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Application of a sedation scoring system in dogs following premedication

Thesis is submitted by Deepti Deshpande

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To Mom, Dad and Prathmesh,

You have been my inspiration and my strength. Thank you for being a part of my life!

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Abstract

Pharmacogenetics is the study of how variations in the genome influence drug pharmacokinetics (the body's effect on the drug) and pharmacodynamics (the drug's effect on the body). The *MDR1* gene codes for a membrane-bound drug transporter protein, P-glycoprotein (P-gp) that transports drugs across the cell membrane using an energy-dependent mechanism. Anecdotal reports in the literature suggested that dogs with a mutation in the *MDR1* gene (*MDR1-1*Δ) show increased sensitivity to routinely used veterinary sedatives such as acepromazine and butorphanol, resulting in increased duration and depth of sedation. This study has 3 aims. First is to gain experience with a sedation scoring system that can be used to assess the level of sedation. The second aim is to assess the difference in sedation of dogs premedicated with dexmetomidine and acepromazine. The third aim is to investigate the effect of acepromazine (n=29) and a combination of acepromazine and butorphanol (n=12) on *MDR1* genotyped rough-coated collies.

In the study assessing the sedation of dogs premedicated with dexmadetomidine and acepromazine, 30 dogs scheduled for orchidectomy were divided into two groups; the DEX group (n=15) and the ACE group (n=15). Dogs in the DEX group received dexmedetomidine (125 μg/m^2) and morphine (0.5 mg/kg) while the dogs in the ACE group received acepromazine (0.04 mg/kg) and morphine (0.5 mg/kg). The dogs were sedation scored at 0, 10, 20 and 30 minute intervals. The dogs in the DEX group had a statistically higher sedation score at 30 minutes than the dogs in the ACE group (*p* value =0.0189). Dogs premedicated with dexmedetomidine had a higher sedation score than dog's premedicated acepromazine at 30 minutes. The heart rate, respiratory rate and mean arterial blood pressure were not different between the DEX and the ACE group at 30 minutes post administration of premedication agent.

The second study investigated the effects of acepromazine and a combination of acepromazine and butorphanol in dogs carrying the *MDR1-1*Δ mutation. Genotyping for the *MDR1-1*Δ mutation was performed in 31 rough-coated collies. Dogs were considered healthy based on clinical history, physical examination, complete blood count, serum chemistry and urinalysis. Twenty-nine of the 31 rough coated collies were deemed healthy and were enrolled in the sedation trial assessing the effects of

acepromazine on the *MDR1-1*Δ mutants. A subset of the 29 rough coated collies was enrolled in the study assessing the effects of combination of acepromazine and butorphanol. The rough coated collies were divided in 3 groups based on their genotype: homozygous mutants, heterozygous carriers and normal group. After administration of acepromazine (0.04 mg/kg, IV) or a combination of acepromazine (0.04 mg/kg) and butorphanol (0.05 mg/kg), sedation scoring was performed at 0, 30 minutes, 60 minutes, 90 minutes, 2 , 2.5 , 3 , 4 and 6 hour intervals by an observer blinded to the results of the *MDR1* genotype. Following administration of acepromazine, homozygous mutant collies (*MDR1* -/-) (n = 10) reached a greater level of sedation and remained sedated for a longer duration as compared to the heterozygous carriers (*MDR1* +/-) (n =10) and wild-type collies (*MDR1* +/+) (n = 9) (p= 0.0176). A subset of 12 dogs was sedated with a combination of acepromazine (0.04 mg/kg) and butorphanol (0.05 mg/kg). Heterozygous carriers (*MDR1* -/+) had significantly higher sedation scores than homozygous mutants (*MDR1* -/-) and normal groups (*MDR1* +/+) when sedated with the combination (p=0.0423). This unexpected result may have been due to the small number of dogs tested. The author recommends lower dosing of acepromazine and butorphanol in dogs that are homozygous mutants to the *MDR1-1*Δ mutation and recommends the constant monitoring of sedation.

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Abbrevations

ABC	ATP Binding Cassette Transporter Proteins
ACE	Acepromazine Group
ATP	Adenosine Triphosphate
AUC	Area Under Curve
AV	Atrioventricular
cAMP	Cyclic Adenosine Monophosphate
CBC	Complete Blood Count
CHO	Chinese Hamster Ovary
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CYP	Cytochrome P450 enzymes
D (1 & 2)	Dopamine Receptors
DD	Deepti Deshpande
DEX	Dexmedetomidine Group
DNA	Deoxyribonucleic acid
GABA	Gamma Amino Butyric Acid
$GABA_A$	Gamma Amino Butryric Acid A Receptors
$GABA_B$	Gamma Amino Butyric Acid B Receptors
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
HR	Heart Rate
IFN-gamma	Interferon gamma
IM	Intramuscular
IV	Intravenous
M (1-5)	Muscarinic Receptors
MAP	Mean Arterial Pressure
MDR1	Multidrug Resistant-1 gene
MDR1-1Δ	Multidrug Resistant-1-1Δ mutation
MDR1a (-/-)	Multidrug Resistant -1a Knockout
MDR1a(+/+)	Multidrug Resistant -1a Wildtype
MUAEC	Massey University Animal Ethics Committee
MUT/MUT	MDR1-1Δ homozygous mutants
MUT/N	MDR1-1Δ heterozygous carriers
MUVTH	Massey University Veterinary Teaching Hospital
N/N	MDR1-1Δ normal
NBD	Nucleotide Binding Domain
NRS	Normal Saline
NZVP	New Zealand Veterinary Pathology
PCR	Polymerase Chain Reaction
P-gp	P-glycoprotein
R	Recalculated Sedation Score
RAI	Relative Adrenal Insufficiency
RBC	Red Blood Cells
RNA	Ribonucleic acid
RYR1	Ryanodine Receptor-1
SA	Sinoatrial
SAP	Systolic Arterial Pressure
SD	Standard Deviation
SDS	Simple Descriptive Systems
TMD	Transmembrane Domain
USA	United States of America
USDA	United States Department of Agriculture
VAS	Visual Analogue Score
VCPL	Veterinary Clinical Pharmacology Laboratory
WA	Washington State

Chapter I:

Introduction and Literature Review

1. Introduction

The origin of human and veterinary anaesthesia is vague. For centuries many ancient cultures all over the world practised the concept of anaesthesia. However in the 19th century it was recognized for the first time the possibility of pain in animals during surgical procedures. The aim to reduce animal suffering resulted in the development of veterinary anaesthesia. In the early 19th century Hickman conducted experiments on mice and dog producing inhalation anaesthesia with the use of carbon dioxide (Clark, 1938). He also demonstrated that surgical anaesthesia can be produced by asphyxia and the inhalation of nitrous oxide (Clark, 1938). Ether, used as a recreational drug, was recognized for its anaesthetic properties in the mid 1800s. Morton, the pioneer of ether anaesthesia in human medicine, was the first to attempt ether anaesthesia in dogs. By the end of the 19th century, various anaesthetic agents such as ether, chloroform and chloral hydrate were used by veterinary surgeons to reduce the pain and agony of animals during surgical procedures. The extraction of cocaine introduced a new form of anaesthesia called, conduction anaesthesia. (Tranquilli, Thurmon, & Grimm, 2007). This lead to advances in regional and local anaesthesia enabling the use of epidural anaesthesia in veterinary practice (Brook, 1935). The advent and development of barbiturates commenced in 1930 which opened a whole gamut of anaesthetic drugs (Jones, 2002). The discovery of the phenothiazine group of drugs made general anaesthesia popular and safer in large animals. Newer drugs such as tranquilizers, opioids, alpha-2 adrenergic agonists, dissociative anaesthetics, muscle relaxants and inhalant anaesthetics made anaesthesia and recovery even safer (Tranquilli et al., 2007).

1.1 Balanced Anaesthesia

In 1954, Hall and Weaver published a paper entitled “Some notes on balanced anaesthesia for dogs and cats” (Hall & Weaver, 1954). This paper described the concept of balanced anaesthesia in veterinary medicine for the first time with the administration of premedication agents, intravenous barbiturates and maintenance with inhalation agent and oxygen (Hall & Weaver, 1954). The authors

observed that balanced anaesthesia techniques improved the chances of survival of patients (Hall & Weaver, 1954). Balanced anaesthesia is the use of multiple drugs to specifically attenuate individual components of the “anaesthetized state”, which are consciousness, analgesia, muscle relaxation and alteration of autonomic reflexes (Lundy, 1926). The advantage of using a balanced anaesthesia technique is that small amounts of each drug can be used without having to contend with the disadvantages of large doses of a single drug.

The balanced anaesthesia technique has three components: premedication, induction and maintenance of anaesthesia. Premedication agents are anxiolytics and pre-emptive analgesics. Premedication agents aid in reducing the magnitude and development of pre-operative stress (Väisänen et al., 2002). Analgesics such as opioids as well as local anaesthetic agents are recommended prior to and during surgery to prevent transmission of painful stimuli. Preemptive analgesics are crucial in the management of peri-operative and post-operative pain (Hellyer et al., 2007). Intravenous anaesthetics such as propofol, thiopentone, ketamine and alfaxalone are recommended for induction of anaesthesia (Bednarski et al., 2011). Maintenance of anaesthesia is possible with either inhalation or intravenous anaesthetics. Inhalant anaesthetic agents have gained popularity for the maintenance of general anaesthesia as they have obvious advantages such as ease of administration, predictable depth of anaesthesia at particular dosages and rapid recovery (Bednarski et al., 2011). Recent studies show that total intravenous anaesthesia (TIVA) with propofol or alfaxalone is an alternative to inhalation anaesthesia (Suarez, Dzikiti, Stegmann, & Hartman, 2012).

Anaesthetic agents induce unconsciousness during which the animal should not perceive or recall noxious stimuli. However, deep levels of anaesthesia may not block all the reactions to noxious surgical stimuli. By definition, general anaesthetic agents block sensory perception of noxious stimuli but may not suppress other somatic and autonomic reflexes evoked by surgery (Antognini, Wang, & Carstens, 1999). If attempts to block these stimuli are made by increasing the dose of inhalant anaesthetics significant cardiovascular and respiratory depression may ensue (Ilkiw, 1999). Lower levels of anaesthesia may result in severe depression in animals with severe systemic diseases thus potentially increasing the morbidity and mortality in these patients (Ilkiw, 1999). Balanced anaesthesia techniques involve using the

lowest possible doses of inhalant anaesthetic along with other drugs which modify the response to noxious stimuli (Ilkiw, 1999).

In current veterinary anaesthesia practice there is no “ideal anaesthetic” agent. However the implementation of balanced anaesthesia techniques by the combination of anticholinergics, sedatives and tranquilizers, volatile anaesthetics, injectable short acting anaesthetics and opioids is a step towards ideal anaesthesia.

1.2 Premedication

Premedication has become a crucial step in anaesthetic protocols. Premedication agents are drugs administered prior to the induction of anaesthesia to provide muscle relaxation, anxiolysis, analgesia and to reduce the doses of drugs required for induction and the maintenance of anaesthesia (Bednarski et al., 2011). The most common premedication agents used in veterinary practice are anticholinergics, analgesics and sedatives (Bednarski et al., 2011).

1.2.1 Anticholinergics

Anticholinergics, also called as parasympatholytics, block the parasympathetic nervous system (Adams, 2001). The two most common anticholinergics used in veterinary medicine are atropine and glycopyrrolate. Both atropine and glycopyrrolate have specific antimuscarinic action and do not block the nicotine cholinergic receptors. There are five types of muscarinic receptors: M1, M2, M3, M4 and M5. The M1 receptors are located in brain, glands and sympathetic ganglia, M2 receptors are located in the heart and hindbrain, M3 receptors are located in the secretory glands, smooth muscles, brain and endothelium, M4 receptors are located in the basal forebrain and striatum and M5 receptors are located in substansia niagra (Caulfield, 1993). The presynaptic muscarinic heteroreceptors are located on the sympathetic nerve terminals and inhibit the release of norepinephrine. The blockade of these receptors facilitates the release of norepinephrine. The presynaptic muscarinic autoreceptors are located on the parasympathetic nerve terminals and normally inhibit the release of acetycholine. However the blockade of these muscarinic receptors by anticholinergics results in the release of acetylcholine (Caulfield, 1993). The net pharmacologic effects of

anticholinergics in different organs are defined by the relative dominance of the parasympathetic or sympathetic tone in that organ. Once the anticholinergics block the cholinergic impulses, the adrenergic impulses dominate and the resultant effect is a sympathomimetic-like effect (Adams, 2001).

A. Atropine

Preoperative anticholinergics are administered to prevent or treat bradycardia due to vagal stimulation and decrease salivary and bronchial secretion (Bednarski et al., 2011). Atropine has the same affinity to all muscarinic receptors (Bräuner-Osborne & Brann, 1996). The blockade of pupillary constrictor muscles results in prolonged mydriasis and reduces lacrimal secretion thus causing corneal drying (Ludders & Heavner, 1979). Atropine increases heart rate by blocking the vagal parasympathetic input to the SA and the AV node. At therapeutic dose (0.04 mg/kg) atropine causes blockade of postsynaptic muscarinic receptors causing increase in sinus rate, acceleration of AV conduction and an increase in atrial contractility (Donald, Samueloff, & Ferguson, 1967). Atropine decreases airway secretions, increases airway diameter and increases anatomical pulmonary dead space (Davis, Roberts, Coleridge, & Coleridge, 1982). Atropine administration causes dramatic gastrointestinal effects causing dose and breed specific inhibition of antral motility. In a study, low doses of atropine (0.02 mg/kg) in beagles inhibited GI motility for 30 minutes whereas high doses of atropine (0.04mg/kg) inhibited GI activity for 3 hours. In Labradors however, the effects of atropine were not dose related and inhibited GI motility for 3 hours at any dose (Burger et al., 2006). Atropine can attain high concentrations in the brain causing sedation (Fassi & Rosenberg, 1979).

B. Glycopyrrolate

Glycopyrrolate is the other common anticholinergic agent and is more potent than atropine (Fassi & Rosenberg, 1979). Similar to atropine, glycopyrrolate has same the affinity to all muscarinic receptors. Administration of glycopyrrolate to dogs with normal intraocular pressure does not alter the pupil diameter and intraocular pressure (Frischmeyer, Miller, Bellay, Smedes, & Brunson, 1993). Glycopyrrolate increases sinus rate and increases cardiac index (Lemke, Tranquilli, Thurmon,

Benson, & Olson, 1993). Glycopyrrolate has similar GI effects to atropine. However unlike atropine, sedation is not observed with glycopyrrolate as glycopyrrolate is a selective peripheral anticholinergic agent which does not cross the blood- brain and placental barrier as well as atropine (Proakis & Harris, 1978). Studies show that the CSF concentration 10 minutes after administration of 0.1 mg/kg intravenously of atropine and glycopyrrolate was 10.3 ng/ml and 0.9 ng/ml respectively (Proakis & Harris, 1978). The placental penetration of atropine is greater than glycopyrrolate with the measured foetal serum concentration of 13 ng/ml and 0.63 ng/ml 10 minutes post intravenous administration of atropine and glycopyrrolate respectively (Proakis & Harris, 1978). Glyccopyrrolate is a more potent antisialogogue than atropine (Fassi & Rosenberg, 1979). Glyccopyrolate is safer than atropine as it has fewer side effects and thus is preferred over atropine as a premedication agent (Fassi & Rosenberg, 1979).

The use of anticholinergics as premedication agents as a part of anaesthesia protocol remains a topic for debate (Bednarski et al., 2011). Anticholinergics were previously used to overcome excessive salivation resulting from irritant inhalant anaesthetics. However, the newer anaesthetics are not particularly irritant and hence do not cause salivation. Thus there is no longer a requisite for pre-emptive anticholinergic drugs (Best, 2001). The chances of tachycardia, increased myocardial oxygen consumption and myocardial hypoxemia are reasons to avoid anticholinergics as a component of anaesthetic protocols (Bednarski et al., 2011). However the pre-emptive use of anticholinergics is warranted for procedures in which there is an increased risk of vagal bradycardia (e.g. ocular surgery) (Bednarski et al., 2011). Brachycephalic airway syndrome is associated with airway obstruction and increased vagal tone. The use of anticholinergics is indicated in dogs with brachycephalic airway syndrome to prevent bradycardia from vasovagal reflex induced suppression (Bednarski et al., 2011). These drugs are potent bronchodilators and increase the dead space ventilation which may accentuate postoperative hypoxemia (Best, 2001). They also paralyze the respiratory epithelial cilia and decrease the tracheobronchial cilliary clearance of mucus for 24 hours post administration (Best, 2001). The use of anticholinergics along with alpha-2 agonists has been controversial as concurrent administration of alpha-2 agonist and anticholinergics results in increased heart rate and blood pressure thus increasing myocardial oxygen demand (Lemke et al., 1993). Hence, the administration of

anticholinergics should be based on the each individual animal's profile, heart rate and blood pressure (Bednarski et al., 2011). Anticholinergics are necessary premedication agents in certain procedures; however they should be used judiciously after taking their potential adverse effects into consideration.

1.2.2 Pre-emptive analgesia

Surgical interventions typically cause significant peripheral tissue injury. Most anaesthetics however are not thought to provide analgesia (Dyson, 2008). They cause unconsciousness but do not prevent the nervous system from the afferent signals resulting from surgical interventions. If analgesia is not adequately provided peri-operatively, this acute, nociceptive and inflammatory pain may be transformed into persistent maladaptive pain post-operatively (Dyson, 2008). Nociceptive signals from damaged tissues once initiated launch a cascade of alterations in the somatosensory system resulting in an increase in the response to subsequent stimuli, thus amplifying the perception of pain (Woolf, 2004). Pre-emptive analgesia is given before a surgical procedure to reduce the physiological consequences of nociceptive transmission provoked by the procedure (Dahl & Møiniche, 2004). Pre-emptive analgesia has a protective effect on the nociceptive pathway thus reducing the post-operative pain and the development of chronic or maladaptive pain (Dahl & Møiniche, 2004).

Neurobiology of pain

Painful or injurious stimuli to the body are detected by free peripheral nerve endings called nociceptors. These nociceptors convert peripheral thermal, chemical and mechanical energy at the site of stimulus to electrical activity and conduct it to the dorsal horn of spinal cord (Besson, 1999). There are different kinds of nociceptors depending on their location in various tissues and their response to stimuli. Myelinated Aδ fibers are specialized for detecting mechanical and thermal injury and for triggering a rapid sharp pain response also called as "First Pain". The unmyelinated C nociceptors respond to strong mechanical, thermal and/or chemical stimuli and mediate a delayed burning response called "Second Pain". Pain signals are transmitted from the nociceptor to the secondary nociceptor neurons in the

dorsal horn in the spinal cord (Woolf, 2004). Two classes of dorsal horn neurons are involved in the signalling of pain sensation and response. The nociceptive specific (NS) neurons respond to signals transmitted by A δ and C nociceptors. The wide-dyanamic range (WDR) neurons respond to both non-nociceptive impulses in A β and nociceptive impulses by A δ and C fibres (Woolf, 2004). Tissue damage results in release of algogenic or pain promoting substances from peripheral nerve endings and extraneural sources such as substance P, prostaglandins, serotonin, bradykinin and histamine (Besson, 1999). These mediators cause *peripheral sensitization* of nociceptors resulting in an altered transduction and increased conduction of nociceptor impulses towards the CNS (Woolf, 2004). Sensitization of nociceptors results in a reduced threshold for activation, an increase in response to given stimulus and the appearance of spontaneous activity. There is an alteration in the responsiveness of the NS and WDR neurons in the dorsal horn resulting in *central sensitization* (Woolf, 2004). The central sensitization causes the signals from the A δ and the C fibres to amplify resulting in hyperalgesia. Aβ fibres detect non-painful stimuli such as a light touch. However, with central sensitization, the Aβ fibres will amplify the perception of pain and will interpret touch as painful stimuli. This is called as allodynia. The central sensitization outlasts the stimuli that triggered the alteration and this is called as “pain memory” (Dahl & Møiniche, 2004). The peripheral and the central sensitization together contribute to post injury pain hypersensitivity. This hypersensitivity of injured tissue results in an increased response to noxious stimuli (hyperalgesia) and a decrease in pain threshold (Woolf, 2004). Hyperalgesia and allodynia occur at the site of tissue injury and also the surrounding tissues (Woolf, 2004).

Current evidence suggests that administration of local anaesthetics and opioids can reduce post-operative pain by preventing central sensitization (Hellyer et al., 2007). The pain perception outlasts the painful stimulus during surgery which is primarily due to central and peripheral sensitization (Dahl & Møiniche, 2004). If pre-emptive analgesic agents are provided pre-operatively and peri-operatively, the phenomenon of peripheral and central sensitization can be prevented. Providing analgesics throughout the painful procedure prevents the acute surgical pain from becoming maladaptive chronic pain (Hellyer et al., 2007). It is now known that pre-emptive analgesia may reduce the risk of developing chronic post-operative pain. Pre-emptive analgesia prevents injury-induced alteration in the CNS and thus

enables better peri-operative and post-operative pain management with lower doses of analgesics (Dahl & Møiniche, 2004).

Anaesthetic agents prevent the perception of pain during the surgical procedure. However anaesthetic agents do not prevent the transmission of noxious stimuli to the CNS. These painful stimuli are induced from the surgical site during the procedure and cause 'wind up' (Besson, 1999). The excessive induction from the pain fibres results in central and peripheral sensitization ultimately causing allodynia and hyperalgesia (Besson, 1999). Thus providing preoperative, perioperative and postopertative analgesia is a part of balanced anaesthetic techniques. Pre-emptive analgesia decreases the anaesthetic requirement, postoperative pain and stress and makes the animal more comfortable (Ilkiw, 1999).

1.2.3 Sedatives

Sedatives are used preoperatively to reduce stress, induce sedation, provide restraint, reduce the dose of injectable or inhalant anaesthetic agents and smooth recovery. Various classes of sedatives are currently available and have different actions. The most common drugs used in veterinary practice are phenothiazines (acepromazine), alpha-2-adrenergic agonists (xylazine, medetomidine, dexmedetomidine) and benzodiazepines (diazepam and midazolam).

A. Phenothiazines

Phenothiazines such as acepromazine are amongst the most commonly used sedatives in veterinary practice. The primary action of phenothiazines is mediated by blockade of dopamine receptors in the basal ganglia and limbic system (Horn & Snyder, 1971). Dopamine is an inhibitory neurotransmitter and plays an important role in the regulation of behaviour, fine motor control, autonomic and endocrine functions. Dopamine exerts its effect through interaction with specific dopaminergic receptors located on the neuronal membrane surface (Horn & Snyder, 1971). There are two subtypes of dopamine receptors: D1 and D2. The D1 receptors are located postsynaptically whereas the D2 receptors have both presynaptic and postsynaptic locations. The activation of D1 receptor increases adenylate cyclase activity and intracellular concentration of cyclic adenosine monophospahate (cAMP) whereas activation of D2 receptors decreases adenylate cyclase activity and intracellular

concentration of cAMP (Vallone, Picetti, & Borrelli, 2000). Phenothiazines exert their action by the blockade of the D2 receptors (Girault & Greengard, 2004). Phenothiazines reduce the conditioned avoidance behaviour in response to aversive stimuli but escape or avoidance to unconditioned behaviour is not inhibited (Brunton, Parker, Blumenthal, & Buxton, 2007). At therapeutic doses phenothiazines decrease spontaneous motor activity (Bhargava & Chandra, 1964). Phenothiazines bind to alpha-1 receptors resulting in peri-operative hypotension (Turner, Ilkiw, Rose, & Warren, 1974). Phenothiazines act as anti-emetics by blocking the dopamine receptors in the chemoreceptor trigger zone (Rosenkilde & Govier, 1957). These drugs also depress the catecholamines in the thermoregulatory zone which can lead to severe hypothermia (Pottie, Dart, Perkins, & Hodgson, 2007). The most common phenothiazine agents used as a sedative in small animal veterinary practice is acepromazine.

Acepromazine

Acepromazine is the most common phenothiazine used in veterinary practice as a sedative. This drug provides reliable sedation and anxiolysis and is among the preferred premedication agents. One of the main requirements of a good sedative is to decrease the anaesthetic doses. Acepromazine decreases halothane and isoflurane requirements. Dogs premedicated with acepromazine with a doses of 0.02 and 0.2 mg/kg had a 34% and 46% decrease in the MAC values of halothane, respectively (Tranquilli et al., 2007). Intramuscular administration of acepromazine at (0.2 mg/kg) decreased the MAC of halothane and Isoflurane by 28% and 48%, respectively (Tranquilli et al., 2007).

The antiemetic and antihistaminic properties of acepromazine, make it an ideal drug to be paired with drugs that cause emesis such as opioids like morphine. Valverde, et.al 2004, showed that the administration of acepromazine 15 minutes prior to opioid administration lower the incidence of vomiting in dogs (Valverde, Cantwell, Hernandez, & Brotherson, 2004).

