EFFICACY OF GASTRIC-ACID LOWERING THERAPY IN THE DOG

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

ALEXA MARIA ELENA BERSENAS

In partial fulfillment of requirements

for the degree of

Master of Science

December, 2005

© Alexa M. E. Bersenas, 2005

Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 978-0-494-17665-8

Our file Notre référence

ISBN: 978-0-494-17665-8

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

EFFICACY OF GASTRIC-ACID LOWERING THERAPY IN THE DOG

Alexa M. E. Bersenas
University of Guelph, 2005

Advisor: Karol A. Mathews
Department of Clinical Studies

This thesis is an investigation of the normal canine gastric acid profile and the degree of gastric acid suppression achieved using antisecretory drugs.

Continuous 24-hour intragastric pH measurements were obtained in fasted and fed dogs. Thereafter, a randomized, multiple cross-over study compared saline IV q12h, ranitidine 2mg/kg IV q12h, famotidine 0.5 mg/kg IV q12h, pantoprazole 1 mg/kg IV q24h, and omeprazole ~ 1mg/kg PO q24h, for 7 days. Recordings were obtained on Day 0, 2, and 6. Next, famotidine 0.5mg/kg IV q8h and omeprazole suspension (~1mg/kg) PO q12h were evaluated in 6 dogs. The median pH and the percent time $\text{pH} \geq 3$ and $\text{pH} \geq 4$ were compared.

Fasting produced greater acid suppression than feeding. Famotidine, pantoprazole and omeprazole showed significant acid suppression based on median 24-h pH and percent time $\text{pH} \geq 3$ and ≥ 4 . Omeprazole suspension q12h was the most effective at suppressing gastric acid secretion.

ACKNOWLEDGEMENTS

I want to take this opportunity to thank my advisor Dr. Karol Mathews who introduced me to critical care, mentored me during my residency and encouraged me to embark on a Master of Science degree at the University of Guelph.

I would like to acknowledge all of the people at the University of Guelph who encouraged me in this endeavour, prior to its inception and throughout the course of the work including Dr. Karol Mathews, Dr. Dana Allen, Dr. Michael O'Grady, Dr. Howard Dobson, Dr. Stephen Kruth, Dr. Julie Armstrong and Dr. Peter Conlon. This project also gave me the privilege to work with individuals at McMaster Health Sciences and I would like to thank Cindy James, Dr. David Armstrong and Dr. Richard Hunt for their input, contributions, time and effort.

This project would not have been possible without the help of all the support staff at the Small Animal Clinic at the University of Guelph. Special thanks to the technical staff in the intensive care unit: Shannon Bolger, Cathy Cavanagh, Deborah-Lynn Cloutier, Deborah Cook, Heather Creasey, Ingrid Danylyk, Melanie Lewis, Andrea Steele, Kathy Taylor; and to the staff in the pharmacy: Nancy Haliburton and Lynn Sorbara, and Dr. Angela Hofstra. Help was provided throughout the experimental studies from my summer students Barbara Alexander and Laine Misch; Annette Morrison at Animal Care Services; and ongoing data and statistical advice from Dan Schnurr, Gabrielle Montieth and Dr. William Sears. Thanks to you all!!!

I also want to express my thanks to the members of my Advisory Committee: Dr. Karol Mathews, Dr. Dana Allen and Dr. Peter Conlon; also to Dr. Andrew Brooks for being a member of my Examination Committee.

Great thanks and appreciation to the Pet Trust Fund for full financial support of this research project.

DECLARATION OF WORK PERFORMED

I declare that with the exception of the items listed below, all work in this thesis was performed by me.

Cindy Adams provided assistance with the use and maintenance of all the equipment.

Assistance for pH probe and meter calibration, dog handling, probe placement, and data downloading was provided by Amanda Hathaway, Laine Misch and Barbara Alexander.

University of Guelph, Laboratory Services, Animal Health Laboratory, provided the blood results (complete blood cell count and biochemical profile), and routine bacterial cultures and urea slant testing.

Helicobacter polymerase chain reaction (PCR) testing was performed by Health Gene, Markham, Ontario.

Radiology assisted with accurate assessment and determination for pH probe placement.

Dan Schnurr provided all computer assistance with retrieval and organization of raw data.

Dr. William Sears provided advice on the statistics applied and Gabrielle Monteith wrote the statistical programs for data analysis.

TABLE OF CONTENTS

Abstract: EFFICACY OF GASTRIC-ACID LOWERING THERAPY IN THE DOG

Acknowledgements.....	i
Declaration of Work Performed.....	iii
List of Tables.....	vii
List of Figures.....	viii

CHAPTER I

INTRODUCTION AND OBJECTIVES.....	1
STATEMENT OF GOALS AND HYPOTHESES.....	3
LITERATURE REVIEW.....	5
1.1 – GASTRIC ANATOMY & PHYSIOLOGY.....	6
1.2 – GASTRIC ACID SECRETION.....	9
• Overview.....	9
• Phases of Gastric Acid Secretion.....	11
• Miscellaneous Causes of Gastric Acid Output.....	12
• Normal Gastric Acid Output.....	13
• Basal Gastric Acid Secretion.....	14
• Stimulated Gastric Acid Secretion.....	18
1.3 – PATHOGENESIS & PATHOPHYSIOLOGY OF GASTRIC ULCERATION.....	20
• Mucosal Defense Mechanisms.....	21
• Predisposing Risk Factors in Veterinary Medicine.....	22

• Helicobacter Infection.....	23
• Role of Acid Suppression.....	25
• Adverse Effects of Acid Suppression.....	27
1.4 – ACID-LOWERING PHARMACOLOGIC AGENTS.....	29
• Histamine Receptor Antagonists (H ₂ RAs).....	29
• Proton Pump Inhibitors (PPIs).....	32
• Omeprazole Suspension.....	36
• PPIs vs H ₂ RAs.....	38
1.5 – VETERINARY STUDIES.....	40
1.6 – MONITORING GASTRIC ACID.....	48
• Probe Placement.....	52
1.7 – CONCLUSION.....	54

CHAPTER II

Effects of ranitidine, famotidine, pantoprazole, and omeprazole on intragastric pH in dogs.....	55
2.1 – Abstract.....	55
2.2 – Introduction.....	57
2.3 – Materials and Methods.....	59
2.4 – Results.....	64
2.5 – Discussion.....	68
2.6 – Tables.....	75
2.7 – Figures.....	81

2.8 – Footnotes.....	83
2.9 – References.....	84

CHAPTER III

Day and night-time analysis of the effects of ranitidine, famotidine, pantoprazole, and omeprazole on intragastric pH in dogs & Number of dogs responding to acid-lowering therapy.....	87
3.1 – Abstract.....	87
3.2 – Introduction.....	89
3.3 – Materials and Methods.....	91
3.4 – Results.....	95
3.5 – Discussion.....	101
3.6 – Tables.....	108
3.7 – Footnotes.....	119
3.8 – References.....	120

CHAPTER IV

GENERAL DISCUSSION.....	123
4.1 – Future Research.....	130
4.2 – Conclusions.....	132
MASTER REFERENCE LIST.....	134

APPENDICES

5.1–1 – List of Abbreviations.....	147
------------------------------------	-----

5.2-1 – 24-Hour Raw Data for Chapters II and III.....	149
5.2-2 – 12-Hour Raw Data for Chapters II and III.....	158

LIST OF TABLES

Table 2.6-1 – Median 24-h pH values of all fasted dogs.....	75
Table 2.6-2 – Median 24-h pH values of all fed dogs.....	76
Table 2.6-3 - Median 24-hour intragastric pH in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 7 days.....	78
Table 2.6-4 - Mean percent time intragastric pH was ≥ 3 in 12 healthy dogs treated with ranitidine, famotidine, pantoprazole, omeprazole, or saline solution for 7 days.....	79
Table 2.6-5 - Mean percent time intragastric pH was ≥ 4 in 12 healthy dogs treated with ranitidine, famotidine, pantoprazole, omeprazole, or saline solution for 7 days.....	80
Table 3.6-1 - Median pH values for the daytime, nighttime, and 24-h period on day 0.....	108
Table 3.6-2 - Median pH values for the daytime, nighttime, and 24-h period on day 2.....	109
Table 3.6-3 - Median pH values for the daytime, nighttime, and 24-h period on day 6.....	110

Table 3.6-4 – Mean percent of time intragastric pH ≥ 3 for the daytime, nighttime and 24-h period on day 0.....	111
Table 3.6-5 – Mean percent of time intragastric pH ≥ 3 for the daytime, nighttime and 24-h period on day 2.....	112
Table 3.6-6 - Mean percent of time intragastric pH ≥ 3 for the daytime, nighttime and 24-h period on day 6.....	113
Table 3.6.7 - Mean percent of time intragastric pH ≥ 4 for the daytime, nighttime and 24-h period on day 0.....	114
Table 3.6-8 - Mean percent of time intragastric pH ≥ 4 for the daytime, nighttime and 24-h period on day 2.....	115
Table 3.6-9 - Mean percent of time intragastric pH ≥ 4 for the daytime, nighttime and 24-h period on day 6.....	116
Table 3.6-10 Number of dogs with a pH ≥ 3 for a given percentage of the 24-h day....	117
Table 3.6-11 Number of dogs with a pH ≥ 4 for a given percentage of the 24-h day....	118

LIST OF FIGURES

Figure 2.7-1 - Median intragastric pH in 12 healthy dogs treated with ranitidine, famotidine, pantoprazole, omeprazole, or saline solution for 2 days.....	81
Figure 2.7-2 - Median intragastric pH in 12 healthy dogs treated with ranitidine, famotidine, pantoprazole, omeprazole, or saline solution for 6 days.....	82

CHAPTER I

INTRODUCTION AND OBJECTIVES

In the study described in this thesis, we evaluated canine gastric acid secretion in normal dogs, both fasted and fed, and subsequently compared the effects of ranitidine, famotidine, omeprazole and pantoprazole on inhibition of canine gastric acid secretion in normal dogs fed daily, standardized meals.

Gastric-acid suppressants are frequently prescribed in small animal medicine for several disorders. Acid-lowering therapies are prescribed for these conditions with little, or no, information published with respect to the effectiveness and duration of action of these drugs given at standard veterinary doses. In dogs, the use of gastric-acid suppressants has been advocated for the prevention or treatment of upper gastrointestinal erosions, ulcers or hemorrhage associated with corticosteroid or non-steroidal anti-inflammatory analgesic (NSAID) administration, hypotension, shock, sepsis, coagulopathies, head trauma, neurological disease, hypergastrinemia, renal failure and liver failure (Matz 1995, Papich 1993). Studies have been limited, and to date have only evaluated the protective nature of acid-lowering therapies with the use of NSAIDs (Cavanagh *et al* 1987, Jenkins *et al* 1991) Otherwise, no clinical studies have evaluated the efficacy of gastric acid-lowering agents on canine gastric pH. Acid-lowering therapy is also indicated for esophageal ulcers and erosions subsequent to esophageal foreign body, gastric reflux, and for chronic vomiting and regurgitation problems (Eddlestone 2002). Again, no veterinary studies have been performed to establish the effectiveness of gastroprotectants when used in these conditions.

Despite the frequent use of gastric acid suppressants in dogs, a paucity of comparative studies evaluating the efficacy of these drugs are available to guide therapy. Ill dogs with feeding tubes placed into the stomach for nutritional support, have given us the rare opportunity to measure gastric pH during administration of acid suppressants. As the pH measurements varied, with some less than 3 depending on the suppressant administered, the efficacy of gastric acid-lowering therapies was questioned and their use warranted further investigation in dogs.

The goal of antisecretory therapy is to reduce gastric acid secretion, prevent damage to the gastric mucosa and allow the regenerative mucosal mechanisms to prevail. Human studies have established that the healing of acid-related disorders (gastric and duodenal ulcers, and erosive esophagitis) is highly correlated with the degree of gastric acid suppression (Huang and Hunt 2001). The degree of gastric acid suppression necessary for treating acid related disease in veterinary medicine has not been established. The goals established for gastric pH for humans may not be the same for the canine patient due to differences in gastric physiology.

The purpose of the study was to identify those gastric acid-lowering agents able to significantly inhibit gastric acid secretion in the healthy dog. Drugs were selected from the two large classes of drugs used for gastric acid suppression in veterinary medicine. Our sample population utilized 12 healthy beagle dogs. Drug efficacy was determined continuously over a 24-hour period.

STATEMENT OF GOALS AND HYPOTHESES

In the first part of the study, the objective was to establish normal baseline gastric acid profiles of 12 healthy beagle dogs and to determine the optimal testing conditions (fasted versus fed) for the drug study to follow. The null hypothesis was that there was no difference in gastric acid profile among fasted and fed dogs.

The second part of the study was designed to evaluate the degree (efficacy and duration of action) of gastric acid suppression achieved in dogs using four currently recommended gastric-acid suppressants at standard veterinary doses. Two histamine₂ receptor antagonists (H₂RA), ranitidine and famotidine, and two proton pump inhibitors (PPIs), omeprazole and pantoprazole, were selected and results were compared to saline and to baseline. This study used the same 12 healthy beagle dogs as in the first part of the study. Drug efficacy was determined over a 24-hour period.

The efficacy and duration of action of each drug was determined by evaluating three separate parameters over a 24-hour period: median gastric pH; and the percentage of time the gastric pH was maintained above or equal to, pH 3, and pH 4, respectively.

The null hypothesis was that each drug will show no difference in gastric pH (as assessed by the three parameters) when compared to saline, and again when compared to baseline. A further null hypothesis, considered that there is no difference among the four evaluated drugs.

Following completion of the drug study, drug efficacy was statistically re-evaluated during a 12-hour 'day-time' interval and a 12-hour 'night-time' interval. The purpose of this comparison was to determine whether differences were noted in the duration of action of each drug and whether dosing schedules may have affected results

in the 24-hour determinations. We were also interested in evaluating whether a circadian rhythm was detected in dogs over a 24-hour period.

LITERATURE REVIEW

Gastric-acid suppressants are frequently prescribed in small animal medicine; despite this, no clinically relevant comparative studies have specifically investigated the efficacy of these therapies reported in dogs. A large body of information exists regarding gastric acid secretion and its suppression. The development of gastric acid-lowering therapies has frequently utilized the dog as a model and these drugs have clearly been shown to have an effect on canine gastric pH.(Akimoto *et al* 2000,Katz *et al* 1987,Kromer *et al* 1990,Larsson *et al* 1983,Larsson *et al* 1985,Postius *et al* 1991b,Yamada *et al* 1989) Regardless, these early investigational studies did not determine the extent or duration of action of acid-lowering therapies in the dog in a physiologic setting. The limitations of the information gained from these early studies include the evaluation of pharmacologically altered canine gastric acid secretion, (i.e. by pentagastrin or histamine)(Katz *et al* 1987,Larsson *et al* 1983,Larsson *et al* 1985,Postius *et al* 1991b), assessment of high drug dosages (i.e. during toxicity testing)(Kromer *et al* 1990,Postius *et al* 1991b) and gastric pH determination times of only a few hours duration.(Akimoto *et al* 2000,Katz *et al* 1987,Larsson *et al* 1985,Yamada *et al* 1989) Although a canine model was utilized during drug development, canine drug doses used in the clinical setting have been extrapolated from human data. As physiologic differences exist between the gastrointestinal tract of humans and dogs, including gastric emptying rate, basal gastric acid secretion and gastric acid output (Dressman 1986,Kararli 1995,Kuna 1964,Lui *et al* 1986), a study evaluating the efficacy of gastric-acid lowering therapies in the clinical veterinary setting is warranted.

1.1- Gastric anatomy & physiology

The stomach of the dog is glandular and is separated into three regions lined by cardiac, gastric (or parietal) and pyloric mucosa. These regions have been further divided, with the proximal portion of the stomach consisting of the cardia, fundus and body; and the distal portion the antrum and pylorus. The cardia is a narrow band of tissue at the entrance to the stomach, which secretes mucus to protect the adjacent esophagus. The fundus and body function to temporarily store food and secrete digestive enzymes. The antrum serves to regulate the release of hydrochloric acid, and grind ingesta. Together, the antrum and pylorus regulate food entry into the duodenum and prevent duodenal reflux. The surface of the stomach is lined by a simple columnar epithelium of surface mucous cells. In the dog, there are numerous invaginations of the surface epithelium, known as gastric pits which further differentiate into gastric glands.(Herdt 1997) These gastric glands are divided into sections: the isthmus, neck and base. While the gastric glands are prevalent throughout all regions of the stomach, the cellular structure differs within regions of the stomach. The cardiac glands primarily contain mucous secreting cells. The glands of the body and fundus contain hydrochloric acid secreting parietal cells, and in close proximity are the histamine-releasing enterochromaffin-like (ECL) cells, the pepsinogen secreting chief cells, mucous-neck cells, and endocrine cells that secrete serotonin.(McColl *et al* 2000) The gastric glands of the distal stomach contain fewer parietal cells and chief cells. Other cells in this region include the mucous neck cells and endocrine cells known as G cells that secrete gastrin. The pyloric glands have no parietal cells, but contain G cells and chief cells.(Herdt 1997) The lifespan of these different cells in the stomach varies. The epithelial cells of the canine gastric mucosa are

rapidly renewed. These surface mucous cells have a lifespan of 3-5 days and are replaced by migration of undifferentiated cells from the walls of the gastric pits, a process that is completed in 3 days. The mucous neck cells do not migrate and they are renewed in 1 week. The same undifferentiated cells from the wall of the gastric pits migrate down to replace the parietal cells and chief cells which turn over more slowly as they have a lifespan of approximately one year.(Guilford and Strombeck 1996)

The key cell in gastric acid secretion is the parietal cell. The parietal cell secretes hydrogen ion via a H^+K^+ ATPase pump. Hydrogen ion secretion is a high energy process, therefore 30-40% of the parietal cell volume consists of mitochondria (more than any other mammalian cell).(Davenport 1982,Simpson 2005) Gastric acid production by parietal cells is mediated by one of two processes: calcium dependent (increases in free cytosolic calcium) or cyclic AMP dependent activation.(Soll and Berglindh 1987)

Histamine stimulates cyclic AMP production in parietal cells via an H_2 receptor.(Davenport 1982,Soll and Berglindh 1987) Gastrin and acetylcholine rely on calcium dependent activation of the proton pump. Based on gastric stimulation, the proton pump proceeds from an inactive to an active state such that over a period of hours, essentially all the ATPase molecules cycle through an acid-producing state. Once activated, the pump actively exchanges one hydrogen ion into the gastric lumen for a potassium ion into the parietal cytoplasm and hydrolyzes one adenosine triphosphate (ATP) to adenosine diphosphate (ADP) in the process.(Herdt 1997,Sachs *et al* 1995)

The accumulation of potassium in the parietal cytoplasm is overcome by the addition of a K^+ and Cl^- cotransport pathway that pumps one K^+ and one Cl^- ion back into the

gastric lumen. The end result is secretion of H^+ and Cl^- and no net movement of K^+ .(Forte and Wolosin 1987)

Hydrogen ions are generated from the dissociation of intracellular carbonic acid (H_2CO_3) resulting in the production of one bicarbonate ion for each hydrogen ion generated. As hydrogen ions are secreted into the gastric lumen, bicarbonate (HCO_3^-) anions accumulate in the cell. The excess HCO_3^- is removed by a $\text{Cl}^-/\text{HCO}_3^-$ exchange on the basolateral membrane. For each hydrogen ion secreted on the luminal surface of the parietal cell, a HCO_3^- ion is generated at the basolateral surface.(Sachs 1987) This transport pathway, along with a Na^+/H^+ exchange at the basolateral membrane, maintains acid–base regulation and together they allow entry of NaCl into the parietal cell.(Forte and Wolosin 1987,Sachs 1987) Also basolateral Na^+/K^+ ATPase and K^+ conductive channels are present to provide the driving force for Cl^- movement.

1.2- Gastric acid secretion

Overview

The amount of acid in gastric contents is determined by: the rate of gastric acid secretion, the neutralization of acid by bicarbonate, the dilution by food and other digestive enzymes, and by back diffusion of acid through the gastric mucosa.(Davenport 1982) Differences in gastric pH occur throughout the stomach. The distribution of the parietal cell in the stomach of the dog accounts for much of the changes in pH, however other factors are also noted. The cardiac region has a higher pH than the remainder of the stomach due to secretion of alkaline mucus which protects the adjacent esophageal mucosa from the acidic stomach contents.(Herdt 1997,Kararli 1995) The fundus and body of the stomach are more acidic due to a local concentration of parietal cells.(Davenport 1982,Herdt 1997) The pH of the gastric antrum is higher than that of the fundus and body due to a reduced number of parietal cells. The pH continues to increase through the pylorus and into the small intestine.

Secretion of hydrochloric acid is initiated via three systems: endocrine, neurocrine and paracrine. In the dog, these systems are mediated by three separate chemical messengers: gastrin, acetylcholine and histamine, respectively. The receptors (gastrin, cholinergic muscarinic type, and histamine type 2 receptors) for each of these three messengers are located on the basolateral membrane of the parietal cell. Gastrin is produced by G cells, in response to food, and is released into the bloodstream where it subsequently binds to the parietal cell's gastrin receptor. Acetylcholine is released from the vagus nerve, and the postganglionic neurons in proximity to the parietal cells. Acetylcholine stimulates gastric acid secretion directly at the cholinergic receptors, and

indirectly stimulates G cells, and the release of gastrin, and enterochromaffin-like (ECL) cells, and the release of histamine.(Herdt 1997,Simpson 2005) In canine glands, cholinergic agents are generally the most effective of the natural secretagogues, producing a sustained response.(Soll and Berglindh 1987) Histamine is stored and released from granules of the ECL cells within the gastric mucosa.(Soll and Berglindh 1987) The ECL cells control gastric acid secretion by mobilizing histamine in response to circulating gastrin. In addition, the ECL cells are thought to operate under nervous control and to be influenced by local inflammatory processes.(Norlen *et al* 2001) There are considerable species differences in the magnitude of the response to histamine.(Soll and Berglindh 1987) These three pathways are interdependent as the messenger substances potentiate one another and augment gastric acid production beyond the sum of their individual responses.(Davenport 1982,Grossman 1981)

Acid provides an optimally low pH for pepsin activity. Pepsinogen, a group of proteolytic enzymes, is converted to pepsin in the presence of an acid. Pepsin is active in the digestion of collagen and is a potent stimulant of gastrin and cholecystokin;(Herdt 1997) however, peptic hydrolysis of protein is not necessary for digestion. Gastric acid also functions to sterilize chyme before it enters the small intestine.(Davenport 1982)

A negative feedback system exists in the stomach whereby an increasingly acidic pH stimulates the release of somatostatin. Somatostatin inhibits both the release of gastrin and gastrin-mediated release of histamine.(Simpson 2005) At a gastric pH of 2, gastrin secretion is depressed, and by pH 1, gastrin release is completely inhibited.(Grossman 1981,Herdt 1997) Gastric acid secretion is also inhibited by epinephrine, norepinephrine,

dopamine, serotonin, secretin, vasoactive intestinal peptide, glucagon, and calcitonin, as well as by prostaglandins E and I.(Grossman 1981,Simpson 2005)

Phases of gastric acid secretion

Ultimately, gastric acid secretion is stimulated by the anticipation of eating and the presence of undigested food in the stomach and small intestine. Acid secretion has been divided into several phases: basal, cephalic, gastric and intestinal.

Basal secretion occurs independent of stimulation by food sight, smell or ingestion. During the basal state, the parietal cells are stimulated by histamine and acetylcholine.(Grossman 1981,Soll and Berglinde 1987) As complete gastric emptying of solid food from the stomach of the dog is approximately 12 hours, basal acid secretion only occurs after a 12 hour fast.

The cephalic phase of gastric acid secretion is evoked by stimuli acting directly on the brain and is mediated entirely by the vagus nerve.(Grossman 1981) The effective stimuli include smell, taste, and chewing of palatable food as well as conditioned reflexes.(Davenport 1982) Hypoglycemia is another initiator of cephalic stimulation.(Grossman 1981)

The gastric phase of acid secretion is initiated by gastric distension and by the protein content of food. Gastric stimulation allows the release of gastrin into the bloodstream, which subsequently acts on the parietal cells at the gastrin receptor and stimulates gastrin-mediated histamine release. The only macronutrient known as a chemical stimulant of acid secretion is protein.(Grossman 1981) Other food types incite

gastric acid secretion by distension alone. Two-thirds of the total gastric acid secretion occurs in the gastric phase.(Moreland 1988)

The intestinal phase of gastric acid secretion is incited by the presence of food in the small intestine. Distension and protein products are again the initiators of the intestinal phase of gastric acid secretion. It is interesting to note that intravenous infusions of a mixture of amino acids stimulates acid secretion at about one third of the maximal acid output; however, the response is vagally independent (McArthur *et al* 1983) and occurs without the stimulation of gastrin.(Davenport 1982,Konturek *et al* 1978,Mariano *et al* 1984) The absorbed amino acids contribute directly to the intestinal phase of gastric acid secretion.(Konturek *et al* 1978) Amino acids infused intravenously cause stimulation of gastric acid secretion by a mechanism sensitive to distension pressure and the pH of gastric content.(Konturek *et al* 1978)

Miscellaneous causes of gastric acid output

Nervous and hormonal stimuli can affect gastric acid production. Stress causes reactions resulting in the secretion or hypersecretion of hydrochloric acid. Increased gastric acid secretions have been reported in conditions such as anxiety (Gray *et al* 1953,Hoffman 1955,Kuna 1964), frustration, (Atwater and Carson 1959) restraint, (Brodie and Marshall 1963,Menguy 1960) and exposure to noise.(Rovelstad *et al* 1952) Anger and fear usually cause a decrease in gastric acid secretion. (Eichhorn and Tracktir 1955) Strong physical activity or pain can also inhibit gastric acid secretion.(Davenport 1982) Various pharmacologic agents have also been implicated in altering gastric acid secretion. Cortisone or anterior pituitary hormone cause an increased secretion of gastric

acid (Engel 1955, Gray *et al* 1951a, Gray *et al* 1951b, Stempien and Dagradi 1954); furosemide lowers gastric acid secretion, (Ayalon *et al* 1981) and there are potential inhibitory effects of sedatives and anesthetics (e.g., acepromazine and thiopentone) on gastric acid secretion. (Bastaki and Waton 1996) In people, infection with *Helicobacter pylori* can alter gastric acid secretion and is associated with gastric ulceration (discussed under *Helicobacter* infection)

Normal gastric acid output

The rate of gastric acid secretion is known to be widely different among species. Canine intragastric pH differs from humans during both basal and maximal acid output. (Akimoto *et al* 2000, Dressman 1986, Dressman and Yamada 1991, Grossman 1981, Kararli 1995, Lin 1995) In the dog, basal gastric acid secretion is low at approximately 0.1 mEq/hr (Grossman 1981) compared to humans where gastric acid secretion in the male is 3.7 $\pm$ 2.1 mEq/hr and in the female is 2.2 $\pm$ 1.8 mEq/hr. (Dotevall 1961) However, once stimulated, peak gastric acid response is considerably higher in the dog. (Dressman 1986, Mulvihill *et al* 1985) The maximal acid output in the dog, when stimulated with histamine, is 39 $\pm$ 5 mEq/hr compared to humans where peak acid output is 23 $\pm$ 7 mEq/hr in the male and 18 $\pm$ 5 mEq/hr in the female. (Dotevall 1961, Emas and Grossman 1967, Mulvihill *et al* 1985) Overall, basal acid secretion in the dog is <1% (0.1 μ mol/min/kg) of maximal capacity (18 μ mol/min/kg.) (Lin 1995)

Basal gastric acid secretion

Basal acid secretion follows a circadian rhythm in humans with the highest secretion rate occurring in the evenings, followed by a gradual decline during the night and the lowest rate in the morning. (Grossman 1981) The cause of the circadian rhythm is unknown. The increase in nocturnal pH is postulated to be due to duodenal gastric reflux or decreased vagal tone.(Fimmel *et al* 1985) No circadian rhythm has been identified in dogs.(Grossman 1981) However, canine nocturnal gastric pH shows a dramatic reduction in acid output (pH range 6-8).(Postius *et al* 1991a)

Dogs have negligible basal acid secretion.(Papich 1993) Numerous studies report only slightly acidic or achlorhydric gastric juice indicating that gastric secretion is not continuous in the canine fasting state.(Akimoto *et al* 2000,Cavanagh *et al* 1987,Kuna 1964,Yamada *et al* 1989) Regardless, other reports continue to suggest that gastric pH usually remains in the acid range.(Lui *et al* 1986,Youngberg *et al* 1985)

Prior studies evaluating the gastric pH of fasted dogs have generated mean fasted gastric pH ranges from 1.5(Youngberg *et al* 1985) to greater than 6.(Akimoto *et al* 2000,Kuna 1964,Yamada *et al* 1989) Several studies support the fasting gastric pH in dogs as being weakly acidic to neutral (higher than pH 6.0):(Akimoto *et al* 2000,Cavanagh *et al* 1987,Kuna 1964,Yamada *et al* 1989)

Kuna recorded basal canine gastric pH over a 30-minute time interval using an *in situ* pH electrode placed perorally, in addition to gastric aspirates when obtainable. The pilot study, involving 19 tests, found that gastric pH was 6.0 or above, and the mean pH recording was 7.2. Subsequently, over 400 tests were performed in 24 dogs, and 77% of all pH recordings obtained were at 6.0 or above. Most dogs in the study had acidic

recordings ($\text{pH} < 6$) one or more times during the course of the study; however, in a few dogs, a neutral pH was obtained in every test during the study period.(Kuna 1964) The limitations to this study were that it was performed over a very short time period (30 minutes), which may not have identified fluctuations in gastric acid secretion over time, and the peroral placement of the gastric probe, although it followed an acclimatization period, may have altered gastric acid secretion.

Akimoto et al. found that the mean fasted pH of eight healthy beagle dogs was 6.8 $\pm$ 0.2, with a range of 2.7-8.3. The authors found that gastric pH was less than 5.0 in only six of the total 40 samples studied. This study collected gastric aspirates using an intubation method and measured gastric pH *ex vivo*. Fasted samples were collected from each dog on five separate occasions.(Akimoto *et al* 2000) The wide range of pH obtained in this study may have been associated with the method of sampling since the location from which the stomach aspirates were collected was not evaluated and aspirates may have been collected from the cardiac or pyloric regions of the stomach and not have been reflective of true gastric, fundic secretion. In addition, gastric acid collection via aspiration, in and of itself, may have incited differing degrees of gastric acid secretion. Finally, single samples, although performed on 5 separate occasions, may not be indicative of canine gastric pH over a full 24-hour period.

Yamada and colleagues evaluated gastric pH profiles in six fasting beagle dogs over an 8-hour period. Results revealed wide inter- and intra-individual variation, with pH ranging from 0.9-7.0. This study monitored gastric pH using an *in situ* pH electrode placed perorally at each time of measurement. Five recordings were collected over the 8-hour time interval.(Yamada *et al* 1989) Again, the wide range in pH may have been

attributed to the peroral insertion of a pH sensor, which may alter gastric acid secretion.

In addition, this study had an intermittent and short sampling period (5 pH determinations in an 8-hour period), which could miss trends in gastric acid secretion.

Simpson et al. evaluated basal gastric acid secretion in six healthy anesthetized beagle dogs using continuous gastric aspiration over a 30-minute period. The pH varied widely among dogs, ranging from 3.3 to 7.8. It was noted that gastric secretion during the basal period was viscous and low in volume and the investigators were therefore unable to reliably determine titratable acidity. In such circumstances, gastric acid secretion was determined by pH measurement alone.(Simpson *et al* 1999) The sedatives and general anesthetics used in the study may have altered gastric acid secretion. Secretion may also have been affected by varying attempts at obtaining gastric aspirates. Finally, in these six dogs, basal gastric acid secretion was only determined for a single 30 minute sample and may not reflect the true gastric pH in a larger sample size over a longer sampling period.

Conversely, Youngberg et al. report that the fasted canine gastric pH remains low.(Youngberg *et al* 1985) Their findings, evaluating gastric pH in four beagle dogs using radiotelemetric pH measurements, on two separate occasions, revealed that the mean fasting canine gastric pH was 1.5 (range of 0.9-2.5). The gastric pH recordings from the radiotelemetric pH capsule were obtained during the initial 30-minute period that the capsule remained in the stomach. (Youngberg *et al* 1985) These investigators used a novel pH recording system that required swallowing of the radiotelemetric Heidelberg capsule. Since swallowing can stimulate the cephalic phase of gastric acid secretion, this would lower pH and therefore may not have accurately documented basal acid secretion. This may account for the lower pH recordings obtained in this study.

Lui and colleagues also evaluated basal gastric acid secretion in four healthy beagle dogs, on three separate occasions, using the same radiotelemetric device (Lui *et al* 1986). The gastric pH in this study averaged 1.8 (range 0.5-3.9). The gastric pH recordings from the radiotelemetric pH capsule were obtained until the pH-monitoring device was emptied from the stomach, which averaged 100 minutes. The investigators defined gastric emptying as the time elapsed between time of capsule administration and the time at which sustained elevation in the pH was observed. The authors assumed that the rise in pH implied movement of the capsule into the small intestine. This may be a false assumption. High pH readings may have been recorded from the stomach and inappropriately identified as recordings from the small intestine generating an inaccurate mean gastric pH value. Another limitation of this study involves validation of the capsule when compared to a pH meter, using standardized buffer solution. Validation identified that, for pH values of approximately 2.5 to 6.5, the capsules tended to underestimate the pH of the buffer solution, and that from pH 7 to 8 the readings were slightly overestimated. The under- and overestimations of pH did not exceed ± 0.5 pH units; however, considering the logarithmic nature of pH, a change in pH of 0.5 reflects a large shift in hydrogen ion concentration. The margin of error is larger using this method than what is considered an acceptable drift of 0.1-0.2 pH units using pH meters.

