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The Use of Macrocyclic Lactones in Veterinary Practice

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Abbreviation

1. ABCB1: ATP Binding Cassette Subfamily B Member 1
2. BBB: Blood-brain barrier
3. CNS: Central Nervous System
4. DNA: Deoxyribonucleic acid
5. GABA: Gamma-aminobutyric acid
6. GluCl: Glutamate-gated Chloride channel
7. ILE: Intravenous Lipid Emulsion
8. LD50: Lethal Dose, 50%
9. Mdr: Multi-drug resistant
10. ML: Macrocyclic lactones
11. PCR: Polymerase chain reaction
12. P-gp: Permeability glycoprotein
13. PO: Per Os
14. SC: Subcutaneous
15. WHO: World Health Organization

1. Abstract

Macrocyclic lactones (MLs) are parasiticides that can kill a variety of arthropods and helminths. They are one of the most important classes of anthelmintics due to our dependence on them for controlling parasitic infections in livestock, companion animals and humans. Despite its importance, the resistance towards MLs, such as ivermectin or moxidectin in dogs, is an increasing concern. There is a known sensitivity of Collies towards MLs, showing neurotoxicity symptoms when administered drugs are in a higher dose than the labelled dose. However, not only Collies but several other dog breeds have shown similar symptoms. A number of researches showed that the neurotoxicity was attributed to the unfunctional blood-brain barrier (BBB) in susceptible dogs, due to having a defect in the ABCB1 gene resulting in lack of functional permeability glycoprotein (P-gp). The current review examines the mechanism of actions, pharmacokinetics, biological toxicity and activity of MLs, in accordance to ML sensitive breeds due to their defect in the gene ABCB1 and the treatment in case of ML toxicosis. Although a number of treatments are suggested as there is no specific antidote for ML toxicosis. In general, MLs have a long half-life, therefore when an overdose occurs, it leads to a long-lasting illness. Therefore, symptomatic care is the most important part of the treatment.

Összefoglalás

A makrociklikus laktonok (MLs) olyan parazitaellenes hatóanyagok, amelyek képesek elpusztítani különböző ízeltlábúakat és férgeket. A parazitaellenes szerek egyik legfontosabb csoportja, használjuk őket élelmiszertermelő állatoknál, társállatoknál és embereknél is. A makrociklikus laktonok gyakran alkalmazott parazitaellenes szerek, ezért egyre többször tapasztalunk rezisztenciát is velük szemben. Juhászkutyáknál ismert a makrociklikus laktonokra való érzékenység: ha a hatóanyagokat nem használati utasítás szerinti dózisban alkalmazzuk, kialakulhatnak idegrendszeri tünetek. Ezen tünetek nemcsak collieknál, hanem más kutyafajtáknál is kialakulhatnak. Számos kutatásban vizsgálták a neurotoxicitás fellépésének okát, mely az ABCB1 gén mutációja miatt alakul ki, amely a P-glikoprotein hiányát eredményezi a vér-agy gátban. Jelenlegi tanulmányunkban az ML-érzékeny fajtáknál vizsgáltuk a csoportba tartozó anyagok hatásmechanizmusát, farmakokinetikáját, biológiai aktivitását és toxicitását, illetve az ABCB1 gén hibája esetén fellépő ML toxikózist, illetve az ilyenkor alkalmazott kezelési lehetőségeket. ML toxikózis

esetén számos típusú, leginkább tüneti terápia alkalmazható, de specifikus antidótum nem létezik ellenük. Általánosságban elmondható, hogy az ML-ok hosszú felezési idővel rendelkeznek, amely túladagolás esetén hosszantartó megbetegedéshez vezethet. A tüneti kezelés a toxikózis terápiájának legfontosabb része.

2. Introduction

Companion animals are subjected to a number of parasitic infections. Many groups of parasites are also transferable from animals to humans and back—in other words, many parasites are zoonotic. Although the connection between our environment and parasitic diseases is not straightforward, it is existing. For example, climate change has the capability to increase parasitic diseases' incidence and prevalence worldwide. Increase in temperature can directly affect the parasites' life cycles, leading to a direct effect to the prevalence of the organisms within the area, due to many parasitic organisms have a temperature-dependent development, regardless of within the host or in the environment (Lafferty, 2009). Although the parasitic disease emergence and re-emergence may not be the most recognized consequences of climate change, it is one of the most concerning.

As parasitic diseases often go deeper than just declining human health, they can promote economic stagnation in communities and countries that are most likely to be affected, and ultimately decrease the overall quality of life. The devitalizing nature of parasitic diseases reduces community productivity and advancement, resulting in losses of millions of working days and reduction in economic growth each year. Therefore, treating parasitic diseases is of huge importance for both human and veterinary medicine (Wells et al., 2014).

The World Health Organization estimates that 2 billion people have been infected by parasites (WHO, 2019), leading to increased morbidity and mortality. Parasitic infections in livestock animals are an important matter in animal welfare and economics, also affecting food production. Needless to say, as parasites can also infect companion animals, pet healthcare market is an essential economic consideration as animal health companies provide several drug discovery programs (Holden-Dye and Walker, 2014). Although parasitic diseases may not always cause high mortality rates, they can cause further negative effects on issues that we are already facing. Therefore, it is essential and commanding to limit and reduce the prevalence and incidence of parasitic diseases on a global scale. Nowadays, there are a variety of medical options for the treatment of parasitic infections, from external parasites to internal parasites (Taylor et al., 2015).

3. Parasiticides: Macrocyclic lactones

3.1 PARASITICIDES IN GENERAL

Veterinary parasiticides, also called as antiparasitics, are products that kill or inhibit the growth of parasitic organisms that infest livestock, pets and other animals. Antiparasitics can be divided into the following categories: endoparasiticides, ectoparasiticides and endectocides. Drugs against external parasites, ectoparasiticides, are also considered as insecticides, acaricides, or repellents. In contrast, drugs against internal parasites can be divided into antiprotozoals or anthelmintics (Taylor et al., 2015).

Anthelmintics may be also called vermifuges or vermicides, whether the drug will paralyze the worm or destroy the helminths. Anthelmintics must be selectively toxic to the parasite, which is achieved by inhibiting the metabolism of worms, or by pharmacokinetic properties of the compounds that allow the higher concentrations of the drug in the worm compared to the parasite host (Holden-Dye and Walker, 2014). An ideal anthelminthic drug would have a broad spectrum of action, large therapeutic index, effective low dose and low cost. Anthelmintics can be further subdivided into antitrematodals, antinematodals and anticestodals; where the target of the drug is flukes, roundworms and tapeworms, respectively (Laing et al., 2017). Several highly effective and selective anthelmintics are available, which are: macrocyclic lactones, benzimidazoles, imidazothiazoles, isoquinoline and benzazepine derivatives, organophosphates, piperazines, tetrahydropyrimidines, salicylanilides and substituted phenols, and other unclassified drugs. The majority of anthelmintics and nematicides are limited in their action between trematodes, cestodes and nematodes; however, benzimidazoles have cross-phyla activity and are more active against nematodes than against cestodes or trematodes (Holden-Dye and Walker, 2014).

3.2 MACROCYCLIC LACTONES

Anthelmintics are separated into classes on the basis of similar chemical structure and mode of action. Macrocyclic lactones are also known as endectocides, which are a group of drugs effective against both endoparasites and ectoparasites. MLs include 2 groups: avermectins and milbemycins, which are known to be potent ectoparasiticides and endoparasiticides, and are products of *Streptomyces* bacteria. Since MLs are commonly used as parasiticides in many species, they are available in a wide array of formulations (Taylor et al., 2015).

Ivermectin, moxidectin, milbemycin, and selamectin are some of the most common small animal veterinary medicine agents that are used for heartworm prevention. Not only for heartworm infection, ivermectin, moxidectin and doramectin are commonly extra-label used for demodectic and sarcoptic mange as well as other ecto- and endoparasites (Plumb, 2005).

3.2.1 Structure and Chemical Properties of Macrocyclic Lactones

Avermectins are a group of drugs that are highly active against a variety of nematodes, produced from *Streptomyces avermectinius* via fermentation (Burg et al., 1979). This group includes ivermectin, abamectin, doramectin, eprinomectin and selamectin.

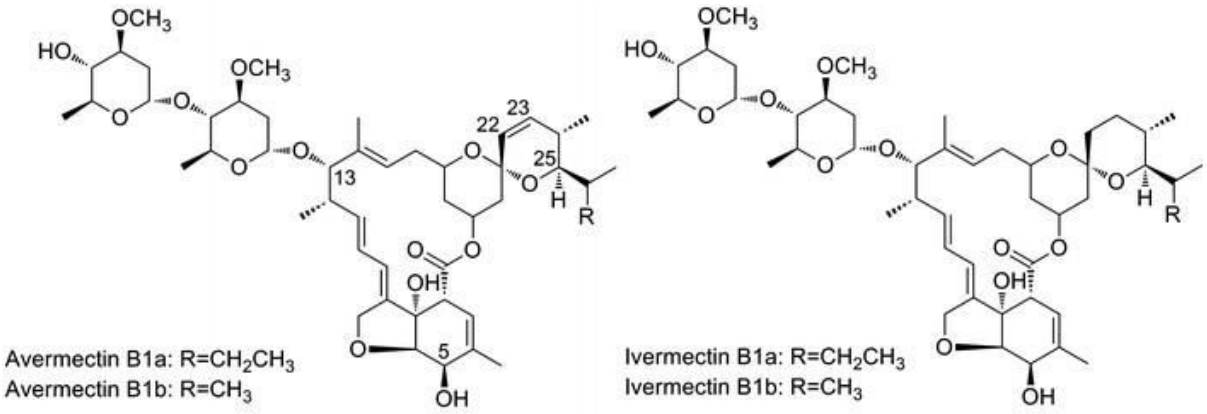

Figure 1: The chemical structure of avermectin and ivermectin. Source: Zhang et al., 2015.

The structures of drugs in the avermectin group share some similarities with antibacterial macrolides and antifungal macrocyclic polyenes; however, they differ in the mechanism of action (Taylor, 2004). Avermectins' structure is also similar to that of milbemycins, although they are different in a way that the bisoleandrosyloxy group is substituting the macrolide ring at position 13 (El-Saber Batiha et al., 2020). Another major difference is that there is an attachment of alkyl group of a different type at C-25 in both classes. When the hydroxyl group at position number 12 of avermectin is deleted, it leads to the production of 13-deoxyavermectin (avermectin aglycone). As this is very similar to several milbemycins' structures, they are also referred to as glycosylated milbemycins (Zhang et al., 2015). Due to its accessibility, the C-4 position of avermectins is often the most studied site for chemical alteration. Without altering the parent drugs' potency, the insertion of different chemical groups such as acyl, amino, or thio groups is performed in order to modify the solubility, stability and distribution of the drug. Also, by modifying the terminal

sugar of avermectins, many different derivatives can be produced in order to improve the potency and efficacy as anthelmintic drugs (Liu et al., 2020).

