

**THE GASTRODUODENAL EFFECTS OF BUFFERED ASPIRIN,  
CARPROFEN, AND ETODOLAC IN THE HEALTHY DOG**

**and**

**COMPARISON OF THE CLOTEST® TO HISTOPATHOLOGIC  
EVALUATION IN IDENTIFYING THE PRESENCE OF *HELICOBACTER*  
*SPP.* IN HEALTHY DOGS**

By

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### (ABSTRACT)

Twenty-four healthy, mixed breed dogs were divided into four groups. Group I received a placebo PO BID, group II received an average 16.5 (range, 15.1-17.8) mg/kg buffered aspirin PO BID, group III received an average 2.2 (range, 2.0-2.4) mg/kg carprofen PO BID, and group IV received an average 12.8 (range, 11.7-13.8) mg/kg etodolac PO QD (with a placebo in the P.M.). All treatments continued for 28 consecutive days. Gastroduodenal endoscopy was performed on days -9, 0, 5, 14 and 28. Multiple gastric biopsies were obtained endoscopically on day -9 to determine each dog's *Helicobacter spp.* status.

Five areas, consisting of four regions in the stomach and one in the proximal duodenum, were evaluated endoscopically, and each was assigned a score from 1 to 11 based on qualitative assessment of submucosal hemorrhage, erosion, or ulceration. These scores for each region were then summed to give a total score for each endoscopic evaluation.

Erosions and submucosal hemorrhages were seen in all dogs receiving aspirin. Only minor gastric lesions were observed in the carprofen, etodolac, and control groups. No adverse clinical signs were noted in any dog given any treatment during the course of the study. There was no predilection site for lesion development in any group. Median total score on days 0, 5, 14, and 28 were as follows: group I, 5.0, 5.0, 5.0, 5.0; group II, 5.0, 27.0, 26.0, 27.5; group III, 5.0, 5.0, 6.0, 5.0; group IV, 5.0, 7.0, 5.0, 5.0, respectively.

There was no significant difference between dogs receiving carprofen, etodolac, or placebo. The administration of carprofen, etodolac, or placebo to healthy dogs resulted in significantly less gastroduodenal lesion development than in dogs receiving buffered aspirin.

# COMPARISON OF THE CLOTEST® TO HISTOPATHOLOGIC EVALUATION IN IDENTIFYING THE PRESENCE OF *HELICOBACTER SPP.* IN HEALTHY DOGS

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## (ABSTRACT)

Thirty healthy, random source, dogs were evaluated to determine the prevalence of *Helicobacter spp.*, and to compare the ‘Campylobacter-like organism’ test (CLOtest®) to histopathologic identification of *Helicobacter spp.* organisms. Gastric mucosal biopsies from each of four gastric regions (cardia, pyloric antrum, greater curvature, and angularis incisura) were obtained endoscopically for use in the CLOtest® and for histopathologic evaluation. Twenty-seven of 30 dogs (90%) were positive for spiral bacteria suspected to be *Helicobacter spp.* by histopathologic evaluation in at least one of the four gastric regions. Three dogs (10%) were negative for *Helicobacter spp.* in all gastric regions by histopathologic evaluation. The CLOtest® was found to have a sensitivity, specificity, and positive predictive value of 84%, 81%, and 92%, respectively, when compared to histopathologic evaluation. When only the angularis incisura was evaluated, the sensitivity, specificity, and positive predictive value increased to 92%, 94%, and 96%, respectively. The angularis incisura had the highest, whereas the pyloric antrum had the lowest, prevalence of positive test results when compared to dogs determined to be overall *Helicobacter spp.* positive (histopathologic positive in at least one gastric region). The results of this study suggest the prevalence of *Helicobacter spp.* in apparently healthy dogs is high. For accurate and economical detection of *Helicobacter spp.* in a dog undergoing upper gastrointestinal endoscopy, a tissue sample should be taken from the angularis incisura for CLOtest® sampling.

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## Literature Review

### Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used for treatment of musculoskeletal disease in veterinary medicine, and are the most common medications used by humans worldwide.<sup>1</sup> They are generally recognized for their analgesic, anti-pyrexia, and anti-inflammatory properties. In humans, NSAID therapy is associated with upper gastrointestinal symptoms in 25% of patients and ulcers and erosions in 40% of patients.<sup>1</sup> NSAID use creates a three to four fold increased risk of ulcer bleeding and perforation.<sup>1</sup> The two most common causes of human ulcer disease include NSAIDs and *Helicobacter pylori*.<sup>2</sup> Side effects of NSAID therapy recognized in the dog include gastrointestinal bleeding, hepatotoxicity, ulceration, and possible nephrotoxicity.<sup>1-4</sup> Clinical signs often seen in dogs with gastric ulcers include vomiting, hematemesis, melena, pallor, weakness, anorexia, and abdominal pain. No statistics are available on the incidence or prevalence of gastrointestinal ulcers in dogs receiving NSAIDs.

In humans, several risk factors are associated with the development of NSAID-induced gastrointestinal toxicity, including old age, previous episode of GI ulceration or bleeding, concurrent administration of other medications, especially corticosteroids or anticoagulants, prior NSAID-induced ulceration, and other concurrent disease.<sup>5</sup> Smoking is considered a risk factor when one of the other risk factors occurs concurrently, but by itself should not increase the risk of NSAID-induced GI ulceration.<sup>1</sup> The risk of developing a bleeding ulcer increases three to four times in people taking NSAIDs.<sup>1</sup>

In a retrospective study, 43 dogs with non-neoplastic causes of gastric and/or duodenal ulcers were reviewed.<sup>3</sup> The two most common predisposing factors were hepatic disease and treatment with NSAIDs. Other predisposing factors recorded with increased frequency included mastocytosis and uremia. Nonregenerative anemia was the most common clinicopathologic finding. Dogs that were receiving NSAIDs were more likely to have pyloric ulcers, whereas dogs with hepatic disease were most likely to have duodenal ulcers.<sup>3</sup>

In a second retrospective study, 240 dogs and cats that had been reported to the Georgia Animal Poison Information Center because of NSAID ingestion over a 19 month period were reviewed.<sup>6</sup> The most common NSAIDs consumed were ibuprofen (120), acetaminophen (94), aspirin (18), and indomethacin (8). Ninety percent of the exposures in dogs were due to accidental ingestion or overdose, whereas all of the exposures in cats were due to inappropriate administration by owners. The most common clinical signs were vomiting, diarrhea, and depression. No follow-up was available to assess the condition of these potential toxicoses.

Renal disease is also often implicated as a cause of gastroduodenal ulceration. Renal failure will cause an increase in serum gastrin levels because the kidneys are responsible for clearing gastrin.<sup>4</sup> Gastrin will increase gastric acidity and when combined with the mucosal

damage of uremic toxins, ulceration occurs. Inhibition of endogenous prostaglandins is believed to be responsible for both the renal and the gastrointestinal side effects that occur with NSAIDs.

### **Prostaglandin Synthesis**

Inflammation, which NSAIDs decrease, is the response of living tissue to injury and is initiated by enzymes, mediators, tissue damage, and tissue repair.<sup>7</sup> The cardinal signs of inflammation include redness, swelling, heat, pain, and loss of function. Inflammation can be initiated by trauma, infectious organisms, and physical and chemical irritants. When cell damage occurs, there is disruption of the phospholipid portion of the cell membrane; phospholipase A<sub>2</sub> is activated and arachidonic acid is released.<sup>8</sup> Arachidonic acid is widely distributed throughout the body and is stored in the cell membrane of most cells.

Arachidonic acid is acted upon by the enzyme cyclooxygenase, the enzyme that NSAIDs inhibit. Initially, cyclooxygenase converts arachidonic acid to intermediate prostaglandins (PGG<sub>2</sub>, PGH<sub>2</sub>), which are subsequently converted to the prostaglandins (PGE<sub>2</sub>, PGD<sub>2</sub>, PGI<sub>2</sub>, PGF<sub>2</sub>) and the thromboxanes (TXA<sub>2</sub> and TXB<sub>2</sub>).<sup>8</sup> Arachidonic acid is also acted upon by the enzyme lipoxygenase. This enzyme converts arachidonic acid to the leukotrienes (LTA<sub>4</sub>, LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>).<sup>8</sup> The products of the cyclooxygenase and the lipoxygenase pathways are considered eicosanoids. Glucocorticoids inhibit phospholipase A<sub>2</sub> and therefore, are dual blockers, inhibiting both the cyclooxygenase and lipoxygenase enzymes, and their metabolites.<sup>8</sup> NSAIDs have no inhibitory effects on the arachidonic acid metabolites that have already been formed, but instead they suppress further formation of the prostaglandins and the leukotrienes.<sup>8</sup>