In summary, significant differences are noted among the studies in the methods used for determining gastric acid secretion as well as the time interval over which samples were collected. The discrepancies in the results may be related to sample size, the duration of pH recording, and the method for pH determination (intermittent versus continuous; gastric aspirates versus pH probes versus radio-telemetry). The studies

evaluated herein have all examined basal acid secretion in the dog after a minimum 12-hour fast. However, where a low pH has been obtained, residual ingesta or acid may remain incompletely evacuated from the previous meal.(Davenport 1982) Overall, reports are conflicting, with many documenting high stomach pH in the fasting dog, and other reports identifying that dogs retain a low gastric pH despite low basal acid output. One reliable finding across many studies is that the gastric pH of beagle dogs is variable, and gastric pH is most variable in the fasting state.(Dressman 1986,Simpson *et al* 1999,Yamada *et al* 1989)

Stimulated gastric acid secretion

Human studies have identified that transient elevations in pH are observed after each meal.(Fimmel *et al* 1985) A meal neutralizes and dilutes acid in the stomach, with a transient increase in pH of approximately one hour's duration (pH 5.0 with a range of 4.3-5.4).(Kararli 1995) However, gastric acid is released in response to eating and the natural stimulus of food causes peak gastric acid output. In people, food can stimulate gastric acid secretion to the same degree as that achieved pharmacologically with maximal histamine stimulation, or a maximal dose of exogenous gastrin.(Davenport 1982,Fordtran and Walsh 1973) In the dog, food stimulates gastric secretion beyond that achieved by pharmacological intervention.(Brooks 1985) As acid secretion proceeds, the pH returns to premeal values over a period of 60-90 minutes.(Dressman 1986) The initial post-prandial pH peak which occurs in humans is blunted in the dog.(Youngberg *et al* 1985)

Youngberg and colleagues determined basal and post-prandial gastric pH, in four beagle dogs, on two separate occasions using continuous recordings from radiotelemetric equipment. Post-prandial gastric pH ranged from 0.5 to 3.5 with a mean of pH 2.1 $\pm$ 0.4;(Dressman 1986) which was higher than at baseline (mean pH 1.5 $\pm$ 0.4). The authors suggested that this was probably due to the buffering action of food. The post-prandial gastric pH profiles illustrated a wave-like pattern; however, the sharp increase in pH followed by a strong downward trend which has been documented in humans, was not observed in the dogs. Statistically there was no change in pH over the first post-prandial hour.(Youngberg *et al* 1985) The lack of marked buffering by food may be attributed to increased gastric acid production in the dog.(Youngberg *et al* 1985)

Previous reports have documented that gastric pH response varies with the type of meal ingested.(Richardson *et al* 1976) The secretory response to food in the stomach is chiefly determined by the protein content of the food. Protein is the most powerful stimulant for gastrin release and, conversely, neutralizes gastric acid by its presence in the stomach.(Brooks 1985,Davenport 1982) Despite glucose and fat both inciting the release of a small amount of gastrin, the effects of glucose on gastric acid secretion are negligible, while fat stimulates powerful inhibitors of secretion with a net decrease in gastric acid production.(Davenport 1982,Richardson *et al* 1976) Distension is also an important stimulant of the acid-secretory response to a meal and this is mediated by a gastrin-independent mechanism.(Richardson *et al* 1976)

1.3- Pathogenesis and pathophysiology of gastric ulceration

Gastrointestinal (GI) ulceration is a disruption in the mucosal lining of the stomach or small intestine. An ulcer is a mucosal defect extending through the lamina muscularis mucosae into the submucosa or deeper, whereas an erosion does not penetrate the lamina muscularis mucosae.(Moreland 1988) Ulcers can occur in any region of the gastrointestinal tract including the gastric fundus, antrum, pylorus or small intestine.(Stanton and Bright 1989) The distribution of GI ulcers is based on the underlying condition. Conflicting results appear in the literature, where in both dogs and humans, some studies report that GI ulceration is most common in the duodenum(Moreland 1988,Soll and Isenberg 1983), and others document that ulcer location is more common in the stomach.(Silverstein *et al* 1981,Stanton and Bright 1989) However, when gastric ulcers develop, these have a predisposition for the gastric fundus, antrum, and pylorus.(Stanton and Bright 1989) Ulcer disease results from an imbalance between gastric acid secretion and the gastric mucosal defense mechanisms. The pathogenesis is multifactorial. Gastric acidity plays a role in ulcerogenesis; however, the degree of gastric acidity is not the only cause of mucosal injury since erosions and ulcers can occur in areas of anacidity. Other factors include increased bile reflux, decreased mucosal perfusion, and decreased delivery of bicarbonate to the protective mucous layer.(Davenport 1982) Acidosis may also play a role in ulcer formation. Systemic acidosis has been shown to increase the hydrogen ion concentration at the mucosal surface.(Soybel *et al* 1996)

Mucosal Defense Mechanisms

Several defense mechanisms exist for protecting the gastrointestinal tract from mucosal damage. Failure of any single defense mechanism has little effect on maintaining mucosal integrity, however, a combination of failures allows extension of a superficial erosion through the muscularis mucosae into the submucosa.(Allen *et al* 1993,Shorrock and Rees 1988)

1. Bicarbonate secretion

Bicarbonate neutralizes hydrochloric acid within the gastric lumen and is produced by the gastric, pyloric and duodenal mucosae.(Feldman 1983) The parietal cells produce bicarbonate and hydrochloric acid via carbonic anhydrase.(Sachs 1987) Bicarbonate secretion is stimulated by prostaglandins (E class), cholinergic agents, and glucagons, and inhibited by NSAIDs, alpha-adrenergic agonists, and bile salts.(Feldman 1983) Bicarbonate diffuses into the gastric lumen down its concentration gradient through large pores into the mucus layer on the surface of the cells where it neutralizes gastric acid and provides a protective barrier.

2. Mucus secretion

Mucus serves to lubricate food and protects against mechanical injury to the stomach.(Florey 1962) In addition, it combines with bicarbonate to form a thick alkaline layer that traps and neutralizes inward moving H^+ ions. Mucus secretion is stimulated by prostaglandins, and inhibited by NSAIDs.(Kerss *et al* 1982)

3. Gastric epithelial barrier

The epithelium is composed of tight junctions and a hydrophobic lipoprotein layer, composed of active phospholipids which prevent back-diffusion of H^+ ions.(Davenport *et al* 1968,Hills *et al* 1983)

4. Mucosal blood flow

A dense microcirculation supplies the surface epithelium. These capillaries supply the bicarbonate that diffuses into the gastric lumen and rapidly removes H^+ ions and inflammatory mediators that may have diffused across the mucous layer.

Maintenance of gastric mucosal bloodflow is essential for ongoing protection and repair of the mucosal layer.(Starlinger *et al* 1981)

5. Rapid cell-turnover

Injured epithelium is repaired in two to four days due to the rapid replication of the mucosal epithelium.(Matz 1995) In addition, restitution, or migration of adjacent healthy epithelial cells, rapidly covers minor damage to the epithelial surface within 15-30 minutes.(Lacy 1987)

6. Production of cytoprotective agents

Prostaglandins E_2 (PGE_2) and I_2 (PGI_2) directly inhibit histamine activation of the parietal cell.(Soll and Berglindh 1987) In addition to modulating gastric acid secretion, PGE_2 increases mucosal blood flow, HCO_3^- and mucus secretion, and cell division and restitution.

Predisposing risk factors in veterinary medicine

In veterinary medicine, GI ulceration is most commonly seen with the use of non-steroidal anti-inflammatory drugs (NSAIDs). Hepatic disease, both acute and chronic, is

the most common medical condition associated with ulcer disease, however many other conditions have also been reported in association with gastroduodenal ulceration.(Stanton and Bright 1989) Gastroduodenal ulceration has been reported with the use of non-steroidal anti-inflammatory drugs (NSAIDs)(Cavanagh *et al* 1987,Jenkins *et al* 1991,Johnston *et al* 1995,Murtaugh *et al* 1993) and corticosteroids.(Boston *et al* 2003,Fenster 1973) Gastrointestinal ulceration has also been noted secondary to stress in humans, this is less well identified in dogs, however canine GI ulceration is documented secondary to shock, multiple trauma and disseminated intravascular coagulation (DIC).(Stanton and Bright 1989) The pathogenesis is secondary to vasoconstriction and ischemia to the gastric mucosa. Neurological disease and intervertebral disc disease, with or without the administration of corticosteroids, are associated with gastric mucosal lesions. (Dowdle *et al* 2003,Neiger *et al* 2000). Other causes include uremia, (Cheville 1979) mast cell tumours (MCT) and gastrinomas, (Brooks and Watson 1997,Fox L.E. *et al* 1990,Howard *et al* 1969,Stanton and Bright 1989) Zollinger-Ellison syndrome, (Happe *et al* 1980) gastroduodenal neoplasia, (Wright 1981) lymphocytic/plasmacytic and eosinophilic gastroenteritis, (Stanton and Bright 1989) hypoadrenocorticism (Addison's disease), (Medinger *et al* 1993) and pancreatitis. Pancreatitis has been reported as an underlying cause of gastroduodenal ulceration in dogs, although few reports exist documenting this association in companion animals.(Middleton *et al* 1983)

Helicobacter infection

Helicobacter organisms are gram-negative, microaerophilic, spiral-shaped bacteria, isolated from the stomach of humans, non-human primates, cats, dogs, ferrets,

pigs, and cheetahs. *Helicobacter spp.* infection is closely associated with gastric ulcer disease in humans.(Pawlik *et al* 2002) In people, *H. pylori* exerts diverse effects on gastric acid secretion. It can result in increased, decreased, or unchanged gastric acid production. The effects on acid secretion depend on the area of stomach affected (fundus or antrum), and the degree of mucosal atrophy produced by the gastritis.(McColl *et al* 2000)

The prevalence of gastric *Helicobacter* in dogs is high with reports ranging from 61-100% in both clinically healthy dogs and in those with gastrointestinal signs.(Eaton *et al* 1996,Geyer *et al* 1993,Happonen *et al* 1996,Yamasaki *et al* 1998) The gastritis observed in infected dogs is less severe than that observed in *H. pylori*-infected humans.(Simpson *et al* 1999) To date, studies have failed to determine the significance of helicobacter infection in dogs.(Simpson *et al* 2000)

Simpson and colleagues evaluated gastric acid secretion in six uninfected beagles and in eight beagles affected with naturally acquired *Helicobacter* infection. Dogs were anesthetized during the study and gastric secretion was determined by orogastric aspiration and *ex vivo* measurement of pH. Basal acid determinations were performed on gastric juice accumulated over a single 30-minute period. Infected dogs had a gastric pH ranging from 1.2-7.9, and uninfected dogs had gastric pH ranging from 3.3-7.8, with no significant difference in basal pH between groups. Subsequently gastric acid secretion was maximally stimulated in both groups of dogs with pentagastrin (a gastrin analogue). Results demonstrated that uninfected dogs had a lower gastric pH during maximal acid output than did infected dogs ($p<0.01$). (Simpson *et al* 1999) The authors reported an overall absence of abnormalities in gastric acid output despite substantial colonization

with *H. felis* and *H. bizzozeronii*-like organisms and argued that a direct inhibitory, or secretory, effect of these bacteria on gastric acid production in the dog was not substantiated. A lack of inflammation in the stomach of affected dogs, based on histopathology, may explain this finding. The authors concluded that infection with *H. felis* and *H. bizzozeronii*-like bacteria was not associated with marked perturbations in the gastric-secretory axis or gastric structure of dogs.(Simpson *et al* 1999)

Regardless of the statements made by these authors, a significant difference was noted in maximal acid stimulation between dogs infected and uninfected with *Helicobacter* organisms, and any study evaluating the effects of gastric acid secretion should, to be prudent, identify the *Helicobacter* status of the dogs under investigation.

Role of acid suppression

Extensive research has been conducted in human medicine evaluating the role of acid suppression for the prevention or healing of gastroduodenal ulceration. Large clinical trials have identified the groups of critically ill patients most at risk for upper gastrointestinal ulceration and those where gastrointestinal prophylaxis confers significant reduction in bleeding episodes.(Cook *et al* 1999,Cook *et al* 1991,Cook *et al* 1994) This information allows a more selective use of gastrointestinal prophylaxis. No such investigations have been performed in veterinary medicine and the use of acid-lowering therapy (as gastroprotectants) is extensive and has been recommended in all conditions where gastrointestinal ulceration has previously been documented in both human and veterinary patients.(Haskins 2000,Matz 1995,Moreland 1988,Richter 1992,Stanton and Bright 1989)

Once ulcer disease is identified, the goal of antisecretory therapy is to reduce gastric acid secretion, prevent damage to the gastric mucosa and allow regenerative mucosal mechanisms to prevail. Human studies have established that the healing of acid-related disorders (gastric and duodenal ulcers and erosive esophagitis) is highly correlated with the degree of gastric acid suppression.(Huang and Hunt 2001) These studies have also identified that the optimal degree of acid suppression will vary with the underlying disease process.(Colin-Jones 1990) The optimal acid intragastric pH for healing of duodenal ulcers in people is above 3 for a period of 18-20 hours per day (~75% of the day); little benefit is noted with more extensive suppression.(Burget *et al* 1990) Gastroesophageal reflux disease is optimally managed with intragastric pH above 4 for 16-22 hours of the day.(Bell *et al* 1992) A critical pH threshold of 4 is also recommended for the prevention of stress-related gastrointestinal ulcers in the critically ill patient as a pH above 4 inhibits pepsin and inactivates fibrinolysis.(Fennerty 2002) Acute gastrointestinal bleeding requires a pH greater than 6 to achieve hemostasis since this allows adequate platelet aggregation and prevents the dissolution of blood clots.(Green, Jr. *et al* 1978,Li *et al* 2000) A recent study demonstrated that platelet aggregation *in vitro* is normal at pH 7.4; however, at pH 6.8 platelet aggregation is inhibited and at pH 6.2 platelet aggregation is abolished.(Li *et al* 2000) In addition, these researchers documented a significant decrease in gastric mucosal bleeding times (GMBT) in rats when the pH levels were above 6.4.(Li *et al* 2000)

The degree of gastric acid suppression necessary for treating acid related disease in veterinary patients has not been established. Studies on platelet aggregation have been evaluated and the results are similar in several non-canine species, they are likely similar

for canine patients as well. As such, a target goal of pH 6 is likely necessary when treating acute gastrointestinal bleeding in the dog. When treating gastrointestinal ulcers, pH goals may differ for the canine patient due to differences in gastric physiology. Regardless, when extrapolating from human patients, it is likely that the degree and duration of gastric acid suppression, and the length of therapy will correlate with ulcer healing. Studies are necessary to identify target pH goals when treating gastrointestinal hemorrhage, erosions, or ulcers.

Adverse effects of acid suppression

While acid suppression has therapeutic benefits, potential adverse effects must be considered. Gastric acid is a primary bactericidal barrier; prolonged gastric acid suppression has demonstrated effects on enteric infections and gastric bacterial colonization.(Ortiz *et al* 1998,Williams 2001) Increased gastric pH allows colonization of the stomach with bacteria, and predisposes the patient to pneumonia should reflux and aspiration of gastric contents occur.(Cook *et al* 1996,Heyland and Mandell 1992) In addition, a hypothesis for acid reduction leading to carcinogenesis has been suggested. High doses of omeprazole result in pronounced acid inhibition and increased plasma concentrations of gastrin in all species studied.(Ekman *et al* 1985) Toxicology studies using omeprazole in rats have identified proliferation of the gastric endocrine cells (ECL cells) with formation of focal gastric carcinoids.(Ekman *et al* 1985) However, sustained hypergastrinemia with excessive and prolonged proton pump inhibitor (PPI) therapy (12 month duration), did not show these same effects in dogs, but resulted in reversible gastric mucosal and muscular hypertrophy.(Ekman *et al* 1985) Subsequent investigations

in people have identified that long-term (11 years) use of PPIs is effective and safe, with no dysplasia or neoplasia observed on gastric biopsies.(Klinkenberg-Knol *et al* 2000) Another identified problem is rebound gastric acid secretion on discontinuation of histamine₂ receptor antagonist (H₂RA) therapy.(Nwokolo *et al* 1991) The mechanism of rebound acid hypersecretion is not clearly understood, but, the phenomenon is not associated with hypergastrinemia.(Nwokolo *et al* 1991)

As a result of some of these concerns, a therapeutic approach advocating moderate acid suppression has been adopted, and optimal acid suppression should be directed towards increasing gastric pH by the minimum required to allow GI repair based on the underlying disease condition. As Burget and colleagues have pointed out, little benefit is noted when gastric pH is maintained at greater than 3 during duodenal ulcer healing.(Burget *et al* 1990) A similar approach should be undertaken in veterinary medicine to decrease exposure of dogs to unnecessary medications. For this to occur target pH goals to optimize healing of gastrointestinal lesions must be identified in addition to the degree of gastric acid suppression required by various gastric-acid lowering medications in order to attain these pH goals.

1.4- Acid-lowering pharmacologic agents

Acid-lowering pharmacologic agents are gastroprotectants that encompass several different classes of drugs used to treat and prevent gastrointestinal ulceration.

Histamine receptor₂ antagonists (H₂RAs)

Cimetidine, ranitidine and famotidine are reversible H₂-specific receptor antagonists. They bind to the H₂ receptor on the basolateral membrane of the parietal cell and competitively inhibit binding of histamine with a resultant decrease in gastric acid secretion. H₂RAs also inhibit gastric acid secretion controlled by gastrin and vagal pathways.(Soll and Berglindh 1987)

The H₂RAs differ slightly in their structure, but have many similarities in their pharmacological properties. Formulations are available for both oral and parenteral administration. H₂RAs can be administered intravenously, as well as intramuscularly and subcutaneously.

H₂RAs differ in their potency (famotidine > ranitidine > cimetidine). Potency of the H₂RAs relates to the competitive nature and the affinity for binding of the H₂ blocker to its receptor.(Huang and Hunt 2001) Ranitidine is four to ten times more potent than cimetidine, and famotidine 20-40 times more potent than cimetidine.(Matz 1995,Schunack 1987) In addition, famotidine has a longer duration of action, especially in hypersecretory states.(Friedman 1987,Schunack 1987) However, increased potency does not correlate to increased efficacy.(Papich 1993) In human medicine, all H₂RAs are about equally effective for treating or preventing GI ulcers when equipotent dosages are administered.(Feldman and Burton 1990,Schunack 1987) A dose response is identified

with the H₂RAs, with higher doses yielding significantly more gastric acid suppression.(Smith *et al* 1985,Soll and Berglindh 1987)

In addition to the H₂RAs anti-secretory effects, some of these drugs have a prokinetic effect on gastrointestinal motility. Ranitidine has prokinetic properties mediated by inhibition of acetylcholinesterase activity. The prokinetic action is not equal among H₂RAs (ranitidine > cimetidine > famotidine).(Parkman *et al* 1998)

Cimetidine, the first H₂RA, is an imidazole derivative.(Schunack 1987) The serum half life is 1.3 hours in the dog.(Plumb 2002) The drug is both metabolized in the liver and excreted unchanged in urine, with more drug excreted via the kidneys during parenteral administration (75%) then during oral dosing (48%). Cimetidine does inhibit the hepatic microsomal enzyme system by binding directly to certain species of cytochrome P-450 thereby altering the metabolic rates of other drugs dependent on this system. Cimetidine reduces the metabolism, prolongs serum half-lives, and increases the serum levels of several drugs including beta blockers, lidocaine, calcium channel blockers, benzodiazepines, ethanol, metronidazole, phenytoin, quinidine, theophylline and warfarin.(Plumb 2002) Cimetidine is recommended at 5-10 mg/kg every 6 to 8 hours. Due to its increased frequency of administration, and its interaction with the cytochrome P450 system, cimetidine's use has been replaced by later generation H₂RAs, which require less frequent administration and have fewer drug interactions.

Ranitidine was the second H₂RA launched on the human market. Ranitidine given at a rate of 2 mg/kg, orally or parenterally, three times a day in dogs has been calculated to inhibit gastric acid secretion by at least 50% throughout the day.(Papich 1993) The drug has a serum half-life of 2.2 hours in dogs, with a duration of action of 8-12 hours. It

is metabolized in the liver to inactive metabolites. Ranitidine reversibly binds to the hepatic cytochrome P-450 enzyme system and can alter the clearance of drugs metabolized by the same route, but this is usually clinically insignificant.(Dowling 1995) Drug elimination is via renal clearance.

Famotidine is a later generation H₂ blocker and is more potent than ranitidine.(Hogan *et al* 1993) It contains a thiazole ring structure which differs chemically from cimetidine and ranitidine.(Friedman 1987) Famotidine, based on its structural class, has exceptional H₂ receptor affinity, and is 8-9 times more potent than ranitidine.(Friedman 1987,Schunack 1987) Famotidine administered parenterally has a serum half-life of 2-3 hours. It has been demonstrated in dogs to have a significantly longer duration of action than ranitidine.(Pendleton *et al* 1983) Famotidine is partially metabolized by the liver (1/3 of the dose if administered intravenously and 2/3 of the dose when administered orally); the remainder is eliminated virtually unchanged in the urine.(Plumb 2002) Famotidine does not appear to have any effects on the cytochrome P-450 enzyme system and therefore does not alter the metabolism of other drugs.(Dowling 1995) Famotidine has previously been recommended at a dose of 0.5 mg/kg orally or parenterally, once to twice a day, again to decrease gastric acid secretion by 50% throughout the day.(Papich 1993) More recently, famotidine has been recommended at 0.1 – 0.2 mg/kg every 12 hours.(Allen *et al* 2005) Famotidine has not been developed for veterinary licensure and as such, dose recommendations are based on extrapolation from early investigational studies using the canine and feline model. Washabau has determined the lower dose for famotidine based on available literature (personal communication). To support a lower recommended dose for famotidine, Washabau cites reports which

document that famotidine at 0.01-0.03 mg/kg demonstrates a dose-dependent inhibition on histamine(Uchida *et al* 1993), and famotidine at 0.13 mg/kg maximally inhibits acid secretion(Katz *et al* 1987).

H₂RAs have minimal adverse reactions and are considered very safe in humans and dogs.(Burek *et al* 1985,Plumb 2002,Schunack 1987) Several problems have been identified with the use of H₂RAs. A lack of response has been noted in some human patients; these patients may have increased hyperacidity associated with increased vagal tone. H₂RAs are not effective in inhibiting meal-stimulated acid secretion.(Netzer *et al* 1999a) As previously discussed, rebound gastric acid hypersecretion and relapse of gastrointestinal ulceration has been reported after drug discontinuation.(Nwokolo *et al* 1991) In human medicine, tachyphylaxis has also been demonstrated with the use of H₂RAs over several days;(Netzer *et al* 1999b,Wilder-Smith and Merki 1992) to date no such reports have been described in veterinary medicine.

Proton pump inhibitors (PPIs)

Omeprazole and pantoprazole are proton pump inhibitors (PPIs) that block the H⁺/K⁺ATPase enzyme pump on the apical surface of the parietal cell. These substituted benzimidazoles are acid-activated sulphydryl (SH) agents that bind irreversibly to the ATPase and block the action of the proton pump.(Pisegna 2002) PPIs must first be absorbed in the duodenum and subsequently secreted into the acidic gastric parietal cell. Once inside the cell, in the presence of acid, these drugs become protonated into their active form and generate sulphydryl (SH)-reactive compounds that are cationic and trapped in the cell. The SH-reagents bind with available SH groups on the H⁺K⁺ATPase

enzyme resulting in its irreversible inhibition.(Sachs 1987). In contrast to receptor antagonists, proton pump inhibitors block histamine-2, gastrin- and cholinergic-mediated sources of acid production, and inhibit gastric acid secretion at the final common pathway of the H^+/K^+ ATPase proton pump.(Pisegna *et al* 1999)

PPIs take several days to reach steady state because they bind only to the proton pumps that are actively secreting acid, sparing inactive pumps which are resting in the cytosol.(Larsson *et al* 1983) Approximately 25% of parietal cells are in a resting phase at any given time and will thus escape inhibition; several days of therapy are required to inactivate all pumps.(Larsson *et al* 1983,Stedman and Barclay 2000) In dogs, 3-5 days are required before maximum action is noted.(Larsson *et al* 1985) PPIs are irreversible inhibitors of the ATPase pump and as such, long-acting *in vivo*. Recovery of acid secretion after PPI inhibition requires *de novo* synthesis of pump protein and/or disulfide reduction, and reactivation of inhibited pumps.(Shin and Sachs 2002) H^+/K^+ ATPase pump half-life has been reported to be approximately 25 hours in various species,(Sachs 1987) and more recently to have a half-life of production of approximately 50 hours.(Sachs *et al* 1995). In humans, the half-life of the inhibitory effect on acid secretion is approximately 28 hours for omeprazole and approximately 46 hours for pantoprazole.(Shin and Sachs 2002) Because of their long-duration of action PPIs are administered once daily in dogs.(Foushee 2000)

In North America, omeprazole is available in an oral formulation only, however a parenteral formulation is available in Europe and has been extensively studied. Pantoprazole is available in oral and parenteral formulations, and is currently the only injectable PPI available in North America.

Omeprazole is recommended at 0.7mg/kg orally once a day in dogs.(Papich 1993,Plumb 2002) Omeprazole is 5-10 times more potent than cimetidine.(Larsson *et al* 1983) The elimination half-life is approximately 1 hour independent of its route of administration.(Larsson *et al* 1983,Larsson *et al* 1985) Elimination is dependent on hepatic metabolism.(Plumb 2002) Omeprazole is metabolized by the cytochrome P-450 enzyme system to inactive metabolites, 80% of the metabolites are recovered in urine, and the remainder excreted in feces.(Foushee 2000) Omeprazole partially inhibits the cytochrome P-450 pathway, and therefore, may alter clearance of other drugs utilizing this pathway for metabolism.(Papich 1993)

Prior to this study, no dosing recommendations were published for pantoprazole in veterinary medicine. Recommendations for dosing in our institution were extrapolated from human dosing recommendations, and from information generated in the development of the drug for human use. Animal pharmacological studies identified that in the dog, pantoprazole showed a median effective dose (ED₅₀) range of 0.2-2.4 mg/kg, and a dose of 1.2 mg/kg significantly elevated gastric pH to values between 4 and 7 after a 5-day dosing regimen.(Panto IV Product Monograph, 2002) Dosing recommendations at the Ontario Veterinary College are 1 mg/kg, max 30 mg/dog, IV every 24 hours. Recently, in the veterinary literature, pantoprazole has been recommended at this dose based on our experience in the ICU.(Allen *et al* 2005) Pantoprazole has an elimination half-life of approximately 0.7 hours in the dog.(Bachouchi 2003) Like all PPIs, it undergoes extensive hepatic metabolism and conjugation. Pantoprazole has minimal, to no, interaction with the cytochrome P-450 system, no food effect, and no active

metabolites.(Kromer *et al* 1990,Pisegna 2002) Drug interactions have not been reported with this medication.(Pisegna 2002,Stedman and Barclay 2000)

When comparing omeprazole to pantoprazole, human and animal studies suggest that pantoprazole and omeprazole have a similar potency on a milligram per milligram basis.(Kromer *et al* 1990,Stedman and Barclay 2000). Differences exist however in their pharmacology and pharmacological effects. In humans the half-life of the inhibitory effect on acid secretion differs between these two compounds (omeprazole 28 hours; pantoprazole 46 hours.)(Shin and Sachs 2002) Their different durations of action may reflect different rates of pump reactivation. Omeprazole's activity (and that of all other PPIs, excluding pantoprazole) relies partially on the reversal of the inhibitory disulfide bond; in contrast, pantoprazole activity depends entirely on new protein synthesis.(Shin and Sachs 2002) These compounds also differ in the pKa at which the drugs become activated. Both maintain a pKa below 5 and are thus active within the acid environment of the parietal cell, and less likely to be trapped in spaces outside the parietal cell. However, omeprazole (pK_a 4) has a relatively higher pKa than pantoprazole and achieves greater stability at neutral pH.(Sachs 1987) Pantoprazole (pK_a 3.96) has a lower pKa, which offers the advantage of being activated only in the highly acidic canalicular space rather than in other tissue sites with a higher pH, such as the kidneys, brains or intestines.(Pisegna 2002) Overall, when compared to omeprazole, pantoprazole has a higher stability at moderately acidic pH values, less inhibitory potential against cytochrome P-450 and a longer duration of action. However, a recent human study evaluated several different orally administered PPIs and results demonstrated that

omeprazole consistently maintained gastric pH above 4 for a longer period of time (11.8 h), than pantoprazole (10.1 h).(Miner, Jr. *et al* 2003)

PPIs have minimal adverse effects. Mild elevations in liver enzymes (alanine aminotransferase (ALT) and aspartate transaminase (AST)) have been reported. Diarrhea has also been reported.(Stedman and Barclay 2000) As previously discussed, increased gastrin levels associated with prolonged PPI use, causes reversible trophic effects on the gastric mucosa and muscularis layers.(Ekman *et al* 1985) Tolerance has not been noted with prolonged PPI therapy.(Klinkenberg-Knol *et al* 2000,Netzer *et al* 1999b)

Omeprazole suspension

As many critically ill patients are not able to take oral medication, the parenteral route for administration is required. However, until recently no parenteral benzimidazole was available in North America. An alternative method for the delivery of this agent was a liquid suspension of omeprazole capsules (Prilosec – USA), or of enteric-coated tablets (Losec – Canada), which could be administered via feeding tube (nasogastric or gastrostomy tube). Administration of omeprazole suspension via feeding tube is commonly performed in human patients.(Balaban *et al* 1997,Larson *et al* 1996,Mohiuddin *et al* 1997,Phillips *et al* 1996,Sharma *et al* 1999) At the time of performing this study, a parenteral PPI was not available in the United States and therefore, evaluation of the omeprazole suspension in dogs was considered necessary.

Benzimidazole derivatives (i.e. omeprazole, pantoprazole), are lipophilic weak bases that are chemically unstable in an acid environment (Larson *et al* 1996,Pilbrant and Cederberg 1985). Drug delivery must withstand the acid environment of the stomach for

subsequent release of the drug in the neutral or alkaline duodenum. Designing a formulation requires that the drug remain protected against degradation in the acid stomach. An intragastric suspension was prepared by mixing the contents of the enteric-coated granules of omeprazole with sodium bicarbonate solution, the latter allows complete dissolution of the granules and appears to protect the drug from destruction by gastric acid.(Lasky *et al* 1998,Phillips *et al* 1996) Simplified omeprazole suspension prevented clinically significant bleeding and successfully inhibited gastric acid secretion.(Balaban *et al* 1997,Larson *et al* 1996,Phillips *et al* 1996,Sharma *et al* 1999)

Other formulations of omeprazole have been evaluated in human medicine.(Larson *et al* 1996,Mohiuddin *et al* 1997) Omeprazole enteric-coated granules mixed with water,(Larson *et al* 1996) orange juice, Alka Seltzer, or apple sauce (Mohiuddin *et al* 1997) have demonstrated comparable gastric acid suppression to that of the intact capsule.

Concerns with formulating suspensions include opening the capsule and releasing the enteric-coated granules, or crushing and effectively removing the enteric-coated protection from omeprazole tablets. Granules may be lost (up to 10%), damaged during nasogastric or gastrostomy tube administration, or may prematurely be degraded on exposure to acid stomach contents.(Larson *et al* 1996) When omeprazole is combined with sodium bicarbonate added issues include the extent to which the bicarbonate contributes to increasing gastric pH, and potential side effects associated with this formulation. Other problems associated with delivery of PPIs through feeding tubes are changes in bioavailability, and/or adherence to the plastic tube.(Pisegna 2002)

PPIs versus H₂RAs

Differences have been noted in the efficacy of PPIs vs. H₂RAs in human and veterinary patients (Huang and Hunt 2001, Jenkins *et al* 1991) and it is these observations that prompted their evaluation in this study. Previous human and animal studies have reported that PPIs, due to their irreversible binding of the proton pump, have proven to be more effective gastric acid suppressants than the H₂RAs. (Fennerty 2002, Kromer *et al* 2000, Larsson *et al* 1983)

In human studies, PPIs result in a prolonged and highly effective inhibition of both basal and stimulated gastric acid secretion, whereas H₂RAs are more effective at inhibiting intragastric acidity during periods of basal acid secretion, and they only partially inhibit the acid stimulated by gastrin. (Huang and Hunt 2001) Both famotidine and ranitidine have demonstrated a slightly higher antisecretory effect in the basal state when compared to that in the presence of food. (Netzer *et al* 1999a) Food intake thus compromises the antisecretory efficacy of both drugs. In addition, there appears to be a shorter duration of action when these drugs are administered during the day. (Fimmel *et al* 1985) For maximal therapeutic efficacy, the last dose of an H₂-receptor antagonists must be taken after the last meal of the day. (Johnston and Wormsley 1988) As the longest period of basal acid secretion occurs at night, dosing after an evening meal or at bedtime is optimal for these agents. For PPIs, efficacy does not appear to be significantly affected by food; (Foushee 2000) and the effects of omeprazole in humans, is the same at night as in the morning. (Sachs 1987) Once daily dosing in the morning is more effective than dosing in the evening with respect to the suppression of gastric acid. (Huang and Hunt 2001)

Ranitidine, appears to be one of the most commonly prescribed gastroprotectants in dogs. In people, ranitidine has been associated with tachyphylaxis, which is reported to occur between 48 and 72 hours post-administration, thus raising the question of potential efficacy beyond this period.(Fennerty 2002) No studies have been performed in dogs to evaluate whether tachyphylaxis develops in this species. Conversely, PPIs are reported to improve gastric acid inhibition after repeated once-daily dosing.(Larsson *et al* 1985,Stedman and Barclay 2000) Human studies have documented that higher doses of omeprazole provide a much more predictable gastric acid inhibition than do lower doses (Huang and Hunt 2001) and, where higher doses are needed, PPIs must be given twice daily to achieve an optimal suppression of 24-hour intragastric acidity.(Huang and Hunt 2001)

1.5- Veterinary studies

There is a paucity of clinical studies evaluating the use of gastroprotectants in veterinary medicine. Few large animal studies have evaluated the effects of different gastroprotectants on gastric pH in llamas, horses and sheep.(Drew *et al* 1992,Sangiah *et al* 1989) A few small animal comparative studies have endoscopically evaluated the effects of different acid-lowering therapies on mechanically- or NSAID-created gastric ulcers.(Cavanagh *et al* 1987,Jenkins *et al* 1991) However, studies evaluating the direct acid-lowering effects of gastric acid suppressants for establishing potential guidelines for therapeutic use in the dog have not been reported. A large body of information exists in the human literature where a canine model was employed during development of these drugs for human use, and results from these studies offer insight into the efficacy and duration of action of gastroprotectants in dogs. However, as the resting gastric pH in dogs is higher than in humans;(Akimoto *et al* 2000,Cavanagh *et al* 1987,Kuna 1964,Yamada *et al* 1989) acid secretion is frequently stimulated in the dog model to mimic the human subject during pharmacological studies. Histamine was previously used at doses determined for maximal acid output. Histamine was subsequently substituted for pentagastrin, a gastrin analogue which contains a C-terminal amino-acid sequence of gastrin, and, unlike histamine, does not exert serious side effects such as vasodilation, tachycardia and vomiting.(Kwiecien and Konturek 2003)

A study using eight healthy fasted beagle dogs compared the effects of water, placebo (gelatin) capsule, pentagastrin and ranitidine at 50 mg/dog (5-6 mg/kg IV) on gastric pH.(Akimoto *et al* 2000) This study determined gastric pH based on intermittent gastric aspirates, with eight samples collected over a four-hour period. Ranitidine

administration successfully raised intragastric pH from a mean fasting pH of 6.8 +/- 0.2 (range 2.7-8.3) to a mean pH of 7.5 (range 7.0-8.6) within one hour of its administration and this effect was maintained during the four-hour sampling period. The authors concluded that ranitidine effectively raised the gastric pH in the dog, as no samples were identified below pH 7.0. This study relied on intermittent aspirates and times of lower pH may have been missed. In fact, results from the dogs receiving water alone showed pH recordings between 6.2 – 8.1, and baseline data from these same authors found that gastric pH was less than 5.0 in only six of 40 samples studied. Closer evaluation of the data argues that the resting pH of dogs is high and although ranitidine, at a high, non-clinically relevant dose, may have elevated the gastric pH somewhat, the dogs had a markedly elevated pH at baseline. Ranitidine's effectiveness, at standard veterinary doses, beyond four hours remains undetermined.

Yamada and colleagues evaluated the effects of omeprazole administered IV, at 1mg/kg, in six healthy beagle dogs.(Yamada *et al* 1989) This study monitored gastric pH using an *in situ* pH electrode placed perorally at each time of measurement, and five recordings were collected over the 8-hour time interval. The pH was consistently maintained at 6.6 +/- 0.2 for a period of at least three hours. In comparison, the fasted baseline recordings noted wide variation in gastric pH ranging from 0.9-7.0. Omeprazole was successful for reliably maintaining gastric pH in the neutral range and consistently increased gastric pH despite the peroral insertion of a pH sensor, which may incite gastric acid secretion. The dose of omeprazole, 1 mg/kg, was based on those reported in human studies, and the dose closely approaches the 0.7 mg/kg currently recommended in the veterinary literature for dogs. Limitations of this study were the short sampling period,

and intermittent sampling (5 pH determinations in an 8-hour period). The effects of omeprazole beyond this short sampling period are necessary.