When comparing the physical and chemical properties of ivermectin and other avermectins, avermectins are less volatile and poorly soluble in water. On the other hand, 50% of ivermectin dissolves in water in less than 6 hours, while 90% of avermectin takes more than 16.8 days to be dissolved in water (El-Saber Batiha et al., 2020). Avermectins are generally soluble in organic solvents such as ethanol, chloroform, diethyl ether, and ethyl acetate. Due to their high absorption coefficient, they are less likely to accumulate in the water column. Ivermectin, on the other hand, has a high affinity to organic matter and hydrophobic properties allow it to accumulate in the environment (Lumaret et al., 2012). It is also known from experiments and research data that ivermectin residues are strongly attached to soil particles (Iglesias et al., 2018). Ivermectin is lipophilic, which may allow the drug to bioaccumulate in animal tissues. However, crossing the biological membrane is prevented due to its high molecular weight, regardless of its lipophilic nature (El-Saber Batiha et al., 2020).

The milbemycin group consists of 3 agents: milbemycin oxime, moxidectin and nemadectin. Similarly, to avermectins, milbemycins are products of fermentation by *Streptomyces* species. Although they both share a similar mechanism of action, milbemycins possess longer half-life than avermectins (Melhorn and Aspock, 2008). However, milbemycins are not glycosylated and differ from avermectin aglycone in which that milbemycins are protonated at C-13 position instead of being hydroxylated (El-Saber Batiha et al., 2020).

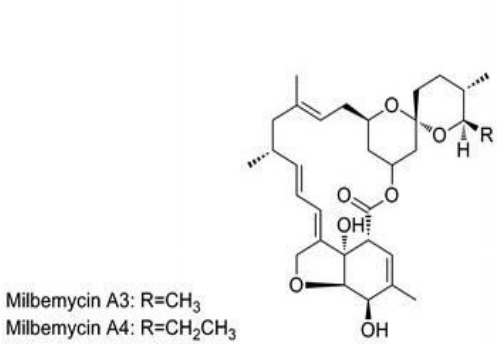

Figure 2: The chemical structure of milbemycins. Source: Zhang et al., 2015.

Milbemycin derivatives also possess different substituents at 5- and 25-positions. Moxidectin, for example, possess methoxime moiety at the 23-position and olefinic side

chain at the 25-position. This is specific in moxidectin, and not seen in any other milbemycins and avermectins (Prichard and Geary, 2019).

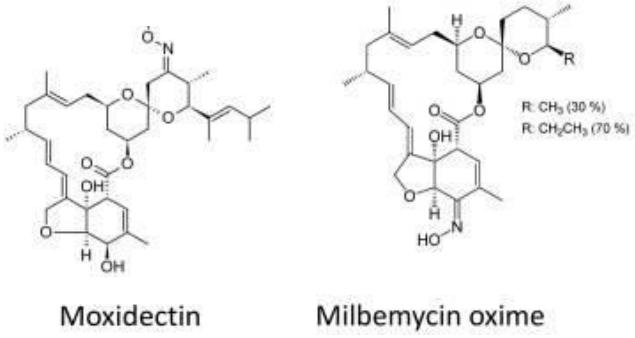

Figure 3: The chemical structure of moxidectin and milbemycin oxime. Source: Prichard and Geary, 2019.

3.2.2 Pharmacokinetics

Fast oral absorption is seen in MLs, but after subcutaneous injection, the absorption rate is more gradual (McKellar and Benchaoui, 1996). They are also lipophilic, and accumulate in fat tissue leading to long elimination half-life, therefore having a long-lasting effect. MLs are known for their wide distributing effect as well (Taylor, 2004).

In avermectins, taking ivermectin as an example, it requires about 4 hours for PO administered ivermectin to reach maximum plasma levels in dogs (t_{max}= 4 hours). Absorption is slower in SC administration, with t_{max}=32-36 hours in dogs and t_{max}=28 hours in cats (Gokbulut et al., 2006; Chittrakarn et al., 2009). The elimination half-life after PO administration is 3.3 days, while after SC administration it is 3.2 days in dogs (Gokbulut et al., 2006) and 3.5 days in cats (Chittrakarn et al., 2009).

Al-Azzam et al. (2007), have performed a study that compares the pharmacokinetics of ivermectin and moxidectin after PO administration. The study showed that moxidectin has a faster absorption compared to ivermectin, as t_{max}=2-3 hours in dogs. Moxidectin has a high bioavailability after PO administration, as about 90% of the drug is absorbed, and the elimination half-life is 13.9 to 25.9 days in dogs (Al-Azzam et al., 2007). Although these parameters were influenced by the body condition of the tested dogs, we can see that moxidectin has a higher absorption rate than ivermectin.

3.2.3 Mechanism of action

MLs exert their effect by binding to ligand-gated chloride channels, resulting in the blockage of transmission of electrical impulses in the muscles and nerves of nematodes (Mealey, 2008). Avermectins bind to glutamate-gated chloride (GluCl) channels which are specific to invertebrates. Binding to GluCl allows the channel to open and leads to an influx of chloride ions. As a result, more chloride ions enter the cells, leading to hyperpolarization and subsequent paralysis of the pharynx, body wall, and uterine muscles in nematodes (Srivastaba et al., 2020).

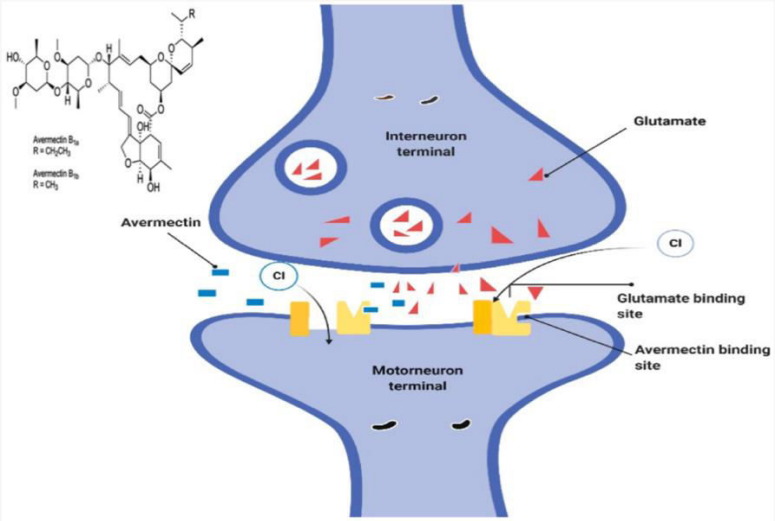

Figure 4: The proposed model to describe mechanisms of ivermectin sensitivity.

Source: El-Saber Batiha et al., 2020.

In nematodes, not only GluCl channels are affected, but avermectins also have a minor effect on GABA receptors. Avermectins can stimulate the release of GABA from nerve endings and enhance the binding of GABA to its receptor (Bloomquist, 2003).

The role of avermectins on chloride channels opening, decreased muscle tone, GABA-like effects and nerve signal transmission has been well studied in several different species, model systems, and different doses (Knipple et al., 1995). One experiment showed that very low dose of avermectin induced an early and fast irreversible inhibition of inhibitory postsynaptic neurotransmitters, delay in reducing the excitatory potential, and increasing the chloride ion entry in the crustacean nerve model. Another study showed that the concentration of avermectin has reversibly increased in the extensor tibiae muscle of *Schistocerca gregaria* by chloride ions in the GABA receptors (Mrozik, 2010). An experiment was conducted by Fisher and Mrozik (1992), designed to differentiate between two different conductance states activated by avermectin. The first model was activated by carbachol, glutamate, ibotenic and

quisqualic acid, and the second was activated by GABA and muscimol (a GABA agonist). In this experiment, avermectin was not able to effectively activate the second conductance like GABA, as it did not require a second messenger pathway due to its direct effect. However, high irreversible increase in the chloride channels opening was observed when the avermectin concentration increased to or above 10 pmol (Fisher and Mrozik, 1992). Based on the mentioned results, it can be established that GABA-insensitive chloride channels can be directly induced by avermectins (Laing et al., 2012; Area et al., 1995). In order to understand the more specific mechanism of avermectins, such as by which avermectin acts against different species of insects, arthropods, nematodes and immature worms, it is necessary to isolate the various species' binding site of avermectins and study how they induce the opening of chloride channel and leads to hyperpolarization (Bloomquist, 2003).

Many pet owners may be concerned about the effect of MLs to their pets. However, the administered dose that leads to the paralysis in nematodes is not toxic for mammals, as they lack the GluCl channels. Although avermectins exert a small effect on GABA receptors, mammalians are only affected by a minor portion. GABA is the chief inhibitory neurotransmitter in the brain in the mammalian CNS; and has 2 receptors, $GABA_A$ being a complex of ligand-gated ion channel complex, and $GABA_B$ metabotropic receptors. The postsynaptic binding of GABA to the receptor serves to reduce the neuronal excitability throughout the nervous system. In mammals, ML binds to GABA type A-gated chloride channels (Lanusse et al., 2009), which are different from where GABA, benzodiazepines, barbiturates, or picrotoxin bind (Arslan, 2015). $GABA_A$ receptors are only present in the CNS, where binding of ML is normally prevented by the blood-brain barrier (BBB). However, when there is enough ML dose to permeate through the BBB, the overdose will allow MLs to bind to $GABA_A$ receptors, as well as to glycine- and voltage-gated chloride ion channels (Trailovic and Nedeljkovic, 2011).

Although avermectins' mechanism of action is well described, that of milbemycins' is not completely understood despite the fact that their anti-parasitic properties have been discovered about 20 years ago. Milbemycins, similarly to avermectins, possess a high affinity for GluCl channels specific to invertebrates. The glutamate-gated binding site is beside the GABA-gated chloride channels, therefore, ML may possibly affect the GABA-gated chloride channels as well (Lanusse et al., 2009).

Nemadectin, the most potent milbemycin, has both insecticidal and nematocidal effects. It also serves as substrate of moxidectin synthesis, another member of milbemycins, which is a commercial endectocide. Moxidectin also binds to myoneural junctions of insects

and the neuronal membrane of nematodes (Njue et al., 2004), and as a result, chloride ion influx occurs and hyperpolarization of the postsynaptic cells leads to decreased resistance in the cell membrane. In the end, the neurotransmission process is disrupted, leading to disabled locomotion and the deaths of parasites (Permberton et al., 2001).

4. Macrocyclic Lactone Sensitivity in Specific Breeds

Specific dog breeds, such as collies, are known to be sensitive when using MLs. This refers to neurotoxicity, due to characteristic defects in the blood-brain barrier (BBB) in susceptible breeds (Dowling, 2006). The reason for the sensitivity can be explained genetically—the genetic mutation of permeability glycoprotein.

4.1 Permeability Glycoprotein

P-gp is a component of the BBB that influences the effects of drugs by preventing their entries to the brain (Merola and Eubig, 2012). It does not have intrinsic metabolic functions; however, it is an important component of the intestinal drug metabolism. P-gp is a protein that affects the pharmacokinetics of many substrates including MLs, by transporting absorbed substrates back across a transmembrane in the body (Mealey, 2008). It is found in all mammalian species, and well distributed throughout the body of dogs and cats (Ginn, 1996; Van Der Heyden et al., 2009).