Acepromazine however produces dramatic cardiovascular effects in both conscious and anaesthetized patients (Nogueira, Fernández del Palacio, López, & Resende, 2012). Acepromazine has antiarrhythmic properties and studies show that at lower doses (0.025 mg/kg) acepromazine prevents epinephrine induced

arrhythmias in dogs anaesthetized with halothane (Dyson & Pettifer, 1997). Acepromazine is known to decrease systemic arterial blood pressure but the cardiac output is maintained by increasing stroke volume. Thus the renal blood flow and glomerular filtration rate (GFR) are not decreased in spite of the low blood pressure (Boström, Nyman, Kampa, Häggström, & Lord, 2003). High concentrations of angiotensin and vasopressin measured after the administration of acepromazine suggest that the blood pressure was low enough to produce a response from compensatory mechanism to maintain GFR (Boström et al., 2003).

Acepromazine can decrease the hematocrit by 22% of the baseline. This decrease in the hematocrit was speculated to be due sequestration of RBCs in the spleen (Wilson, Evans, E, & Mullineaux, 2004). A study showed that the decrease in hematocrit was not directly related to the splenic size. The significant decrease in hematocrit and a mild increase in splenic size, on administration of low doses of acepromazine may relate to the sequestration of RBCs in other organs such as liver skin or muscles and not just spleen (Wilson et al., 2004).

Acepromazine is thought to lower the seizure threshold and hence has been contraindicated in dogs with the history of seizures (Tranquilli et al., 2007). However, one study refuted this contraindication and proved that there is no correlation between the acepromazine administration to dogs with a history of seizures and the recurrence of seizures during hospitalization (McConnell, Kirby, & Rudloff, 2007).

B. Alpha-2-adrenergic agonist

Alpha-2-agonists are widely used in veterinary medicine and induce reliable dose dependant sedation, analgesia and muscle relaxation. The advantage of using alpha-2-agonists for sedation is that they can be easily reversed by alpha-2 antagonists such as atipamizole and are ideal for short diagnostic and surgical procedures (VÄHÄ-Vahe, 1990).

Alpha-2 receptors are located pre and post-synaptically in the neuronal and non-neuronal tissues and extrasynaptically in the vascular endothelium and the platelets (D. B. Bylund, 1985). Norepinephrine is the endogenous ligand of the alpha-2 receptors. In the nervous system the alpha-2 receptors are located presynaptically on the sympathetic nerve endings and on noradrenergic neurons where they inhibit the release of noradrenaline. The stimulation of alpha-2

adrenoreceptors by alpha-2 agonists results in inhibition of adenyl cylase. It also activates G_i protein gated potassium ion channel, which cause hyperpolarisation of neuronal cell. This hyperpolarization of the cell causes a decrease in the rate of firing of excitable cells in the CNS. The stimulation of adrenoreceptors also results in inhibition of calcium ion conduction. This cascade of events on stimulation of the adrenoreceptors by the alpha-2 agonists results in inhibition of neurotransmitter noradrenaline release (Khan, Ferguson, & Jones, 1999).

Three distinct subtypes of alpha-2 receptors have been identified – a, b and c. Alpha-2a receptors are located in the cerebral cortex, locus coeruleus and platelets and are responsible for sedation, supraspinal analgesia, centrally mediated bradycardia and hypotension. Alpha-2b receptors are located in the dorsal root ganglia of spinal cord and vascular endothelium and are responsible for spinal analgesia, vasoconstriction and peripherally mediated reflex bradycardia. Alpha-2c receptors are located in the dorsal root ganglia of spinal cord and are responsible for spinal analgesia, hypothermia and modulation of dopaminergic activity (Scheinin et al., 1994). All the alpha-2 agonists have similar affinities to the different alpha-2 adrenergic receptor subtypes (D. Bylund, 1992).

The sedative and anxiolytic effects of alpha-2 agonists are mediated by activation of supraspinal receptors or the postsynaptic receptors located in the pons (locus coeruleus). The analgesic effects of alpha-2 receptors are mediated by the activation of receptors in the dorsal horn of spinal cord (Scheinin et al., 1994)

Alpha-2 agonists have been used synergistically with opioids, acepromazine and ketamine for short surgical procedures. On administration of alpha-2 agonists an initial period of vasoconstriction and reflex bradycardia is typically observed followed by decreased sympathetic tone, heart rate and blood pressure. Alpha-2 agonists have the potential to sensitize the myocardium to epinephrine-induced arrhythmias (Tranquilli et al., 2007).

Alpha-2 agonists bind to the adrenoreceptor preventing the release of norepinephrine. Norepinephrine is necessary for arousal. The blockade of norepinephrine release results in sedation. However pre-existing pain, fear and stress can increase the endogenous catecholamine concentrations and interfere with alpha-2 agonist mediated inhibition to the release of neurotransmitters. Sedation with alpha-2 agonists thus can be consistently achieved when administered to dogs in a quiet environment with minimal stimulation.

The most common alpha-2 agonists used in veterinary medicine are xylazine, medetomidine and dexmedetomidine. Xylazine is a potent alpha-2 agonist but also has some alpha-1 adrenergic effects. Due to severe cardiovascular effects of xylazine and the association of high mortality rate, the use of xylazine in veterinary practice has diminished (Dyson, Maxie, & Schnurr, 1998). Medetomidine is a highly selective alpha-2 agonist and is a racemic mixture of two optical enantiomers. Medetomidine has been frequently used in veterinary practice for its reliable sedation, muscle relaxation and analgesic effects (Adams, 2001). Dexmedetomidine is an active enantiomer of medetomidine and hence is more potent than medetomidine.

Dexmedetomidine

Dexmedetomidine is the most potent and selective alpha-2 agonist available in veterinary medicine. Dexmedetomidine is known for its multiple uses. It provides reliable sedation and good analgesia (Gomez-Villamandos et al., 2006). Studies show that dexmedetomidine CRI is a reliable and a valuable adjunct to isoflurane in maintaining surgical anaesthesia in healthy dogs (Uilenreef, Murrell, McKusick, & Hellebrekers, 2008). It has been used as a sedative, peri-operative CRI and as an analgesic. Dexmedetomidine has anti-myocardial ischemic effects in dogs and humans (Willigers, Prinzen, Roekaerts, de Lange, & Durieux, 2003). It has been used for prophylaxis and adjuvant treatment of peri-operative myocardial ischemia in humans (Gertler, Brown, Mitchell, & Silvius, 2001).

C. Benzodiazepines

Benzodiazepine agonists produce most of their pharmacological effects by modulating GABA-mediated neurotransmission. GABA is a primary inhibitory neurotransmitter in the mammalian nervous system, cell membrane of most CNS neurons and autonomic ganglia (Haefely, 1990). There are two main types of GABA receptors: $GABA_A$ and $GABA_B$. Activation of $GABA_A$ receptor increases chloride conductance and generates fast inhibitory postsynaptic potentials. Activation of $GABA_B$ increases potassium conductance and generates slow inhibitory postsynaptic potential. Benzodiazepines bind to the $GABA_A$ receptor complex and increase the frequency of chloride channel opening (Haefely, 1990).

Benzodiazepines produce sedation, anxiolysis, spinal cord mediated skeletal muscle relaxation and are anticonvulsants. Due to the activity of benzodiazepines on $GABA_A$ receptors in the CNS, these drugs cause retrograde amnesia. Diazepam and midazolam are the most commonly used benzodiazepines in veterinary medicine. Both these drugs are unreliable sedatives in dogs but are good muscle relaxants and anticonvulsants. They produce reliable sedation in old, young and debilitated dogs as they have limited side effects and produce limited effects on cardiorespiratory and pulmonary function (Tranquilli et al., 2007). Benzodiazepines have anticonvulsant activity and are useful for treatment of status epilepticus (Adams, 2001). Diazepam has been routinely used as an induction agent in combination with ketamine (White, Shelton, & Taylor, 2001). However, benzodiazepine drugs as a premedication agent in healthy dogs frequently cause excitation (Adams, 2001). Thus benzodiazepines have limited application as a premedication agent in healthy adult dogs but are safe sedatives in neonatal, geriatric and critically ill patients (Tranquilli et al., 2007).

D. Opioids

Opioids have been used for centuries for medical and recreational purposes. Opioids can be divided into four groups (McDonald & Lambert, 2011):

1. Naturally occurring endogenous peptides e.g. dynorphin, met-enkephalin.
2. Opium alkaloids such as morphine, purified from the poppy *Papaverum somniferum.*
3. Semisynthetic opioids which are a modification of naturally occurring morphine e.g. diacetylmorphine (heroin)
4. Synthetic derivatives with structure related to morphine e.g. pethidine, fentanyl, methadone, pentazocine and buprenorphine.

Opioids have many versatile applications and continue to be a cornerstone of effective management of pain in dogs from acute trauma, surgical procedures, painful medical conditions and chronic pain (Hellyer et al., 2007). Opioids are frequently used in dogs for their analgesic effects, sedative effects and as an adjunct to anaesthesia. Exogenously administered opioids bind to specific opioid receptors and mimic the naturally occurring endogenous opioid peptides (McDonald & Lambert, 2011). There are three well defined opioid receptors- δ (delta), κ (kappa) and μ (mu), now reclassified as (DOP) or OP_1, (KOP) or OP_2 and (MOP) or OP_3, respectively. A fourth receptor (NOP/OP_4) or ORL-1 has recently been identified

which is responsible for increased awareness to pain rather than analgesia (McDonald & Lambert, 2011).

The opioid receptors are located in the nociceptors, sensory nerves, brain and spinal cord (Pleuvry, 2005). In the periphery μ, δ and κ receptors are located primarily in the unmyelinated primary sensory neurons. Large myelinated nociceptors, which are responsible for response to injury, express low levels of opioid receptors. Hence opioids suppress persistent pain but do not hamper responses to new injury (Pleuvry, 2005). The μ receptor mediate most of the analgesic effects of opioids and also all the adverse effects. The dorsal horn of the spinal cord mostly expresses μ receptors, however κ and δ are also present (Pleuvry, 2005). In the CNS these opioid receptors have been identified in supraspinal sites including the mesencephalic reticular formation, periaqueductal grey, nucleus raphe magnus, various nuclei of rostral ventromedial medulla, thalamus and cortex (Pleuvry, 2005).

Opioid receptors are membrane bound receptors that are coupled with G proteins. Once an opioid binds to the receptor it inhibits adenyl cyclase activity which opens the potassium ion currents and suppresses voltage gated calcium ion currents. The hyperpolarization of the neurons results in decreased release of pain neurotransmitters: glutamate and substance P. The binding of opioids to the various receptors throughout the body results in systemic analgesia (Pleuvry, 2005).

Opioid pharmacodynamics can be described by using certain specific terms (Table: 1.1). *Affinity* of a drug describes is ability to bind to receptors within the body, whereas *activity* of a drug describes its ability to cause action in or on the cell where it resides (Adams, 2001). *Full Agonists* have both affinity and activity for one or more opioid receptors displaying full efficacy (Adams, 2001). *Partial Agonists* have an affinity and activity for one or more opioid receptors but the efficacy of these drugs is limited as compared to full agonists (Adams, 2001). *Agonist-Antagonist* opioids act as agonists at some opioid receptors and antagonists at some other opioid receptors (Adams, 2001). *Antagonists* have affinity but no activity at opioid receptors. They are used as reversal agents for agonists (Adams, 2001).

Activity	Opioid	Receptor
Opioid Agonist	Morphine	μ, κ, δ
	Codeine	μ
	Hydromorphone	μ
	Oxymorphone	μ
	Meperidine	μ
	Methadone	μ
	Fentanyl	μ
	Sufentanil	μ
	Alfentanil	μ
	Carfentanil	μ
	Remifentanil	μ
	Etorphine	μ
	Propoxyphene	μ
Opioid Partial Agonist	Buprenorphine	μ
	Tramadol	Weak μ,δ and κ agonist, inhibits neuronal reuptake and releases noradrenaline and 5-HT
Opioid Agonist-Antagonist	Butorphanol	K-agonist, μ-antagonist
	Nalbuphine	K-agonist, μ-antagonist
	Pentazocine	K-agonist, μ-antagonist
Opioid Antagonist	Naloxone	μ, κ, δ

Table 1.1: Opioid Receptor Activity (Adams, 2001), (Pleuvry, 2005)

Table 1.1 shows the activity of different opioids on endogenous opioid receptors.

The most common side effects of opioids in dogs are sedation, CNS depression, hypothermia, nausea, vomiting, mydriasis and dose-dependent respiratory depression (Tranquilli et al., 2007). Opioids have variable effects on GI motility and have both emetic and anti-emetic effects (Blancquaert, Lefebvre, & Willems, 1986). Opioids also decrease the GI motility and the effects depend on dose, route and the opioid itself (Bardon & Ruckebusch, 1985).

1.3 Sedation: clinical implications

Animals in veterinary clinics are often stressed and sedation forms an important component of veterinary care, welfare and safety of the staff. The environment in veterinary clinics can be very distressing to the animals and judicious use of sedatives, tranquilizers and general anaesthetics can help reduce the potential anxiety and stress of hospitalization (Karas, 1999). Short surgical procedures and diagnostic investigations warrant the use of short acting sedatives to reduce stress and pain. The choice of sedative depends on the behaviour of the patient and the desired endpoint i.e. anxiolysis versus deep sedation with analgesia. Judicious use of sedatives, tranquilizers and general anaesthetics enhances the quality of diagnostic procedures such as radiography, ultrasonography, biopsy procedures etc in a quiet relaxed patient (Karas, 1999). Sedation is useful when a thorough exam cannot be performed because of the patient's uncooperative nature. Clinical examinations such as orthopaedic, ocular, aural and oral examinations are distressing for the patient and often difficult to perform without appropriate sedation. Other procedures such as biopsy of skin or bone, aspiration of bone marrow, laceration repair, lancing of abscesses, wound care, placement of intravenous catheters, urinary catheterization in females, diagnostic procedures, passage of an orogastric tube, blood and urine sampling, grooming bathing and nail trimming may all be better performed under sedation (Karas, 1999). The use of sedatives for performing stressful procedures on animals prevents accidents, makes them comfortable and prevents potential future behavioural problems.

There is growing evidence that the neuro-endocrine stress response to severe injury or illness may become sufficiently intense that it contributes to morbidity and mortality. Thus the use of sedatives and analgesics is warranted in emergency and critically ill patients as administration of these agents improves the outcome and decreases mortality (Hansen, 2005). Several veterinary studies suggest that the stress response contributes to myocardial infarction, immune dysfunction, thrombosis, excess haemorrhage, pneumonia, impaired respiration, infections and other complications and thus the stress response is best moderated (Siracusa et al., 2008). Since providing optimal comfort for critically ill patients remains the main goal in emergency and critical care, the use of sedatives and analgesics to control pain and stress in these patients is a fundamental necessity. Currently no veterinary

studies have been published which address the impact of the stress response on the recovery of animals from injury. However sufficient human literature is available to support the contention that stress prolongs recovery (Curtis Sessler, Grap, & Ramsay, 2008).

In veterinary hospitals, animals' behavioural manifestations of distress such as struggling, pacing vocalizing and failure to sleep are best treated with physical comfort and sedation (Hansen, 2005). Sedation is not a substitute for restorative sleep but can be used to reduce anxiety and distress while promoting sleep in animals in intensive care units (Hansen, 2005). Restful sleep is one of the therapeutic goals in the acute phase of any illness. Animals are frequently sleep deprived in hospitals due to frequent medical interventions, activity in busy hospitals, anxiety from being in an unfamiliar environment, frequent handling by staff and separation anxiety. A combination of environmental control, calming techniques, gentle handling and pharmacological therapy are necessary to try to comfort critically ill patients. Distressed animals divert their energy into coping behaviours (Hansen, 2005). Hence it has been recommended that using sedatives with some potential side effects is preferable to a sleep deprived and restless patient.

Critically ill patients need sleep to cope with the illness and restorative sleep is crucial for a patient. Studies in rats have shown that alpha-2 agonists such as dexmedetomidine results in sedation which is similar to normal sleep (Nelson et al., 2003). The studies suggest that endogenous sleep pathways are involved in dexmedetomidine induced sedation. Thus dexmedetomidine sedation can be used in intensive care units to provide animals with restful sleep (Nelson et al., 2003).

1.4 Sedation Scoring Scales

In veterinary clinics, the use of sedatives, tranquilizers and anaesthetics in emergency care patients is crucial but can become challenging due to the potential side effects of these drugs. Though evidence suggests that stress of handling and hospitalization can increase the morbidity and mortality in critically ill patients, sedation in these patients can potentially lead to further complications. The adverse effects of these drugs may discourage their use. A sedation scoring system can be used to aid clinicians in the determination of a patient targeted sedation protocol. A

sedation scale can be used to quantify anxiety and the depth of sedation. Patients needs differ as the clinical circumstances and therapeutic targets for patients change over time. Thus assessing the sedation score allows clinicians to make rational decisions on the choice of drugs. Inadequate sedation and anxiety are undesirable. However over sedation can be equally problematical. Hence a scale to quantify sedation will aid in preventing under- sedation as well as over- sedation.

Many different sedation-agitation scales have been used in human Intensive Care Units. These include the Ramsey Sedation Scale, Sedation Agitation Scale, Motor Activity Assessment Scale, Vancouver Interactive and Calmeness Scale, Richmond Agitation-Sedation Scale, Adaptive to Intensive Care Environment Instrument and Minnesota Sedation Assessment Tool (Curtis Sessler et al., 2008). These scales are implemented in human ICUs to ensure patient comfort (Curtis Sessler et al., 2008). However the use of sedation scales in veterinary Intensive Care units has not been established. The current use of sedation scales has been limited to academic institutes to compare the sedative effects of various anaesthetics and premedication agents. The adoption of sedation scales in general practice should aid in a greater frequency of reaching an appropriate sedation level, lower the incidence of over sedation, reduce the doses of sedative and analgesic drugs, achieve patient comfort and safety and accurately measure pain and agitation, .

An appropriate level of sedation prior to surgery reduces the total anaesthetic dose required, which allows for a safer and balanced anaesthesia (Bednarski et al., 2011). The risks of cardiovascular depression are reduced if the patient is sufficiently sedated. Not only does appropriate sedation enable safer anaesthesia but it also reduces the total cost of anaesthesia. Using a sedation scoring system to target a level of sedation prior to induction of anaesthesia helps achieve optimal sedation. Assessing the level of anxiety and providing sedation to attain a particular sedation score should enable appropriate sedation.

Sedation Scales must be easy to interpret and recall, have well defined and discrete criteria allocated for each level of sedation, ability to assess anxious behaviours, inter-rater reliability and finally have evidence of validity in the relevant population (Curtis Sessler et al., 2008). A sedation endpoint can be regularly redefined for individual patients through the use of a sedation scales. Sedation scales assess levels of consciousness ranging from alert to unresponsive and must have a subdomain of arousal or awakeness in response to stimuli (Curtis Sessler et

al., 2008). Cognition in animals can be assessed by response to being called by name. The sedation scale must include recognisable criteria which determine the degrees of anxiety. Behaviours such as vocalization, continuous movement, dilated pupils continuous struggling on restraint reflect anxiety in dogs. Sedation scales may also aid in early detection of adverse drug reactions or inter-individual variability in response to different sedatives and anaesthetics.

The clinical applications of sedation scoring systems are numerous. The incorporation of sedation scoring scales in veterinary practice should enable an appropriate level of sedation and a predicable degree of comfort for each patient. The ability to alter the level of sedation based on application of a numerical scale is a first step towards individualized pharmacotherapy. Appropriate sedation and pain management along with compassionate care may help to achieve an optimal standard veterinary care in companion animal practice. Table: 1.2 shows an example of a sedation scoring scale that can be incorporated in veterinary practice.

Observation	Description	Score
Vocalization	Quiet	0
	Whining softly but quiets with soothing touch	-1
	Whining continuously	-2
	Barking Continuously	-3
Posture	Lateral Recumbence	3
	Sternal Recumbence	2
	Sitting or ataxic while standing	1
	Standing	0
	Moving continuously	-1
Appearance	Eyes sunken, glazed or unfocused; ventromedial rotation	3
	Eyes glazed but follow movement	2
	Protrusion of nictitating membrane; normal visual responses	1
	Normal Appearance	0
	Pupils dilated; Abnormal facial expression	-1
Interactive	Recumbent; no response to voice or touch	3
Behaviour	Recumbent; lifts head in response to voice or touch	2
	Recumbent but stands in response to voice or touch	1
	Moves towards/ away from voice or touch; appears anxious	0
	Wags tail/ excited/Growls or hisses when approached or touched	-1
	Very excited/ jumps/Bites or swats when approached	-2
Restraint	Lies on floor ,no restraint required	2
	Lies on floor with light restraint of head or neck	1
	Sits up on floor; with light restraint	0
	Requires regular correction	-1
	Struggles continuously against restraint	-2
Response	No response to whistle	3
to Noise	Minimal response to whistle	2
	Slow or moderate response to whistle	1
	Brisk response to whistle; raises head with eyes open	0

Table 1.2: Sedation scoring system (Hofmeister, Chandler, & Read, 2010).

Table 1.2 shows an example of a sedation scoring system that can be used to assess the level of sedation in dogs.

Various factors of common occurrence affect the pharmacodynamics and pharmacokinetics of the different sedatives and anaesthetic drugs thus affecting the degree of sedation in a particular animal. These factors include age, rate of administration of drugs, concentration of drugs, physical status of the animal, muscular development, adiposity, respiratory and circulatory status of the animals, drug permeability coefficient, prior or concurrent drug administration, fear, recent feeding, metabolic state, disease conditions etc (Tranquilli et al., 2007). The partial coefficient, ionization and the protein binding of sedatives and anaesthetics significantly affect the permeation of the drug in the blood brain barrier. Factors that increase metabolic rate such large meals, hyperthyroidism etc can also increase the sedative and anaesthetic requirement (Tranquilli et al., 2007).

Certain breeds of dogs are considered at a high sedation and anaesthetic risk due to higher incidence of disease or anatomical abnormality. Brachycephalic dogs are prone to anaesthetic and sedation complications. These dogs are prone to cardiac arrests and respiratory obstructions due to brachycephalic airway syndrome. Brachycephalic dogs can have a high vagal tone which makes them prone to cardiac arrhythmias (Tranquilli et al., 2007). Hounds such as whippet, Afghan, borzoi, wolfhound and Saluki have a low fat-to-body weight ratio and a low muscle-to-body mass ratio. This consequently leads to an increase in the blood levels of unbound drug when anaesthetized with barbiturates resulting in prolonged sedation, rougher recoveries and occasional fatalities (Tranquilli et al., 2007).

An important factor for inter-individual variability in pharmacodynamics and pharmacokinetics of a specific drug is genetics. Genetic variations and mutations play a crucial role in the drug absorption, disposition and drug response in an animal. Various mutations and genetic variations have recently been discovered which are responsible for adverse drug reactions in animals (Mosher, 2010).

1.5 Pharmacogenetics

In the year 1957 a concept was proposed that inheritance might explain the individual variation in drug efficacy and susceptibility to adverse drug reactions (Mealey, 2006a). In 1959 the term pharmacogenetics was introduced which involves the studying of the impact of genetic variations on drug effects (Mealey, 2006a). It

was proposed that the inter-individual variability in drug responses may primarily be due to genetic differences between individuals (Mealey, 2006a). The ultimate goal of pharmacogenetics has been to realize the idea of personalized medicine. The Human Genome Project in 1990 resulted in a renewed interest in the field of pharmacogenetics and this field has remarkably expanded ever since (Mealey, 2006a). Currently the field of veterinary pharmacogenetics is in its preliminary stages. However with the complete genome sequencing of different species of animals now available, veterinary pharmacogenetics is fast gaining pace.

Basic Genetics Concepts

The genome is the entirety of the animal's hereditary information contained in many genes. A gene is a molecular unit of heredity information which contains a specific sequence of DNA coding for a particular protein. When a gene is expressed the DNA is transcribed to RNA which is then further translated to make different proteins. The combination of three nucleotides makes a specific codon and each codon specifies a particular amino acid or an amino acid termination called as stop codon (Newman, 2010). The genetic code has redundancy, that is two or more codons may code for a single amino acid (Newman, 2010). Mutations in specific genes result in the variations of individuals in a population. A mutation alters the DNA sequence, which in turn alters the RNA creating a different codon. If the original codon and the mutated codon happen to code for the same amino acid then the mutation is silent. However if the mutated codon, codes for a different amino acid then the change in protein may be deleterious (Newman, 2010). A specific location in the gene or the gene locus in every individual has potentially two alleles, one from each parent. An allele is a DNA sequence at a given gene location on the chromosome. If an individual has two identical alleles then he is said to have a homozygous genotype, whereas if an individual has two different alleles then he is said to have a heterozygous genotype (Newman, 2010). The outward manifestation of a genotype is called the phenotype. The alleles in a gene interact in different ways at a functional level resulting in variations in the type of dominance. This results in different phenotypic effects in different allelic combinations (Newman, 2010). The phenotypic expression may be as obvious as coat colour or something inconspicuous as drug efficacy and sensitivity.

Before the discovery of molecular biological techniques, the study of pharmacogenetics relied heavily on phenotypic observations (Newman, 2010). However the rapid advancement of molecular biology has made it possible to correlate phenotypes to a particular genotype. Modern pharmacogenetics involves a systematic search to identify sequences of DNA that may play a role in the drug pharmacokinetics and pharmacodynamics (Newman, 2010). Understanding the effects of genetic variation on drug disposition takes pharmacogenetics closer to the goal of individual drug therapy.