Prostaglandin E<sub>2</sub> is the predominant eicosinoid detected during inflammatory conditions.<sup>7</sup> PGE<sub>2</sub> is a potent dilator of vascular smooth muscle and increases vascular permeability, which accounts for the rubor that occurs with inflammation.<sup>7</sup> This vasodilation also increases blood flow through the damaged tissues and adds to the edema caused by other agents, such as bradykinins.<sup>7</sup> PGE<sub>2</sub> acts with other mediators of inflammation to produce pain and pyrexia.<sup>9</sup> Although other prostanoids, including PGF<sub>2α</sub>, prostacyclin (PGI<sub>2</sub>), and TXA<sub>2</sub>, are present in an inflammatory reaction, they are usually present at less than 25% of the concentration of PGE<sub>2</sub>.<sup>9,10</sup> It is also a potent vasodilator as well as a potent inhibitor of platelet aggregation.<sup>11</sup> Both PGE<sub>2</sub> and PGI<sub>2</sub> can also cause smooth muscle relaxation.<sup>11</sup> PGI<sub>2</sub> and TXA<sub>2</sub>, as well as PGE<sub>2</sub> and PGF<sub>2α</sub>, often have directly opposite physiologic effects in the body.<sup>11</sup>

### **Cyclooxygenase**

Recently, it has been discovered that there are two forms of the cyclooxygenase enzyme, cyclooxygenase -1 (COX-1) and cyclooxygenase -2 (COX-2). COX-1 is known as the “constitutive” form and is considered important in normal physiologic roles. It is thought to perform “housekeeping” functions to synthesize prostaglandins that are responsible for cytoprotection of the gastric mucosa, regulation of renal blood flow, and platelet aggregation.<sup>12</sup>

COX-1, is present in most cells and tissues, including platelets, kidneys, synovium, and gastric mucosa.<sup>1</sup>

COX-2 is considered the “inducible” form that is associated with inflammation. COX-2 is not detectable in most tissues at a resting state, however it increases dramatically with inflammation and the presence of inflammatory mediators, such as cytokines.<sup>7,12</sup> COX-2 is expressed in inflamed tissues, activated monocytes, macrophages, fibroblasts, synoviocytes, and chondrocytes.<sup>1,5</sup> Therefore, if a drug can selectively inhibit COX-2, and not affect the physiologic functions of COX-1, common side effects such as gastrointestinal ulceration can be avoided.

The severity of side effects of NSAIDs can be variable and the inhibition of either COX-1 or COX 2 may be responsible. It has been found that there are differences in the inhibition of these enzymes when different NSAIDs are tested *in vitro*. These data are then expressed as a ratio of the amount of drug necessary to inhibit 50% (IC<sub>50</sub>) of the activity of each enzyme.<sup>5,13</sup> This activity is reported in a ratio of COX-2 (IC<sub>50</sub>): COX-1(IC<sub>50</sub>), in which a ratio of one or more means that less COX-2 than COX-1 is inhibited by a given drug concentration.<sup>5,13</sup> (See Table 1) Therefore, NSAIDs with a high COX-2 (IC<sub>50</sub>): COX-1(IC<sub>50</sub>) ratio are generally associated with a higher incidence of gastrointestinal side effects, and NSAIDs with a low COX-2 (IC<sub>50</sub>): COX-1(IC<sub>50</sub>) ratio generally have a better safety profile.<sup>5,7,13</sup> Examples of drugs with a high ratio include aspirin, piroxicam, and indomethacin, whereas ibuprofen, carprofen, etodolac, and naproxen generally have a low ratio.<sup>5,7,13</sup> Meloxicam is considered a selective inhibitor of COX-2. Both etodolac and carprofen have been reported to have low COX 2: COX 1 ratios from *in vitro* studies; etodolac has a favorable ratio of less than one, while carprofen has a ratio of approximately one.<sup>5,12,14</sup> Aspirin has a consistently high COX-2: COX-1 ratio.<sup>12,14</sup> When the activity of carprofen and etodolac were evaluated against isoenzymes of canine COX-1 and COX-2, carprofen was found to potently inhibit canine COX-2.<sup>15</sup> Etodolac did not have selectivity for canine COX-2, and was only marginally selective for canine COX-1.<sup>15</sup> Because different biologic systems are used to determine COX-2 (IC<sub>50</sub>): COX-1(IC<sub>50</sub>) ratios, it will be difficult to compare data from multiple studies until a standard test is established.<sup>5,7</sup>

## Nitric Oxide

Nitric oxide synthase (NOS) and its role in inflammation has recently been investigated. NOS is the enzyme that catalyzes the amino acid L-arginine to nitric oxide (NO). NO is responsible for many diverse physiologic and pathologic conditions. Similar to the different isoenzymes of COX, there are two forms of NOS, the constitutive and inducible forms. The constitutive form then has two forms based on location of action: neuronal cNOS (nNOS, NOS-1) works as a neurotransmitter for central and peripheral nerves and endothelial cNOS (eNOS, NOS-3), acts as a potent vascular mediator with actions on platelets and vascular tone.<sup>16</sup> The constitutive forms are regulated by calcium fluxes within a cell and are produced in small amounts.<sup>18</sup> The inducible form of NOS (NOS-2 or iNOS) can be induced by endotoxins,

cytokines, interleukins, and growth factors.<sup>16,18</sup> It is expressed in many cells including endothelial cells, smooth muscle cells, and macrophages.<sup>19</sup>

Nitric oxide is thought to be favorable when produced in small amounts by the constitutive NOS, whereas larger quantities produced by the iNOS can be cytotoxic and cause pathological consequences.<sup>16,19,20</sup> It is believed that iNOS is induced in inflammatory tissues, concurrently with COX-2.<sup>16,20</sup> NO has also been found to activate COX-2, thereby increasing prostaglandin production and further enhancing inflammation.<sup>19-21</sup> NO is thought to be cytotoxic, whereas prostaglandins are not.<sup>19</sup>

The inhibition of iNOS and NO production by NSAIDs may partially explain their anti-inflammatory actions, in addition to their inhibition of cyclooxygenase and prostaglandins.<sup>22</sup> It has been reported that aspirin, ibuprofen, indomethacin, and sodium salicylate will inhibit the iNOS in rat alveolar macrophages.<sup>21</sup> Dexamethasone is an example of a dual iNOS and COX-2 inhibitor.<sup>19</sup> Therefore, drugs that can be produced that selectively inhibit iNOS and COX-2, while preserving the constitutive NOS and COX-1, can significantly decrease severe side effects. It is important that as these drugs are manufactured, the inhibition of the endothelial NOS not be inhibited, as it could cause severe vascular compromise. The protective role of nitric oxide in the gastrointestinal tract will be discussed later.

## **Prostaglandins**

Prostaglandins (PG) occur naturally in the body and are not always associated with an inflammatory stimulus. In the kidney, prostaglandins are important in regulating renal hemodynamics; PGE<sub>2</sub> is the major vasodilatory PG and is synthesized primarily in the renal medulla.<sup>23</sup> In addition to vasodilation, PG's increase renal blood flow and glomerular filtration rate, inhibit sodium reabsorption in the distal tubule, and increase renin release.<sup>23,24</sup> PGE<sub>2</sub> is released in response to many agonists including angiotensin II, reduced renal arterial pressure, norepinephrine, and furosemide.<sup>25</sup> PGI<sub>2</sub> is stimulated in response to angiotensin II.<sup>25</sup> COX-1 has been found in renal arteries and arterioles, glomeruli, and collecting ducts, however, none was found in the proximal or distal collecting tubules, Henle's loop, or the macula densa.<sup>24</sup> COX-2, found primarily in the macula densa, plays a minor role in the kidney under basal conditions.<sup>24</sup>

NSAIDs inhibit production of renal vasodilatory prostaglandins, which leads to constriction of the afferent arteriole and a decline in renal function. Administration of NSAIDs to healthy human patients appears to have negligible effects on renal function, however, these drugs may alter renal function in those patients with renal compromise.<sup>23,26-28</sup> There is evidence that in healthy dogs, effects of NSAIDs are negligible, however these drugs may significantly alter renal function in patients with renal disease.<sup>23,26-28</sup>

In the gastrointestinal tract, prostaglandins of the E, F, and I types are responsible for enhancing mucosal resistance to injury.<sup>29</sup> Prostaglandins have several effects including the

inhibition of gastric acid secretion, stimulation of gastric and duodenal mucus secretion, stimulation of gastric and duodenal bicarbonate secretion, enhancement of gastric mucosal blood flow, and promotion of healing of ulcers and erosions.<sup>29</sup> These activities contribute to the role prostaglandins play in gastric 'cytoprotection'. This term describes the protective effect prostaglandins play on the gastric mucosa.<sup>30</sup>