Juliano and Ling (1976), were the first to isolate P-gp from chemotherapeutic drug-resistant Chinese hamster ovary cells that were selected for colchicine resistance. Isolated as a membrane glycoprotein, they have analysed this protein is one of the major factors that limits permeability into the ovary cell, allowing the multidrug resistance function. Later, cDNA was shown to encode P-gp, where it has been isolated from a multidrug resistance carcinoma cell line, selected for being resistant to colchicine, vinblastine, and doxorubicin (Chen et al., 1986). Afterwards, the name MDR1 was decided for the gene, and was classified as the ATP Binding Cassette Subfamily B Member 1 (ABCB1) (Dean et al., 2001). As P-gp was firstly focused on its function as chemotherapeutic resistance of tumour cells, the first P-gp substrate drugs identified were cytostatic drugs (Sarkadi et al., 2006). Today it is known that P-gp transports a wide variety of structurally unrelated drugs such as chemotherapeutic drugs, antibiotics, non-steroids, corticosteroids. Currently known drugs are listed below:

Chemotherapeutics	Cardiac drugs	Antimicrobials/ Antifungals	Steroids	Immuno-suppressants	H1-antihistamines	H2 antihistamines	Miscellaneous
Doxorubicin	Digoxin	Doxycycline	Itraconazole	Cyclosporine	Fexofenadine	Cimetidine	Amitriptyline
Mitoxantrone	Diltiazem	Erythromycin	Ketoconazole	A	Terfenadine	Ranitidine	Butorphanol
Paclitaxel	Losartan		Rifampin	Tacrolimus			Ivermectin
Vinblastine	Quinidine		Tetracycline	Antiemetics			Morphine
Vincristine	Verapamil			Domperidone			Moxidectin
				Ondansetron			Phenothiazines
							Phenytoin
							Selamectin

Table 1: Currently known Permeability Glycoprotein substrate drugs. Source: Dowling, 2006.

4.1.1 Location of P-gp

As previously mentioned, P-gp is highly expressed in neoplastic tissues. Apart from tumour cells, P-gp is normally located along with the apical border of cell types that serves as barrier function such as intestinal epithelial cells, brain capillary endothelial cells, biliary canalicular cells, renal proximal tubular epithelial cells, testes capillary endothelial cells, placenta, adrenal cortex and CD34+ hematopoietic stem cells (Geyer and Janko, 2012). According to this expression, P-gp can be seen as a protective protein due to its function of limiting entry of substrates into internal compartments (Dowling, 2006). Not only limiting drug entry into the gastrointestinal tract, P-gp also promotes drug elimination in the liver, kidney, and intestines (Martinez et al., 2008).

P-gp's drug limiting function is essential in the brain. P-gp is expressed on brain capillary endothelial cells and functions as part of the BBB to extrude endogenous substances and xenobiotics, such as drugs, from the brain into the circulation (Dowling, 2006). Also, brain endothelial cells are special as they lack pinocytotic vacuoles and fenestrations in their plasma membranes, making the blood barrier selectively permeable (Bernacki, 2008). Substances entering the brain must diffuse through the endothelial cells or be actively transported into endothelial cells by uptake transporters (Urquhart and Kim, 2009). When substances enter the brain, they are potentially extruded back to the apical membrane by P-gp and other efflux proteins (Bernacki, 2008; Urquhart and Kim, 2009).

4.1.2 Mechanism of P-gp

For drug metabolism, cytochrome P450, family 3, subfamily A (CYP3A) is the major enzyme for phase 1 drug metabolizing family. Both CYP3A and P-gp are highly expressed in enterocytes in the gastrointestinal tract, and since substrates of CYP3A are often substrates of

P-gp, they work together in order to prevent oral absorption of many drugs. When a substrate drug enters the intestinal tract, passive processes occur and it is absorbed into the enterocytes. Once inside the enterocyte, the drug may be metabolized by CYP3A, or may enter the systemic circulation, or may be removed by P-gp back into the intestinal lumen which can allow another access to a different enterocyte at a more distal location. Therefore, non-P-gp substrate drugs only pass the enterocytes once, while P-gp substrate drugs have more than one chance to enter the enterocyte and may continuously cycle between the enterocyte and the intestinal lumen. This can result in repeated P-gp efflux, leading to repeated access of CYP3A to the drug molecule or faecal excretion of the drug. Due to many drugs being substrates for CYP3A and p-gp, it is difficult to estimate the individual effects of each protein to reduced oral drug absorption (Dowling, 2006).

The P-gp can be controlled. For example, the antifungal agent ketoconazole inhibits P-gp efflux activity and CYP3A metabolic activity, therefore when administered simultaneously with cyclosporine, it can increase the oral bioavailability of cyclosporine. Although concurrent administration of P-gp substrate drugs and inhibitor drugs can be utilized for effective oral administration for drugs having poor bioavailability, it must be done carefully as toxicity can occur (Dowling, 2006).

4.2 IVERMECTIN-SENSITIVE COLLIE LINEAGES AND THE GENE MUTATION

To treat mammals with MLs, the important role of P-gp in protecting the brain from the penetration of drugs across the BBB cannot be ignored. As previously stated, in lower organisms such as parasites, MLs bind to glutamate-gated and GABA-gated chloride ion channels with high affinity, resulting in an inhibition of nerve activity. However, this occurrence does not take place in mammals as neuronal glutamate-gated chloride channels and GABA-gated chloride channels are restricted to the CNS (Huang and Casida, 1997). The channels are protected from ML binding by the P-gp mediated drug efflux at the BBB (Merola and Eubig, 2012). Therefore, functionally active P-gp is essential in mammals that require ML treatment or any other P-gp substrate drugs.

4.2.1 Mutation

Some dog breeds, for example Collies, are widely known to have ivermectin sensitivity; in other words, having a low threshold for toxicosis due to overdose. The sensitivity of these breeds is attributed to missing one gene, the previously mentioned

ABCB1 gene. As ABCB1 gene encodes P-gp, the base pair deletion in the ABCB1 gene (ABCB1-1Δ) produces a frame shift that generates a premature stop codon in the ABCB1 gene, resulting in a non-functional P-gp (Mealey et al., 2001).

Due to the major role of P-gp in drug disposition, ABCB1-1Δ mutation can lead to improved oral bioavailability and reduced drug elimination through the liver, kidneys, and the gastrointestinal tract. Moreover, it can result in an accumulation of P-gp substrates in the brain that would be normally removed by P-gp (Mealey, 2008). In dogs with this defect, treatment with MLs with doses above the normal dose may result in their accumulation in the CNS, causing neurologic effects. When overdosed amount of MLs is administered, adverse effects can occur in dogs even without the defect, likely due to saturation of P-gp's transport capacity. Dogs may be homozygous or heterozygous for this defect, although homozygous dogs have a higher risk of developing toxicosis from ML ingestion (Mealey, 2008).

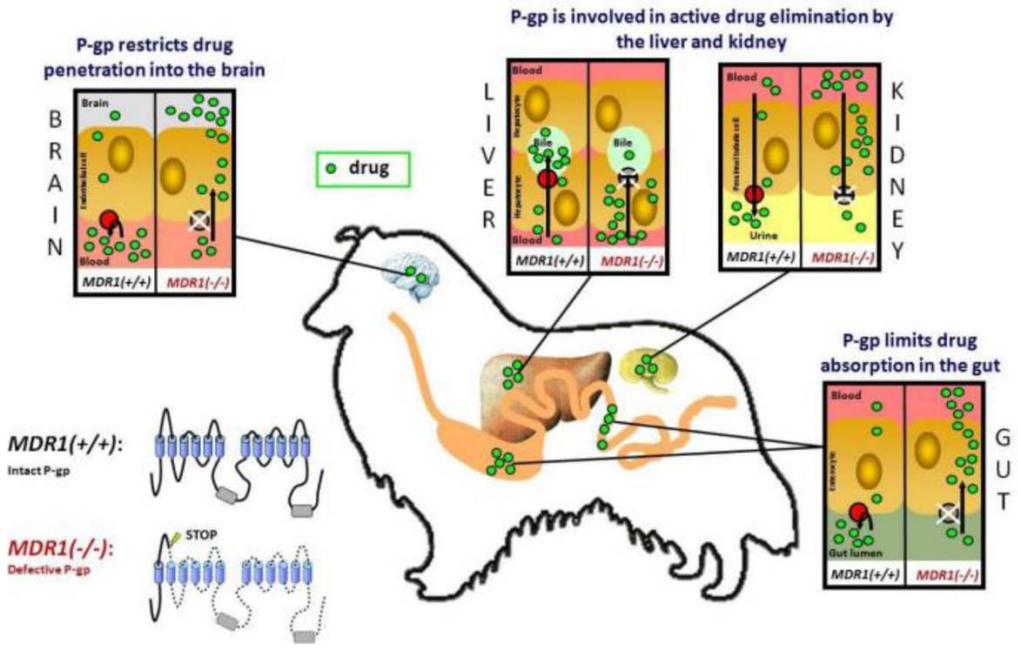

Figure 5: The role of P-gp in drug disposition. P-gp (shown in red) is an ATP-driven efflux transporter which pumps its substrates out of the cell. The intact P-gp limits drug entry into the organism after the oral administration, promotes drug elimination into the bile and urine, and restricts drug penetration across the BBB. In MDR (-/-) (ABCB1-1Δ defect) dogs which do not express a functional P-gp, enteral drug absorption is enhanced, biliary and urinary drug elimination is reduced, and the permeation of blood-tissue barriers is increased at the blood-brain barrier, blood-testis barrier and blood-placenta barrier. As a consequence, P-gp transported drugs can cause adverse effects in these dogs. Source: Geyer and Janko, 2012.

Several experiments were performed in a genetically engineered knockout mouse in order to analyse drug interactions with P-gp. Mice are peculiar in a way that the ABCB1 gene

is duplicated in them, referred to as ABCB1a and ABCB1b (also referred as mdr1a and mdr1b, respectively) (Pastan and Gottesman, 1991). According to Schinkel et al. (1994), in an experiment causing insertional mutagenesis in both ABCB1a and ABCB1b genes, no obvious phenotype nor abnormal physiological changes were observed. However, in this case, it must be emphasized that the laboratory mice grew up in a toxic-free and well-controlled environment. Another experiment was conducted on ABCB1a knockout mice with mite infection. The mice were treated with diluted ivermectin solution spray, and in mice with ABCB1a mutation, paralytic symptoms such as immobility, recumbency, and comatose states were observed. After this experiment, a more detailed analysis was developed, which showed that mice with an ABCB1 mutation have 50-100-fold sensitivity towards ivermectin due to an increased accumulation (Schinkel et al., 1994). The LD_{50}, the dose required to kill 50% of the test population, was 600-800 µg/kg in knockout mice, while in mice without the defect was 50-60 mg/kg (Schinkel et al., 1997). Based on these experiments, it is clearly shown that P-gp expression at the BBB is essential to determine the safety and toxicity margin of MLs, including ivermectin in mammals.

4.2.2 Breed predisposition of the mutation

Although many people believe that Collies, specifically Scotch collies, are the only sensitive breeds towards MLs, the following 12 breeds share the same feature: Australian Shepherd, Border Collie, English Shepherd, German Shepherd, Longhaired Whippet, McNab, Miniature Australian Shepherd, Old English Sheepdog, Shetland Sheepdog, Silken Windhound, and White Swiss Shepherd (Neff et al., 2004). However, ivermectin sensitivity is not present in all individuals of the breed, and it is not related to sex, collie-eye anomaly, or hair coat type (Tranquili et al., 1987).

