ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“DISSERTAÇÃO DO ESTÁGIO CURRICULAR DOS CICLOS DE ESTUDO CONDUCENTES AO GRAU DE MESTRE EM MEDICINA VETERINÁRIA DA EUVG”

Resumo

O gene da multirresistência aos fármacos 1 (MDR1) é responsável por codificar uma proteína transportadora, designada glicoproteína-P (P-gp). A P-gp encontra-se presente em diferentes tecidos, tais como, células endoteliais dos vasos cerebrais, células dos túbulos renais, intestino, pele, entre outros. Está também presente em alguns tipos de células neoplásicas, onde se encontra frequentemente sobre-expressa. É uma proteína transmembranar glicosada, que tem a função de transportar para o exterior das células diversos compostos anfipáticos e hidrofóbicos tais como toxinas e xenobióticos, entre os quais vários fármacos usados correntemente na prática veterinária.

Em algumas raças de cães é frequente a existência de uma mutação do gene MDR1, que origina a síntese de uma P-gp não funcional. A ausência da P-gp na barreira hematoencefálica permite a acumulação dos seus substratos no sistema nervoso central, originando neurotoxicidade. Atualmente são reconhecidas várias moléculas como substratos da P-gp. Esta mutação é classicamente associada à neurotoxicidade por ivermectina em cães das raças Collie e seus cruzamentos. No entanto, esta mutação está também descrita em diversas outras raças de cães, maioritariamente de pastoreio, e tem sido associada a toxicidade provocada por outros fármacos e toxinas.

Este trabalho relata um caso clínico de um cão de raça Rough Collie, que foi assistido na Clínica Veterinária VetCondeixa com um quadro clínico de sintomatologia neurológica ligeira, incluindo depressão, desorientação, ataxia, tremores musculares, hipersalivação, midríase bilateral e alterações propriocetivas. Tratava-se de um cão de 21 meses de idade, que tinha sido desparasitado cerca de oito horas antes do aparecimento dos sinais clínicos citados, com uma administração oral de emodepside e praziquantel (Profender®), e vacinado com CaniLeish®. A dose administrada do desparasitante interno correspondia a cerca do dobro da dose de tratamento recomendada. Face aos sinais clínicos observados, foi instituído tratamento de suporte e efetuado um teste de DNA, que revelou uma mutação homozigótica no gene MDR1.

A neurotoxicidade evidenciada neste caso clínico leva-nos a suspeitar que poderá ter sido provocada por sobredosagem de emodepside num cão de raça Collie portador de uma mutação homozigótica no gene MDR1. Apesar da neurotoxicidade associada à sobredosagem de emodepside ser reconhecida pela Agência Europeia do Medicamento, este é, de acordo com o nosso conhecimento, o primeiro caso clínico descrito de uma suspeita de intoxicação por emodepside.

Considerando a importante função da P-gp na distribuição dos fármacos no organismo, seria importante proceder ao despiste da mutação do gene MDR1, sobretudo em cães de raças em que esta mutação é frequente, de forma a optar por fármacos seguros no tratamento destes animais.

Abstract

The multi-drug resistance gene 1 (MDR1) is responsible for encoding an efflux transport protein designated P-glycoprotein (P-gp). The P-gp is expressed in various tissues such as the capillary endothelial cells of the brain, renal tubular cells, intestinal cells, skin, among others. It is also present in some types of neoplastic cells, where it is often overexpressed. It is a glycosylated transmembrane protein that transports several amphipathic and hydrophobic molecules such as toxins and xenobiotics, including drugs commonly used in veterinary practice.

A mutation in MDR1 gene is frequent in some dog breeds, and encodes the synthesis of a non-functional P-gp. The absence of P-gp in the blood-brain barrier may originate the accumulation of its substrates in the central nervous system, leading to neurotoxicity. Currently, a wide variety of molecules are known to be substrates of P-gp. This mutation has been classically associated with ivermectin neurotoxicity in dogs of Collie breeds. However, this mutation has been described in several other dog breeds, mainly herding breeds, and associated with toxicity of other drugs and toxins.