A study by Simoens and colleagues evaluated the effects of omeprazole on post-prandial gastric acid secretion in 8-10 beagle dogs with a gastric fistula. Gastric acid secretion was evaluated following feeding of canned dog food and hog liver boiled in water. Gastric secretions were collected by gravity from the gastric fistula and pH was recorded *ex vivo*. The study reported that omeprazole at 2 mg/kg IV, raised intragastric pH within one hour of administration and maintained gastric pH above 5.5 for the full 11-hour study period. (Simoens *et al* 1989) The results of this study indicate that omeprazole IV will be effective in the clinical setting, however evaluation on the duration of action beyond 11 hours is required

Postius *et al.* compared the effects of famotidine, pantoprazole, and the two combined, under maximal, pentagastrin-induced, gastric acid stimulation in six dogs for a full 24-hour period. The purpose of the study was to determine whether the efficacy of PPIs is influenced by the secretory state of the stomach. In the study, an indwelling gastric pH electrode continuously monitored pH starting at 8 am. (Postius *et al* 1991b) Food was offered once a day at 10 am and pentagastrin infusion was begun at 12 noon. Pentagastrin in the control group produced a constant median intragastric pH of 1.17 (1.12-1.48, 25 and 75% quartiles). Pantoprazole (10 $\mu\text{mol/kg}$; 4.324 mg/kg) IV administered at 3 pm was effective at raising the intragastric pH by greater than 1 pH unit for about 14 hours. Subsequently famotidine (0.75 $\mu\text{mol/kg}$; 0.25 mg/kg) IV administered at 2 pm and again at 4 pm produced a transient increase in pH (median pH approximately 2.5). The effect was more pronounced after the second dose; however, increases in gastric

pH had completely disappeared by 6-7 hours. Doubling the dose of famotidine (1.5 umol/kg; 0.50 mg/kg) was more effective at raising pH (median pH approximately 4) however its duration of effect was unchanged. Concomitant treatment with both drugs, (i.e. famotidine at 2 and 4 pm and pantoprazole at 3 pm) significantly shortened the duration of elevated intragastric pH from 14 hours (pantoprazole alone) to approximately 8 hours when pantoprazole and the lower dose of famotidine were used, and to approximately 6 hours when pantoprazole and the higher dose of famotidine were used. The study demonstrated that pantoprazole has an improved effect when administered in an acidic environment as pre-treatment with an H₂RA did not prolong but actually shortened the duration of the pH-elevating effect of pantoprazole. This study had several excellent points. It monitored gastric pH over a long duration and used an indwelling pH probe to allow many pH determinations in the recording period. Dogs were fed in this study, which, based on pH versus time curves, allowed visualization of a transient increase in gastric pH after meal ingestion. However, after feeding, pentagastrin was initiated prior to drug administration, which prevented assessment of the effects of drug on the postprandial state. The study demonstrated that pantoprazole is effective at raising gastric pH in the dog; it does, however, suggest that although pantoprazole may have a relatively long duration of action, the extent of change in pH is underwhelming as the authors documented a raise in pH by only 1 unit. The famotidine portion of the study documents a dose-dependent effect on gastric acid suppression. However, the use of two doses in close proximity does not allow evaluation of this drug in a clinical setting. Finally, the results of concomitant administration of an H₂RA and a PPI is very interesting as it suggests that the combination in fact limits the actions of the PPI; this

finding is relevant should this combination be considered in the clinical setting. Further studies without the use of pentagastrin are warranted to see the effects of these drugs, alone and in combination, in a clinical setting.

Kromer, Postius and Riedel evaluated the effects of orally administered omeprazole 2 mg/kg (two times the recommended clinical dose) or ranitidine at 8.5 mg/kg (four times the recommended single dose) and 15 mg/kg (eight times the recommended dose) under maximal acid stimulation (with pentagastrin or carbachol) in dogs with a gastric fistula. (Kromer *et al* 2000) Intragastric pH was continuously monitored over a 24-hour period by means of a glass pH electrode placed via the gastric fistula. The pH recordings commenced at 8 am, secretagogue infusion was initiated at 12 noon, and the experimental drug administered at 2 pm with the effects of the drug evaluated from 2 pm to 8 am the following morning (18 hour drug data). Ranitidine's duration of action was approximately 7 hours and its effect was extremely variable. By contrast, a single oral dose of omeprazole elevated intragastric pH for an estimated 14 hours based on the pH versus time curves published. These authors comment that stimulation of gastric acid with pentagastrin or carbachol was necessary in order to prevent spontaneous pH fluctuations observed in dogs in both the basal or fed condition. The use of a secretagogue, as well as the high doses of ranitidine, limit the clinical relevance of the study; its results can only confirm that ranitidine has an effect on gastric acid secretion but fails to identify the extent of this effect in the clinical setting. However, the study confirms that the duration of ranitidine, an H₂RA, remains short at 7 hours despite large increases in the dose administered. The study also demonstrates that omeprazole is a better acid inhibitor than H₂RAs in the dog.

A further study by Kromer, Postius and Riedel evaluated the effects of omeprazole and pantoprazole in beagles equipped with a Heidenhain pouch.(Kromer *et al* 1990) This is a surgically prepared, vagally denervated fundic mucosal pouch, which allows collection of gastric secretions without contamination by saliva, food, etc. Gastric acid secretion was stimulated with impromidine, a specific, partial, histamine-H₂-receptor agonist.(Molina *et al* 1980) Omeprazole at 0.03, 0.06 and 0.12 mg/kg (0.09, 0.18, and 0.35 umol/kg) was administered to each dog and results were compared to pantoprazole at 0.15, 0.28, and 0.56 mg/kg (0.33, 0.65, and 1.3 umol/kg). This 4-hour study was divided into a 30-minute basal period, followed by impromidine infusion for 90-minutes to achieve a stable plateau prior to test drug administration. The test drug effect was subsequently evaluated for the remaining 2-hour period. Gastric acid was collected in 15-minute fractions and titrated with standard base. A dose dependent and long-lasting (2-hour) inhibition was noted at all dosages. No difference was noted in duration of action between both drugs. Overall, the authors concluded that both compounds had similar potency and efficacy. This investigation is markedly limited by its short study period; therefore, no conclusions can be made regarding the duration of action of these drugs. Further studies are necessary to evaluate the duration of action and potential differences between the two drugs in a more clinically relevant model.

Another comparative study on acid suppression evaluated the effects of a novel H₂RA (ORF 17583) and compared this to cimetidine, ranitidine, famotidine and omeprazole at incrementally increasing doses in dogs.(Katz *et al* 1987) Gastric secretions were obtained via a gastric fistula or a Heidenhain pouch. Gastric acid secretion was stimulated by histaminergic (betazole hydrochloride), and non-histaminergic (tetragastrin

and 2-deoxy-D-glucose, meal) stimulants. Gastric secretions were collected and titrated with known base over a 3-hour period. Each compound inhibited acid secretion in a dose-related manner, and exhibited a similar ability to maximally suppress acid secretion. The order of potency was famotidine = ORF = omeprazole > ranitidine > cimetidine.(Katz *et al* 1987) This study compared the potency of several H₂RAs to a PPI and confirmed findings of several other studies. Again this study was of short duration and the drugs require further investigation over longer time periods.

Alternative types of studies have investigated the efficacy of acid suppressants on healing of gastric ulcers. Comparative studies have incorporated endoscopic evaluation of iatrogenically induced gastric lesions. A study was performed evaluating the efficacy of 3 different H₂RAs (cimetidine, ranitidine and a novel drug BMY-2527) at reducing aspirin-induced gastric mucosal injury.(Kromer *et al* 2000) Aspirin was administered at 100 mg/kg and gastric ulcers were confirmed endoscopically within 2 hours. Gastric pH was also determined by collection and titration of gastric secretions at the time of endoscopy. Aspirin administration decreased gastric pH to approximately 3. Pretreatment with each of the three H₂RAs prevented the aspirin-induced decrease in gastric pH in a dose-related manner. The results demonstrated a dose-related antagonism of aspirin-induced gastric mucosal injury and that acid inhibition is an important component of the mechanisms whereby H₂RAs protect the gastric mucosa from aspirin-induced damage in the dog.(Cavanagh *et al* 1987) This study is the only study in the dog which demonstrates that acid inhibition is an important mechanism for gastric ulcer prevention.

Subsequently Jenkins and colleagues compared endoscopically the effects of cimetidine and omeprazole on aspirin-induced gastritis and on mechanically-created

gastric ulcers in 17 healthy dogs.(Jenkins *et al* 1991) Gastric acid was not evaluated in the study. The results revealed that treatment with omeprazole appeared to be superior to treatment with cimetidine, although the differences were not significant. Based on previous laboratory studies where omeprazole appeared to be a better acid inhibitor than an H₂RA in the dog (Kromer *et al* 2000) the results from this study are potentially discouraging and suggest that omeprazole may not be more effective in a clinical model. This latter study demonstrates the ongoing need for further research to evaluate gastric acid-lowering therapies in clinically ill veterinary patients.

These studies identify that an effect has been documented in the canine model with ranitidine,(Akimoto *et al* 2000,Daly *et al* 1981,Katz *et al* 1987,Kromer *et al* 2000) famotidine,(Katz *et al* 1987,Postius *et al* 1991b) omeprazole,(Katz *et al* 1987,Kromer *et al* 1990,Kromer *et al* 2000,Larsson *et al* 1983,Simoens *et al* 1989,Yamada *et al* 1989) and pantoprazole.(Kromer *et al* 1990,Postius *et al* 1991b) However they also suggest that differences exist between the different anti-secretory drugs and their effects. Apparent limitations in these studies include higher drug doses than clinically recommended, short examination periods, differing methods for determination of gastric acid secretion, intermittent pH determinations, and the use of gastric acid stimulants that alter the normal physiologic state of the dog. Pharmacologic stimulation of canine gastric acid secretion confirms drug effect in the face of maximal acid output, however, this does not evaluate the effect of these drugs in a potential clinical setting.

These limited studies emphasize the requirement for more comprehensive and veterinary directed studies evaluating the effects of gastric acid-lowering therapy on gastric acid secretion in the dog.

1.6- Monitoring gastric acid

Gastric acidity is based on two variables: the amount of acid secreted in a given time and the acidity of a particular sample. The latter recognizes that there are other constituents (sodium, chloride, mucus, bicarbonate, etc.) in gastric 'juice' which alter the final acidity of the solution. Studies have identified that gastric acid concentration can be independent of its rate of secretion and concentration and volume are not dependent on one another.(Davenport 1982)

Gastric acidity can be determined by measuring pH or titratable acid, or both. These variables are not interchangeable and recognition must be made as to what each is measuring.

The concentration of acid is estimated by titration with a standard base. The ionized hydrogen ions, the majority of hydrogen ions in gastric secretions, are neutralized first at a pH of approximately 3.5 and termed 'free acid'. When titration is continued to near neutral pH, the buffered hydrogen ions (buffered by proteins and other non-acid gastric secretions) are neutralized and 'total titratable acid' is determined. Titratable acidity is expressed in milliequivalents per litre (mEq/L) and represents the sum of the hydrogen ion concentration and the un-ionized hydrogen ion concentration. The problems with determining the amount of acid by titration is that all of the acid produced may not be accounted for due to loss of gastric secretions through the pylorus, through back-diffusion of acid across the mucosa, and through neutralization by bicarbonate.(Davenport 1982)

The pH is the measure of hydrogen ion activity. The acidity of a solution is the *activity* of hydrogen ions and not their concentration.(Davenport 1982) pH is the negative

logarithm to the base 10 of the *activity* of hydrogen ions (a_{H^+}) in solution ($pH = -\log_{10} a_{H^+}$). (Davenport 1982) However, when hydrogen ions are infinitely dilute in solution, for example in blood, the activity of hydrogen ions is exactly equal to the concentration (i.e. $[H^+] = a_{H^+}$), hence the familiar formula $pH = -\log_{10} [H^+]$. When the concentration of H^+ is increased, for example in gastric secretions, hydrogen ion activity is usually less than its concentration; and an *activity coefficient* must be determined to accurately calculate *activity* of an acid ($pH = -\log_{10} f[H^+]$). (Davenport 1982) This coefficient factor depends both on the hydrogen ion concentration and the sum of the concentrations of sodium and potassium ions, this factor approaches but does not equal 1; therefore, pH only relates closely, but not exactly, to intragastric hydrogen ion concentration. (Walt 1986) When using pH to evaluate a wide range of pH values, pH is unable to account for changes in intragastric volume. Under these circumstances, intragastric pH changes cannot be taken to reflect underlying changes in acid secretion. (Armstrong *et al* 1989) pH is thus a measure that allows evaluation of gastric acid in the dynamic environment of the stomach. While not being able to measure the exact amount of acid being secreted, the gastric 'juice' pH changes with increased or decreased H^+ secretion, as well as with secretion, absorption and mixing of stomach contents.

Physiologically gastric acidity can be determined by removing gastric secretions continuously (for titration and determination of total acid production) or intermittently (for titration or pH recordings *ex vivo*). Finally, a monitoring system has been developed that allows continuous intragastric pH measurements. Advantages and disadvantages exist for all mechanisms of determining gastric acidity.

When comparing gastric aspiration techniques to indwelling, ambulatory, long-term recording devices, several issues arise. Gastric aspiration requires removal of gastric secretions, which may alter the rate of further gastric acid production in the stomach.(Fimmel *et al* 1985) This fluid may, however, be returned to the stomach if gastric acid is measured using pH rather than titration with standard base. Intermittent gastric aspiration techniques have limitations to the number of samples obtained and generally do not produce more than a few hundred single pH values throughout a 24-hour period. In addition, difficulties in retrieving gastric secretions have been noted during periods of anacidity (during the night and during pharmacologic acid inhibition)(Kuna 1964,Simpson *et al* 1999) and after meal ingestion.(Fimmel *et al* 1985) Also, gastric aspiration techniques require manipulation, which can stimulate gastric acid secretion, and alter the subject's gastric acidity. This was shown by Kuna when higher acidity was documented from aspirated gastric juice than recorded by electrode *in situ* prior to removal of the stomach fluid and the greatest difference was noted in those in whom difficulty had been encountered during gastric aspiration.(Kuna 1964) Finally gastric aspiration techniques hinder normal daily activity and may add a component of stress. Anxiety and restraint have both been implicated in increased gastric acid secretion.(Brodie and Marshall 1963,Gray *et al* 1953,Hoffman 1955,Kuna 1964,Menguy 1960)

Indwelling ambulatory long-term recording devices using pH electrodes and data storage capability provide a physiological examination where subjects are able to move freely and daily activity is not impeded. Gastric juice is not removed from the stomach and the inherent changes in gastric acid secretion due to its removal are avoided.

However, these devices are limited to measuring gastric pH; quantitation of H^+ (i.e. titratable acidity) is not possible. In addition, the pH probe is present within the gastric lumen, and may cause irritation to the gastric mucosa. This may incite further gastric acid secretion. Concerns have been raised as to whether the pH electrodes measure acidity in the gastric juice or acidity at the mucosal surface as differences in pH have been noted based on probe placement. Despite these concerns studies have documented that the pH obtained on aspirated samples compares favourably to the pH recorded *in situ* using the pH electrode device.(Fimmel *et al* 1985,Savarino *et al* 1989) The evolution of gastric pH monitoring has resulted in the continuous pH monitoring system becoming the gold standard for evaluating intragastric pH. Long-term gastric pH monitoring devices have been validated by studies comparing nasogastric aspiration versus continuous intragastric pH monitoring.(Fimmel *et al* 1985,Mela and Savarino 1990) Continuous pH monitoring systems have been shown to be more precise than gastric aspiration; more physiologic, because acid is not removed from the stomach; and more reliable, because of their sampling frequency.(Fimmel *et al* 1985,Mela and Savarino 1990,Savarino *et al* 1989) Glass pH probes have been validated and their use has become common place (Armstrong *et al* 2003,Fimmel *et al* 1985,Savarino *et al* 1989) Our sampling system using the Digitrappor Mark III and glass pH electrodes has been validated and used extensively.(Armstrong *et al* 2003,Mela *et al* 1992)

The use of long-term ambulatory recording devices record gastric pH every 4-6 seconds over a 24 hour period, generating 14400 to 21600 data points for each subject for each day of evaluation. Once recordings are obtained a method is required to store and analyse such a large volume of data. For graphical display, data reduction techniques are

utilized; 5-20 minute intervals are recommended.(Armstrong *et al* 1989) For graphical display of groups of recordings, the mean or the median can be used.(Armstrong *et al* 1989) The median is theoretically more robust since it is less susceptible to outlying values.(Armstrong *et al* 1989,Emde 1990) For statistical analysis 24-hour analyses are frequently reduced to a single summary variable (ie. 24-hour mean, 24-hour median, percentage of time gastric pH greater than 3). Generally, no single parameter will be adequate and therefore multiple calculations are needed.(Rohmel *et al* 1990)

Theoretically, the mean, median or mode may all be suitable summary variables, but all are affected differently.(Armstrong *et al* 1989) Again, the median is less susceptible to outliers and has been recommended and widely used.(Armstrong personal communication)(Emde 1990,Rohmel *et al* 1990) In addition, the time spent above a given pH threshold has been shown to be a better tool than the mean or the median in representing the results of continuous 24-h gastric pH metry studies.(Mela *et al* 1992)

For statistical analysis, acidity may be expressed as pH or H^+ concentration and there is no universally acceptable recommendation for measure. (Armstrong *et al* 1989,Emde *et al* 1987) Despite the transformation, neither measure of acidity is generally normally distributed.(Rohmel *et al* 1990) Considering the wide range in H^+ concentration, it remains convenient to retain the logarithmic representation.

Probe placement

As different regions in the stomach have different degrees of acidity, the ideal placement for the pH probe is within the gastric fundus and body where the highest concentration of parietal cells is located. Studies in humans have evaluated proximal

versus distal probe placements and found that, in the fasting state, fundic and antral mean pH are similar; however, after a meal, fundic pH rises more than antral pH.(Fimmel *et al* 1985) In addition, pH is lower when the pH probe is adjacent to gastric mucosa versus when it is placed within gastric secretions; however, once the pH probe is wedged into the mucosa the pH dramatically increases.(Fimmel *et al* 1985) As pH can markedly vary between different parts of the stomach, it is vital that the electrode is maintained in a known and reproducible position.(Emde *et al* 1987) Positioning of the probe under fluoroscopy is highly recommended.(Emde *et al* 1987) Probe location should ideally be determined frequently. Initial radiology cannot always ensure accurate position, especially in the antrum or after food intake.(Emde *et al* 1987) For accurate placement of the pH probe in humans, the electrode is passed transnasally until an acidic pH is recorded. The electrode is then positioned in the fundic region of the stomach under fluoroscopic guidance. The distance of the electrode tip to the nares is recorded and kept constant in each subject.(Fimmel *et al* 1985) Ideally, probe positioning in the canine model should include fluoroscopy for accurate placement and probe location should be re-evaluated frequently.

1.7- Conclusion

In summary, ranitidine, famotidine, omeprazole and pantoprazole have all been determined to show an effect on gastric acid secretion in the dog, however, the extent of gastric acid inhibition of these drugs at recommended veterinary doses in normal dogs, without surgical, pharmacological or other intervention has not been determined. Based on the widespread use of gastric acid-lowering therapies in the dog, the evaluation of these drugs, in a clinically applicable setting, is warranted. We propose to explore this in the studies presented in this thesis.

CHAPTER II

Effects of ranitidine, famotidine, pantoprazole, and omeprazole on intragastric pH in dogs

2.1 Abstract

Objective—To identify the normal gastric acid secretion profile in dogs and determine the degree of gastric acid suppression associated with four gastric acid suppressants.

Animals—12 healthy Beagles.

Procedure—Intragastric pH was measured continuously for 24-hour periods with a digital recording system placed via a gastrostomy tube. Baseline measurements were obtained when food was withheld and when dogs were fed a standard diet. Dogs were then treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 7 days, and intragastric pH was recorded on days 0, 2, and 6. Subsequently, the effects of administering famotidine (0.5 mg/kg, IV, q 8 h; 6 dogs) and omeprazole as a suspension (1 mg/kg, PO, q 12 h; 6 dogs) were evaluated. Median 24-hour intragastric pH, percentage of time pH was ≥ 3 , and percentage of time pH was ≥ 4 were determined.

Results—Median pH, percentage of time pH was ≥ 3 , and percentage of time pH was ≥ 4 were all significantly higher when food was withheld than when dogs were fed.

Famotidine, pantoprazole, and omeprazole significantly suppressed gastric acid secretion, compared with saline solution, as determined on the basis of median 24-hour pH and percentages of time pH was ≥ 3 or ≥ 4 . However, ranitidine did not. Omeprazole suspension suppressed gastric acid secretion.

Conclusions and Clinical Relevance—Results suggest that in healthy dogs, famotidine, pantoprazole, and omeprazole significantly suppress gastric acid secretion. Omeprazole, administered twice daily as a suspension, was the only regimen tested that approached the potential therapeutic efficacy for acid-related disease when assessed by criteria for human patients.

2.2- Introduction

A large body of information exists regarding gastric acid secretion and its suppression in humans and dogs,(Akimoto *et al* 2000,Katz *et al* 1987,Kromer *et al* 1990,Larsson *et al* 1983,Larsson *et al* 1985,Postius *et al* 1991,Yamada *et al* 1989) and several drugs have been shown in experimental studies to have an effect on intragastric pH in dogs. However, previous studies involving dogs were most often performed as an adjunct to development of gastric acid suppressants for use in humans. Thus, these studies were not specifically aimed at determining the extent or duration of gastric acid suppression in dogs in clinically relevant settings. Earlier studies, for instance, involved measuring effects in dogs after gastric acid secretion was altered pharmacologically with pentagastrin or histamine,(Katz *et al* 1987,Kromer *et al* 1990,Larsson *et al* 1983,Larsson *et al* 1985,Postius *et al* 1991) were focused on determining toxic effects following administration of extremely high dosages,(Kromer *et al* 1990,Postius *et al* 1991) or involved measuring intragastric pH for short periods or only intermittently.(Akimoto *et al* 2000,Katz *et al* 1987,Larsson *et al* 1983,Yamada *et al* 1989) As a result, little is known about the effects in dogs of commonly used gastric acid suppressants administered as recommended in the veterinary literature.

In addition, despite the common use of gastric acid suppressants in dogs, to our knowledge there are no published comparative studies specifically investigating the efficacy of gastric acid suppression in the veterinary literature. In humans, healing of gastric and duodenal ulcers and erosive esophagitis is highly correlated with the degree of gastric acid suppression.(Huang and Hunt 2001) For people with duodenal ulcers, for instance, optimal treatment involves maintaining an intragastric pH ≥ 3 for 18 to 20 hours

a day (i.e., approximately 75% of the day), and little benefit is associated with more extensive gastric acid suppression.(Burget *et al* 1990) In people with gastroesophageal reflux disease, intragastric pH should be ≥ 4 for 16 to 22 hours a day.(Bell *et al* 1992) A critical intragastric pH threshold of 4 is also recommended for prevention of gastrointestinal tract ulcers in critically ill patients, because pH values > 4 inhibit pepsin and inactivate fibrinolysis.(Fennerty 2002) Finally, an intragastric pH > 6 is necessary to achieve hemostasis with acute gastrointestinal tract bleeding, as pH values > 6 allow adequate platelet aggregation and prevent dissolution of blood clots.(Fennerty 2002)

The optimal degree of gastric acid suppression necessary for treating acid-related diseases in dogs has not been established, and critical intragastric pH thresholds in dogs may differ from those established for humans because of differences in gastric physiology. Regardless, it seems likely that the degree and duration of gastric acid suppression will correlate with ulcer healing in dogs as it has in humans.(Burget *et al* 1990) The purposes of the study reported here were to identify the normal gastric acid secretion profile in healthy dogs that were fed and from which food had been withheld and to determine the degree and duration of gastric acid suppression associated with four gastric acid suppressants (two histamine₂ [H₂] receptor antagonists and two proton pump inhibitors) commonly prescribed in dogs.

2.3- Material and Methods

Dogs—Twelve purpose-bred sexually intact Beagles (5 males and 7 females) were used in the study. Dogs ranged from 7 months to 5 years old (median, 14 months; mean, 23 months) and weighed between 7.5 and 12.7 kg (median, 9.8 kg; mean, 9.9 kg). All dogs were determined to be healthy on the basis of results of a general physical examination, CBC, and serum biochemical profile. In all dogs, a percutaneous, endoscopically-placed gastrostomy (PEG) tube was inserted in the gastric fundus at least 2 weeks prior to the start of the study. Multiple biopsy specimens were obtained from the fundus of the stomach at the time of PEG tube placement and submitted for *Helicobacter* screening. All dogs were negative for *Helicobacter* spp on the basis of results of a urea slant test, routine bacterial culture, and a generic polymerase chain reaction assay. General physical health of the dogs was subsequently monitored by means of weekly physical examinations, at which time body weight was also recorded.

Measurement of intragastric pH—Intragastric pH was recorded continuously with a digital recording system; dogs were fully ambulatory while intragastric pH was recorded. In brief, intragastric pH was measured with a single-channel, internally referenced glass pH probe^a attached to a portable data recorder.^b At the beginning of each recording session, the electrode was calibrated with commercial buffer solutions^c of pH 1.7 and pH 7 at 21°C; a drift of 0.1 pH units was tolerated. Following calibration, the probe was passed into the stomach through the PEG tube and secured with adhesive tape. The probe extended 5 to 7 cm beyond the PEG tube into the fundus of the stomach. Prior to the study, appropriate probe positioning was confirmed in 2 dogs by means of fluoroscopy,

and this same positioning was used for all dogs in the study. During recording sessions, intragastric pH was recorded every 4 seconds by the data recorder. Following a recording session, pH data were downloaded to a data file^d and stored for later analysis.

Determination of normal gastric acid secretion profile—In all dogs, the normal gastric acid secretion profile was determined while food was withheld, and subsequently while dogs were fed. To determine gastric acid secretion while food was withheld, dogs were fed at 8:00 PM the night prior to a recording session. Food was then withheld overnight, and beginning at 9 AM the next morning, intragastric pH was measured continuously for 24 hours. Food was withheld during the entire 24-hour recording session.

One week later, gastric acid secretion was measured while dogs were fed. Briefly, intragastric pH was measured continuously for 24 hours, beginning at 9 AM. Dogs were fed $\frac{3}{4}$ of a cup of kibble^e at 10:00 AM, 3 dog treats^f at 3:00 PM, and $\frac{3}{4}$ of a cup of kibble at 8:00 PM and again at 8:00 AM the following morning. Probes were removed at 9:00 AM. Water was available ad libitum during recording sessions.

Effects of H₂ receptor antagonists and proton pump inhibitors—Following determination of the normal gastric acid secretion profile, a randomized controlled trial was performed to determine the effects of two H₂ receptor antagonists (ranitidine and famotidine) and two proton pump inhibitors (pantoprazole and omeprazole). The study was constructed as a randomized Latin square design with repeated measures over days (days 0, 2 and 6).(Jones and Kenward 1989) The feeding schedule used during determination of the normal gastric acid secretion profile was maintained throughout this

portion of the study. Dogs were treated with ranitidine^g (2 mg/kg, IV, q 12 h), famotidine^h (0.5 mg/kg, IV, q 12 h), pantoprazoleⁱ (1 mg/kg, IV, q 24 h), omeprazole^j (approx 1 mg/kg, PO, q 24 h), or saline (0.9% NaCl) solution (3 mL, IV, q 12 h). Drugs were administered for one week, and intragastric pH was measured for 24 hours immediately prior to the initiation of each treatment and on days 2 and 6 of each treatment period. All dogs received all five treatments. In each dog, treatments were administered in random order with 1 week between treatments; randomization schedules were developed with a random number generator.(Fishman and Moore 1982) Any complications or adverse effects identified during treatment periods were recorded.

Dosages of ranitidine, famotidine, and omeprazole were selected on the basis of published recommendations.(Haskins 2000,Matz 1995,Plumb 2002) The highest recommended dosages of ranitidine and famotidine were selected in an attempt to maximally inhibit gastric acid secretion. A 12-hour dosing frequency was selected, as we believed that compliance with more frequent administration guidelines tends to be poor in clinical practice. For administration of omeprazole, 10-mg tablets were administered as necessary to approximate the target dosage; the actual dosage administered ranged from 0.8 to 1.3 mg/kg. The dosage of pantoprazole was selected on the basis of information generated in dogs during development of the drug for human use.(2002,Kromer *et al* 1990,Sabata 1999) Pantoprazole solution was prepared by injecting 10 mL of saline solution into the vial containing the dry powder to produce a final concentration of 4 mg/mL. Pantoprazole was used within four hours after preparation. All injectable drugs were prepared daily by technical staff unrelated to the project. Syringes were labeled #1-6 to identify the recipients. Drug volumes were all made up to 3mL by the addition of

saline. All dogs receiving injectable medication received an injection of drug or saline at 9 AM and 9 PM daily. Injectable drugs and saline solution were administered intravenously over 15 minutes. Personnel administering drugs and downloading pH determinations were blinded to the drugs delivered. Omeprazole was excluded from the blinding as it was administered orally as a single 10 mg tablet. The drug schedule was unmasked on completion of data collection.

Effects of dosing frequency—In six dogs, a study was performed to determine whether increasing the frequency of famotidine administration would more effectively reduce gastric acid secretion than twice daily administration. In those dogs, famotidine was administered at a dosage of 0.5 mg/kg, IV, every 8 hours for 1 week. Intragastric pH was measured for 24 hours on days 2 and 6 of the treatment period.

In six dogs, a study was performed to determine whether increasing the frequency of omeprazole administration from once a day to twice daily administration, with omeprazole formulated into a suspension, would have a significant effect on gastric acid secretion. Omeprazole suspension (2 mg omeprazole/mL of 8.4% sodium bicarbonate) was prepared as described (Phillips *et al* 1996) and administered at a dosage of 1 mg/kg every 12 hours for 1 week. The omeprazole suspension was administered as a bolus through the PEG tube; the tube was flushed with 10 mL of water following drug administration. Intragastric pH was measured for 24 hours on days 2 and 6 of the treatment period.

Statistical analyses—For each 24-hour recording of intragastric pH, median pH, percentage of time intragastric pH was ≥ 3 , and percentage of time intragastric pH was ≥ 4 were determined. Paired *t* tests were used to compare values obtained for normal gastric acid secretion when dogs were fed with values obtained when food was withheld.

For evaluation of the effects of ranitidine, famotidine, pantoprazole, and omeprazole, ANOVA for repeated measures was performed with main effects being treatment and day and controlling for the effects of dog, drug carryover, and week. The Dunnett test was used to compare, for each day, values obtained for each of the 4 drugs with values obtained during administration of saline solution. The Tukey test was used to compare, for each drug, values obtained each day and, for each day, values obtained for the 4 drugs.

For evaluation of the effect of dosing frequency of famotidine on gastric acid secretion, ANOVA was used to analyze data obtained. Post hoc pairwise *t* tests were performed to determine significant differences.

For all analyses, assumptions of normality were tested by means of the Shapiro-Wilk test; equality of variance assumptions were assessed by plotting residuals against explanatory variables and against the predicted value. Logarithmic transformations were performed as necessary. All statistical analyses were performed with standard software.(Cary 1999) Values of $P \leq 0.05$ were considered significant. 95% confidence intervals (CI) were all calculated.

2.4- Results

Normal gastric acid secretion profile—Median 24-hour intragastric pH when food was withheld from the dogs (mean, 4.44; CI, 3.33 to 5.92) was significantly ($P < 0.001$) higher than median 24-hour intragastric pH when dogs were fed (1.30; CI, 1.11 to 1.53). Percentage of time that intragastric pH was ≥ 3 was significantly ($P < 0.001$) higher when food was withheld (mean, 66.0%; CI, 52.4% to 83.0%) than when dogs were fed (13.3%; CI, 8.9% to 19.8%). Similarly, percentage of time that intragastric pH was ≥ 4 was significantly ($P < 0.001$) higher when food was withheld (63.9%; CI, 47.4% to 80.4%) than when dogs were fed (12.8%; CI, 7.3% to 18.3%).

Inter- and intra-dog variability - The fasted median gastric pH varied among the 12 dogs from pH 0.9 to 7.0; this was based on $n=24$ recordings (combining baseline (fasted) Week 0 and Week 6, Day 0 recordings; Table 2.6-1). Sixteen of the 24 recordings (67%) maintained a median gastric pH > 5.5 . When dogs were fed the median pH for the 12 dogs ranged from pH 0.9 to 6.1, this was based on $n=82$ recordings (combining baseline (fed) recordings, all Day 0 recordings and saline recordings; Table 2.6-2). Conversely, in fed dogs, a total of 67 out of 82 recordings (82%) had an acidic gastric pH with a median pH < 3 (only one dog had a median pH > 5.5).

Intra-dog variation was also recorded. When dogs were fasted (two recordings per dog), median pH varied between the two days, despite similar conditions, with some dogs displaying wide variation (i.e. Dog #11 median pH 1.6 vs. 6, and Dog #8 median pH 2.7 vs. 6.7); and other dogs maintaining consistency and repeatability (Dog #9 median pH 6.4 and 6.7, or Dog #7 median pH 6.6 on both occasions; Table 2.6-1). Again during the fed state, individual dogs demonstrated markedly different median pH values (i.e. Dog #3 pH

6.1, 2.7 and 4.6; and Dog #10 pH 2.9, 4.8 and 1.3) on three separate days under similar conditions. Only four dogs (#1, 5, 6, and 9) consistently maintained a median gastric pH < 3 on all fed, non-drug, recording days. See Table 2.6-2.

Effects of ranitidine, famotidine, pantoprazole, and omeprazole—One dog developed a gastrointestinal tract disturbance and vomited several times during the week that saline solution was administered. Therefore, values for saline solution were based on data for only 11 dogs.

All saline recordings (days 0, 2, and 6) were compared with each other and with the baseline (day 0) recordings using the pre-determined outcome measures (median pH, percentage of time intragastric pH was ≥ 3 , and percentage of time intragastric pH was ≥ 4). No significant differences were detected.

Due to study design, Day 0 recordings were limited to recording gastric pH over 23-hours rather than a full 24-hour period. Small discrepancies were noted on statistical comparisons between [drug and placebo] versus [drug and baseline] and subsequently placebo (saline) was used for drug to “no drug” comparisons.

Values for median 24-hour intragastric pH on days 2 and 6 were significantly higher when dogs were given famotidine, pantoprazole, or omeprazole than when they were given saline solution (Table 2.6-3). However, when given ranitidine, values obtained on days 2 and 6 were not significantly different from values obtained when dogs were given saline solution.

On days 2 and 6, when dogs were given famotidine, pantoprazole, or omeprazole, the percentage of time that intragastric pH was ≥ 3 was significantly greater than that of

dogs given saline solution (Table 2.6-4). However, when given ranitidine, the percentage of time that intragastric pH was ≥ 3 was not significantly different from that of dogs given saline solution.

When dogs were given omeprazole, the percentage of time that intragastric pH was ≥ 4 was significantly greater on day 2 than when dogs were given saline solution. However, on day 6, dogs given famotidine, pantoprazole, or omeprazole all had a greater percentage of time with intragastric pH ≥ 4 than dogs given saline solution (Table 2.6-5). When plotting the results of all 4 drug regimens, as well as saline, over a 24-hour period, gastric pH followed a similar pattern, however the differences in the percentage of time that the pH was ≥ 3 or ≥ 4 among drugs was apparent (Figures 2.7-1 and 2.7-2).

