proceedings of the National Academy of Science of the United States of America* **101**, 11725-11730

Whilst completing my residency at the University of Bristol and in collaboration with Washington State University, we performed a study investigating the prevalence of the MDR-1 mutation in the UK. In total 642 dogs were tested and we found the mutation in 6 breeds (Rough Collies, Smooth Collies, Shetland Sheepdogs, Australian Shepherds, Old English Sheepdogs, Border Collies). These results are similar to previous studies and suggest these breeds have a similar ancestry. In the study 249 Shetland sheepdogs were tested, revealing 12% were affected, 47% were carriers and 41% were normal. This paper is currently pending publication in the Journal of Small Animal Practice.

Breed	Allele %	Mutant/Mutant	Mutant/Normal	Normal/Normal	Total
Australian shepherd	46.4	7 (25%)	12 (43%)	9 (32%)	28
Border collie	2.3	0	2 (5%)	41 (95%)	43
Old English sheepdog	10.6	0	7 (21%)	26 (79%)	33
Rough collie	70.7	15 (52%)	11 (38%)	3 (10%)	29
Shetland sheepdog	35.7	6 (12%)	23 (47%)	20 (41%)	49
Smooth collie	72.7	5 (45%)	6 (55%)	0	11

**Figure 2:** Frequencies of the MDR1 mutation in affected breeds (Tappin *et al*, 2008)

The only way of knowing if a dog has normal or abnormal p-glycoprotein is a DNA based test run from a cheek swab, or in some cases a blood sample, and costs about £70. Cheek swabs are easy to take and it's not a painful procedure for your dog.



**Figure 3:** A DNA brush. To take samples the upper lip is lifted the brush rolled against the lip, allowed to dry and sent back to the laboratory for analysis.

By knowing the MDR status, it is possible to predict the MDR status of progeny. While it is ideal to use only "Normal/Normal" breeding pairs, one must always consider other genetic factors in addition to the MDR1 gene. Because the MDR1 gene is present in such a large percentage of Collies and Australian Shepherds, it may be necessary to breed "Normal/Mutant" dogs in order to maintain a large enough pool of good breeding stock. By using thoughtful breeding strategies including these guidelines, future generations of dogs will have a substantial decrease in the frequency of the mutant MDR1 gene. Figure 4 provides guidelines for consideration when owners are contemplating breeding dogs that may be affected by the MDR1 mutation.

	<b>Normal/Normal Male</b>	<b>Normal/Mutant* Male</b>	<b>Mutant/Mutant Male</b>
<b>Normal/Normal Female</b>	100% Normal/Normal puppies	Normal/Normal and/or Normal/Mutant puppies	100% Normal/Mutant puppies
<b>Normal/Mutant* Female</b>	Normal/Normal and/or Normal/Mutant puppies	Any combination of puppies	Normal/Mutant and/or Mutant/Mutant puppies
<b>Mutant/Mutant Female</b>	100% Normal/Mutant puppies	Normal/Mutant and/or Mutant/Mutant puppies	100% Mutant/Mutant puppies

**Figure 4:** MDR1 Breeding combinations and outcomes.\*Normal/mutant is the same as mutant/normal and "heterozygote"

Further information about the MDR-1 mutation is available from:

<http://www.laboklin.co.uk/laboklin/showGeneticTest.jsp?testID=8032>

<http://www.vetmed.wsu.edu/depts-VCPL/>

## **Frequency of the mutant MDR1 allele in dogs in the United Kingdom**

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### **Keywords**

Multi drug resistance gene (MDR1), ABCB1 gene, p-glycoprotein, drug sensitivity

**Summary:**

**Objectives:** To investigate the presence and prevalence of the *MDR1* mutation within different breeds of dog in the United Kingdom (UK).

**Methods:** DNA was collected, with owner consent, from buccal swabs or residual blood samples from 642 dogs of 40 different dog breeds including 250 dogs representing eight pastoral breeds. The *MDR1* gene was amplified using polymerase chain reaction (PCR) and reaction products were analysed by amplified fragment length polymorphism (AFLP) to determine MDR1 genotype.

**Results:** The *MDR1* mutation was found in six pastoral breeds with varying allelic frequency; Smooth collies (allelic frequency 72.7%), Rough collies (70.7%), Australian Shepherds (46.4%), Shetland Sheepdogs (35.7%), Old English Sheepdogs (10.6%) and Border collies (2.3%). The mutation was not identified in dogs of the other 34 breeds tested.

**Clinical Significance:** These results estimate the prevalence of the *MDR1* mutation within pastoral canine breeds in the UK. These data may guide therapeutic decisions in dogs of these breeds and suggest that further screening of dogs of these breeds is justified.