### **Gastric Mucosal Defense**

There are many factors, other than prostaglandins, that protect the stomach and duodenum from injury: the 'alkaline mucus' layer, mucosal blood flow, surface hydrophobicity of membrane phospholipids, epithelial regenerative capacity, and synthesis of protective nitric oxide and growth factors, such as epidermal growth factor.<sup>29,31-36</sup> The epithelium in the stomach is very resistant to the high acid concentrations that it is continually exposed. Sometimes, the pH of gastric acid is as low as 1.0. The gastric mucosal barrier consists of a layer of mucus, the cell membranes of the gastric epithelial cells, and the subepithelial vasculature.<sup>31</sup> There is an 'alkaline mucus' layer that is adherent to the surface epithelium that can serve as a protective barrier as it adheres tenaciously.<sup>29</sup> The substance that makes up the mucus layer is composed of 5% glycoprotein molecules and 95% water and will prevent pepsin permeation and diffusion of hydrogen ions.<sup>29</sup> The mucus, therefore, is not by itself a barrier, but the alkaline secretions contained within the mucus help protect against the hydrogen ions. It is this bicarbonate rich fluid derived from the plasma and the oxyntic cells that neutralizes the hydrogen ions.<sup>31</sup>

Factors that will increase mucus secretion include luminal acid and prostaglandins, whereas agents like aspirin, steroids, ethanol, and bile salts will decrease mucus secretion.<sup>29</sup> The bicarbonate that is secreted by the surface epithelial cells are stimulated and inhibited by similar agents as the mucus.<sup>29,32</sup> As the luminal acid output increases, there is a corresponding increase in bicarbonate production. Therefore, it is suggested that the 'alkaline mucus' layer, including both mucus and bicarbonate secretion, plays a role in mucosal defense. This is primarily due to the pH gradient that occurs at this mucus layer to protect the surface epithelial cells. While the luminal acid may have a pH of 2, a slightly alkaline pH of 7.3 has been reported at the cell membrane.<sup>29</sup> The ability of the mucosa to maintain an alkaline zone adjacent to the epithelium is severely compromised when the luminal pH decreases to 1.5<sup>29</sup> or 1.8.<sup>32,36</sup>

The cytoprotective mucosal barrier is also dependent on normal gastric mucosal blood flow. It has been shown that normal gastric circulation will protect surface epithelium from noxious stimuli, whereas under the same conditions, reduced mucosal blood supply will not.<sup>29</sup> Exposure of the gastric epithelium to various irritants will result in an increased blood flow (known as protective hyperemia), which will provide substances, such as plasma bicarbonate, as well as remove products, such as back diffused hydrogen ions.<sup>36</sup> Therefore, adequate blood flow is necessary for the mucosa to resist challenges. It is thought that blood flow protects the gastric mucosa by also providing adequate oxygen, nutrients, and energy sources which can then allow more rapid elimination and buffering of back diffused hydrogen ions.<sup>29,36</sup> Prostaglandins

of the E and I class, as well as increases in acid secretion have been found to enhance mucosal blood flow, whereas agents such as NSAIDs will inhibit mucosal blood flow.<sup>29,32</sup>

Another important factor regarding mucosal blood flow is the maintenance of the intramucosal acid-base neutrality. When the luminal pH is greater than 2, the secretion of mucus and bicarbonate will form the alkaline mucus layer that can buffer the acid back diffusion, with any remaining hydrogen ions that gain entry into the tissue being buffered by the bicarbonate in the blood. Acid back diffusion occurs when there is damage to the surface cells, and acid diffuses into the submucosa.<sup>3</sup> This causes mucosal damage. Therefore, if blood supply is limited, there will be an accumulation of hydrogen ions within the gastric mucosa, which can lead to cell damage and tissue necrosis.<sup>36</sup> Back diffusion of hydrogen ions occurs because of increased permeability, secondary to an irritant, which can stimulate mucosal blood flow.<sup>29</sup> When systemic acidosis occurs with a blood pH of less than 7.0, gastric ulcers can develop because there is not enough bicarbonate in circulation to neutralize the back diffusing hydrogen ions, even if the luminal pH is 3.5.<sup>31</sup>

The third mechanism responsible for protecting the gastric epithelium against injury is its ability to proliferate rapidly. The gastric epithelium is a rapidly proliferating tissue in the body only second to the bone marrow.<sup>29</sup> The surface epithelial cells are renewed every three days.<sup>29,31</sup> Epithelial restitution is the process by which surface epithelial cells are rapidly repaired and can take as little as thirty minutes to occur.<sup>31</sup> Epithelial cells that are either adjacent to or below damaged cells will migrate over the basement membrane to cover the defect. A luminal pH of 3.0 or less will inhibit restitution from occurring, unless a high bicarbonate level is accessible on the basal side of the cell. The damaged mucosa will release increased levels of bicarbonate into the lumen to assist restitution.<sup>31</sup>

If the microvascular supply of the mucosa is intact after injury to the surface epithelium, there is cell migration from the gastric pits along the denuded epithelium, and within a few minutes (in rats, slower in humans) a new surface epithelium is formed.<sup>37</sup> A 'mucoïd cap', consisting of gelatinous layer of mucus and proteinaceous exudate, is formed as a protective covering so that restitution can occur.

The gastric surface also has a very high capacity for repelling acid because of its hydrophobic property. The 'mucoïd cap' not only coats the epithelium but protects it from the cytotoxic effects of the luminal acid.<sup>35,38,39</sup> It has been suggested that the hydrophobicity of the mucus gel layer in the stomach is the primary barrier to damage induced by acid.<sup>1</sup>

Besides prostaglandins, growth factors such as epidermal growth factor (EGF), are present in the gastric mucosa and are thought to be protective.<sup>2,38</sup> The major endogenous source of EGF are the salivary glands, and excision of these will decrease cytoprotection and augment development of gastric lesions.<sup>33,34,38,40</sup> It has been determined that areas of mucosal ulceration have an increased concentration of endogenous EGF.<sup>38</sup> EGF in the stomach is thought to come primarily from the swallowed saliva produced by the salivary glands, whereas

EGF in the duodenum is thought to be produced locally and is not affected by salivary gland removal.<sup>38</sup>

EGF is important in the maintenance and repair of the gastric mucosa, and has been shown to speed repair of experimental ulcers.<sup>31,35</sup> When EGF was given parenterally or orally, it enhanced ulcer healing in rats with intact salivary glands and increased the rate of healing in sialoadenectomized rats.<sup>38</sup>

Nitric oxide (NO), also known as Endothelium Derived Relaxing Factor, has several protective actions in the stomach similar to prostaglandins. As previously discussed, NO is thought to be favorable when produced in small amounts by the constitutive NOS, whereas larger quantities produced by the iNOS can be cytotoxic and cause pathological consequences. Both prostaglandins and NO are capable of enhancing mucosal blood flow, mucus production, and repair of the mucosal epithelium.<sup>39,41,42</sup> NO, released by endothelial cells, causes relaxation of vascular smooth muscles in large vessels.<sup>41,42</sup> Although NO appears to play a protective role in gastric microcirculation, it may also be involved in pathologic events in the gastric mucosa. NO has the ability to reduce the adherence of neutrophils to vascular endothelium<sup>39</sup>, which appears to be an important event in the pathogenesis of NSAID induced gastropathy. This will be discussed in more detail later in this manuscript.

### **Duodenal Mucosal Defense**

The duodenum mucosal defense is similar to that of the stomach. The duodenum of the dog has been shown to produce and secrete a bicarbonate rich fluid.<sup>29</sup> The proximal duodenum secretes twice as much bicarbonate as the distal duodenum.<sup>29</sup> The duodenum also has a clearly defined mucus layer, adherent to the mucosa. Similar to the stomach, mucus and bicarbonate secretion are stimulated by luminal acid and prostaglandins.<sup>29</sup> Prostaglandins of the E, F, and I types, providing a similar protective mechanism as in the stomach, are found within the duodenal mucosa as well.<sup>29</sup>

### **NSAID Gastroduodenopathy**

Two mechanisms by which NSAIDs damage the gastroduodenal mucosa include an acute local effect that causes topical irritation to the epithelium, and NSAIDs ability to decrease prostaglandin synthesis.<sup>1,39</sup> Because most NSAIDs are weak acids (aspirin), they have the ability to accumulate in the gastric epithelial cells due to ion trapping.<sup>39</sup> At a low pH, NSAIDs become lipid soluble, diffuse into the gastric epithelial cells, and then can not escape. This causes damage to the surface epithelial cells, causing a breakdown in the mucosal barrier, and allowing hydrogen ions and acid to back diffuse the mucosa, leading to injury.<sup>1</sup> The damage produced by topical irritation from NSAIDs is not the major contributory factor to severe mucosal injury. In fact, when NSAIDs have been formulated with slow release enteric coatings, the incidence of ulceration was comparable to standard NSAIDs.<sup>39</sup>

It is thought that the major contribution of NSAID induced gastrointestinal injury is due to its inhibition of prostaglandin synthesis. Many of the normal protective mechanisms of the gastric mucosa are prostaglandin dependent. Systemic inhibition of prostaglandins will decrease mucus production, decrease bicarbonate secretion, and decrease mucosal blood flow. NSAIDs will also inhibit platelet aggregation through the inhibition of thromboxane synthesis.<sup>1,39</sup> This may increase preexisting gastrointestinal bleeding or exacerbate bleeding of NSAID induced ulceration. However, it has been suggested that prostaglandin inhibition alone is insufficient to account for all mucosal injury. NSAIDs can also affect gastric mucosal defenses directly.