This paper describes a clinical report of a 21-month old male Rough Collie dog that presented to VetCondeixa Veterinary Clinic due to development of mild neurological signs, including depression, disorientation, ataxia, muscle tremors, hypersalivation, bilateral mydriasis and proprioceptive deficits. The dog was dewormed eight hours previously to development of clinical signs with an oral administration of emodepside and praziquantel (Profender®), and vaccinated with Canileish®. The dose administered was nearly the double of the recommended treatment dose. The clinical signs were interpreted as associated to an emodepside toxicosis, and appropriated supportive treatment instituted. A DNA-test was performed, and an homozygous mutation in the MDR1 gene detected.

Here is described a clinical case of a strong suspicion of neurotoxicity associated with an overdose of emodepside in a Collie dog carrier of an homozygous mutation in the MDR1 gene. Although the neurotoxicity associated with the overdose of emodepside is recognized by the European Medicines Agency, this is, to our best knowledge, the first clinical report of a possible emodepside toxicosis.

Considering the relevance of P-gp function in drug disposition, it is of paramount importance to screen the MDR1 gene mutation, especially in dogs breeds where this mutation is frequent, so that safe drugs are used in the treatment of these animals.

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Lista de Abreviaturas

ATP - Adenosine Triphosphate
ABC - ATP-binding cassette
ABCB1 - ATP-binding cassette B1 gene
BBB - Blood Brain Barrier
bp - Base Pair
CBC - Complete Blood Count
CNS - Central Nervous System
del - Deletion
DNA - Deoxyribonucleic Acid
EMA - European Medicine Agency
h - Hour
IV - Intravenous
K - Potassium
kDa - Kilodalton
Kg - Kilogram
MDR1 - Multi-drug resistance 1 gene
mg - Milligram
mL - Milliliter
nt - Nucleotide
NSW - North South West
PCR - Polymerase Chain Reaction
P-gp - P - Glycoprotein
RTD - Recommended Treatment Dose
SLO-1 - Gene which encodes the Calcium-Activated Potassium Channels
® - Registered Trademark
% - Percentage
(+/+) - Homozygous normal for the gene mutation
(-/+) - Heterozygous for the gene mutation
(-/-) - Homozygous affected for the gene mutation

Possible emodepside toxicosis in a Collie with MDR1 gene mutation

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Abstract

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A mutation in MDR1 gene is frequent in some dog breeds, and encodes the synthesis of a non-functional P-gp. The absence of P-gp in the blood-brain barrier may originate the accumulation of its substrates in the central nervous system, leading to neurotoxicity. Currently, a wide variety of molecules are known to be substrates of P-gp. This mutation has been classically associated with ivermectin neurotoxicity in dogs of Collie breeds. However, this mutation has been described in several other dog breeds, mainly herding breeds, and associated with toxicity of other drugs and toxins.

This paper reports a clinical case of a strong suspicion of neurotoxicity associated with an overdose of emodepside in a Collie dog carrier of an homozygous mutation in the MDR1 gene. Although the neurotoxicity associated with the overdose of emodepside is recognized by the European Medicines Agency, this is, to our best knowledge, the first clinical report of a possible emodepside toxicosis.

Considering the relevance of P-gp function in drug metabolism, it is of paramount importance to screen the MDR1 gene mutation, especially in dogs breeds where this mutation is frequent, so that safe drugs are used in the treatment of these animals.

Keywords: Collie, Emodepside, Adverse Drug Reactions, MDR 1 gene mutation, ABCB1 gene, P-Glycoprotein

Background

The ATP-binding cassette-B1 (ABCB1) gene, also designated as multi-drug resistance 1 (MDR1) gene encodes an adenosine triphosphate (ATP) driven efflux pump called P-glycoprotein (P-gp). This is a 170 kDa transmembrane protein which belongs to the family of membrane bound ATP-binding cassette (Dean *et al.*, 2001). The P-gp was reported for the first time in 1976, in ovary cells of Chinese hamsters, where it was largely expressed and responsible for the resistance to colchicine and other chemotherapeutic agents (Juliano & Ling, 1976 cit. by Martinez *et al.*, 2008). Later, in the 1980s, the gene who codes the P-gp was identified and designated as the MDR1 gene due to its overexpression in multi-drug resistant tumor cells (Ueda *et al.*, 1987).