When comparing the effects of the 4 drugs using median 24-hour intragastric pH; on day 2, the only significant difference among the drugs was that of omeprazole resulting in a significantly higher pH than ranitidine; whereas on day 6 famotidine, pantoprazole, and omeprazole all resulted in a significantly higher pH than ranitidine. (Table 2.6-3) Similar results were obtained when drug comparisons were made for the percentage of time pH was ≥ 3 for both days 2 & 6. (Table 2.6-4) However when examining the percentage of time pH was ≥ 4 , the only significant finding was on day 6 when omeprazole resulted in a significantly higher pH than ranitidine. (Table 2.6-5)

Significant drug carryover effects were identified when evaluating median 24-hour intragastric pH and percentage of time intragastric pH was ≥ 3 . This implied that potential drug interactions were evident, such that measured drug effects on intragastric pH were significantly dependent on the order in which drugs were administered. Famotidine and pantoprazole had more effects on subsequent measures than did

ranitidine. These drug carryover effects were controlled for in study design and statistical analyses.

Effects of dosing frequency—No significant differences in median 24-hour intragastric pH, percentage of time intragastric pH was ≥ 3 , or percentage of time intragastric pH was ≥ 4 were found when famotidine was administered every 8 hours rather than every 12 hours.

Mean values for median 24-hour intragastric pH on days 2 and 6 when dogs were given omeprazole suspension were 4.7 (CI, 4.0 to 5.6) and 4.9 (CI, 4.1 to 5.8). Mean percentages of time intragastric pH was ≥ 3 were 90.9% (CI, 80.5% to 101.2%) on day 2 and 78.4% (CI, 68.1% to 88.8%) on day 6. Mean percentages of time that intragastric pH was ≥ 4 were 78.3% (CI, 63.8% to 92.8%) on day 2 and 70.7% (CI, 56.2% to 85.3%) on day 6. Although none of the dogs vomited on days that intragastric pH was recorded, vomiting (4 dogs) and diarrhea (4 dogs) were seen at various times after administration of the omeprazole-sodium bicarbonate suspension.

2.5- Discussion

Previous studies have shown that the gastric acid secretion profile in Beagles is variable.(Akimoto *et al* 2000,Yamada *et al* 1989) For this reason, we elected to measure intragastric pH continuously for a 24-hour period to capture the fluctuations in intragastric pH.

One important limitation of the present study was that intragastric pH, rather than gastric acid secretion, was measured. Intragastric pH relates closely, but not exactly, to intragastric hydrogen ion concentration,(Walt 1986) but does not take into account changes in intragastric volume. Therefore, intragastric pH cannot be taken to reflect underlying changes in acid secretion.(Armstrong *et al* 1989) Other limitations of the continuous intragastric pH monitoring technique used in the present study include variations in pH resulting from the location of the probe within the stomach, the need for the probe to be in contact with the gastric mucosa or fluid, and the inability to identify episodes of gastroduodenal reflux.(Fimmel *et al* 1985) Despite these limitations, continuous pH monitoring systems have become the gold standard for monitoring intragastric pH. These monitoring systems have been shown to be more precise than gastric aspiration; more physiologic, because acid is not removed from the stomach; and more reliable, because of their sampling frequency.(Chiverton *et al* 1989,Mela and Savarino 1990,Savarino *et al* 1989)

Previous reports have noted considerable inter- and intra-dog variability in gastric pH both in the fasted and fed states.(Dressman 1986,Simpson *et al* 1999,Yamada *et al* 1989,Youngberg *et al* 1985) Our study confirms this variability. Despite this finding, we demonstrate definite trends during the fasted and fed states. The majority of the study

dogs maintained an elevated pH (a pH >5.5) during the fasted state with occasional outliers, which were not repeatable on retesting. Conversely, during the fed state, most dogs maintained a low pH (pH < 3) and, although outliers were noted, again these were not repeatable.

As previously reported, basal gastric acid secretion in dogs is low when compared with humans.(Kararli 1995) However, peak gastric acid secretion is considerably higher in dogs than in people. Results of the present study are in agreement with these findings. For example, the median pH was 4.44 in the fasted state, but feeding a standard diet at fixed times was effective at stimulating gastric acid secretion resulting in a median pH of 1.30. It is for this reason that we elected to feed dogs in the present study when evaluating the effects of gastric acid suppressants.

The present study examined the efficacy of acid suppressants available in North America at the time of investigation. Two commonly used H₂ receptor antagonists (ranitidine and famotidine - both parenteral formulations) were selected, and 2 proton pump inhibitors (pantoprazole (parenteral formulation) and omeprazole (oral formulation, as this is the only formulation available in North America)). Omeprazole was selected as it is frequently prescribed for dogs. Because oral drug administration will itself cause gastric acid secretion, by stimulating the cephalic phase of acid production,(Akimoto *et al* 2000) use of omeprazole tablets may have influenced the pH measurements obtained in the present study. Although we considered giving placebo tablets to dogs during the weeks that they were receiving injectable medications, we thought that this would further confound our results.

Gastric parietal cells are stimulated to produce hydrochloric acid via three

separate messengers (acetylcholine, gastrin, and histamine).(Soll and Berglindh 1987) All 3 messengers act to stimulate the H^+/K^+ ATPase enzyme (proton pump) responsible for hydrogen secretion. The H_2 receptor antagonists block gastric acid secretion at the level of the H_2 receptor on gastric parietal cells, and even though the acetylcholine and gastrin pathways remain intact, inhibition of the histamine pathway affects the potentiation of the other two pathways and leads to significant gastric acid inhibition.(Soll and Berglindh 1987) In contrast, proton pump inhibitors block gastric acid secretion at the level of the H^+/K^+ ATPase enzyme itself.

Dosages of gastric acid suppressants currently recommended in the veterinary literature have been extrapolated from dosages recommended for humans and have been calculated to inhibit maximally stimulated acid secretion by at least 50% throughout the day.(Papich 1993) However, the degree of gastric acid suppression necessary for various therapeutic effects in dogs is still undetermined.

In the present study, we found that administration of famotidine, pantoprazole, or omeprazole significantly suppressed gastric acid secretion, compared with administration of saline solution. No significant differences were noted in efficacy of the drugs when comparing measurements obtained on day 2 with those obtained on day 6. Conversely, ranitidine had no significant effect on gastric acid secretion, compared with saline solution, suggesting that at the dosage used, it would be unlikely to have any beneficial effects on gastric ulcer prophylaxis or treatment. Increasing the dosing frequency (i.e., administration every 8 hours instead of every 12 hours), or dose may improve its effects, as plasma ranitidine concentration has been shown to have a significant linear correlation with gastric acid inhibition in people.(Mignon *et al* 1982)

A placebo was included in this study, as well as baseline recordings (Day 0) for each week. Small discrepancies were noted during statistical comparison of [drug to saline] and [drug to baseline] likely due to inherent variability. As Day 0 recordings were recorded over 23-hours rather than a full 24-hour period the authors elected to use placebo for all comparisons against drug effect. Placebo was felt to be a more accurate comparison because it involved 24-hour comparisons and because placebo recordings were maintained throughout the study weeks ensuring matched testing conditions on all recording days.

In the present study, when comparing antisecretory drugs against each other, only omeprazole showed a significant improvement in gastric acid inhibition compared to both H₂RAs on day 2, as assessed by the percentage of time that the pH was maintained ≥ 3 . By day 6, no significant differences were found in any outcome measure (median intragastric pH, percentage of time intragastric pH was ≥ 3 , and percentage of time intragastric pH was ≥ 4) among famotidine, pantoprazole, and omeprazole. Previous studies have reported that proton pump inhibitors are more potent at inhibiting gastric acid secretion than are H₂ receptor antagonists;(Fennerty 2002,Kromer *et al* 2000,Larsson *et al* 1983) however, we did not find this to be true in the present study. Potency of the H₂ receptor antagonists relates to the extent to which they bind with the H₂ receptor,(Huang and Hunt 2001) and famotidine is reported to be 8 to 9 times as potent as ranitidine.(Friedman 1987,Schunack 1987) In the present study, at the dosages used, famotidine increased intragastric pH to a greater extent and for a longer time than did ranitidine.

Drug carryover effects were identified in the present study, with famotidine and

pantoprazole having more pronounced carry-over effects than ranitidine. For pantoprazole, a proton pump inhibitor, carryover effects were not unexpected, as this drug binds irreversibly to the proton pump.(Larsson *et al* 1985) However, carryover effects with the H₂ receptor antagonists were unexpected and difficult to explain, and it is possible that other factors unrelated to drug administration (i.e., environmental factors and acclimation of the dogs) may have accounted for the carryover effects. Regardless, our findings suggest that washout periods longer than 1 week be used for future studies. The 1-week washout period used in the present study was selected because it represented a minimum of 20 half-lives for all four drugs and full restoration of gastric acid secretion has been demonstrated one week after administration of proton pump inhibitors have been discontinued.(Huang and Hunt 2001,Larsson *et al* 1985) While the carry-over effect was identified, the comparative aspects of the present study were not affected, as these carry-over effects were controlled for in the study design and statistical analyses.

At the end of the randomized controlled trial, after unblinding the drug randomization schedule, we found that none of the four drugs resulted in an intragastric pH ≥ 3 for at least 75% of the time and additional studies were performed to determine whether modifications in the administration protocol would improve gastric acid suppression. In particular, we assumed that gastric acid suppressants would be used most frequently in critically ill dogs that would not be receiving oral medications; therefore, the effects of an increased dosing frequency of famotidine, and twice daily omeprazole administered as a suspension through a feeding tube, were examined. Omeprazole tablets were dissolved in bicarbonate, as previously described,(Phillips *et al* 1996) to prevent degradation in the acid environment of the stomach since omeprazole is absorbed in the

small intestine.(Larson *et al* 1996) Omeprazole tablets^j were used since they were available in Canada at the time of the study, but omeprazole capsules^l would have been an alternative.

Increasing the frequency of famotidine administration from two to three times a day did not have any significant effects on suppression of gastric acid secretion in the present study. In contrast, when omeprazole was administered twice daily as a suspension, mean percentages of time that intragastric pH values were ≥ 3 and ≥ 4 were 90.9% and 78.3%, respectively. Omeprazole, administered twice daily as a suspension, was the only drug regimen that approached the potential therapeutic efficacy for healing of gastric-related disease when assessed by criteria for human patients (pH ≥ 3 / pH ≥ 4 for approximately 75% of the day). Studies in people have documented that higher dosages of omeprazole provide a more predictable inhibition of gastric acid secretion.(Huang and Hunt 2001) In addition, when higher doses are needed, twice daily administration is recommended to achieve optimal gastric acid suppression.(Huang and Hunt 2001)

Adverse gastrointestinal tract effects (vomiting and diarrhea) were seen in multiple dogs in the present study when omeprazole suspension was administered every 12 hours, but were not observed with any of the other drug formulations. These adverse effects may have been related to the greater frequency of omeprazole administration, although this has not been identified in toxicology studies during which much higher dosages were administered.(Ekman *et al* 1985) Alternatively, the formulation of omeprazole, the addition of sodium bicarbonate, or the route or rate of drug administration may be the cause as one dog vomited immediately after receiving the omeprazole suspension. Gastroenteritis could not be ruled out; however, gastroenteritis

was unlikely as the dogs remained bright, the adverse effects were seen randomly throughout the week, and all gastrointestinal problems resolved when drug administration was discontinued. Interestingly, vomiting was not reported in a study involving human patients receiving this same formulation.(Phillips *et al* 1996)

Dogs in the present study were healthy and were fed a standard daily diet. Thus, results should be extrapolated with caution to ill, possibly anorexic, dogs that may have altered gastric acid secretion. It is also important to recognize that gastric acid suppressants have been shown to act differently depending on whether gastric acid secretion is stimulated. In humans, proton pump inhibitors result in prolonged and highly effective inhibition of both basal and stimulated gastric acid secretion, whereas H₂ receptor antagonists were more effective at inhibiting intragastric acidity during periods of basal acid secretion.(Huang and Hunt 2001)

Finally, the optimal degree of gastric acid suppression in dogs has not been established. It is likely that, in dogs and humans, gastric acid suppression should be tailored to manage the acid-related condition, with consideration given to the extent and severity of the condition. Results of the present study suggest that various gastric acid suppressants, when administered at currently recommended dosages, have different degrees of gastric acid suppression in dogs.

2.6 Tables

Table 2.6-1-- Median 24-hour intragastric pH values of all fasted dogs

	Dog #	Dog Name	Drug	Week	Day	Median pH
1	#1	Babs	None	0	Baseline	Fasting 6
2	#4	Honey	None	0	Baseline	Fasting 5.4
3	#5	Ike	None	0	Baseline	Fasting 1.9
4	#6	Kenny	None	0	Baseline	Fasting 2.4
5	#3	Patsy	None	0	Baseline	Fasting 5.6
6	#2	Trakey	None	0	Baseline	Fasting 6
7	#7	Angelina	None	0	Baseline	Fasting 6.6
8	#12	Blossom	None	0	Baseline	Fasting 5.9
9	#8	Champagne	None	0	Baseline	Fasting 2.7
10	#9	Gilmour	None	0	Baseline	Fasting 6.4
11	#10	Kyle	None	0	Baseline	Fasting 2.9
12	#11	Lemieux	None	0	Baseline	Fasting 6
13	#1	Babs	None	6	0	Fasting 6.6
14	#4	Honey	None	6	0	Fasting 0.9
15	#5	Ike	None	6	0	Fasting 4.4
16	#6	Kenny	None	6	0	Fasting 6.2
17	#3	Patsy	None	6	0	Fasting 6.2
18	#2	Trakey	None	6	0	Fasting 6.1
19	#7	Angelina	None	6	0	Fasting 6.6
20	#12	Blossom	None	6	0	Fasting 7
21	#8	Champagne	None	6	0	Fasting 6.7
22	#9	Gilmour	None	6	0	Fasting 6.7
23	#10	Kyle	None	6	0	Fasting 6.6
24	#11	Lemieux	None	6	0	Fasting 1.6

n= 16 with
pH > 5.5

Table 2.6-2 --Median 24-hour intragastric pH values of all fed dogs

	Dog #	Dog Name	Drug	Week	Day	State	Median pH
1	#1	Babs	None	1	Baseline	Fed	1
2	#2	Trakey	None	1	Baseline	Fed	1.3
3	#3	Patsy	None	1	Baseline	Fed	1.3
4	#4	Honey	None	1	Baseline	Fed	1.1
5	#5	Ike	None	1	Baseline	Fed	1.1
6	#6	Kenny	None	1	Baseline	Fed	1
7	#7	Angelina	None	1	Baseline	Fed	1.2
8	#8	Champagne	None	1	Baseline	Fed	1.7
9	#9	Gilmour	None	1	Baseline	Fed	1.2
10	#10	Kyle	None	1	Baseline	Fed	1.2
11	#11	Lemieux	None	1	Baseline	Fed	2.4
12	#12	Blossom	None	1	Baseline	Fed	1.6
13	#1	Babs	None	2	0	Fed	2.9
14	#2	Trakey	None	2	0	Fed	1.3
15	#3	Patsy	None	2	0	Fed	1.5
16	#4	Honey	None	2	0	Fed	3.6
17	#5	Ike	None	2	0	Fed	1.2
18	#6	Kenny	None	2	0	Fed	1.4
19	#7	Angelina	None	2	0	Fed	1
20	#8	Champagne	None	2	0	Fed	1
21	#9	Gilmour	None	2	0	Fed	1.1
22	#10	Kyle	None	2	0	Fed	2.9
23	#11	Lemieux	None	2	0	Fed	1.5
24	#12	Blossom	None	2	0	Fed	1.2
25	#1	Babs	None	3	0	Fed	1.2
26	#2	Trakey	None	3	0	Fed	1.1
27	#3	Patsy	None	3	0	Fed	1.4
28	#4	Honey	None	3	0	Fed	3.9
29	#5	Ike	None	3	0	Fed	1.3
30	#6	Kenny	None	3	0	Fed	1
31	#7	Angelina	None	3	0	Fed	1.1
32	#8	Champagne	None	3	0	Fed	1.2
33	#9	Gilmour	None	3	0	Fed	1
34	#10	Kyle	None	3	0	Fed	1.5
35	#11	Lemieux	None	3	0	Fed	1.4
36	#12	Blossom	None	3	0	Fed	1.8
37	#1	Babs	None	4	0	Fed	1.1
38	#2	Trakey	None	4	0	Fed	1.4
39	#3	Patsy	None	4	0	Fed	6.1
40	#4	Honey	None	4	0	Fed	3.2
41	#5	Ike	None	4	0	Fed	1.2
42	#6	Kenny	None	4	0	Fed	2.6
43	#7	Angelina	None	4	0	Fed	3.6
44	#8	Champagne	None	4	0	Fed	1.1

	Dog #	Dog Name	Drug	Week	Day	Score	Median pH
45	#9	Gilmour	None	4	0	Fed	1.2
46	#10	Kyle	None	4	0	Fed	1.1
47	#11	Lemieux	None	4	0	Fed	3.9
48	#12	Blossom	None	4	0	Fed	2.5
49	#1	Babs	None	5	0	Fed	1.5
50	#2	Trakey	None	5	0	Fed	4.3
51	#3	Patsy	None	5	0	Fed	3
52	#4	Honey	None	5	0	Fed	2
53	#5	Ike	None	5	0	Fed	1.7
54	#6	Kenny	None	5	0	Fed	2.4
55	#7	Angelina	None	5	0	Fed	3.3
56	#8	Champagne	None	5	0	Fed	2.8
57	#9	Gilmour	None	5	0	Fed	2.2
58	#10	Kyle	None	5	0	Fed	0.9
59	#11	Lemieux	None	5	0	Fed	3.8
60	#12	Blossom	None	5	0	Fed	4.9
61	#1	Babs	Saline	1	2	Fed	1.1
62	#1	Babs	Saline	1	6	Fed	0.9
63	#3	Patsy	Saline	4	2	Fed	2.7
64	#3	Patsy	Saline	4	6	Fed	4.6
65	#4	Honey	Saline	3	2	Fed	3.4
66	#4	Honey	Saline	3	6	Fed	1.2
67	#5	Ike	Saline	2	2	Fed	1.4
68	#5	Ike	Saline	2	6	Fed	1.2
69	#6	Kenny	Saline	1	2	Fed	0.9
70	#6	Kenny	Saline	1	6	Fed	1.2
71	#7	Angelina	Saline	5	2	Fed	1.7
72	#7	Angelina	Saline	5	6	Fed	1.6
73	#8	Champagne	Saline	4	2	Fed	1.4
74	#8	Champagne	Saline	4	6	Fed	3.6
75	#9	Gilmour	Saline	5	2	Fed	1.3
76	#9	Gilmour	Saline	5	6	Fed	2.7
77	#10	Kyle	Saline	2	2	Fed	4.8
78	#10	Kyle	Saline	2	6	Fed	1.3
79	#11	Lemieux	Saline	3	2	Fed	1.2
80	#11	Lemieux	Saline	3	6	Fed	1.4
81	#12	Blossom	Saline	1	2	Fed	2.1
82	#12	Blossom	Saline	1	6	Fed	1.2
							n = 67 with pH < 3

Table 2.6-2 -- continued

Table 2.6-3—Median 24-hour intragastric pH in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 7 days.

Day	Saline solution pH	Ranitidine pH	Famotidine pH	Pantoprazole pH	Omeprazole pH
Day 0	1.97 (1.54–2.52)	1.61 (1.27–2.03)	1.68 (1.32–2.12)	1.78 (1.41–2.26)	1.52 (1.20–1.92)
Day 2	1.78 (1.39–2.28)	2.53 (2.00–3.20)	2.83* (2.23–3.59)	3.11* (2.45–3.93)	3.86* [†] (3.05–4.89)
Day 6	1.67 (1.31–2.14)	2.05 (1.62–2.60)	3.68* [†] (2.91–4.66)	3.49* [†] (2.76–4.42)	4.09* [†] (3.23–5.17)

Data are mean of the median (95% confidence interval)

*Significantly ($P < 0.05$) different from value obtained when saline solution was administered.

[†]Significantly ($P < 0.05$) different from value obtained when ranitidine was administered.

Table 2.6-4—Mean percent of time intragastric pH was ≥ 3 in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 7 days.

Day	Saline solution %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
Day 0	36.4 (25.9–46.9)	27.7 (17.6–37.8)	27.8 (17.7–37.9)	31.6 (21.5–41.7)	26.2 (16.2–36.3)
Day 2	33.3 (22.7–43.8)	44.6 (34.5–54.7)	48.9* (38.8–59.0)	54.2* (44.1–64.3)	66.9* ^{†,¶} (56.8–77.0)
Day 6	29.1 (18.6–39.6)	37.2 (27.2–47.3)	60.1* [†] (50.0–70.2)	59.1* [†] (49.0–69.2)	70.2* [†] (60.1–80.2)

Data are mean percent (95% confidence interval).

*Significantly ($P < 0.05$) different from value obtained when saline solution was administered.

[†]Significantly ($P < 0.05$) different from value obtained when ranitidine was administered.

[¶]Significantly ($P < 0.05$) different from value obtained when famotidine was administered.

Table 2.6-5 Mean percent of time intragastric pH was ≥ 4 in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 7 days.

Day	Saline solution	Ranitidine	Famotidine	Pantoprazole	Omeprazole
	%	%	%	%	%
Day 0	20.7 (14.0–30.7)	16.5 (11.4–24.1)	14.8 (10.2–21.6)	18.8 (12.9–27.4)	13.1 (9.0–19.1)
Day 2	22.6 (15.3–33.4)	26.8 (18.4–39.0)	30.5 (20.9–44.5)	34.7 (23.9–50.6)	44.9* (30.9–65.4)
Day 6	17.2 (11.6–25.4)	25.3 (17.4–36.9)	48.4* (33.2–70.5)	40.9* (28.1–59.6)	52.3* [†] (35.9–76.2)

Data are mean percent (95% confidence interval).

*Significantly ($P < 0.05$) different from value obtained when saline solution was administered.

[†]Significantly ($P < 0.05$) different from value obtained when ranitidine was administered.

[¶]Significantly ($P < 0.05$) different from value obtained when famotidine was administered.

2.7 – Figures

Figure 2.7-1—Median intragastric pH in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution for 2 days. Drugs were administered at 9:00 AM and 9:00 PM (open arrows). Dogs were fed 3/4 cup of kibble at 10:00 AM the day of recording and 8:00 AM the following morning (B), 3 treats at 3:00 PM (T), and 3/4 cup of kibble at 8:00 PM (D).

Figure 2.7-2—Median intragastric pH in 12 healthy dogs treated with ranitidine, famotidine, pantoprazole, omeprazole, or saline solution for 6 days. Drugs were administered at 9:00 AM and 9:00 PM (open arrows). Dogs were fed 3/4 cup of kibble at 10:00 AM the day of recording and 8:00 AM the following morning (B), 3 treats at 3:00 PM (T), and 3/4 cup of kibble at 8:00 PM (D).

2.8 - Footnotes

^aIngold bipolar electrode, MIC Medical Instruments Corp, Solothurn, Switzerland.

^bGastrograph III, MIC Medical Instruments Corp, Solothurn, Switzerland.

^cBuffer solutions, Fisher Scientific, Nepean, ON, Canada.

^dWinreflux, MIC Medical Instruments Corp, Solothurn, Switzerland.

^eIams maintenance dry original formula, Iams Co, Dayton, Ohio.

^fIams biscuits small original, Iams Co, Dayton, Ohio.

^gZantac, GlaxoSmithKline Inc, Mississauga, ON, Canada.

^hPepcid IV, Merck Frosst Canada Ltd, Kirkland, QC, Canada.

ⁱPantoIV, Altana Pharma Inc, Oakville, ON, Canada.

^jLosec, AstraZeneca Canada Inc, Mississauga, ON, Canada.

^kSabata M, Byk Canada Inc, Oakville, ON, Canada: Personal communication, 1999.

^lPrilosec, AstraZeneca Int, Wilmington, Del.

2.9 – References

- Akimoto M, Nagahata N, Furuya A, Fukushima K, Higuchi S, Suwa T (2000), Gastric pH profiles of beagle dogs and their use as an alternative to human testing, *Eur.J.Pharm.Biopharm.* 49: 99-102
- Armstrong D, Emde C, Blum AL (1989), Twenty-four-hour intragastric pH-metry studies: easy to do but difficult to analyse, *Eur.J.Gastroenterol.Hepatol.* 1: 167-174
- Bell NJ, Burget D, Howden CW, Wilkinson J, Hunt RH (1992), Appropriate acid suppression for the management of gastro-oesophageal reflux disease, *Digestion* 51 Suppl 1: 59-67
- Burget DW, Chiverton SG, Hunt RH (1990), Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression, *Gastroenterology* 99: 345-351
- Cary NC (1999), *SAS/STAT user's guide: version 8.*, SAS Institute Inc,
- Chiverton SG, Burget DW, Hunt RH (1989), Validation of pH dataloggers for pharmacologic studies, *Scand.J.Gastroenterol.* 24: 975-981
- Dressman JB (1986), Comparison of canine and human gastrointestinal physiology, *Pharmaceutical Research* 3: 123-131
- Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C (1985), Toxicological studies on omeprazole, *Scand.J.Gastroenterol.Suppl.* 108: 53-69
- Fennerty MB (2002), Pathophysiology of the upper gastrointestinal tract in the critically ill patient: rationale for the therapeutic benefits of acid suppression, *Crit.Care Med.* 30: S351-S355
- Fimmel CJ, Etienne A, Cilluffo T, von Ritter C, Gasser T, Rey JP, Caradonna-Moscatelli P, Sabbatini F, Pace F, Buhler HW (1985), Long-term ambulatory gastric pH monitoring: validation of a new method and effect of H₂-antagonists, *Gastroenterology* 88: 1842-1851
- Fishman GS, Moore LR (1982), A statistical evaluation of multiplicative congruential generators with modulus ($2^{31}-1$), *J.Am.Stat.Assoc.* 77: 129-136
- Friedman G (1987), GI drug column, Famotidine, *The American Journal of Gastroenterology* 82: 504-506
- Haskins S (2000), Therapy for shock, in *Kirk's current veterinary therapy XIII, Small Animal Practice*, ed. Bonagura JD and Kirk RW, WB Saunders Co, Philadelphia p 140-147

- Huang JQ, Hunt RH (2001), Pharmacological and pharmacodynamic essentials of H(2)-receptor antagonists and proton pump inhibitors for the practising physician, *Best.Pract.Res.Clin.Gastroenterol.* 15: 355-370
- Jones B, Kenward MG (1989), *Design and analysis of cross-over trials*, Chapman and Hall, London p 189-200
- Kararli TT (1995), Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, *Biopharm.Drug Dispos.* 16: 351-380
- Katz LB, Tobia AJ, Shriver DA (1987), Effects of ORF 17583, other histamine H2-receptor antagonists and omeprazole on gastric acid secretory states in rats and dogs, *J.Pharmacol.Exp.Ther.* 242: 437-442
- Kromer W, Postius S, Riedel R (2000), Animal pharmacology of reversible antagonism of the gastric acid pump, compared to standard antisecretory principles, *Pharmacology* 60: 179-187
- Kromer W, Postius S, Riedel R, Simon WA, Hanauer G, Brand U, Gonne S, Parsons ME (1990), BY 1023/SK&F 96022 INN pantoprazole, a novel gastric proton pump inhibitor, potentially inhibits acid secretion but lacks relevant cytochrome P450 interactions, *J.Pharmacol.Exp.Ther.* 254: 129-135
- Larson C, Cavuto NJ, Flockhart DA, Weinberg RB (1996), Bioavailability and efficacy of omeprazole given orally and by nasogastric tube, *Dig.Dis.Sci.* 41: 475-479
- Larsson H, Carlsson E, Junggren U, Olbe L, Sjostrand SE, Skanberg I, Sundell G (1983), Inhibition of gastric acid secretion by omeprazole in the dog and rat, *Gastroenterology* 85: 900-907
- Larsson H, Mattson H, Sundell G, Carlsson E (1985), Animal pharmacodynamics of omeprazole. A survey of its pharmacological properties in vivo, *Scand.J.Gastroenterol.Suppl.* 108: 23-35
- Matz ME (1995), Gastrointestinal ulcer therapy, in *Current Veterinary Therapy XII, Small animal practice*, ed. Bonagura JD and Kirk RW, WB Saunders Co, Philadelphia p 706-710
- Mela GS, Savarino V (1990), Inaccuracy of hourly sampled pH measurements in describing the effect of antisecretory drugs on circadian gastric acidity, *J.Clin.Pharmacol.* 30: 45-49
- Mignon M, Chau NP, Nguyen-Phuoc BK, Sauvage M, Leguy F, Bonfils S (1982), Ranitidine upon meal-induced gastric secretion: oral pharmacokinetics and plasma concentration effect relationships, *Br.J.Clin.Pharmacol.* 14: 187-193
- PantoIV [product monograph]. 2002. Oakville, ON, Canada, Altana Pharma Inc.

- Papich MG (1993), Antiulcer therapy, *Vet.Clin.North Am.Small Anim Pract.* 23: 497-512
- Phillips JO, Metzler MH, Palmieri MT, Huckfeldt RE, Dahl NG (1996), A prospective study of simplified omeprazole suspension for the prophylaxis of stress-related mucosal damage, *Crit. Care Med.* 24: 1793-1800
- Plumb DC (2002), *Veterinary drug handbook*, Iowa State University Press, Ames, Iowa
- Postius S, Brauer U, Kromer W (1991), The novel proton pump inhibitor pantoprazole elevates intragastric pH for a prolonged period when administered under conditions of stimulated gastric acid secretion in the gastric fistula dog, *Life Sci.* 49: 1047-1052
- Sabata M. personal communication. 1999. Oakville, Ontario, Canada, Byk Canada Inc.
- Savarino V, Mela GS, Zentilin P, Magnolia MR, Scalabrini P, Valle F, Moretti M, Bonifacino G, Celle G (1989), Gastric aspiration versus antimony and glass pH electrodes. A simultaneous comparative in vivo study, *Scand.J.Gastroenterol.* 24: 434-439
- Schunack W (1987), What are the differences between the H₂-receptor antagonists?, *Aliment.Pharmacol.Ther.* 1 Suppl 1: 493S-503S
- Simpson KW, Strauss-Ayali D, McDonough PL, Chang YF, Valentine BA (1999), Gastric function in dogs with naturally acquired gastric *Helicobacter* spp. infection, *J.Vet.Intern.Med.* 13: 507-515
- Soll AH, Berglindh T (1987), Physiology of isolated gastric glands and parietal cells: receptors and effectors regulating function, in *Physiology of the Gastrointestinal Tract*, ed. Johnson LR, Raven Press, New York p 883-906
- Walt R (1986), Twenty four hour intragastric acidity analysis for the future, *Gut* 27: 1-9
- Yamada I, Mizuta H, Goda T, Haga K, Ogawa K (1989), Gastric pH profile and its control in fasting beagle dogs, *Chem.Pharm.Bull.* 37: 2539-2541
- Youngberg C, Wlodyga J, Schmaltz S, Dressman JB (1985), Radiotelemetric determination of gastrointestinal pH in four healthy beagles, *American Journal of Veterinary Research* 46: 1516-1521

CHAPTER III

Day and night-time analysis of the effects of ranitidine, famotidine, pantoprazole, and omeprazole on intragastric pH in dogs

&

Number of dogs responding to acid-lowering therapy

3.1 Abstract

Objective—To identify differences in “day” and “night”-time gastric acid inhibition associated with four gastric acid suppressants and to determine the number of test dogs responding to acid-suppression therapy in a 24-hour period.

Animals—12 healthy Beagle dogs

Procedure—Intragastric pH was measured continuously for 24-hour periods with a digital recording system placed via a gastrostomy tube. Dogs were treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (approximately 1 mg/kg, range 0.8 to 1.3 mg/kg PO, q 24 h), or saline solution for 7 days, and intragastric pH was recorded on days 0, 2, and 6. The 24-hour recordings were divided into two 12-hour periods [(‘day’ 9 AM to 9 PM) and (‘night’ 9 PM to 9 AM)]. The 12-hour ‘day’ and ‘night’ median pH, percentage of time pH was ≥ 3 , and percentage of time pH was ≥ 4 were determined. Thereafter, the number of dogs responding to gastric acid suppression was determined based on the ability of each drug to maintain gastric pH at or above pH 3 for 66% and 75% of the 24-hour day and subsequently at or above pH 4 for 66% of the 24-hour day.

Results—**12-hour analysis** On Day 2, during the day, famotidine was unable to significantly increase gastric pH compared to saline (median pH and %pH ≥ 3); and,

during the night, pantoprazole was unable to increase gastric pH compared to saline (median pH and %pH $\geq$ 3). Pantoprazole, like omeprazole, was able to increase gastric pH $\geq$ 4 during the day only. By Day 6, pantoprazole and famotidine showed a significant effect when compared to saline, over each 12-hour segment, and were comparable to omeprazole. A small but significant effect was noted with ranitidine by Day 6 at night, when ranitidine showed an increase in median gastric pH compared to saline. Otherwise, ranitidine showed no difference when compared to saline.

Dog responders - None of the drugs evaluated were able to consistently maintain gastric pH $\geq$ 3 or pH $\geq$ 4 for 66% to 75% of the day.

Conclusions and Clinical Relevance—12-hour analysis - Results suggest that in healthy dogs, famotidine, pantoprazole, and omeprazole significantly suppress gastric acid secretion when compared to saline. Famotidine is a better gastric acid inhibitor during the night, during minimal secretory states. During early therapy (day 2) pantoprazole's effects are diminished during the night, suggesting that the dosing schedule for this drug should potentially be altered when commencing therapy. **Dog responders** - Omeprazole consistently has the most dogs responding to gastric acid suppression. Ranitidine has the same number of dog responders as placebo and is ineffective as a gastric acid suppressant in the regimen used in this study. At less stringent pH cut-offs (pH $\geq$ 3 for 50-66% of the day), pantoprazole has more responders than famotidine. Once more stringent acid suppression is considered (pH $\geq$ 3 for 75% of the day, or pH $\geq$ 4 for 66% of the day) famotidine, pantoprazole and omeprazole all have only 2-3 dog responders.

3.2 Introduction

Having completed the study evaluating the effects of four commonly used gastric acid lowering therapies at standard veterinary doses, several questions remained unanswered. As assessed by 24-hour median pH and the percentage of time that gastric pH was maintained above, or equal to, 3 and 4, famotidine, pantoprazole and omeprazole all showed significant effects on gastric pH when compared to saline. However, further evaluation was required to determine whether these drugs reached maximal therapeutic effect evenly throughout the 24-hour recording interval, or whether changes to the dosing regimens should be considered. In addition, we wanted to determine the number of study dogs responding to acid-lowering therapy, a clinically relevant piece of information since patients are treated individually and not as a group.

The dosing frequency of gastric acid suppressants has previously been based on pharmacodynamic, as well as pharmacokinetic, features of each drug including its volume of distribution, and drug clearance. In order to evaluate the appropriateness of dosing frequency, *a posteriori* statistical analysis divided data obtained over a full 24-hour period into two 12 hour time intervals ‘day’ (from 9 AM to 9 PM) and ‘night’ (from 9 PM to 9 AM) to evaluate whether the drugs administered as recommended had an equal effect during these two time periods. Although these drugs were administered at differing frequencies, their effect over a full 24-hour period, and subsequently over two 12-hour increments were compared to determine whether current recommendations achieve gastric acid inhibition equally over the 24-hour day, and whether acid suppression is equally achieved in the basal state (overnight) versus the fed state (during normal eating hours). Differences in effect of histamine₂ receptor antagonists (H₂RAs) and proton pump

inhibitors (PPIs) have been noted based on the time of administration in human medicine with H₂RAs showing the greatest effect when administered in the evening after the last meal, and PPIs showing the best response when administered once a day in the morning.

