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BRIEF REPORT

Prevalence of the ABCB1-1Δ gene mutation in a sample of New Zealand Huntaway dogs

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ABSTRACT

Aims: To determine the prevalence of the ATP Binding Cassette Subfamily B Member 1-1Δ mutation (ABCB1-1Δ; previously Multidrug Resistance 1 (MDR1) mutation) in a cohort of New Zealand Huntaway dogs.

Materials and methods: Samples were opportunistically collected from Huntaway dogs (n = 189) from throughout New Zealand. Buccal swabs were collected from 42 Huntaways from the Wairarapa region and 147 blood samples from Huntaways from the Gisborne, Waikato, Manawatū/Whanganui, Hawkes Bay, Canterbury and Otago regions. DNA was extracted from all samples and tested for the presence of the ABCB1-1Δ allele.

Results: Of 189 Huntaway dogs that were tested, two were found to be heterozygous carriers of the ABCB1-1Δ allele and the remaining 187/189 dogs were homozygous for the wild type allele. No dogs homozygous for the mutation were identified.

Conclusions and clinical relevance: The results of this study show that the ABCB1-1Δ allele is present in Huntaway dogs. The low prevalence in this convenience sample suggests that the prevalence of this allele in the Huntaway population is likely to be low. We recommend that veterinary clinicians discuss the potential for this mutation in Huntaways with dog owners including the clinical implications for dogs that are homozygous for the mutated allele and the potential for testing for the mutation, as they would do for other known mutations.

Abbreviations: ABCB1: ATP Binding Cassette Subfamily B Member 1; CNS: Central nervous system; MDR1 test: Multidrug resistance 1 gene test

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Introduction

The New Zealand Huntaway is characterised as a working farm dog, and as such is bred for traits suitable to their work of moving stock (sheep and cattle) on New Zealand farms. The Huntaway is typically used to drive stock forwards and has been bred and selected for its physical endurance and a deep, loud bark, rather than to conform to an arbitrary set of physical characteristics (Oliver *et al*. 2009). The Huntaway has become an iconic part of New Zealand society, but to date little is known of their history and genetics. A major assumption regarding the pedigree of the Huntaway is that the breed is descended from herding dog breeds, including Border Collie and Rough Coated Collie breeds (Anonymous 2022). Additional breeds thought to be progenitors of the Huntaway include Labrador, Rottweiler, Harrier Hound, Gordon Setter and Smithfield Collie (Dogs New Zealand 2022).

The potential for Huntaways to be descended from Collie breeds is notable because Collies have been shown to carry a mutation in the ABCB1 (ATP Binding Cassette Subfamily B Member 1) gene, which encodes P-glycoprotein, an ATP-dependent drug transporter that moves several substances across the blood–brain barrier and protects the brain from xenobiotic toxicity (Mealey *et al.* 2001; Glavinas *et al.* 2004). ABCB1 was previously known as the multi drug resistance (MDR1) gene. The mutation, denoted ABCB1-1Δ, is a nonsense mutation, consisting of a 4-bp deletion in the fourth exon in the ABCB1 gene (Neff *et al.* 2004). The ABCB1-1Δ allele is present in 4.8–61.2% of some Collie populations in Germany (Geyer *et al.* 2005a; Gramer *et al.* 2011), Brazil (Monobe *et al.* 2015), Israel (Dekel *et al.* 2017) and the USA (Neff *et al.* 2004) that have been tested, and can also be found in breeds descended from Collies, such as the Australian Shepherd (Neff *et al.* 2004). Neff *et al.* (2004) hypothesised that as the ABCB1-1Δ allele was conserved among different breeds, it was identical by descent and originated in an ancestral dog that occurred in the UK around 1873, indicating that all modern dogs containing the mutation are potentially related.

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The P-glycoprotein encoded by ABCB1 is a large transmembrane efflux protein located in the brain, and in the intestine, bile canaliculi and renal tubules (Mealey 2008). Many drugs that are substrates for P-glycoprotein are in common use in veterinary medicine. Macrocyclic lactones are one such drug family to which Huntaways may be exposed either as a treatment or inadvertently (Mealey 2008; Parton *et al.* 2012).

