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ORIGINAL

Analysis of *mdr1-1Δ* mutation of *MDR1* gene in the "Cimarron Uruguayo" dog

Análisis de la mutación *mdr1-1Δ* del gen *MDR1* en el perro Cimarrón Uruguayo

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ABSTRACT

Objective. The aim of this paper is to analyze the frequency of the mdr1-1D mutation of the MDR1 gene in a dog sample of the Uruguayan Cimarron breed with the objective of increasing the knowledge of this breed's genome. **Materials and methods.** Thirty-six animals of this breed were analyzed. The MDR1 gene region, which includes the location where the mutation would be present, was amplified by PCR. **Results.** The mutation was not detected in any of the analyzed Uruguayan Cimarron. **Conclusions.** The lack of described ivermectin intoxication cases in veterinary clinic in this breed is explained by the lack of the mutation object of this study. The sequence studied in Cimarron dogs is kept compared to other breeds, except Collies and related breeds (Border Collie, Bearded Collie, Old English sheepdog).

Key words: Dogs, mutations, pharmacogenetics (*Source:CAB*).

RESUMEN

Objetivo. El propósito de este trabajo fue analizar la frecuencia de la mutación mdr1-1D del gen MDR1 en una muestra de perros de raza Cimarrón Uruguayo con el fin de profundizar en el conocimiento del genoma del animal. **Materiales y métodos.** Se analizaron treinta y seis animales de la raza mencionada. La región del gen MDR1 que incluiría la mutación se amplificó por PCR. **Resultados.** En los perros cimarrones analizados no se detectó la mutación mencionada. **Conclusiones.** La ausencia de la mutación estudiada explicaría el hecho de que no se hayan descrito casos de intoxicación por ivermectina en esta raza. La secuencia en el perro Cimarrón es conservada respecto de otras razas caninas, excepto Collie y sus derivadas.

Palabras clave: Farmacogenética, mutaciones, perros (*Fuente:CAB*).

INTRODUCTION

Cimarron dogs are the only native canine breed of Uruguay. It is believed that they are descendants of Mastiffs and Greyhounds (war and hunting dogs) introduced by colonizers and conquerors, that were used for game hunting and as shepherd dogs (1). In 1989 the Cimarron breed was officially recognized by the Rural Association of Uruguay (ARU) and the Uruguayan Kennel Club (KCU). At that moment, the Breeders Society of Uruguayan Cimarron dog was created which, along with the KCU, made the first official registry of the breed. Today, this registry has more than 6000 inscriptions in the genealogical records of the KCU. On February 21, 2006, the World Canine Organisation recognized the Uruguayan Cimarron as a breed in primary form, standard FCI nº 353 / 10.04.2006 / E (2). At the beginning of 2011 these animals were recognized definitely as a breed. Today, these animals have an important function in security and protection, as well as in working with cattle and in game hunting (2).

Currently, parasite control is achieved through the use of pharmaceutical products. Among these, the group of avermectins has received great acceptance due to its wide action spectrum both on endoparasites and ectoparasites and its high pharmacologic potency (doses are in the order of mg/kg). Ivermectin was introduced as an antiparasitic drug in 1981. It has had a wide diffusion and use in different species of domestic animals worldwide. Its use is considered safe because its action sites are limited to the central nervous system (CNS), and so they are protected by the blood-brain barrier. For intoxication to take place, doses of about mg/kg of weight are needed in most species. The symptomatology that appears during intoxication cases is due to a larger passage of the drug into the CNS. This was demonstrated in affected canines by finding high ivermectin concentrations at this level (3-5).

The *MDR1* gene ("multiple drug resistance gene"), part of the ABC gene family ("ATP binding cassette"), codifies for P glycoprotein. This glycoprotein, among other functions, is responsible for limiting drug entry into the CNS. In Collie related breeds (Border Collie, Bearded Collie, English Shepherd, Australian Shepherd, Scottish Shepherd) and long-hair Whippet and Silken Windhounds, the *mdr1-1*Δ mutation for this gene was described, which produces the a frame-shift followed by the formation of a stop codon, which leads to the formation of a protein that completely loses its function (3-6). Homozygote animals for this mutation have a phenotype sensible to ivermectin, which predisposes them to a potentially lethal neurotoxicosis (3-5, 7). In our country, in general, it is recommended not to use this drug in susceptible breeds. This makes its use limited, not being possible to take advantage of the benefits of this drug. In addition, there are other drugs (corticosteroids, chemotherapeutic agents) used in veterinary clinic that also interact with the *mdr1-1*Δ mutation and cause toxic reactions in canines, including heterozygote animals (4).

Very little is known about Cimarron dog's genome. The aim of this paper is to analyze the frequency of the *mdr1-1*Δ mutation of the *MDR1* gene in a sample of canines of the Uruguayan Cimarron breed with the objective of increasing the knowledge of *MDR1* locus in this breed.