3.3 Material and Methods

A posteriori statistical analyses were performed on data obtained from a randomized, multiple cross-over, double-blind study in 12 healthy, intact, beagle dogs (7 females, 5 males; age 7 months – 2 years) equipped with a previously placed gastrostomy tube (a minimum of two weeks prior to data collection). Each dog received five drug regimens in random order. The regimens were: ranitidine^a (2 mg/kg IV q 12 h), famotidine^b (0.5 mg/kg IV q 12 h), pantoprazole^c (1 mg/kg IV q 24 h), omeprazole^d (approximately 1 mg/kg PO q 24 h), or saline (control) IV q 12 h. Omeprazole was excluded from the blinding as it was administered orally as a single 10 mg tablet, the actual dosage of omeprazole administered to the 12 dogs ranged from 0.8 to 1.3 mg/kg. Drugs were administered for 1 week, with a 1-week washout period between tests. All injectable drugs were prepared daily and syringes were labelled by staff unrelated to the project to ensure blinding. Drug volumes were all made up to 3 mL by the addition of saline. All dogs administered injectable medication received an injection of drug or saline at 9 AM and 9 PM daily. Injectable drugs and saline solution were administered intravenously over 15 minutes. Personnel administering drugs and downloading pH determinations were blinded to the drug treatments.

Intragastric acidity was measured by means of a single channel, internally referenced glass pH electrode^e attached to a portable Gastrograph III pH data recorder.^f The intragastric pH was monitored continuously for 24 hours with sequential gastric pH values recorded every 4 seconds by the data recorder. Recordings were obtained on Day 0 (weekly baseline), Day 2 and Day 6 of each treatment. The 24-hour data was downloaded to a pH data file^g and stored for analysis at a later date.

Electrodes and recorders were calibrated at room temperature (22°C), prior to, and on completion of each study, using commercial buffer solutions^h of pH 1.7 and pH 7. A drift of 0.1 pH units was tolerated. After calibration, the probe was passed via a pre-placed percutaneously-endoscopically placed gastrostomy (PEG) tube, into the fundus of the stomach and secured with adhesive tape. The probe extended 5-7cm beyond the PEG tube into the fundus of the stomach. Probe positioning was predetermined by fluoroscopic examination in two dogs prior to study commencement and was maintained for all dogs based on similar body size and weight (range 7.5 and 12.7 kg; median weight 9.8 kg; mean weight 9.9kg)

During the test days, the dogs were fully ambulatory throughout the pH recordings. Dogs underwent a 12-hour fast prior to initiating each study. Subsequently meals were standardized. The feeding regimen consisted of $\frac{3}{4}$ cup dry dog foodⁱ at 10:00 AM, 3 treats^j at 3:00 PM, $\frac{3}{4}$ cup dry dog food at 8:00 PM and again at 08:00 AM the following morning. This feeding schedule was maintained throughout the study period. Water was available ad libitum. Any complications or side effects during the drug treatments were recorded.

Data recordings were obtained from 9:00 AM until 9:00 AM the following morning. In determining drug effects, outcome measures used for comparison among the treatments were the median pH-values covering the entire 24-hour period and for the time from 9:00 AM to 9:00 PM ('day') and from 9:00 PM to 9:00 AM ('night'), respectively. Other measured variables included the percentage of time that the gastric pH was maintained above or equal to 3 ($\text{pH} \geq 3$) and above or equal to 4 ($\text{pH} \geq 4$) during these same time segments.

Statistical analyses

The 24-hour data were subdivided into two 12-hour increments and all statistical analyses were performed on the 12-hour drug data ('day' and 'night'). The median pH, the percentage of time that the intragastric pH was greater than or equal to 3 ($\text{pH} \geq 3$), and greater than or equal to 4 ($\text{pH} \geq 4$), were derived for each 12-hour recording interval. The statistical design was a randomized latin square, with repeated measures over days.(Jones and Kenward 1989) Randomization was performed using a random number generator.(Fishman and Moore 1982) For evaluation of the effects of ranitidine, famotidine, pantoprazole, and omeprazole, on days 0, 2 and 6, ANOVA for repeated measures was performed. Possible effects of 'dog', 'drug carry-over' and 'week' effect were controlled for. Multiple means comparisons were applied using Dunnett's test when comparing drugs to saline (for each day), and Tukey's test was performed when comparing days against each other (for each drug), and when comparing drugs amongst each other (for each day).

All significance values were based on a Type I error of $\alpha \leq 0.05$. ANOVA assumptions of normality were tested using the Shapiro-Wilk test; the equality of variance assumptions were assessed by plotting residuals against explanatory variables and against the predicted value. Various error structures were examined amongst those offered by SAS (AR1, toep, toeph, etc) to accommodate evaluation of repeated measures over days. Based on these residual analyses, no transformations were applied to 'day' or 'night' $\text{pH} \geq 3$. Analysis of the 'day' residuals revealed that the data was not normally distributed; however, there was no improvement by applying a transformation (log or logit) and these data were therefore not transformed. Conversely, analysis of the 'night'

residuals met all criteria for normality. Log transformations were necessary for 12-hour median pH, both 'day' and 'night'; and logit transformations were necessary for 'day' and 'night' $\text{pH} \geq 4$. For the 'day' $\text{pH} \geq 4$ assumptions of analysis of variance were adequately met after logit transform was applied, except that the residuals were non-normally skewed. All statistical analyses were performed using SAS, a commercially available software program.(Cary 1999)

3.4 Results

12-hour analyses

All 12 dogs completed the study; however, one dog did not complete the saline placebo week. This dog had a gastrointestinal disturbance with vomiting several times during the recording period; therefore, data for the saline group includes only 11 dogs.

Saline 'day' results (days 0, 2 and 6) were compared with each other and with all baseline 'day' results and no significant differences were noted. Saline 'night' results (nights 0, 2, and 6) were also compared against one another and against all baseline 'night' recordings. No statistical significance was noted when any of the above comparisons were made with respect to 12-hour median pH, percent time pH ≥ 3 or percent time pH ≥ 4 . Subsequently, all 12-hour drug data were compared to 12-hour saline controls.

Daytime measurements

Median pH – The 'day' median pH was evaluated on days 0, 2 and 6, (Tables 3.6-1, 3.6-2, 3.6-3). On day 2, the 'day' median pH for pantoprazole (pH 3.24) and omeprazole (pH 4.39) were significantly higher than saline (pH 1.85); ranitidine and famotidine showed no difference when compared to saline. By day 6, the median pH for famotidine (pH 3.27), pantoprazole (pH 3.73) and omeprazole (pH 4.22) were significantly greater than for saline (pH 1.56). Ranitidine showed no significant difference when compared to saline.

Duration pH ≥ 3 - The percentage of time that each test drug maintained gastric pH at, or above, 3 during the 12-hour day on days 2 and 6 are listed in Tables 3.6-5 and 3.6-6. On day 2, the length of time the pH was ≥ 3 was significantly greater for pantoprazole (62.0%) and omeprazole (75.7%) than for saline (31.2%). By day 6, the percent time that the pH was ≥ 3 was significantly greater for famotidine (55.3%), pantoprazole (64.3%) and omeprazole (80.0%) than for saline (28.3%). Ranitidine showed no significant difference in the duration of gastric acid suppression (pH ≥ 3) when compared to saline.

Duration pH ≥ 4 - The percentage of time that each test drug maintained the gastric pH at, or above 4 during the 12-hour day period (days 0, 2 and 6) are given in Tables 3.6-7, 3.6-8, and 3.6-9, respectively. Results on day 2 showed that pantoprazole (51.6%) and omeprazole (62.5%) produced a significantly greater duration of acid suppression when compared to saline (21.4%). By day 6, famotidine (47.4%), pantoprazole (47.5%) and omeprazole (72.7%) produced significantly more time with a pH ≥ 4 than did saline (17.0%). Ranitidine produced no significant difference in the extent of gastric acid suppression achieved when compared to saline.

Night-time measurement

Median pH – The night median pH was evaluated on days 0, 2 and 6 (Tables 3.6-1, 3.6-2, 3.6-3). On day 2, the night median pH for famotidine (pH 3.17), and omeprazole (pH 3.33) were significantly higher than that for saline (pH 1.88). By day 6, the median pH for ranitidine (pH 2.61), famotidine (pH 4.03), pantoprazole (pH 3.26) and omeprazole (pH 3.51) were significantly greater than that for saline (pH 1.67).

Duration pH ≥ 3 – The percentages of time that each test drug maintained the gastric pH at, or above, 3 during the 12-hour night on days 2 and 6 are listed in Tables 3.6-5 and 3.6-6. Results from day 2 showed that the length of time that the pH ≥ 3 was significantly greater for famotidine (54.3%) and omeprazole (58.0%) than for saline (35.4%); whereas results from ranitidine and pantoprazole were no different from saline. On day 6, the percent time that the pH was ≥ 3 was significantly greater for famotidine (64.9%), pantoprazole (53.9%) and omeprazole (60.3%) compared to saline (30.0%). Ranitidine showed no significant difference in the duration of gastric acid suppression (pH ≥ 3) when compared to saline.

Duration pH ≥ 4 – The percentage of time that each test drug maintained the gastric pH at, or above 4 during the 12-hour night period is given in Tables 3.6-7, 3.6-8, and 3.6-9. Results on day 2 showed that no drug had a significant effect on gastric acid inhibition when compared to saline. By day 6, famotidine (54.8%), pantoprazole (40.3%) and omeprazole (49.7%) produced significantly more time with a pH ≥ 4 than did saline (14.4%). Ranitidine resulted in no significant difference in the extent of gastric acid suppression achieved when compared to saline.

The inter-drug comparison revealed that, on day 2, the median 12-hour ‘day’ pH for omeprazole (pH 4.39) was significantly higher than for ranitidine (pH 2.29) and famotidine (2.53). By the ‘night’ 12-hour recording, famotidine (pH 3.17) had reached a significantly greater median pH than did saline (pH 1.88), and no further difference could be appreciated between famotidine (pH 3.17) and omeprazole (pH 3.33) (Table 3.6-2).

Similarly, during day 2, omeprazole resulted in a $\text{pH} \geq 3$ for 75.7% of the 12-hour period, which was significantly greater than for ranitidine (42.0%) and famotidine (43.5%).

During the night recording again, famotidine (pH 54.3%) reached statistically higher pH values when compared to saline (35.4%), and no difference in gastric acid inhibition was noted between famotidine (54.3%) and omeprazole (58.0%) (Table 3.6-5).

By day 6, the 12-hour day median pH for famotidine (pH 3.27), pantoprazole (pH 3.73) and omeprazole (pH 4.22) were significantly higher than ranitidine (pH 1.77); but during the night this significant difference was no longer present (Table 3.6-3). Similarly, during the day, the length of time that the pH reached ≥ 3 was significantly greater for famotidine (55.3%), pantoprazole (64.3%) and omeprazole (80.0%) than for ranitidine (29.9%). By the night period, only famotidine remained significantly better than ranitidine (pH >3 [64.9% vs. 44.6% respectively])(Table 3.6-6). When evaluating the percent time that the gastric pH was ≥ 4 , on day 2 there were no significant inter-drug differences during the day or night. By day 6, during the day, famotidine (47.4%), pantoprazole (47.5%) and omeprazole (72.7%) were significantly better than ranitidine (18.5%) at raising the pH. By the night period, there was no longer any significant difference among all four drugs evaluated (Table 3.6-9).

The ANOVA analysis revealed that, when evaluating the night $\text{pH} \geq 4$, there was no drug effect and no drug-by-day effect. The ANOVA analysis also demonstrated a significant 'week' effect for all parameters evaluated (median pH, and percent time that the pH was ≥ 3 and 4). In addition, a drug 'carry-over' effect was noted for the 12-hour analyses of median pH (day and night), the percent time that the pH ≥ 3 (day and night) and the percent time that the pH ≥ 4 (night only). Carry-over implies that potential drug

interactions were evident such that drugs' abilities to increase gastric pH were statistically different depending on the drug order. Drug randomization controlled for the 'carry-over' effect and the ANOVA statistically adjusted for this effect.

Number of dogs responding to acid-lowering therapy

In this study, a mean of 2.6 of 11 placebo treated dogs maintained gastric $\text{pH} \geq 3$ for at least 50% of the day, and only one of 11 dogs maintained a gastric $\text{pH} \geq 3$ or 4 for more than 66% of the day (Tables 3.6-10 and 3.6-11). Ranitidine also showed only two of 12 dogs responding with a $\text{pH} \geq 3$ for 66% of the day on day 2, and no dogs responding on day 6; one of 12 dogs responding with a $\text{pH} \geq 3$ for more than 75% of the day on day 2 and none on day 6. Omeprazole consistently had the most dogs responding on days 2 and 6 in all categories ($\text{pH} \geq 3$ for 50%, 66% and 75% of the day; and $\text{pH} \geq 4$ for 50% and 66% of the day) (Tables 3.5-10 and 3.5-11). On day 2, when famotidine and pantoprazole were compared, pantoprazole had more dogs (9 vs. 6, respectively) with a $\text{pH} \geq 3$ for at least 50% of the day and at least 66% of the day (5 vs. 2, respectively) than did famotidine; these drugs were evenly matched once pH elevation was evaluated over 75% on day 2 of therapy. These drugs again showed similar response rates after 6 days of treatment. When a $\text{pH} \geq 4$ was evaluated, pantoprazole had more dogs with a $\text{pH} \geq 4$ for 50% of the day than famotidine (5 vs. 2 respectively); however, when longer acid suppression times were evaluated (66 and 75% of the day), these drugs were evenly matched in maintaining gastric $\text{pH} \geq 4$. Overall, only two to three of 12 dogs attained a $\text{pH} \geq 3$ for 75% of the day when using famotidine, pantoprazole or omeprazole; and again

only three out of 12 dogs attained a $\text{pH} \geq 4$ for 66% of the day using famotidine and pantoprazole and four out of 12 dogs when using omeprazole.

3.5 Discussion

12-hour analyses

Results from this work demonstrate that famotidine has improved effects during the night, and that pantoprazole, early in therapy, shows superior effects during the day; both drugs demonstrate more pronounced effects with continued daily therapy.

Omeprazole early in therapy is more effective than ranitidine and famotidine (during the day) and more effective than ranitidine (at night). After nearly one week of therapy famotidine, pantoprazole and omeprazole are all able to significantly increase the median gastric pH and the length of time that gastric pH is maintained above three, during both the day and night-time periods, when compared to saline. The study also identified that the drugs evaluated have little effect at raising gastric pH beyond four during the nighttime interval. Finally, the results in this chapter support previous findings from the 24-hour analyses that ranitidine consistently achieves poor gastric acid inhibition.

The 12-hour analyses were performed to evaluate whether drugs had similar effects during the 'day' and 'night'-time periods and allowed assessment of the time of greatest effect of the drugs investigated. In this study, ranitidine and famotidine were administered every 12 hours, whereas omeprazole and pantoprazole were administered every 24 hours. We had concerns regarding whether drugs administered once a day were in fact lowering gastric pH during the entire 24-hour period, and whether drugs administered every 12 hours had a different effect between day treatment and night treatment, as has been observed in people taking H₂RAs.(Savarino *et al* 1992,Wilder-Smith and Merki 1991)

The two 12-hour intervals separated the 24-hour recordings into a 'day' period, evaluating recordings from 9 AM to 9 PM when the dogs were exposed to light, activity, and feeding (3 standardized meals per day), and a 'night' period, evaluating recordings from 9 PM to 9 AM the following morning when dogs were housed in the dark, and sleeping. The authors acknowledge that these time intervals were chosen *a posteriori*, after inspection of data, and therefore result in a descriptive study. However, this follows the dosing schedule and a typical day-night period.

This study found that day and night effects were most prevalent early in the course of therapy (on day 2). Analysis of the 12-hour data revealed that famotidine's effect was only apparent during the night rather than during the entire 24-hour period (based on median pH and percent of time that the pH was ≥ 3), and conversely, pantoprazole's effect was noted during the day rather than the entire 24-hour period. After several days of therapy, these differences became negligible and were no longer statistically significant. The 12-hour analysis also revealed that pantoprazole was able to raise intragastric pH ≥ 4 when compared to saline during the day, but this effect was lost on evaluation of the entire 24-hour period.

The increased effect of famotidine at night is in accordance with previous findings in the human literature where H₂RAs are most effective at night when secretory effects are minimized.(Wilder-Smith and Merki 1991) As in people, famotidine in dogs appears to be more effective in suppressing basal acid secretion when food is not inciting gastric acid secretion.(Netzer *et al* 1999a,Savarino *et al* 1992)

The decreased effect of pantoprazole at night suggests that its effects early in treatment do not withstand acid suppression over the extended 24-hour day. Dosing

changes are, therefore, recommended for pantoprazole. Suggestions might include an increase in the once daily dose or twice a day administration. Further investigation is necessary to confirm such an adjustment in dose or dosing frequency in dogs.

Interestingly, recommendations in the human literature include using higher doses of PPIs to provide more predictable gastric acid inhibition, or twice a day dosing of PPIs to achieve optimal acid suppression in the early phase of therapy.(Huang and Hunt 2001)

Based on the findings that famotidine is an excellent nocturnal gastric acid suppressant and that, early in therapy, PPI administration has an improved effect during the day, concomitant use of PPIs with an H₂RA for maximal acid suppression might be considered. Night-time H₂RAs have been shown to be effective in reducing residual nocturnal acid secretion in human patients receiving PPIs.(Peghini *et al* 1998) This, however, must be considered cautiously since a previous study in dogs using concomitant treatment with famotidine and pantoprazole (i.e. famotidine at 2 and 4 PM and pantoprazole at 3 PM), demonstrated a shortened duration of the pH-elevating effect of pantoprazole. (Postius *et al* 1991) Further studies are necessary before such combination therapy can be either recommended or discouraged.

The 24-hour statistical analysis demonstrated that omeprazole was the only drug able to increase gastric pH ≥ 4 compared to placebo on day 2. The 12-hour analysis identified that this effect was most prominent during the day, but imperceptible overnight. This portion of the study demonstrated that no drug was able to increase gastric pH beyond that of placebo during the night early in therapy, thus suggesting that no drug is beneficial in this time frame at the doses currently recommended.

By day 6 famotidine, pantoprazole and omeprazole significantly increased intragastric pH when compared to saline, both during the day and at night; no significant difference could be appreciated among the drugs. This study demonstrated that the PPIs, omeprazole, and particularly pantoprazole, had consistently improved effects on gastric acid inhibition by day 6, thus supporting findings of previous studies demonstrating improved gastric acid inhibition after repeated once-daily dosing with a PPI.(Larsson *et al* 1985,Stedman and Barclay 2000) Famotidine also displayed enhanced gastric acid suppression after several days of therapy and again demonstrated good nocturnal acid suppression. After six days of therapy, famotidine, at night, was the only drug that statistically was able to outperform ranitidine. This study has demonstrated that tolerance does not develop with famotidine in the dog; this is in contrast to reports in humans that have demonstrated the development of tachyphylaxis with the use of H₂RAs over several days.(Netzer *et al* 1999b,Wilder-Smith and Merki 1992)

During the 24-hour evaluations, ranitidine did not show any significant increase in gastric pH compared to saline. The 12-hour analysis is the sole method by which ranitidine was shown to have some effect on gastric acid inhibition at night by day 6. In summary, at the current recommended doses, ranitidine is a poor gastric acid suppressant.

The ANOVA analysis revealed that there was no drug effect and no drug-by-day effect when evaluating the percent time that gastric pH was greater than, or equal to, 4 during the night. This finding suggests that statistically, there were no differences among any of the drugs on any of the days in their ability to raise gastric pH beyond 4. Regardless, differences of the least square means were generated to look at the differences between each drug on each day. There were no differences noted among any

of the treatments (including placebo) on day 2; however, a small number of statistically significant differences were noted on day 6, namely famotidine, pantoprazole and omeprazole were each significantly better at raising intragastric pH ≥ 4 than was saline. This finding had previously been noted in the 24-hour analysis.

The ANOVA analysis also revealed a consistent 'week' effect, with gastric pH increasing throughout the 10-week study period, as well as a 'carry-over' effect. A 'carry-over' effect implies that potential drug interactions were evident such that the drugs' abilities to increase gastric pH were statistically different depending on the drug order. We propose that acclimation of the dogs to the facility and study procedures may have contributed to the effects noted in the ANOVA. Previous studies have documented that nervous and hormonal stimuli can affect gastric acid production. Increased gastric acid secretion has been reported in conditions such as anxiety (Gray *et al* 1953, Hoffman 1955, Kuna 1964), restraint, (Brodie and Marshall 1963, Menguy 1960) and noise. (Rovelstad *et al* 1952) Decreasing or eliminating stress conditions by conditioning should result in decreasing acidity of gastric juice (Kuna 1964) Since acclimatization takes 2 or 3 weeks for dogs to return to normal gastrointestinal physiology (Dressman and Yamada 1991) it is likely that acclimatization played a role in lowering gastric acid secretion throughout the study period. Drug randomization controlled for the 'week-effect' and the 'carry-over' effect and the ANOVA statistically adjusted for these effects.

Number of dogs responding to acid-lowering therapy

This portion of the study identified that treatment with pantoprazole caused more dogs to respond to therapy than did famotidine when lower pH thresholds (pH ≥ 3) or

shorter durations of elevated pH (50% or 66% of the 24-hour period) were evaluated. Once the more stringent human criteria for acid suppression were applied: higher pH thresholds (pH $\geq$ 4 for 66% of the day) or longer acid suppression (pH $\geq$ 3 for more than 75% of the 24-hour recording), then these two drugs were evenly matched in the number of responders. Regardless, few dogs (only two to three out of 12) met these criteria after almost one week of therapy. Omeprazole had the highest number of dogs responding to acid suppression; however, again few dogs (3-4 out of 12) met the stringent human criteria.

There is considerable inter- and intra-dog variability in gastric pH.(Dressman 1986,Simpson *et al* 1999,Yamada *et al* 1989) With such variability, determining which dogs are responding to gastric acid lowering therapy is difficult. Critical pH values have been determined in human medicine and these include a pH $>$ 3 for 18 hours of the day (i.e. approximately 75% of the day) for duodenal ulcer healing(Burget *et al* 1990) and a pH $>$ 4 for a minimum of 16 hours of the day (i.e. 66% of the day) for treatment of gastroesophageal reflux disease(Bell *et al* 1992), or for prevention of gastrointestinal ulceration in the critically ill.(Fennerty 2002). A pH $>$ 6 is necessary to achieve hemostasis with acute gastrointestinal tract bleeding.(Fennerty 2002)

In this study, pH thresholds of 3 and 4 were set as criteria for successful acid inhibition and, based on this, the percentage of time that the dogs maintained these thresholds were calculated. Considering that few dogs attained these critical values for a large portion of the day, the number of dogs maintaining these pH values for a minimum of 50% of the day, as well as for 66% and 75% of the day, were calculated.

A small number of dogs receiving placebo (saline) maintained a gastric pH ≥ 3 , for 50% to 75% of the day. However, this study documented that dog responders were most consistently noted with omeprazole where the majority of dogs reached a gastric pH ≥ 3 for 50-66% of the day. At similar cut-offs (pH ≥ 3 , for 50-66% of the day), pantoprazole had more responders than famotidine. Once further acid-suppression was evaluated (a pH ≥ 4 or suppression for a minimum of 75% of the day) then famotidine, pantoprazole and omeprazole were all similar. Ranitidine treatment produced no more dogs responding to acid suppression than did saline at any cut-off.

These results suggest that the current treatment recommendations for dogs do not increase gastric pH adequately, and certainly not to the levels anticipated by many veterinarians. Despite not having critical values identified for the healing of esophageal and gastroduodenal ulcers in dogs, the pH values obtained with the current dosing recommendations cannot attain a persistent pH of 6, which is necessary for platelet aggregation and to prevent clot dissolution in the face of acute gastrointestinal hemorrhage in several species.(Green, Jr. *et al* 1978,Li *et al* 2000)

If healing of gastrointestinal ulcers in dogs follows a similar pattern to that in humans (Burget *et al* 1990), then evaluation into the degree and duration of acid suppression for various conditions in the dog is necessary. At currently recommended doses, dogs are not meeting the human criteria for healing of GI related ulcers.

3.6- Tables

Table 3.6-1

Median pH values for the daytime, nighttime, and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 0.

Period of evaluation	Saline pH	Ranitidine pH	Famotidine pH	Pantoprazole pH	Omeprazole pH
pH 9:00-21:00 h	1.93	1.58	1.64	1.82	1.70
daytime	(1.52-2.46)	(1.25-2.00)	(1.30-2.07)	1.44-2.30)	(1.35-2.15)
pH 21:00-9:00h	2.12	1.66	1.83	1.71	1.41
Night-time	(1.59-2.81)	(1.26-2.18)	(1.39-2.41)	(1.30-2.25)	(1.07-1.85)
24-h pH	1.97	1.61	1.68	1.78	1.52
	(1.54-2.52)	(1.27-2.03)	(1.32-2.12)	(1.41-2.26)	(1.20-1.92)

Data are mean of the median (95% confidence interval)

Table 3.6-2

Median pH values for the daytime, nighttime, and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 2.

Period of evaluation	Saline pH	Ranitidine pH	Famotidine pH	Pantoprazole pH	Omeprazole pH
pH 9:00-21:00 h	1.85	2.29	2.53 ♦	3.24*	4.39*¶§
daytime	(1.45-2.35)	(1.82-2.89)	(2.01-3.20)	(2.57-4.09)	(3.48-5.54)
pH 21:00-9:00h	1.88	2.69	3.17*	2.65 ♦	3.33*
Night-time	(1.41-2.50)	(2.04-3.53)	(2.41-4.17)	(2.01-3.48)	(2.53-4.38)
24-h pH	1.78	2.53	2.83*	3.11*	3.86*¶
	(1.39-2.28)	(2.00-3.20)	(2.23-3.59)	(2.45-3.93)	(3.05-4.89)

Data are mean of the median (95% confidence interval)

*Significantly (P<0.05) different compared to saline

¶Significantly different compared to ranitidine

§Significantly different compared to famotidine

♦ Denotes changes apparent on 12-hour analysis that were unapparent on 24-hour

Table 3.6-3

Median pH values for the daytime, nighttime, and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 6.

Period of evaluation	Saline pH	Ranitidine pH	Famotidine pH	Pantoprazole pH	Omeprazole pH
pH 9:00-21:00 h	1.56	1.77	3.27*¶	3.73*¶	4.22*¶
daytime	(1.23-2.00)	(1.40-2.24)	(2.59-4.13)	(2.96-4.71)	(3.34-5.32)
pH 21:00-9:00h	1.67	2.61*♦	4.03*	3.26*	3.51*
Night-time	(1.25-2.22)	(1.99-3.43)	(3.06-5.30)	(2.48-4.29)	(2.67-4.61)
24-h pH	1.67	2.05	3.68*¶	3.49*¶	4.09*¶
	(1.31-2.14)	(1.62-2.60)	(2.91-4.66)	(2.76-4.42)	(3.23-5.17)

Data are mean of the median (95% confidence interval)

*Significantly (P<0.05) different compared to saline

¶Significantly different compared to ranitidine

♦ Denotes changes apparent on 12-hour analysis that were unapparent on 24-hour

Table 3.6-4

Mean percent of time intragastric pH $\geq$ 3 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 0.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	35.3	23.7	23.0	28.1	26.4
daytime	(23.1-47.5)	(12.0-35.4)	(11.3-34.8)	(16.3-39.8)	(14.7-38.1)
pH 21:00-9:00h	37.4	31.6	32.3	34.7	26.0
Night-time	(25.8-49.0)	(20.4-42.7)	(21.1-43.4)	(23.6-45.9)	(14.9-37.1)
24-h pH	36.4	27.7	27.8	31.6	26.2
	(25.9-46.9)	(17.6-37.8)	(17.7-37.9)	(21.5-41.7)	(16.2-36.3)

Data are mean percent (95% confidence interval).

Table 3.6-5

Mean percent of time intragastric pH ≥ 3 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 2.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	31.2	42.0	43.5 ♦	62.0*	75.7*¶§
daytime	(19.0-43.4)	(30.3-53.7)	(31.7-55.3)	(50.3-73.7)	(64.0-87.5)
pH 21:00-9:00h	35.4	47.3	54.3*	46.3 ♦	58.0*
Night-time	(23.8-47.0)	(36.2-58.4)	(43.2-65.5)	(35.2-57.4)	(46.9-69.1)
24-h pH	33.3	44.6	48.9*	54.2*	66.9*¶§
	(22.7-43.8)	(34.5-54.7)	(38.8-59.0)	(44.1-64.3)	(56.8-77.0)

Data are mean percent (95% confidence interval)

*Significantly ($p < 0.05$) different from value obtained when saline solution administered.

¶Significantly different compared to ranitidine

§Significantly different compared to famotidine

♦Denotes changes apparent on 12-hour analysis that were unapparent on 24-hour

Table 3.6-6

Mean percent of time intragastric pH ≥ 3 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 6.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	28.3	29.9	55.3*¶	64.3*¶	80.0*¶§
daytime	(16.0-40.5)	(18.2-41.7)	(43.6-67.1)	(52.5-76.0)	(68.3-91.7)
pH 21:00-9:00h	30.0	44.6	64.9*¶	53.9*♦	60.3*♦
Night-time	(18.4-41.6)	(33.5-55.7)	(53.8-76.1)	(42.7-65.0)	(49.2-71.4)
24-h pH	29.1	37.2	60.1*¶	59.1*¶	70.2*¶
	(18.6-39.6)	(50.0-70.2)	(50.0-70.2)	(49.0-69.2)	(60.1-80.2)

Data are mean percent (95% confidence interval)

*Significantly ($p < 0.05$) different from value obtained when saline solution administered.

¶Significantly different compared to ranitidine

§Significantly different compared to famotidine

♦ Denotes changes apparent on 12-hour analysis that were unapparent on 24-hour

Table 3.6.7

Mean percent of time intragastric pH $\geq$ 4 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 0.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	24.9	12.7	9.7	12.2	10.4
daytime	(12.2-44.3)	(5.9-25.1)	(4.5-19.9)	(5.7-24.3)	(4.8-21.1)
pH 21:00-9:00h	22.0	21.7	19.2	24.2	15.2
Night-time	(11.3-38.4)	(11.4-37.2)	(9.9-33.7)	(13.0-40.6)	(7.7-27.8)
24-h pH	20.7	16.5	14.8	18.8	13.1
	(14.0-30.7)	(11.4-24.1)	(10.2-21.6)	(12.9-27.4)	(9.0-19.1)

Data are mean percent (95% confidence interval).

Table 3.6-8

Mean percent of time intragastric pH ≥ 4 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 2.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	21.4	30.6	30.2	51.6*♦	62.5*
daytime	(11.3-36.8)	(17.6-47.7)	(17.3-47.3)	(34.0-68.8)	(44.6-77.5)
pH 21:00-9:00h	25.7	32.4	36.2	28.0	35.8♦
Night-time	(13.5-43.4)	(18.3-50.6)	(20.9-54.9)	(15.4-45.5)	(20.6-54.4)
24-h pH	22.6	26.8	30.5	34.7	44.9*
	(15.3-33.4)	(18.4-39.0)	(20.9-44.5)	(23.9-50.6)	(30.9-65.4)

Data are mean percent (95% confidence interval)

*Significantly ($p < 0.05$) different from value obtained when saline solution administered.

Table 3.6-9

Mean percent of time intragastric pH $\geq$ 4 for the daytime, nighttime and 24-h period in 12 healthy dogs treated with ranitidine (2 mg/kg, IV, q 12 h), famotidine (0.5 mg/kg, IV, q 12 h), pantoprazole (1 mg/kg, IV, q 24 h), omeprazole (1 mg/kg, PO, q 24 h), or saline solution on day 6.

Period of evaluation	Saline %	Ranitidine %	Famotidine %	Pantoprazole %	Omeprazole %
pH 9:00-21:00 h	17.0	18.5	47.4*¶♦	47.5*¶♦	72.7*¶
daytime	(10.1-27.2)	(11.4-28.8)	(33.6-61.6)	(33.7-61.6)	(60.0-82.6)
pH 21:00-9:00h	14.4	34.3	54.8*	40.3*	49.7*♦
Night-time	(7.1-27.1)	(10.6-52.8)	(36.0-72.2)	(24.0-59.1)	(31.5-67.9)
24-h pH	17.2	25.3	48.4*	40.9*	52.3*¶
	(11.6-25.4)	(17.4-36.9)	(33.2-70.5)	(28.1-59.6)	(35.9-76.2)

Data are mean percent (95% confidence interval).

*Significantly ($p < 0.05$) different from value obtained when saline solution administered.

¶Significantly different compared to ranitidine

Table 3.6-10

Number of dogs with a pH > 3 for a given percentage of the 24-hour day

	% of the day	Saline n=11	Ranitine n=12	Famotine n=12	Pantoprazole n=12	Omeprazole n=12
Day 0	>50%	4	1	2	3	3
	>66%	1	1	0	1	0
	>75%	1	1	0	0	0
Day 2	>50%	2	3	6	9	10
	>66%	0	2	2	5	9
	<u>>75%</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>2</u>	<u>3</u>
Day 6	>50%	2	2	7	9	12
	>66%	0	0	5	5	9
	<u>>75%</u>	<u>0</u>	<u>0</u>	<u>3</u>	<u>2</u>	<u>3</u>

Underlined values represent those dogs meeting a gastric pH >3 for a minimum of 75% of the day; criteria established for the healing of gastroduodenal ulceration in humans.

Table 3.6-11**Number of dogs with a pH > 4 for a given percentage of the 24-hour day**

	% of the day	Saline n=11	Ranitidine	Famotidine	Pantoprazole	Omeprazole
Day 0	>50%	1	1	0	2	0
	>66%	1	1	0	0	0
	>75%	1	1	0	0	0
Day 2	>50%	1	3	2	5	6
	<u>>66%</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>2</u>	<u>4</u>
	>75%	0	1	1	0	1
Day 6	>50%	1	1	7	6	8
	<u>>66%</u>	<u>0</u>	<u>0</u>	<u>3</u>	<u>3</u>	<u>4</u>
	>75%	0	0	0	1	1

Underlined values represent those dogs meeting a gastric pH >4 for a minimum of 66% of the day; criteria established for the treatment of gastroesophageal reflux disease in humans and for prophylaxis against gastrointestinal ulceration in the critically ill human patient.

3.7 - Footnotes

^aZantac, GlaxoSmithKline Inc, Mississauga, ON, Canada.

^bPepcid IV, Merck Frosst Canada Ltd, Kirkland, QC, Canada.

^cPantoIV, Altana Pharma Inc, Oakville, ON, Canada.

^dLosec, AstraZeneca Canada Inc, Mississauga, ON, Canada.

^eIngold bipolar electrode, MIC Medical Instruments Corp, Solothurn, Switzerland.

^fGastrograph III, MIC Medical Instruments Corp, Solothurn, Switzerland.

^gWinreflux, MIC Medical Instruments Corp, Solothurn, Switzerland.

^hBuffer solutions, Fisher Scientific, Nepean, ON, Canada.

ⁱIams maintenance dry original formula, Iams Co, Dayton, Ohio.

^jIams biscuits small original, Iams Co, Dayton, Ohio.