The ABCB1-1Δ mutation was identified through signs of central nervous system (CNS) dysfunction first observed in dogs that developed neurotoxicity following treatment with ivermectin, a macrocyclic lactone used to treat a variety of endo- and ecto-parasites (Seward 1983). Ivermectin and other macrocyclic lactone drugs bind to chloride channels in the nervous system of nematodes and arthropods, causing an influx of chloride ions; this suppresses synaptic transmission and leads to paralysis and death of the invertebrate. Mammals have homologous target molecules, however these are restricted to the CNS and protected by the blood–brain barrier. P-glycoprotein is a component of the blood–brain barrier and transports a broad array of substrates, thus protecting the brain from neurotoxic substances. Individuals homozygous for the ABCB1-1Δ mutation do not express functionally intact P-glycoprotein. Studies in knockout mice lacking the ABCB1 gene found increased ivermectin levels in brain tissue and a decrease in drug elimination (Schinkel *et al.* 1994).

Subsequently it has been shown that several drugs that are normally exported from the CNS via the P-glycoprotein can also be toxic at normally therapeutic doses in dogs homozygous for the ABCB1-1Δ mutation (Henik *et al.* 2006; Krugman *et al.* 2012; Campbell *et al.* 2017). Dogs with this mutation show increased sensitivity to many P-glycoprotein-transported drugs in addition to ivermectin, including moxidectin, milbemycin oxime, acepromazine, butorphanol, digoxin, vincristine and loperamide (Mealey 2008). Furthermore, studies have shown an intermediate phenotype for heterozygous dogs (Mealey and Meurs 2008; Coelho *et al.* 2009) for some P-glycoprotein substrates, however this has not been established for macrocyclic lactones. Macrocyclic lactones are widely used on farms in New Zealand and toxicity in dogs has been previously recorded (Parton *et al.* 2012). Thus, any increased susceptibility to toxicosis in working farm dogs is especially salient.

Given that Collies and other herding dogs may be progenitors of the Huntaway, it is important to determine if the ABCB1-1Δ allele is present in the population of Huntaway dogs. There is an obvious interest in testing various herding dogs descended from Collie breeds; some investigation of the ABCB1-1Δ allele in other potential Huntaway progenitor breeds (Rottweiler and Labrador) has occurred without the allele having so far been found (Mealey and Meurs 2008; Bissonnette *et al.* 2009). To date, no examination of the Huntaway breed for the presence of the ABCB1-1Δ mutation has occurred. The aim of this study was therefore to determine the prevalence of the ABCB1-1Δ mutation in a sample of New Zealand Huntaway dogs.

Materials and methods

Two cohorts of dogs were examined in this study. The first was an opportunistic collection of 42 buccal swabs obtained from Huntaway dogs from farms in the Wairarapa region of New Zealand between January and March 2019. The dogs in this study were sampled from five farms with 2–15 dogs sampled per farm. Swabs were stored for approximately 1 month at −20°C prior to extraction. The second cohort was also an opportunistic collection, comprising 147 blood samples originally collected for a different study between January 2019 and April 2021; these were all working Huntaway dogs from farms in the following regions: Gisborne, Waikato, Manawatū/Whanganui, Hawke's Bay, Canterbury and Otago. Blood was collected into vacutainers containing EDTA and stored for 1–3 months at −80°C prior to DNA extraction. The dogs in this study were sampled from 112 farms with 1–10 dogs sampled per farm. Both collections were approved by the Massey University Animal Ethics Committee (approval numbers 19/74 and 18/27 respectively).

DNA from the buccal swabs was extracted using a Chelex protocol (Martín-Platero *et al.* 2010). DNA from the blood samples was extracted with the NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. DNA from both extraction protocols was quantified using a NanoDrop 2000 (ThermoFisher, Waltham, MA, USA).

DNA from all samples was submitted to InfoGeneNZ at Massey University (Palmerston North, NZ) for their canine genetic health screening MDR1 test. The MDR1 test uses a protocol developed by Geyer *et al.* (2005b), which amplifies a 138-bp fragment from the wild-type ABCB1 gene and a 134-bp fragment from the ABCB1-1Δ allele that has the 4-bp deletion. Visualisation of the amplified product occurs via fluorescence using an Applied Biosystems 3500xL Genetic Analyzer (ThermoFisher, Waltham, MA, USA). Results of this analysis for a normal (wild type) individual is a 138-bp amplicon; a carrier will have both a 138-bp and a 134-bp amplicon, while individuals homozygous for the mutant allele will have a single amplicon of 134 bp. The MDR1 test only identifies the presence or absence of the ABCB1-1Δ allele and no other potential variants of the ABCB1 gene.