The most prominent effect of NSAIDs is to make the gastric mucosa more susceptible to the damaging effects of the acid in the lumen. When a sufficient amount of unbuffered acid diffuses from the lumen into the mucosa, necrosis occurs. NSAIDs will decrease bicarbonate production and inhibit mucus secretion,<sup>43</sup> thereby decreasing natural defenses.

NSAIDs will cause a decrease in gastric mucosal blood flow<sup>37</sup>, and recently it has been discovered that NSAIDs damage the vascular endothelium very early after administration.<sup>1,39</sup> Neutrophils have been reported to play a critical role as mediators of this endothelial damage.<sup>39,44-47</sup> The number of neutrophils adhering to the vascular endothelium in the gastric and mesenteric microcirculation is increased following NSAID administration (mechanism unclear). This is often observed within thirty minutes of NSAID administration, similar timing to the inhibition of prostaglandin synthesis by the same drugs.<sup>39,48</sup> It is thought that NSAID administration results in upregulation of ICAM-1, an endothelial adhesion molecule in the gastric microcirculation, thereby contributing to the adhesion of neutrophils.<sup>49</sup>

In one study, the severity of NSAID (naproxen and indomethacin) gastropathy was reduced in rats rendered neutropenic by either anti-neutrophil serum or methotrexate.<sup>46</sup> A similar study using aspirin yielded the same results and it was concluded that gastric mucosal injury is a neutrophil dependent process.<sup>45</sup> In another study, gastric mucosal prostaglandins were found to be deficient in neutropenic rats after aspirin administration, and yet no gastric lesions developed.<sup>45</sup> This suggests that a deficiency in mucosal prostaglandins alone is not sufficient to cause mucosal damage. It has also been shown that administering prostaglandins at doses known to prevent gastric injury will prevent the leukocyte adherence to vascular endothelium.<sup>39</sup> Prostaglandins of the E and I series have both been shown to inhibit neutrophil adherence and chemotaxis in response to various agents.<sup>44</sup> Prostacyclin (PGI<sub>2</sub>), derived from the endothelium, may modulate neutrophil function and when inhibited by NSAIDs, may contribute to neutrophil adherence.<sup>37</sup>

The neutrophil mediated mucosal injury is thought to be due to several processes. There may be direct plugging of the microvasculature by aggregates of neutrophils resulting in hypoperfusion or there may be vascular damage mediated by the leukocyte adhesion.<sup>45</sup> Inflammatory mediators, such as proteases and oxygen-derived free radicals, may be released by activated neutrophils and cause direct damage to the endothelium.<sup>44,46</sup> Neutrophils may also produce focal ischemia.<sup>46</sup> Prostaglandins have been shown to inhibit neutrophil activation,<sup>46</sup>

therefore NSAIDs while inhibiting prostaglandins may actually activate the circulating neutrophils. However, all this work has been done in vitro and whether neutrophils contribute to NSAID induced injury in humans or animals is still unknown. Interestingly, one study in humans noted that an increased white blood cell count was a risk factor for the development of ulcers.<sup>39</sup>

### **Gastric Adaptation**

It is well documented that aspirin induced lesions begin to heal spontaneously despite aspirin administration. This is called gastric adaptation; the process by which visible gastric mucosal lesions lessen, and may resolve despite continued administration of an injury causing substance.<sup>50</sup> The gastrointestinal tract is constantly exposed to various noxious substances, including acid secretion, and constantly adapts to survive.

In one study of human patients receiving aspirin, submucosal hemorrhages and/or focal erosions were present within 24 hours of administration. The mucosal injury was maximal at 3 days and then lessened by 7 days.<sup>51</sup> Resolution of the mucosal injuries was longer following one day of aspirin administration than after 7 days of aspirin administration.<sup>51</sup> Submucosal hemorrhages were produced within only two hours of aspirin administration.<sup>51</sup> Similar results have been found in dogs receiving aspirin and cinchophen; gastric lesions, including erosions, returned to normal mucosa within 5-12 days despite continued aspirin administration.<sup>51,52</sup> In another study of human patients, etodolac caused less mucosal damage than naproxen, with maximum damage occurring within 24 hours in both groups.<sup>53</sup> Adaptation occurred in both groups with resolution beginning at 7 days and being complete by 28 days.<sup>53</sup> Gastric adaptation has also been shown to enhance mucosal resistance to further injury induced by more noxious agents.<sup>54</sup>

The mechanism of gastric adaptation is unknown. It is thought that it is direct contact of the drug with the gastric mucosa that is responsible for this phenomenon, rather than NSAIDs systemic effects. One study suggests that if antacids are provided, adaptation will not occur.<sup>37</sup> Mucosal adaptation is thought to occur in two phases: an initial stabilization phase (injury stops) followed by a resolution phase (lesions resolve).<sup>50</sup> The first phase occurs rapidly with lesions demonstrable within two hours of aspirin administration. During this time, the gastric mucosa becomes infiltrated with neutrophils adherent to endothelium. As the stomach develops tolerance to aspirin, there is a decrease in neutrophil-endothelial adherence and a rise in epithelial cell proliferation, suggesting that the activation of neutrophils plays a role in development of lesions as well as adaptation.<sup>37</sup>

Gastric adaptation has been shown to be independent of prostaglandin synthesis.<sup>55</sup> The effects of mucosal blood flow, although thought to be decreased by NSAIDs, is somewhat controversial regarding its importance in mucosal protection; some studies report a decrease while others report an increase.<sup>56</sup> It is also possible that widespread initial injury caused by NSAIDs may increase the ability of the mucosa to tolerate further insult without serious

injury.<sup>37</sup> In summary, gastric mucosal blood flow, neutrophil activation, and growth factors all may play a role in gastric adaptation, but no clear mechanism has been elucidated.

### **NSAIDs in the Colon**

NSAIDs are frequently associated with injury to the gastroduodenal portions of the GI tract, but rarely with the colon. There are a number of clinical and experimental observations that suggest that the side effects of NSAIDs on the gastrointestinal tract are selective and site specific for the gastroduodenal mucosa. However, there have been many case reports in the human literature supporting an association between NSAIDs and colonic ulceration, colonic stricture formation,<sup>57</sup> and colonic perforation. Drug formulations, such as enteric coating and buffered products, may result in a reduction of upper gastrointestinal clinical signs, however, there may be an increase in the number of distal intestinal manifestations. There are thought to be four toxicologic manifestations associated with NSAIDs in the colon: *de novo* toxicity, hypersensitivity, suppository induced, and reactivation of bowel disease.<sup>58</sup>

*De novo* toxicity, or disease attributed to NSAID use in an otherwise normal colon, has been described in the literature since 1966 in almost 200 cases in humans.<sup>58</sup> Many types of NSAIDs have been implicated. In one report, fifteen humans with signs of colitis were found to have been taking NSAIDs for less than 4 weeks.<sup>58</sup> When the NSAIDs were discontinued, rapid improvement in clinical signs occurred without any other therapy.<sup>58</sup> Case reports of benign colonic ulcers, primarily seen in the cecum and ascending colon, have been reported with diclofenac, indomethacin, aspirin, ibuprofen, and naproxen.<sup>59</sup> A higher number of cases appear to be caused by mefenamic and flufenamic acid.<sup>60</sup> Clinical signs generally include diarrhea, hematochezia, and weight loss.<sup>60</sup> Generally, endoscopy is normal, but both inflammation or ulceration have been reported. Similarly, histologic changes include nonspecific mild colitis or an ulcerative colitis in more severe cases.<sup>60</sup> Diarrhea usually ceases within days of discontinuation of the drug.<sup>60</sup>

There have been several cases of acute colitis associated with salicylate sensitivity.<sup>58</sup> Patients in whom this occurred had a history of aspirin hypersensitivity. Colitis was resolved after the drug was discontinued.