MATERIALS AND METHODS

Analysed samples. Thirty-six not related, Uruguayan Cimarron dogs from different regions of Uruguay, were analyzed. Blood samples were extracted from the anterobrachial vein or from the saphenous in asepsis conditions with EDTA anticoagulant.

DNA isolation. Isoamilic phenol-chloroform-alcohol and the Chelex 100 DNA extraction techniques were used, as they were previously described (1, 8).

PCR amplification. The region of the *MDR1* gene, which would include the *mdr1-1*Δ mutation (Figure 1) was amplified using a couple of primers according to Neff et al (4) (amplification program used: denaturalization: 95ºC, 4 minutes; 35 cycles of 95ºC, 1 minute, 56ºC, 30 seconds, 72ºC, 45 seconds). The amplifications were made using the thermocycler Multi Gene II from Labnet.

181 atgtttcgct attcaaatt**g gcttgatagg ttgtatatgt tggtg**gggac aatggctgcc

241 atcatccatg gagctgcact ccctctcatg atgctggttt ttggaaacat gacag**atag**c

301 tttgcaaatg caggaatttc aa**gaaacaaa actttccag ttataat**taa tgaaagtatt

Figure 1. Sequence of a region from *MDR1* gene. **Bolds**: Regions where primers anneal. The deletion is marked.

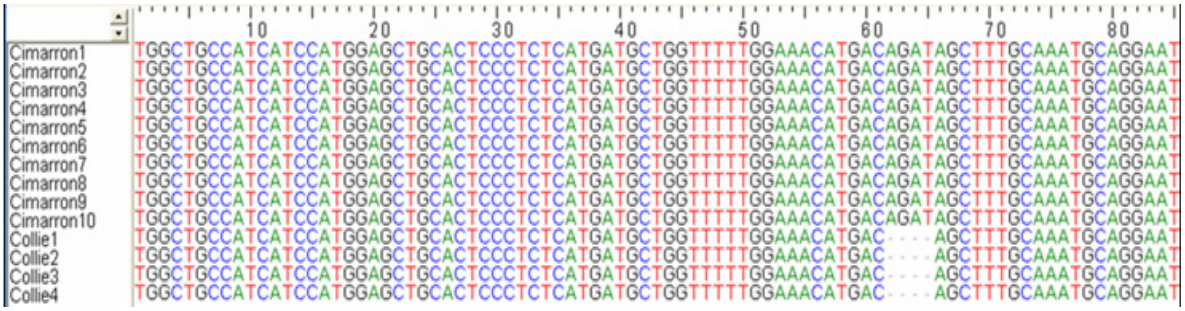

Figure 2. Sequence of the analyzed region of *MDR1* gene. Samples with the mutation are Collie dogs (boxes).

Primers:
Forward: GGC TTG ATA GGT TGT ATA TGT TGG TG
Reverse: ATT ATA ACT GGA AAA GTT TTG TTT C

Sample analysis. The analyzed samples were sequenced in a sequencer 3730 XL DNA Analyzer (AB USA) in Macrogen Inc sequencing service, Korea, and the results were read with the BioEdit program (free distribution) (9).

RESULTS

A fragment of 154bp was obtained from the PCR amplification. The analysis of this region where the mdr1-*1*Δ mutation should be present showed that the studied animals did not have it. In figure 2 the obtained sequences for 10 of the 36 analyzed Cimarron dogs are shown, along with 4 Collie samples, which have the mutation.

DISCUSSION

The *mdr1-1*Δ mutation is present in several canine breeds with different frequencies (1, 3, 4, 10).

The results found in this work are those expected if we consider the fact that Cimarron dogs come from breeds of Spanish origin, among which there are no Collies (English origin). However, since little is known about this breed, as mentioned above, it is interesting to discard the presence of *mdr1-1*Δ mutation in the breed. In previous studies (4) a frequency of the *mdr1-1*Δ mutation between 4% and 50% has been found in different breeds. Considering the variable frequency in which this mutation appears in the different breeds, we cannot discard the appearance of such mutation as the number of Cimarron dogs increases (1, 3, 4). On the other side, it should be taken into account that it was only in the 1970`s decade that the recuperation of the breed began in Cerro Largo (North-eastern province of Uruguay). From this point on, the animals were spread to the rest of the country (1), and in 1988 the Cimarron Breeders Society was founded. Before this, these dogs had little breeding control, being mainly used to work with cattle (1). In rural areas, they could have bred with other breeds. The fact of not finding the mutation could be considered as proof to discard crossbreeding with breeds that do have the mutation. On the other hand, due to the frequencies in which the mutation was described previously (0.6-54.6%) (1, 3, 4, 10) and considering that the number of animals sequenced in this work is relatively low (N=36), we consider that it is important to continue increasing the population sample. Another factor to be taken into account and that would justify the continuity of this study, is the interaction between the mutation analyzed and the different drugs used in Veterinary Clinic.

It may be concluded that the lack of described ivermectin intoxication cases in veterinary clinic in this breed is explained by the absence of the mutation analyzed. On the other side, the sequence studied in Cimarron dogs is conserved compared to other breeds, except Collie and related animals.

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