In the past several years, genotyping analysis of ABCB1-1Δ mutation among different breeds was conducted on more than 15,000 dogs worldwide (Gramer et al., 2011). In the previously stated 12 dog breeds that are affected by this gene deletion mutation, the ABCB1 gene deletion is widespread in Collies, with 35% being homozygous and 42% being heterozygous. Its frequency is much lower in other breeds of collie lineage, such as in Shetland Sheepdogs (8.4%), or Old English Sheepdogs (3.6%). It is suspected that deletion occurs to the Longhaired Whippet and the Silken Windhound due to mutation being introduced by crossing with Shetland Sheepdogs (Dowling, 2006). Purebred dogs of other breeds have also been tested, where it has been found that none of them has the gene defect (Merola and Eubig, 2012). Apart from purebred dogs, high frequency values were also found

in herding breed mixes and unclassified breed mixed dogs (Mealey et al., 2008; Gramer et al., 2011). In contrary, few dog breeds that have a close genetic relationship or share the breeding history with the previously mentioned 12 dog breeds were demonstrated as not having the ABCB1-1Δ mutation. Such dog breeds include Bearded Collie, Anatolian Shepherd Dog, Greyhound, Belgian Tervuren, Kelpie, Borzoi, Australian Cattle Dog and Irish Wolfhound (Neff et al., 2004; Mealey et al., 2008; Gramer et al., 2011).

However, a recent report showed that other gene mutations can also produce ivermectin sensitive phenotype—an ABCB1 mutation that is different from the ABCB1-1Δ mutation was found in an ivermectin-sensitive Border Collie (Han et al., 2010). Therefore, there is no guarantee that dogs without ABCB1-1Δ mutation will tolerate a higher dosage of MLs.

The gene defect can be easily found in dogs (Mealey et al., 2008), although it is difficult to understand whether the frequencies of the gene defect in the tested animal population are representative to that of the general population. Although there are a lot of data about ABCB1 genotyping, it is difficult for veterinarians and dog owners to recognize if this gene mutation is relevant for the patient on a practical basis.

4.2.3 Testing for ABCB1-1Δ gene mutation

Genotyping method for the ABCB1 gene status of individual dogs could be used to avoid cases of acute neurotoxicity and to choose safe therapeutic medication. Several methods using PCR for allelic differentiation are used, but have not reached to the point that may be used as a point-of-care test at clinics. Ideally, a bedside testing for allele-specific DNA amplification would be non-invasive DNA sampling and require minimal hands (Stiedl and Weber, 2017).

Samples using blood, buccal swab and dewclaw samples can be used for testing. DNA from the samples are extracted and stored at -20°C until further processing; the DNA amplification. According to Stiedl and Weber (2017), the total reaction volume of 20µl including 10µl of PCR master mix, 5µl of template, 4 µl of water, 0.5µl of allele-specific forward primer and 0.5 µl of reverse primer is needed for allele-specific PCR. The amplification is performed on a thermocycler with the following protocol: hot start at 96°C for 5 minutes, then 45 cycles of denaturation at 98°C for 5 seconds, followed by annealing at 50°C for 5 seconds, synthesis at 68°C for 10 seconds, and lastly, extension at 72°C for 1 minute. The reaction is completed in 35 minutes . After the DNA amplification process, PCR

products are visualized on 2% agarose gel under ultraviolet light at 312 nm (Baars et al., 2008).

After the genotyping test, there will be 3 patterns of ABCB1 gene results. First result will be “Normal/Normal” which indicates that the dog would not be expected to have adverse reactions to normal doses of drugs. “Normal/Abnormal” result indicates that the dog is a carrier, meaning that the dog may pass on the mutant gene to their offspring and may experience adverse reactions to normal doses of drugs. Lastly, “Abnormal/Abnormal” result indicates that the dog will pass on the mutant gene to their offspring and the dog is expected to experience drug toxicity to normal doses (Mealey et al., 2008).

4.3 TREATMENT OF ABCB1-1Δ DOGS WITH MACROCYCLIC LACTONES; AND ITS INTOXICATION OCCURRENCE

As ABCB1-1Δ dogs are prone to neurotoxicity caused by MLs due to the lack of P-gp expression at the BBB, can we not treat parasitic infections with MLs in sensitive breeds? The answer is no, as treatment of these dogs with MLs does not inevitably result in neurological signs.

Due to the common use of MLs as parasiticides, they are available in a wide range of formulations (Taylor, 2004). For small animals, there are products that include ivermectin, moxidectin, or milbemycin oxime in tablets, or topical products including selamectin are available. Ivermectin, moxidectin, milbemycin oxime and doramectin are also used off-label for various indications (Plumb, 2005), which we will discuss later in this chapter. There also may an accidental exposure of dogs and cats to large animal products, which many formulations intended for large animals are concentrated and easier for accidental overdoses to occur. The safety of treatment depends on the following factors: dosage/treatment indication, route of application, individual compound, heterozygous or homozygous genotype of the dog.

Dosage of MLs is crucial for the occurrence and outcome of the intoxication, regardless of whether the ML was therapeutically applied or accidentally ingested. According to Geyer et al. (2007), after the SC application of doramectin at 600 µg/kg to ABCB1-1Δ dogs, severe neurological symptoms were observed, but both dogs fully recovered within 14 days (Geyer et al., 2007). On the other hand, two ABCB1-1Δ homozygous dogs died within 5-6 days after the application of 1 mg/kg, which is just slightly higher than the other case (Geyer and Janko, 2012).

Route of application is also an important factor for the safety of treatment with MLs. According to Geyer et al. (2007), this particularly applies to topical application of moxidectin and selamectin. Moxidectin and selamectin are widely used for ecto- and endoparasitic diseases, and should not be orally applied to ABCB1-1Δ homozygous dogs (Geyer et al., 2007). Similarly, Paul et al. (2004) have stated that spot-on applications of ivermectin did not induce neurotoxicity in ABCB1-1Δ homozygous dogs at dosages up to 1.0 mg/kg; however, the oral application of the equivalent dose would lead to severe toxicity for ABCB1-1Δ dogs (Paul et al., 2004). Apart from spot-on and oral applications, ivermectin is often extra-label used as SC injection at 400 µg/kg for the treatment of mange disease, while this route of application in ABCB1-1Δ dogs generally results in less severe toxicity although the signs are long-lasting compared to the oral route of application (Hopper et al., 2002).

Individual compounds of MLs are a key factor in neurotoxicity. Ivermectin and doramectin have similar neurotoxicological potential compared to other members of MLs, as they can induce severe toxicity at dosages above 200 µg/kg (Merola and Eubig, 2012). On the other hand, other MLs such as selamectin, moxidectin and milbemycin oxime appear to be safer than ivermectin and doramectin in treatment of ABCB1-1Δ homozygous dogs. Especially selamectin and milbemycin oxime seem to have less neurotoxicological potential and have only shown mild toxicity at dosages above 5 mg/kg in ABCB1-1Δ dogs (Merola and Eubig, 2012; Novotny et al., 2000). Regardless of their mentioned safety, it must be emphasized that the in treatment of ABCB1-1Δ dogs, the safety margin is drastically reduced if P-gp expression is lacking in the BBB. Therefore, the treatment of ABCB1-1Δ dogs with any ML compound requires particular caution.

Lastly, the genotype of the dog takes a major role in the safety of treatment with MLs. Systematic studies on ML application to ABCB1-1Δ homozygous or ABCB1-1Δ heterozygous dogs have not been performed for any compound; however, according to Sartor et al. (2004), although the genetic ABCB1 status was unknown, many clinical cases with ML intoxication in Collies or related breeds resulted in two types of reaction. The first type of reaction is mild ataxia with CNS depression and quick recovery, and the second type with more severe and long-lasting intoxications. Presumably, the first type is ABCB1-1Δ heterozygous dogs and the second type is ABCB1-1Δ homozygous dogs (Sartor et al., 2004; Nakai et al., 1990). In addition, it was shown that ABCB1-1Δ heterozygous dogs can tolerate the oral doses of ivermectin up to 600 µg/kg, which is an equivalent to therapeutic dose in normal ABCB1 dogs. Therefore, ABCB1-1Δ heterozygous dogs can be regarded as having an intermediate ML sensitivity, and it is unlikely that these dogs will suffer from coma or death even in high ML dosages unlike ABCB1-1Δ homozygous dogs (Bissonnette et al., 2009).

4.4.1 Intoxication with MLs

Generally, ML intoxication signs are related to the CNS; such as neurologic depression, ataxia, mydriasis, blindness, tremors, and hypersalivation. As the signs progress, the animal can become comatose. The signs are similar in both dogs and cats for all of the ML substances; however, the breed can affect the length of the toxicosis. Depending on the

dose and the breed, the toxicosis may persist for days to weeks, due to the long half-life of these agents (Kenny et al., 2008).

Agent	Formulations	Therapeutic dosages (labeled and off-label) (mg/kg)	Acute, subacute or chronic dosages published as safe (mg/kg)	Toxic dosages ML sensitive dogs (mg/kg)	Acute toxic dosage normal dog/cat (mg/kg)
Ivermectin	Tablets, oral liquid, oral paste, feed premix, injectable, topical, otic	0.006–0.6 PO D 0.024 PO C 0.2–0.4 SC D, C	0.5 PO daily × 12 weeks D 0.06 PO Collies 0.2–1.33[a]PO or SC C 0.72 PO C	0.1–0.4 PO 0.2–0.25 SC	0.2–2.5 PO D 0.3 SC C
Selamectin	Topical	6 topical D, C	6 PO D, C 40 topical Collies 72–114 topical D 236–367 topical C	5 PO	None found
Moxidectin	Tablets, oral drench, injectable, topical	0.003 PO D 0.17 sustained release SC D 2.5 topical D 1 topical C	1.15 PO daily × 1 year D 0.09 PO Collies 0.85 SC D, Collies	1 PO	1.9–2.8 PO D 1 PO C
Doramectin	Injectable, pour-on	0.6 SC D, C	0.5–1 PO daily × 91 days D 0.2 SC C	0.2–0.7 SC	None found
Milbemycin	Tablets	0.5—2 PO D 2 PO C	10 PO Collies 10 PO C	5–10 PO 0.8 PO × 2 days 1.5 PO × 13 days	None found

Table 2: Therapeutic, nontoxic, and toxic dosages of macrocyclic lactones in both normal and sensitive dogs and in cats. Based on Merola and Eubig, 2012. Abbreviation: D = dogs, C= cats.

4.3.2 Ivermectin

Indication	Drug	Dosage	Label	ABCB1 normal dogs	ABCB1-1Δ mutant dogs
Heartworm prevention	Ivermectin	6-12 µg/kg PO once monthly	Heartgard®	+	+
Generalized demodicosis		400 – 600 µg/kg PO daily	Extra-label	+	-
Other ectoparasitic and endoparasitic infections		50 – 200 µg/kg PO once	Extra-label	+	+/- Toxic at >100 µg/kg
		300 – 400 µg/kg PO or SC weekly	Extra-label	+	-

Table 3: Treatment safety of ectoparasitic and endoparasitic infections with MLs in ABCB1 normal dogs and ABCB1-1Δ mutant dogs; ivermectin column. Source: Geyer and Janko, 2012. Abbreviation: PO, oral application, SC, subcutaneous application, "+" tolerated, "-" not tolerated, may induce neurotoxicosis.