3.8- References

- Bell NJ, Burget D, Howden CW, Wilkinson J, Hunt RH (1992), Appropriate acid suppression for the management of gastro-oesophageal reflux disease, *Digestion* 51 Suppl 1: 59-67
- Brodie DA, Marshall RW (1963), Gastric content of fasted primates: a survey, *Science* 141: 174-175
- Burget DW, Chiverton SG, Hunt RH (1990), Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression, *Gastroenterology* 99: 345-351
- Cary NC (1999), *SAS/STAT user's guide: version 8.*, SAS Institute Inc,
- Dressman JB (1986), Comparison of canine and human gastrointestinal physiology, *Pharmaceutical Research* 3: 123-131
- Dressman JB, Yamada K (1991), Animal models for oral drug absorption, in *Pharmaceutical bioequivalence*, ed. Dighe SV, Tse FLS, and Welling PG, Dekker, New York p 235-266
- Fennerty MB (2002), Pathophysiology of the upper gastrointestinal tract in the critically ill patient: rationale for the therapeutic benefits of acid suppression, *Crit.Care Med.* 30: S351-S355
- Fishman GS, Moore LR (1982), A statistical evaluation of multiplicative congruential generators with modulus ($2^{31}-1$), *J.Am.Stat.Assoc.* 77: 129-136
- Gray SJ, Ramsey C, Reifenstein RW, Benson JA, Jr. (1953), The significance of hormonal factors in the pathogenesis of peptic ulcer, *Gastroenterology* 25: 156-172
- Green FW, Jr., Kaplan MM, Curtis LE, Levine PH (1978), Effect of acid and pepsin on blood coagulation and platelet aggregation. A possible contributor prolonged gastroduodenal mucosal hemorrhage, *Gastroenterology* 74: 38-43
- Hoffman CR (1955), The causes and prevention of recurrences in peptic ulcer disease, *Am.J.Dig.Dis.* 22: 17-20
- Huang JQ, Hunt RH (2001), Pharmacological and pharmacodynamic essentials of H(2)-receptor antagonists and proton pump inhibitors for the practising physician, *Best.Pract.Res.Clin.Gastroenterol.* 15: 355-370
- Jones B, Kenward MG (1989), *Design and analysis of cross-over trials*, Chapman and Hall, London p 189-200

- Kuna S (1964), The pH of gastric juice in the normal resting stomach, *Arch.Int.Pharmacodyn.Ther.* 152: 79-97
- Larsson H, Mattson H, Sundell G, Carlsson E (1985), Animal pharmacodynamics of omeprazole. A survey of its pharmacological properties in vivo, *Scand.J.Gastroenterol.Suppl.* 108: 23-35
- Li Y, Sha W, Nie Y, Wu H, She Q, Dai S, Jia L, Yu W (2000), Effect of intragastric pH on control of peptic ulcer bleeding, *J Gastroenterol.Hepatol.* 15: 148-154
- Menguy R (1960), Effects of restraint stress on gastric secretion in the rat, *Am.J.Dig.Dis.* 5: 911-916
- Netzer P, Eschenmoser K, Halter F, Inauen W (1999a), Impact of food intake on the antisecretory effect of low-dose ranitidine and famotidine, *Aliment.Pharmacol.Ther.* 13: 407-412
- Netzer P, Gaia C, Sandoz M, Huluk T, Gut A, Halter F, Husler J, Inauen W (1999b), Effect of repeated injection and continuous infusion of omeprazole and ranitidine on intragastric pH over 72 hours, *Am.J.Gastroenterol.* 94: 351-357
- Peghini PL, Katz PO, Castell DO (1998), Ranitidine controls nocturnal gastric acid breakthrough on omeprazole: a controlled study in normal subjects, *Gastroenterology* 115: 1335-1339
- Postius S, Brauer U, Kromer W (1991), The novel proton pump inhibitor pantoprazole elevates intragastric pH for a prolonged period when administered under conditions of stimulated gastric acid secretion in the gastric fistula dog, *Life Sci.* 49: 1047-1052
- Rovelstad RA, Owen CA, Jr., Magath TB (1952), Factors influencing the continuous recording of in situ pH of gastric and duodenal contents, *Gastroenterology* 20: 609-624
- Savarino V, Mela GS, Zentilin P, Vigneri S, Cutela P, Mele MR, Di Mario F (1992), Mealtime versus nighttime acid inhibition. A clinical pharmacological study with ranitidine, *Dig.Dis.Sci.* 37: 1368-1372
- Simpson KW, Strauss-Ayali D, McDonough PL, Chang YF, Valentine BA (1999), Gastric function in dogs with naturally acquired gastric *Helicobacter* spp. infection, *J.Vet.Intern.Med.* 13: 507-515
- Stedman CA, Barclay ML (2000), Review article: comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors, *Aliment.Pharmacol.Ther.* 14: 963-978
- Wilder-Smith CH, Merki HS (1991), Is the time of dosing of a potent long-acting H₂-receptor antagonist critical? Twenty-four hour pH measurements with SKF-94482, *Scand.J.Gastroenterol.* 26: 501-507

Wilder-Smith CH, Merki HS (1992), Tolerance during dosing with H₂-receptor antagonists. An overview, *Scand.J.Gastroenterol.Suppl.* 193: 14-19

Yamada I, Mizuta H, Goda T, Haga K, Ogawa K (1989), Gastric pH profile and its control in fasting beagle dogs, *Chem.Pharm.Bull.* 37: 2539-2541

CHAPTER IV

GENERAL DISCUSSION

The use of acid-lowering therapy in veterinary medicine tends to follow guidelines established in human medicine. The standard of care regarding gastroprotectant use in human medicine continues to evolve, with the type of drug, dose and delivery being studied on an ongoing basis. Recently, the use of acid-lowering therapy as prophylactic treatment for stress-ulcer prophylaxis in the critical care setting has been questioned and guidelines developed for their limited use.(Cook *et al* 1994,Cook *et al* 1996) There are few studies in the veterinary literature investigating the appropriate use of acid-lowering therapy.

Our research confirms, as has been previously reported, that there is considerable inter- and intra-dog variability in gastric pH in both the fasted and fed states.(Dressman 1986,Simpson *et al* 1999,Yamada *et al* 1989,Youngberg *et al* 1985). However, despite wide ranges in pH in healthy dogs, this study demonstrates that the majority of dogs maintained a high pH during the fasted state with a small number of outliers contributing to a wide pH range; and a greater number of dogs maintained a low pH during the fed state, with outliers again causing wide changes in pH. Hypotheses for the outliers with a high pH during the fed state include incorrect probe placement in the cardiac or pyloric regions of the stomach, or episodes of gastroduodenal (biliary) reflux. Proposed mechanisms for low pH recordings during the fasted state include the presence of hormonal or environmental stimuli such as anxiety, or noise, which can incite gastric acid secretion. Overall, inherent variability in canine gastric pH must be anticipated.

Results from this work demonstrate that gastric acid secretion is much lower in the basal state than in the fed state. We have shown that feeding stimulates gastric acid secretion in the majority of dogs tested and that fasting is one of the most successful approaches to inhibiting gastric acid secretion over a 24-hour period.

The drug portion of this study demonstrates that antisecretory drugs, at currently recommended doses, have differing degrees of acid suppression. Ranitidine was ineffective at raising gastric pH when compared to placebo. Famotidine, pantoprazole and omeprazole all showed significant improvement in the degree and duration of gastric acid inhibition when compared to saline. Omeprazole was consistently the most effective gastric acid inhibitor. Overall, the degree of gastric acid inhibition attained using famotidine, pantoprazole and omeprazole was low. Based on arbitrary human pH thresholds, none of the drugs evaluated were able to consistently maintain gastric pH at ≥ 3 or ≥ 4 for 66% to 75% of the day. The results from this study should, however, aid the practitioner in determining the extent of acid inhibition that can be expected with antisecretory drugs administered at standard veterinary doses.

Previous studies have demonstrated that H₂RAs produce an effect on gastric acidity for six to seven hours.(Kromer *et al* 2000,Postius *et al* 1991) Despite these earlier results, no improvement in gastric acid inhibition was noted when famotidine was administered every eight hours instead of every 12 hours, thus suggesting that despite its short duration of action an increase in dosing frequency would not improve the effects of famotidine over the 24-hour period. In fact, famotidine has been promoted for its potential as a once a day treatment in dogs (0.5 mg/kg once a day) despite its short serum half-life (2-3 hours)(Pendleton *et al* 1983,Plumb 2002) and its short duration of action.

Such a dosing schedule requires further evaluation, but, considering that twice a day therapy was ineffective at raising intragastric pH above 3 or 4 for 66% of the day in our study, one can conclude that once a day therapy will not be any more effective.

However, famotidine demonstrated a significantly improved effect over the one-week treatment period. This is in contrast to reports in humans that have demonstrated the development of tachyphylaxis with the use of H₂RAs over several days.(Netzer *et al* 1999,Wilder-Smith and Merki 1992) This study found that tolerance does not develop with famotidine in the dog.

This is the first study documenting the effects of gastric acid-lowering therapy for clinical use in dogs without using artificial means to increase gastric acid secretion as used in previous studies. The authors acknowledge several limitations in this study. This research was conducted in healthy dogs fed a standard daily diet, therefore, the results cannot be directly extrapolated to inappetent, sick or critically ill dogs, nor to other species with different gastrointestinal physiology. The dogs did undergo percutaneous, endoscopically-placed gastrostomy (PEG) tube insertion prior to study commencement. The use of a PEG tube was selected for comfort for the dogs during the study procedure and for ease of pH recording. Reports have documented delayed postoperative restoration of circadian rhythm in canine gastric acidity if a gastropexy occurs at an untolerated position. The latter may result in unphysiological distensions in the gastric wall with subsequent continuous stimulation of acid secretion.(Postius and Brauer 1995) It is apparent that no method for evaluation of gastric pH in dogs is without potential effects on gastric acid secretion. We postulate that the insertion of a well-placed PEG tube, as previously described (Mathews and Binnington 1986), and subsequent probe placement

via this tube, causes less stress or potential gastric stimulation when compared to per oral or per nasal probe placement. In conclusion, no method is able to guarantee continuous ideal probe placement within the stomach and, therefore, gastric acid secretion may be altered unintentionally and intermittently.

Another potential factor that may have altered results in this study involved placement of the pH probe within the stomach. Fluoroscopic evaluation of two dogs prior to study commencement confirmed that extending the probe 5 cm beyond the tip of the PEG tube placed the probe within the body of the stomach for accurate pH recording. However, unexpected elevated pH recordings on probe insertion prompted repeat fluoroscopy in two dogs halfway through the study to confirm that the probes in fact extended beyond the lumen of the PEG tube in the empty stomach. This was confirmed; however, thereafter probes were inserted up to 7.5 cm beyond the PEG tube tip to ensure the probe's position beyond the PEG tube. Despite fluoroscopic confirmation that probes were located in the body of the stomach, the possibility could not be excluded that probes were intermittently able, once the stomach had emptied, to measure cardiac or pyloric or potentially duodenal pH during the 24-hour monitoring.

On evaluating the study design, dogs underwent five treatment regimens, with saline included as a placebo. A Day 0 baseline recording was added to ensure that dogs returned to a predrug state prior to initiating subsequent drug therapy. Drug administration could therefore be statistically compared to baseline or to placebo. Due to study design, Day 0 recordings were limited to recording gastric pH over 23-hours rather than a full 24-hour period. Small discrepancies were noted on statistical comparisons between [drug and placebo] and [drug and baseline] and subsequently placebo was

elected for all comparisons. Placebo was felt to be a more accurate comparison because it involved 24-hour comparisons and because placebo recordings were maintained throughout the study weeks, thus ensuring matched testing conditions on all recording days.

On completion of statistical analyses 'carry-over' effects and 'week' effects were consistently identified. The 'carry-over' effects were noted after famotidine and pantoprazole administration. Based on famotidine's short serum half-life of 2-3 hours,(Pendleton *et al* 1983) and virtually no accumulation in plasma after repeated dosing,(Lin 1991) it is unlikely that famotidine truly exhibited a 'carry-over' effect in our study, but rather that some other underlying effect(s) on gastric acid secretion prevailed. Conversely, pantoprazole, a PPI which binds irreversibly to the proton pump (Shin and Sachs 2002) is more likely to have lingering effects beyond the 7 day washout period. Nonetheless, it is puzzling that pantoprazole should have such an effect when omeprazole, a similar PPI, did not have this effect. We suspect that other underlying causes were responsible for the apparent 'carry-over' effect identified in the study. The authors postulate that the 'carry-over' and 'week' effects may have been attributed to decreasing or eliminating stress conditions by conditioning the dogs, with a resultant decrease in acidity of gastric juice. This has previously been demonstrated in dogs and humans. (Davenport 1982,Kuna 1964) We postulate that environmental adaptation may have taken place over the course of the study, with the dogs becoming more accustomed to procedures and thereby contributing to a 'week' effect that was independent of the drugs administered.

Despite the possibility that other factors unrelated to drug may have accounted for the ‘carry over’ and ‘week’ effects, our findings suggest that the washout period should be extended to ensure that drug interaction is not the underlying source of these effects. Despite previous human studies which used a one-week washout period when examining PPIs, (Sugiyama *et al* 1995) more recent human studies have extended the washout period to two weeks.(Armstrong *et al* 2003) Considering that PPIs are irreversible inhibitors of the gastric parietal ATPase enzyme, recovery of the H⁺K⁺ATPase pump depends on new protein synthesis, and the half-life of the ATPase protein is approximately 30 hours(Sachs 1987), ten half lives for *de novo* protein synthesis would require 12.5 days. Thus a two-week washout period may be more appropriate in ensuring that all of the effects of the PPIs have been effectively eliminated.

If a true drug interaction was noted in this study, and drugs from the previous week were not fully eliminated prior to subsequent drug administration, then based on drug randomization, the effects of all five tested regimens should be altered equally and their pH effects should be greater than otherwise expected. In that event, the true effects of each regimen would have lower pH values than those recorded, and acid-lowering therapy would have less effect than suggested in this study. The results of our study already indicate that the antisecretory drugs tested at current veterinary doses demonstrate little to no effect in inhibiting gastric acid production. In conclusion, it must be emphasized that the comparative aspects of the present study are unaffected, and the ‘carry-over’ effect and ‘week’ effect were controlled for in the study design and statistical analyses.

All 12 beagle dogs were negative for *Helicobacter spp.* infection. These results were surprising considering that previous reports have documented the incidence of *Helicobacter* infection as being from 67-100% in healthy pet dogs, and 100% in healthy laboratory dogs.(Eaton *et al* 1996,Happonen *et al* 1998,Yamasaki *et al* 1998). The high number of *Helicobacter* negative test dogs is likely due to their source and housing conditions. These dogs were all sourced from the same breeding colony, and although they were not specific pathogen free (SPF), they were housed at a research facility with minimal environmental exposure. Alternatively, faulty testing or lack of appropriate testing may have missed positive *Helicobacter* dogs. The probability of the latter was minimized by evaluating multiple gastric fundic biopsy samples and using a combination of diagnostic methods as currently recommended(Simpson *et al* 1999) including urease production, culture using Trypticase Soy Broth (TSB) and polymerase chain reaction (PCR) testing. Histopathology was not performed due to cost-constraints.

4.1 – Future research

The research herein only begins to evaluate gastric acid secretion in the dog. Further research should aim to determine the secretory state of the stomach in ill dogs, in patients that are eating, and in those that have become inappetant or anorexic. This is critical since differences in acid secretion have been noted in healthy versus ill human subjects, where critically ill patients often have a diminished acid secretion and hypersecretion is rare.(Fennerty 2002) Once the extent of acid secretion is established in the ill dog, then the response to gastroprotectant therapy in the ill population can be determined.

It is likely that ulcer healing in dogs, like people, has a significant predictable relationship with parameters of antisecretory therapy, namely the degree and duration of gastric acid suppression and the length of treatment. However, the degree of acid suppression necessary for the optimal healing of gastroduodenal ulcers has not been determined in veterinary patients. Further studies are required to determine threshold pH values necessary to allow optimal healing of esophageal ulcers, gastric ulcers and duodenal ulcers. Research involving sequential endoscopic evaluation of the gastrointestinal tract as well as simultaneous pH recordings will be necessary to identify pH goals of antisecretory therapy.

Considering that the degree of acid suppression in our study was underwhelming when studying standard gastric acid-lowering therapies at currently recommended veterinary doses, alternative protocols need to be evaluated. More aggressive protocols, such as constant rate infusions of both H₂RAs and PPIs need to be evaluated for patients with overt gastric hemorrhage as acute gastrointestinal bleeding requires a pH greater

than 6 to achieve platelet aggregation and prevent clot dissolution.(Green, Jr. *et al* 1978,Li *et al* 2000)

Gastric acid suppression must be evaluated in light of its necessity for prophylaxis versus ulcer healing. In human medicine, few critically ill patients have clinically important gastrointestinal bleeding. Large meta-analyses have demonstrated that prophylaxis against stress ulcers can be safely withheld from critically ill patients unless they have coagulopathy or require mechanical ventilation.(Cook *et al* 1994) The incidence of clinically significant bleeding, as well as the impact on morbidity and outcome associated with such events need to be determined in the canine patient. Animals with low risk of bleeding might not derive benefit from prophylactic measures to protect the gastric mucosa from acid and the costs associated with such widespread use of antisecretory drugs may be unnecessary.(Steinberg 2002)

Finally, this research has demonstrated that gastric acid production and its suppression are not equal among species and that extrapolation of findings to other species is not appropriate. The results from this study are therefore not necessarily applicable to other small animals; for example, gastric acid production in the cat, an obligate carnivore with significant differences in gastric physiology, will need to be studied separately.

4.2 – Conclusions

Recommendations that can be considered based on results from this study include replacing ranitidine with famotidine since famotidine proved to be a much more effective H₂ antagonist for gastric acid suppression. In addition, famotidine demonstrated improved effects overnight and more pronounced effects over time. It is a good drug for routine prophylaxis where cost is a concern and where an injectable drug is preferred. Famotidine is recommended for dogs requiring extended therapy (a minimum of one week), since its effects will improve with time and become comparable to the more expensive PPIs.

Pantoprazole was a good inhibitor of acid production when compared to saline. Early in therapy, it demonstrated improved effects during the initial 12 hours of therapy, but at the dose used (1 mg/kg) could not maintain acid inhibition throughout the 24 day; therefore, an alteration in dose or dosing frequency should be considered for this drug, at least for the first few days of therapy. Pantoprazole consistently had more dogs responding to therapy than did famotidine when lower pH thresholds or shorter durations of elevated pH were considered. Like famotidine, pantoprazole demonstrated more pronounced effects with continued daily therapy. After one week of therapy, pantoprazole was comparable to famotidine and omeprazole.

Omeprazole was consistently the most potent inhibitor of gastric acid production and had the highest number of dogs responding to acid suppression. It is the drug of choice for acid suppression and is the most appropriate drug, based on its effect on pH. Due to cost, other antisecretory drugs are more appropriate for prophylaxis. Omeprazole can be formulated into an effective suspension and administered via a feeding tube for

dogs unable to take oral medication. Parenteral omeprazole may soon be available in North America and would be a welcome addition to the current selection of gastric acid-lowering therapies.

The most important clinical findings in this study are that in dogs, fasting and increasing the standard dose of omeprazole from once to twice-a-day administration produces higher gastric acid suppression than any previously recommended antisecretory therapy. Ultimately drug selection should be based on the underlying disease process and its severity, if the patient can tolerate oral medication, the cost, and the ease of administration.

MASTER REFERENCE LIST

- Akimoto M, Nagahata N, Furuya A, Fukushima K, Higuchi S, Suwa T (2000), Gastric pH profiles of beagle dogs and their use as an alternative to human testing, *Eur.J.Pharm.Biopharm.* 49: 99-102
- Allen A, Flemstrom G, Garner A, Kivilaakso E (1993), Gastroduodenal mucosal protection, *Physiol.Rev.* 73: 823-857
- Allen DG, Dowling PM, Smith DA (2005), *Handbook of Veterinary Drugs*, Lippincott Williams & Wilkins, Philadelphia
- Armstrong D, Bair D, James C, Tanser L, Escobedo S, Nevin K (2003), Oral esomeprazole vs. intravenous pantoprazole: a comparison of the effect on intragastric pH in healthy subjects, *Aliment.Pharmacol.Ther.* 18: 705-711
- Armstrong D, Emde C, Blum AL (1989), Twenty-four-hour intragastric pH-metry studies: easy to do but difficult to analyse, *Eur.J.Gastroenterol.Hepatol.* 1: 167-174
- Atwater JS, Carson JM (1959), Therapeutic principles in management of peptic ulcer. 3. Anticholinergic-tranquilizer combination, *Am.J.Dig.Dis.* 4: 1055-1065
- Ayalon A, Devitt PG, Guzman S, Suddith RL, Rayford PL, Thompson JC (1981), Suppression of gastric secretion by furosemide in dogs, *Proc.Soc.Exp.Biol.Med.* 167: 343-345
- Bachouchi S. Pantoprazole and Dogs; Altana Pharma Inc. 2003. Personal Communication
- Balaban DH, Duckworth CW, Peura DA (1997), Nasogastric omeprazole: effects on gastric pH in critically ill patients, *Am.J.Gastroenterol.* 92: 79-83
- Bastaki SM, Waton NG (1996), Effects of anaesthetics and trauma on stimulated gastric acid secretion in chronic fistula dogs, *Clin.Exp.Pharmacol.Physiol.* 23: 532-536
- Bell NJ, Burget D, Howden CW, Wilkinson J, Hunt RH (1992), Appropriate acid suppression for the management of gastro-oesophageal reflux disease, *Digestion* 51 Suppl 1: 59-67
- Boston SE, Moens NM, Kruth SA, Southorn EP (2003), Endoscopic evaluation of the gastroduodenal mucosa to determine the safety of short-term concurrent administration of meloxicam and dexamethasone in healthy dogs, *Am.J.Vet.Res.* 64: 1369-1375
- Brodie DA, Marshall RW (1963), Gastric content of fasted primates: a survey, *Science* 141: 174-175
- Brooks D, Watson GL (1997), Omeprazole in a dog with gastrinoma, *J.Vet.Intern.Med.* 11: 379-381

- Brooks FP (1985), Effect of diet on gastric secretion, *Am.J.Clin.Nutr.* 42: 1006-1019
- Burek JD, Majka JA, Bokelman DL (1985), Famotidine: summary of preclinical safety assessment, *Digestion* 32 Suppl 1: 7-14
- Burget DW, Chiverton SG, Hunt RH (1990), Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression, *Gastroenterology* 99: 345-351
- Cary NC (1999), *SAS/STAT user's guide: version 8.*, SAS Institute Inc,
- Cavanagh RL, Buyniski JP, Schwartz SE (1987), Prevention of aspirin-induced gastric mucosal injury by histamine H2 receptor antagonists: a crossover endoscopic and intragastric pH study in the dog, *J.Pharmacol.Exp.Ther.* 243: 1179-1184
- Cheville NF (1979), Uremic gastropathy in the dog, *Vet.Pathol.* 16: 292-309
- Chiverton SG, Burget DW, Hunt RH (1989), Validation of pH dataloggers for pharmacologic studies, *Scand.J.Gastroenterol.* 24: 975-981
- Colin-Jones DG (1990), Acid suppression: how much is needed?, *BMJ* 301: 564-565
- Cook D, Heyland D, Griffith L, Cook R, Marshall J, Pagliarello J (1999), Risk factors for clinically important upper gastrointestinal bleeding in patients requiring mechanical ventilation. Canadian Critical Care Trials Group, *Crit.Care.Med.* 27: 2812-2817
- Cook DJ, Fuller HD, Guyatt GH, Marshall JC, Leasa D, Hall R, Winton TL, Rutledge F, Todd TJ, Roy P (1994), Risk factors for gastrointestinal bleeding in critically ill patients. Canadian Critical Care Trials Group, *N.Engl.J.Med.* 330: 377-381
- Cook DJ, Reeve BK, Guyatt GH, Heyland DK, Griffith LE, Buckingham L, Tryba M (1996), Stress ulcer prophylaxis in critically ill patients. Resolving discordant meta-analyses, *JAMA* 275: 308-314
- Cook DJ, Witt LG, Cook RJ, Guyatt GH (1991), Stress ulcer prophylaxis in the critically ill: a meta-analysis, *Am.J.Med.* 91: 519-527
- Daly MJ, Humphray JM, Bunce KT, Stables R (1981), The effect of ranitidine on gastric acid secretory response curves to histamine, pentagastrin or bethanechol in the dog with a Heidenhain pouch, *Agents Actions* 11: 160-164
- Davenport HW (1982), Gastric secretion, digestion, and absorption, in *Physiology of the digestive tract*, ed. Davenport HW, Year Book Medical Publishers, Inc., Chicago p 113-142
- Davenport HW, Warner HA, Code CF (1968), Functional significance of gastric mucosal barrier to sodium, *Gastroenterology* 54: Suppl-11

- Dotevall G (1961), Gastric secretion of acid in diabetes mellitus during basal conditions and after maximal histamine stimulation, *Acta.Med.Scand.* 170: 59-69
- Dowdle SM, Joubert KE, Lambrechts NE, Lobetti RG, Pardini AD (2003), The prevalence of subclinical gastroduodenal ulceration in Dachshunds with intervertebral disc prolapse, *J S.Afr. Vet.Assoc* 74: 77-81
- Dowling PM (1995), Therapy of gastrointestinal ulcers, *Can.Vet.J.* 36: 276-277
- Dressman JB (1986), Comparison of canine and human gastrointestinal physiology, *Pharmaceutical Research* 3: 123-131
- Dressman JB, Yamada K (1991), Animal models for oral drug absorption, in *Pharmaceutical bioequivalence*, ed. Dighe SV, Tse FLS, and Welling PG, Dekker, New York p 235-266
- Drew ML, Ramsay E, Fowler ME, Kass PH (1992), Effect of flunixin meglumine and cimetidine hydrochloride on the pH in the third compartment of the stomach of llamas, *J Am.Vet.Med.Assoc* 201: 1559-1563
- Eaton KA, Dewhirst FE, Paster BJ, Tzellas N, Coleman BE, Paola J, Sherding R (1996), Prevalence and varieties of Helicobacter species in dogs from random sources and pet dogs: animal and public health implications, *J.Clin.Microbiol.* 34: 3165-3170
- Eddlestone SM (2002), Drug therapies used in gastrointestinal disease, *Compendium on continuing education for the practicing veterinarian* 24: 452-467
- Eichhorn R, Tracktir J (1955), The effect of hypnotically induced emotions upon gastric secretion, *Gastroenterology* 29: 432-438
- Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C (1985), Toxicological studies on omeprazole, *Scand.J.Gastroenterol.Suppl.* 108: 53-69
- Emas S, Grossman MI (1967), Comparison of gastric secretion in conscious dogs and cats, *Gastroenterology* 52: 29-34
- Emde C (1990), Graphical display and statistical evaluation of data gained by long-term ambulatory intragastric pH monitoring, *Dig.Dis.* 8 Suppl 1: 82-86
- Emde C, Garner A, Blum AL (1987), Technical aspects of intraluminal pH-metry in man: current status and recommendations, *Gut* 28: 1177-1188
- Engel FL (1955), Addison's disease and peptic ulcer, *J Clin.Endocrinol.Metab* 15: 1300-1307
- Feldman M (1983), Gastric secretion, in *Gastrointestinal Disease: Pathophysiology, Diagnosis, Management*, ed. Sleisenger MH and Fordtran JS, W.B. Saunders Co, Philadelphia p 541-558

- Feldman M, Burton ME (1990), Histamine₂-receptor antagonists. Standard therapy for acid-peptic diseases. 1, *N.Engl.J Med.* 323: 1672-1680
- Fennerty MB (2002), Pathophysiology of the upper gastrointestinal tract in the critically ill patient: rationale for the therapeutic benefits of acid suppression, *Crit.Care.Med.* 30: S351-S355
- Fenster LF (1973), The ulcerogenic potential of glucocorticoids and possible prophylactic measures, *Med.Clin.North.Am.* 57: 1289-1294
- Fimmel CJ, Etienne A, Cilluffo T, von Ritter C, Gasser T, Rey JP, Caradonna-Moscatelli P, Sabbatini F, Pace F, Buhler HW (1985), Long-term ambulatory gastric pH monitoring: validation of a new method and effect of H₂-antagonists, *Gastroenterology* 88: 1842-1851
- Fishman GS, Moore LR (1982), A statistical evaluation of multiplicative congruential generators with modulus ($2^{31}-1$), *J.Am.Stat.Assoc.* 77: 129-136
- Florey HW (1962), The secretion and function of intestinal mucus, *Gastroenterology* 43: 326-329
- Fordtran JS, Walsh JH (1973), Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *J Clin.Invest.* 52: 645-657
- Forte G, Wolosin JM (1987), HCl secretion by the gastric oxyntic cell, in *Physiology of the gastrointestinal tract*, ed. Johnson LR, Raven Press, New York p 853-862
- Foushee LL (2000), Omeprazole, *Compendium on continuing education for the practicing veterinarian* August: 746-749
- Fox L.E., Rosenthal RC, Twedt D.C., Dubleizig RR, MacEwen EG, Grauer GF (1990), Plasma Histamine and Gastrin Concentrations in 17 Dogs with Mast Cell Tumors, *J.Vet. Intern.Med.* 4: 242-246
- Friedman G (1987), GI drug column, Famotidine, *The American Journal of Gastroenterology* 82: 504-506
- Geyer C, Colbatzky F, Lechner J, Hermanns W (1993), Occurrence of spiral-shaped bacteria in gastric biopsies of dogs and cats, *Vet.Rec.* 133: 18-19
- Gray SJ, Benson JA, Jr., Reifenshtein RW (1951a), Chronic stress and peptic ulcer. I. Effect of corticotropin (ACTH) and cortisone on gastric secretion, *J.Am.Med.Assoc.* 147: 1529-1537
- Gray SJ, Benson JA, Jr., Spiro HM, Reifenshtein RW (1951b), Effects of ACTH and cortisone upon the stomach: its significance in the normal and in peptic ulcer, *Gastroenterology* 19: 658-673

- Gray SJ, Ramsey C, Reifstein RW, Benson JA, Jr. (1953), The significance of hormonal factors in the pathogenesis of peptic ulcer, *Gastroenterology* 25: 156-172
- Green FW, Jr., Kaplan MM, Curtis LE, Levine PH (1978), Effect of acid and pepsin on blood coagulation and platelet aggregation. A possible contributor prolonged gastroduodenal mucosal hemorrhage, *Gastroenterology* 74: 38-43
- Grossman MI (1981), Regulation of gastric acid secretion, in *Physiology of the Gastrointestinal Tract*, ed. Johnson LR, Raven Press, New York p 659-671
- Guilford WG, Strombeck DR (1996), Gastric Structure and Function, in *Strombeck's Small Animal Gastroenterology*, ed. Guilford WG, Center SA, Strombeck DR, Williams DA, and Meyer DJ, W.B. Saunders Co., Philadelphia p 239-253
- Happe RP, van dG, I, Lamers CB, van Toorenburg J, Rehfeld JF, Larsson LI (1980), Zollinger-Ellison syndrome in three dogs, *Vet.Pathol.* 17: 177-186
- Happonen I, Saari S, Castren L, Tyni O, Hanninen ML, Westermarck E (1996), Occurrence and topographical mapping of gastric Helicobacter-like organisms and their association with histological changes in apparently healthy dogs and cats, *Zentralbl.Veterinarmed.A* 43: 305-315
- Haskins S (2000), Therapy for shock, in *Kirk's current veterinary therapy XIII, Small Animal Practice*, ed. Bonagura JD and Kirk RW, WB Saunders Co, Philadelphia p 140-147
- Herdt T (1997), Secretions of the digestive tract, in *Textbook of Veterinary Physiology*, ed. Cunningham JG, W.B.Saunders, Philadelphia p 290-299
- Heyland D, Mandell LA (1992), Gastric colonization by gram-negative bacilli and nosocomial pneumonia in the intensive care unit patient. Evidence for causation, *Chest* 101: 187-193
- Hills BA, Butler BD, Lichtenberger LM (1983), Gastric mucosal barrier: hydrophobic lining to the lumen of the stomach, *Am.J.Physiol* 244: G561-G568
- Hoffman CR (1955), The causes and prevention of recurrences in peptic ulcer disease, *Am.J.Dig.Dis.* 22: 17-20
- Hogan DL, McQuaid KR, Koss MA, Crombie DL, Hunter S, Metz C, Euler AR, Isenberg JI (1993), Gastric acid suppression is greater during intravenous ranitidine infusion versus bolus injections of famotidine, *Aliment.Pharmacol.Ther.* 7: 537-541
- Howard EB, Sawa TR, Nielsen SW, Kenyon AJ (1969), Mastocytoma and gastroduodenal ulceration. Gastric and duodenal ulcers in dogs with mastocytoma, *Pathol.Vet.* 6: 146-158

- Huang JQ, Hunt RH (2001), Pharmacological and pharmacodynamic essentials of H(2)-receptor antagonists and proton pump inhibitors for the practising physician, *Best.Pract.Res.Clin.Gastroenterol.* 15: 355-370
- Jenkins CC, DeNovo RC, Patton CS, Bright RM, Rohrbach BW (1991), Comparison of effects of cimetidine and omeprazole on mechanically created gastric ulceration and on aspirin-induced gastritis in dogs, *Am.J.Vet.Res.* 52: 658-661
- Johnston DA, Wormsley KG (1988), The effect of food on ranitidine-induced inhibition of nocturnal gastric secretion, *Aliment.Pharmacol.Ther.* 2: 507-511
- Johnston SA, Leib MS, Forrester SD, Marini M (1995), The effect of misoprostol on aspirin-induced gastroduodenal lesions in dogs, *J Vet.Intern.Med.* 9: 32-38
- Jones B, Kenward MG (1989), *Design and analysis of cross-over trials*, Chapman and Hall, London p 189-200
- Kararli TT (1995), Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, *Biopharm.Drug Dispos.* 16: 351-380
- Katz LB, Tobia AJ, Shriver DA (1987), Effects of ORF 17583, other histamine H2-receptor antagonists and omeprazole on gastric acid secretory states in rats and dogs, *J.Pharmacol.Exp.Ther.* 242: 437-442
- Kerss S, Allen A, Garner A (1982), A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents, *Clin.Sci.(Lond)* 63: 187-195
- Klinkenberg-Knol EC, Nelis F, Dent J, Snel P, Mitchell B, Prichard P, Lloyd D, Havu N, Frame MH, Roman J, Walan A, Group LT (2000), Long-term omeprazole treatment in resistant gastroesophageal reflux disease: efficacy, safety, and influence on gastric mucosa, *Gastroenterology* 118: 661-669
- Konturek SJ, Tasler J, Cieszkowski M, Jaworek J (1978), Comparison of intravenous amino acids in the stimulation of gastric secretion, *Gastroenterology* 75: 817-824
- Kromer W, Postius S, Riedel R (2000), Animal pharmacology of reversible antagonism of the gastric acid pump, compared to standard antisecretory principles, *Pharmacology* 60: 179-187
- Kromer W, Postius S, Riedel R, Simon WA, Hanauer G, Brand U, Gonne S, Parsons ME (1990), BY 1023/SK&F 96022 INN pantoprazole, a novel gastric proton pump inhibitor, potently inhibits acid secretion but lacks relevant cytochrome P450 interactions, *J.Pharmacol.Exp.Ther.* 254: 129-135
- Kuna S (1964), The pH of gastric juice in the normal resting stomach, *Arch.Int.Pharmacodyn.Ther.* 152: 79-97

- Kwiecien S, Konturek SJ (2003), Gastric analysis with fractional test meals (ethanol, caffeine, and peptone meal), augmented histamine or pentagastrin tests, and gastric Ph recording, *J.Physiol.Pharmacol.* 54 Suppl 3: 69-82
- Lacy ER (1987), Gastric mucosal defense after superficial injury, *Clin.Invest Med.* 10: 189-200
- Larson C, Cavuto NJ, Flockhart DA, Weinberg RB (1996), Bioavailability and efficacy of omeprazole given orally and by nasogastric tube, *Dig.Dis.Sci.* 41: 475-479
- Larsson H, Carlsson E, Junggren U, Olbe L, Sjostrand SE, Skanberg I, Sundell G (1983), Inhibition of gastric acid secretion by omeprazole in the dog and rat, *Gastroenterology* 85: 900-907
- Larsson H, Mattson H, Sundell G, Carlsson E (1985), Animal pharmacodynamics of omeprazole. A survey of its pharmacological properties in vivo, *Scand.J.Gastroenterol.Suppl.* 108: 23-35
- Lasky MR, Metzler MH, Phillips JO (1998), A prospective study of omeprazole suspension to prevent clinically significant gastrointestinal bleeding from stress ulcers in mechanically ventilated trauma patients, *J Trauma* 44: 527-533
- Li Y, Sha W, Nie Y, Wu H, She Q, Dai S, Jia L, Yu W (2000), Effect of intragastric pH on control of peptic ulcer bleeding, *J.Gastroenterol.Hepatol.* 15: 148-154
- Lin JH (1991), Pharmacokinetic and pharmacodynamic properties of histamine H₂-receptor antagonists: relationship between intrinsic potency and effective plasma concentrations, *Clin.Pharmacokinet.* 20: 218-236
- Lin JH (1995), Species similarities and differences in pharmacokinetics, *Drug Metab Dispos.* 23: 1008-1021
- Lui CY, Amidon GL, Berardi RR, Fleisher D, Youngberg C, Dressman JB (1986), Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans, *J.Pharm.Sci.* 75: 271-274
- Mariano EC, Deak S, Reddell MT, Landor JH (1984), Mechanisms of protein activation of the intestinal phase of gastric secretion, *Surgery* 95: 492-496
- Mathews KA, Binnington AG (1986), Percutaneous incisionless placement of a gastrostomy tube utilizing a gastroscope: preliminary observations., *Journal of the American Animal Hospital Association* 22: 601-610
- Matz ME (1995), Gastrointestinal ulcer therapy, in *Current Veterinary Therapy XII, Small animal practice*, ed. Bonagura JD and Kirk RW, WB Saunders Co, Philadelphia p 706-710

- McArthur KE, Isenberg JI, Hogan DL, Dreier SJ (1983), Intravenous infusion of L-isomers of phenylalanine and tryptophan stimulate gastric acid secretion at physiologic plasma concentrations in normal subjects and after parietal cell vagotomy, *J Clin Invest* 71: 1254-1262
- McColl KE, el Omar E, Gillen D (2000), *Helicobacter pylori* gastritis and gastric physiology, *Gastroenterol Clin North Am* 29: 687-703, viii
- Medinger TL, Williams DA, Bruyette DS (1993), Severe gastrointestinal tract hemorrhage in three dogs with hypoadrenocorticism, *J Am Vet.Med.Assoc* 202: 1869-1872
- Mela GS, Savarino V (1990), Inaccuracy of hourly sampled pH measurements in describing the effect of antisecretory drugs on circadian gastric acidity, *J.Clin.Pharmacol.* 30: 45-49
- Mela GS, Savarino V, Vigneri S (1992), Optimizing the information obtained from continuous 24-hour gastric pH monitoring, *Am.J.Gastroenterol.* 87: 961-966
- Menguy R (1960), Effects of restraint stress on gastric secretion in the rat, *Am.J.Dig.Dis.* 5: 911-916
- Middleton DJ, Watson AD, Vasak E, Culvenor JE (1983), Duodenal ulceration associated with gastrin-secreting pancreatic tumor in a cat, *J.Am.Vet.Med.Assoc.* 183: 461-462
- Mignon M, Chau NP, Nguyen-Phuoc BK, Sauvage M, Leguy F, Bonfils S (1982), Ranitidine upon meal-induced gastric secretion: oral pharmacokinetics and plasma concentration effect relationships, *Br.J.Clin.Pharmacol.* 14: 187-193
- Miner P, Jr., Katz PO, Chen Y, Sostek M (2003), Gastric acid control with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole: a five-way crossover study, *Am J Gastroenterol.* 98: 2616-2620
- Mohiuddin MA, Pursnani KG, Katzka DA, Gideon RM, Castell JA, Castell DO (1997), Effective gastric acid suppression after oral administration of enteric-coated omeprazole granules, *Dig.Dis.Sci.* 42: 715-719
- Molina E, Rentz J, Hirschowitz BI (1980), Use of impromidine to define specific histamine H-2 effects on gastric secretion, heart rate and blood pressure in conscious dogs, *J Pharmacol.Exp.Ther.* 214: 483-487
- Moreland K (1988), Ulcer disease of the upper gastrointestinal tract in small animals: pathophysiology, diagnosis, and management, *Compendium on continuing education for the practicing veterinarian* 10: 1265-1279
- Mulvihill SJ, Pappas TN, Garcia R, Debas HT. Interspecies variation in acid secretion: a comparative study. *Gastroenterology* 88, 1511. 1985.