The concept of individualization of drug therapies has two important clinical implications. The first approach is to focus on the use of pharmacogenetics for the prediction of adverse drug reaction or non responsiveness to certain drugs (Mosher, 2010). This may have legal implications. In human medicine some drugs such as abacavir cannot be given to individuals without being genotyped. The use of this drug without genotyping the patient is considered a malpractice due to 100% sensitivity of predicting the occurrence of adverse reactions in people with HLAB*5701 single nucleotide polymorphism (Panel, 2013). The second implication is the use of these genetic mutations for the benefit of the patient. Pharmacogenetics can be used to predict patients that are more likely to benefit from a particular drug due to appropriate receptor interactions. Some animals with specific mutations may be non-responders to particular drugs (Mosher, 2010). Thus instead of using a trial and error approach, pharmacogenetics may help clinicians make a better choice of drug for the individual patient.

Various pharmacogenetically based differences in drug absorption, distribution, metabolism, excretion and drug receptor interactions have been identified in veterinary medicine. The following are the different genetic variations responsible for alteration of drug disposition in dogs known currently.

CYP2D15 in Beagles

While studying the pharmacokinetics of celecoxib, a cyclooxygenase-2 inhibitor, a bimodal distribution of drug clearance in a colony of 242 beagles was observed (Paulson et al., 1999). Of these beagles, 45% had extensive metaboliser phenotype (plasma half life 1.72 hours, clearance 18.2 ml/min/kg) whereas 53 % had poor metaboliser phenotype (plasma half life 5.18 hours, clearance 7.12 ml/min/kg)

on intravenous dosing of celecoxib (5 mg/kg) (Paulson et al., 1999). There was equal distribution of the extensive metaboliser phenotype and poor metaboliser phenotype within each sex. The difference in the two populations was shown to be due to difference in the rate of metabolism of celecoxib by the liver enzymes CYPs, which are cytochrome P450 enzymes (Paulson et al., 1999). CYP2D15 plays a crucial role in the metabolism of celecoxib however the contribution of other CYPs in the polymorphism cannot be denied (Paulson et al., 1999).

CYP2D11 in greyhounds

It has long been known that greyhounds recover relatively slowly from the effects of thiopentone anaesthesia, an ultra short acting barbiturate. The low body fat in greyhounds resulting in slower redistribution of drug from the central compartment to the adipose tissue was considered the cause for delayed recovery from thiopentone anaesthesia. However recent studies suggest that this slower recovery from thiopentone in greyhounds is due to slower hepatic metabolism of thiopentone (Court, Hay-Kraus, Hill, Kind, & Greenblatt, 1999). The elimination of several drugs has been reported to be significantly lower in greyhounds. These include propofol, antipyrine, ketoconazole, celecoxib, methadone, morphine and succinylcholine (Court et al., 1999). The molecular basis has been explored for the slower hepatic metabolism in greyhounds. Studies suggest that a reduced expression of CYP2B11, a major isoform of cytochrome P450 in Greyhounds, is responsible for the delayed metabolism (Court et al., 1999).

Thiopurine Methyltransferase (TPMT) in Giant Schnauzers and Alskan Malamutes

The enzyme TPMT(Thiopurine Methyltransferase) metabolises a number of immunosuppressant drugs such as 6-mercaptopurine and azathioprine (Kidd et al., 2004). Azathioprine is used in dogs to treat immune mediated diseases including immune mediated haemolytic anaemia, immune mediated thrombocytopenia, rheumatoid arthritis, immune mediated polyarthritis, inflammatory bowel disease and dermatologic diseases (Kidd et al., 2004). TPMT is an enzyme present in the RBCs and aids in metabolizing azathioprine to 6-mercaptopurine. The inability to metabolize thiopurines results in adverse effects such as bone marrow suppression (leukopenia and thrombocytopenia with or without anaemia) (Kidd et al., 2004). A study with 177 dogs was published by

Kidd in 2004 observed that Giant Schnauzers have low TPMT activity (2.7 fold lower than population mean) whereas the Alaskan malamutes had a relatively high activity (3.3 fold higher than population mean) (Kidd et al., 2004). Low TPMT activity in humans has been associated with increased toxicity (Kidd et al., 2004). The canine TPMT gene was sequenced and a total of 9 polymorphisms were identified including 6 SNPs and 3 insertion/deletion variants (Kidd et al., 2004). Further studies are warranted to prove the association between the genetic mutation and the clinical outcome.

Malignant Hyperthermia

Malignant Hyperthermia is an inherited condition of the muscles. Dogs susceptible to malignant hyperthermia release excessive calcium from the sarcoplasmic reticulum on the exposure to volatile anaesthetics and depolarising muscle relaxants such as succinyl choline (Roberts et al., 2001). Malignant hyperthermia susceptible dogs show tachycardia, hyperthermia, elevated carbon dioxide production and death if the anaesthetic is not discontinued. Medical interventions by using of calcium channel antagonist dantrolene has been efficacious at reversing malignant hyperthermia (Roberts et al., 2001). Malignant hyperthermia is more common in humans and swine (Roberts et al., 2001). However, a recent mutation has been discovered in dogs which make them susceptible to malignant hyperthermia. The V547A mutation on the RYR1 gene (ryanodine receptor) has been associated with malignant hyperthermia in dogs (Roberts et al., 2001). This mutation causes the ryanodine receptors to open for a longer interval in the presence of inhalant anaesthetics and depolarising muscle relaxants resulting in excessive release of calcium from the sarcoplasmic reticulum. Elevated resting sarcoplasmic calcium causes muscle contraction, excessive ATP hydrolysis, accelerated metabolism and hyperthermia, all of which are characteristics of malignant hyperthermia (Roberts et al., 2001).

***MDR1* Gene**

The multidrug resistant-1 or the *MDR1* gene encodes for a protein called the P-glycoprotein (P-gp) (Martinez et al., 2008). The P-gp is a transmembrane protein pump that actively pumps out the substrate from inside of the cell into the

extracellular compartment. A genetic mutation in the *MDR1* gene in herding dogs causes premature termination of protein translation resulting in a non-functional P-gp pump (Martinez et al., 2008). This genetic mutation makes herding dogs potentially more sensitive to many therapeutic drugs. The *MDR1-1*Δ mutation is probably the most well known and well studied genetic mutation in veterinary pharmacogenetics.

Overview of P-glycoprotein

P- glycoprotein was first identified in 1976 in Chinese hamster ovary (CHO) cells that were selected in culture for colchicine resistance (Riordan & Ling, 1979). The CHO colchicine resistant cells expressed large quantities of a 170 KDa protein that conferred upon the cells resistance to colchicine and other drugs. This protein was later called the P-glycoprotein since it is a plasma membrane glycoprotein (Riordan & Ling, 1979). In 1980 the gene encoding for P-gp was sequenced and was named multidrug resistance-1 (*MDR1*) since over-expression of P-gp in tumour cells conferred resistance against many chemotherapeutic drugs (Chen et al., 1986). Most of the research in the 1980's focused on multidrug resistant tumour cells and this lead to a further understanding of P-gp and identification of other P-gp substrates. In the early 1990's areas of intense P-gp research focused on using pharmaceutical agents to inhibit the function of P-gp and thus overcoming the resistance caused due to over-expression of P-gp. Many drugs were identified as inhibitors of P-gp and researchers were optimistic that modulation of P-gp could overcome multidrug resistance in tumour cells (Mealey, 2004). However many other factors conferring resistance to chemotherapeutics in cancer cells were identified and were found to be more clinically relevant than over-expression on P-gp (Mealey, 2004). Thus the interest in developing P-gp inhibitors waned over time.

In the mid 1990's a serendipitous finding renewed the interest in P-gp. A group of investigators studying P-gp had genetically engineered mice that did not express P-gp (*MDR1*a -/- mice) (Schinkel et al., 1994). The researchers were disappointed on seeing that *MDR1a -/-* mice were healthy, fertile and lived a normal life span. They thus concluded that the P-gp was not essential for the survival of mice. Various tests such as complete blood cell count, biochemistry profiles and other physiological parameters were performed to identify the role of P-gp. The researchers did not find any anatomical or physiological abnormalities in these

MDR1a (-/-) knockout mice (Schinkel et al., 1994). Their interest in these mice would have faded, had it not been for a mite infestation in the colony. The mite infestation was treated as per the laboratory protocol by topical ivermectin. It was observed that within 24 hours nearly all the mice with *MDR1*a (-/-) knockout mice were dead whereas all the wild type animals that expressed P-gp survived. The researchers found that the P-gp plays an important role in the blood brain barrier in mice and that the *MDR1*a (-/-) knockout mice had a defective blood brain barrier. This resulted in a 100 fold greater concentration of ivermectin in the brain of *MDR1a* (-/-) knockout mice than the wild types (Schinkel et al., 1994). This study paved the way to many studies investigating the role and significance of P-gp.

ABC transporters

P-gp is a member of the family of transporter proteins called ATP binding cassette transporter proteins (ABC) (Cascorbi, 2006). The mammalian ABC super family is an extensive and functionally diverse family of proteins. Most of the efflux transporters belong to the ABC super family and these transporters play an important role in the protection of the cell from harmful xenobiotics, metabolites and other endogenous compounds (Cascorbi, 2006). The translocation of substrates across the biomembranes by these transporter proteins is an active process and requires energy that is generated by the hydrolysis of ATP and intermediate phosphorylation of the transporter. This ATP dependant process enables transport of substrates even against steep concentration gradients (Cascorbi, 2006).

Structure of P-glycoprotein

P-gp is a large 170 kD protein. It consists of four distinct domains: two highly hydrophobic integral membrane domains or transmembrane domains (TMD) and two hydrophilic nucleotide binding domains (NBDs) located at the cytoplasmic face of the membrane (Figure: 1.1) (Higgins, Callaghan, Linton, Rosenberg, & Ford, 1997). P-gp can be viewed as two half molecules with each half consisting of one integral membrane domain and one nucleotide-binding domain. The two half molecules are separated by a ‘linker’ region. The linker region is highly charged and

phosphorylated at several sites by protein kinase C. Phosphorylation of the 'linker' aids P-gp to regulate heterologous ion channels (Higgins et al., 1997).

The integral membrane domain or the transmembrane domain (TMD) of P-gp has two central roles in the transport process (Higgins et al., 1997). The first is to form a pathway through which solute can be translocated across the membrane. The second is to provide amino acid residues which interact directly with substrates and form substrate binding sites (Higgins et al., 1997). Each integral membrane domain of a P-gp consists of six membrane spanning α-helices that are separated by hydrophilic loops. Thus there are 12 membrane spanning α-helices in each P-gp molecule which is consistent with the 'six-plus-six' P-gp model (Higgins et al., 1997).

The two NBDs of the P-gp share 30-40% amino acid sequence with each other and with other ABC transporters (Higgins et al., 1997). These NBDs also carry a significant additional amino acid sequence which is the 'signature' motif that defines the NBDs of ABC transporters (Higgins et al., 1997). The NBDs are located on the cytoplasmic face of the membrane and provide the energy for active transport of the solutes across the membrane. They bind by hydrolyzing the ATP (Higgins et al., 1997).

The structure of P-gp though reflected as a static conformation is actually dynamic. The P-gp transmembrane domain undergoes significant conformational changes with transportation of solutes and hydrolysis of ATP (Higgins et al., 1997). These conformational changes are restricted to the secondary structural elements or domains and there are no gross perturbations in the structures. The primary structures such as the α-helix and the β-sheet remain constant (Higgins et al., 1997). The binding of the ATP to the NBD results in the conformational changes in the transmembrane domain of the P-gp. It is the energy of this binding rather than the hydrolysis of ATP molecule which provides the initial energy for translocation of substrate (Martinez et al., 2008). The substrate binds to the TMD resulting in conformational changes in the TMD substrate binding site. These conformational changes decrease the affinity of the substrates to the binding sites. This TMD binding site reorientation causes release of the substrate at the basal membrane surface. Subsequent hydrolysis of ATP aids in the return of transporter protein to its original configuration (Martinez et al., 2008).

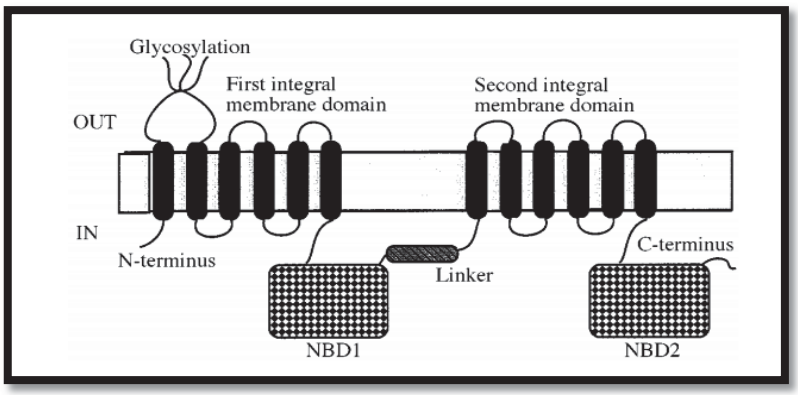

Figure 1.1: Topological map and domain organisation of P-gp, predicted from its primary sequence (Higgins et al., 1997).

Function of P-glycoprotein

P-gp is normally expressed in various mammalian tissues such as the apical border of intestinal epithelial cells, brain capillary endothelial cells, biliary canalicular cells, renal proximal tubular epithelial cell, placenta and testes. P-gp actively effluxes xenobiotics from the intestine, brain capillary, bile canaliculus and renal tubules. Its function is to protect the cell against the exposure and toxic effects of xenobiotics.

There is a constant chemical warfare in nature. Plants produce noxious or toxic compounds to prevent themselves being eaten by animals ranging from worms and insects to larger animals. They also produce compounds that would protect them from bacterial and fungal infections. Animals have hence evolved with a mechanism to cope with this exposure to toxins which allows them to feed on certain plants that otherwise couldn't be eaten. P-gp plays an important role in the protection against these xenobiotics (Schinkel, 1997). It offers generalized protection against amphipathic compounds that otherwise enter the body through passive diffusion through the intestinal lumen (Schinkel, 1997). P-gp offers protection at several levels (Figure: 1.2). The primary site where the xenobiotics may enter the body is the intestine. The intestine expresses P-gp which actively effluxes these xenobiotics out into the lumen. The blood brain barrier protects the brain from the toxic effects of xenobiotics and forms the second line of defence of the P-gp by preventing xenobiotics from achieving high concentrations in the brain. P-gp is also expressed in the liver and the kidneys and aids in excretion of xenobiotics and P-gp substrates

in the blood (Schinkel, 1997). The expression of P-gp in these organs prevents the toxic effects caused by xenobiotics and P-gp substrates (Schinkel, 1997).

P-gp is not only present in mammals but homologues also exist in micro-organisms such as *Plasmodium falciparum*, *Candida albicans*, *Saccharomyces cerevisiae* and *Lactococcus lactis* (Mealey, 2004). The expression of P-gp in these microorganisms confers resistance to different drugs. P-gp expression in *Plasmodium falciparum* confers resistance to chloroquin and P-gp expression in *Candida albicans* and *Lactococcus lactis* confers resistance to azoles. P-gp homologue expression in helminths and nematodes also contribute to resistance against antiparasitics (Mealey, 2004).

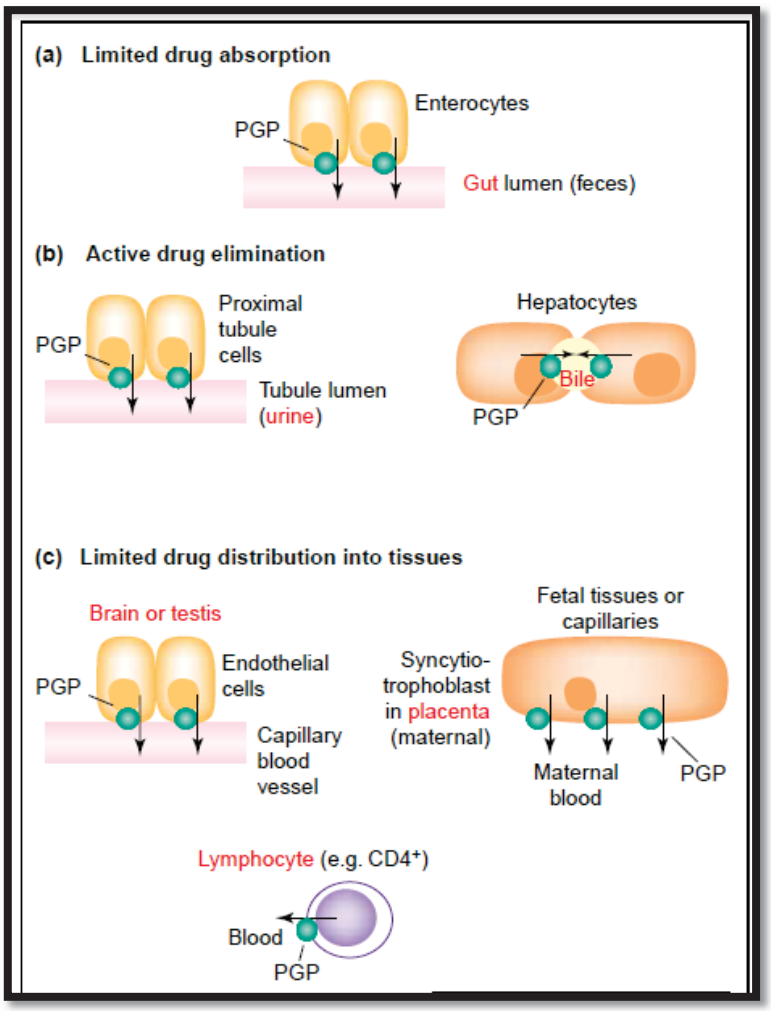

Figure 1.2: P-glycoprotein expression and function in various tissues (Fromm, 2004).

P-gp functions as an ATP-dependent efflux transporter, which pumps its substrates out of cells. (a) P-gp limits drug entry into the body after oral drug administration as a result of its expression in the luminal (apical) membrane of enterocytes. (b) P-gp promotes drug elimination into urine and bile as a result of its expression in the luminal membrane of proximal tubule cells in the kidneys and the canalicular membrane of hepatocytes, respectively. (c) Once a xenobiotic has reached the systemic blood circulation, P-gp limits drug penetration into sensitive tissues (e.g. into the brain, testis and fetal circulation) and into lymphocytes.

Role of P-glycoprotein in intestinal drug absorption

P-gp is expressed on the apical border of the intestine (Lin, 2003). This transmembrane pump is known to transport substrates from the cytoplasm back into the lumen and thus limit the absorption of P-gp substrates. P-gp is not uniformly distributed along the intestinal epithelial villi. It is expressed in the apical surface of columnar epithelial cells but not in the crypts (Lin, 2003). The distribution of P-gp is not uniform along the length of the intestine either. The expression of P-gp increases progressively from the stomach to the colon. P-gp expression is the least in the stomach, intermediate in jejunum and highest in the colon (Lin, 2003). The oral bioavailability of P-gp substrates is severely affected by the P-gp expression in the intestine (Lin, 2003). Drugs potentially influenced by intestinal P-gp include paclitaxel, digoxin, cyclosporine A, dexamethasone, opioids, fluoroquinolones, ivermectin, beta-adrenergic antagonists and certain antiviral compounds (Martinez et al., 2008).

Evidence that P-gp plays an important role in drug absorption was first demonstrated in Caco-2 cells with high P-gp expression. In these cells it was observed that the basolateral-to-apical transport of vinblastine and docetaxel was 10 and 20 fold, respectively, greater than the apical-to-basolateral transport. In the presence of verapamil the apical-to-basolateral transport of vinblastine and docetaxel was enhanced (Lin, 2003). Verapamil is a P-gp inhibitor thus altering the normal functioning of P-gp. This observation concluded that P-gp plays an important role in the efflux of P-gp substrates by pumping the substrates from the cytoplasm back into the lumen. In vitro studies suggested that P-gp played a significant role in the absorption of drugs by limiting their transport across the enterocytes in the intestine (Lin 2003)

In vivo studies in *MDR1* (-/-) knockout mice provided the evidence that P-gp plays an important role in intestinal absorption of drugs. The oral absorption of paclitaxel was studied in *MDR1* (-/-) and *MDR1* (+/+) mice. It was observed that the plasma area under curve (AUC) of paclitaxel was 3 fold higher in *MDR1* (-/-) than in *MDR1* (+/+) mice (Lin, 2003). The theory that P-gp has an efflux function is further supported by the observation that paclitaxel was excreted from the blood circulation into the intestinal lumen of the mice after intravenous injection. P-gp mediated intestinal excretion of digoxin is also observed in mice. It was observed that 16% of

intravenous dosing of digoxin was excreted through the intestinal lumen in *MDR1* (-/-) mice after 90 minutes (Lin, 2003). This interestingly, raises questions about considering an additional pathway for elimination through the intestinal epithelium, especially for drugs which are substrates of P-gp.

Recent studies show that there are two types of P-gp substrates. The first is the vinblastine type, in which the intestinal absorption is affected by P-gp. The second is the verapamil type for which the intestinal absorption is unaffected by P-gp (Ogihara et al., 2006). The vinblastine type has low membrane permeability and high P-gp affinity whereas the verapamil type has high membrane permeability and high P-gp affinity. It has been observed that the vinblastine type substrates are easily captured by the P-gp in the intestinal epithelial cells before they transport to the basolateral side as they have low permeability. Thus the oral absorption of vinblastine type substrates is poor (Ogihara et al., 2006). The drugs which have high affinity towards P-gp and low permeability have poor oral bioavailability and are unfavourable for development of oral drugs (Ogihara et al., 2006).

In veterinary medicine, little information is available about the role of P-gp in drug disposition and the role of P-gp modulation. Prednisolone is a known substrate of P-gp (Van der Heyden et al., 2012). It has been traditionally used as a treatment for various enteropathies, immune mediated diseases and as an anticancer therapy. A recent study evaluated the effect of P-gp modulation on the plasma prednisolone concentration and prednisolone pharmacokinetics in dogs (Van der Heyden et al., 2012). The study evaluated the effect of rifampicin, a P-gp inducer on the plasma AUC of prednisolone. Rifampicin induces an increase in the intestinal expression on P-gp. The increased expression of P-gp in the enterocytes results in a reduced plasma AUC of prednisolone (Van der Heyden et al., 2012). On the other hand ketoconazole is an antifungal and also an inhibitor of P-gp. It was used in this study to modulate the plasma AUC of prednisolone. Ketoconazole decreased the *MDR1* expression in enterocytes and slightly increased the plasma AUC on oral administration of prednisolone. Ketoconazole, however, is an inhibitor of P-gp and a cytochrome P450 enzyme called CYP3A (Van der Heyden et al., 2012). Thus concomitant administration of substrates for ketoconazole and P-gp or CYP3A should be avoided.

Role of P-glycoprotein in drug disposition

P-gp plays an important role in the drug disposition in the body and it protects various organs against the toxic effects of xenobiotics (Mealey, 2004). The P-gp pump is located in the blood-brain barrier, blood-testes barrier and blood-placenta barrier and is crucial in preventing exposure of the brain, testes and foetus to toxic substances (Mealey, 2004).

There are two primary interfaces between the peripheral circulation and the brain, the first is the blood-brain barrier and the second is blood-cerebrospinal fluid (CSF) barrier. Since the surface area of the BBB is approximately 5000 times greater than blood-CSF barrier, BBB is considered the main route for trafficking of endogenous substances and xenobiotics out of the brain (Kusuhara & Sugiyama, 2001).

The blood-brain barrier is a functional selective barrier between the blood and the brain. It functions to protect and maintain the homeostasis of the brain parenchymal microenvironment (Perrière et al., 2007). The blood brain barrier is formed by the endothelial cells lining the cerebral capillaries ensheathed by the astrocytic endfeet. The brain endothelial cells are distinguished from the endothelial cells of other organs by interendothelial tight junctions linked to transendothelial electrical resistance and a paucity of pinocytic vesicles (Perrière et al., 2007). In contrast to the BBB capillaries, the capillaries in the blood-CSF barrier are leaky, but the epithelial cells in the blood-CSF barrier are connected to each other by tight junctions (Kusuhara & Sugiyama, 2001). These anatomical barriers prevent the transport of xenobiotics via the paracellular route. Hence xenobiotics circulating in the blood must be transported across the transcellular route. The xenobiotics thus need to have low molecular weight and high lipophilicity to enter the brain. It has been observed that some xenobiotics which are highly lipophilic and low molecular weight did not achieve high concentrations in the brain. The poor distribution of these drugs in the brain leds to the conclusion that there are efflux transporters like the P-gp, which actively eliminate these drugs from the brain (Kusuhara & Sugiyama, 2001).