Physiologically, this P-gp transports a wide range of structurally diverse compounds that are amphipathic and hydrophobic such as toxins, xenobiotics and many drugs to the outside of the cells (Borst & Oude-Elferink, 2002; Mealey, 2004), except in skin cells, where P-gp exerts its transport function in the reverse direction, acting as an influx pump (Ito *et al.*, 2008; Hashimoto *et al.*, 2013). The P-gp is present in several tissues with secretory and excretory functions such as the canalicular membrane of hepatocytes, in the luminal membrane of the proximal tubules of the kidney, in the brush border membrane of enterocytes, in capillary endothelial cells of the brain and testis, in trophoblast cells, in the cortex and medulla of adrenal glands and in hematopoietic stem cells (Thiebaut *et al.*, 1987; Cordon-Cardo *et al.*, 1989; Chaudhray & Robinson, 1991). P-gp limits drug absorption in the luminal membrane of enterocytes after oral administration, promotes its elimination from the body into bile and urine through its expression in the hepatocytes and proximal tubular kidney cells, and prevents the accumulation of its substrates in the central nervous system (CNS) (Fromm, 2004). It is also present, and frequently overexpressed, in some types of neoplastic cells, rendering them multi-drug resistant (Thiebaut *et al.*, 1987; Cordon-Cardo *et al.*, 1989; Chaudhray & Robinson, 1991).

In 1996, Schinkel *et al.* reported that homozygous affected (-/-) mices for the MDR1 gene mutation had increased penetration of some drugs into the CNS when compared with wild type mices, suggesting that P-gp is fundamental to the functional integrity of the blood-brain barrier (BBB).

Mealey *et al.* (2001) identified a 4-base pair deletion in the fourth exon of the MDR1 gene in ivermectin-sensitive Collies, which was referred as MDR1 nt230(del4). The deletion mutation causes a frameshift, which generates multiple stop codons that give origin to a truncated P-gp with less than 10% of the wild type amino acid sequence. This allele was associated to loss of the P-gp function (Roulet *et al.*, 2003; Mealey, 2004).

Ivermectin has been the drug more frequently associated to neurotoxicity in dogs with the MDR1 mutation, especially in Collies and its crosses (Seward, 1983 cit. by Monobe *et al.*, 2013; Paul *et al.*, 1987 cit. by Mealey, 2004; Hopper *et al.*, 2002). Ivermectin is a P-gp substrate, and when this glycoprotein is absent, ivermectin penetrates the BBB and accumulates in the CNS, causing different levels of neurotoxicity according to the dose administered to the dog (Geyer & Janko, 2012). The clinical signs associated include vomiting, ataxia, mydriasis, muscle tremors, hypersalivation, seizures, blindness, ptyalism, stupor, coma and death (Hooper *et al.*, 2002; Nelson *et al.*, 2003; Sartor *et al.*,

2004; Geyer *et al.*, 2005a; See *et al.*, 2009). Beyond macrocyclic lactones and chemotherapeutic agents, several other drugs were identified as substrates of P-gp, some of them routinely used in veterinary practice (Table 1).

Although Collie breeds have been the most frequent breeds related to this mutation, Neff *et al.* (2004) showed that eight more breeds could also be associated. Six of them were from the Collie lineage, and interestingly the other two were breeds of the sighthound group. Although these two sighthound breeds didn't belong to the herding group of the Collie lineage, it was concluded that all the dogs included in the study that carried the mutant allele were descendants from the same dog that lived in Great Britain before the first records of genetic isolation breeds, in 1870. Since then, MDR1 mutation has been reported in several other dog breeds (Table 2).