- Murtaugh RJ, Matz ME, Labato MA, Boudrieau RJ (1993), Use of synthetic prostaglandin E1 (misoprostol) for prevention of aspirin-induced gastroduodenal ulceration in arthritic dogs, *J.Am.Vet.Med.Assoc* 202: 251-256
- Neiger R, Gaschen F, Jaggy A (2000), Gastric mucosal lesions in dogs with acute intervertebral disc disease: characterization and effects of omeprazole or misoprostol, *J Vet.Intern.Med.* 14: 33-36
- Netzer P, Eschenmoser K, Halter F, Inauen W (1999a), Impact of food intake on the antisecretory effect of low-dose ranitidine and famotidine, *Aliment.Pharmacol.Ther.* 13: 407-412
- Netzer P, Gaia C, Sandoz M, Huluk T, Gut A, Halter F, Husler J, Inauen W (1999b), Effect of repeated injection and continuous infusion of omeprazole and ranitidine on intragastric pH over 72 hours, *Am.J.Gastroenterol.* 94: 351-357
- Norlen P, Bernsand M, Konagaya T, Hakanson R (2001), ECL-cell histamine mobilization in conscious rats: effects of locally applied regulatory peptides, candidate neurotransmitters and inflammatory mediators, *Br.J Pharmacol.* 134: 1767-1777
- Nwokolo CU, Smith JT, Sawyerr AM, Pounder RE (1991), Rebound intragastric hyperacidity after abrupt withdrawal of histamine H2 receptor blockade, *Gut* 32: 1455-1460
- Ortiz JE, Sottile FD, Sigel P, Nasraway SA (1998), Gastric colonization as a consequence of stress ulcer prophylaxis: a prospective, randomized trial, *Pharmacotherapy* 18: 486-491
- PantoIV [product monograph]. 2002. Oakville, ON, Canada, Altana Pharma Inc.
- Papich MG (1993), Antiulcer therapy, *Vet.Clin.North Am.Small Anim Pract.* 23: 497-512
- Parkman HP, Pagano AP, Ryan JP (1998), Ranitidine and nizatidine stimulate antral smooth muscle contractility via excitatory cholinergic mechanisms, *Dig.Dis.Sci.* 43: 497-505
- Pawlik T, Konturek PC, Konturek JW, Konturek SJ, Brzozowski T, Czesnikiewicz M, Plonka M, Bielanski W, Areny H (2002), Impact of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on gastric ulcerogenesis in experimental animals and in humans, *Eur.J.Pharmacol.* 449: 1-15
- Peghini PL, Katz PO, Castell DO (1998), Ranitidine controls nocturnal gastric acid breakthrough on omeprazole: a controlled study in normal subjects, *Gastroenterology* 115: 1335-1339

- Pendleton RG, Torchiana ML, Chung C, Cook P, Wiese S, Clineschmidt BV (1983), Studies on MK-208 (YM-11170) a new, slowly dissociable H₂-receptor antagonist, *Arch.Int.Pharmacodyn.Ther.* 266: 4-16
- Phillips JO, Metzler MH, Palmieri MT, Huckfeldt RE, Dahl NG (1996), A prospective study of simplified omeprazole suspension for the prophylaxis of stress-related mucosal damage, *Crit.Care Med.* 24: 1793-1800
- Pilbrant A, Cederberg C (1985), Development of an oral formulation of omeprazole, *Scand.J.Gastroenterol.Suppl.* 108: 113-120
- Pisegna JR (2002), Pharmacology of acid suppression in the hospital setting: focus on proton pump inhibition, *Crit.Care Med.* 30: S356-S361
- Pisegna JR, Martin P, McKeand W, Ohning G, Walsh JH, Paul J (1999), Inhibition of pentagastrin-induced gastric acid secretion by intravenous pantoprazole: a dose-response study, *Am.J.Gastroenterol.* 94: 2874-2880
- Plumb DC (2002), *Veterinary drug handbook*, Iowa State University Press, Ames, Iowa
- Postius S, Brauer U, Kromer W. Determination of the duration of action of gastric anti-secretory drugs by 24-hour pH-metry in the dog. *Canadian Journal of Gastroenterology* . 1991a.
- Postius S, Brauer U, Kromer W (1991b), The novel proton pump inhibitor pantoprazole elevates intragastric pH for a prolonged period when administered under conditions of stimulated gastric acid secretion in the gastric fistula dog, *Life Sci.* 49: 1047-1052
- Postius S, Brauer U (1995), Loss of circadian rhythm in luminal acidity of canine stomach by implantation of a gastric fistula, *Digestion* 56: 35-40
- Richardson CT, Walsh JH, Hicks MI, Fordtran JS (1976), Studies on the mechanisms of food-stimulated gastric acid secretion in normal human subjects, *J.Clin.Invest.* 58: 623-631
- Richter KP (1992), Therapy for vomiting patients with gastrointestinal ulcers, *Veterinary Medicine* August: 819-824
- Rohmel J, Merki HS, Wilder-Smith CH, Walt RP (1990), Analysis and statistical evaluation of continuous pH recordings, *Dig.Dis.* 8 Suppl 1: 87-96
- Rovelstad RA, Owen CA, Jr., Magath TB (1952), Factors influencing the continuous recording of in situ pH of gastric and duodenal contents, *Gastroenterology* 20: 609-624
- Sabata M. personal communication. 1999. Oakville, Ontario, Canada, Byk Canada Inc.
- Sachs G (1987), The gastric proton pump: the H⁺,K⁺-ATPase, in *Physiology of the Gastrointestinal Tract*, ed. Johnson LR, Raven Press, New York p 865-879

- Sachs G, Shin JM, Briving C, Wallmark B, Hersey S (1995), The pharmacology of the gastric acid pump: the H⁺,K⁺ ATPase, *Annu.Rev.Pharmacol.Toxicol.* 35: 277-305
- Sangiah S, MacAllister CC, Amouzadeh HR (1989), Effects of misoprostol and omeprazole on basal gastric pH and free acid content in horses, *Res.Vet.Sci.* 47: 350-354
- Savarino V, Mela GS, Zentilin P, Magnolia MR, Scalabrini P, Valle F, Moretti M, Bonifacino G, Celle G (1989), Gastric aspiration versus antimony and glass pH electrodes. A simultaneous comparative in vivo study, *Scand.J.Gastroenterol.* 24: 434-439
- Schunack W (1987), What are the differences between the H₂-receptor antagonists?, *Aliment.Pharmacol.Ther.* 1 Suppl 1: 493S-503S
- Sharma VK, Vasudeva R, Howden CW (1999), The effects on intragastric acidity of per-gastrostomy administration of an alkaline suspension of omeprazole, *Aliment.Pharmacol.Ther.* 13: 1091-1095
- Shin JM, Sachs G (2002), Restoration of acid secretion following treatment with proton pump inhibitors, *Gastroenterology* 123: 1588-1597
- Shorrock CJ, Rees WD (1988), Overview of gastroduodenal mucosal protection, *Am.J.Med.* 84: 25-34
- Silverstein FE, Gilbert DA, Tedesco FJ, Buenger NK, Persing J (1981), The national ASGE survey on upper gastrointestinal bleeding. II. Clinical prognostic factors, *Gastrointest.Endosc.* 27: 80-93
- Simoens C, Woussen-Colle MC, De Graef J (1989), Effect of acute suppression of acid secretion by omeprazole on postprandial gastrin release in conscious dogs, *Gastroenterology* 97: 837-845
- Simpson K, Neiger R, DeNovo R, Sherding R (2000), The relationship of *Helicobacter* spp. infection to gastric disease in dogs and cats, *J.Vet.Intern.Med.* 14: 223-227
- Simpson KW (2005), Diseases of the Stomach, in *Textbook of Veterinary Internal Medicine Volume 2*, ed. Ettinger SJ and Feldman EC, Elsevier Saunders, St. Louis, Missouri p 1310-1331
- Simpson KW, Strauss-Ayali D, McDonough PL, Chang YF, Valentine BA (1999), Gastric function in dogs with naturally acquired gastric *Helicobacter* spp. infection, *J.Vet.Intern.Med.* 13: 507-515
- Smith JL, Gamal MA, Chremos AN, Graham DY (1985), Famotidine, a new H₂-receptor antagonist. Effect on parietal, nonparietal, and pepsin secretion in man, *Dig.Dis.Sci.* 30: 308-312

- Soll AH, Berglindh T (1987), Physiology of isolated gastric glands and parietal cells: receptors and effectors regulating function, in *Physiology of the Gastrointestinal Tract*, ed. Johnson LR, Raven Press, New York p 883-906
- Soll AH, Isenberg JI (1983), Duodenal ulcer diseases, in *Gastrointestinal Disease: Pathophysiology, Diagnosis, Management*, ed. Sleisenger MH and Fordtran JS, W.B. Saunders Co., Philadelphia p 624-672
- Soybel DI, Klingensmith ME, Cima RR (1996), Effects of serosal-side acidosis on cell pH (pHi) and membrane electrical properties in gastric mucosa, *J Surg.Res.* 63: 147-151
- Stanton ME, Bright RM (1989), Gastroduodenal ulceration in dogs. Retrospective study of 43 cases and literature review, *J.Vet.Intern.Med.* 3: 238-244
- Starlinger M, Jakesz R, Matthews JB, Yoon C, Schiessel R (1981), The relative importance of, *Gastroenterology* 81: 732-735
- Stedman CA, Barclay ML (2000), Review article: comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors, *Aliment.Pharmacol.Ther.* 14: 963-978
- Steinberg KP (2002), Stress-related mucosal disease in the critically ill patient: risk factors and strategies to prevent stress-related bleeding in the intensive care unit, *Crit.Care Med.* 30: S362-S364
- Stempien SJ, Dagradi A (1954), The histamine response of the gastric mucosa in a patient with adrenal insufficiency: effect of cortisone administration, *Gastroenterology* 27: 358-362
- Sugiyama M, Aoki T, Matsuo Y (1995), Determining the optimal dose of lansoprazole injection by 24-hour intragastric pH monitoring, *J.Clin.Gastroenterol.* 20: S14-S16
- Uchida M, Ohba S, Ikarashi Y, Misaki N, Kawano O (1993), Effect of the novel histamine H₂-antagonist 5,6-dimethyl-2-[4-[3-(1-piperidinomethyl)phenoxy]-(z)-2-butenylamino]-4(1H)-pyrimidine dihydrochloride on histamine-induced gastric acid secretion in Heidenhain pouch dogs, *Arzneimittelforschung.* 43: 873-876
- Walt R (1986), Twenty four hour intragastric acidity analysis for the future, *Gut* 27: 1-9
- Wilder-Smith CH, Merki HS (1991), Is the time of dosing of a potent long-acting H₂-receptor antagonist critical? Twenty-four hour pH measurements with SKF-94482, *Scand.J.Gastroenterol.* 26: 501-517
- Wilder-Smith CH, Merki HS (1992), Tolerance during dosing with H₂-receptor antagonists. An overview, *Scand.J.Gastroenterol.Suppl* 193: 14-19
- Williams C (2001), Occurrence and significance of gastric colonization during acid-inhibitory therapy, *Best.Pract.Res.Clin.Gastroenterol.* 15: 511-521

Wright RP (1981), Malignant gastric ulcer associated with adenocarcinoma of the gastric fundus in a dog, *Vet.Med.Small Anim.Clin.* 76: 845-848

Yamada I, Mizuta H, Goda T, Haga K, Ogawa K (1989), Gastric pH profile and its control in fasting beagle dogs, *Chem.Pharm.Bull.* 37: 2539-2541

Yamasaki K, Suematsu H, Takahashi T (1998), Comparison of gastric lesions in dogs and cats with and without gastric spiral organisms, *J.Am.Vet.Med.Assoc.* 212: 529-533

Youngberg C, Wlodyga J, Schmaltz S, Dressman JB (1985), Radiotelemetric determination of gastrointestinal pH in four healthy beagles, *American Journal of Veterinary Research* 46: 1516-1521

APPENDICES

Appendix 5.1-1 - List of Abbreviations

Cl^- - chloride ion

COX – 1 – cyclooxygenase 1

COX – 2 – cyclooxygenase 2

GBMT – gastric mucosal bleeding time

GI – gastrointestinal

H^+ - hydrogen ion

HCO_3^- – bicarbonate

H^+K^+ ATPase – hydrogen potassium ATPase

H_2RA – histamine₂ receptor antagonist

ICU – intensive care unit

IV - intravenous

MCT – mast cell tumour

Na^+ - sodium

NaCl – sodium chloride

NSAIDs – non-steroidal anti-inflammatory drugs

PCR – polymerase chain reaction

PEG – percutaneously endoscopically-placed gastrostomy

PGE_2 – Prostaglandins E_2

PGI_2 - Prostaglandins I_2

pH – negative log of the H^+ ion concentration

$\text{pH} \geq 3$ – pH greater than or equal to 3

$\text{pH} \geq 4$ - pH greater than or equal to 4

PO – per os

PPI – proton pump inhibitor

SPF – specific pathogen free

SRMD – stress-related mucosal disease

TSB – trypticase soy broth

ul – microliter

°C – degrees celsius

% pH $\geq$ 3 – percent time pH greater than or equal to 3

% pH $\geq$ 4 - percent time pH greater than or equal to 4

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#1	Babs	None	Fasting	0	Baseline	5.6794	6	90.2222	95.7917
#4	Honey	None	Fasting	0	Baseline	4.5262	5.4	66.3750	72.5602
#5	Ike	None	Fasting	0	Baseline	2.9566	1.9	32.4722	37.2361
#6	Kenny	None	Fasting	0	Baseline	2.9470	2.4	25.5556	38.6898
#3	Patsy	None	Fasting	0	Baseline	4.9701	5.6	75.1481	84.4213
#2	Trakey	None	Fasting	0	Baseline	4.7638	6	65.3426	70.5972
#7	Angelina	None	Fasting	0	Baseline	6.5722	6.6	100.0000	100.0000
#12	Blossom	None	Fasting	0	Baseline	4.1786	5.9	56.8796	58.4630
#8	Champagne	None	Fasting	0	Baseline	3.5499	2.7	38.9630	46.8148
#9	Gilmour	None	Fasting	0	Baseline	6.3077	6.4	100.0000	100.0000
#10	Kyle	None	Fasting	0	Baseline	3.4121	2.9	38.0833	49.3380
#11	Lemieux	None	Fasting	0	Baseline	5.1573	6	77.3750	84.0648
#1	Babs	None	Fasting	6	0	5.6254	6.6	80.1102	85.0428
#4	Honey	None	Fasting	6	0	2.3002	0.9	24.7645	27.9627
#5	Ike	None	Fasting	6	0	4.2148	4.4	52.1040	59.4908
#6	Kenny	None	Fasting	6	0	4.9157	6.2	61.4619	71.7571
#3	Patsy	None	Fasting	6	0	4.6973	6.2	62.5827	66.5491
#2	Trakey	None	Fasting	6	0	4.2481	6.1	56.2008	57.8289
#7	Angelina	None	Fasting	6	0	5.4267	6.6	73.8683	77.3226
#12	Blossom	None	Fasting	6	0	6.6226	7	93.7678	94.7872
#8	Champagne	None	Fasting	6	0	6.2539	6.7	90.8401	94.3814
#9	Gilmour	None	Fasting	6	0	4.8170	6.7	64.7326	66.4090
#10	Kyle	None	Fasting	6	0	5.5603	6.6	81.9363	83.0958
#11	Lemieux	None	Fasting	6	0	2.8022	1.6	33.0686	38.3397
#1	Babs	None	fed	1	Baseline	1.4179	1	6.6204	7.1343
#4	Honey	None	fed	1	Baseline	1.8501	1.1	12.8194	14.2963
#5	Ike	None	fed	1	Baseline	1.6264	1.1	7.1019	8.9306
#6	Kenny	None	fed	1	Baseline	1.3108	1	4.5880	5.4352

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#3	Patsy	None	fed	1	Baseline	1.6239	1.3	4.3981	5.9398
#2	Trakey	None	fed	1	Baseline	2.0234	1.3	12.1528	15.0231
#7	Angelina	None	fed	1	Baseline	1.9668	1.2	12.1944	13.1620
#12	Blossom	None	fed	1	Baseline	2.4167	1.6	19.8704	28.8287
#8	Champagne	None	fed	1	Baseline	2.2463	1.7	16.5509	22.7269
#9	Gilmour	None	fed	1	Baseline	1.8117	1.2	10.6528	12.9583
#10	Kyle	None	fed	1	Baseline	1.8629	1.2	11.0000	14.2454
#11	Lemieux	None	fed	1	Baseline	3.0311	2.4	35.9398	43.8287
#1	Babs	None	fed	2	0	3.0651	2.9	37.4976	49.7681
#4	Honey	None	fed	2	0	3.9755	3.6	48.0290	57.8164
#5	Ike	None	fed	2	0	1.7786	1.2	9.4300	13.7440
#6	Kenny	None	fed	2	0	1.7667	1.4	6.0338	13.8406
#3	Patsy	None	fed	2	0	2.5622	1.5	22.2560	23.5652
#2	Trakey	None	fed	2	0	2.4144	1.3	20.7391	23.3671
#7	Angelina	None	fed	2	0	1.6531	1	9.4546	12.2953
#12	Blossom	None	fed	2	0	2.6107	1.2	28.5473	34.8616
#8	Champagne	None	fed	2	0	1.1990	1	2.8849	4.2766
#9	Gilmour	None	fed	2	0	2.0845	1.1	18.0927	20.3005
#10	Kyle	None	fed	2	0	3.4238	2.9	43.2436	49.6449
#11	Lemieux	None	fed	2	0	2.4447	1.5	21.3016	25.0411
#1	Babs	None	fed	3	0	2.0569	1.2	16.6965	18.9043
#4	Honey	None	fed	3	0	3.9894	3.9	49.2729	58.5825
#5	Ike	None	fed	3	0	1.5364	1.3	2.2030	8.5222
#6	Kenny	None	fed	3	0	1.1688	1	1.2271	1.5363
#3	Patsy	None	fed	3	0	2.4200	1.4	22.5958	23.6901
#2	Trakey	None	fed	3	0	1.9429	1.1	15.5563	17.6675
#7	Angelina	None	fed	3	0	1.3601	1.1	3.5171	5.4399
#12	Blossom	None	fed	3	0	2.2749	1.8	11.3342	26.9730

Appendix 5.2-1 - 24-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#8	Champagne	None	fed	3	0	1.3648	1.2	1.6909	2.6185
#9	Gilmour	None	fed	3	0	1.4848	1	7.7397	8.6456
#10	Kyle	None	fed	3	0	2.4276	1.5	23.1895	29.0739
#11	Lemieux	None	fed	3	0	1.8109	1.4	9.8652	12.3919
#1	Babs	None	fed	4	0	1.8611	1.1	13.5804	19.8126
#4	Honey	None	fed	4	0	3.5177	3.2	38.1419	53.4035
#5	Ike	None	fed	4	0	1.7225	1.2	8.9908	14.6819
#6	Kenny	None	fed	4	0	3.1656	2.6	32.9871	44.2437
#3	Patsy	None	fed	4	0	5.8255	6.1	96.6762	97.5023
#2	Trakey	None	fed	4	0	2.2362	1.4	16.5080	20.8271
#7	Angelina	None	fed	4	0	3.4672	3.6	46.8380	55.5776
#12	Blossom	None	fed	4	0	3.2669	2.5	32.0176	43.6177
#8	Champagne	None	fed	4	0	2.0571	1.1	21.6581	27.0738
#9	Gilmour	None	fed	4	0	2.3334	1.2	20.9769	26.7742
#10	Kyle	None	fed	4	0	2.1758	1.1	20.6087	22.8357
#11	Lemieux	None	fed	4	0	3.6690	3.9	49.9541	63.5055
#1	Babs	None	fed	5	0	2.3141	1.5	17.4471	28.5334
#4	Honey	None	fed	5	0	2.7051	2	27.5231	35.6491
#5	Ike	None	fed	5	0	2.3908	1.7	20.8416	31.2624
#6	Kenny	None	fed	5	0	2.8732	2.4	29.1029	41.1759
#3	Patsy	None	fed	5	0	3.3861	3	43.4224	51.2005
#2	Trakey	None	fed	5	0	3.8587	4.3	57.1815	67.9067
#7	Angelina	None	fed	5	0	3.1054	3.3	41.1034	53.9688
#12	Blossom	None	fed	5	0	4.7126	4.9	74.5737	88.6275
#8	Champagne	None	fed	5	0	2.9320	2.8	28.2429	48.7753
#9	Gilmour	None	fed	5	0	2.7203	2.2	20.7044	37.1041
#10	Kyle	None	fed	5	0	1.5773	0.9	10.4739	13.8461
#11	Lemieux	None	fed	5	0	3.7548	3.8	47.6013	63.9693

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#12	Blossom	Saline	fed	1	2	2.9471	2.1	29.9398	39.0185
#1	Babs	Saline	fed	1	2	1.6296	1.1	10.3843	11.2824
#6	Kenny	Saline	fed	1	2	1.5057	0.9	8.3565	11.1806
#5	Ike	Saline	fed	2	2	2.0224	1.4	12.2037	17.7963
#10	Kyle	Saline	fed	2	2	4.2541	4.8	55.2379	64.7825
#4	Honey	Saline	fed	3	2	3.8031	3.4	46.6528	53.5278
#11	Lemieux	Saline	fed	3	2	2.0841	1.2	14.0602	18.9074
#3	Patsy	Saline	fed	4	2	3.2563	2.7	38.2870	47.7731
#8	Champagne	Saline	fed	4	2	2.3883	1.4	26.9120	34.6111
#7	Angelina	Saline	fed	5	2	2.4270	1.7	24.9954	35.7870
#9	Gilmour	Saline	fed	5	2	2.2328	1.3	18.6759	21.8843
#12	Blossom	Saline	fed	1	6	1.7277	1.2	8.1343	14.0694
#1	Babs	Saline	fed	1	6	1.5916	0.9	13.9306	14.3889
#6	Kenny	Saline	fed	1	6	1.4356	1.2	2.9120	5.4630
#5	Ike	Saline	fed	2	6	1.6811	1.2	8.7500	9.4398
#10	Kyle	Saline	fed	2	6	2.3205	1.3	21.5972	26.9306
#4	Honey	Saline	fed	3	6	1.9586	1.2	12.4259	17.5509
#11	Lemieux	Saline	fed	3	6	2.3006	1.4	18.5694	24.8426
#3	Patsy	Saline	fed	4	6	3.9389	4.6	53.6944	57.1296
#8	Champagne	Saline	fed	4	6	3.2154	3.6	40.9676	59.6713
#7	Angelina	Saline	fed	5	6	2.5501	1.6	23.6642	33.6420
#9	Gilmour	Saline	fed	5	6	2.9939	2.7	35.9583	47.8426
#3	Patsy	Famo	fed	1	2	3.3098	2.9	33.1898	49.4491
#9	Gilmour	Famo	fed	1	2	3.4038	3	38.1435	50.5787
#4	Honey	Famo	fed	2	2	3.6192	2.8	44.3102	47.7315
#2	Trakey	Famo	fed	2	2	3.6722	3.3	38.0046	55.2500
#8	Champagne	Famo	fed	2	2	1.7285	1.4	4.6667	6.1620
#1	Babs	Famo	fed	3	2	2.6170	1.8	18.9769	30.9630

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#2	Trakey	Famo	fed	3	2	3.1352	2.6	25.7454	41.9722
#7	Angelina	Famo	fed	3	2	2.3371	2	12.5787	34.3056
#10	Kyle	Famo	fed	3	2	3.8225	3.5	45.1157	59.9306
#5	Ike	Famo	fed	4	2	2.4614	1.7	18.9303	30.0764
#12	Blossom	Famo	fed	4	2	5.0408	5.5	78.7222	84.2083
#6	Kenny	Famo	fed	5	2	4.8001	5.4	74.0093	80.0046
#11	Lemieux	Famo	fed	5	2	3.2973	3.2	40.3287	52.7361
#3	Patsy	Famo	fed	1	6	3.0434	2.2	30.5463	41.3611
#9	Gilmour	Famo	fed	1	6	4.6848	5.7	69.0787	75.2870
#4	Honey	Famo	fed	2	6	4.0625	4.3	52.5509	61.6250
#8	Champagne	Famo	fed	2	6	4.5720	5.4	62.4861	72.3750
#1	Babs	Famo	fed	3	6	2.2736	1.3	17.3472	21.7269
#2	Trakey	Famo	fed	3	6	3.4589	2.8	41.3796	48.2824
#7	Angelina	Famo	fed	3	6	4.8048	5.7	69.3056	78.1898
#10	Kyle	Famo	fed	3	6	3.4089	2.8	35.8426	47.0278
#5	Ike	Famo	fed	4	6	3.3424	2.7	30.1481	44.6065
#12	Blossom	Famo	fed	4	6	4.4674	4.8	72.1528	79.5046
#6	Kenny	Famo	fed	5	6	4.1069	4.2	54.8843	68.2685
#11	Lemieux	Famo	fed	5	6	3.9537	4.6	55.4259	64.4583
#5	Ike	Omeprazole	fed	1	2	4.5532	5.1	66.0231	74.5926
#11	Lemieux	Omeprazole	fed	1	2	4.4024	4.6	71.5880	85.1759
#1	Babs	Omeprazole	fed	2	2	2.8821	2.2	27.3733	38.8163
#7	Angelina	Omeprazole	fed	2	2	2.9583	2.6	19.5139	38.3611
#12	Blossom	Omeprazole	fed	2	2	3.6134	3.8	44.5926	74.2917
#6	Kenny	Omeprazole	fed	3	2	3.4895	3.6	34.0509	71.3426
#9	Champagne	Omeprazole	fed	3	2	3.6342	3.8	48.6157	59.5509
#4	Honey	Omeprazole	fed	4	2	4.1736	4.3	56.3102	71.6389
#2	Trakey	Omeprazole	fed	4	2	5.0006	5.2	83.6667	89.6435

Appendix 5.2-1 - 24-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	24 Hour 9AM-9AM			
						Mean	Median	%≥4	%≥3
#9	Gilmour	Omep	fed	4	2	3.9076	4	52.0556	69.2778
#3	Patsy	Omep	fed	5	2	4.5181	5.5	66.3148	69.1204
#10	Kyle	Omep	fed	5	2	3.4515	3.5	27.4444	65.9074
#5	Ike	Omep	fed	1	6	4.1793	4.6	68.3426	74.2917
#11	Lemieux	Omep	fed	1	6	4.5058	5	61.9861	72.4352
#1	Babs	Omep	fed	2	6	3.1532	3.1	29.6991	55.5185
#7	Angelina	Omep	fed	2	6	3.9338	3.8	45.9167	66.4491
#12	Blossom	Omep	fed	2	6	3.6678	3.4	40.1065	59.1713
#6	Kenny	Omep	fed	3	6	3.7835	4	50.2176	61.7407
#9	Champagne	Omep	fed	3	6	3.8519	4.1	54.8611	71.9444
#4	Honey	Omep	fed	4	6	3.2800	3.5	35.0389	65.9575
#2	Trakey	Omep	fed	4	6	5.0178	4.9	90.2731	92.8148
#9	Gilmour	Omep	fed	4	6	4.3416	4.5	66.0694	82.6759
#3	Patsy	Omep	fed	5	6	3.7109	4.5	58.3565	66.1852
#10	Kyle	Omep	fed	5	6	4.3263	4.5	66.8935	77.6991
#4	Honey	Panto	fed	1	2	3.1193	3.2	34.1250	54.5509
#10	Kyle	Panto	fed	1	2	3.8438	4.1	56.1389	74.4861
#6	Kenny	Panto	fed	2	2	1.6435	1.3	7.0741	7.8935
#9	Gilmour	Panto	fed	2	2	4.1219	4.1	57.0000	85.2083
#3	Patsy	Panto	fed	3	2	2.1386	1.5	12.4907	15.0741
#12	Blossom	Panto	fed	3	2	3.4075	3	38.3704	50.7500
#1	Babs	Panto	fed	4	2	3.0207	2.6	29.1343	42.6389
#7	Angelina	Panto	fed	4	2	3.3583	3.5	43.7726	53.8985
#11	Lemieux	Panto	fed	4	2	4.0768	4.6	59.0556	69.4074
#5	Ike	Panto	fed	5	2	4.2194	5.2	66.7870	70.6991
#2	Trakey	Panto	fed	5	2	4.3296	4.6	69.4630	83.9074
#8	Champagne	Panto	fed	5	2	3.4482	4	51.1944	63.0509
#4	Honey	Panto	fed	1	6	3.5879	3.6	44.2778	60.3472

Appendix 5.2-1 - 24-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	24 Hour			
						Mean	Median	%≥4	%≥3
#10	Kyle	Panto	fed	1	6	2.8612	2.6	25.4259	44.4861
#6	Kenny	Panto	fed	2	6	2.9936	2.8	22.4630	45.8843
#9	Gilmour	Panto	fed	2	6	3.6924	3.6	41.8426	69.6343
#3	Patsy	Panto	fed	3	6	2.6099	1.6	20.8889	22.6574
#12	Blossom	Panto	fed	3	6	3.5455	3.3	35.1296	62.9352
#1	Babs	Panto	fed	4	6	4.4040	4.9	67.7269	75.8148
#7	Angelina	Panto	fed	4	6	4.2411	5	66.6574	73.6389
#11	Lemieux	Panto	fed	4	6	4.2002	4.3	60.9907	72.1713
#5	Ike	Panto	fed	5	6	3.5884	4.5	53.4676	59.0463
#2	Trakey	Panto	fed	5	6	4.5232	4.8	75.6574	82.8194
#8	Champagne	Panto	fed	5	6	3.7684	4.5	54.9491	61.1111
#2	Trakey	Rani	fed	1	2	2.6938	2	19.2546	33.3796
#7	Angelina	Rani	fed	1	2	2.2898	1.7	10.9769	30.3935
#8	Champagne	Rani	fed	1	2	4.1701	4.4	54.2593	63.8333
#3	Patsy	Rani	fed	2	2	3.0826	2.6	26.9861	45.9259
#11	Lemieux	Rani	fed	2	2	2.4221	1.7	16.5694	26.9306
#5	Ike	Rani	fed	3	2	2.8921	2.3	22.7917	36.1713
#9	Gilmour	Rani	fed	3	2	4.0930	4.6	56.7685	65.5185
#6	Kenny	Rani	fed	4	2	2.0239	1.4	12.1111	20.5694
#10	Kyle	Rani	fed	4	2	2.5119	1.9	18.6799	28.4854
#1	Babs	Rani	fed	5	2	2.8285	2.5	31.6435	43.8889
#4	Honey	Rani	fed	5	2	2.9126	2.6	34.8845	45.2933
#12	Blossom	Rani	fed	5	2	5.8697	6.2	95.4722	99.2593
#2	Trakey	Rani	fed	1	6	2.4979	1.8	17.2176	30.2037
#7	Angelina	Rani	fed	1	6	2.0146	0.9	19.3704	21.1065
#8	Champagne	Rani	fed	1	6	2.5598	1.8	17.4098	28.3187
#3	Patsy	Rani	fed	2	6	2.7368	1.9	22.2731	33.0926
#11	Lemieux	Rani	fed	2	6	3.8431	3.4	45.6620	53.1713