Ivermectin is the most common avermectin derivative and is available in numerous forms and ways of administration. It is available in form of chewable tablets for heartworm prevention, ectoparasite treatment, and as microfilaricide. Ivermectin is used for heartworm prevention at dosages of 6-12 µg/kg in dogs and 24 µg/kg in cats. It is also used extra-label in dogs as a microfilaricide at 50-200 µg/kg, and to treat ectoparasites at 300-400 µg/kg (Plumb, 2005). As tablets for heartworm prevention range from 68 to 272 µg of ivermectin per tablet, and 100-400 µg/kg PO would be the toxic dosage for ivermectin-sensitive dogs, toxicosis is rare even when small animals ingest several of these pills. Intoxications can occur accidentally, such as miscalculation of dosage when using off-label products, exposure to large animals' formulation (e.g. horse dung) which are relatively high in concentrations.

Clinical signs were seen in sensitive breeds with a history of ivermectin sensitivity at a dose ranging from 80 to 340 µg/kg (Houston et al., 1987; Merola et al., 2009). However, ABCB1 gene deletion was not tested in any of the dogs. In breeds considered having a normal response to ivermectin, mild clinical signs have been observed at dosages above 200 µg/kg, in 1 to 2.5 mg/kg or greater, more severe signs have been observed (Merola et al., 2009; Houston et al., 1987). Some dogs showed signs at relatively low ivermectin dosages in one retrospective study (Merola et al., 2009). German shepherds also showed adverse effects, and this reaction may be explained as a small percentage of this breed carry the ABCB1 gene defect.

It must be emphasized that sensitivity reactions are not expected with standard heartworm preventative dosages even in ABCB1-1Δ dogs. No signs have developed when ivermectin-sensitive Collies were treated with 10 times the heartworm preventative dosage (60 µg/kg). This indicates that even in ABCB1-1Δ dogs, low dose ivermectin is a safe heartworm preventative (Nakai et al., 1990). However, problems can occur in ABCB1-1Δ dogs or even in dogs with normal ABCB1 genotype, when ivermectin is used at higher dosages as a microfilaricide or for demodicosis. Clinical signs have been seen in ivermectin-sensitive Collies, with dosages as low as 0.1 mg/kg (Tranquilli et al., 1987). When overdosed, most frequent clinical signs observed were lethargy, ataxia, hypersalivation, tremors, mydriasis, blindness, and bradycardia. However, in ivermectin intoxication, blindness is typically temporary and is associated with retinal oedema and electroretinogram abnormalities. In severely affected animals, coma, seizures, and death have been reported (Lewis, 1994; Kenny et al., 2008).

4.3.3 Selamectin

Indication	Drug	Dosage	Label	ABCB1 normal dogs	ABCB1-1Δ mutant dogs
Heartworm prevention	Selamectin	6 mg/kg spot on monthly	Stronghold, Revolution	+	+
Other ectoparasitic and endoparasitic infection					

Table 4: Treatment safety of ectoparasitic and endoparasitic infections with MLs in ABCB1 normal dogs and ABCB1-1Δ mutant dogs; selamectin column. Source: Geyer and Janko, 2012. Abbreviation: "+" tolerated, "-" not tolerated, may induce neurotoxicosis.

Selamectin is available for dogs and cats in forms of topical products which are labelled for prevention of heartworm and for killing fleas and ear mites. Other treatments include sarcoptic mange and tick infestation in dogs and ascarids and hookworms in cats (Krautmann et al., 2000). The minimum dosage as a parasiticide is 6 mg/kg, in concentrations of 60 mg/mL and 120 mg/mL. In the same dosages, it may also be used to treat sarcoptic mange and tick infestation in dogs and hookworms and ascarids in cats (Plumb, 2005). Due to the lack of more concentrated forms, intoxication by overdose is less common. The most common clinical signs due to selamectin exposure include vomiting, drooling, itching, lethargy, agitation, anorexia, and ataxia; many of them resulting from inadvertent oral exposure or administration (Merola and Eubig, 2014).

4.3.4 Doramectin

Doramectin has been used off-label to treat demodicosis in dogs and cats at 0.6 mg/kg SC once weekly (Plumb, 2005). It is also available as an injectable formulation at 10 mg/mL for ruminants and pigs and pour-on for cattle at 5 mg/mL (Lanusse et al., 2009). Small animals are less frequently exposed to doramectin compared to some of the more common MLs, however, toxicosis can easily occur due to products available in high concentrations.

One reported toxicosis was in a collie given 0.2 mg/kg doramectin SC, with clinical signs of blindness, restlessness, CNS depression, recumbency, hypersalivation, tremors, tachypnea, ataxia, head pressing, disorientation, lack of menace response, and bradycardia (Yas-Natan, 2003). However, the particular dose to be termed for overdose has not been determined yet (Geyer et al., 2007).

4.3.5 Moxidectin

Indication	Drug	Dosage	Label	ABCB1 normal dogs	ABCB1-1Δ mutant dogs
Heartworm prevention	Moxidectin	170 µg/kg SC every six months	ProHeart	+	+
		2.5 mg/kg moxidectin + 10 mg/kg imidacloprid spot on monthly	Advocate, Advantage mutli	+	+
Generalized demodicosis		2.5 mg/kg moxidectin + 10 mg/kg imidacloprid spot on monthly	Advocate, Advantage multi	+	+
		200 – 400 µg/kg PO daily	Extra-label	+	-
Other ectoparasitic and endoparasitic infections		250 µg/kg PO or SC weekly	Extra-label	+	?
		400 µg/kg PO every 3-4 days for 3-6 weeks	Extra-label	+	-
		2.5 mg/kg moxidectin + 10 mg/kg imidacloprid spot on monthly	Advocate, Advantage multi	+	+

Table 5: Treatment safety of ectoparasitic and endoparasitic infections with MLs in ABCB1 normal dogs and ABCB1-1Δ mutant dogs; moxidectin column. Source: Geyer and Janko, 2012. Abbreviation: PO, oral application, SC, subcutaneous application, "+" tolerated, "-" not tolerated, may induce neurtoxicosis.

Moxidectin is available in many forms including topical products, SC injection, and monthly tablets for heartworm prevention in small animals. Similarly to ivermectin, moxidectin products intended for use in equines and ruminants are available in relatively high concentrations (0.5%-2% or 5-20 mg/mL), therefore it is possible for small animals to be exposed to high doses from small amounts of these products. As with ivermectin, another potential exposure to moxidectin for dogs could be via horse dung (Perez et al., 2001). In dogs, moxidectin is used for heartworm prevention at 0.17 mg/kg sustained-release SC injection every 6 months. As a topical preparation for heartworm prevention, it can be used for both dogs and cats, with an amount of 2.5 mg/kg and 1 mg/kg, respectively (Plumb, 2005).

Marketed moxidectin containing products are some of the few drugs that are approved for canine generalized demodicosis. Generalized demodicosis caused by *Demodex canis* mites is a very common skin disease and moxidectin, amitraz (an alpha-adrenergic receptor agonist) and isoxazolines are the possible treatment options (Six et al., 2016). Although other MLs are often used in the treatment of generalized demodicosis, it is an extra-labelled product, meaning that they are not approved for this indication. Extra-label use of moxidectin for the treatment of demodicosis is performed at a dosage of 200-400 µg/kg PO, which is well tolerated in normal dogs, however, not in ABCB1-1Δ homozygous dogs. As 400 µg/kg PO application to ABCB1-1 homozygous dogs would lead to neurotoxicosis, it cannot be applied; therefore, to treat generalized demodicosis in ABCB1-1Δ homozygous dogs, spot on preparations of moxidectin and isoxazolines are the current treatment options (Heine et al., 2005).

Clinical signs such as ataxia, tremor, seizure, hyperthermia, tachycardia, blindness, hypersalivation, bradycardia, coma, and respiratory depression were seen in dogs exposed to equine moxidectin dewormers (See et al., 2009).

4.3.6 Milbemycin oxime

Indication	Drug	Dosage	Label	ABCB1 normal dogs	ABCB1-1Δ mutant dogs
Heartworm prevention	Milbemycin oxime	500 µg/kg milbemycin oxime + 5 mg/kg praziquantel PO monthly	Milbemax	+	+
		500 – 990 µg/kg PO monthly	Interceptor	+	+
Generalized demodicosis		0.5 – 2.0 mg/kg PO daily	Extra-label	+	+
Other ectoparasitic and endoparasitic infections		500 µg/kg milbemycin oxime + 5 mg/kg praziquantel PO monthly	Milbemax	+	+
		500 – 990 µg/kg PO monthly	Interceptor	+	+

Table 6: Treatment safety of ectoparasitic and endoparasitic infections with MLs in ABCB1 normal dogs and ABCB1-1Δ mutant dogs; milbemycin oxime column. Source: Geyer and Janko, 2012. Abbreviation: PO, oral application, SC, subcutaneous application, "+" tolerated, "-" not tolerated, may induce neurotoxicosis.

Milbemycin oxime is available for dogs and cats in forms of oral chewable tablets (2.3-27mg) for heartworm prevention and as an antihelmintic agent; and 0.1% otic solution for treating ear mites (Plumb, 2005). Since it is not available in more concentrated dosage forms, overdoses are relatively rare. The therapeutic dosages of milbemycin for heartworm prevention are 0.5 mg/kg in dogs and 2 mg/kg in cats. When ivermectin-sensitive breeds were dosed at 5 -10 mg/kg, clinical signs of ataxia, hypersalivation, mydriasis, and lethargy have been seen (Tranquilli et al., 1991). Mild clinical signs have been reported to develop in normal dogs at 10-20 mg/kg, and the most common signs reported include ataxia, tremor, lethargy, vomiting, mydriasis, disorientation, and hypersalivation (Merola and Eubig, 2014).

When dosages of 0.5-2.5 mg/kg milbemycin oxime were applied PO to Collies, no clinical symptoms of neurotoxicity were observed (Tranquilli et al., 1991). However, it has to be assumed that these dogs, although of the Collie breed, were not affected by the ABCB1-1Δ mutation. Barbet et al. (2009) performed the analysis of the safety of milbemycin oxime treatment in dogs with generalized demodicosis, including two ABCB1-1Δ homozygous and ABCB1-1Δ heterozygous dogs. No adverse drug reactions were seen in dogs with normal ABCB1 status and ABCB1-1Δ heterozygous dogs. On the other hand, although the treatment has started with a low dose of 0.3 mg/kg/day, both ABCB1-1Δ dogs showed signs of ataxia following an increase of the dose to 1.5 mg/kg/day. When the dose was reduced to 0.6

mg/kg/day, both dogs have recovered (Barbet et al., 2009). This study shows that for treating dogs diagnosed with generalized demodicosis, milbemycin oxime may be a safer choice than ivermectin or doramectin for ABCB1-1Δ homozygous dogs.

5. Treatment of ML toxicosis

As there are no specific antidotes for ML toxicosis, decontamination and appropriate symptomatic and supportive care are the keys of the treatment. According to Merola and Eubig (2012), it is possible for even severely intoxicated animals to completely recover with committed treatment. As some patients would require hospitalization for several days, it is important to advise the animal owners beforehand.