It has been estimated that 10-30% of human patients that use NSAID suppositories have side effects, and usually this is dose dependent.<sup>58,60</sup> There are cases of proctitis, as well as strictures and ulcerations, occurring after suppository use. Suppositories are thought to produce high rectal concentrations of the drug, which may induce colonic damage.<sup>58</sup> In one study of 43 human patients taking 100 mg indomethacin suppositories, 49% stopped treatment due to inability to retain the suppository, discomfort, irritation, pain, or bleeding.<sup>60</sup>

There have been over one hundred cases of patients with reactivation of bowel disease attributable to NSAID use.<sup>58</sup> Many types and classes of NSAIDs were accountable. Four patients with quiescent inflammatory bowel disease had rapid exacerbations when they were

given NSAIDs.<sup>61</sup> In a controlled prospective study of 92 human patients receiving a variety of NSAIDs, it was found that 31 patients admitted with complications of diverticular disease were receiving NSAIDs.<sup>60</sup> This was significantly higher than the control population.<sup>60</sup> Nineteen of the 31 patients presented with perforation, whereas only 8 of the 61 patients not receiving NSAIDs presented this way.<sup>60</sup>

A retrospective study of 268 cases of colonic or small bowel perforations, revealed a threefold higher rate of large intestinal perforation compared with small intestinal perforation in patients taking NSAIDs.<sup>62,63</sup> In another study, NSAID use was strongly associated with an increased risk of both upper and lower GI perforation, with no predilection for site.<sup>64</sup> In a prospective study, it was found that patients taking NSAIDs were five times more likely to develop colonic inflammation than the general population.<sup>65</sup> Ten percent of patients with newly diagnosed colonic inflammation may be related to NSAID administration.<sup>65</sup>

The mechanism of injury in the large intestine is not completely known. It is speculated that increased intestinal permeability may allow entrance of toxins, bacteria, and exogenous antigens into the mucosa, which then causes inflammation.<sup>58,59,66</sup> Impaired prostaglandin synthesis has also been speculated. Prostaglandins, including PGE<sub>2</sub>, are synthesized by colonic tissue, and are thought to act primarily as cytoprotective agents rather than proinflammatory agents.<sup>66</sup> Inhibition of prostaglandins by NSAIDs may act to initiate or aggravate intestinal inflammation.

There is also reported damage to the small intestine in rats and humans as well. The pathogenesis of NSAID induced small intestinal damage is unclear and poorly understood. It has been proposed that the suppression of prostaglandin synthesis by NSAIDs is not a major component to the injury induced.<sup>49,67,68</sup> There is temporal support that the intestinal damage occurs many hours after effects on prostaglandin inhibition have subsided.<sup>49</sup> The primary event in the pathogenesis of small intestinal damage may be an increase in intestinal epithelial permeability.<sup>49</sup> The increase in permeability allows the diffusion of bacteria, toxins, and antigens into the lamina propria, thereby inducing an inflammatory reaction.<sup>49</sup> The role of neutrophils is also thought to be less critical role in the intestine than in the stomach.<sup>49</sup>

It has also been suggested that because most NSAIDs have extensive enterohepatic circulation, the intestinal epithelium is continually exposed to a bile-NSAID mixture which may be toxic to the intestinal epithelial cells.<sup>49,68</sup> It has been found that when the bile duct of a rat is ligated, NSAID induced small intestinal injury does not occur.<sup>49</sup> This supports that bile is critical for injury to occur. Aspirin, which does not undergo enterohepatic circulation, does not cause intestinal injury.<sup>49</sup> In a recent study, the drug nabumetone, which does not undergo enterohepatic circulation, did not produce significant intestinal damage.<sup>49</sup> A study in rats using diclofenac, which undergoes enterohepatic circulation, and nitrofenac, which does not undergo enterohepatic circulation, found that nitrofenac produced less intestinal damage than diclofenac.<sup>69</sup>

It is thought that there may be an important role for enteric bacteria in NSAID induced intestinal injury.<sup>49,68</sup> Various antibiotics, when given concurrently with NSAIDs, have been shown to decrease the severity of the intestinal injury as well as decrease the permeability changes.<sup>68</sup> Studies have shown that luminal bacteria counts are markedly elevated in rats that have been given NSAIDs.<sup>49</sup> Currently, it is not clear if the bacteria counts are increased prior to injury or as a consequence of injury.

### ***Helicobacter***

*Helicobacter pylori* infection, in people, is associated with a wide variety of clinical entities ranging from asymptomatic chronic gastritis to gastric carcinomas.<sup>70,71</sup> *Helicobacter* species are thought to naturally inhabit the stomach of many mammalian species including man,<sup>72</sup> dogs,<sup>73,74</sup> cats,<sup>74,75</sup> cheetahs,<sup>76</sup> ferrets,<sup>76</sup> and non-human primates.<sup>76</sup> *Helicobacter* spp. infections have been found to infect between 20-90% of people, with the majority of these cases being subclinical.<sup>72,76</sup> *Helicobacter pylori* has been found to infect 90-100% of human patients with duodenal ulcers and 70-90% of patients with gastric ulcers.<sup>77</sup>

The reported prevalence of *Helicobacter* spp. in healthy animals is high. In one report, 100% of healthy laboratory and shelter dogs and 67% of pet dogs (with non gastric diseases) were colonized with gastric spiral bacteria similar to *H. felis* or *H. heilmannii*.<sup>78</sup> Other studies report 82% of dogs (100/122) and 76% of cats (96/127)<sup>79</sup> and 100% of dogs (10/10) and 60% of cats (6/10)<sup>74</sup> were infected with *Helicobacter* -like organisms. In a post mortem study of 55 random source cats, *Helicobacter*-like organisms were identified in 70% of juvenile cats and 97% of adult cats.<sup>72,76</sup>

Spiral bacteria (originally *Campylobacter*) were first recognized in the stomach of dogs in 1889, 50 years before they were reported in humans in 1939.<sup>76</sup> It was not until 1983 that an association between the spiral bacteria and clinical disease was reported in human medicine. *Helicobacter* organisms are gram negative, microaerophilic bacteria with flagellae. They have urease activity and are motile in the viscous gastric mucus. At least thirteen species of *Helicobacter* have been identified in animals, however not all the species are pathogenic.<sup>76</sup> The two most common species in dogs and cats are *Helicobacter felis* and *Helicobacter heilmannii*.<sup>74,76,78,79</sup> *Helicobacter pylori*, the most common organism in humans, is rarely found to inhabit domestic animals.<sup>70,78</sup> In 1994, one study documented isolation of *H. pylori* from domestic cats, suggesting possible zoonotic potential.<sup>71</sup>

*Helicobacter* spp. have the unique ability to survive in the highly acidic environment of the stomach and several mechanisms have been proposed. They are generally found in the mucus layer overlying the mucosa in the stomach. Urease production is a very important factor that enables the bacterium to live in the upper gastrointestinal tract. The urease enzyme is intimately associated with the cell wall within the organism and is responsible for hydrolyzing urea, which is present in gastric juice, to ammonium and bicarbonate.<sup>80</sup> It was presumed that the organism

was able to hydrolyze enough urea in its immediate environment ( perhaps intracellularly ) to neutralize the hydrogen ions that were penetrating its cell wall.<sup>80</sup> It is suggested that it is the urease activity that allows *Helicobacter* to colonize the stomach environment by producing a protective “alkaline cloud”.<sup>81</sup> Many of the *Helicobacter*-like organisms that are found in domestic animals are ultrastructurally and antigenically similar to *H. pylori*, the organism which has been studied extensively.<sup>80</sup> In one study, it was found that at a pH <2, *H. pylori* was able to generate ammonium in order to maintain itself in the environment.<sup>80</sup>

The ammonia that is initially produced from the urease enzyme, before its transformation to ammonium, may be partly responsible for damage to the gastric mucosa. In epithelial cells, ammonia can cause histologic changes and vacuolization.<sup>76</sup> This injures the gastric mucosal barrier, which potentiates erosion and ulceration. In addition, disruption to the mucus layer can occur with the *Helicobacter spp.* organisms. When the bacteria attach to the mucus producing cells lining the stomach, they can decrease the release of mucus into the lumen.<sup>76</sup>

A second adaptation that may help *Helicobacter spp.* survive in its environment is its ability to regulate gastric acid secretion. Decreased gastric acid output, or hypochlorhydria, has been observed in the early stages of infection with *Helicobacter spp.*<sup>76,81</sup> There is often a superficial gastritis seen along with the hypochlorhydria. The mechanism by which the bacterium causes this change is unknown, however, it is hypothesized that the parietal cell function is directly affected.<sup>81</sup> The decreased gastric acid output may be partly responsible for facilitating the early colonization of *Helicobacter* organisms within the stomach.

Another mechanism that enables *Helicobacter spp.* to survive in its environment is its flagellae, which allows it to move through the viscous mucus within the stomach. The flagellae facilitate movement of the organism through the gastric mucosal pH gradient that ranges from a pH of 2 on the luminal side to a neutral pH closer to the epithelial surface. The movement produced by the flagellae prevent clearance by the normal peristaltic waves in the stomach and thereby allow adherence to the mucosal epithelial cell.<sup>81</sup> However, the organism must use some of the aforementioned defenses in order to survive the acidic mucus, initially.

*Helicobacter spp.* may produce cytotoxins that directly affect the gastric epithelial cells and cause an inflammatory reaction.<sup>76</sup> Approximately 50% of *H. pylori* strains can express cytotoxic activity in vitro with some infected humans are able to produce IgG specific for this cytotoxin.<sup>76</sup> *H. pylori* that have the cytotoxic activity have a certain gene (vac A) which has been identified and is termed type I; human patients with duodenal ulcerations are always infected with type I bacteria.<sup>76</sup> Bacteria without this gene are termed type II *H. pylori*.