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24 Hour				
						Mean	Median	%≥3	%≥4	
#5	Ike	Rani	fed	3	3	6	2.4155	1.7	15.9213	27.1481
#9	Gilmour	Rani	fed	3	3	6	2.3848	1.3	19.3981	22.0139
#6	Kenny	Rani	fed	4	4	6	3.4886	2.7	42.3796	47.9537
#10	Kyle	Rani	fed	4	4	6	2.7633	2.2	28.6574	40.4306
#1	Babs	Rani	fed	5	5	6	3.5251	3.7	45.5370	60.7269
#4	Honey	Rani	fed	5	5	6	2.1975	1.6	15.3355	26.9806
#12	Blossom	Rani	fed	5	5	6	3.8283	4.2	52.2963	60.0833
#1	Babs	Fam3	fed	6	6	2	3.8198	4.1	51.9306	65.9861
#4	Honey	Fam3	fed	6	6	2	3.2531	2.8	35.3657	47.7778
#5	Ike	Fam3	fed	6	6	2	3.0037	3.1	28.0463	54.6667
#6	Kenny	Fam3	fed	6	6	2	3.7591	3.8	48.2963	61.0509
#3	Patsy	Fam3	fed	6	6	2	4.2304	4.9	65.4630	71.8380
#2	Trakey	Fam3	fed	6	6	2	3.9617	4	51.4444	73.7176
#1	Babs	Fam3	fed	6	6	6	3.4748	3.3	41.7593	53.8750
#4	Honey	Fam3	fed	6	6	6	3.5738	3.3	34.4769	63.1620
#5	Ike	Fam3	fed	6	6	6	2.4763	1.6	22.7546	40.4676
#6	Kenny	Fam3	fed	6	6	6	3.1851	3	36.5417	51.1157
#3	Patsy	Fam3	fed	6	6	6	3.7958	3.9	49.4722	65.9120
#2	Trakey	Fam3	fed	6	6	6	4.2340	4.1	52.8102	70.1759
#7	Angelina	Ome2	fed	6	6	2	4.8896	5	80.9102	93.8730
#12	Blossom	Ome2	fed	6	6	2	5.9082	5.7	100.0000	100.0000
#8	Champagne	Ome2	fed	6	6	2	4.3726	4.4	75.5941	93.5852
#9	Gilmour	Ome2	fed	6	6	2	4.6521	4.6	80.4980	88.5673
#10	Kyle	Ome2	fed	6	6	2	4.2364	4.2	65.1268	87.9014
#11	Lemieux	Ome2	fed	6	6	2	4.4103	4.7	67.6405	81.3810
#7	Angelina	Ome2	fed	6	6	6	4.2404	4.7	74.2382	79.5313
#12	Blossom	Ome2	fed	6	6	6	5.3717	5.6	82.2688	88.7119
#8	Champagne	Ome2	fed	6	6	6	3.3655	3.9	49.3194	61.4388

**Appendix 5.2-1 - 24-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	24-Hour			
						Mean	Median	%≥4	%≥3
#9	Gilmour	Ome2	fed	6	6	4.4792	5.2	68.3393	76.5849
#10	Kyle	Ome2	fed	6	6	4.3322	4.7	68.4952	76.7471
#11	Lemieux	Ome2	fed	6	6	4.9424	5.3	81.8093	87.5180

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3
#1	Babs	None	Fasting	0	Baseline	6.1007	6.1	98.5556	99.5093	5.2580	5.8	81.8889	92.0741				
#4	Honey	None	Fasting	0	Baseline	5.0887	5.7	80.4259	85.2593	3.9638	4.4	52.3241	59.8611				
#5	Ike	None	Fasting	0	Baseline	4.0199	4.6	54.4167	60.3241	1.8934	1.5	10.5278	14.1481				
#6	Kenny	None	Fasting	0	Baseline	3.0518	2.1	28.2870	35.3241	2.8422	2.6	22.8241	42.0556				
#3	Patsy	None	Fasting	0	Baseline	5.2907	6	81.2963	88.6481	4.6494	5.2	69.0000	80.1944				
#2	Trakey	None	Fasting	0	Baseline	6.0258	6.6	87.1944	91.1944	3.5018	2.95	43.4907	50.0000				
#7	Angelina	None	Fasting	0	Baseline	6.6336	6.6	100.0000	100.0000	6.5109	6.5	100.0000	100.0000				
#12	Blossom	None	Fasting	0	Baseline	6.5928	6.7	98.8426	99.5926	1.7643	0.8	14.9167	17.3333				
#8	Champagne	None	Fasting	0	Baseline	3.3380	2.6	34.6759	44.0463	3.7618	2.9	43.2500	49.5833				
#9	Gilmour	None	Fasting	0	Baseline	6.2270	6.4	100.0000	100.0000	6.3884	6.5	100.0000	100.0000				
#10	Kyle	None	Fasting	0	Baseline	4.2124	4.3	52.4630	61.2037	2.6117	1.8	23.7037	37.4722				
#11	Lemieux	None	Fasting	0	Baseline	5.5132	6.3	81.7778	83.6296	4.8015	5.1	72.9722	84.5000				
#1	Babs	None	Fasting	6	0	6.6577	6.8	97.6061	99.2828	4.6791	5.8	64.0707	71.9881				
#4	Honey	None	Fasting	6	0	3.2986	1.9	41.6263	44.6465	1.3850	0.8	9.3064	12.6678				
#5	Ike	None	Fasting	6	0	5.5126	6.7	74.6061	78.3434	3.0249	1.8	31.4751	42.2076				
#6	Kenny	None	Fasting	6	0	6.0507	6.7	85.1919	91.0707	3.8752	3.1	39.7074	54.0513				
#3	Patsy	None	Fasting	6	0	5.6478	6.6	80.7475	82.0505	3.8259	3.4	45.9302	52.3382				
#2	Trakey	None	Fasting	6	0	6.1402	6.8	87.3434	89.2424	2.5135	0.8	27.6507	29.0305				
#7	Angelina	None	Fasting	6	0	6.7442	7	97.3939	97.8586	4.2188	4.7	52.3011	58.4962				
#12	Blossom	None	Fasting	6	0	7.0624	7.1	100.0000	100.0000	6.2195	6.8	88.0544	90.0083				
#8	Champagne	None	Fasting	6	0	6.2375	6.7	89.1919	93.8889	6.2689	6.8	92.3511	94.8329				
#9	Gilmour	None	Fasting	6	0	6.8029	6.8	100.0000	100.0000	2.9965	1.3	32.4011	35.6144				
#10	Kyle	None	Fasting	6	0	6.5132	6.7	98.1616	99.3939	4.6867	6.3	67.0618	68.1545				
#11	Lemieux	None	Fasting	6	0	4.1521	4.4	53.5779	62.3610	1.5461	0.6	13.9848	15.9880				
#1	Babs	None	fed	1	Baseline	1.9095	1.2	13.0278	13.8796	0.9263	0.8	0.2130	0.3889				
#4	Honey	None	fed	1	Baseline	1.9613	1.2	12.3796	13.1667	1.7389	0.9	13.2593	15.4259				
#5	Ike	None	fed	1	Baseline	1.5372	1	5.8056	8.1944	1.7156	1.2	8.3981	9.6667				
#6	Kenny	None	fed	1	Baseline	1.2981	1	4.5093	4.5463	1.3235	1	4.6667	6.3241				

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3
#3	Patsy	None	fed	1	Baseline	1.7263	1.4	4.9352	6.5833	1.5215	1.3	3.8611	5.2963				
#2	Trakey	None	fed	1	Baseline	2.0113	1.4	11.6574	14.3704	2.0356	1.2	12.6481	15.6759				
#7	Angelina	None	fed	1	Baseline	1.6714	1.1	8.2593	9.4722	2.2622	1.3	16.1296	16.8519				
#12	Blossom	None	fed	1	Baseline	3.1044	2.2	36.2778	43.8056	1.7290	1.3	3.4630	13.8519				
#8	Champagne	None	fed	1	Baseline	2.3331	1.8	13.0648	22.8056	2.1595	1.5	20.0370	22.6481				
#9	Gilmour	None	fed	1	Baseline	1.6116	1.3	5.1759	8.9537	2.0118	1.2	16.1296	16.9630				
#10	Kyle	None	fed	1	Baseline	1.7692	1.2	9.0000	10.5370	1.9566	1.3	13.0000	17.9537				
#11	Lemieux	None	fed	1	Baseline	3.0285	2.7	36.2778	47.1481	3.0338	1.9	35.6019	40.5093				
#1	Babs	None	fed	2	0	3.9522	4.1	52.5051	72.3939	2.2519	1.3	23.7407	29.0278				
#4	Honey	None	fed	2	0	3.6493	3.3	37.6970	56.2626	4.2745	5.7	57.5000	59.2407				
#5	Ike	None	fed	2	0	1.4006	1.1	2.0808	6.0707	2.1252	1.3	16.1667	20.7778				
#6	Kenny	None	fed	2	0	1.5508	1.4	1.5758	6.5253	1.9646	1.4	10.1204	20.5463				
#3	Patsy	None	fed	2	0	1.6030	1.4	3.7677	4.6768	3.4414	1.7	39.2037	40.8796				
#2	Trakey	None	fed	2	0	1.3641	1.2	2.4949	2.9293	3.3771	2	37.4630	42.1019				
#7	Angelina	None	fed	2	0	1.1353	1	1.3636	1.9192	2.1278	1	16.8719	21.8076				
#12	Blossom	None	fed	2	0	3.2498	2.9	39.1111	49.6162	2.0247	1	18.8629	21.3353				
#8	Champagne	None	fed	2	0	1.3637	1	5.3647	7.3045	1.0480	1	0.6113	1.5006				
#9	Gilmour	None	fed	2	0	1.1313	1	1.4242	1.5556	2.9584	1.4	33.3735	37.4850				
#10	Kyle	None	fed	2	0	2.7848	1.9	31.4040	37.7677	4.0096	5	54.0976	60.5334				
#11	Lemieux	None	fed	2	0	2.0775	1.5	13.9408	17.0320	2.7813	1.6	28.0489	32.3826				
#1	Babs	None	fed	3	0	1.6162	1.2	5.7677	7.4141	2.4609	1.2	26.7154	29.4379				
#4	Honey	None	fed	3	0	3.5799	3.4	41.0404	57.1212	4.3648	5.4	56.8201	59.9222				
#5	Ike	None	fed	3	0	1.7230	1.4	3.5657	15.2424	1.3654	1.2	0.9538	2.3613				
#6	Kenny	None	fed	3	0	1.1255	1	0.1313	0.2323	1.2085	1	2.2317	2.7317				
#3	Patsy	None	fed	3	0	1.4884	1.3	2.8597	3.5709	3.2763	1.7	40.7371	42.1836				
#2	Trakey	None	fed	3	0	1.2923	1.1	1.1515	1.9091	2.5395	1.2	28.7619	32.1141				
#7	Angelina	None	fed	3	0	1.2258	1.1	1.8081	4.7879	1.4833	1.1	5.0838	6.0376				
#12	Blossom	None	fed	3	0	2.5974	2.6	8.9726	36.0720	1.9784	1.2	13.5049	18.6092				

12 Hour													
Dog #	Dog	Drug	State	Week	Day	9AM-9PM			9PM-9AM				
						Mean	Median	%≥4	Mean	Median	%≥4		
#8	Champagne	None	fed	3	0	1.5769	1.4	3.3535	5.0808	1.1704	1.1	0.1667	0.3611
#9	Gilmour	None	fed	3	0	1.2611	1	4.2820	4.6452	1.6904	1	10.9179	12.3227
#10	Kyle	None	fed	3	0	3.1830	2.7	36.8283	46.4848	1.7352	1.1	10.6862	13.1123
#11	Lemieux	None	fed	3	0	2.0459	1.5	13.5657	16.1111	1.5955	1.3	6.4728	8.9823
#1	Babs	None	fed	4	0	1.7407	0.9	11.8586	18.6768	1.9714	1.2	15.1588	20.8538
#4	Honey	None	fed	4	0	2.6148	2.3	17.4141	41.0101	4.3454	4.9	57.1442	64.7653
#5	Ike	None	fed	4	0	1.3908	1.2	1.6566	6.1313	2.0266	1.2	15.7144	22.5206
#6	Kenny	None	fed	4	0	2.8819	2.4	28.0101	37.2424	3.4256	3	37.5498	50.6621
#3	Patsy	None	fed	4	0	5.7182	5.8	98.7576	99.4949	5.9239	6.3	94.7680	95.6755
#2	Trakey	None	fed	4	0	1.8930	1.5	8.6869	11.3131	2.5508	1.2	23.6781	29.5490
#7	Angelina	None	fed	4	0	3.1401	3.1	45.6263	51.5859	3.7672	3.8	47.9489	59.2370
#12	Blossom	None	fed	4	0	2.4080	1.8	19.4767	29.1545	4.0542	3.5	43.5133	56.8756
#8	Champagne	None	fed	4	0	2.1706	1.3	21.9293	29.6566	1.9531	1.1	21.4094	24.7060
#9	Gilmour	None	fed	4	0	1.5157	1	6.7879	12.6667	3.0831	1.7	33.9846	39.7074
#10	Kyle	None	fed	4	0	1.3370	1	4.6566	7.9899	2.9448	1.3	35.2315	36.4444
#11	Lemieux	None	fed	4	0	3.2691	3.5	43.7071	54.3636	4.0357	4.1	55.6811	71.8863
#1	Babs	None	fed	5	0	1.9834	1.2	12.4356	22.2952	2.6832	2.1	23.0388	35.4937
#4	Honey	None	fed	5	0	2.9983	2.6	31.2424	42.5859	2.4363	1.4	24.1133	29.2897
#5	Ike	None	fed	5	0	2.5549	2.2	24.2222	41.5758	2.2404	1.2	17.7424	21.8076
#6	Kenny	None	fed	5	0	2.3426	1.7	17.9495	26.5152	3.3598	3.2	39.3277	54.6162
#3	Patsy	None	fed	5	0	3.6456	4.3	52.2727	56.4242	3.1482	2.6	35.3088	46.4117
#2	Trakey	None	fed	5	0	3.5288	4.1	51.7879	63.8283	4.1610	4.4	62.1261	71.6455
#7	Angelina	None	fed	5	0	2.7319	2.6	37.4747	45.5556	3.4478	3.8	44.4300	61.6816
#12	Blossom	None	fed	5	0	4.6929	4.8	83.5455	95.5051	4.7306	4.9	66.3487	82.3224
#8	Champagne	None	fed	5	0	2.8448	3.2	28.7172	54.8384	3.0119	2.3	27.8081	43.2170
#9	Gilmour	None	fed	5	0	2.3219	2.2	7.8075	29.4598	3.0866	2.3	32.5591	44.1307
#10	Kyle	None	fed	5	0	1.3678	0.9	5.9798	12.7576	1.7693	0.9	14.5939	14.8440
#11	Lemieux	None	fed	5	0	2.9252	3	27.7475	51.6162	4.5153	4.7	65.8024	75.2940

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	%≥4		%≥3	Mean	Median		%≥3	Mean	Median	%≥4
#12	Blossom	Saline	fed	1	1	2	2.9899	2.2	28.6389	39.2963	2.9043	2.1	31.2407	38.7407			
#1	Babs	Saline	fed	1	1	2	1.7245	1.1	12.4167	12.8796	1.5347	1.1	8.3519	9.6852			
#6	Kenny	Saline	fed	1	1	2	1.6706	1	11.8704	12.6481	1.3408	0.9	4.8426	9.7130			
#5	Ike	Saline	fed	2	2	2	2.0650	1.5	9.3981	18.6019	1.9799	1.3	15.0093	16.9907			
#10	Kyle	Saline	fed	2	2	2	3.8330	3.6	48.9153	54.1999	4.6748	5.4	61.5541	75.3543			
#4	Honey	Saline	fed	3	3	2	2.9774	2.2	28.0000	36.5463	4.6288	5.9	65.3056	70.5093			
#11	Lemieux	Saline	fed	3	3	2	1.8781	1.2	10.8056	13.0833	2.2900	1.3	17.3148	24.7315			
#3	Patsy	Saline	fed	4	4	2	2.7731	2.1	26.0833	37.7315	3.7396	4	50.4907	57.8148			
#8	Champagne	Saline	fed	4	4	2	3.0016	2.7	39.0093	48.7315	1.7751	1.2	14.8148	20.4907			
#7	Angelina	Saline	fed	5	5	2	2.9770	2.9	32.2407	49.6852	1.8770	1.2	17.7500	21.8889			
#9	Gilmour	Saline	fed	5	5	2	2.0304	1.3	11.9537	14.6481	2.4352	1.2	25.3981	29.1204			
#12	Blossom	Saline	fed	1	6	6	2.2124	1.4	13.7593	24.2870	1.2431	1	2.5093	3.8519			
#1	Babs	Saline	fed	1	6	6	2.2574	1.1	26.7593	27.0926	0.9257	0.8	1.1019	1.6852			
#6	Kenny	Saline	fed	1	6	6	1.7585	1.4	5.7222	10.8148	1.1127	1.1	0.1019	0.1111			
#5	Ike	Saline	fed	2	6	6	1.7658	1.2	9.4722	10.0463	1.5963	1.1	8.0278	8.8333			
#10	Kyle	Saline	fed	2	6	6	1.4620	1	4.6019	12.0093	3.1790	1.8	38.5926	41.8519			
#4	Honey	Saline	fed	3	6	6	1.9177	1.3	10.9352	13.9444	1.9995	1	13.9167	21.1574			
#11	Lemieux	Saline	fed	3	6	6	2.1383	1.3	15.3241	18.0463	2.4629	1.6	21.8148	31.6389			
#3	Patsy	Saline	fed	4	6	6	3.3178	2.2	42.4815	46.2037	4.5599	5.3	64.9074	68.0556			
#8	Champagne	Saline	fed	4	6	6	3.5585	3.7	41.8148	69.6111	2.8723	2.8	40.1204	49.7315			
#7	Angelina	Saline	fed	5	6	6	2.4227	1.6	18.9294	31.9596	2.6775	1.5	28.3981	35.3241			
#9	Gilmour	Saline	fed	5	6	6	2.7376	2.1	30.3519	42.0648	3.2502	3.4	41.5648	53.6204			
#3	Patsy	Famo	fed	1	2	2	3.0384	2.7	26.3981	47.0093	3.5812	3.1	39.9815	51.8889			
#9	Gilmour	Famo	fed	1	2	2	2.3833	1.5	14.4259	31.1204	4.4242	4.4	61.8611	70.0370			
#4	Honey	Famo	fed	2	2	2	3.0445	2	35.1389	36.6852	4.1939	4.5	53.4815	58.7778			
#2	Trakey	Famo	fed	2	2	2	3.6494	3.3	38.1667	56.1852	3.6950	3.2	37.8426	54.3148			
#8	Champagne	Famo	fed	2	2	2	2.0209	1.6	8.9630	9.4907	1.4361	1.1	0.3704	2.8333			
#1	Babs	Famo	fed	3	2	2	2.2732	1.7	11.9907	19.8333	2.9608	2.1	25.9630	42.0926			

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3
#2	Trakey	Famo	fed	3	3	2.8418	2.4	20.0741	32.5278	3.4287	3	31.4167	51.4167				
#7	Angelina	Famo	fed	3	3	2.1346	2	8.3704	27.2778	2.5396	2.3	16.7870	41.3333				
#10	Kyle	Famo	fed	3	3	3.4523	3	34.4815	52.4722	4.1927	4.3	55.7500	67.3889				
#5	Ike	Famo	fed	4	4	2.3294	1.5	18.5271	26.0028	2.5934	1.9	19.3333	34.1481				
#12	Blossom	Famo	fed	4	4	5.1813	5.6	79.3241	86.8519	4.9003	5.4	78.1204	81.5648				
#6	Kenny	Famo	fed	5	5	4.5089	5.3	67.1944	74.3981	5.0913	5.4	80.8241	85.6111				
#11	Lemieux	Famo	fed	5	5	3.5787	3.5	45.2315	55.0648	3.0160	3	35.4259	50.4074				
#3	Patsy	Famo	fed	1	6	2.6867	1.9	25.4167	34.4074	3.4000	2.8	35.6759	48.3148				
#9	Gilmour	Famo	fed	1	6	4.6436	5.8	65.0185	72.7963	4.7261	5.6	73.1389	77.7778				
#4	Honey	Famo	fed	2	6	4.1777	4.3	55.0000	65.6389	3.9474	4	50.1019	57.6111				
#8	Champagne	Famo	fed	2	6	4.3967	4.9	63.0741	71.5648	4.7474	6.1	61.8981	73.1852				
#1	Babs	Famo	fed	3	6	1.8857	1.2	10.0370	12.5648	2.6614	1.7	24.6574	30.8889				
#2	Trakey	Famo	fed	3	6	3.5990	2.5	45.0278	46.7407	3.3188	2.9	37.7315	49.8241				
#7	Angelina	Famo	fed	3	6	4.1015	4.1	52.9630	65.4630	5.5082	6	85.6481	90.9167				
#10	Kyle	Famo	fed	3	6	2.7309	1.9	22.0648	32.9537	4.0869	3.8	49.6204	61.1019				
#5	Ike	Famo	fed	4	6	3.0286	2.2	24.6667	35.2500	3.6561	3.3	35.6296	53.9630				
#12	Blossom	Famo	fed	4	6	4.7942	5.2	79.1204	83.1019	4.1406	4.6	65.1852	75.9074				
#6	Kenny	Famo	fed	5	6	3.6203	3.5	45.0000	55.6204	4.5935	4.7	64.7685	80.9167				
#11	Lemieux	Famo	fed	5	6	4.0622	4.8	59.4444	64.5833	3.8452	4	51.4074	64.3333				
#5	Ike	Omeprazole	fed	1	2	4.9384	5.4	75.2315	86.2407	4.1680	4.8	56.8148	62.9444				
#11	Lemieux	Omeprazole	fed	1	2	4.7453	4.8	91.6667	95.4722	4.0596	4	51.5093	74.8796				
#1	Babs	Omeprazole	fed	2	2	3.7860	3.5	42.9444	57.9537	1.9776	1.4	11.7936	19.6683				
#7	Angelina	Omeprazole	fed	2	2	3.1163	2.9	19.2685	47.3519	2.8003	2.3	19.7593	29.3704				
#12	Blossom	Omeprazole	fed	2	2	3.8218	4.3	62.4815	77.7778	3.4051	3.4	26.7037	70.8056				
#6	Kenny	Omeprazole	fed	3	2	3.9523	4.1	54.3889	88.3611	3.0267	3.1	13.7130	54.3241				
#9	Champagne	Omeprazole	fed	3	2	4.4671	5.3	70.6204	73.2685	2.8014	2.6	26.6111	45.8333				
#4	Honey	Omeprazole	fed	4	2	3.9265	4.3	54.7407	62.5648	4.4206	4.3	57.8796	80.7130				
#2	Trakey	Omeprazole	fed	4	2	4.9035	5	83.7037	93.7593	5.0977	5.3	83.6296	85.5278				

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

12 Hour															
Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM	
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3		
#9	Gilmour	Omep	fed	4		2	4.6999	5.2	74.6759	82.3889	3.1152	3.4	29.4352	56.1667	
#3	Patsy	Omep	fed	5		2	4.1469	5.1	63.2130	65.8796	4.8892	5.8	69.4167	72.3611	
#10	Kyle	Omep	fed	5		2	4.0008	3.7	38.3056	79.8889	2.9023	3	16.5833	51.9259	
#5	Ike	Omep	fed	1		6	5.4399	5.4	99.3796	100.0000	2.9188	2.8	37.3056	48.5833	
#11	Lemieux	Omep	fed	1		6	4.5650	4.8	67.3333	81.1852	4.4465	5.8	56.6389	63.6852	
#1	Babs	Omep	fed	2		6	4.3283	4.2	56.5648	88.6481	1.9780	1.8	2.8333	22.3889	
#7	Angelina	Omep	fed	2		6	4.5182	4.4	65.5833	84.9815	3.3494	2.9	26.2500	47.9167	
#12	Blossom	Omep	fed	2		6	3.6665	3.6	41.5556	69.4259	3.6690	2.9	38.6574	48.9167	
#6	Kenny	Omep	fed	3		6	3.6597	3.3	42.3889	54.0741	3.9072	4.3	58.0463	69.4074	
#9	Champagne	Omep	fed	3		6	2.6678	2.7	10.0463	43.9074	5.0361	4.9	99.6759	99.9815	
#4	Honey	Omep	fed	4		6	3.9633	4	51.3981	83.7130	2.5953	2.9	18.6433	48.1626	
#2	Trakey	Omep	fed	4		6	4.9926	4.8	99.1389	99.6667	5.0430	5.1	81.4074	85.9630	
#9	Gilmour	Omep	fed	4		6	4.7247	4.8	84.4259	90.8796	3.9586	3.9	47.7130	74.4722	
#3	Patsy	Omep	fed	5		6	4.1010	4.6	70.5185	78.1852	3.3207	3.5	46.1944	54.1852	
#10	Kyle	Omep	fed	5		6	4.7214	4.9	78.6481	87.2685	3.9313	4.1	55.1389	68.1296	
#4	Honey	Panto	fed	1		2	3.7363	3.9	48.3796	78.0648	2.5022	1.8	19.8704	31.0370	
#10	Kyle	Panto	fed	1		2	4.9533	4.8	92.7222	99.9907	2.7344	2.9	19.5556	48.9815	
#6	Kenny	Panto	fed	2		2	1.9791	1.4	12.2870	12.6944	1.3079	1.1	1.8611	3.0926	
#9	Gilmour	Panto	fed	2		2	4.5379	4.4	82.7130	99.0093	3.7059	3.7	31.2870	71.4074	
#3	Patsy	Panto	fed	3		2	2.1967	1.6	12.3796	14.6852	2.0805	1.4	12.6019	15.4630	
#12	Blossom	Panto	fed	3		2	3.2194	3	34.1852	50.6481	3.5956	3	42.5556	50.8519	
#1	Babs	Panto	fed	4		2	2.5669	2.5	18.6759	35.4167	3.4746	2.9	39.5926	49.8611	
#7	Angelina	Panto	fed	4		2	3.2345	2.9	40.6649	49.9907	3.4821	3.7	46.8796	57.8056	
#11	Lemieux	Panto	fed	4		2	4.1292	4.6	66.2963	75.9352	4.0244	4.4	51.8148	62.8796	
#5	Ike	Panto	fed	5		2	4.0149	5.1	66.4815	69.8241	4.4240	5.4	67.0926	71.5741	
#2	Trakey	Panto	fed	5		2	4.9725	4.9	92.5833	98.9259	3.6867	3.8	46.3426	68.8889	
#8	Champagne	Panto	fed	5		2	4.0426	4.3	62.8333	78.2407	2.8539	2.3	39.5556	47.8611	
#4	Honey	Panto	fed	1		6	4.0675	3.9	49.4537	65.5833	3.1082	3.4	39.1019	55.1111	

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3
#10	Kyle	Panto	fed	1	6	3.2713	3.4	32.8889	66.0926	2.4511	1.7	17.9630	22.8796				
#6	Kenny	Panto	fed	2	6	3.5444	3.5	32.5278	66.4074	2.4427	2.2	12.3981	25.3611				
#9	Gilmour	Panto	fed	2	6	3.4773	3.6	40.8148	71.2130	3.9074	3.7	42.8704	68.0556				
#3	Patsy	Panto	fed	3	6	2.0839	1.5	11.1204	12.5741	3.1360	1.8	30.6574	32.7407				
#12	Blossom	Panto	fed	3	6	3.8384	3.7	43.8056	71.7222	3.2526	3.1	26.4537	54.1481				
#1	Babs	Panto	fed	4	6	4.0888	4.9	69.3611	74.1759	4.7192	5	66.0926	77.4537				
#7	Angelina	Panto	fed	4	6	4.3712	5.2	72.5370	75.9537	4.1111	4.6	60.7778	71.3241				
#11	Lemieux	Panto	fed	4	6	3.6966	4	53.8981	68.9630	4.7039	5.6	68.0833	75.3796				
#5	Ike	Panto	fed	5	6	3.7478	4.7	57.2222	61.7500	3.4290	3.9	49.7130	56.3426				
#2	Trakey	Panto	fed	5	6	4.5925	4.8	82.0556	86.5185	4.4539	4.9	69.2593	79.1204				
#8	Champagne	Panto	fed	5	6	4.0332	5	60.3056	69.7963	3.5035	3.8	49.5926	52.4259				
#2	Trakey	Rani	fed	1	2	2.6625	2	19.3796	32.2222	2.7251	2	19.1296	34.5370				
#7	Angelina	Rani	fed	1	2	2.1172	1.6	9.1204	26.6111	2.4623	1.9	12.8333	34.1759				
#8	Champagne	Rani	fed	1	2	3.6226	3.4	43.2593	56.9630	4.7176	5.7	65.2593	70.7037				
#3	Patsy	Rani	fed	2	2	2.5817	1.8	15.2500	31.3981	3.5834	3.5	38.7222	60.4537				
#11	Lemieux	Rani	fed	2	2	1.8944	1.5	9.7037	10.9444	2.9498	2.7	23.4352	42.9167				
#5	Ike	Rani	fed	3	2	2.7843	1.9	22.5278	31.0278	2.9999	2.7	23.0556	41.3148				
#9	Gilmour	Rani	fed	3	2	5.1909	5.5	82.0370	94.5185	2.9951	1.7	31.5000	36.5185				
#6	Kenny	Rani	fed	4	2	2.3181	1.5	20.4537	26.1019	1.7296	1.4	3.7685	15.0370				
#10	Kyle	Rani	fed	4	2	2.2290	1.6	12.8509	20.5689	2.7947	2.3	24.5068	36.3990				
#1	Babs	Rani	fed	5	2	2.8438	2.5	33.4259	43.6574	2.8132	2.4	29.8611	44.1204				
#4	Honey	Rani	fed	5	2	2.5367	2.2	25.2593	39.4259	3.2886	3.1	44.5124	51.1624				
#12	Blossom	Rani	fed	5	2	5.9292	6.2	97.0648	99.8333	5.8103	6.2	93.8796	98.6852				
#2	Trakey	Rani	fed	1	6	2.4136	1.9	13.1574	27.3333	2.5821	1.8	21.2778	33.0741				
#7	Angelina	Rani	fed	1	6	2.0975	0.8	20.5185	21.1574	1.9318	0.9	18.2222	21.0556				
#8	Champagne	Rani	fed	1	6	1.9372	1.7	5.9826	14.1971	3.1823	2.1	28.8360	42.4391				
#3	Patsy	Rani	fed	2	6	2.2112	1.6	15.0185	23.5000	3.2624	2.4	29.5278	42.6852				
#11	Lemieux	Rani	fed	2	6	3.0322	2	31.4907	39.3426	4.6540	6	59.8333	67.0000				

Appendix 5.2-2 - 12-h Raw Data from Chapters II and III

12 Hour														
Dog #	Dog	Drug	State	Week	Day	9AM-9PM			12 Hour			9PM-9AM		
						Mean	Median	%≥4	%≥3	Mean	Median	%≥4	%≥3	Mean
#5	Ike	Rani	fed	3	6	2.1999	1.6	11.3519	19.3704	2.6310	2	20.4907	34.9259	
#9	Gilmour	Rani	fed	3	6	1.9423	1.3	11.8611	12.1574	2.8274	1.7	26.9352	31.8704	
#6	Kenny	Rani	fed	4	6	3.0845	2.1	32.8796	39.5185	3.8926	4.3	51.8796	56.3889	
#10	Kyle	Rani	fed	4	6	2.3517	1.9	23.5741	34.8704	3.1748	2.6	33.7407	45.9907	
#1	Babs	Rani	fed	5	6	3.0435	3.1	29.2315	53.1852	4.0067	4.4	61.8426	68.2685	
#4	Honey	Rani	fed	5	6	1.9338	1.3	11.5370	18.0833	2.4614	2.4	19.1349	35.8803	
#12	Blossom	Rani	fed	5	6	3.9542	4.1	52.0185	66.0093	3.7024	5.05	52.5741	54.1574	
#1	Babs	Fam3	fed	6	2	4.2644	4.5	63.4907	80.7130	3.3753	3	40.3704	51.2593	
#4	Honey	Fam3	fed	6	2	3.2811	2.9	37.2130	49.9722	3.2250	2.6	33.5185	45.5833	
#5	Ike	Fam3	fed	6	2	3.3337	3.4	30.4167	67.8611	2.6737	2.1	25.6759	41.4722	
#6	Kenny	Fam3	fed	6	2	3.7491	3.7	45.7222	59.3981	3.7691	4	50.8704	62.7037	
#3	Patsy	Fam3	fed	6	2	4.4569	5.1	71.4907	77.6944	4.0039	4.6	59.4352	65.9815	
#2	Trakey	Fam3	fed	6	2	4.4480	4.2	68.7500	96.1852	3.4754	3	34.1389	51.2500	
#1	Babs	Fam3	fed	6	6	3.3935	3.5	42.0741	54.1852	3.5561	3.3	41.4444	53.5648	
#4	Honey	Fam3	fed	6	6	3.9153	3.7	39.8333	78.8426	3.2323	2.9	29.1204	47.4815	
#5	Ike	Fam3	fed	6	6	2.0884	1.5	14.6944	34.7222	2.8642	2.5	30.8148	46.2130	
#6	Kenny	Fam3	fed	6	6	3.5550	3.6	47.5833	58.0463	2.8153	2.6	25.5000	44.1852	
#3	Patsy	Fam3	fed	6	6	3.8059	4	51.7963	70.6852	3.7858	3.7	47.1481	61.1389	
#2	Trakey	Fam3	fed	6	6	3.8847	3.8	45.9167	69.8056	4.5834	4.6	59.7037	70.5463	
#7	Angelina	Ome2	fed	6	2	4.7966	5	84.6949	91.6048	4.9811	4.6	77.1857	96.1051	
#12	Blossom	Ome2	fed	6	2	5.4669	5.5	100.0000	100.0000	6.3257	6.7	100.0000	100.0000	
#8	Champagne	Ome2	fed	6	2	4.4763	4.4	91.9491	94.6606	4.2690	4.2	59.2483	92.5104	
#9	Gilmour	Ome2	fed	6	2	4.9304	4.9	88.8606	96.5537	4.3811	4.1	72.3547	80.7904	
#10	Kyle	Ome2	fed	6	2	4.8363	4.5	86.6949	99.7882	3.6655	3.9	44.5982	76.5876	
#11	Lemieux	Ome2	fed	6	2	4.5295	5	70.5648	84.6854	4.2960	4.2	64.8384	78.2147	
#7	Angelina	Ome2	fed	6	6	5.1113	5.2	89.8490	93.9400	3.4121	4.2	59.3909	65.8273	
#12	Blossom	Ome2	fed	6	6	4.6138	5.5	68.1733	77.4194	6.1244	6.4	96.2659	99.9255	
#8	Champagne	Ome2	fed	6	6	3.6343	4.4	57.9600	65.8816	3.1108	3.8	41.1315	57.2288	

**Appendix 5.2-2 - 12-h Raw Data from
Chapters II and III**

Dog #	Dog	Drug	State	Week	Day	9AM-9PM				12 Hour				9PM-9AM			
						Mean	Median	% $\geq$ 4		% $\geq$ 3	Mean	Median		% $\geq$ 4		% $\geq$ 3	
#9	Gilmour	Ome2	fed	6	6	4.3393	5	66.7639		81.0053	4.6151	5.3		69.8693		72.2918	
#10	Kyle	Ome2	fed	6	6	4.4709	4.9	69.1727		78.7256	4.1981	4.6		67.8400		74.8337	
#11	Lemieux	Ome2	fed	6	6	4.8376	5.2	83.3551		90.9176	5.0466	5.6		80.2726		84.1383	