5.1 Usage of Emetics and Adsorbents

Following oral ingestion of MLs, inducing emesis for drug removal may be the initial therapy considered if the ingestion was recent and the animal is asymptomatic. Even gastric lavage may be considered if the emetics are not effective. In ML toxicosis, there are no established criteria for when to induce or avoid emesis (Merola and Eubig, 2012).

However, emesis is not a perfect solution. According to Wyse et al. (2003), as the liquid or paste formulations will empty from the stomach faster than solid formulations, it is anticipated that the emesis will not be quick enough. In occurrence of toxicosis, administration of adsorbents is also advised; however, inducing emesis would result in the delay of administration. Also, subsequent administration of adsorbents, may be vomited if emesis is induced. In addition, in patients showing neurological signs, aspiration must be avoided, therefore emesis is not suggested in those patients showing signs of tremors, seizures, or CNS depression. In conclusion, inducing emesis is best if the ingestion occurred within the past 30 to 60 minutes. Emesis may also be considered if a large meal was taken prior to ingesting MLs (Merola and Eubig, 2012).

Adsorbents, such as activated charcoal, can discourage the enteral absorption of drugs and improve the elimination through forces. Their usage is recommended in case of several types of toxicosis. It is likely to be beneficial if given in the first 4 hours, and repeated every 8 hours for 2 days in case of ivermectin toxicosis (Merola et al., 2009; Lovell, 1990), although the exact efficiency of activated charcoal in case of ML toxicosis has not been yet proved. As with emesis, the possibility of aspiration would increase when administering adsorbents to symptomatic patients, especially to those in a comatose state. Therefore, administration is prohibited in patients lacking a gag reflex. Other complications of adsorbent administration include hypernatremia and hypermagnesemia, as the free water would be osmotically be drawn into the gastrointestinal lumen; although the incidence has not yet been reported in small animals (Dorrington et al., 2003).

In addition, repeated administration of activated charcoal may not be much of a benefit in dogs with ABCB1-1Δ mutation due to non-functional P-gp may reduce the biliary elimination of P-gp substrate drugs. However, this has not been proven, as the amount of ML eliminated in bile has not been evaluated yet (Geyer and Janko, 2012).

5.2 SYMPTOMATIC AND SUPPORTIVE CARE

During ML toxicosis, supportive care and monitoring electrolytes, fluid balance, blood pressure, heart rate, body temperature, blood gases and respiratory function are particularly important. Parenteral alimentation may also be needed. In case of pronounced respiratory depression, oxygen, intubation and mechanical ventilation are required. Atropine or glycopyrrolate may be administered in a pre-anaesthetic dose in case of bradycardia (Merola and Eubig, 2012). Histamine H_2 receptor blockers or proton pump inhibitors may also be administered in order to prevent gastric acid secretion, with the intention to decrease gastric irritation. However, omeprazole and pantoprazole are more recommended in ABCB1-1Δ mutant dogs, as cimetidine is a P-gp substrate drug (Campbell and Chapman, 2000). Sucralfate may also be used for gastric protection, as sucralfate may increase gastric mucosal prostaglandins, which further leads to increasing the output of soluble mucous (Quadros et al., 1987).

Neurotoxicosis can lead to unresponsiveness, and in such circumstances, physostigmine can be administered at a total dose of 40 µg/kg intravenously twice a day (Nelson et al., 2003). Physostigmine is a cholinesterase inhibitor that increases the acetylcholine concentration at the synapses in the peripheral and central nervous system. As acetylcholine regulates the inhibitory GABAergic and excitatory glutamatergic neuronal firing, physostigmine may improve the clinical signs. However, the duration of improvement is for a short time only, physostigmine effects last only 30-90 minutes (Tranquilli et al., 1987). Physostigmine does not accelerate the recovery of the patient, nor improve the general outcome. Also, the administration must be done carefully, as physostigmine overdose can lead to bradycardia and development of convulsion. Glycopyrrolate administration may be suggested prior to physostigmine administration in order to avoid side effects such as bradycardia. Despite its side effects, physostigmine is a helpful premedication during recovery, either to arouse a patient for to walk and eat or to visually encourage an owner that recovering is still possible (Hopper et al., 2002).

For treating tremors or seizures which often occur as late-onset symptoms resulting from neurotoxicity, benzodiazepine drugs such as diazepam should be avoided. According to Snowden et al. (2006), in clinical cases, diazepam administration resulted in transient improvement of CNS stimulation, which was subsequently followed by severe CNS depression (Snowden et al., 2006). This is due to the enhancement of benzodiazepine drugs binding to GABA receptors potentiated by MLs, and further enhancement of GABAergic activity, causing CNS depression (Campbell and Chapman, 2000). Some state that propofol may be an appropriate medication in this phase of neurotoxicosis (Snowden et al., 2006; Gallagher, 2010), however, propofol also binds GABA receptors (Trailovic and Nedeljkovic, 2011). Therefore, not only diazepam but propofol may be cautiously used in an attempt to control tremors or seizures.

The possible antidote had been previously discussed - picrotoxin, a GABA antagonist that blocks GABA-activated chloride ion channels. It has been intravenously infused in few cases in treating neurotoxicosis resulting from ivermectin, at a dosage rate of 1 mg/min for 8 minutes (Sivine et al., 1985). At first, picrotoxin seemed to reverse CNS depression; however, 30 minutes after administration, violent clonic seizures were observed. Due to this occurrence, picrotoxin is no longer recommended its use as a routine antidote for ivermectin intoxication (Sivine et al., 1985).

Flumazenil is also a GABA antagonist that was a potential antidote for ivermectin toxicosis, in an experimental model of drug interactions in rodents (Trailovic and Nedeljkovic, 2011). However, flumazenil is a GABA antagonist at the binding sites of benzodiazepines, rather than the binding site of GABA (D'Hulst et al., 2009). Therefore, flumazenil is more likely to prevent benzodiazepines from binding, not directly influencing the effect of GABA. If flumazenil was clinically beneficial, the effects would be similar to that of physostigmine, both transiently improving clinical signs (Gwaltney-Brant and Rumheiha, 2002).

Till the current day, the assessment of GABA receptors is very challenging. It is known that there are several different binding sites of GABA receptors, where each of them binds to different types of drugs. These different binding sites interact allosterically, by binding compounds to other sites, which influence the opening of the channels leading to chloride ion influx (Arslan, 2015; D'Hulst et al., 2009). The relationship of different binding sites and the relationships between MLs and drugs that bind to GABA receptors requires more investigation. Until the investigation has identified the clear view of the allosteric

relationship, drugs such as diazepam and propofol may be used with caution to control tremors or seizures resulting from ML intoxication (Geyer and Janko, 2012).

5.3 TREATING IVERMECTIN TOXICOSIS WITH ILE

In case of ivermectin toxicosis, veterinary patients can result in death without aggressive treatment, and severe toxicosis often requires mechanical ventilation and intensive care; particularly in ABCB1-1Δ patients. Treatment called Intravenous Lipid Emulsion has been specifically used to remove ivermectin toxicosis. Although the exact mechanism is unknown, novel ILE treatment (also known as Lipid Resuscitation Therapy) has been effective in human patients with lipid-soluble drug toxicosis (Clarke et al., 2011; Neal et al., 2018).

5.3.1 Mechanism of ILE

There are several theories about the mechanism of action of ILE. One theory was proposed by Weinberg et al. (1998), that a lipid compartment gets created in the blood into which the lipophilic drug may dissolve, thereby removal of the drug is possible with lipids from the aqueous plasma circulation. Another theory proposed by Fettiplace et al. (2018), relating ILE as “lipid shuttle/subway”. Lipid compartment would roam for the lipid-soluble drug in high blood organ, then redistributes to muscles for storage and the liver for detoxification (Neal et al., 2018). Addition to redistributing the drug, cardiotonic and postconditioning effects from ILE have been shown in both animal and human models. As cardiac contractility is directly increased by lipids, ILE will allow the increase in cardiac output and preload in single volume expansion (Neal et al., 2019; Fettiplace et al., 2018). Separately from the cardiac mechanisms, Fettiplace et al. (2018), have also mentioned the lipid-induced effect on the improvement of vascular tone and contractility. Though the exact mechanisms are still unclear, these cardiovascular benefits improve blood pressure and cardiac output (Neal et al., 2018; Fettiplace et al., 2018).

5.3.2 Clinical reports of ivermectin toxicosis patients treated with ILE

In veterinary patients to treat ivermectin toxicosis, 1.5 ml/kg of a 20% sterile lipid solution is administered intravenously over 10 minutes, followed by constant rate infusion of 0.25 mg/kg/min over 60 minutes (Clarke et al., 2011). In the case report of Clarke et al.

(2011), the patient treated by ILE for ivermectin toxicosis was discharged from the hospital 48 hours after admission and was clinically healthy within 4 days after ivermectin ingestion. However, further diagnostic examinations found that the patient in this case was unaffected with ABCB1-1Δ gene mutation.

ILE treatments are not always successful. 3 dogs with ivermectin toxicosis had been treated with a 1.5 ml/kg of 20% formulation of ILE intravenous bolus, followed by a slow intravenous infusion (0.25 – 0.5 ml/kg/m) over 30 minutes. All 3 dogs were homozygous for ABCB1-1Δ gene defect. Initial clinical signs were tremors, ptyalism, and central nervous signs; and these neurological signs were not improving after the treatment (Wright et al., 2011). As ILE treatment was ineffective in these ABCB1-1Δ homozygous mutant dogs, further investigation is needed in order to investigate why ILE treatment was unsuccessful; and if further use of ILE may yield better outcomes.

In summary, ILE appears to be a helpful and safe treatment for intoxicated veterinary patients. However, ILE effects are only guaranteed in specific toxicosis, although adverse events relating to ILE are infrequent. Standard therapy and symptomatic treatment shall be performed before considering ILE, and the potential side effects shall be assessed prior to the administration as a potential antidote for ML intoxication.

6. Conclusions

Although there is a proven correlation between ABCB1-1Δ gene mutation and the ivermectin sensitivity of certain breeds, MLs can still be used for treatment with caution. The MLs reviewed in this literature review (ivermectin, selamectin, doramectin, moxidectin and milbemycin oxime) are effective and safe drugs for heartworm prophylaxis with a broad spectrum against helminths and a variety of routes of application. All of the drugs can be safely administered to ABCB1-1Δ homozygous mutant dogs, with correct dosage and indication. However, it must be emphasized that doses higher than the heartworm preventatives, ABCB1-1Δ mutant dogs will show neurological toxicity with any of the ML substances.

Toxicosis becomes more likely when higher, extra-label dosages are administered to dogs with the ABCB1-1Δ gene mutation or when pet animals are accidentally exposed to, or iatrogenically overdosed with concentrated ML-containing products intended for large animals use. Drug interactions between ML and other P-gp substrates might also result in ML toxicosis.

The knowledge of ABCB1 genotype is essential in order to achieve a ML dosage regimen that is tolerated without causing life-threatening adverse drug effects. Therefore, prior to administration of P-gp substrate drugs, genotyping of at-risk breeds for ABCB1 mutation status is recommended, rather than avoiding the use of drug in collies and other affected breed. A simple blood or cheek swab test is available for this determination of genotyping. On the other hand, ABCB1 genotyping is not absolutely necessary in other purebred dogs not listed previously.