Humans infected with *H. pylori* have been found to have increased basal secretions of serum gastrin levels as well as increased post prandial gastrin levels.<sup>81</sup> The etiology of the hypergastrinemia is unclear, but it has been hypothesized that the ammonia produced as a result of urea breakdown by urease may produce an alkaline environment in the region of the G cells, and therefore stimulate gastrin secretion.<sup>76,81</sup> A second hypothesis suggests that because

gastrin is inhibited by somatostatin, perhaps somatostatin levels are decreased. In several studies, antral mucosal somatostatin concentrations and the number of D cells (responsible for producing somatostatin) were decreased in human patients with *H. pylori* infection.<sup>76,81,82</sup> Because hypergastrinemia will increase the normal output of gastric acid up to three times in *H. pylori* infected humans,<sup>76</sup> it is conceivable that ulceration could occur due to an increased acid load in the stomach and the duodenum. Decreased gastrin levels and increased numbers of D cells can be found after eradication of the bacterium.<sup>76,81</sup>

In humans, *H. pylori* is classified as a carcinogen and has been associated with gastric adenocarcinoma and B-cell lymphoma.<sup>76</sup> Infection with the bacterium may quadruple the risk of developing gastric neoplasia.<sup>76</sup> Thirty one per cent-52% of gastric cancers may be attributable to *H. pylori*.<sup>83</sup> It is unknown how the bacteria induces the change, but it is thought that metaplasia of the epithelial cells may occur directly due to the presence of the organism or that the mucosa is more susceptible to carcinogens because it has been altered by the bacteria.<sup>76</sup> Another theory suggests that ammonia promotes cell division, increasing the risk of mutation.<sup>83</sup> Ascorbic acid, an important antioxidant responsible for scavenging oxygen free radicals, has been found to be decreased in the gastric fluid of people with *H. pylori* infection, and this may increase the risk for cancer as well.<sup>83</sup>

Diagnosis and treatment of *H. pylori* in people has been extensively investigated because of the risks associated with the development of peptic ulcer disease and cancer. Many infected animals and humans are asymptomatic. Clinical signs in dogs and cats thought to be associated with *Helicobacter spp.* include chronic intermittent vomiting and less frequently inappetence, pica, weight loss, and fever may be seen. The clinical significance of infection in dogs and cats is unclear. In humans, post prandial discomfort and heartburn are frequently attributed to *Helicobacter spp.*

The diagnosis of *Helicobacter spp.* can include invasive and noninvasive tests. Invasive tests use endoscopically obtained biopsy specimens and include histopathological examination, rapid urease tests, and culture.<sup>76</sup> Organisms can be visualized in the gastric tissue under microscopic evaluation; special stains such as the Warthin Starry silver stain can be used to enhance visualization of the organisms.<sup>76,84</sup> This test has high sensitivity (91-99%)<sup>84-86</sup> and specificity (95-100%)<sup>84-86</sup> in humans, but false negatives are possible because of a patchy distribution of the organism in the stomach. It is recommended that two specimens from each region in the stomach (antrum, cardia, angularis incisura, and fundus) be evaluated.<sup>76</sup> This technique also allows determination of the extent of the inflammatory changes.

The staining techniques needed to visualize the spiral organisms have been studied. Hematoxylin and Eosin (H & E) was studied against the Warthin Starry silver impregnation technique, and in only one instance (of ten cases), did H&E staining fail to identify the organism.<sup>87</sup> A modified Diff-Quik stain can be used to demonstrate the organism as well.<sup>88</sup>

Rapid urease testing can be performed with an endoscopically obtained biopsy sample. Because the organism is a potent producer of urease, the sample is placed in a broth containing urea and a pH indicator. A color change is seen as the urease breaks down the urea into ammonia and raises the pH. The CLOtest® is one example of a rapid urease test and was used in this study. The sensitivity of the test in humans has been reported as 70-98%,<sup>76,84-86,89</sup> and the specificity as 93-100%.<sup>84-86,89</sup> Because this test is read within a 24 hour period (ideally at 20 minutes, 1 hour, 3 hours, and 24 hours), it enables a quick diagnosis. By one hour, 80-85% of positive patients<sup>86,90</sup>, and by 3 hours, 90% of positive patients will be detected, by the CLOtest®.<sup>86</sup> False negatives can occur when very low numbers of the organism are detectable or there is a patchy area of distribution of the organism.<sup>86</sup> Therefore, it has been recommended that more than one biopsy specimen be evaluated. The CLOtest® is less sensitive after the patient has received antibiotics or bismuth, because the organism may be killed or be present in smaller numbers. False positives are very rare and can occur when gastric acid is absent (patients taking H2 antagonists or proton pump inhibitors), because commensal organisms such as *Proteus sp.* can grow in the stomach and produce the urease enzyme.<sup>86</sup>

Although the company that produces the CLOtest® recommends that the test be incubated in a warm place (30-40°C), one study investigated the difference between samples kept at 37° C versus room temperature (22-24° C). The study concluded that incubation at 37° C hastened the time to a positive test result, but the time saved was less than an hour.<sup>91</sup> Therefore, room temperature is adequate. It has also been concluded that biopsy size is not critical; a small biopsy specimen will have the same diagnostic yield for detecting *Helicobacter spp.* by rapid urease test as a large biopsy sample.<sup>92</sup> However, doubling the amount of gastric tissue in the CLOtest will hasten the development of a positive test by 1.5-2 hours.<sup>93</sup>

Culture of the *Helicobacter spp.* organism is the least sensitive of the above techniques. It has a sensitivity of 70%-77%<sup>84,86</sup> and a specificity of 100% in humans<sup>84,86</sup> Many *Helicobacter spp.*, such as *H. heilmannii*, have not yet been successfully cultured.<sup>76</sup>

Brush cytology is an easy and quick diagnostic tool for the detection of the *Helicobacter spp.* Brush cytology was the most sensitive method for detecting spiral organisms, with a sensitivity of 100%, in a limited study of dogs and cats.<sup>87</sup> Brush cytology has the advantage that a large area or multiple areas of the stomach can be tested. When a rapid staining agent, such as the Romanovsky type stain, is used it allows for a very rapid result.

Non-invasive tests, such as serology and urea breath tests, have not been used clinically in the veterinary species as of yet, but are currently being investigated. These tests are generally used in human patients that are asymptomatic or as a method to monitor response to therapy. Serologic testing for the IgG antibodies to *Helicobacter spp.* organisms has a high sensitivity (88-99%) and specificity (86-95%) in humans.<sup>76,84</sup> It is commonly used as an initial screening test. However, it has very limited usefulness in monitoring response to therapy since titers often do not decrease until 6-12 months after eradication.<sup>76,84,94</sup>

The urea breath test utilizes a meal that has been labeled with a radiolabeled carbon isotope. Urea is metabolized to ammonia and bicarbonate by the organism. The bicarbonate is then excreted as a labeled carbon dioxide in the breath.<sup>76,84</sup> The level of radioactivity is measured. This method has a sensitivity of 90-100% and a specificity of 98-100% in humans and is a very quick, noninvasive test to perform.<sup>84</sup> The urea breath test is often used as a confirmatory test in asymptomatic patients if the serology was positive. This test is also the test of choice to monitor response to therapy or it is used commonly as a screening test for multiple people in a household that may be infected.<sup>94</sup>

Treatment for *Helicobacter spp.* has been thoroughly investigated in human patients because of the severity of clinical disease associated. Once *Helicobacter spp.* infection is eradicated, ulcers recur in less than 2% of human patients.<sup>76</sup> No ideal treatment has been identified, however, it has been determined that monotherapy is ineffective. Combination therapies often include an H<sub>2</sub> receptor blocker, a bismuth based compound, with or without an antibiotic. The most common bismuth triple therapy consists of bismuth, metronidazole, and tetracycline or amoxicillin for two weeks. In veterinary medicine, this triple therapy has been adopted and is generally given for 2-3 weeks. Little information regarding eradication rates is available because of financial constraints of the owner and the need for general anesthesia to perform endoscopy.