## **Introduction**

Administration of ivermectin causes neurotoxicosis in some, but not all Rough Collies, and toxicity in these dogs occurs at doses much lower than those required to cause toxicity in other breeds (Pulliam and others 1985, Paul and others 1987). This susceptibility to ivermectin is caused by a frame-shift mutation within the multi-drug resistance (*MDR1*) gene (also referred to as *ABCB1*), which leads to the generation of a premature stop codon and the production of incomplete P-glycoprotein (Mealey and others 2001, Roulet and others 2003). P-glycoprotein is an ATP-dependent drug transporter, which forms an essential part of the blood-brain barrier (Fromm 2000). Expressed on the luminal side of brain capillary endothelial cells, P-glycoprotein functions to transport a variety of substrates (including ivermectin) entering brain tissue, back into the capillary lumen.

Many drugs are transported by P-glycoprotein and the toxic potential in dogs with the *MDR1* mutation is not limited to ivermectin, and has been reported with a wide variety of P-glycoprotein substrates including vincristine and doxorubicin (Mealey and others 2003, Mealey and others 2008), loperamide (Sartor and others 2004), moxidectin (Geyer and others 2005a), digoxin and mexiletine (Henik and others 2006). Although most dogs with adverse reactions to such drugs have been homozygous for the *MDR1* mutation, heterozygote animals have been shown to have increased haematological toxicity to vincristine, leading to treatment delays and increased morbidity and mortality (Mealey and others 2008).

The prevalence of Rough Collies with heterozygous *MDR1* mutations is high, with the frequency of affected dogs varying slightly between geographical locations. Studies have revealed an allelic frequency of 64% in Rough Collies in France (Hugnet and others 2004), 60% in the United Kingdom (UK; Neff and others 2004), 56% in the United States (Mealey and Meurs 2008) and 55% in Germany (Geyer and others 2005b). The *MDR1* mutation documented in Rough Collies has since been discovered within several other related breeds including Australian Shepherd Dogs (Nelson and others 2003), Border Collies (Geyer and others 2005), Old English Sheepdogs, Shetland sheepdogs (Neff and others 2004) and more recently German Shepherd dogs (Mealey and Meurs 2008).

Information from breed histories and linkage disequilibrium studies have suggested that the *MDR1* mutation arose in the UK during the 19<sup>th</sup> Century (Neff and others 2004). Neff and others (2004) found higher frequencies of the *MDR1* mutant allele in British Rough Collies, Old English Sheepdogs and Shetland Sheepdogs compared with populations from the USA, thus the aim of this study was to further investigate the presence and prevalence of the *MDR1* mutation within canine breeds in the UK.

### **Materials and Methods**

DNA was collected from buccal swabs or residual blood obtained from a total of 642 dogs. Dogs from eight pastoral (n=250 dogs), nine gundog (n=156 dogs), six hound (n=53 dogs), three terrier (n=51 dogs), four working (n=49 dogs), five toy (n=48 dogs) and five utility breeds (n=35 dogs) were investigated.

One hundred and ninety three dogs were recruited actively to the study by approaching members of breed societies from which the *MDR1* mutation had been documented previously (Rough, Smooth and Border collies, Old English and Shetland Sheepdogs and Australian shepherds). Buccal DNA samples were taken by the owners of these dogs and submitted via the post. Only one sample was submitted per household in an effort to reduce the possibility of bias from sampling large numbers of dogs within the same family.

In addition, 449 samples were derived from residual blood samples taken during routine investigations at the Small Animal Hospital, School of Veterinary Sciences, University of Bristol. These samples were from breeds in which *MDR1* mutations had not been reported previously. DNA was extracted from 200µl of EDTA anticoagulated, residual whole blood using the Macherey-Nagel, NucleoSpin 96 Blood Kit (ABgene Ltd., Epsom, Surrey, UK) as per the manufacturer's protocol.

DNA from cheek swab samples was extracted using methods published previously (Richards and others 1993). For the 25 µL polymerase chain reaction (PCR), 1 µL of the extracted DNA was used as template for PCR amplification under the following conditions: initial denaturation at 93 °C for 2 min; 31 cycles consisting of 93 °C × 20 sec,

55 °C × 20 sec, 72 °C × 1 min; followed by a 4 °C hold. Each PCR reaction tube contained 1× reaction buffer (Promega, Madison, WI, USA), 0.2 mM each dNTP (MBI, Hanover, MD, USA), 2.0 mM MgCl<sub>2</sub>, 0.5 μM each primer, 0.5 U *Taq* polymerase (Promega). The primers (IDT, Coralville, IA, USA) used for amplified fragment length polymorphism (AFLP) spanned the mutation area, base pairs 294–297 of the canine *MDR1* gene (GenBank [AF045016](#)), generating 148 and 144 bp products for the wild type and mutant alleles, respectively. The primer sequences were as follows: forward primer (*MDR1* base pairs 200–225) 5'-GGC TTG ATA GGT TGT ATA TGT TGG TG; reverse primer, which was 5-FAM labeled, (*MDR1* base pairs 347–323), 5'-ATT ATA ACT GGA AAA GTT TTG TTT.