Bibliography

1. Al-Azzam, S.I., Fleckenstein, L., Cheng, K.-J., Dzimianski, M.T. and McCall, J.W. (2007). Comparison of the pharmacokinetics of moxidectin and ivermectin after oral administration to beagle dogs. *Biopharmaceutics & Drug Disposition*, 28(8), pp.431–438.
2. Arena, J.P., Liu, K.K., Paress, P.S., Frazier, E.G., Cully, D.F., Mrozik, H. and Schaeffer, J.M. (1995). The Mechanism of Action of Avermectins in Caenorhabditis Elegans: Correlation between Activation of Glutamate-Sensitive Chloride Current, Membrane Binding, and Biological Activity. *The Journal of Parasitology*, 81(2), p.286.
3. Arslan, A. (2015). Distinct roles of gamma-aminobutyric acid type A receptor subtypes: A focus on phasic and tonic inhibition. *The Journal of Neurobehavioral Sciences*, 2(2), p.72.
4. Baars, C., Leeb, T., von Klopmann, T., Tipold, A. and Potschka, H. (2008). Allele-specific polymerase chain reaction diagnostic test for the functional MDR1 polymorphism in dogs. *The Veterinary Journal*, 177(3), pp.394–397.
5. Barbet, J.L., Snook, T., Gay, J.M. and Mealey, K.L. (2009). ABCB1-1Δ (MDR1-1Δ) genotype is associated with adverse reactions in dogs treated with milbemycin oxime for generalized demodicosis. *Veterinary Dermatology*, 20(2), pp.111–114.
6. Bernacki, J., Dobrowolska, A. and Nierwinska, K. (2008). Physiology and pharmacological role of the blood-brain barrier. *Pharmacological Reports*, 65, pp.600–22.
7. Bissonnette, S., Paradis, M., Daneau, I. and Silversides, D.W. (2009). The ABCB1-1Δmutation is not responsible for subchronic neurotoxicity seen in dogs of non-collie breeds following macrocyclic lactone treatment for generalized demodicosis. *Veterinary Dermatology*, 20(1), pp.60–66.
8. Bloomquist, J.R. (1993). Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 106(2), pp.301–314.
9. Bloomquist, J.R. (2003). Chloride channels as tools for developing selective insecticides. *Archives of Insect Biochemistry and Physiology*, 54(4), pp.145–156.
10. Burg, R.W., Miller, B.M., Baker, E.E., Birnbaum, J., Currie, S.A., Hartman, R., Kong, Y.-L., Monaghan, R.L., Olson, G., Putter, I., Tunac, J.B., Wallick, H., Stapley, E.O., Oiwa, R. and Omura, S. (1979). Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism and Fermentation. *Antimicrobial Agents and Chemotherapy*, [online] 15(3), pp.361–367. Available at: https://aac.asm.org/content/aac/15/3/361.full.pdf [Accessed 13 Apr. 2020].
11. Campbell, A. and Chapman, M. (2000). *Handbook of poisoning in dogs and cats*. Oxford ; Malden, Ma: Blackwell Science.
12. Chen, C., Chin, J.E., Ueda, K., Clark, D.P., Pastan, I., Gottesman, M.M. and Roninson, I.B. (1986). Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell*, 47(3), pp.381–389.
13. Chittrakarn, S., Janchawee, B., Ruangrut, P., Kansenalak, S., Chethanond, U., Kobasa, T. and Thammapalo, S. (2009). Pharmacokinetics of ivermectin in cats receiving a single subcutaneous dose. *Research in Veterinary Science*, 86(3), pp.503–507.

14. Clarke, D.L., Lee, J.A., Murphy, L.A. and Reineke, E.L. (2011). Use of intravenous lipid emulsion to treat ivermectin toxicosis in a Border Collie. *Journal of the American Veterinary Medical Association*, 239(10), pp.1328–1333.
15. ffrench-Constant, R.H., Anthony, N., Aronstein, K., Rocheleau, T. and Stilwell, G. (2000). Cyclodiene Insecticide Resistance: From Molecular to Population Genetics. *Annual Review of Entomology*, 45(1), pp.449–466.
16. Dean, M., Hamon, Y. and Chimini, G. (2001). The Human ATP-Binding Cassette (ABC) Transporter Superfamily. *Genome Research*, 11(7), pp.1156–1166.
17. D'Hulst, C., Atack, J.R. and Kooy, R.F. (2009). The complexity of the GABAA receptor shapes unique pharmacological profiles. *Drug Discovery Today*, 14(17–18), pp.866–875.
18. Dorrington, C.L., Johnson, D.W. and Brant, R. (2003). The frequency of complications associated with the use of multiple-dose activated charcoal. *Annals of Emergency Medicine*, 41(3), pp.370–377.
19. Dowling, P. (2006). Pharmacogenetics: it's not just about ivermectin in collies. *Can Vet J.*, 47(12), pp.1165–1168.
20. El-Saber Batiha, G., Alqahtani, A., Ilesanmi, O.B., Saati, A.A., El-Mleeh, A., Hetta, H.F. and Magdy Beshbishy, A. (2020). Avermectin Derivatives, Pharmacokinetics, Therapeutic and Toxic Dosages, Mechanism of Action, and Their Biological Effects. *Pharmaceuticals*, 13(8), p.196.
21. Fisher, M.H. and Mrozik, H. (1992). The Chemistry and Pharmacology of Avermectins. *Annual Review of Pharmacology and Toxicology*, 32(1), pp.537–553.
22. Geyer, J. and Janko, C. (2012). Treatment of MDR1 Mutant Dogs with Macrocyclic Lactones. *Current Pharmaceutical Biotechnology*, 13(6), pp.969–986.
23. Geyer, J., Klintzsch, S., Meerkamp, K., Wohlke, A., Distl, O., Moritz, A. and Petzinger, E. (2007). Detection of the nt230(del4) MDR1 mutation in White Swiss Shepherd dogs: case reports of doramectin toxicosis, breed predisposition, and microsatellite analysis. *Journal of Veterinary Pharmacology and Therapeutics*, 30(5), pp.482–485.
24. Ginn, P.E. (1996). Immunohistochemical Detection of P-glycoprotein in Formalin-fixed and Paraffin-embedded Normal and Neoplastic Canine Tissues. *Veterinary Pathology*, 33(5), pp.533–541.
25. Gokbulut, C., Karademir, U., Boyacioglu, M. and McKellar, Q.A. (2006). Comparative plasma dispositions of ivermectin and doramectin following subcutaneous and oral administration in dogs. *Veterinary Parasitology*, 135(3–4), pp.347–354.
26. Gramer, I., Leidolf, R., Döring, B., Klintzsch, S., Krämer, E.-M., Yalcin, E., Petzinger, E. and Geyer, J. (2011). Breed distribution of the nt230(del4) MDR1 mutation in dogs. *The Veterinary Journal*, 189(1), pp.67–71.
27. Gwaltney-Brant, S.M. and Rumbeiha, W.K. (2002). Newer antidotal therapies. *Veterinary Clinics of North America: Small Animal Practice*, 32(2), pp.323–339.
28. Han, J.-I., Son, H.-W., Park, S.-C. and Na, K.-J. (2010). Novel insertion mutation ofABCB1gene in an ivermectin-sensitive Border Collie. *Journal of Veterinary Science*, 11(4), p.341.
29. Heine, J., Krieger, K., Dumont, P. and Hellmann, K. (2005). Evaluation of the efficacy and safety of imidacloprid 10% plus moxidectin 2.5% spot–on in the treatment of generalized demodicosis in dogs: results of a European field study. *Parasitology Research*, 97(S1), pp.S89–S96.

30. Holden-Dye, L. and Walker, R.J. (2014). Anthelmintic drugs and nematicides: studies in Caenorhabditis elegans. *WormBook*, pp.1–29.
31. Hopper, K., Aldrich, J. and Haskins, S.C. (2002). Ivermectin Toxicity in 17 Collies. *Journal of Veterinary Internal Medicine*, 16(1), pp.89–94.
32. Houston, D.M., Parent, J. and Matushek, K.J. (1987). Ivermectin toxicosis in a dog. *Journal of the American Veterinary Medical Association*, 191, pp.78–80.
33. Huang, J. and Casida, J.E. (1997). Avermectin B1a binds to high- and low-affinity sites with dual effects on the gamma-aminobutyric acid-gated chloride channel of cultured cerebellar granule neurons. *Journal of Pharmacology and Experimental Therapeutics*, 281(1), pp.261–66.
34. Iglesias, L.E., Saumell, C., Sagüés, F., Sallovitz, J.M. and Lifschitz, A.L. (2017). Ivermectin dissipation and movement from feces to soil under field conditions. *Journal of Environmental Science and Health, Part B*, 53(1), pp.42–48.
35. Juliano, R.L. and Ling, V. (1976). A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 455(1), pp.152–162.
36. Kenny, P.J., Vernau, K.M., DVM, B.P. and Maggs, D.J. (2008). Retinopathy associated with ivermectin toxicosis in two dogs. *Journal of the American Veterinary Medical Association*, 233(2), pp.279–284.
37. Knipple, D.C., Henderson, J.E. and Soderlund, D.M. (1995). Structural and Functional Characterization of Insect Genes Encoding Ligand-Gated Chloride-Channel Subunits. *ACS Symposium Series*, pp.205–215.
38. Krautmann, M.J., Novotny, M.J., De Keulenaer, K., Godin, C.S., Evans, E.I., McCall, J.W., Wang, C., Rowan, T.G. and Jernigan, A.D. (2000). Safety of selamectin in cats. *Veterinary Parasitology*, 91(3–4), pp.393–403.
39. Lafferty, K.D. (2010). The ecology of climate change and infectious diseases. *Ecology*, 91(3), pp.928–929.
40. Laing, R., Gillan, V. and Devaney, E. (2017). Ivermectin – Old Drug, New Tricks? *Trends in Parasitology*, 33(6), pp.463–472.
41. Lanusse, C.E., Lifschitz, A.L. and Imperiale, F.A. (2009). Macrocyclic lactones: endectocide compounds. *Veterinary pharmacology and therapeutics*, 9, pp.1119–44.
42. Lewis, D.T., Merchant, S.R. and Neer, T.< (1994). Ivermectin toxicosis in a kitten. *Journal of the American Veterinary Medical Association*, 205, pp.584–6.
43. Liu, M., Panda, S.K. and Luyten, W. (2020). Plant-Based Natural Products for the Discovery and Development of Novel Anthelmintics against Nematodes. *Biomolecules*, 10(3), p.426.
44. Lovell, R.A. (1990). Ivermectin and Piperazine Toxicoses in Dogs and Cats. *Veterinary Clinics of North America: Small Animal Practice*, 20(2), pp.453–468.
45. Lumaret, J.-P., Errouissi, F., Floate, K., Rombke, J. and Wardhaugh, K. (2012). A Review on the Toxicity and Non-Target Effects of Macrocyclic Lactones in Terrestrial and Aquatic Environments. *Current Pharmaceutical Biotechnology*, 13(6), pp.1004–1060.
46. Martinez, M., Modric, S., Sharkey, M., Troutman, L., Walker, L. and Mealey, K. (2008). The pharmacogenomics of P-glycoprotein and its role in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics*, 31(4), pp.285–300.
47. McKellar, Q.A. and Benchaoui, H.A. (1996). Avermectins and milbemycins. *Journal of Veterinary Pharmacology and Therapeutics*, 19(5), pp.331–351.
48. Mealey, K.L. (2008). Canine ABCB1 and macrocyclic lactones: Heartworm prevention and pharmacogenetics. *Veterinary Parasitology*, 158(3), pp.215–222.