Table 1 - Examples of P-glycoprotein substrates

Chemotherapeutics	**Antimicrobial agents**	**Opioids**	**Macrocyclic Lactones**
Actinomycin D	Doxycycline	Butorphanol	Ivermectin*
Docetaxel[¥]	Erythromycin*,[¥]	Fentanyl	Milbemycin Oxime
Doxorubicin	Grepafloxacin	Loperamide	Moxidectin
Etoposide[¥]	Itraconazole*,[¥]	Methadone*	Selamectin
Imatinib	Ketoconazole*	Morphine	
Mitoxantone	Levofloxacin	Pentazocine*	**Miscellaneous**
Paclitaxel	Sparfloxacin		Bromocriptine*
Vinblastine[¥]	Tetracycline	**Antiemetics**	Chlorpromazine*
Vincristine[¥]		Domperidone	Colchicine
	Immunosuppressants	Ondansetron	Emodepside
Steroid hormones	Cyclosporine A*,[¥]		Flesinoxam
Aldosterone	Tacrolimus*,[¥]	**H1-antihistamines**	Grapefruit Juice*
Cortisol[¥]		Fexofenadine	Phenytoin
Dexamethasone[¥]	**Cardiac drugs**	Terfenadine	Rhodamine 123
Estradiol	Amiodarone		Terfenadine
Hydrocortisone	Carvedilol*	**H2-antihistamines**	Vecuronium
Methylprednisolone	Digitoxin	Cimetidine	
Progesterone	Digoxin	Ranitidine	
	Diltiazem[¥]		
Antidepressants	Losartan		
Amitriptyline	Nicardipine*		
Fluoxetine*	Quinidine*		
Paroxetine*	Talinolol		
St John's Wort*	Verapamil*,[¥]		

* P-glycoprotein inhibitors

[¥] Substrate of Cytochrome P450 3A

Adapted from (Sakaeda *et al.*, 2002; Mealey, 2004; Geyer, *et al.*, 2005a; Dowling, 2006; Linardi & Natalini, 2006; Mealey *et al.*, 2008; Martinez *et al.*, 2008, http://www.vetmed.wsu.edu/depts-vcpl/drugs.aspx)

