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Awareness of the MDR-1 gene mutation in owners of sheep herding breeds related to collie lineage

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Abstract: The research focuses on the MDR-1 gene mutation, predominantly found in Collies and other related breeds, which affects the functionality of P-glycoprotein, a crucial component of the blood-brain barrier in dogs. This mutation results in heightened sensitivity to certain drugs, most notably ivermectin, which can lead to severe neurotoxic effects. The mutation is inherited in an autosomal recessive pattern, and it's discovery is linked to the introduction of ivermectin in the 1980s. This study aims to assess the awareness among dog owners of purebred dogs concerning the MDR-1 gene mutation and its implications. The study was conducted by distributing a survey to dog owners, focusing on their knowledge about the mutation, as well as their choices regarding genetic testing. The findings indicate that while a substantial number of owners are aware and tested their dogs, a considerable portion remains uninformed about the mutation and the associated risks.
The obtained results underscore the need for increase education on the MDR-1 gene mutation and advocate for genetic testing to prevent health risks. This would not only safeguard the health of individual dogs but also guide breeding decisions to prevent the spread of this deleterious allele.

Key Words: dog; MDR-1 mutation; owners; questionnaire.

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Introduction

Dogs have shared their lives with humans for thousands of years. The attachment bond that has been created between these two species (Mariti et al., 2013a,b; 2018; 2020) requires careful management of the dog's welfare.

The gene MDR-1 (multidrug resistance-1) or ABCB-1 gene, located on chromosome 14 in dogs (Geyer et al., 2005), encodes for P-glycoprotein ATP-dependent, which is found as a component of the blood-brain barrier with the role of transporting numerous substrates (Neff et al., 2004), both hydrophilic and amphiphilic (Fromm, 2004), including many drugs used in veterinary medicine (Mealey, 2004). The mutation nt230(del4) causes a frameshift accompanied by numerous premature stop codons and results in a truncated P-glycoprotein composed of only 10% of the original amino acid sequence, thus affecting its functionality (Neff et al., 2004). This mutation exhibits autosomal recessive inheritance with the possibility of determining an altered phenotype in heterozygous individuals, although in attenuated form (Monobe et al., 2013). Its discovery is linked to the introduction in the 1980s of a new antiparasitic drug based on ivermectin, which causes lethal paralysis in nematode and arthropod parasites while its use in mammals is safe as the active ingredient's homologous target is protected by the blood-brain barrier (Shan et al., 2001). However, clinical cases of neurotoxicosis have been observed in some patients with elevated concentrations of ivermectin in the central nervous system (Neff et al., 2004). Moreover, Mealey et al. (2001) discovered a pre-existing deletion in homozygosity of 4 bp in the fourth exon in the Collie breed. It is now known that more than twenty drugs are substrates of P-glycoprotein, as it has subsequently been discovered that other drugs interact with MDR-1, demonstrating that the latter defines a multi-drug sensitivity (Mealey et al., 2003).

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The fact that the same mutated allele has been found in different dog breeds apparently not sharing the same descent, suggested that it was a mutation preceding contemporary breeds so, the breeds related to Collie have been studied according to phylogeny and phenotype in relation to drug sensitivity (Neff et al., 2004). These studies highlighted that the breeds showing the mutated allele are seven belonging to the Collie lineage (Collie, Shetland sheepdog, Australian shepherd and Miniature Australian shepherd, Old English sheepdog and English sheepdog, McNab) and two not belonging to the shepherd's lineage (Longhaired Whippet and Silken Windhound) (Neff et al., 2004). The frequencies in each of them varied depending on the geographical area, with high frequencies being recorded in both British and American Collies (Neff et al., 2004).