Further studies demonstrated that P-gp is located in the luminal and abluminal plasma membranes of the brain capillary endothelial cells (Bendayan, Ronaldson, Gingras, & Bendayan, 2006). P-gp is also located in the astrocytes and the

pericytes. P-gp has also been observed in the nuclear envelope Golgi apparatus, smooth endoplasmic reticulum and rough endoplasmic reticulum. P-gp is also localized in caveolea, which are invaginated plasma membranes and play an important role in endocytosis. The expression of P-gp in caveolea suggests that it prevents the uptake of P-gp substrates through endocytosis (Bendayan et al., 2006).

It has been assumed that P-gp plays a role in the efflux of P-gp substrates in both the BBB and blood-CSF barrier (Mealey et al., 2008a). A recent paper challenged this assumption. ^{99m}Tc-sestamibi, a radio-labelled P-gp substrate was injected in *MDR1* wildtype and *MDR1* knockout canine models. Serial nuclear scintigraphy images were obtained to assess the uptake of ^{99m}Tc-sestamibi in the brain tissue as also direct measurement of the radioactivity in the blood and CSF was assessed by taking serial blood and CSF samples. It was observed that the ^{99m}Tc-sestamibi uptake in the brain was higher in the *MDR1* knockout dogs than the *MDR1* normal dogs. The ^{99m}Tc-sestamibi activity in the blood samples did not differ between the *MDR1* normal and *MDR1* knockout dogs. This suggests that the P-gp in BBB limits the accumulation of P-gp substrates in the brain concluding that differences in the uptake of ^{99m}Tc-sestamibi in the brain are due to differences in the functioning of P-gp in the BBB between the *MDR1* normal and *MDR1* knockout dogs (Mealey et al., 2008a). Interestingly it was noted that the ^{99m}Tc-sestamibi activity did not differ between the CSF samples of the *MDR1* normal and *MDR1* knockout dogs. This may suggest that P-gp does not play a role in the efflux of xenobiotics in the blood-CSF barrier as was previously thought (Mealey et al., 2008a).

P-gp efflux pump in the BBB protects the brain against several drugs. Many studies in *MDR1* knockout mice have shown that P-gp substrates such as ivermectin, vinblastine, doxorubicin, methadone, ritonavir, verapamil, quinidine, paclitaxel, saquinovir, loperamide, digoxin etc. achieve high concentrations in the brain as compared to *MDR1* wildtype mice (Martinez et al., 2008).

P-gp plays a crucial role in the preventing exposure of the foetus to xenobiotics (Ceckova-Novotna, Pavek, & Staud, 2006). P-gp is expressed in the trophoblast in mice and humans. The expression of P-gp in the trophoblast of the placenta was confirmed by mRNA and protein expression in all phases of pregnancy in humans (Ceckova-Novotna et al., 2006). In vitro and in vivo studies have proved that the P-gp efflux pump is functionally active transporter in maternal-foetal drug transport (Ceckova-Novotna et al., 2006). No studies in dogs are currently published

localizing the expression of P-gp in canine placenta. The P-gp efflux pump actively pumps out xenobiotics from the trophoblast cells back to maternal circulation providing protection to the foetus (Ceckova-Novotna et al., 2006). As the majority of the placenta is from foetal origin, it is not surprising that the P-gp genotype of the foetus and not the dam is critical in determining the degree of foetal exposure to p-gp substrates (Lankas, Wise, Cartwright, Pippert, & Umbenhauer, 1998). In a study with CF-1 strain of mice, the foetus of these mice were divided in three P-gp genotypes: homozygous positive (+/+), heterozygous (+/-) and homozygous negative (-/-) (Lankas et al., 1998). The foetuses were exposed to P-gp substrate, L-652,280 which is a naturally occurring avermectin and is known to produce cleft palate as a developmental toxicity in mice. It was observed that only foetuses that were heterozygous (+/-) or homozygous (-/-) P-gp genotypes were sensitive to cleft palate induction by the compound. This suggests that the genotype of the foetus plays a role in the P-gp expression in the placenta (Lankas et al., 1998). There is however no current study published in dogs proving the same. More studies assessing the role of the foetal genotype in dogs are warranted. The foetal genotype can have clinical implications when prescribing drugs to the dam during pregnancy or during parturition. The foetal genotype may play a significant while choosing premedication and anaesthetic agents during a caesarean section.

Role of P-glycoprotein in excretion

Drugs are excreted from the body by the liver and kidneys either as metabolites or unchanged molecules. P-gp expression in the liver and kidneys plays an important role in the active excretion of xenobiotics and P-gp substrate (Mealey, 2004).

The bile is formed in the hepatocytes and is excreted into the bile canaliculus. The canaliculi are small tubules formed with tight apical junctions of adjacent hepatocytes. The tight apical junctions separate the hepatocyte into two domains: apical and basolateral domain (Martinez et al., 2008). Bile is produced by osmosis and is secreted into the canaliculus by active transport by the ABC transporter proteins and specially P-gp (Martinez et al., 2008). There are multiple paths through which solutes can be transported through the liver. The solutes can either move across the sinusoidal membrane from the portal blood to the metabolizing site and

ultimately to the bile canaliculi from the hepatocyte membrane, or the solutes can move from the hepatocyte across the canalicular membrane into the bile or finally the solutes can move from the hepatocyte across the lateral membrane into the sinusoid. P-gp however is located exclusively on the canalicular membrane where it is responsible for excretion of P-gp substrates into the bile (Martinez et al., 2008).

A characteristic feature of P-gp efflux transporters is that their expression and activity can be modulated by various factors including cytokines (Kawaguchi, Matsui, Watanabe, & Takakura, 2004). A study was published investigating the effect of interferon-γ (IFN- γ) on the transport activity and expression of P-gp in mice (Kawaguchi et al., 2004). The mice were injected with intraperitoneal interferon-γ and the pharmacokinetics of intravenous digoxin, a P-gp substrate was examined. Mice pre-treated with interferon had a decreased plasma elimination of digoxin and had a concomitant increase in the tissue digoxin levels in the liver, kidney and intestine. It was observed that the excretion of digoxin in the liver and bile, but not in the intestinal lumen, was decreased. The urinary and biliary excretion clearance in IFN-gamma treated mice was 65% and 55%, respectively, of those clearances in untreated mice (Kawaguchi et al., 2004). CYP3A, a cytochrome P450 enzyme, shares many common substrates with P-gp, with digoxin being one of them. In the above study it was found that though the excretion of digoxin was drastically low in IFN- γ treated mice, the P-gp expression was reduced only by 20 – 30%. Thus the lower excretion clearance of digoxin in IFN- gamma mice must be due to concurrent down regulation of CYP3A by 20-30% (Kawaguchi et al., 2004).

P-gp expression has been identified in the proximal tubules, mesangial cells, thick limb of Henle's loop and collecting ducts in the kidney (Martinez et al., 2008). The density of P-gp in the proximal tubules is crucial since it plays an important role in the active elimination of toxins and xenobiotics (Martinez et al., 2008). Tubular secretion in the kidneys involves both the uptake transporters located at the basolateral and the efflux transporters localized in the apical border of proximal convoluted tubules. P-gp is the main efflux transporter located in the proximal convoluted tubule where it primarily transports cations (Martinez et al., 2008).

The role of P-gp in the kidneys can be modulated by various P-gp inhibitors such as verapamil and quinidine. A study was published investigating the role of P-gp inhibitors such as verapamil and quinidine on the elimination of a P-gp substrate,

digoxin. It was observed that in porcine kidney cells the efflux of digoxin was decreased in the presence of P-gp inhibitors (Tanigawara et al., 1992).

PSC 833 is an inhibitor of P-gp. A study showed that PSC 833 decreased the excretion of vincristine and digoxin in rats. Administration of PSC 833 significantly decreased the renal and biliary excretion of both vincristine and digoxin, thus increasing the plasma concentration of these drugs (Song, Suzuki, Kawai, & Sugiyama, 1999).

Anticancer Agents	Opioids
Doxorubicin	Loperamide
Docetaxel	Morphine
Vincristine	
Vinblastine	Cardiac drugs
Etoposide	Digoxin
Mitoxantrone	Diltiazem
Actinomycin D	Verapamil
	Talinolol
Steroid Hormones	
Aldosterone	Immunosuppressants
Cortisol	Cyclosporine
Dexamethasone	Tacrolimus
Methylprednisolone	
	Miscellaneous
Antibiotics	Ivermectin
Erthromycin	Amitryptiline
Ketaconazole	Terfenadine
Itraconazole	Ondansetron
Tetracycline	Domperidone
Doxycycline	Phenothiazines
Levofloxacin	Vercuronium
Sparfloxacin	Moxidectin
	Selamectin

Table1.3: Selected P-gp substrates (Martinez et al., 2008).

This table shows a list of all published P-gp substrates.

*MDR1-1*Δ Mutation in Dogs

Ivermectin is a semisynthetic lactone in the avermectin family and a commonly used antiparasitic in veterinary medicine. It is frequently used to treat and control nematode and arthropod parasites. Ivermectin causes tonic paralysis in invertebrate organisms by potentiating glutamate-gated chloride channels and/or gamma amino butyric acid (GABA)-gated chloride channels of the peripheral nervous system (Mealey, Bentjen, Gay, & Cantor, 2001). Mammals are protected from the toxic effects of ivermectin as the blood brain barrier prevents access of ivermectin to the central nervous system. The GABA receptors in mammals are restricted to within the central nervous system. Thus mammals are protected from the neurologic effects of ivermectin (Mealey et al., 2001).

However a subpopulation of rough coated collies were shown to be extremely sensitive to neurotoxic effects of ivermectin (Paul, Tranquilli, Seward, Todd Jr, & DiPietro, 1987). Despite numerous studies and investigations the mechanism for ivermectin sensitivity in collies remained unknown. It was hypothesized that since the *MDR1* knockout mice are sensitive to ivermectin toxicity due to lack of P-gp, a similar defect in the *MDR1* gene may be responsible for the ivermectin sensitivity in some collies (Roulet et al., 2003).

In 2001, Mealey reported a 4 base pair deletion mutation on the *MDR1* gene which was associated with the ivermectin sensitivity in collies (Mealey et al., 2001). This 4 base pair deletion causes a frame shift in the mRNA reading frame, generating premature stop codons that result in premature termination of P-gp translation and a severely truncated protein. According to the amino acid sequence, the synthesized P-gp is less than one-tenth its original size (Mealey et al., 2001). The normal functioning of P-gp depends on the ATP binding site, substrate binding site, phosphorylation site and multiple membrane spanning proteins (Figure: 1.4). Since none of these sites are present in the truncated P-gp, it can be assumed that the functional form of P-gp does not exist in dogs homozygous for this mutation (Roulet et al., 2003).

The specific cause of the mutation remains unknown. However, Mealey reported a palindromic sequence (GGTTTTTGG) nine bases upstream of the *MDR1* 4 base pair deletion site (Mealey et al., 2001) (Figure: 1.3). Palindromic DNA sequences are made up of nucleic acids within the DNA double helix that is the same

when read from 5' to 3' on one strand and 3' to 5' on the other complementary strand. These are unusual DNA structures which promote genetic instability (Lewis, AkgÜN, & Jasin, 1999). Unusual DNA structures like these can cause the DNA polymerase to pause and disrupt DNA replication. Mutations can occur in both the palindromic sequences and also in the vicinity of the palindrome. The identification of a palindromic sequence in close proximity to the *MDR1*-1Δ mutation may in fact be the cause of the mutation (Mealey et al., 2001).

The inheritance pattern of *MDR1*-1Δ is found to be consistent with autosomal recessive inheritance pattern (Mealey et al., 2001). The collies that are homozygous for the mutation are sensitive to ivermectin toxicity while the heterozygous genotype collies are not sensitive to ivermectin neurotoxicosis (Mealey et al., 2001).

```
275 TGGTTTTTGGAAACATGACAGATAGCTTTGCAAATGCAGGAATTTCAAGAAACAAAACTTTT 336
    TGGTTTTTGGAAACATGAC----AGCTTTGCAAATGCAGGAATTTCAAGAAACAAAACTTTT

337 CCAGTTATAATTAATGAAAGTATTACGAACAATACACAACATTTCATCAACCATCTGGAGGA 398
    CCAGTTATAATTAATGAAAGTATTACGAACAATACACAACATTTCATCAACCATCTGGAGGA

399 GGAAATGACCACGTATGCCTATTATTACAGTGGGATCGGTGCTGGCGTGCTGGTGGCTGCTT 460
    GGAAATGACCACGTATGCCTATTATTACAGTGGGATCGGTGCTGGCGTGCTGGTGGCTGCTT

461 ACATCCAGGTTTCATTCTGGTGCCTGGCAGCAGGAAGACAGATACTCAAAATTAGAAAACAA 522
    ACATCCAGGTTTCATTCTGGTGCCTGGCAGCAGGAAGACAGATACTCAAAATTAGAAAACAA

523 TTTTTTCATGCTATCATGCGACAGGAGATTGGCTGGTTTGACGTGCATGACGTTGGGGAGCT 584
    TTTTTTCATGCAATCATGCGACAGGAGATTGGCTGGTTTGACGTGCATGACGTTGGGGAGCT

585 TAACACCCGGCTCACAGACGATGTCTCCAAAATCAATGAAGGAATTGGCGACAAAGTTGGAA 646
    TAACACCCGGCTCACAGACGATGTCTCCAAAATCAATGAAGGAATTGGCGACAAAATTGGAA

647 TGTTCTTTCAATCAATAGCAACATTTTTCACCCGGTTTTATAGTGGGGGTTTACACGTGGTT 708
    TGTTCTTTCAATCAATAGCAACATTTTTCACCCGGTTTTATAGTGGGGGTTTACACGTGGTT
```

Figure 1.3: Partial (bases 275±708) sequence comparison of wild-type (top) and mutant (bottom) *MDR1* cDNAs.

A 4-base pair deletion is present in the mutant cDNA. The remainder of the *MDR1* cDNA sequence was similar for ivermectin-sensitive and non-sensitive collies. Codons in the vicinity of the deletion are indicated by brackets for both the wild-type and mutant cDNAs. Bold letters indicate stop codons created in the mutant cDNA as a result of the frame shift. The dashed box indicates the palindromic sequence in the vicinity of the deletion mutation (Mealey et al., 2001).

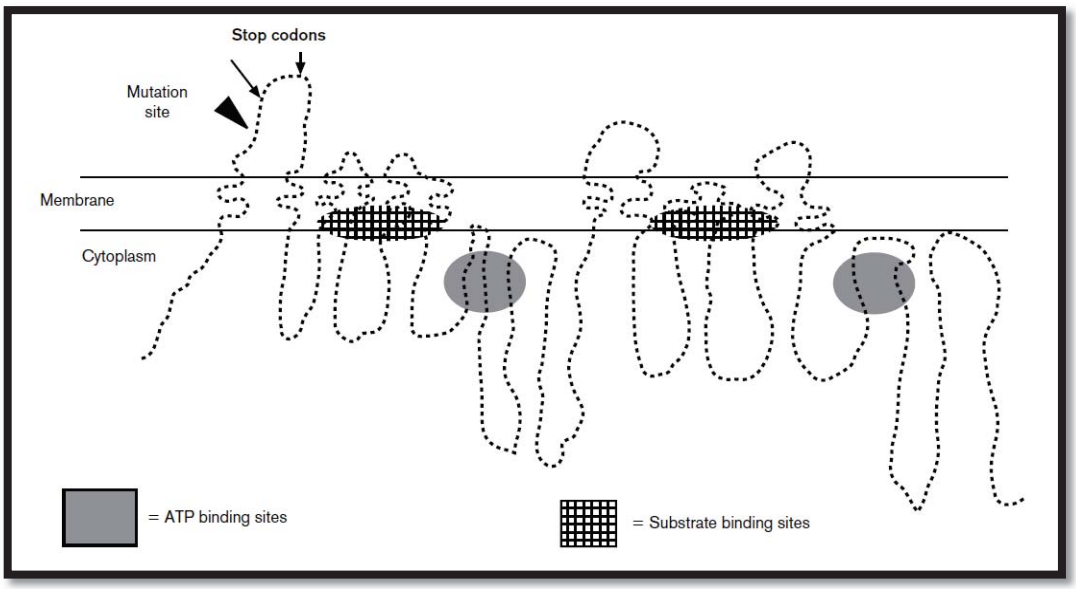

Figure 1.4: Diagrammatic representation of the transmembrane structure of P-glycoprotein (P-gp) (Mealey et al., 2001).

(modified from Gottesman and Pastan, 1993). The mutation site occurs at amino acid 75, resulting in a frame shift that generates several downstream stop codons, the first two of which occur at amino acid positions 91 and 111. More than 90% of the protein is predicted to be truncated in dogs homozygous for the mutant allele.

Breed Distribution of *MDR1-1*Δ mutation

The *MDR1*-1Δ mutation can be traced to one dog that lived in Great Britain in 1800s. Before 1870 there was no formal registry for sheep dogs and only regional varieties of working dogs that had adapted to the terrain that existed. With industrialization in the 19th century the role of working dogs in society diminished and this led to the disappearance of many local breeds of dogs. With the aim to preserve and restore the existing breeds of dogs, came the advent of dog shows. The first formal breeds to originate from working sheepdog populations were the collies, old English sheepdogs and Shetland sheepdogs. All of these herding dogs share the working collie lineage suggesting that the *MDR1-1*Δ allele must have been present in the collies since 1890. The *MDR1-1*Δ in old English sheepdogs, Australian shepherds, Shetland sheepdogs, English shepherds, Border collies and McNabs can be traced back to a single ancestral mutation that has been inherited by descent.

The *MDR1-1*Δ mutation has also been identified in 2 sighthound breeds: longhaired whippets and silken windhound. The Longhaired whippets are an ancient variety that was restored in the 1950s by a single breeder who also bred Shetland sheepdogs. The Longhaired whippets were interbred with Shetland sheepdogs for their longer coat. It has been speculated that the *MDR1-1*Δ allele accompanies the long hair phenotype allele. The silken windhound has been recently developed in 1980s by the crossing of various sighthounds such as the Borzoi, whippets and longhaired whippets. The silken windhound may have received the *MDR1*-1Δ mutation from the longhaired whippets (Neff et al., 2004).

A study in 2008 reported that the *MDR1-1*Δ mutation is most commonly observed in rough coated collies and Australian shepherds. The other breeds that exhibit the mutation are English shepherds, old English sheepdogs, Shetland sheepdog, longhaired whippet, silken windhound, McNab and very rarely Border collies. The diversity of haplotypes exhibited by the herding dogs (collies, old English sheepdogs, Australian shepherds, Shetland sheepdogs, English shepherds, Border collies and McNabs) in the collie lineage is consistent with prolonged segregation of *MDR1-1*Δ gene. This suggests that the *MDR1-1*Δ mutation in the 7 herding dog breeds has been present for many generations. However the longhaired whippets and the silken windhounds segregate only a few haplotypes suggesting a recent occurrence in these two breeds (Neff et al., 2004). A recent study identified the occurrence of *MDR1-1*Δ in German shepherds (Mealey & Meurs, 2008b). Unlike the other herding dogs and the two sighthounds, the ancestry of the German shepherd breed could not be traced to the working dogs in the collie lineage. The authors also noticed that most German shepherds carrying at least one allele were white-factored dogs, i.e. had either parents or grandparents had a white coat (Mealey & Meurs, 2008b). However whether the *MDR1-1*Δ mutation accompanies the white coat is still unknown (Mealey & Meurs, 2008b).

Drug Toxicities Associated with *MDR1-1*Δ Mutation

The *MDR1-1*Δ mutation is associated with many adverse drug reactions. Ivermectin sensitivity in collies has been recognized since the introduction of ivermectin in veterinary practice (Mealey, 2008). Studies show that dogs homozygous for *MDR1-1*Δ mutation develop adverse neurologic effects after single

dose of ivermectin of 120 µg/kg. Heterozygous and wild types do not show sensitivity to ivermectin at 120 µg/kg (Mealey, 2008). However heterozygotes may experience neurotoxicity if ivermectin is given at doses greater than 120 µg/kg and especially if given daily as per protocols for treatment of demodectic mange (Mealey, 2008). Dogs with *MDR1-1*Δ mutation are also so sensitivity to other macrocyclic lactones such as milbemycin, selamectin and moxidectin (Mealey, 2008).

Loperamide is an opioid antidiarrheal devoid of CNS activity. It is excluded from the brain by P-gp. However in dogs with *MDR1-1*Δ mutation loperamide enters the CNS causing neurotoxicity. Loperamide toxicity has been reported in a collie that had *MDR1-1*Δ homozygous genotype (Sartor, Bentjen, Trepanier, & Mealey, 2004). The dog received 0.14 mg/kg of loperamide, PO q12 and showed neurologic signs such as rear limb weakness, difficulty in holding the head up, vocalization, disorientation and ataxia (Sartor et al., 2004).

Digoxin toxicity has been documented in collies that are homozygous for the *MDR1*-1Δ mutation (Henik, Kellum, Bentjen, & Mealey, 2006). Deficiency of P-gp in the intestine and kidneys due to the *MDR1-1*Δ mutation in collies resulted in increased absorption and decreased excretion, respectively of digoxin. This resulted in high plasma concentrations of digoxin causing digoxin toxicity (Henik et al., 2006). The clearance of digoxin is primarily through renal excretion. Fifty percent of the digoxin excretion is through active tubular secretion. Since the P-gp is deficient in *MDR1-1*Δ homozygous dogs, it resulted in decreased urinary and increased serum digoxin concentrations (Henik et al., 2006).

A case report, published by Mealey in 2003 suggests that dogs with the *MDR1-1*Δ mutation are sensitive to toxic effects of doxorubicin and vincristine (Mealey, Northrup, & Bentjen, 2003). A 4 year old, rough coated collie was diagnosed with lymphoma and was treated with the modified University of Wisconsin canine lymphoma protocol. The protocol involved a combination of drugs such as vincristine (0.5 mg/m^2, IV), doxorubicin (30 mg/m^2, IV), prednisone, L-aparginase (400 U/kg, SC), cyclophosphamide (250 mg/m^2, IV), vinblastine (2 mg/m^2) and were given either alone or in combinations over 179 days. It was observed that throughout the treatment protocol the dog developed myelosuppression and gastrointestinal adverse effects whenever vincristine and doxorubicin were administered but did tolerate cyclophosphamide well. Doxorubicin and vincristine are known substrates of

P-gp. The alteration of P-gp functioning in these dogs may be responsible for delayed biliary and renal excretion resulting in toxicity.

*MDR1-1*Δ: Sedation and Anaesthesia

The non-functioning of the P-gp leads to increased exposure of the CNS, foetus, testes, epithelial cells and other tissues to toxic xenobiotics. Thus it has been hypothesized that P-gp deficient dogs have increased disease susceptibility. In 2007, Mealey published a study indicating that dogs with *MDR1-1*Δ mutation had relative adrenal insufficiency (RAI) and had a low basal cortisol concentration and low plasma cortisol concentration post ACTH stimulation test. The absence of the P-gp efflux pump in the BBB in dogs with *MDR1-1*Δ mutation results in increased concentration of endogenous corticosteroids in the hypothalamus causing suppression of the hypothylamus-pituitary access. This results in relative adrenal insufficiency in dogs homozygous to the *MDR1-1*Δ mutation.

Dogs carrying the *MDR1-1*Δ mutation are susceptible to toxicities from P-gp substrate drugs even at normal dosages. The non-functional P-gp allows high concentrations of P-gp substrate drugs due to increased oral absorption, delayed elimination from bile and urine and high concentrations of these drugs in the CNS, placenta and testis. Some anecdotal reports suggest that dogs homozygous to the *MDR1-1*Δ mutation show prolonged recovery following sedation and anaesthesia. Dogs carrying *MDR1-1*Δ mutation when sedated with acepromazine and butorphanol are anecdotally thought to have prolonged sedation and increased CNS depression (Mealey, 2006b).

Acepromazine and butorphanol are routinely used sedatives in veterinary practice as a part of an anaesthesia protocol or for sedation. Acepromazine belongs to the phenothiazine group of drugs which is a large group containing drugs with diverse chemical structures. Phenothiazines are found to be inducers, substrates as well as inhibitors of P-gp. The exact nature of association of acepromazine and P-gp is currently unknown. Butorphanol on the other hand is an opioid and hence a potential P-gp substrate. It asserts its primary effect on kappa receptors in the brain, spinal cord and sensory neurons. Acepromazine is used alone or in combination with butorphanol or other opioids in veterinary practice. If acepromazine is a P-gp substrate it may attain a high concentration in the CNS of dogs that have no

functional P-gp. The combination of acepromazine with butorphanol, which is a potential P-gp substrate or other P-gp substrates, may heavily sedate *MDR1-1*Δ homozygous mutants more than the normal dogs.

Aims of this study

This study has three goals. The first is to gain experience with the sedation scoring system that would allow comparison of different dogs given the same sedative. The second aim is to compare the sedation in dogs given a combination of dexmedetomidine and morphine to a combination of acepromazine and morphine. The third aim is to determine the effects *MDR1-1*Δ mutation on the level of sedation by acepromazine and a combination of acepromazine and butorphanol in rough coated collies assessed by using the validated sedation scoring system.

