Canine multi-drug resistance-1 mutation prevalence: A South African perspective

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The multi-drug resistance (mdr-1) gene mutation is a phenomenon well known to current veterinary practitioners. The mutation causes a predisposition for, amongst other phenomena, macrocyclic lactone-induced neurotoxicosis in affected canines, a condition that can be fatal. Various herding dog breeds can be heterozygous or homozygous for the mutation, and prevalence differs only slightly in dog populations between geographical regions. This report provides prevalence data of the canine mdr-1 mutation in 306 South African dogs.

The multi-drug resistance (mdr-1 or ABCB1) gene encodes for the large transmembrane protein, P-glycoprotein, which is, amongst other things, an integral part of the blood–brain barrier (Geyer & Janco 2012). A mutation that occurs in the gene is associated with increased sensitivity towards certain drugs, typically observed in herding dog breeds.

The effects of the mutation in the mdr-1 gene are well known to veterinary practitioners and are often the reason for non-utilisation of certain drugs, in particular the avermectins (macrocyclic lactones). Clinical signs of macrocyclic lactone-induced neurotoxicosis are associated with diffuse cerebral and cerebellar dysfunction caused by increased gamma-aminobutyric acid (GABA) inhibitory activity. The clinical presentation varies and is generally dose dependent (Geyer & Janco 2012). There is currently no specific safe and effective antidote available to treat this neurotoxicity and symptomatic and supportive treatment is indicated (Geyer & Janco 2012). Toxicity related to the mdr-1 gene mutation should not be confused with the subchronic neurotoxicity that could follow macrocyclic lactone administration in all dogs (Bissonnette *et al.* 2008).

The consequences of this mutation can have far-reaching effects, but not all drugs transported by P-glycoprotein cause neurotoxicity in affected dogs. Drugs that have been documented in literature to cause toxicoses in dogs with the mdr-1 mutation include ivermectin (dose dependent; doses used to treat heartworm seem to be safe, but doses used to treat mange will cause toxicity in homozygous mutant dogs), other macrocyclic lactones related to ivermectin (higher risk of causing neurotoxicity in homozygous mutant dogs than in normal individuals; use strictly according to recommended doses), acepromazine and butorphanol (cause a more profound and prolonged sedation in affected individuals; when used, a reduction in dose is recommended), vincristine, vinblastine and doxorubicin (increased likelihood of causing adverse drug effects in affected dogs), as well as erythromycin and loperamide (Dowling 2006).

Although any breed of dog, or even mixed breeds, can be homozygous for the mutation, breeds considered most likely to be affected by the mutation include Smooth and Rough Collies, Australian Shepherds (including miniature breeds), German Shepherds, White Swiss Shepherds, Long-haired Whippets, Old English Sheepdogs, Shetland Sheepdogs, Silken Windhounds, McNab Shepherds and Border Collies, as well as herding breed crosses (Geyer & Janco 2012). Breed predisposition frequency values from various countries have been reported over the past few years. As more dogs are being tested, more breeds will probably be added to the list. It further seems likely that the mdr-1 mutation is not only present in domesticated canine species in South Africa (Lobetti & Caldwell 2012).

Since the discovery of the gene mutation, present knowledge and understanding have improved significantly and practitioners no longer have to adhere to the principle 'if white feet don't treat'. MDR-1 gene mutation testing is now readily available through many diagnostic laboratories and allows veterinary practitioners to select drugs for safe use based on accurate molecular testing. The purpose of this communication is to give veterinary practitioners an indication of the prevalence of the canine mdr-1 mutation in dogs in South Africa and to encourage genetic testing, not only to minimise the risk of adverse effects with certain drugs, but also to ensure responsible breeding practices.

The mdr-1 gene is inherited as an autosomal recessive trait (Dowling 2006). This implies that an animal needs two mutant alleles to express the symptoms (known as homozygous for the mutation; mdr1-1Δ/mdr1-1Δ). Affected dogs will pass on the mutant allele to all offspring and have a very high probability to display the symptoms. A dog could also be heterozygous for the mutation (a carrier; mdr1-1Δ/N). Carrier dogs can produce clear, carrier or even affected puppies, depending on the other parent's genotype. The mutation follows a Mendelian inheritance pattern and therefore two seemingly healthy dogs that are both carriers of the mutation can produce puppies that are affected by the mutation. Normal or clear dogs (homozygous without the mutation; N/N) will not display the symptoms and cannot pass a mutant allele to the offspring.