Today, there are numerous studies conducted in different geographical areas highlighting how the mutation has also been identified in mixed breeds (Gramer, 2010) or in other breeds although with a lower incidence than Collie, such as in the case of the White Swiss Shepherd (Geyer et al., 2007). The Border Collie is a suspected breed where heterozygous and homozygous dogs has been found affected, even though with a low frequency. For this reason, it is advisable that drug administration should be done cautiously or after a genetic testing (Geyer et al., 2005). The same Author (Geyer et al., 2007) reported two cases of neurotoxicosis in White Swiss Shepherds following the administration of doramectin for the treatment of dermatological diseases; until then, there were no reports in the literature of this dog breed affected by the mutation.

The study aims to verify the awareness of dog purebred owners on the MDR-1 mutation and about which drugs could be harmful on affected dogs, and their choices regarding genetic testing. Additionally, it examines their perception of associated risks and evaluates potential strategies they could adopt to address this issue.

Material and methods

A questionnaire was developed at the Vet-Hospital H24 in Florence (Tuscany) and piloted on a total of 101 owners subdivided in 39 owners of Collies, 50 of Australian Shepherds, and 12 of Swiss Shepherds. The research was carried out during the year 2023 only on owners of dogs who were followed within the structure, in order to ensure complete information of the dogs.

Owners were contacted by phone or mail to inquire about their willingness to participate in the survey by responding to a questionnaire. The questionnaire included five multiple-choice questions on knowledge of the MDR-1 gene mutation:

- Question 1: Have you ever heard of the MDR-1 gene mutation that affects sheep herding breeds related to collie lineage?
- Question 2: If yes, do you know which drugs could be harmful to your dog?
- Question 3: Would you still have adopted the same dog, knowing this problem?
- Question 4: Have you tested the dog for the mutation of MDR-1 gene, or do you intend to?
- Question 5: If the dog was tested, which was the outcome?

Descriptive statistic of data was carried out using Excel software.

Results and discussion

The sample comprises 49 respondents, including owners of 19 Rough Collies (RoC), 23 Australian Shepherds (AuS), and seven White Swiss Shepherds (WSS). The age of the dogs varies from one to nine years. The majority of the dogs come from large-scale breeding farms (53%), while 31% and 16% come from amateur breeding and other sources, respectively.

Analyzing Table 1, we observe that 25% of the respondents are unaware of the existence of the MDR-1 mutation. Looking into the individual breeds, it is possible to notice that only one out of

seven of White Swiss Shepherds owner is aware of the mutation while, regarding the Collie and the Australian Shepherd breed, the majority of respondents (89% and 74% respectively) are aware of the MDR-1 gene mutation, and they learnt about it in different ways.

Table 1. Answers to question 1 - Have you ever heard of the MDR-1 gene mutation that affects sheep herding breeds related to collie lineage?

	Yes, from farmer		Yes, from veterinarian		Yes, from my knowledge		Yes, vaguely		No	
	N	%	N	%	N	%	N	%	N	%
RoC	4	21	4	21	7	23	2	11	2	11
AuS	9	39	5	22	3	23	0	-	6	26
WSS	0	-	1	14	0	-	2	29	4	57
Total	13	26	10	20	10	20	4	8	12	25

RoC: Rough Collie; AuS: Australian Shepherd; WSS: White Swiss Shepherds

In cases where the answer to question 1 was affirmative (37 out of 49 respondents), this topic was further explored by asking respondents about their awareness of the effects of drugs on animals with the mutation (Table 2).

Table 2. Answers to question 2 - If yes, do you know which drugs could be harmful to your dog?

	Yes, only parasiticides		Yes, also other drugs		No	
	N	%	N	%	N	%
RoC	2	12	11	65	4	23
AuS	2	12	11	65	4	23
WSS	0	-	0	-	3	100
Total	4	12	22	59	11	29

RoC: Rough Collie; AuS: Australian Shepherd; WSS: White Swiss Shepherds

The majority of respondents (59%) have a good understanding of the drugs that interact with the MDR-1 gene mutation, 12% are aware of the parasiticides only, while 29% have no knowledge at all (Table 2). Specifically, owners of Collies and Australian Shepherds are familiar with the issue, whereas none of the Swiss Shepherd owners is informed.