If acepromazine is a P-gp substrate, it can be hypothesized that acepromazine will cause prolonged sedation and more CNS depression in homozygous mutant dogs than normal dogs. If acepromazine is not a P-gp substrate the author does not expect to see any difference in the level of sedation between the three genotype groups. However butorphanol being an opioid is assumed to be a P-gp substrate. Thus the combination of acepromazine and butorphanol is hypothesized to cause maximum sedation in dogs that are homozygous mutants compared to the normal and the heterozygous mutant dogs.

Chapter II:

Material and Methods

2. Material and Methods:

2.1 Comparison of sedation in dogs following administration of dexmedetomidine plus morphine to acepromazine plus morphine

2.1.1 Animals

A total of 30 client owned male dogs weighing 5 to 50 kg of different ages, which were scheduled for routine orchidectomy, were enrolled in the study. The control group of 15 dogs received acepromazine and morphine in their premedication while the treatment group of 15 dogs received dexmedetomidine and morphine in their premedication. The study was approved by the Massey University Animal Ethics Committee - MUAEC Protocol 13/19.

2.1.2 Pre-surgical work up

The dogs had a thorough physical examination including, packed cell volume and total proteins (PCV/TP) performed as a part of pre-surgical work up. The PCV/TP of all 30 dogs was within the normal published reference range (Ettinger & Feldman, 2010). The PCV was performed by using micro-hematocrit tubes, centrifuged for 5 minutes at 3000 rpm and measured by using a PCV graph. The total serum protein was obtained by using a refractometer. The dogs were admitted the day before surgery at the Massey University Veterinary Teaching Hospital. The dogs were fed the evening before the surgery and fasted overnight. They had free access to water until the time of sedation. Prior to sedation all dogs were sedation scored by the same observer.

2.1.3 Sedation Scoring

On the day of surgery, treatment dogs (DEX) were randomly selected by tossing a coin, to have intramuscular injections (IM) of dexmedetomidine (Zoetis Animal Health New Zealand Limited) at 125 μg/m^2 and morphine (Hospira Australia PTY Ltd, Mulgrave, VIC Australia) at 0.5 mg/kg while control dogs (ACE) received acepromazine (0.04 mg/kg) and morphine (0.5 mg/kg) IM. The test drug combination was administered 30 minutes prior to induction of anaesthesia. The dogs were

sedation scored by an observer (DD) who was blinded to the genotypes and closely observed till induction. The dogs were sedation scored at 0 , 10 , 20 and 30 minute intervals following injection of the premedication agents.

2.1.4 Anaesthesia and Surgery protocol

Thirty minutes after administration of the premedication agent, anaesthesia was induced with propofol (Norbrook NZ Ltd) and maintained by inhalant isoflurane (Bayer New Zealand Limited) and medical oxygen as per anaesthesia protocols followed at Massey University Veterinary Teaching Hospital (MUVTH). All dogs received intra-operative fluid therapy with lactated ringer's solution (Baxter Healthcare PTY, NSW, Australia) at a dose of 10 mg/ml. All dogs were monitored intra-operatively by SurgiVet V900 Advisor Vital Signs Multi-Parameter Monitor and the heart rate, respiratory rate, mean arterial blood pressure, temperature, oxygen saturation, end tidal carbon dioxide, isoflurane concentration were measured. The orchidectomy was performed as per MUVTH protocol using a prescrotal approach.

2.1.5 Recovery

Post surgical pain scoring was performed and the dogs were monitored till recovery. All dogs received a combination of buprenorphine and a non-steroidal anti-inflammatory agent as part of postoperative pain management. The dogs were closely monitored by well trained staff till recovery. During the study none of the dogs received antisedan (atipamizole), an alpha-2-adrenegic agonist reversal agent. Atipamizole reverses the sedation and cardiovascular effects of dexmedetomidine and may be used in cases of profound cardiovascular depression. All the dogs were observed overnight at the Massey University Veterinary Teaching Hospital and were monitored for any complications.

2.1.6 Sedation System

The sedation scoring system used in the *MDR1* sedation study and the dexmedetomidine sedation study was the same (Table: 3.1). Sedation scores for each dog were recorded by the same observer (DD). The sedation scale was a modification of the scale used by Hofmeister (Hofmeister et al., 2010). The sedation

scoring system was modified from the original scale used by Hofmeister in 2010 by adding gait as the 7th parameter and scoring posture one minute after assessing the gait. The scale scores sedation based on 7 parameters which are vocalization, appearance, interactive behaviour, restraint, response to noise, gait and posture.

A. Vocalization

This parameter assesses anxiety in a dog. Excessive vocalization maybe observed in dogs with dysphoria or delirium. Depending on their degree of anxiety and excitement they were scored quiet (0); whining softly but soothes on touch (-1); whining continuously (-2); barking continuously (-3).

B. Appearance

The demeanour of the animal and the appearance of the eyes were taken into consideration while scoring the appearance parameter. The appearance is scored as follows: Eyes sunken/glazed/unfocused/ventromedial rotation (3); eyes glazed but follow movement (2); protrusion of nictitating membrane with normal visual responses (1); normal appearance (0); pupils dilated with abnormal facial expression (-1).

C. Interactive Behaviour

The interactive behaviour of the dog was scored based on the response of the animal to voice and touch of the observer. The dogs were scored as follows: Recumbent with no response to voice or touch (3); recumbent but lifts head in response to voice or touch (2); recumbent but stands in response to voice or touch (1); moves towards or away from voice or touch and appears anxious (0); wags tail/excited/growls or hisses when approached or touched (-1); Very excited/jumps/bites/ snaps when approached (-2).

D. Response to sound

Response to sound was assessed by the response of the dog to a clap. The dogs were scored as follows: no response to clap (3); minimal response to clap (2); slow response to clap (1); brisk response to clap (0).

E. Restraint during instrumentation

Restraint during instrumentation was assessed while restraining the dogs for blood pressure, heart rate and respiratory rate recordings. During the sedation trial assessing the difference between dexmedetomidine and acepromazine, blood pressure, heart rate and respiratory rate were not noted prior to induction of anaesthesia. Hence during the dexmedetomidine trial or at time intervals when the blood pressure, heart rate and the respiratory rate readings were not to be noted the dogs were restrained in the same position for instrumentation and scored. The dogs were scored as follows: lies on the floor and no restraint required (2); lies on the floor with light restraint on head or neck (1); sits up on the floor with light restraint (0); requires regular correction (-1); struggles continuously against restraint (-2).

F. Gait

The gait was assessed by walking the dogs on leash in the same room. The dogs were scored as follows: unable to get up (5); sits up on the floor but unable to stand/falls when attempts to stand (4); gait very unsteady/standing but ataxic (3); gait very unsteady (2); mild aberrations in gait/wobbly (1); walks normally (0).

G. Posture

The posture was assessed one minute after assessing the gait. The dogs were scored as follows: lateral recumbence (3); sternal recumbence (2); sitting or ataxic while standing (1); standing (0); moving continuously (-1).

The sedation scores for each parameter were determined and a total sedation score was obtained. The least possible score on the sedation scale was -9 and the maximum sedation score on the scale was 19, with -9 being very anxious dogs and 19 being heavily sedated dogs.

Observation	Description	Score
Vocalization	Quiet	0
	Whining softly but quiets with soothing touch	-1
	Whining continuously	-2
	Barking Continuously	-3
Appearance	Eyes sunken, glazed or unfocused; ventromedial rotation	3
	Eyes glazed but follow movement	2
	Protrusion of nictitating membrane; normal visual responses	1
	Normal Appearance	0
	Pupils dilated; Abnormal facial expression	-1
Interactive	Recumbent; no response to voice or touch	3
Behaviour	Recumbent; lifts head in response to voice or touch	2
	Recumbent but stands in response to voice or touch	1
	Moves towards/ away from voice or touch; appears anxious	0
	Wags tail/ excited/Growls or hisses when approached or touched	-1
	Very excited/ jumps/Bites or swats when approached	-2
Restraint	Lies on floor ,no restraint required	2
during	Lies on floor with light restraint of head or neck	1
instrumentation	Sits up on floor; with light restraint	0
	Requires regular correction	-1
	Struggles continuously against restraint	-2
Response	No response to whistle	3
to Noise	Minimal response to whistle	2
	Slow or moderate response to whistle	1
	Brisk response to whistle; raises head with eyes open	0
Gait	Unable to get up	5
	Sits up on the floor but unable to stand, falls when attempts to stand	4
	Gait very unsteady, standing but ataxic	3
	Gait clearly unsteady	2
	Mild aberrations in gait, wobbly	1
	Walks normally	0
Posture	Lateral Recumbence	3
(1 min after	Sternal Recumbence	2
Assessing	Sitting or ataxic while standing	1
gait)	Standing	0
	Moving continuously	-1

Table 2.1: Sedation Scoring System, modified from (Hofmeister et al., 2010)

2.2 Pharmacogenetic effects of *MDR1-1*Δ mutation on sedation of rough coated collies with acepromazine and a combination of acepromazine and butorphanol.

The following studies were conducted at Massey University Veterinary Teaching Hospital, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, New Zealand from Jan 2013 to Feb 2014.

2.2.1 Animals

Thirty-one rough coated collies, 15 males and 16 females were genotyped. Out of these 31 dogs that were genotyped in the study, only 29 were enrolled in the sedation trial. The inclusion criteria for the enrolment of the dogs in the study were: purebred rough coated collie dogs, dogs aged between 1-10years, healthy based on history, Complete Blood Count, serum chemistry, Urine Analysis and physical exam. The exclusion criteria for dogs in the study were: dogs not able to be handled or injected, anxious dogs, dogs not deemed healthy based on history, Complete Blood Count, serum chemistry, Urine Analysis and physical exam. Two dogs out of 31 were disqualified from the study as one of them was too old for the study (11 years) and the other was diagnosed with renal insufficiency.

All dogs in this study were client owned dogs, residing in or near Palmerston North, New Zealand. The study was approved by the Massey University Animal Ethics Committee - MUAEC Protocol 12/63. All owners provided informed consents prior to enrolling their dogs in the study.

2.2.2 Buccal Swabs

Thirty-one rough coated collies were genotyped for the *MDR1-1*Δ mutation by obtaining buccal swabs from each dog. The cheek swab collection brushes and sample collection instructions were provided by the Veterinary Clinical Pharmacology Laboratory (VCPL), Washington State University, USA.

The cheek swabs were collected as per the instructions in the *MDR1* test kit from VPCL. Two brushes were used per dog and the samples were obtained as follows: The inner surface of the upper lip was rolled outwards by inserting a finger at

the corner of the mouth and pulling the lip upwards. Each brush was held between the thumb and the forefinger and the bristles of the brush were then placed on the inner surface of the cheek, releasing the lip and leaving the brush inside the mouth. The dog's mouth was closed with gentle pressure on the muzzle. The brush was then gently moved back and forth at a short distance between the inner lip and the gums. The brush was twirled gently as it is moved back and forth brush against the surface of the cheek scrapping off some superficial cheek cells. The brushing was continued for 20 seconds with gentle pressure. The same process was repeated on the other cheek with the second cheek brush. Each brush was packed in packaging provided for the brush. The cheek swabs were labelled with the name of the dog and name of the owner. All 31 samples along with their submission forms, name, age, sex of the dog, were shipped to Veterinary Clinical Pharmacology Laboratory, Washington State University, Pullman, WA 99164, USA. These samples were packaged and shipped as per the USDA Guidelines for Importation #1102.

2.2.3 Genotyping

The *MDR1-1*Δ mutation was determined from the buccal swabs at the Veterinary Clinical Pharmacology Laboratory, Washington State University, USA. Polymerase chain reaction (PCR) was performed pursuant to an agreement with Roche Molecular Systems, Incorporation for genotyping the dogs. (USA patents 6,790,621 and 7,393,643; Australian patent 2002249946; European patent 1389240)

Thirty-one dogs were genotyped for the *MDR1-1*Δ mutation. However 2 of the 31 were disqualified from the study, as one was too old and the other was diagnosed with renal insufficiency. The remaining twenty-nine rough coated collies enrolled in the study were divided in 3 groups based on their genotypes. The numbers of dogs in each genotype group were: 10 homozygous mutants (MUT/MUT), 10 heterozygous carrier (MUT/N) and 9 normal/normal (N/N).

2.2.4 Pre-sedation work up

Prior to the sedation scoring trial, all 31 dogs had a pre-sedation work up which involved taking a clinical history from the owner of the dog, followed by blood tests, urine analysis and physical examination of all the dogs.

A. History

A thorough recent and past history of the dogs was obtained from the owners. The parentages of the dogs were noted to identify if any dogs from the study were related and thus share the same genetics profile. Since dogs with *MDR1-1*Δ mutation are predisposed to certain diseases such as relative adrenal insufficiency (Mealey, Gay, Martin, & Waiting, 2007). A detailed history of these dogs regarding previous history of adrenal insufficiency such as vomiting, diarrhoea, lethargy, waxing and waning of disease was recorded.

B. Blood Testing

Complete blood count (CBC) and serum biochemistry profile was performed by the New Zealand Veterinary Pathology (NZVP) laboratory at Massey University. CBC and biochemistry was performed using the Roche Sysmex and Roche modular P-800, respectively. The electrolytes were performed using Roche modular ISE 900. The CBC was performed to rule out abnormalities in the red blood cells (RBC), white blood cells (WBC) and platelets. The serum biochemistry profile included glucose, blood urea nitrogen, creatinine, serum total protein, serum albumin, serum alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. The serum biochemistry profile was performed specifically to assess the functioning of the liver and kidneys and rule out concurrent diseases. Since the liver and kidneys play an important role in the metabolism of drugs the normal function of the liver and kidney was crucial for the study. The values provided by the pathology laboratory were considered as the reference range while interpreting the blood chemistry values.

C. Urine analysis

Free catch mid stream urine samples were collected from the dogs and were analyzed. The urine analysis involved assessing the urine specific gravity and urine dipstick analysis and all dogs in the study had normal urine analyses.

D. Physical Examination

All dogs enrolled in the study had a physical examination during the pre-sedation work up and were assessed for any clinical abnormalities. Rectal temperature of all dogs was recorded. Auscultation of the lungs and the heart for any cardio-respiratory abnormality was performed. Palpation of the abdomen for any abnormality was also performed. The demeanour and the temperament of the dogs were assessed prior to the sedation trial. All dogs that were enrolled in the study had normal physical examination.

Out of the 31 dogs that were genotyped, 2 dogs were excluded from the study as one was too old, 11 years, and the other was diagnosed with renal insufficiency. For the remaining 29 dogs where the presedation workup suggested they were healthy and hence enrolled in the sedation scoring trial.

2.2.5 Sedation Scoring

A. Sedation scoring following administration of acepromazine alone

The sedation scoring trial was performed in a consult room with not more than four dogs at once. The dogs were fasted overnight but had access to water throughout the study. The dogs were allowed to acclimatize to the environment in the consult room for two hours prior to sedation. The sedation scoring system used in this study is the same as the one used in the dexmedetomidine trial (Table 2.1). During these two hours of acclimatisation the dog's level of sedation was scored at 0, 30 minutes, 1 and 2 hour intervals. The heart rate, respiratory rate and mean arterial blood pressure were measured at 0 , 1 and 2 hour intervals during the acclimatisation period. The blood pressure was monitored using a non invasive blood pressure monitor SurgiVet V900 Advisor Vital Signs Multi-Parameter Monitor. At the end of 2 hours of acclimatisation each dog was injected with acepromazine (Delvet Pty Ltd NSW Australia) at 0.04 mg/kg Intravenous (IV). Acepromazine was diluted with 0.5 ml of normal saline 0.9% and given slow IV into the cephalic vein. The IV administration was confirmed by drawing back blood in the syringe before injecting acepromazine. The dogs were then scored at 30 minutes, 60 minutes, 90 minutes and 2, 2.5, 3, 4 and 6 hour intervals according to the sedation score criteria.

All the dogs were taken out for a walk at 3 hours after sedation. The dogs were sedation scored at all time intervals by the same observer (DD) who was blinded to the genotypes of the dogs. Heart rate, respiratory rate and blood pressure were recorded at 30 minutes, 60 minutes, 90 minutes, 2 hours, 2.5 hours, 3 hours, 4 hours and 6 hours intervals.

All dogs were given 2 hours of acclimation to reduce the error due to individual personalities on the sedation scoring. The sedation score at the end of two hours was considered the baseline sedation for that individual dog. The sedation scores were recalculated by subtracting the sedation score at the end of 2 hours of acclimatization period from the sedation scores at 0 hours, 30 minutes, 1 , 1.5 , 2 , 2.5 , 3 , 4 and 6 hour intervals following administration of acepromazine. The recalculated sedation score (R) was the sedation score used to compare the sedation scores between the 3 genotypes: homozygous mutants, heterozygous carriers and normal dogs.

B. Sedation scoring following administration of a combination of acepromazine and butorphanol

A minimum of 7 days wash out period for the dogs was allowed between the two trials:

i. Sedation scoring following administration of acepromazine alone.
ii. Sedation scoring following administration of a combination of acepromazine and butorphanol

Out of the 29 rough coated collies enrolled in the previous study a subset of 12 dogs were enrolled in the second study. Since the dogs were client owned only 12 of the 29 clients were willing to permit second sedation for the second study. Hence a total of 12 dogs, 4 in each of the three different genotype groups, were enrolled in the study. Thus there were 4 dogs in each genotype group: mutant/mutant; mutant/normal and normal/normal.

The sedation scoring trial was performed in a consult room with not more than four dogs at once. The dogs were fasted overnight but had access to water throughout the study. The dogs were allowed to acclimatise to the environment in the consult room for two hours prior to sedation. The sedation scoring system used in

this study is the same as the one used in the dexmedetomidine trial (Table 2.1). During these two hours of acclimatisation the dog's level of sedation was scored at 0, 30 minutes, 1 and 2 hour intervals. The heart rate, respiratory rate and mean arterial blood pressure were measured at 0, 1 and 2 hours interval during the acclimatisation period. The blood pressure was monitored using a non invasive blood pressure monitor SurgiVet V900 Advisor Vital Signs Multi-Parameter Monitor. At the end of 2 hours of acclimatization each dog was injected intravenously with acepromazine (Delvet Pty Ltd NSW Australia) at 0.04 mg/kg and butorphanol at 0.05 mg/kg. Acepromazine was diluted with 0.5 ml of normal saline 0.9% and given slow IV into the cephalic vein. The dogs were then sedation scored at 30 minutes, 60 minutes, 90 minutes and 2, 2.5, 3, 4 and 6 hour intervals. All the dogs were taken out for a walk at 3 hours after sedation. The dogs were sedation scored at all time intervals by the same observer (DD) who was blinded to the genotypes of the dogs. Heart rate, respiratory rate and blood pressure were recorded at 30 minutes, 60 minutes, 90 minutes, 2 , 2.5 , 3 , 4 and 6 hour intervals

All dogs were given 2 hours of acclimation to reduce the error due to individual personalities on the sedation scoring. The sedation score at the end of two hours was considered the baseline sedation for that individual dog. The sedation scores were recalculated by subtracting the sedation score at the end of 2 hours of acclimatization period from the sedation scores at 0 hours, 30 minutes, 1 , 1.5 , 2 , 2.5 , 3 , 4 and 6 hour intervals following administration of acepromazine. The recalculated sedation score (R) was the sedation score used to compare the sedation scores between the 3 genotypes: homozygous mutants, heterozygous carriers and normal dogs.

2.3 Statistical Analysis:

A. Comparison of sedation in dogs following administration of dexmedetomidine plus morphine to acepromazine plus morphine

Statistical analysis was performed using Minitab 16 computer software. The data of 30 dogs was available, 15 dogs premedicated with dexmedetomidine (125 $\mu g/m^2$) and morphine (0.5 mg/kg) (DEX) and 15 dogs premedicated with acepromazine (0.04 mg/kg) and morphine (0.5 mg/kg) (ACE). The sedation scores of

the 30 dogs scored at 0, 10, 20 and 30 minutes interval post premedication was available. Mann-Whitney test was used to determine the difference between sedation scores in DEX and ACE group at 0, 10, 20 and 30 minutes interval. Bonferroni statistical correction was performed while comparing the sedation scores between DEX and ACE group at 0, 10, 20 and 30 minutes interval. Statistical significance was defined as $p<0.05$. Descriptive data was presented as mean ± SD.

B. Pharmacogenetic effects of MDR1-1Δ on the level of sedation following administration of acepromazine alone.

The sedation score at the end of 2 hour acclimatisation prior to injection of acepromazine, was considered the base sedation score for that particular dog. All the sedation scores were recalculated (R) by subtracting the sedation score at 2 hours of acclimatization from the respective sedation scores at 30 minutes, 1, 1.5, 2, 2.5, 3, 4 and 6 hours interval. Commercially available software was used to assess the data by construction of a linear mixed-effects model. A linear mixed effect model was used with genotypes considered as fixed effects and the 29 dogs as random effects. Age, sex, bodyweight and body condition score were the covariates. Descriptive data was obtained as mean ± SD. Area Under Curve (AUC) of the sedation score was calculated for each of the 29 dogs. A two-sample Test was performed to compare the AUC between the 3 different genotypes: homozygous mutants (MUT/MUT), heterozygous carriers (MUT/N) and normal (N/N).

C. Pharmacogenetic effects of MDR1-1Δ on the level of sedation following administration of combination of acepromazine and butorphanol.

The sedation score at 2 hours of acclimatizing period prior to injecting combination of acepromazine and butorphanol was considered the base sedation score for that particular dog. All the sedation scores were recalculated (R) by subtracting the sedation score at 2 hours of acclimatization from the respective sedation scores at 30 minutes, 1, 1.5, 2 hours, 2.5 hours, 3 hours, 4 hours and 6 hours interval. Commercially available software was used to assess the data by construction of a linear mixed-effects model. A linear mixed effect model was used with genotypes considered as fixed effects and the 29 dogs as random effects. Age, sex, bodyweight and body condition score were the covariates. Descriptive statistics was obtained as mean ± SD.

Chapter III:

Dexmedetomidine Sedation trial

3. Results:

Comparison of sedation in dogs following administration of dexmedetomidine plus morphine to acepromazine plus morphine

Thirty healthy, ASA 1 (American Society of Anaesthesiologists physical status classification system), male dogs of various breeds were enrolled in the study (Tables: 3.2 & 3.3). The dogs were randomly divided in two groups. The DEX group received a combination of dexmedetomidine (125 μg/m^2) and morphine (0.5 mg/kg) and the ACE group received a combination of acepromazine (0.04 mg/kg) and morphine (0.5 mg/kg). The dose of dexmedetomidine (125 μg/m^2) was as per the manufacturer's recommendation whereas the dose of acepromazine (0.04 mg/kg) was based on standard published premedication recommendations (Bednarski et al., 2011). Both groups received the same dose of morphine. The mean (± SD) age of the dogs in the DEX group and ACE group was 1.73 yrs ± 1.7 and 2.19 yrs ± 2.15 respectively. The mean (± SD) weight of the dogs in DEX group and ACE group was 22.17 kg ± 11.98 and 21.79 kg ± 14.78 respectively (Table: 3.1). Various breeds enrolled in the study are listed in Tables 3.2 & 3.3.

The DEX and ACE dogs were sedation scored at 0, 10, 20 and 30 minute intervals post premedication with dexmedetomidine and acepromazine respectively. The sedation score in both the DEX and the ACE group increased with time. The median sedation scores of DEX and ACE groups at 10 minutes post premedication were 11 and 9 respectively. The median sedation scores of DEX and ACE groups at 20 minutes post premedication were 18 and 11 respectively. The median sedation scores of DEX and ACE groups at 30 minutes post premedication were 19 and 12 respectively. At 30 minutes the median sedation score of DEX group was significantly higher than ACE group (p =0.0189) (Figure: 3.1). The heart rate, respiratory rate and systolic arterial blood pressure did not vary between the DEX and the ACE group (p>0.05). A boxplot was plotted comparing the heart rate and systolic arterial pressure for the first 20 minutes post induction of the dogs with propofol (Figures: 3.2 & 3.3). Heart rate and systolic arterial pressure did not vary between the DEX and the ACE group (p>0.05).

	DEX	ACE
Age (years)	1.73 ± 1.7	2.19 ± 2.15
Body weight (kg)	22.17 ± 11.98	21.79 ± 14.78

Table 3.1: Mean + SD Age (years) and Bodyweight (Kg) of dogs in DEX and ACE groups.

Breed	Number of Dogs in DEX group
Fox terrier-Chihuahua	1
Golden retriever	1
Poodle X	1
English pointer	1
Labrador retriever	1
Shitzu X	1
Doberman	1
German shepherd	1
Blue heeler X	1
Greyhound	1
Labrador retriver X Staffordshire bull terrier	1
Border collie	1
Cavalier king charles spaniel	1
Labrador X	1
Longhaired standard daschund	1

Table 3.2: Breeds of dogs enrolled in DEX groups.

Fifteen dogs were enrolled in the DEX group belonging to the above listed breeds.

X= Crossbreed

Breed	Number of Dogs in ACE Group
Labrador retriever	1
German shepherd	1
Greyhound	1
Labrador retriever X Staffordshire bull terrier	1
Border collie	1
Rotweiler	1
Jack russel X	1
Cavalier king charles spaniel	1
Tibetan spaniel X	1
Leonburger	1
Bull mastiff X Staffordshire bull terrier	1
Retriever X	1
Griffon	1
Boxer	1
Spaniel	1

Table 3.3: Breeds of dogs enrolled in ACE groups.