Table 2 - Genotyping studies on frequency of MDR1 mutation

		US[a]	US[b]	North-west US[c]	UK[d]	UK[b]	France[e]	Germany[f]	Germany[g]	Japan[h]	Australia[i]
Collie	MDR I (+/+)	22.6%	26.0%	22.5%	7.1%	14.9%	20.0%	23.9%	50.0%	25.0%	12.1%
	MDR I (+/-)	42.0%	46.0%	42.5%	40.5%	51.1%	32.0%	43.1%	50.0%	33.3%	63.6%
	MDR I (-/-)	35.4%	28.0%	35.0%	52.4%	34.0%	48%	33.0%	0%	41.7%	24.3%
	n	1424	161	40	42	94	25	578	14	12	33
	AF	56%	51%	56%	73%	60%	64%	55%	25%	58%	56%
Shetland Sheepdog	MDR I (+/+)	88.2%	84.2%		40.8%			45.7%	33.3%	97.6%	57.1%
	MDR I (+/-)	10.5%	14.7%		47.0%			48.6%	0%	2.4%	42.9%
	MDR I (-/-)	1.3%	1.1%		12.2%			5.7%	66.7%	0%	0%
	n	448	190		49			140	3	42	7
	AF	7%	8%		36%			30%	67%	1%	21%
Australian Shepherd	MDR I (+/+)	53.0%	68.5%		32.1%			67.9%	33.3%	44.4%	35.7%
	MDR I (+/-)	37.0%	29.8%		42.9%			25.2%	66.7%	44.4%	42.8%
	MDR I (-/-)	10.0%	1.7%		25.0%			6.9%	0%	11.2%	21.5%
	n	1421	178		28			333	3	9	14
	AF	29%	17%		46%			20%	33%	33%	43%
Border Collie	MDR I (+/+)	98.4%	100%		95.3%			99.1%	87.5%		
	MDR I (+/-)	1.3%	0%		4.7%			0.6%	12.5%		
	MDR I (-/-)	0.3%	0%		0%			0.3%	0%		
	n	306	222		43			334	8		
	AF	1%	0%		2%			1%	6%		
Old English Sheepdog	MDR I (+/+)	97.5%	92.7%		78.8%			87.5%			
	MDR I (+/-)	2.5%	7.3%		21.2%			12.5%			
	n	40	151		33			24			
	AF	1%	4%		11%			6%			
Australian Shepherd Miniature	MDR I (+/+)	63.1%	51.8%								
	MDR I (+/-)	33.7%	44.6%								
	MDR I (-/-)	3.2%	3.6%								
	n	285	56								
	AF	20%	26%								
Longhaired Whippet	MDR I (+/+)	41.7%	32.6%								
	MDR I (+/-)	58.3%	51.7%								
	MDR I (-/-)	0%	15.7%								
	n	24	89								
	AF	29%	42%								
McNab	MDR I (+/+)		68.6%								
	MDR I (+/-)		28.6%								
	MDR I (-/-)		2.8%								
	n		35								
	AF		17%								
Silken Windhound	MDR I (+/+)	68.8%	65.5%								
	MDR I (+/-)	31.2%	33.3%								
	MDR I (-/-)	0%	1.2%								
	n	16	84								
	AF	16%	18%								
German Shepherd	MDR I (+/+)	89.8%	100%								
	MDR I (+/-)	8.4%	0%								
	MDR I (-/-)	1.8%	0%								
	n	166	95								
	AF	6%	0%								
English Shepherd	MDR I (+/+)	100%	85.7%								
	MDR I (+/-)	0%	14.3%								
	n	28	91								
	AF	0%	7%								
Wäller	MDR I (+/+)							62.9%			
	MDR I (+/-)							37.1%			
	n							62			
	AF							19%			

a - Mealey and Meurs, 2008

b - Neff et al., 2004

c - Mealey et al., 2002

d - Tappin et al., 2008

e - Hugnet et al., 2004

f - Geyer et al., 2005b

g - Baars al., 2008

h - Kawabata et al., 2005

i - Mealey et al., 2005

n - number of dogs studied; AF - allelic frequency for the mutant MDR1(-) allele.

Adapted from (Gramer *et al*, 2011)

Case presentation

A 21-month-old male Rough Collie, weighing 17.5 Kg, was presented to VetCondeixa veterinary clinic presenting mild neurological clinical signs, eight hours after administration of oral Profender® (Bayer HealthCare, Pymble, NSW, Australia) and vaccination with CaniLeish® (Virbac, Carros, France). Recommended treatment doses (RTD) of emodepside and praziquantel for dogs are of 1 mg/kg and 5 mg/Kg, respectively. The Profender® tablet administered contained 30 mg of emodepside and 150 mg of praziquantel, which corresponds to approximately the double of the RTD.

On physical examination, the dog presented mild neurologic clinical signs, including ataxia, depression, disorientation, muscle tremors, hypersalivation and bilateral mydriasis. Also presented proprioceptive deficits in all four limbs, although the interpretation was difficult due the dog's overall weakness. The dog was completely reluctant to eat or drink and it had been necessary to encourage him to walk. The dog's vital signs were within normal limits.

Blood samples were collected for a complete blood count and serum biochemistry which results were within normal limits. Later, an MDR1 genotyping by PCR method was requested. The MDR1 genotyping detected a recessive homozygous mutation for that gene (Appendix 1).

Supportive treatment was instituted with intra-vascular (IV) fluid therapy with Ringer Lactate solution at an infusion rate of 2.5 mL/kg/h, and administration of diazepam (0.5 mg/Kg) and vitamins of B complex (association of thiamine, pyridoxine and cyanocobalamin). Although the clinical signs were not suggestive, a possible immunologic reaction to CaniLeish® vaccine was considered, and a single IV methylprednisolone (2 mg/Kg) was administered.