In question 3 (Table 3), all respondents were asked if they would still choose the same dog, considering the effects of the mutation. It emerges that only 2% of respondents would not choose the same dog, while 27% was uncertain.

Table 3. Answers to question 3 - Would you still have adopted the same dog, knowing this problem?

	Yes		I do not know		No	
	N	%	N	%	N	%
RoC	14	74	4	21	1	5
AuS	17	74	6	26	0	-
WSS	4	57	3	43	0	-
Total	35	71	22	27	1	2

RoC: Rough Collie; AuS: Australian Sheperd; WSS: White Swiss Shepherds

Table 4 reports the data collected regarding question 4, which focuses on the eventual choice to undergo genetic testing for MDR-1. Among the owners, 19% affirms they tested their dog, while 14% stated that the dog was tested by the breeder before being adopted.

Table 4. Answers to question 4 - Have you tested the dog for the mutation of MDR-1 gene or do you intend to?

	Yes, I done		Already tested by the breeder		I wasn't aware of the existence of the test		No	
	N	%	N	%	N	%	N	%
RoC	8	42	1	5	2	11	8	42
AuS	9	39	5	22	3	13	6	26
WSS	2	29	1	14	3	43	1	14
Total	19	39	7	14	8	16	15	31

RoC: Rough Collie; AuS: Australian Sheperd; WSS: White Swiss Shepherds

It is important to note that among the respondents, 16% are not aware of the existence of the test. Another 31% believes the test is unnecessary, arguing that it suffices to avoid administering certain drugs. This belief is most prevalent among Collie owners, at 42%.

Generally, genetic tests should be conducted when the dog is still a puppy, or at least before it reaches reproductive age. This approach provides early knowledge about whether the animal will be affected by certain conditions, some of which may manifest later in life. It also allows for the creation of an appropriate breeding plan that prioritizes the well-being of future puppies. In the specific case of the MDR-1 gene, testing is especially recommended as a precautionary measure in case the dog requires urgent treatment. It enables veterinarians to devise the most effective therapy during emergencies, considering that the effects of the mutation might not become apparent until the dog needs certain medications for the first time.

Table 5 presents the results obtained from responses to question 5, which was directed at all participants who undergone genetic testing for the MDR-1 gene mutation (47% corresponding to 23/49 dogs). This question specifically asked about the outcome of the test.

Table 5. Answers to question 5 - If the dog was tested, which was the outcome?

	Clear		Affected		Carrier	
	N	**%**	**N**	**%**	**N**	**%**
RoC	4	57	2	29	1	14
AuS	9	64	3	22	2	14
WSS	2	100	0	-	0	-
Total	15	65	5	22	3	13

RoC: Rough Collie; AuS: Australian Sheperd; WSS: White Swiss Shepherds.

Despite the limited number of animals tested, we can highlight that five out of 23 exhibit the mutated allele in homozygosity, while three in heterozygosity. Therefore, the allelic frequency in the total sample is equal to 28.5%, with variations among breeds probably due to the small number of tested animals. In fact, the absence of affected Swiss Shepherds in the sample could be attributed to this condition.

One parameter to consider, which could increase the frequency of deleterious alleles (Cecchi et al., 2020a; Cecchi et al., 2020b), is certainly inbreeding, especially in pure breeds. In selection, inbreeding is utilized to consolidate desirable traits found in the finest representatives of a breed. Moreover, this breeding practice leads to inbreeding depression (Leroy et al., 2011), a condition characterized by reduced performance among inbred animals both in reproductive (Chu et al., 2019) and productive parameters such as morphological traits (Cecchi et al., 2018). For this reason, it is important to analyse the genetic variability of a population using pedigree data (Cecchi et al., 2016), and arrange mating avoiding close relationships.