Fifteen dogs were enrolled in the ACE group belonging to the above listed breeds.

X= crossbreed

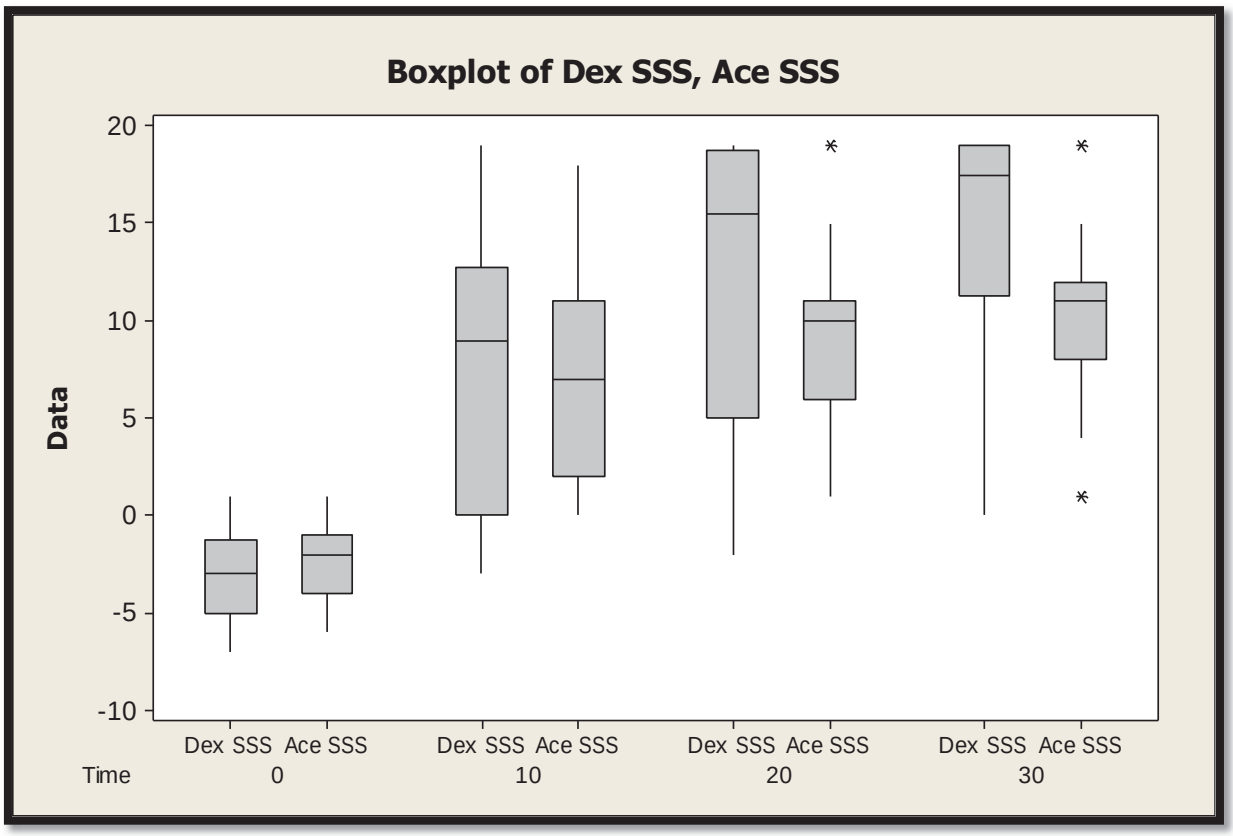

Figure 3.1: Comparison of sedation scores between dogs premedicated with dexmedetomidine (DEX) and acepromazine (ACE).

X axis represents the sedation score. Y axis represents time in minutes. Dex SSS: Sedation scores of dogs in DEX group, Ace SSS: Sedation scores of dogs in ACE group. Sedation scores of both groups – DEX and ACE at 0 minutes, 10 minutes, 20 minutes and 30 minutes increased with time. At 30 minutes the median sedation score of the DEX group was significantly higher than the ACE group (*p* value =0.0189).

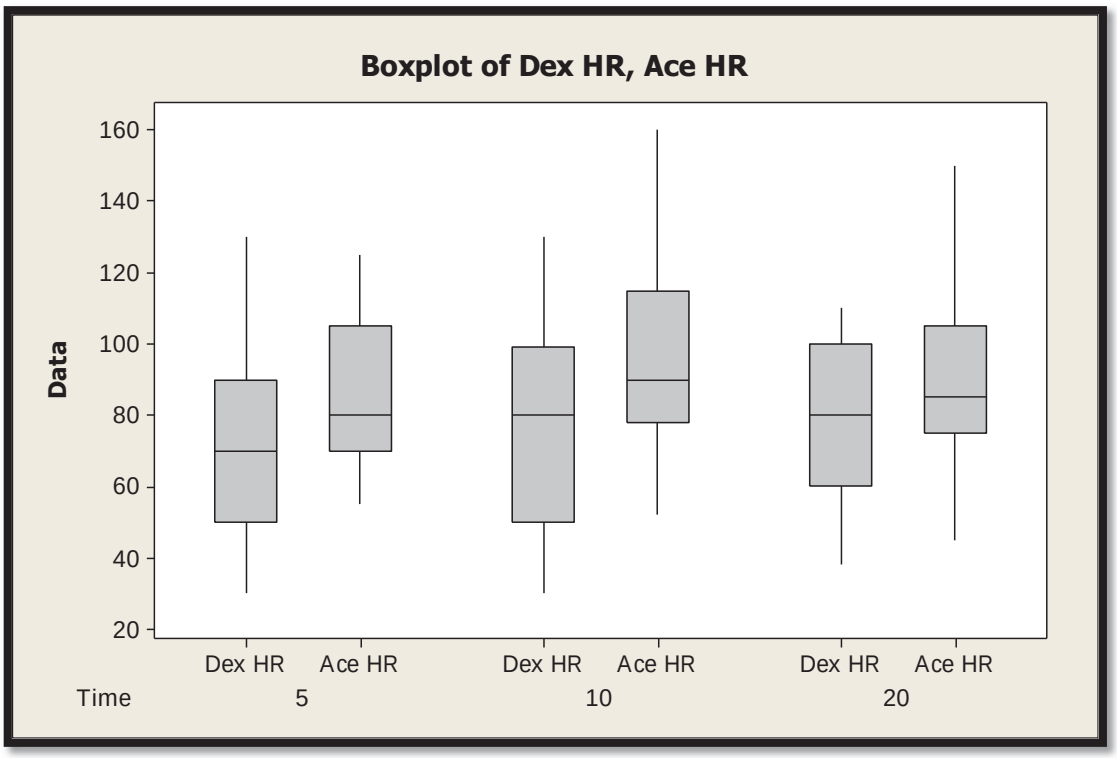

Figure 3.2: Comparison of heart rates (HR) between dogs in DEX and ACE group following induction with propofol.

X axis represents heart rate. Y axis represents time in minutes. Dex HR: Heart rates of dogs in DEX group, Ace HR: Heart rates of dogs in ACE group. There is no statistical difference between the heart rates of the DEX and ACE group during the 20 minutes after induction.

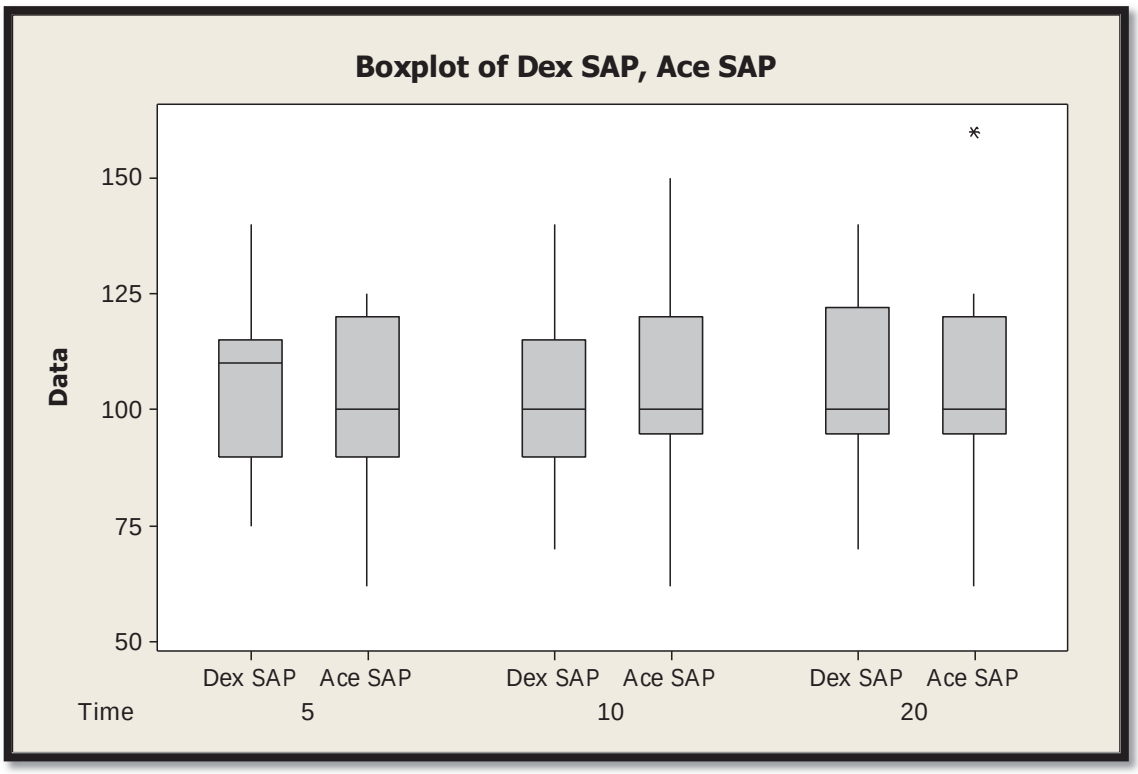

Figure 3.3: Comparison of systolic arterial blood pressure (SAP) between dogs in DEX and ACE following induction with propofol.

X axis represents sedation score. Y axis represents time in minutes. Dex SAP: systolic arterial blood pressure of dogs in DEX group. Ace SAP: systolic arterial blood pressure of dogs in ACE group. There is no statistical difference between the mean arterial blood pressures of the DEX and ACE group during the 20 minutes after induction.

Chapter IV:

Results

4. Results:

4.1 Pharmacogenetic effects of *MDR1-1*Δ mutation on sedation of rough coated collies with acepromazine .

As described in the Methods chapter 29 rough coated collies were enrolled in the study and were divided in 3 groups based on their genotypes homozygous mutant (MUT/MUT), heterozygous carrier (MUT/N) and homozygous normal (N/N) (Table: 4.2).

As described in the Methods chapter, all dogs had a 2 hour acclimation period prior to dosing with acepromazine. The sedation scores of all 29 rough coated collies increased during the acclimation period (Figure: 4.1). The 29 dogs were sedation scored at 0 , 0.5 , 1, 1.5, 2, 2.5, 3, 4 and 6 hour intervals following IV dosing of acepromazine. The sedation scores at each of these time intervals were recalculated (R) by subtracting the sedation score after the 2 hours acclimation period. The sedation scores of individual dogs are plotted in Figure 4.2 and the medians of the recalculated (R) sedation scores are then plotted in a graph (Figure: 4.3). The dogs that were homozygous mutants (MUT/MUT) had higher sedation scores than the heterozygous carriers (MUT/N) and homozygous normal (N/N) over the six hour time intervals (p=0.0176). The sedation scores of heterozygous mutants (MUT/N) were slightly more than the normal (N/N) group over the time intervals measured (p=0.0512).

The median heart rate, respiratory rate and mean arterial blood pressure for MUT/MUT, MUT/N and N/N groups are plotted over the time intervals (Figures: 4.4 - 4.6). No statistical correlation was observed between the heart rates, respiratory rates and mean arterial blood pressure in relation to the genotypes (p>0.05). Age, body weight, body condition score and sex were covariates. No statistical correlation was observed between the covariates and the sedation scores of individual genotypes (p>0.05).

The Area Under the Curve (AUC) for each dog was calculated using the recalculated (R) sedation scores. A two sample T-Test was performed to compare the AUC between the three genotypes. The AUCs of homozygous mutant (MUT/MUT) and heterozygous carrier (MUT/N) were significantly higher than the normal group (N/N) (p=0.014 and p=0.049 respectively)

Count	Genotype	Dog Name	Breed	Age (years)	Sex
1		Briar	Rough Coated Collie	11	F
2		T.T.	Rough Coated Collie	5	M
3	MUT/MUT	Dream	Rough Coated Collie	0.5	F
4	MUT/MUT	Keysha	Rough Coated Collie	10.5	F
5	MUT/MUT	Max	Rough Coated Collie	4.5	M
6	MUT/MUT	Oscar	Rough Coated Collie	3.5	M
7	MUT/MUT	Ferne	Rough Coated Collie	2	F
8	MUT/MUT	Rowan	Rough Coated Collie	2	F
9	MUT/MUT	Paige	Rough Coated Collie	2	F
10	MUT/MUT	Brock	Rough Coated Collie	7	M
11	MUT/MUT	Jasper	Rough Coated Collie	2	M
12	MUT/MUT	Bella	Rough Coated Collie	1	F
13	MUT/N	Jamahl	Rough Coated Collie	6	M
14	MUT/N	Travis	Rough Coated Collie	5	M
15	MUT/N	Zara	Rough Coated Collie	1.5	F
16	MUT/N	Toby	Rough Coated Collie	1	M
17	MUT/N	Sasha	Rough Coated Collie	5	F
18	MUT/N	Joy	Rough Coated Collie	4	F
19	MUT/N	Honey	Rough Coated Collie	7	F
20	MUT/N	Shadow	Rough Coated Collie	0.5	M
21	MUT/N	Jasper	Rough Coated Collie	6	M
22	MUT/N	Danni	Rough Coated Collie	6	F
23	N/N	Pandora	Rough Coated Collie	2.5	F
24	N/N	Ollie	Rough Coated Collie	3	M
25	N/N	Mac	Rough Coated Collie	0.8	M
26	N/N	Savana	Rough Coated Collie	5	F
27	N/N	Matt	Rough Coated Collie	10	M
28	N/N	Tamzin	Rough Coated Collie	3	F
29	N/N	Goldie	Rough Coated Collie	0.5	M
30	N/N	Secret	Rough Coated Collie	0.5	F
31	N/N	Striker	Rough Coated Collie	0.5	M

Table 4.1: Name, age, sex and genotype data of 31 rough coated collies in the study.

	MUT/MUT	MUT/N	N/N
Number of Dogs	10	10	9
Males	4	5	5
Females	6	5	4
Age (Mean) *in years*	3.4 ± 3.1	4.3 ± 2.4	3.9 ± 3.8
Weight (Mean) *In kgs*	22.3 ± 4.9	21.45 ± 3.2	18.93 ± 3.4

Table 4.2: Number of dogs, sex, age and weight data from 29 rough coated collies in the trial assessing the effect of *MDR1-1*Δ mutation on the level of sedation following the IV administration of acepromazine alone.

MUT/MUT: homozygous mutant; MUT/N: heterozygous carrier; N/N: homozygous normal.

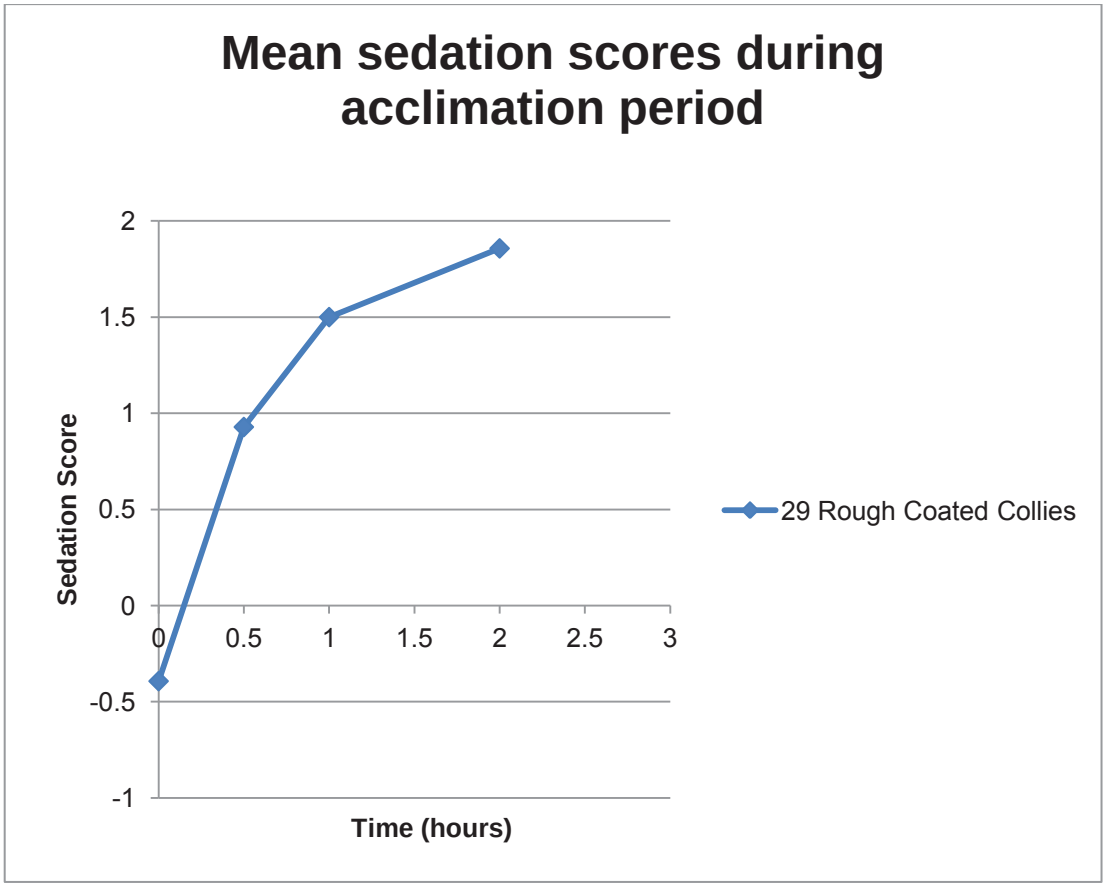

Figure 4.1: Mean sedation scores during acclimation period.
The sedation scores of all 29 rough coated collies increased during the 2 hour acclimation period.

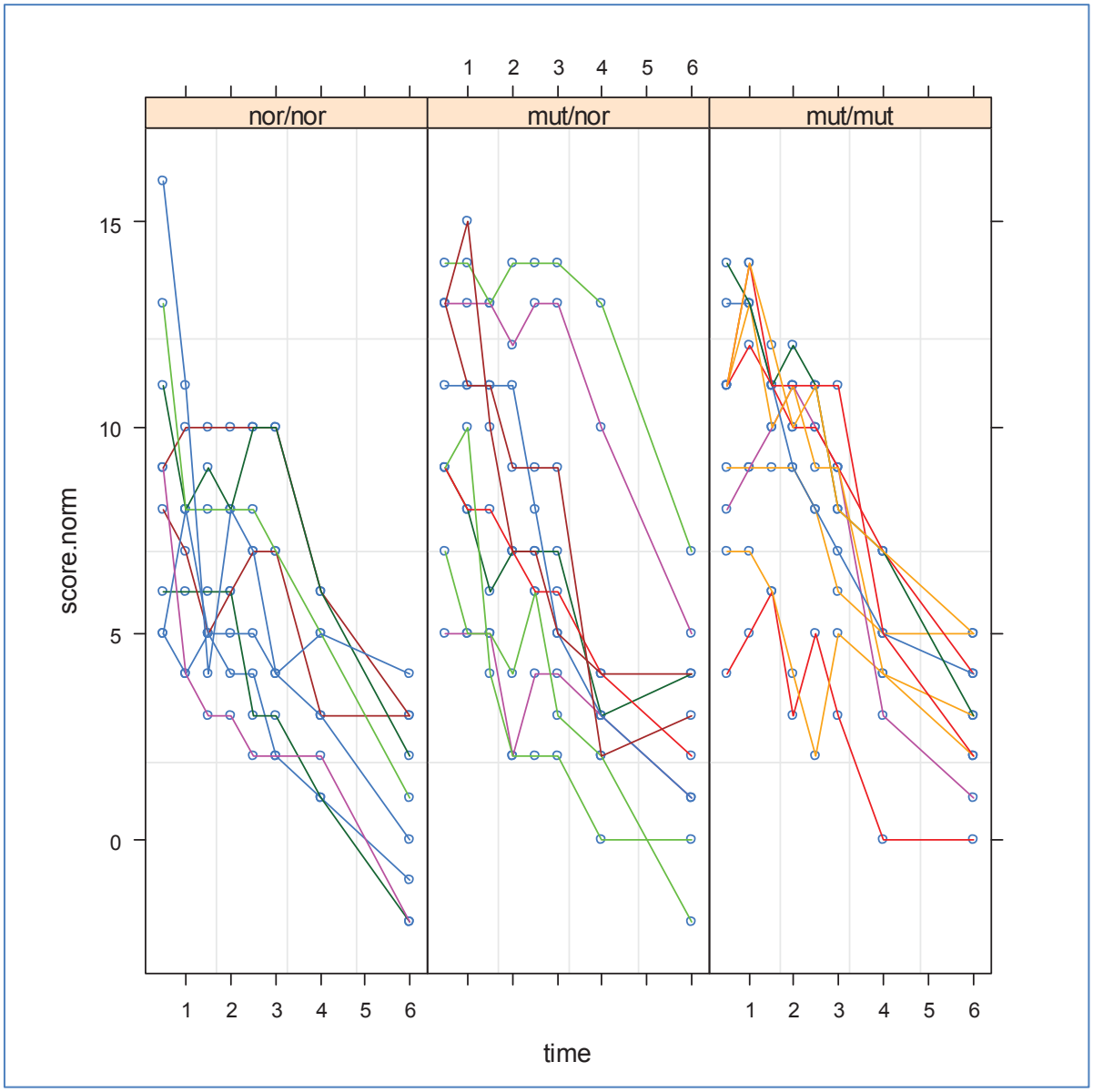

Figure 4.2: Individual sedation scores of 29 rough coated collies.

The graph shows individual sedation scores of the rough coated collies belonging to the three genotypes- N/N, MUT/N, MUT/MUT. The sedation scores of the dogs clustered into their own genotype group suggesting a phenotypic effect to the genotype.

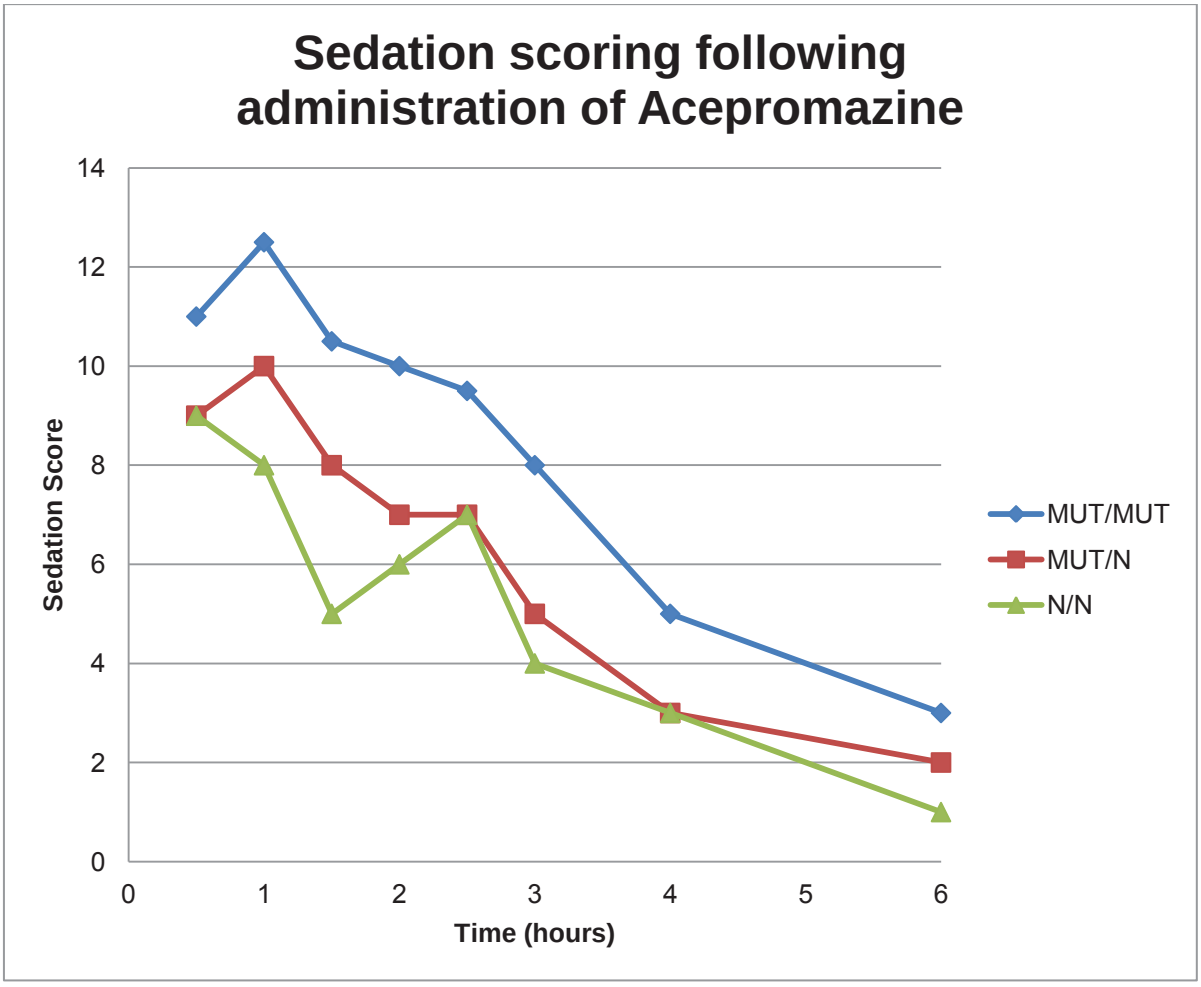

Figure 4.3: Comparison of the median recalculated (R) sedation scores and genotypes in the trial assessing the effect of MDR1-1Δ mutation on the level of sedation following IV administration of acepromazine alone.