*Helicobacter pylori* was identified in a group of research cats<sup>71</sup> and from naturally infected cats.<sup>95</sup> Generally, cats are infected with either *H. felis* or *H. heilmannii*. Therefore, it has been suggested that domestic cats may harbor the organism and transmission to humans is a possibility. An epidemiological study was performed on people who do and do not own cats evaluating *H. pylori* antibodies. It was found that cat owners were at no greater risk of infection than people who do not own cats.<sup>76</sup>

### ***NSAIDs and Helicobacter***

So, how are non-steroidal anti-inflammatory drugs associated with *Helicobacter spp.*, or are they? Because both NSAIDs and *Helicobacter pylori* are considered leading causes of ulcer disease in humans, many studies have been performed to see if the *Helicobacter* organism contributes to the ulcer disease seen with NSAIDs. It has been concluded in one study that NSAID-associated gastric ulcers do not require the *H. pylori* organism to develop.<sup>96</sup> More people that were not NSAID users had *H. pylori* than people with NSAID-associated gastric ulcers.<sup>96</sup> It has also been suggested that NSAID induced damage to the mucosa in the stomach and the duodenum does not increase the susceptibility to *H. pylori* infection.<sup>2</sup> Hemorrhages and erosions associated with NSAIDs, were more frequent in those without *H. pylori* than those with the infection.<sup>2,77,97</sup> It has now been concluded that eradicating the *Helicobacter* organism offers no advantage to healing ulcers due to NSAID administration.<sup>98</sup> In a prospective study to evaluate the development of gastric and duodenal ulceration in humans, *H. pylori* status was determined and subjects were given NSAIDs for a period of three months.<sup>99</sup> The results of this study suggested that *H. pylori* did not confer an increased risk of ulceration when given with

NSAIDs.<sup>99</sup> Another study supports that a history of NSAID use is more common in ulcer patients who have a normal non-infected stomach.<sup>100</sup>

However, the recurrence of bleeding in humans is virtually abolished in patients that receive treatment to eradicate *H. pylori*.<sup>77</sup> In a study that analyzed the literature data (consisting of 27 studies) concerning ulcer relapse rates after cure of *H. pylori* infection, it was found that there was a decreased rate of relapse when the organism had been eradicated.<sup>101</sup>

It still remains unclear, as there is conflicting evidence, whether *H. pylori* is a contributory or independent cause of ulcer disease in humans. It is possible that there are many variables, including bacterial and inflammatory factors, host factors, and hormonal factors that contribute to the development of ulcer disease in humans. In veterinary medicine, the role of *Helicobacter spp.* in animals receiving NSAIDs who develop gastric ulceration is unclear. In fact, the significance of *Helicobacter spp.*, when they are found in dogs, is unclear.

## Aspirin

Humans consume more than 40 billion aspirin tablets worldwide per year and it is the most commonly used over-the-counter NSAID.<sup>1</sup> In 1975, the recommended maintenance dosage for aspirin in the dog was 25-35 mg/kg every eight hours, which is currently the dose used to induce gastrointestinal ulceration in the dog.<sup>102,103</sup> Clinically, the recommended dosage for dogs is 10-25 mg/kg two to three times per day.<sup>104</sup>

Aspirin, or acetylsalicylic acid, is a weak acid with a pKa of 3.5.<sup>104,105</sup> When absorbed, the drug is hydrolyzed to salicylic acid and then widely distributed throughout the body.<sup>104</sup> Salicylate is metabolized in the liver primarily by conjugation by the enzyme, glucuronyl transferase. Cats are deficient in this enzyme, therefore, the half-life is prolonged. High levels of aspirin are maintained in the liver, heart, lungs, renal cortex, and plasma.<sup>104</sup> Salicylate and its metabolites are excreted by the kidneys.

As previously discussed, the mucosal barrier in the normal stomach prevents back diffusion of hydrogen ions from the lumen into the gastric mucosa and the diffusion of sodium from the gastric mucosa into the lumen. Aspirin disrupts the gastric mucosal barrier and increases its permeability, thereby hydrogen ions diffuse into the gastric mucosa.<sup>106</sup> A second mechanism that may cause damage by aspirin to the stomach mucosa is by inhibition of active transport of hydrogen ions into the lumen.<sup>106</sup> In actuality, a combination of the two mechanisms maybe responsible for the damage with an inhibition of ion transports being followed by an increase in permeability.<sup>106</sup>

A study to compare the effects of plain, buffered, and enteric coated aspirin on the gastric mucosa, as well as to determine serum salicylate levels was performed.<sup>107</sup> It was found that only dogs receiving plain aspirin at 25 mg/kg every eight hours developed gastric lesions which consisted of mild petechiations to linear hemorrhages, primarily in the fundus and antrum.<sup>107</sup> In

order to determine whether this finding was due to the local effects or different systemic levels of drug, serum aspirin levels were determined for each group. However, plain, enteric, and buffered aspirin administered at 25 mg/kg every eight hours produced and maintained therapeutic levels.<sup>107</sup> The dogs receiving the enteric coated aspirin at 25 mg/kg every eight hours had the most fluctuations in serum levels. Enteric formulations are designed to resist dissolution in gastric fluids and dissolve readily in intestinal fluids. Because of unreliable dissolution, unpredictable and inconsistent absorption often occurs. In a human study, users of enteric coated and buffered aspirin were at three times more risk to experience a gastrointestinal bleeding episode.<sup>108</sup>

Aspirin is a potent NSAID, known to cause severe gastroduodenal ulceration and perforation. It has a COX-2: COX-1 ratio of approximately 166<sup>14</sup>, meaning that it inhibits the COX-1 enzyme 166 more times than it does the COX-2 enzyme. It is used as a model for gastrointestinal ulceration and to provide a comparison for GI toxicity.<sup>103,109</sup> A study of dogs comparing ketoprofen, indomethacin, prednisone with cinchophen, aspirin (15 mg/kg PO BID), and placebo, found that dogs receiving aspirin has significantly more severe gastric lesions than all other groups.<sup>103,109</sup>

### **Carprofen**

Carprofen is a member of the propionic class of NSAIDs that include ibuprofen, naproxen, and ketoprofen, and is a reversible inhibitor of cyclooxygenase. In standard animal models, carprofen is approximately 35 times less likely to cause gastric ulceration than aspirin.<sup>110</sup> In other animal models, the potency of carprofen as both an antiinflammatory agent and an analgesic is comparable to indomethacin and is greater than aspirin and phenylbutazone.<sup>110</sup> Carprofen is 90% absorbed after oral administration with peak plasma concentrations at 1-3 hours in dogs.<sup>110,111</sup> The mean half life for elimination in dogs is approximately 8 hours (range, 4.5-9.8 hours) after single oral administration.<sup>110,111</sup> Signs most frequently reported as possible adverse reactions to carprofen are vomiting, diarrhea, lethargy, and increased or decreased appetite.<sup>110</sup> No effect on pregnant dogs has been established.<sup>111</sup>

Carprofen is eliminated in dogs primarily by hepatic biotransformation, followed by excretion of the metabolites in the feces (70-80%) and urine (10-20%). Some enterohepatic circulation may also occur.<sup>110,111</sup> The COX-2/COX-1 ratio is favorably low at 1.0.<sup>14</sup>

In 1974, Baruth and Randall found that carprofen (carbazole,C5720) had the same anti-inflammatory activity as indomethacin and was more potent than phenylbutazone and aspirin.<sup>112</sup> Carprofen, in the same study, was also found to be 15 times less ulcerogenic than indomethacin. Strub, in a series of laboratory tests, showed that with regard to anti-inflammatory properties, carprofen was more potent or equipotent to indomethacin and diclofenac sodium.<sup>113</sup> With regards to analgesia, carprofen induced rapid and long lasting pain relief which was equipotent to diclofenac sodium and three times the normal dosage of phenylbutazone. However, the anti-

inflammatory and analgesic dosages of carprofen did not affect prostaglandin synthetase (cyclooxygenase). Strub reported in 1982 that carprofen was a weak inhibitor of cyclooxygenase.<sup>113</sup> In 1983, Hope found that a portion of the anti-inflammatory activity of carprofen may be due to its ability to inhibit phospholipase A<sub>2</sub>. In further support, McKellar concluded that the principal mode of action of carprofen must be by mechanisms other than cyclooxygenase or 12-lipoxygenase inhibition, and are possibly due to the inhibition of phospholipase A<sub>2</sub>.<sup>114</sup> A recent study in 1998 that evaluated the activity of carprofen and other NSAIDs against isoenzymes COX-1 and COX-2 concluded that carprofen was a potent inhibitor of COX-2.<sup>10</sup>

Several studies have been performed looking at the therapeutic efficacy of carprofen. Holsinger compared the acute relief of carprofen with that of a placebo in a double blinded randomized clinical study.<sup>115</sup> Two hundred and nine dogs (mixed breed, gender, and weight) were included in the study. They were documented to have both radiographic and clinical signs of degenerative joint disease. Dogs were given carprofen at 2.2 mg/kg, orally, twice daily for 14 days. The dogs were evaluated on multiple days throughout the study by both the clinical investigator (veterinarian) and the owner for a positive (improvement in lameness) or negative response (no improvement in lameness) to the medication. It was found that dogs treated with carprofen were 24.8 times more likely to receive a positive evaluation by the veterinarian than dogs that were treated with the placebo. When evaluated by the owners, dogs were 13.4 times more likely to receive a positive evaluation than dogs treated with the placebo. Seventy seven dogs (79%) that received carprofen received a positive evaluation by both the owner and the veterinarian. However, this was a very subjective study, with no kinetic evaluation or use of force plate analysis. Adverse side effects were noted in 6 dogs receiving carprofen (decreased appetite, 2; vomiting, 3; vomiting with diarrhea, 1). Dogs receiving the placebo also experienced adverse effects (decreased appetite, 3; anorexia/lethargy, 1; vomiting, 3; vomiting with diarrhea, 1; diarrhea alone, 4).