Comparing our data with that reported in the literature, we notice that the frequencies vary depending on the breed, the population, its genetic makeup, and the selection process. In the study conducted by Marelli et al. (2020) involving 811 subjects across 32 populations (including pure breeds and mixed breeds) the mutated allele has been found in 9 pure breeds and in mixed breeds. This study highlighted that the breeds with the highest mutated allele frequency are Smooth Collie (even though only six dogs were tested) and Rough Collie, with 75 percent and 66 percent of the MDR1 mutated allele, respectively. In Rough Collie, 11% were affected dogs, and 45% were carriers. On the other hand, the Australian Shepherd recorded an allele frequency of 35%, including 41% affected dogs (homozygotes) and 48% carriers (heterozygotes) (Marelli et al., 2020). Regarding the Swiss Shepherd in Italy, the presence of the mutated allele was recorded at 7%, while in mixed breeds, the frequency of the mutated gene was recorded at 15% (Marelli et al., 2020).

Comparing our data with those reported in other geographical areas, we can identify many similarities but also noteworthy differences. Specifically, in the case of the Collie, it is evident that in all studies conducted outside of Italy, the mutation is prevalent compared to what was observed in other breeds with allele frequencies ranging from 55% in Germany (Geyer et al., 2005), to 73% in Great Britain (Tappin et al., 2008), and 56% in both Australia (Mealey et al., 2005) and Northwestern America (Gramer et al., 2010). It is well-documented that the Collie is the breed most affected by this mutation worldwide. For the Australian Shepherd, the literature shows allele frequencies of 43% in Australia (Mealey et al., 2005), 33% in Great Britain (Tappin et al., 2008), and 20% in Germany (Gramer et al., 2010).

Conclusions

The studies illustrate the widespread impact of the MDR-1 gene mutation across various dog breeds, notably the Collie and Australian Shepherd, highlighting important implications for veterinary medicine and breeding strategies. The revelation of this mutation necessitates a broader understanding and awareness among dog owners, especially those of breeds known to carry the mutation.

Despite the genetic predisposition being clearly established, our study reveals a notable variance in owner awareness and response to the mutation. A considerable portion of dog owners remains unaware of the mutation and its potential consequences, particularly in breeds with lower historical incidence, such as the White Swiss Shepherds. This lack of awareness underscores the critical need for enhanced educational efforts aimed at informing dog owners about the risks associated with the MDR-1 mutation and the benefits of pre-emptive genetic testing.

Genetic testing and monitoring inbreeding levels emerge as key tools not only for managing the welfare risks associated with the mutation but also for guiding breeding decisions to prevent the propagation of the deleterious allele. Encouragingly, a substantial number of owners are proactive about testing; however, there remains a considerable percentage who either underestimate the necessity of such measures or are unaware of their availability.

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Conflicts of interest

The authors declare there to be no conflicts of interest.

Authorship Statement

All authors equally conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Consapevolezza della mutazione del gene MDR-1 nei proprietari di razza Collie e affini

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Sintesi

La ricerca si concentra sulla mutazione del gene MDR-1, prevalentemente riscontrata nei Collie e in altre razze affini, che influisce sulla funzionalità della P-glicoproteina, un componente cruciale della barriera emato-encefalica nei cani. Questa mutazione determina una maggiore sensibilità a certi farmaci, in particolare all'ivermectina, che può causare gravi effetti neurotossici. La mutazione è ereditata con un modello autosomico recessivo e la sua scoperta è collegata all'introduzione dell'ivermectina negli anni '80. Questo studio mira a valutare la consapevolezza dei proprietari di cani di razza riguardo alla mutazione del gene MDR-1 e alle sue implicazioni. Lo studio è stato condotto tramite la distribuzione di un questionario a proprietari di cani, chiedendo informazioni sulla loro conoscenza della mutazione così come sulle loro scelte riguardo ai test genetici.

I risultati indicano che, sebbene un numero consistente di proprietari sia informato e abbia testato i propri cani, una parte considerevole rimane ignara della mutazione e dei rischi associati. I risultati ottenuti sottolineano la necessità di aumentare le conoscenze sulla mutazione del gene MDR-1 e promuovere i test genetici per prevenire i rischi per la salute dei cani e gestire in maniera appropriata gli accoppiamenti tra riproduttori.