N/N: homozygous normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant.

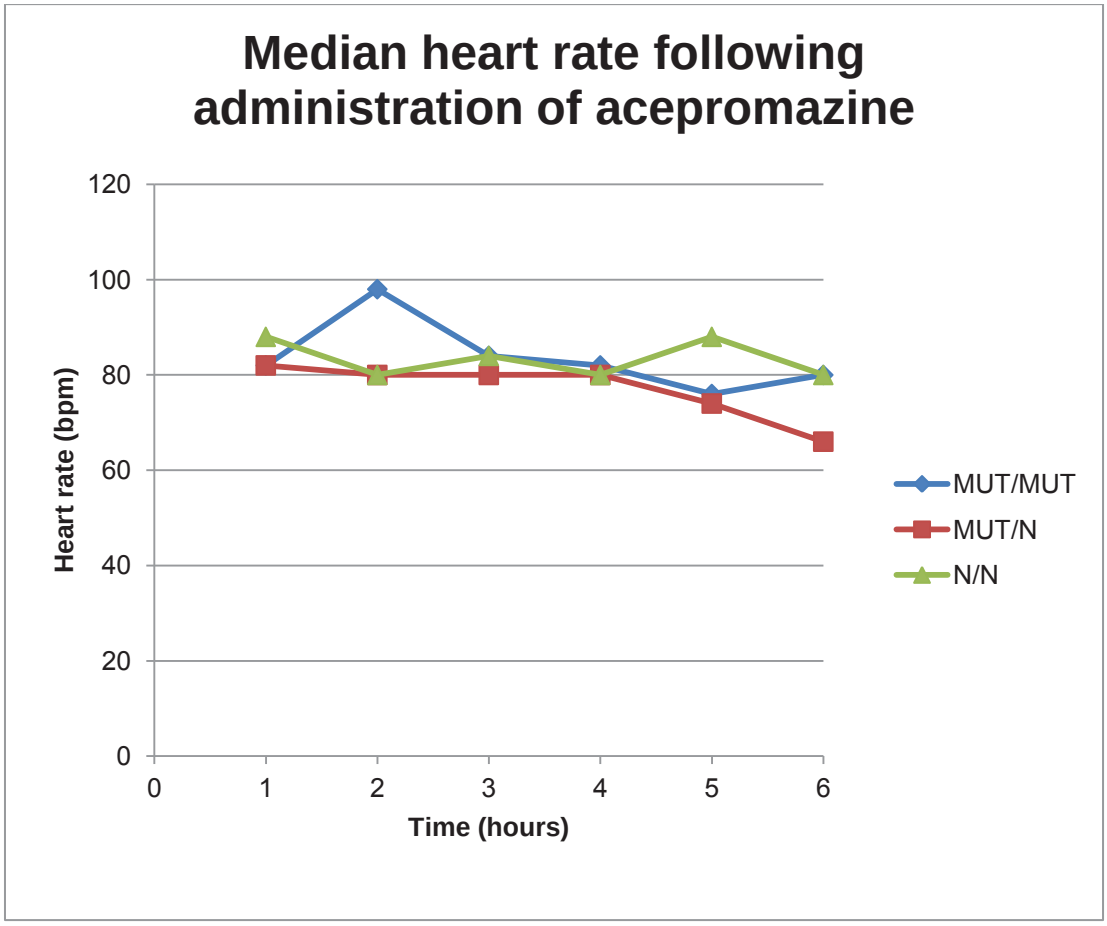

Figure 4.4: Comparison of the median heart rate and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of acepromazine alone.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median heart rates of the dogs in the three genotype groups (p >0.05).

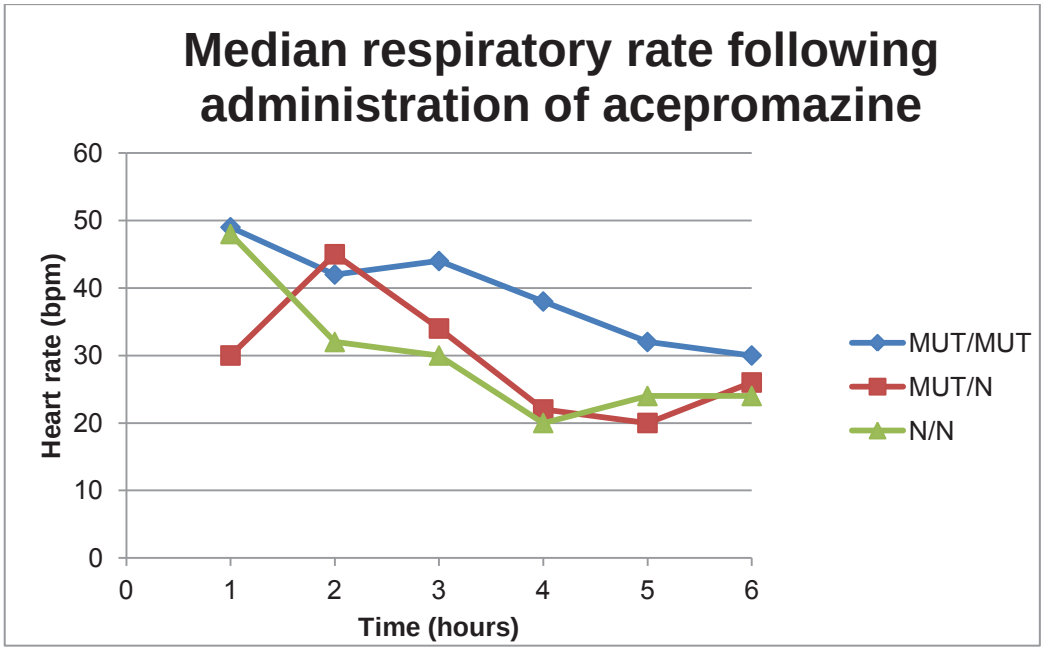

Figure 4.5: Comparison of the median respiratory rate and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of acepromazine alone.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median respiratory rates of the dogs in the three genotype groups (p >0.05).

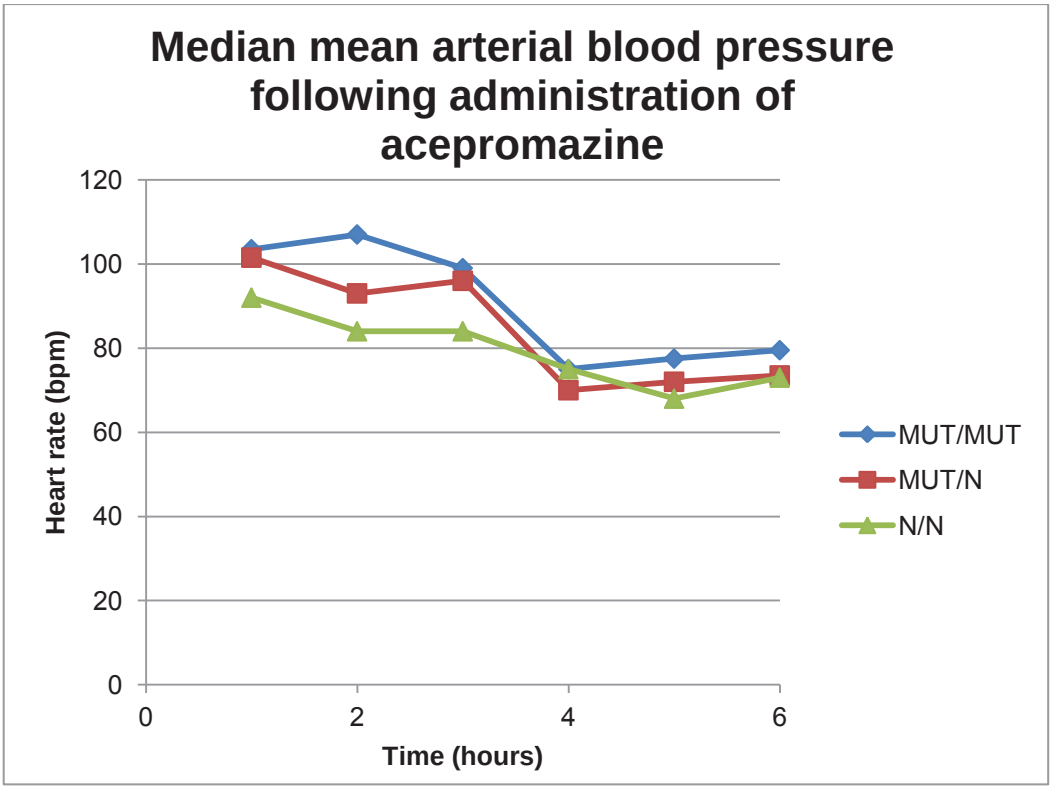

Figure 4.6: Comparison of the median mean arterial blood pressure and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of acepromazine alone.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median mean arterial blood pressure of the dogs in the three genotype groups (p >0.05).

4.2 Pharmacogenetic effects of *MDR1-1*Δ mutation on sedation of rough coated collies with combination of acepromazine and butorphanol.

As described in the Methods chapter a subset of 12 collies from the original 29 collies was enrolled in this study and divided in 3 groups based on their genotypes as: homozygous mutant (MUT/MUT), heterozygous carrier (MUT/N) and normal (N/N) group (Table: 4.3).

	MUT/MUT	MUT/N	N/N
Number of Dogs	4	4	4
Males	1	2	4
Females	3	2	0
Age (Mean) *in years*	2.3 ± 1.7	3.3 ± 3.3	1.2 ± 1.2
Weight (Mean) *In kgs*	21.3 ± 4.6	21.6 ± 4.4	18.9 ± 2.6

Table 4.3: Number of dogs, sex, age and weight data from 12 rough coated collies in the trial assessing the effects of *MDR1-1*Δ mutation on the level of sedation following IV administration of acepromazine and butorphanol combination.

MUT/MUT: Homozygous mutant; MUT/N: heterozygous carrier; N/N: Normal.

All dogs had a 2 hour acclimation period prior to IV dosing with acepromazine and butorphanol. The sedation scores were found to increase during the 2 hours acclimation period (Figure: 4.7). The 12 dogs were sedation scored at 0 , 0.5 , 1, 1.5, 2 hours, 2.5, 3, 4 and 6 hour intervals following IV dosing of a combination of acepromazine (0.04 mg/kg) and butorphanol (0.05 mg/kg). The sedation scores at each of these time intervals were recalculated by subtracting the sedation scores after the 2 hours acclimation period. The medians of the recalculated sedation scores (R) were then plotted in a graph (Figure: 4.8). The dogs that were heterozygous carriers (MUT/N) had higher sedation scores than homozygous mutants (MUT/MUT) and the homozygous normal group (N/N) over the time interval measured (p=0.0423). It was noted that some dogs when given a combination of acepromazine and butorphanol had dyphoria which was characterized by vocalisation and staring.

The median heart rate, respiratory rate and mean arterial blood pressure for MUT/MUT, MUT/N and N/N group were plotted over the time intervals (Figures: 4.9 - 4.11). No statistical correlation using the linear mixed effects model was observed

between the heart rates, respiratory rates and mean arterial blood pressure in relation to the genotypes (p>0.05). Age, body weight, body condition score and sex were covariates. No statistical correlation using the linear mixed effects model was observed between the covariates and the sedation scores of individual genotypes (p>0.05).

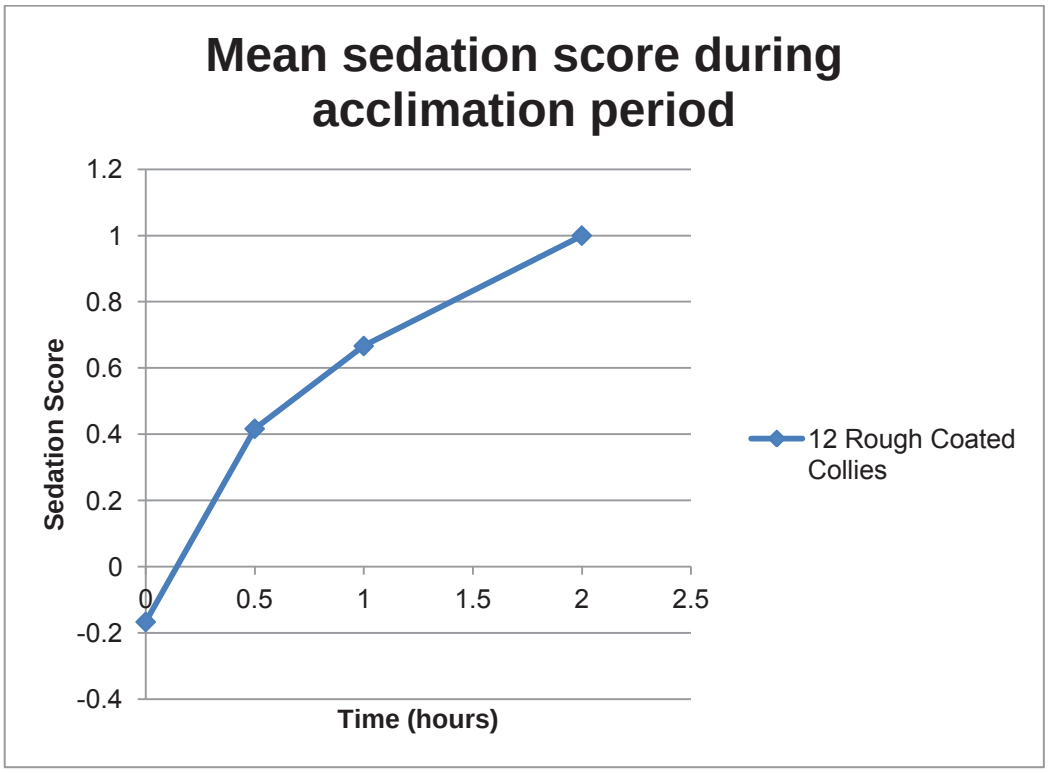

Figure 4.7: Mean sedation score during acclimation period.
All dogs had 2 hours acclimation period prior to dosing with combination of acepromazine and butorphanol. The sedation scores of all 12 rough coated collies increased during the 2 hour acclimation period.

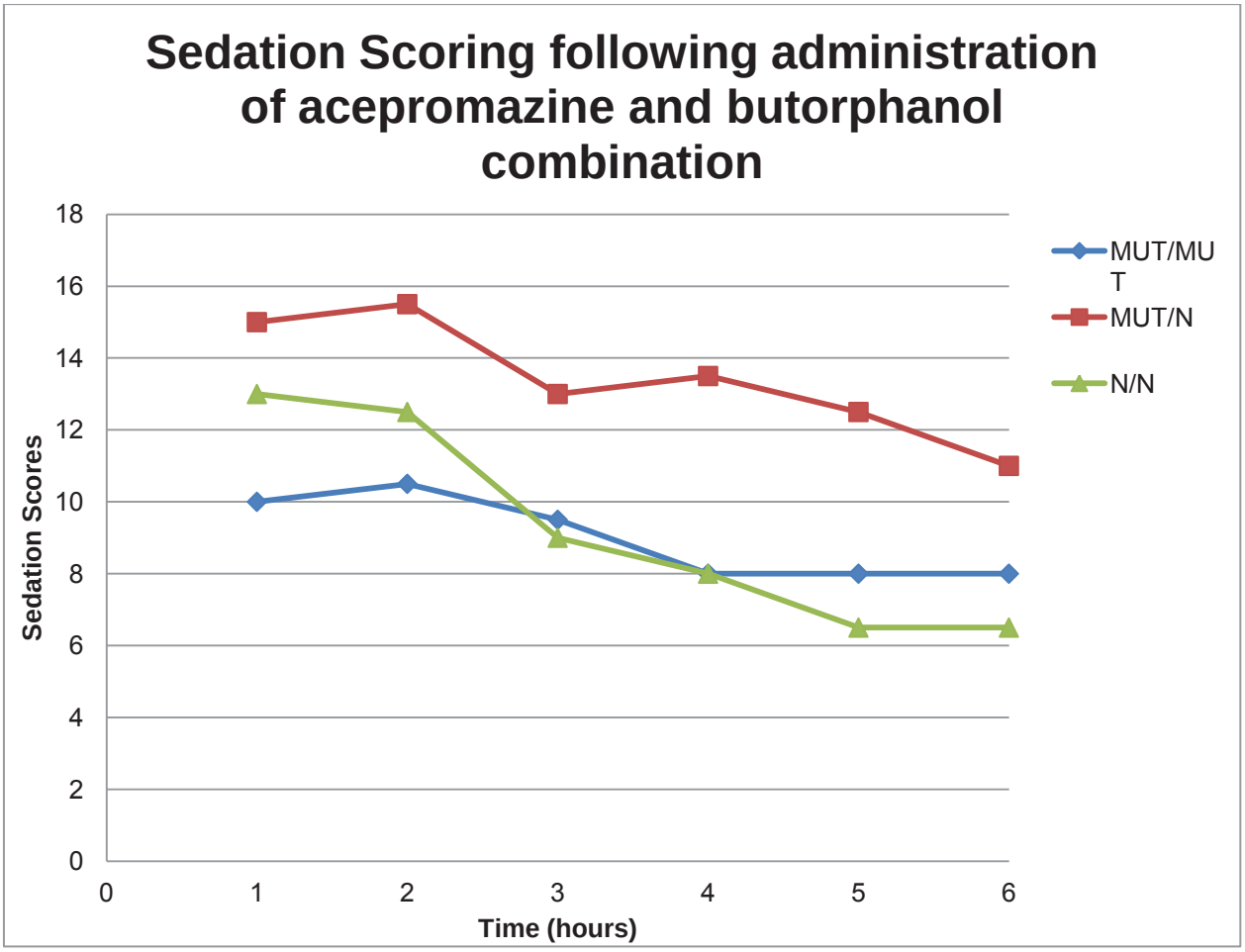

Figure 4.8: Comparison the median recalculated (R) sedation scores and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of combination of acepromazine and butorphanol.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. The data was recalculated by considering the sedation score at the end of 2 hours acclimation period as the base sedation score for that dog. The base sedation score was subtracted from each of the sedation scores at all time intervals and the recalculated sedation score (R) was obtained. The sedation scores of MUT/N are higher than MUT/MUT and N/N (p =0.423).

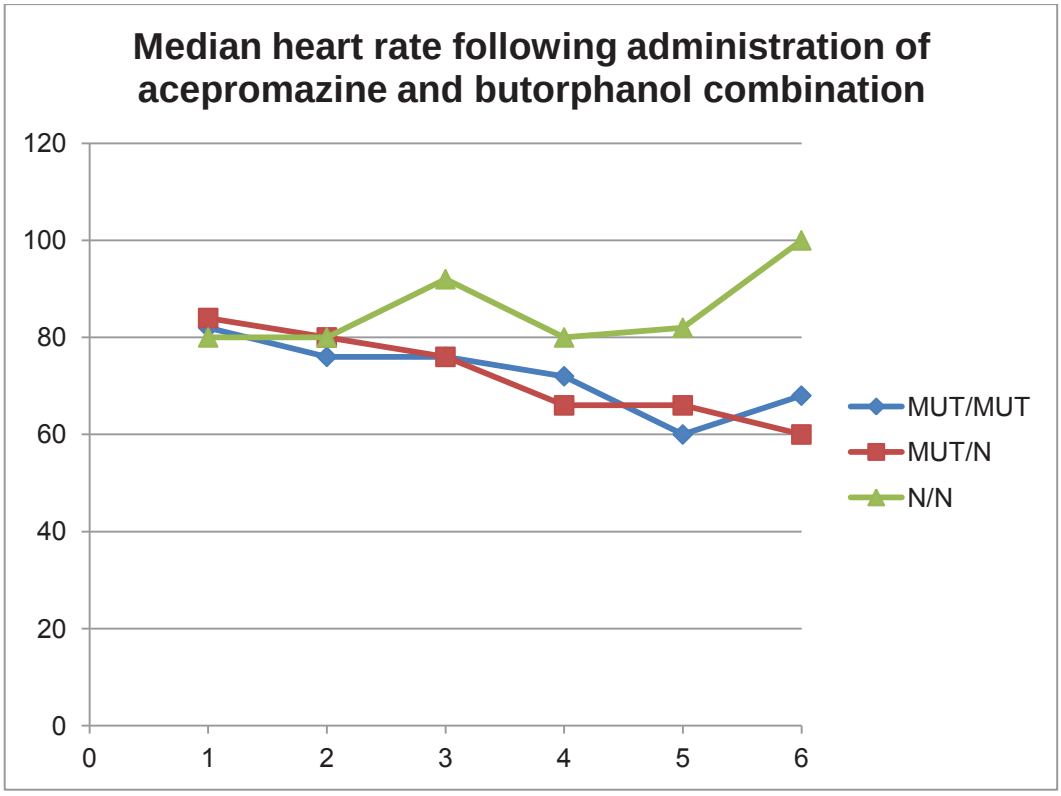

Figure 4.9: Comparison of the median heart rate and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of a combination of acepromazine and butorphanol.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median heart rates of the dogs in the three genotype groups (p >0.05).

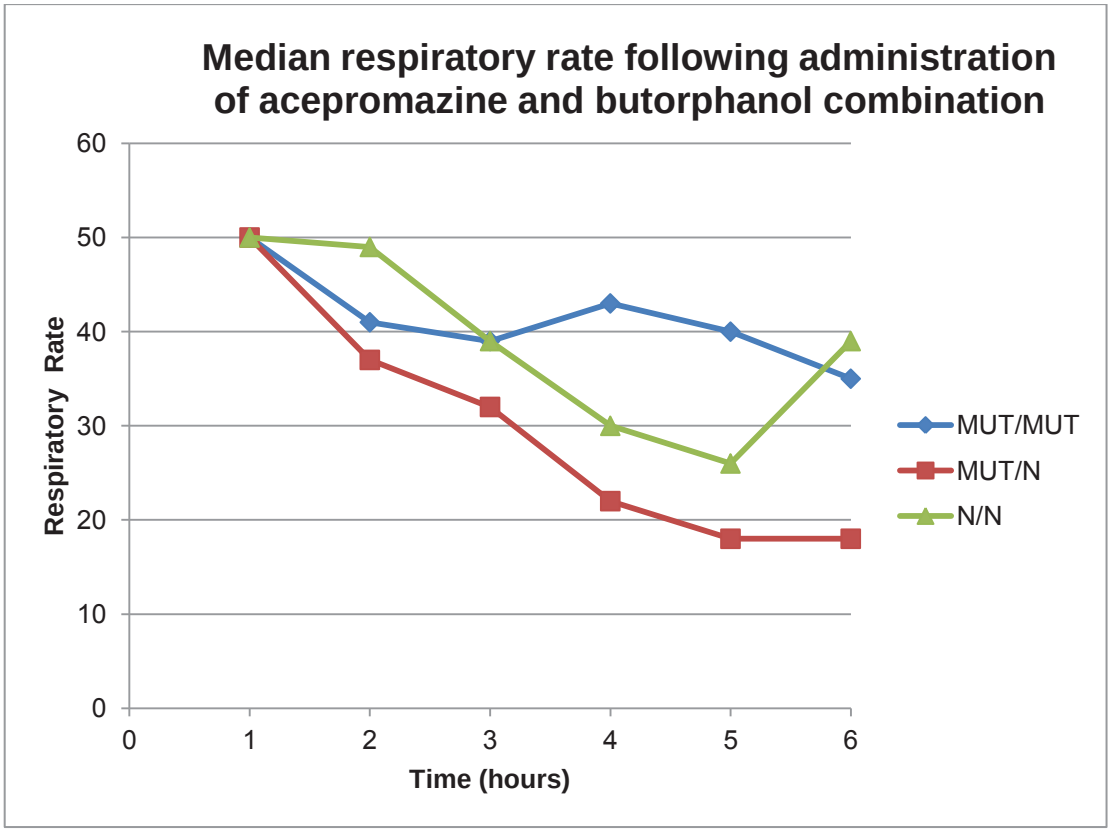

Figure 4.10: Comparison of the median respiratory rates and genotypes in the study assessing the effect of *MDR1-1*Δ mutation on the level of sedation following IV administration of a combination of acepromazine and butorphanol.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median respiratory rates of the dogs in the three genotype groups (p >0.05).