49. Mealey, K.L., Bentjen, S.A., Gay, J.M. and Cantor, G.H. (2001). Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics*, 11(8), pp.727–733.
50. Mealey, K.L., Greene, S., Bagley, R., Gay, J., Tucker, R., Gavin, P., Schmidt, K. and Nelson, F. (2008). P-Glycoprotein Contributes to the Blood-Brain, but Not Blood-Cerebrospinal Fluid, Barrier in a Spontaneous Canine P-Glycoprotein Knockout Model. *Drug Metabolism and Disposition*, 36(6), pp.1073–1079.
51. Mehlhorn, H. and Aspöck, H. (2008). *Encyclopedia of parasitology : with 205 tables / 2 N - Z.* Berlin: Springer.
52. Merola, V.A., Khan, S. and Gwaltney-Brant, S. (2009). Ivermectin Toxicosis in Dogs: A Retrospective Study. *Journal of the American Animal Hospital Association*, 45(3), pp.106–111.
53. Merola, V.M. and Eubig, P.A. (2012). Toxicology of Avermectins and Milbemycins (Macrocylic Lactones) and the Role of P-Glycoprotein in Dogs and Cats. *The Veterinary clinics of North America. Small animal practice*, [online] 42(2), pp.313–vii. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4152460/#R37 [Accessed 16 Oct. 2020].
54. Mrozik, H. (2010). ChemInform Abstract: Advances in Research and Development of Avermectins. *ChemInform*, 25(16), p.no-no.
55. Nakai, M., Kawamura, T., Matsuoka, N., Katae, H. and Ohishi, I. (1990). Estimation of the Safety of Ivermectin in Collies. *Journal of the Japan Veterinary Medical Association*, 43(5), pp.360–366.
56. Neal, J.M., Barrington, M.J., Fettiplace, M.R., Gitman, M., Memtsoudis, S.G., Mörwald, E.E., Rubin, D.S. and Weinberg, G. (2018). The Third American Society of Regional Anesthesia and Pain Medicine Practice Advisory on Local Anesthetic Systemic Toxicity. *Regional Anesthesia and Pain Medicine*, 43(2), pp.113–123.
57. Neff, M.W., Robertson, K.R., Wong, A.K., Safra, N., Broman, K.W., Slatkin, M., Mealey, K.L. and Pedersen, N.C. (2004). Breed distribution and history of canine mdr1-1 , a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. *Proceedings of the National Academy of Sciences*, 101(32), pp.11725–11730.
58. Nelson, O.L., Carsten, E., Bentjen, S.A. and Mealey, K.L. (2003). Ivermectin Toxicity in an Australian Shepherd Dog with the MDR1 Mutation Associated with Ivermectin Sensitivity in Collies. *Journal of Veterinary Internal Medicine*, 17(3), pp.354–356.
59. Njue, A.I., Hayashi, J., Kinne, L., Feng, X.-P. and Prichard, R.K. (2004). Mutations in the extracellular domains of glutamate-gated chloride channel alpha3 and beta subunits from ivermectin-resistant Cooperia oncophora affect agonist sensitivity. *Journal of Neurochemistry*, 89(5), pp.1137–1147.
60. Novotny, M.J., Krautmann, M.J., Ehrhart, J.C., Godin, C.S., Evans, E.I., McCall, J.W., Sun, F., Rowan, T.G. and Jernigan, A.D. (2000). Safety of selamectin in dogs. *Veterinary Parasitology*, 91(3–4), pp.377–391.
61. Pastan, I. and Gottesman, M.M. (1997). Multidrug resistance. *Annual Review of Medicine*, 42, pp.277–86.
62. Paul, A.J., Hutchens, D.E., Firkins, L.D. and Keehan, C.M. (2004). Effects of dermal application of 10.0% imidacloprid-0.08% ivermectin in ivermectin-sensitive Collies. *American Journal of Veterinary Research*, 65(3), pp.277–278.
63. Pemberton, D.J., Franks, C.J., Walker, R.J. and Holden-Dye, L. (2001). Characterization of Glutamate-Gated Chloride Channels in the Pharynx of Wild-Type

and Mutant Caenorhabditis elegansDelineates the Role of the Subunit GluCl-α2 in the Function of the Native Receptor. *Molecular Pharmacology*, 59(5), pp.1037–1043.
64. Perez, R., Cabezas, I., Sutra, J.F., Galtier, P. and Alvinerie, M. (2001). Faecal Excretion Profile of Moxidectin and Ivermectin after Oral Administration in Horses. *The Veterinary Journal*, 161(1), pp.85–92.
65. Plumb, D.C. (2005). *Veterinary drug handbook*. Ames, Iowa: Iowa State Press.
66. Prichard, R.K. and Geary, T.G. (2019). Perspectives on the utility of moxidectin for the control of parasitic nematodes in the face of developing anthelmintic resistance. *International Journal for Parasitology: Drugs and Drug Resistance*, 10, pp.69–83.
67. Quadros, E., Ramsamooj, E. and Wilson, D.E. (1987). Role of mucus and prostaglandins in the gastric mucosal protective actions of sucralfate against ethanol-induced injury in the rat. *The American Journal of Medicine*, 83(3), pp.19–23.
68. Sarkadi, B., Homolya, L., Szakács, G. and Váradi, A. (2006). Human Multidrug Resistance ABCB and ABCG Transporters: Participation in a Chemoimmunity Defense System. *Physiological Reviews*, 86(4), pp.1179–1236.
69. Sartor, L.L., Bentjen, S.A., Trepanier, L. and Mealey, K.L. (2004). Loperamide Toxicity in a Collie with the MDR1 Mutation Associated with Ivermectin Sensitivity. *Journal of Veterinary Internal Medicine*, 18(1), pp.117–118.
70. Schinkel, A.H. (1997). The physiological function of drug-transporting P-glycoproteins. *Seminars in Cancer Biology*, 8(3), pp.161–170.
71. Schinkel, A.H., Smit, J.J.M., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A.A.M., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P.J., Berns, A.J.M. and Borst, P. (1994). Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell*, 77(4), pp.491–502.
72. See, A., McGill, S., Raisis, A. and Swindells, K. (2009). Toxicity in three dogs from accidental oral administration of a topical endectocide containing moxidectin and imidacloprid. *Australian Veterinary Journal*, 87(8), pp.334–337.
73. Sivine, F., Plume, C. and Ansay, M. (1985). Picrotoxin, the antidote to ivermectin in dogs? *Veterinary Record*, 116(7), pp.195–196.
74. Snowden, N.J., Helyar, C.V., Platt, S.R. and Penderis, J. (2006). Clinical presentation and management of moxidectin toxicity in two dogs. *Journal of Small Animal Practice*, 47(10), pp.620–624.
75. Srivastava, P.K., Singh, V.P., Singh, A., Singh, S., Prasad, S.M., Tripathi, D.K. and Chauhan, D.K. (2020). *Pesticides in crop production : physiological and biochemical action*. Hoboken, Nj: John Wiley & Sons, Inc.
76. Stiedl, C. and Weber, K. (2017). Fast and simple detection methods for the 4–base pair deletion of canine MDR1/ABCB1 gene by PCR and isothermal amplification. *Journal of Veterinary Diagnostic Investigation*, 29(2), pp.176–180.
77. Taylor, M. (2004). Macrocyclic Lactones in Antiparasitic Therapy. *The Veterinary Journal*, 167(2), p.120.
78. Taylor, M.A., Coop, R.L. and Wall, R. (2016). *Veterinary parasitology*. Chichester: Wiley Blackwell.
79. Trailovic, S.M. and Nedeljkovic, J.T. (2011). Central and Peripheral Neurotoxic Effects of Ivermectin in Rats. *Journal of Veterinary Medical Science*, 73(5), pp.591–599.
80. Tranquilli, W.J., Paul, A.J., Seward, R.L., Todd, K.S. and Dipietro, J.A. (1987). Response to physostigmine administration in collie dogs exhibiting ivermectin toxicosis. *Journal of Veterinary Pharmacology and Therapeutics*, 10(1), pp.96–100.

81. Tranquilli, W.J., Paul, A.J. and Todd, K.S. (1991). Assessment of toxicosis induced by high-dose administration of milbemycin oxime in collies. *American Journal of Veterinary Research*, 52(7), pp.1170–2.
82. Urquhart, B.L. and Kim, R.B. (2009). Blood−brain barrier transporters and response to CNS-active drugs. *European Journal of Clinical Pharmacology*, 65(11), pp.1063–1070.
83. Van Der Heyden, S., Chiers, K. and Ducatelle, R. (2009). Tissue Distribution of P-Glycoprotein in Cats. *Anatomia, Histologia, Embryologia*, 38(6), pp.455–460.
84. Weinberg, G.L., VadeBoncouer, T., Ramaraju, G.A., Garcia-Amaro, M.F. and Cwik, M.J. (1998). Pretreatment or Resuscitation with a Lipid Infusion Shifts the Dose-Response to Bupivacaine-induced Asystole in Rats. *Anesthesiology*, 88(4), pp.1071–1075.
85. Wells, K., O'Hara, R.B., Morand, S., Lessard, J.-P. and Ribas, A. (2014). The importance of parasite geography and spillover effects for global patterns of host-parasite associations in two invasive species. *Diversity and Distributions*, 21(4), pp.477–486.
86. World Health Organization: WHO (2019). *Soil-transmitted helminth infections*. [online] Who.int. Available at: https://www.who.int/en/news-room/fact-sheets/detail/soil-transmitted-helminth-infections [Accessed 30 Sep. 2020].
87. Wright, H.M., Chen, A.V., Talcott, P.A., Poppenga, R.H. and Mealey, K.L. (2011). Intravenous fat emulsion as treatment for ivermectin toxicosis in three dogs homozygous for the ABCB1-1Δ gene mutation. *Journal of Veterinary Emergency and Critical Care*, 21(6), pp.666–672.
88. Wyse, C.A., McLellan, J., Dickie, A.M., Sutton, D.G.M., Preston, T. and Yam, P.S. (2003). A Review of Methods for Assessment of the Rate of Gastric Emptying in the Dog and Cat: 1898-2002. *Journal of Veterinary Internal Medicine*, 17(5), pp.609–621.
89. Yas-Natan, E., Shamir, M. and Kleinbart, S. (2003). Doramectin toxicity in a collie. *Veterinary Record*, 153, pp.718–20.
90. Zhang, J., Yan, Y.-J., An, J., Huang, S.-X., Wang, X.-J. and Xiang, W.-S. (2015). Designed biosynthesis of 25-methyl and 25-ethyl ivermectin with enhanced insecticidal activity by domain swap of avermectin polyketide synthase. *Microbial Cell Factories*, 14(1).

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