5-FAM-labeled amplicons were detected using AFLP as follows. Briefly, 2 μL of PCR product was mixed with 5 μL of loading buffer containing fluorescent ladder (Applied Biosystems, Foster City, CA, USA), denatured for 2 min at 95 °C, and maintained at 5 °C. Aliquots (1.5 μL) were loaded onto a polyacrylamide gel, subjected to electrophoresis, and analyzed on an ABI 377 instrument (Applied Biosystems). DNA fragment analysis was performed using Genescan 3.1.2 software (Applied Biosystems).

All samples were taken with owner consent and the results of the buccal swabs were reported to the owners. This study was approved by the institutional ethics committees.

## Results

The *MDR1* mutation was found in six breeds (Rough, Smooth and Border collies, Old English and Shetland Sheepdogs and Australian shepherds) which are all pastoral breeds of collie descent (Table 1). The allelic frequency of the *MDR1* mutation varied between these breeds; it was highest for Rough (70.7%) and Smooth (72.7%) collies, followed by Australian shepherds (46.4%), Shetland sheepdogs (35.7%), Old English sheepdogs (10.6%) and Border collies (2.3%). Homozygous mutant *MDR1* alleles were found in 52% of the Rough collies, 45% of the Smooth collies, 25% of the Australian shepherds and 12% of the Shetland sheepdogs. No homozygous mutant *MDR1* alleles were found in the 43 Border collies or 33 Old English sheepdogs included in this study indicating a low incidence of the *MDR1* mutation in these breeds.

The *MDRI* mutation was not found in German Shepherd dogs (n=41), a breed in which the mutation has been documented recently at a low allelic frequency (6%) in the USA (Mealey and Meurs 2008). Similar to previous studies, the *MDRI* mutation was not found in the Bearded Collie, despite it being a British descendant of working sheepdogs. The mutation was not present in any of the remaining 32 breeds included in this study (Table 2).

## **Discussion**

This study documents the *MDRI* mutation within six UK pastoral (Herding) breeds. These comprise the Rough, Smooth and Border collies, Old English and Shetland Sheepdogs and Australian shepherds. Documentation of the *MDRI* mutation was expected within these breeds as they had been shown in previous studies to carry the mutation (Neff and others 2004, Geyer and others 2005, Mealey and Meurs 2008). The present study did not document the *MDRI* mutation in the Bearded collie, which shares a close breeding history to other herding breeds that do contain the mutation, or any other breeds which share a close genetic relationship (Parker and others 2004).

The *MDRI* mutation was not found in UK German Shepherd dogs, but has been documented at low allelic frequency (6%) in this breed in the USA (Mealey and Meurs 2008). German shepherd dogs are not thought to be of collie descent, in comparison with all other breeds in which the *MDRI* mutation has been documented. However the European breed of white Swiss Shepherds are thought to have descended from the collie and have been found to carry the *MDRI* mutation (Geyer and others 2005b). As white Swiss Shepherds are not a defined breed in the USA and all of the dogs reported by Mealey and Meurs had white coats or were born of white-coated parents, it may be that the *MDRI* mutation is linked to the white coat colour. No white-coated German shepherd dogs were sampled during the course of our study and further work is needed to establish this link.

The allelic frequency of the mutant *MDRI* gene appears slightly higher for all breeds when compared with previous studies from the USA, France, Germany and Japan and Australia (Table 3). Previous studies have suggested that the *MDRI* mutation arose in the

herding dog population of the UK in the late 19<sup>th</sup> century (Neff and others 2004). Thus it is likely that the higher numbers found in this study reflect a higher ancestral concentration of the *MDR1* mutation within the UK. Due to the study design, recruitment of affected breeds was based on owner interest rather than randomised sampling; so it is possible that a bias was introduced during recruitment. Although this may overestimate the prevalence within the study populations, this recruitment bias is likely to be similar to previous studies where samples were taken either for diagnostic purposes or through owner interest.