The animal was regularly monitored and an improvement in his clinical condition was observed in the following hours. Twelve hours after admission, the neurologic status was stabilized and the animal accepted a low volume of food and water without vomiting. Twenty-four hours after hospitalization the dog manifested only a slight ataxia. The dog was monitored in the following days, and clinical signs resolved completely without relapses.

Due to the overdose of Profender® administered, the clinical signs presented, the response to supportive treatment and the detection of the homozygous mutation in the MDR1 gene in the genotyping PCR test, a strong suspicion of an emodepside toxicosis associated with a P-gp dysfunction was considered as the cause of development of the clinical signs.

Discussion

This article describes a clinical case of a strong suspicion of an emodepside intoxication in a Rough Collie dog carrier of an homozygous MDR1 gene mutation. The dog was presented to VetCondeixa veterinary clinic due to development of mild neurologic clinical signs eight hours after oral administration of Profender® (Bayer HealthCare, Pymble, NSW, Australia) and vaccination with CaniLeish® (Virbac, Carros, France). The tablet ingested was manufactured for dogs weighing 30 Kg (30 mg emodepside, 150 mg praziquantel), nearly the double of the dog's weight (17.5 Kg), and the neurotoxicity evidenced by the animal was considered to be attributable to the overdose of emodepside administered.

At the time of admission, the first clinical suspicion was of an immunologic reaction against the CaniLeish® vaccine. Although the clinical signs were not strongly suggestive, the immunologic reaction was considered and an IV administration of methylprednisolone (2 mg/Kg) was given. No clinical improvement was detected after methylprednisolone administration.

Adverse reactions to CaniLeish® are reported by EMA (2011) in the European Public Assessment Report on CaniLeish, and have also been described in clinical practice (Coedo, 2013). Most adverse reactions reported are related with temporary local reactions such as swelling, nodule development, pain on palpation or erythema, and resolve spontaneously within two days to two weeks. Other temporary signs commonly described following vaccination can also occur such as hyperthermia, apathy and digestive disorders lasting one to six days. Allergic-type reactions are uncommon (EMA, 2011; Coedo, 2013). Vasovagal shock has also been reported in clinical practice, but is usually mild and resolves within a few minutes (Coedo, 2013).

In fact clinical signs presented by the animal were rather compatible with neurotoxicosis than with the adverse reactions described to the vaccine. In addition, the lack of clinical response to methylprednisolone administration led clinicians to focus on Profender® administration.

Profender® is an endectocide used for prevention and treatment of gastrointestinal parasitism in dogs. The active constituents are emodepside and praziquantel. Emodepside belongs to a new chemical group of depsipeptides, being a semi-synthetic compound produced by fermentation of the fungus *Mycelia sterilia,* and is a substrate of P-gp (EMA, 2008). Emodepside is effective against nematodes, and acts by two different metabolic pathways at neuromuscular junctions. Directly in pre- and post-synaptic calcium-activated potassium SLO-1 gene channels in the muscle and neurons, and indirectly through the pre-synaptic latrophilin receptors (Martin *et al.,* 2012). This mechanism of action of emodepside leads to inhibition of pharyngeal pumping, paralysis and death of the parasites. Praziquantel is effective against trematodes and cestodes, and is an acylated pyrazino-isoquinoline derivative. Its action is consistent with an effect on the b-subunit of voltage-gated-calcium channels (VGCC). It destroys the parasite integument by altering the intracellular calcium concentrations, causing paralysis, contraction and disruption of metabolism leading to the death of parasites (Maddison *et al*., 2008:216-217; EMA, 2008).