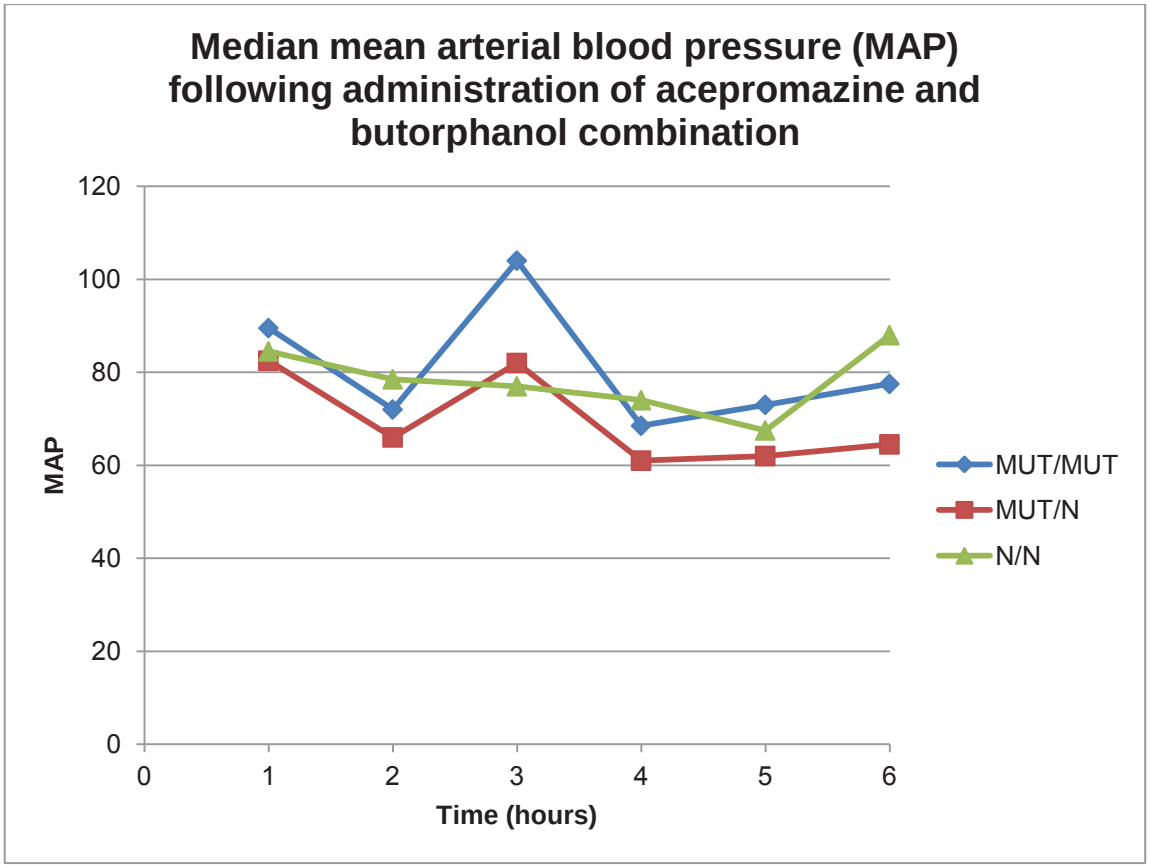

Figure 4.11: Comparison of the median mean arterial blood pressure and genotypes in the study assessing the effect of MDR1-1Δ mutation on the level of sedation following IV administration of a combination of acepromazine and butorphanol.

N/N: normal; MUT/N: heterozygous carrier; MUT/MUT: homozygous mutant. There was no statistical difference in the median mean arterial blood pressures of the dogs in the three genotype groups (p >0.05).

Chapter V:

Discussion

5. Discussion:

5.1 Comparison of sedation in dogs following administration of dexmedetomidine plus morphine to acepromazine plus morphine

Dogs that were premedicated with dexmedetomidine had higher sedation scores at 30 minutes post sedation than the dogs that were premedicated with acepromazine (*p* value=0.0189). Both acepromazine and dexmedetomidine premedicated dogs showed signs of sedation 10 minutes post premedication. The dexmetomidine premedicated dogs consistently had higher sedation scores at 10 minutes, 20 minutes and 30 minutes. Statistical correlation was observed at 30 minutes when the sedation score of the dogs premedicated with dexmedetomidine was significantly higher than the dogs premedicated with acepromazine ($p<0.05$) .

Dexmedetomidine is an alpha-2 adrenergic agonist causing profound sedation and analgesia. Dexmedetomidine asserts its major alpha-2 adrenergic agonist effects on the locus cereulus, a nor-adrenergic region of the neurons in the CNS in the upper brainstem (Kuusela et al., 2001). The locus coeruleus is an important modulator of wakefulness and hence a main site for the sedative action of dexmedetomidine (Kuusela et al., 2001). Acepromazine however asserts its pharmacological effects in part by antagonism of the dopaminergic pathways in the basal ganglia and limbic system in the forebrain (Girault & Greengard, 2004). Acepromazine is considered to have mild to moderate sedation and the sedation with acepromazine does not increase with an increase in dose but may intensify the adverse effects (Monteiro, Junior, Assis, Campagnol, & Quitzan, 2009). Studies show that dexmedetomidine causes profound sedation within 2-5 minutes post administration (Alvaides, Neto, Aguiar, Campagnol, & Steagall, 2008). The level of sedation with dexmedetomidine compared to acepromazine is reported to be higher (Alvaides et al., 2008). A statistical correlation ($p<0.05$) was not observed between the sedation scores of dogs premedicated with dexmedetomidine and acepromazine at 10 and 20 minutes post sedation. However the current study supports the observation that dogs premedicated with dexmedetomidine have a higher sedation score compared to acepromazine at 30 minutes post administration of premedication agent.

Dexmedetomidine is known to have significant cardiovascular effects (Kuusela et al., 2001). Dexmedetomidine causes a biphasic blood pressure response, with a decrease in heart rate and cardiac index and an increase in blood pressure and central venous pressure (Kuusela et al., 2001). However no pulmonary arterial pressure and pulmonary wedge pressure changes have been reported. Studies show that the dexmedetomidine causes bradycardia with the lowest heart rate recorded 2 minutes post administration of dexmedetomidine and lasting for 1 hour. Dexmedetomidine also causes an increase in mean arterial blood pressure with the highest blood pressure reported after 2 minutes post administration of dexmedetomidine and lasting for 20 minutes (Alvaides et al., 2008).

The current study observed no difference in the heart rate, respiratory rate and systolic arterial blood pressure between the dexmedetomidine group and acepromazine group. Dexmedetomidine which is an alpha-2 adrenergic agonist is known to elevate the systolic blood pressure and cause bradycardia whereas acepromazine has alpha-1 antagonist effects that can cause hypotension (Alvaides et al., 2008). However no such effects were noted at the time of intubation which was approximately 30 minutes post IM dosing of the premedication agent.

The probable reason for no difference in the heart rate and mean arterial blood pressure between the DEX and the ACE group is because of the cardiovascular effects of dexmedetomidine and acepromazine are suggested to wear off in 30 minutes. Studies suggest that the cardiovascular effects of dexmedetomidine are pronounced but last for 20 minutes and then wear off (Alvaides et al., 2008). The peak effect of acepromazine also lasts for 30 minutes and then starts wearing off (Monteiro et al., 2009). Therefore, by the time the dogs were ready to intubate, which was 30 minutes after premedication their cardiovascular effects had worn off. Thus there was no difference in the cardiovascular parameters between the DEX and the ACE group.

The dogs that were included in this study belonged to different breeds. Though the mean age and body weight of the dogs belonging to the DEX and ACE groups was similar, the diverse population of dogs enrolled in this study has varied normal reference values for the heart rate depending on the breed. A dog belonging to a small breed will have a higher heart rate but a large breed dog will have a lower heart rate (Ettinger & Feldman, 2010). Comparing the heart rates of dogs belonging to different breeds of dogs may result in erroneous statistical correlations. One of the

reasons for not observing any difference in the heart rate and mean arterial blood pressure between the DEX and the ACE group could be due to a diverse population with varied breeds enrolled in the study. Further studies with more dogs and dogs belonging to the same breed are warranted.

Several sedation scoring systems can be used to assess the level of sedation in dogs. These include the Visual Analogue Score and Simple Descriptive System(Hofmeister et al., 2010). In this study we used a modified sedation scoring system published by Hofmeister in 2010 (Hofmeister et al., 2010). This sedation scoring system is a subjective scale based on behaviour assessment. The sedation scores may be affected by the individual temperament of the dog. In order to minimize the error due to individual temperaments the sedation score at 0 minutes was used as the base sedation score for that dog. The sedation score was then recalculated by subtracting the sedation at 0 minutes from the sedation scores at 10 minutes, 20 minutes and 30 minutes. The recalculated sedation scores were used to the compare the level of sedation between the DEX and the ACE group.

In a previous study by Hofmeister, it was observed that dogs that were injected with isotonic saline (NRS) as a control to acepromazine and hydromorphone, found all dogs that were injected NRS had increased sedation scores overtime (Hofmeister et al., 2010). This is probably because the dogs acclimatise to the environment and thus reduce their anxiety and excitement. However dogs that are inherently calm will not have a profound increase in sedation scores over time when injected with NRS. Since the inherent behaviour affects the sedation score a control is essential to minimize error. In this study the sedation score at 0 was considered the base sedation score and the sedation scores were recalculated by subtracting the sedation at 0 minutes from sedation scores at 10, 20 and 30 minutes. The dogs in this study did not require acclimation as they were left in the same runs they had been in overnight, once the premed was given.

In conclusion, dogs premedicated with dexmedetomidine had a higher sedation score than dog's premedicated with acepromazine at 30 minutes. The heart rate, respiratory rate and mean arterial blood pressure were not different between the DEX and the ACE group post induction. However more studies with more dogs belonging to the same breed are warranted to confirm this finding.

5.2 Pharmacogenetic effects of *MDR1-1*Δ mutation on sedation of rough coated collies with acepromazine .

This study demonstrates the effect of *MDR1-1*Δ mutation on sedation of 29 rough coated collies with intravenous acepromazine. Dogs that were homozygous mutant (MUT/MUT) for the *MDR1-1*Δ mutation had higher sedation scores than the dogs that were heterozygous carriers (MUT/N) or normal animals (N/N) following intravenous administration of acepromazine (0.04 mg/kg) (p=0.0176). The sedation scores of heterozygous mutants (MUT/N) were slightly more than the normal (N/N) group following intravenous administration of acepromazine (p=0.0512).

Out of the 29 rough coated collies that were enrolled in the study, 10 dogs belonged to the homozygous mutant group, 10 to the heterozygous carrier group and 9 to the normal group. The allelic distribution of the *MDR1-1*Δ mutation observed in these 29 rough coated collies is similar to the published allelic distribution. However the sample size of the current study was small, so the allelic distribution in the current study may not be considered a representative for the rough coated collies in New Zealand (Table: 5.1).

Study	No. Of Dogs	MUT/MUT	MUT/N	N/N
Current Study	29	34.5 %	34.5 %	31 %
(Mealey & Meurs, 2008b)	1424	35 %	42 %	23 %
(Neff et al., 2004)	263	31.2 %	46.8 %	22 %

Table 5.1: Table shows the allelic distribution of *MDR1-*Δ mutation published in other studies and in the current study.

The *MDR1-1*Δ mutation in rough coated collies results in a non functional P-gp in homozygous mutants (Mealey et al., 2001). P-gp acts as a vacuum pump at the blood-brain barrier, blood-testis barrier and blood-placenta barrier decreasing the concentration of P-gp substrates in the brain, testes and placenta respectively (Martinez et al., 2008). P-gp also promotes excretion of P-gp substrates by pumping

out the drug through bile in the liver and urine in the kidneys (Martinez et al., 2008). Recent veterinary and human literature suggests that there is a dilemma as to whether phenothiazines are substrates or inhibitors of P-gp (Martinez et al., 2008). Since acepromazine is a member of the phenothiazine group it is unclear whether it is a substrate or an inhibitor of P-gp transport pump.

In this study it was observed that dogs that were homozygous mutants of the *MDR1-1*Δ mutation had prolonged sedation and higher sedation scores compared to the heterozygous mutants and the normal group. Acepromazine probably attains a high concentration in the CNS of the homozygous mutant dogs due to the non functional P-gp. Acepromazine is metabolized by the liver and excreted as uncongugated and conjugated metabolites which are excreted in the urine (Tranquilli et al., 2007). The elimination of acepromazine was probably lower in the homozygous mutant dogs than the normal dogs due to the non functional P-gp. Hence it can be assumed that acepromazine was a substrate of P-gp and thus the dogs that have non functional P-gp have prolonged level and duration of sedation than the heterozygous carrier and the normal dogs.

Acepromazine was the sole sedative agent used in this study to assess the level of sedation in dogs that carry the *MDR1-1*Δ mutation. The prolonged sedation and higher sedation scores in the *MDR1-1*Δ mutants were observed at the low acepromazine dose of 0.04 mg/kg. The non-functional P-gp possibly causes accumulation of high concentrations of acepromazine in the CNS even at low dosages. Acepromazine has a ceiling effect i.e. acepromazine does not cause increased dose related sedation (Monteiro et al., 2009). Increasing the dose of acepromazine will intensify the adverse effects but will not increase the sedation. The effects of higher doses of acepromazine in the dogs with *MDR1-1*Δ mutation were not evaluated in this study.

There are several sedation scoring systems that can be used to assess the level of sedation including the visual analogue score (VAS) and Simple Descriptive System (SDS). The sedation scoring system that has been adopted in this study is a modified sedation scoring system published by Hofmeister, 2010. Sedation Scales must be easy to interpret and recall, have well defined and discrete criteria allocated for each level of sedation, ability to assess anxious behaviours, inter-observer

reliability and finally have evidence of validity in the relevant population (CN Sessler, 2005). The sedation scale published by Hofmeister in 2010 fulfils the criteria mentioned above therefore this sedation scale was adopted for the current study.

On the day of the sedation trial all collies were allowed to acclimatise to the environment in a consult room for 2 hours. Sedation scores of all 29 dogs increased with time during the acclimation period. Most dogs scored low and even negative sedation score at 0 hours of the acclimation period. The low and negative scores were due to anxiety and excitement in the dogs. The level of anxiety and excitement of individual dogs depends on the innate behaviour and temperament of that dog. Some dogs appeared to be more anxious and excited while others were more calm and sedate. As the dogs acclimatised to the environment their sedation scores increased irrespective of their genotypes. The sedation scoring system is based on subjective assessment of the dogs behaviour and the scores may vary depending on the individual temperament. In order to minimize the effect of individual temperament on the sedation score post IV dosing of acepromazine, all dogs were given an acclimation period of 2 hours. The median sedation score of all 29 dogs at 0 hours of acclimation period was 0 and the median sedation score of all 29 dogs at the end of acclimation period at 2 hours interval increased had to 2. This increase in the sedation score suggests that an acclimation period allows the dogs to settle in the new environment and is necessary to minimize the error due to temperament differences between dogs.

In a previous study by Hofmeister, it was observed that dogs that were injected Normal Saline (NRS) as a control to acepromazine and hydromorphone, all dogs that were injected NRS had increased sedation scores overtime (Hofmeister et al., 2010). This is probably because the dogs acclimatize to the environment and thus reduce their level of anxiety and excitement. However dogs that are inherently calm will not have a profound increase in sedation scores over time when injected with NRS. Since the inherent behaviour affects the sedation score a control is essential to minimize error.

The 2 hour sedation scores of all dogs were considered the baseline sedation score for that individual dog. All the sedation scores were recalculated by subtracting the sedation score at the end of 2 hours of acclimatization period from the sedation scores at 0.5 , 1 , 1.5 , 2 , 2.5 , 3 , 4 and 6 hour intervals following administration of

acepromazine. The recalculated sedation scores (R) were used to compare the sedation scores between the 3 genotypes: homozygous mutants, heterogygous carriers and the normal dogs. The statistical tests were performed on the recalculated sedation scores (R) which was done to reduce error due to individual behaviour.

Acepromazine asserts its pharmacological effects by blockade of dopamine receptors (Horn & Snyder, 1971). The dopamine receptors are located primarily in the basal ganglia, limbic system, hypothalamus and chemoreceptor trigger zone (Horn & Snyder, 1971). The dopamine blockade in the basal ganglia and limbic system are responsible for the behavioural effects such as inhibition of conditioned avoidance behaviour and decreased spontaneous motor activity. The blockade of the dopamine receptors in the hypothalamus is responsible for its thermoregulatory effects and the blockade of the chemoreceptor trigger zone is responsible for its antiemetic effects (Tranquilli et al., 2007). Since the dopamine receptors are present in the centres of the brain that are not responsible for cardiovascular functioning, it can be concluded that the non-functional P-gp in the brain of homozygous mutant dogs probably has no effect on the cardiovascular function of the dogs.

However, acepromazine has significant cardiovascular effects primarily due to its affinity to alpha-1 receptors (Boström et al., 2003). The blockade of alpha-1 receptors is responsible for hypotension in dogs. In this study it was observed that post IV dosing of acepromazine the systolic arterial blood pressure in all twenty-nine dogs dropped significantly. There was however no change in the heart rates of the 29 dogs. The respiratory rate of all 29 decreased over time. This was probably due to the dogs becoming less anxious and the sympathetic tone dropping over the 6 hour time interval. This was an expected response due to the published alpha-1 blockade effects of acepromazine (Boström et al., 2003). It has been previously shown that acepromazine causes no change in heart rate and respiratory rate (Boström et al., 2003). Our results found that there were no differences in the mean arterial blood pressure between the three genotypes.

The median heart rate, respiratory rate and mean arterial blood pressure were compared between the three genotypes following administration of acepromazine. No statistical difference was observed between the genotypes and the median heart

rate, respiratory rate and mean arterial blood pressure. Acepromazine exerts its cardiovascular effects mostly by alpha-1 adrenergic blockade. Alpha-1 adrenergic receptors are primarily located in the smooth muscles of the vasculature and the blockade of these receptors, in the vasculature, results in hypotension (Boström et al., 2003).

The P-gp receptors are primarily located in the apical border of intestinal epithelial cells, brain capillary endothelial cells, biliary canalicular cells, renal proximal tubular epithelial cell, placenta and testes but not in the cardiovascular and respiratory system. Hence no cardiovascular differences were expected between the dogs belonging to the three different genotypes. In this study no difference in heart rate, respiratory rate and mean arterial blood pressure between the genotypes were noted.

In conclusion the dogs that were homozygous mutants of the *MDR1*-1Δ mutation have prolonged and higher sedation scores than the heterozygous carriers and the normals. The heterozygous carriers did not sedate significantly more than the normal dogs. However the doses of acepromazine used in these studies were low (0.04 mg/kg) and the effect of higher doses of acepromazine on the homozygous mutants as well as heterozygous carriers were not evaluated. It is thus recommended to genotype all dogs belonging to the collie lineage prior to sedation or anaesthesia. The author also recommends lowering the dose of acepromazine in dogs that are homozygous mutants to the *MDR1-1*Δ mutation and recommends constant monitoring of sedation. Since the homozygous mutants sedate for a longer duration than the heterozygous carriers and normal group the authors recommends monitoring for at least 6 hours post sedation.

More studies assessing the effects of drug combinations on the dogs carrying the *MDR1-1*Δ mutation are warranted. Knowing the effects of combination of anaesthetic drugs is important to have an anaesthetic protocol for the dogs carrying the *MDR1-1*Δ mutation.

A limitation of this study is the small number of dogs enrolled in the study. Further studies with more number of dogs are warranted to support the current findings.

5.3 Pharmacogenetic effects of *MDR1-1*Δ mutation on sedation of rough coated collies with a combination of acepromazine and butorphanol.

As acepromazine was the sole sedative agent used, further studies assessing the combination of acepromazine with other drugs are required to assess their effects on *MDR1-1*Δ mutation dogs. Acepromazine is frequently combined with other drugs such as butorphanol and morphine as a part of routine anaesthesia protocol, with butorphanol and morphine being potential P-gp substrates.

In this study it was observed that dogs that were heterozygous carriers (MUT/N) of the *MDR1-1*Δ mutation had higher sedation scores than homozygous mutants (MUT/MUT) and the normal group (N/N) over the time interval measured (p=0.0423). It was hypothesized that the dogs that were homozygous to the *MDR1-1*Δ mutation would have higher sedation scores than the heterozygous carrier and the normal group. However the observed results were otherwise.

One of the possible explanations for these unexpected results could be an error due to small sample size. The small sample size may have resulted in erroneous statistical correlation between the heterozygous carriers and high sedation scores. The 12 rough coated collies in this study were chosen as a subset from the original 29 rough coated collies from the previous study. A subset from the original population of dogs was selected for practical reasons. The dogs were client owned and hence only owners willing to enrol their dogs in the second acepromazine and butorphanol combination study were included.

Some of the dogs sedated with butorphanol and acepromazine combination became dysphoric. The dysphoria was probably from the butorphanol and was characterized with vocalization and staring. Butorphanol has been known to cause dysphoria in cats but little is known about adverse dysphoria from butorphanol in dogs (Lascelles & Robertson, 2004). The dysphoria that was observed in some of the dogs receiving a combination of butorphanol and acepromazine was primarily due to butorphanol as none of the 29 rough coated from the original population had dysphoria with administration with acepromazine alone. The adverse dysphoric effects of butorphanol made sedation scoring of these dogs difficult. The sedation scoring system was unable to score the degree of dysphoria and thus could not

appropriately assess the degree of sedation on administration of combination of butorphanol and acepromazine.

The other possible explanation for the unexpected result in this study could be due to the impact of dysphoria on the sedation scoring system. Since dyphoria was characterized by vocalisation the sedation scoring system scored these dogs low. Thus if dogs that were homozygous mutants were more dysphoric than the heterozygous carriers, the sedation scoring system would score the homozygous mutants lower than the heterozygous carriers.

Similar to the previous study where acepromazine was the sole sedative agent used in the current study the sedation scores of all 12 dogs increased with time during the acclimation period. Most dogs scored low and even negatively at 0 hours of the acclimation period. The low and negative scores were due to anxiety and excitement. As the dogs got used to the environment they became more comfortable and their sedation scores increased irrespective of their genotypes. The median sedation score of all 12 dogs at 0 hours of acclimation period was 0 . The median sedation score of all 12 dogs at the end of acclimation period at 2 hours interval increased to 2 . The rise in the sedation scores suggest the acclimation period allowed the dogs to settle in the new environment.

The median heart rate in the 12 rough coated collies did not alter significantly post IV dosing of the combination of acepromazine and butorphanol. The median respiratory rate and mean arterial blood pressure decreased slightly post IV dosing of combination of acepromazine and butorphanol. The decrease in respiratory rate post IV dosing of combination of butorphanol and acepromazine was expected as butorphanol is a respiratory depressant (Tranquilli et al., 2007). The drop in the mean arterial blood pressure post IV dosing of combination of acepromazine and butorphanol was not as significant as the drop in mean arterial blood pressure in the previous study with IV dosing of acepromazine alone. Alpha-1 blockade from aceromazine is known to cause hypotension and thus the drop in mean arterial blood pressure was expected due to the alpha-1 blockade effects of acepromazine (Boström et al., 2003).

The median heart rate, respiratory rate and mean arterial blood pressure was compared between the three genotypes following administration of acepromazine.

No statistical correlation was observed between the genotypes and the median heart rate, respiratory rate and mean arterial blood pressure. The P-gp receptors are primarily located in the apical border of intestinal epithelial cells, brain capillary endothelial cells, biliary canalicular cells, renal proximal tubular epithelial cell, placenta and testes. They are not located in the cardiovascular and respiratory system. Thus no difference in heart rate, respiratory rate and mean arterial between the genotypes was observed.

In our previous study with IV dosing of acepromazine alone, it was observed that dogs homozygous mutants to *MDR1-1*Δ had prolonged and higher sedation scores than the normal and heterozygous group. In the combination study of acepromazine and butorphanol a potentially erroneous association between heterozygous genotype and higher sedation score was observed. Hence more studies are warranted with combinations of acepromazine and butorphanol to make further recommendations about their use in *MDR1-1*Δ homozygous mutant dogs.

5.4 Conclusion

The *MDR1-1*Δ mutation has significant pharmacogenetic effects on dogs carrying this mutation. The effects of this mutation can cause adverse drug reactions that can be fatal. Hence more studies understanding the P-gp structure and function are crucial to our understanding of the *MDR1* gene and thus the *MDR1-1*Δ mutation. More research in this field would be a step towards individualised veterinary medicine enabling tailored veterinary therapy, based on the *MDR1* genotype of the animal.

Chapter VI: References

6. References

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