Results

Amplicons of the expected sizes were successfully amplified with the MDR1 test from all DNA samples (n = 189), allowing identification of individuals carrying

the ABCB1-1Δ allele. In total 2/189 dogs were found to be carriers of the ABCB1-1Δ mutation, one from the Wairarapa region (cohort 1) and the other from Hawke's Bay (cohort 2). No dogs homozygous for the mutation were identified.

Discussion

Although the exact ancestry of the New Zealand Huntaway is unknown, it is thought to include Collie dogs that had originated in the UK. Given the high prevalence of the ABCB1-1Δ mutation in Collies worldwide, (Neff *et al*. 2004; Monobe *et al*. 2015; Dekel *et al*. 2017), it was hypothesised that the prevalence of this mutation in Huntaways might also be high. While we did identify the ABCB1-1Δ mutation in the sample of dogs included in this study, it was at a very low rate. As this study did not test a systematic random sample of Huntaways from the population in New Zealand, we cannot be certain the mutation is present at similarly low prevalence in the population as a whole. Presuming it is, it is unknown if the low presence of this mutation is due to a fortuitous founder effect during the initial breeding of Huntaway progenitors in New Zealand, or due to the inadvertent removal of dogs containing the mutation due to unreported attrition. However, the low frequency of the mutation found in this study is of some comfort to Huntaway owners.

Macrocyclic lactones have been used in dogs for treatment of a range of endo- and ectoparasites, including gastrointestinal nematodes (e.g. *Ancylostoma* spp., *Toxocara* spp.), mites (e.g. *Demodex canis*, *Otodectes cynotis, Sarcoptes scabei*), fleas, and some species of ticks (Nolan and Lok 2012). In addition, inadvertent exposure can occur; for example, farm dogs may come into contact with livestock treated with pour-on formulae (Parton *et al*. 2012). Therefore, it was important to establish the susceptibility of the uniquely New Zealand Huntaway breed to toxicosis. Although toxicosis was not found to be a very common reason for presentation to veterinary practices in a survey of working farm dog diseases (Cave *et al*. 2009), it was considered possible that toxicity resulting in the death of dogs on-farm might not be reported to veterinarians. Based on this research, while it is possible for any individual Huntaway to be homozygous for the ABCB1-1Δ mutation, this should be considered very unlikely.

While this study did include a moderate number of dogs, we used an opportunistic rather than a systematic random sampling strategy, and dogs were sourced from predominantly the North Island. Therefore, as mentioned above, the allele prevalence found in this sample of dogs cannot be extrapolated to the whole population of Huntaway dogs in New Zealand. The Huntaway has far more diverse phenotypic characteristics than other breeds, and there may be geographical regions where dogs are genetically different to those included in this study. However, it is argued that trading of dogs, transport of dogs for planned matings, and movement of farmers and their animals around the country continue to homogenise the genetic pool beyond simple geographic regions, and increase the confidence of this inference.

Our data suggest that while the ABCB1-1Δ mutation is present in the New Zealand Huntaway population, its prevalence in the population as a whole is likely to be very low; dogs homozygous for this allele, and therefore susceptible to macrocyclic lactone toxicosis, are likely to be even rarer. A thorough investigation into the genetic diversity of Huntaway dogs throughout New Zealand would be beneficial for better understanding the population. The New Zealand Huntaway is a recently defined dog breed, with much of its pedigree either unknown or historical hearsay. Additional investigations into the genetics of this breed would be beneficial for understanding disease in the population and to provide historical provenance. Testing of dogs for the mutation is sensible prior to the administration of drugs with a low therapeutic window that are transported by P-glycoprotein, (e.g. macrocyclic lactones), particularly those which have been reported to affect heterozygotes, such as vincristine (Mealey and Meurs 2008). However, unlike the Collie breeds, the results of this study do not suggest that the Huntaway is at particular risk relevant to other breeds. Testing for the ABCB1-1Δ mutation is now easily and inexpensively performed in New Zealand through InfoGeneNZ.

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