According to Guest *et al.* (2007) and the European Medicines Agency (EMA, 2008), Profender® is accepted as a safe product when is administered to the dogs at the RTD, but when the RTD is overlapped, some neurological signs are expected, including muscle tremors, incoordination and behavioral changes. Without treatment, clinical signs are transient and self-limiting within eight hours (EMA, 2008). Furthermore, the data provided by EMA also describes that a study was performed to investigate the safety of Profender® in Collie dogs with homozygous mutation of the MDR1 gene, which demonstrated that Profender® was well tolerated in those dogs when emodepside was administered up to doses of 1.6 mg/Kg, and praziquantel up to doses of 8 mg/Kg. Mild and transient neurological symptoms as muscle tremors, ataxia, hypersalivation and in rare cases seizures developed in dogs with the MDR1 gene mutation when active substances were administered at a dose twice or more times higher than the RTD (EMA, 2008). In the present case report, a dose nearly the double of the RTD was administered to a Rough Collie carrier of a MDR1 gene mutation. The neurological symptoms developed were mild and transient. Supportive treatment was instituted, and 12-hours later the dog manifested a significant improvement in clinical signs. Twenty-four hours after administration of Profender®, the dog manifested only a slight ataxia, which resolved completely in a few days.

In our opinion, intoxication due to praziquantel is unlikely in this case since praziquantel is an "old" drug authorized since 1975, and has a safety margin up to five fold of the RTD (5 mg/Kg) (EMA, 1996; Maddison *et al*., 2008:216). The dose of praziquantel administered in this case (150 mg) was approximately 1.7 times higher than the RTD, but significantly lower than the toxic dose. In addition, praziquantel is not considered a P-gp substrate, as recently shown by Dupuy *et al.* (2010) in an *in vitro* study. Furthermore, to our best knowledge, there are no reports of development of neurologic clinical symptoms related with adverse reactions to praziquantel in dogs.

In this case, a dose of emodepside approximately 1.7 times higher than the RTD was administered to a Rough Collie carrier of an homozygous mutation of the MDR1 gene, which in our opinion was the probable cause of the clinical status presented by the animal. The absence of P-gp in liver, intestine, and kidneys may increase the oral bioavailability of P-gp substrates, such as emodepside, also reducing its elimination from the organism (Thiebaut *et al.*, 1987, Mealey *et al.*, 2005). Simultaneously, P-gp absence in the BBB may allow emodepside accumulation and binding to receptors in the CNS, which causing the neurologic symptoms. Clinical signs associated with emodepside intoxication might also be related to the possible binding of emodepside to the potassium SLO-1 channels, also present in mammals, which are reported to be associated to hormone release, regulate neurosecretion, muscle contraction and neuronal excitability (Crisford *et al.*, 2011).

Conclusions

Profender® is considered a safe and effective endectocide for prevention and treatment of gastrointestinal parasitism in dogs when administered at the RTD. Nonetheless, emodepside, a P-gp substrate, has a reduced margin of safety in dogs with the MDR1 gene mutation. Administration of dosages above the RTD might originate the development of neurological clinical symptoms, which are in most cases mild, transient and self-limiting, but in rare cases severe clinical signs like seizures can develop. Thus, Profender® should be administered consciously at the RTD, mainly in dogs with confirmed MDR1 mutation and in dogs of breeds in which this mutation is frequent. For the same reason, all P-gp substrates are recommended to be used with care, in homozygous but also heterozygous dogs for the MDR1 gene mutation.

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Appendix

Dados animal	NOME: Baltique		
Espécie	Raça	Sexo	D. N.
Felina	Rough Collie	M	10/02/2011
Proprietário: Carlos Cortes			

Dados Clínica	Associação Cognitária São Jorge Milreu
ID	IDVet-067-C- 00002
Med. Vet.: Dr. Carlos Dias	
Data: 04/07/2013	

Resultados MDR – Hipersensibilidade à Ivermectina

MDR, Defeito (Hipersensibilidade à Ivermectina)

Mutação: Homozigotico
Genótipo: -/-

O animal é portador do genótipo homozigoto para la mutação nt230 de MDR1.
Carece de um sistema funcional de transporte.
O defeito terá sido herdado dos seus pais e será transmitido à sua descendência.
Certas drogas não devem ser usadas no tratamento deste animal.

PCR

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