In order to test an animal for the mdr-1 gene mutation at one of the South African diagnostic laboratories performing the test, a 0.5 mL – 1 mL whole ethylenediaminetetraacetic acid (EDTA) blood sample must be collected and submitted for polymerase chain reaction (PCR) analysis and deoxyribonucleic acid (DNA) sequencing. Some laboratories also make use of Whatman® FTA filter paper cards, allowing for easy blood collection and sample storage. Although buccal swabs could be used to acquire a DNA sample for PCR, the laboratories that have provided data for this trial do not use these samples because of their poor DNA concentration yields.

Data used in this report include results from 306 dogs tested for the mdr-1 gene mutation during the period June 2009 to December 2012 by two laboratories. Samples tested were received from veterinary practices and breeders from various parts of South Africa. Where breed information could not be verified (by the presenting veterinarian, or pedigree evaluation in the case of registered dogs), the results were excluded. Pedigree data of the dogs tested were not available, therefore relatedness between the dogs could not be established.

The highest mdr-1 mutant homozygous cohort in the South African dog breeds was the Rough Collie (53% of dogs tested were homozygous for the mutation and 35% heterozygous), followed by the Australian Sheepdog (16% of dogs tested were homozygous for the mutation and 56% heterozygous). Despite popular belief in the high incidence of 'ivermectin sensitivity' in Border Collies, only one of the 47 Border Collies tested by these two laboratories during the time period had the mdr-1 gene mutation (Table 1).

Breed predisposition and prevalence in canines reported from the United States of America, Canada, European countries, Australia and Japan show similar patterns, with up to 70% of Rough Collies affected, up to 50% of Australian Sheepdogs and up to 10% of German Shepherds. Only 7% of collie-type cross breeds were affected and less than 5% of Border Collies (Geyer & Janko 2012).

A downside of this South African survey is the very high numbers of certain dog breeds tested, with other breeds under-represented. Rough Collies and Australian Sheepdog associations request testing before breeding and the majority of the results displayed here emanated from dogs tested by breeders for breeding purposes. The very low numbers of other dog breeds such as German Shepherds tested are a concern, as it is assumed that these breeds are not at risk. It is possible that financial constraints of pet owners in South Africa might prevent testing. In addition, it is perceived that certain herding dog breeds are affected and genetic testing is therefore considered futile. A follow-up report with a larger sample size and breed variety, sampled over a longer period of time, is required to obtain a more statistically relevant picture of the South African dog population. Pedigree data should also be included in the study.

Because of the high frequency of the mutation in certain breeds, chances are high that two carriers could be mated; therefore it is of the utmost importance that breeders of the breeds known to be affected are informed of the disorder and know the status of their dogs before mating. In the event of a mating between a clear and carrier individual, all puppies must be tested to ensure the new owners are aware of the dog's status. Affected dogs should not be used for breeding under any circumstances.

Personal communication with South African veterinary practitioners in metropolitan areas revealed that more than 90% of dogs are tested to determine avermectin sensitivity before treatment for *Spirocerca lupi* infection with doramectin

TABLE 1: Multi-drug resistance-1 genotype frequency in different dog breeds in South Africa.

Breed	N/N	N/MDR	MDR/MDR	Total tested
Rough Collie	4	12	18	34
Australian Sheepdog	33	65	19	117
Border Collie	46	-	1	47
Belgian Shepherd	7	-	-	7
German Shepherd	6	1	-	7
White Swiss Shepherd	11	2	-	13
Shetland Sheepdog	2	-	-	2
Anatolian Sheepdog	2	-	-	2
Cross breed	8	1	-	9
Other†	68	-	-	68

N, Normal or clear dogs; MDR, multi-drug resistance.
†, Other dog breeds tested include Golden Retrievers, Labrador Retrievers, Bouvier des Flanders, Australian Cattledogs, Dobermans, Huskies and Dachshunds.

commences. Other reasons for testing include avermectin sensitivity for generalised demodicosis and for dogs being considered for certain chemotherapeutic protocols. The Australian Shepherd cohort in particular was often tested for breeding purposes, a practice that is currently underutilised by many other breeders of at-risk breeds. It is the opinion of the authors that all herding breeds with reported incidence of the mutations should be tested before treatment with relevant compounds and before breeding. The use of certain avermectins in dogs and for particular indications is off-label, and owner consent to treatment should be obtained after a detailed discussion of potential risks and benefits.

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Competing interests

The first author has no competing interest. The second author is employed by Inqaba Biotechnical Industries, one of the companies that supplied data.

Authors' contributions

L.l.R.-P. (University of Pretoria) was the study leader and main author. H.v.d.Z. (Inqaba Biotechnical Industries) assisted in interpretation of the data and made conceptual contributions.

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