The numbers present in this study are relatively small and do not achieve numbers needed to accurately estimate breed prevalence. However, the results obtained document the presence of the *MDR1* mutation within specific breeds and give a starting point for prevalence studies within the UK. The prevalence of the *MDR1* mutation within herding breeds in the UK highlights to clinicians that determining *MDR1* status in suspected breeds is important and in the absence of this knowledge this may impact on therapeutic decision making.

### **Acknowledgements**

The authors would like to thank Steve Bentjen for his help processing the samples.

### **Conflicts of Interest**

The authors declare that a patent for this technology is held by Washington State University listing one of the authors (KLM) as inventor, which makes her eligible for royalty payments.

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<b>Breed</b>	<b>Allele %</b>	<b>Mutant/Mutant</b>	<b>Mutant/Normal</b>	<b>Normal/Normal</b>	<b>Total</b>
<b>Australian shepherd</b>	46.4	7 (25%)	12 (43%)	9 (32%)	28
<b>Border collie</b>	2.3	0	2 (5%)	41 (95%)	43
<b>Old English sheepdog</b>	10.6	0	7 (21%)	26 (79%)	33
<b>Rough collie</b>	70.7	15 (52%)	11 (38%)	3 (10%)	29
<b>Shetland sheepdog</b>	35.7	6 (12%)	23 (47%)	20 (41%)	49
<b>Smooth collie</b>	72.7	5 (45%)	6 (55%)	0	11

**Table 1:** Frequencies of the MDR1 mutation in affected breeds

<b>Breed</b>	<b>No.</b>	<b>Breed</b>	<b>No.</b>
Basset Hound	6	Great Dane	12
Bearded Collie	16	Greyhound	7
Bichon Frise	4	Irish Setter	9
Border Terrier	5	Irish Wolfhound	6
Boxer	18	Jack Russell Terrier	24
Bull Terrier	7	Labrador Retriever	47
Cavalier King Charles Spaniel	14	Lurcher	13
Cocker Spaniel	21	Newfoundland	7
Dachshund	7	Rottweiler	9
Dalmatian	7	Shih Tzu	5
Doberman	10	Staffordshire Bull Terrier	12
English Springer Spaniel	30	Standard Poodle	5
Flat coated retriever	7	Toy Poodle	11
German Shepherd Dog	41	Weimaraner	4
German Short Haired Pointer	5	Whippet	14
Gordon Setter	1	West Highland White Terrier	20
Golden Retriever	32	Yorkshire Terrier	13

**Table 2:** Breeds tested in which no MDR mutations were found

		USA <sup>a</sup>	Germany <sup>b</sup>	Australia <sup>c</sup>	Japan <sup>d</sup>	France <sup>e</sup>
<b>Collie</b>	AF	56%	55%	56%	58%	64%
	MDR1 (+/+)	22.6%	23.9%	12.1%	25.0%	20.0%
	MDR1 (+/-)	42.0%	43.1%	63.6%	33.3%	32.0%
	MDR1 (-/-)	35.4%	33.0%	24.3%	41.7%	48.0%
	n	1424	578	33	12	25
<b>Shetland Sheepdog</b>	AF	7%	30%	21%	1%	
	MDR1 (+/+)	88.2%	45.7%	57.1%	97.6%	
	MDR1 (+/-)	10.5%	48.6%	42.9%	2.4%	
	MDR1 (-/-)	1.3%	5.7%	0%	0%	
	n	448	140	7	42	
<b>Australian Shepherd</b>	AF	29%	20%	43%	33%	
	MDR1 (+/+)	53.0%	67.9%	35.7% <sup>1</sup>	44.4%	
	MDR1 (+/-)	37.0%	25.2%	42.8%	44.4%	
	MDR1 (-/-)	10.0%	6.9%	21.5%	11.2%	
	n	1421	333	14	9	
<b>Border Collie</b>	AF	1%	1%			
	MDR1 (+/+)	98.4%	99.1%			
	MDR1 (+/-)	1.3%	0.6%			
	MDR1 (-/-)	0.3%	0.3%			
	n	306	334			
<b>Old English Sheepdog</b>	AF	1%	6%			
	MDR1 (+/+)	97.5%	87.5%			
	MDR1 (+/-)	2.5%	12.5%			
	MDR1 (-/-)	0%	0%			
	n	40	24			

**Table 3:** Frequency of the MDR1 mutation as reported in previous studies. AF = Allelic frequency, n = number of dogs included in the study. <sup>a</sup>Mealey and Meurs 2008, <sup>b</sup>Geyer and others 2005b, <sup>c</sup>Mealey and others 2005, <sup>d</sup>Kawabata and others 2008, <sup>e</sup>Hugnet and others 2004