Eleven (11.3%) of the 97 dogs treated with carprofen showed elevations of alanine aminotransferase (ALT). Minor transient elevations of liver enzymes have been reported in several published trials of carprofen.<sup>115,116</sup> Pfizer released a statement in July 1997 reporting that approximately 750 reports of side effects of any kind had occurred in the first six months of marketing. An acute hepatopathy, characterized histologically by hepatic necrosis, was diagnosed in 21 dogs (primarily labrador retrievers) that were receiving carprofen.<sup>11</sup> Most dogs had resolution of clinical signs after discontinuation of the drug. Acute liver injury has been associated with NSAID use in humans with the incidence being 3.7 per 100,000 NSAID users.<sup>117</sup>

In 1995, Vasseur performed a multicenter, controlled, randomized trial to compare the efficacy of carprofen against a placebo, which included a subjective analysis from the veterinarian and the owner and a force plate analysis.<sup>118</sup> Dogs were included in the study if clinical lameness and radiographic evidence of degenerative joint disease were present. Dogs were given carprofen 2.2 mg/kg, orally, twice daily, for 14 days or a placebo. According to the owner, dogs receiving carprofen were 4.2 times more likely to have a positive response than the placebo group. According to the veterinarians, the odds ratio was 3.5. On the basis of force plate

analysis, 29 of 36 dogs received a positive evaluation with an odds ratio of 3.3. One dog that received carprofen had an elevation in ALT post treatment. Five dogs in each group had results of a pretreatment fecal occult blood that were negative turn positive. Six of the 36 dogs that received carprofen had adverse effects (vomiting, 2; one each: diarrhea, constipation, eating grass, and personality change). However, four dogs receiving the placebo also experienced adverse effects including diarrhea (3 dogs) and aggression (1 dog).

It was the conclusion in both these studies that most dogs that received carprofen had a positive response and that carprofen had a low prevalence of adverse effects. Six to 16% of cases in the above studies had clinical signs that may have been associated an adverse drug reaction, however, of dogs that received the placebo in either study, adverse effects were seen in 10-12%. These studies suggest that short term use of carprofen (i.e.  $\leq 2$  weeks), has minimal or no harmful side effects on the gastrointestinal tract in dogs.

A study published in 1998 tested the administration of carprofen preoperatively and postoperatively for the prevention of pain in dogs undergoing ovariohysterectomy.<sup>119</sup> The dogs given carprofen preoperatively had lower pain scores than the postoperative or placebo groups. It was concluded that carprofen provided effective analgesia after ovariohysterectomy, and preoperative administration of carprofen had greater analgesic effect than postoperative administration.<sup>119</sup>

In several unpublished laboratory safety studies performed by Pfizer,<sup>110</sup> carprofen has been demonstrated to be well tolerated in the labeled dosage of 2.2 mg/kg, orally, twice daily. The first study used 48 beagles and gave the dogs up to five times the labeled dose (0, 2.2, 6.6, and 11 mg/kg), orally, twice daily for 6 weeks. The study tested the toxicity of 10x the label dose (22 mg/kg), orally, twice daily for 2 weeks. In the six week study, no dogs showed adverse effects related to drug administration with regard to clinical observation, food consumption, body weight, urinalysis results, fecal occult blood tests results, or changes in ophthalmologic, neurologic, hematologic, or ECG examinations. Two of the eight dogs receiving the 10x dosage had mild hypoalbuminemia. Six episodes of black or bloody appearing feces were observed in 3 dogs during days 6-9 of treatment (one episode in 1x, 2 episodes in 3x, and 3 episodes in 10x). Necropsy was performed on all dogs. Five dogs in the 10x group had grossly visible red areas in the intestinal mucosa. Histopathologically, there was no evidence of ulceration, and 2 of the 5 dogs had congestion and/or villous atrophy of the lamina propria. The conclusion was that carprofen given at the recommended clinical dose of 2.2 mg/kg, twice daily, was not associated with systemic toxicosis.

The second study<sup>110</sup> was divided into 4 groups that gave 72 beagles up to 5.7 times the labeled dosage of carprofen (0, 0.45x, 1.6x, and 5.7x; 0, 2, 7, and 25 mg/kg, respectively) for 26 and 52 weeks. Six dogs from each group were necropsied at 26 weeks. No dogs showed adverse effects related to drug administration with regard to food consumption, body weight, urinalysis results, or changes in ophthalmologic, neurologic, ECG, gross or histopathologic examinations. There were reported increases in the serum ALT concentrations (approximately 20 U) in the dogs receiving 5.7 times the recommended dosage (25 mg/kg/d). The prevalence of emesis in this study ranged from 0.4% in control dogs to 1.7% in dogs received carprofen, 25 mg/kg/d. Dogs that were receiving 2 and 7 mg/kg/d had overall prevalence of emesis of 0.8%. It was

stated that several dogs in each of the treatment groups (none in the control group) had a mild, nonspecific dermatitis characterized by slight redness or rash. However, no dose relationship was determined. The conclusion was that no significant clinical, gross, or histologic changes occurred in dogs receiving carprofen at 5.7 times the recommended label dose (25 mg/kg/d).

In the third study,<sup>110</sup> three beagles were given increasing dosages of carprofen, orally, once daily, for 5 days (5, 10, 20, 40, and 80 mg/kg). Two days after administration of the 80 mg/kg dose, a single dose of 160 mg/kg was administered. No abnormal clinical signs were observed in the dogs on the days when 5 and 40 mg/kg of carprofen was given. On the days 10 and 20 mg/kg were given, 1 dog and 2 dogs, respectively, had loose stools. When 80 mg/kg was given, 1 dog vomited and serum ALT concentration was elevated in all three dogs. When 160 mg/kg was given, clinical signs were observed the following day characterized by loose feces and vomiting and were seen on the fourth day after administration also. ALT concentrations returned to normal in 2 of the 3 dogs after 7 days. The packed cell volume and hemoglobin values were found to be decreased after the 80 mg./kg dose was given and remained low after 7 days. The conclusion was that exposure to high dosages of carprofen resulted in few clinical signs of toxicity.

### **Etodolac**

In 1991 etodolac became the first NSAID of the pyranocarboxylic class to be approved in the United States for use in human patients with osteoarthritis.<sup>120</sup> It is characterized by potent analgesic activity and has been shown to have minimal side effects.

Peak plasma concentrations in the dog following oral administration occur between 30-60 minutes with an elimination half life of 10-14 hours.<sup>5</sup> Enterohepatic circulation has been reported in the dog,<sup>5</sup> however, if present is not extensive in humans. In humans, etodolac is extensively metabolized in the liver, with renal elimination being the primary route of excretion.

Etodolac has been shown to have a 10 fold selectivity to preferentially inhibit COX-2,<sup>12,121</sup> which may explain its favorable margin of safety. However, the COX -2 / COX-1 ratio has been reported to be 0.8, which would classify etodolac as an equipotent inhibitor of the cyclooxygenase enzyme<sup>122</sup> Etodolac has been found to be more potent in chronic models of inflammation as opposed to acute models of inflammation.<sup>123</sup>

Etodolac has been shown to be as effective, in therapeutic trials for human osteoarthritis, as ibuprofen<sup>124</sup>, piroxicam<sup>125</sup>, diclofenac<sup>120</sup>, sulindac<sup>126</sup>, aspirin<sup>126</sup>, nabumetone<sup>120</sup>, and naproxen<sup>120</sup>. Etodolac has been found to have greater efficacy than diclofenac and indomethacin in other reports<sup>120</sup>. When etodolac was compared to ibuprofen, a higher incidence of gastrointestinal bleeding was seen in human patients receiving ibuprofen.<sup>124</sup> When piroxicam was compared to etodolac in a human study, there were no significant differences obtained in the incidence of any specific adverse effect.<sup>125</sup> Etodolac was found to be less ulcerogenic than six other anti-inflammatory drugs (indomethacin, diclofenac, piroxicam, naproxen, ketoprofen, and aspirin) in a human trial.<sup>127</sup> One report in humans suggests an acute colitis may be associated

with the administration of etodolac; both cases had resolution of clinical signs after discontinuation of etodolac.<sup>128</sup>

Dogs given etodolac for treatment of coxofemoral osteoarthritis demonstrated improvement in objective measurement of gait function.<sup>129</sup> This study used two dosages of etodolac (high, 10-15 mg/kg; low 2-4 mg/kg) given to dogs orally, once daily, for 8 days. Ground reaction forces and a subjective scoring system were used. The dogs in the group receiving the higher dosages of etodolac had improved ground reaction forces. Etodolac was approved for use in the dog in the United States in October 1998.

Table 1: Mean IC50 values ((g/ml) of selected NSAIDs

| <b>Drug</b>  | <b>Ratio COX-2/COX-1</b> |
|--------------|--------------------------|
| Piroxicam    | 600                      |
| Aspirin      | 166                      |
| Indomethacin | 60                       |
| Carprofen    | 1                        |
| Etodolac     | 0.7                      |

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**THE GASTRODUODENAL EFFECTS OF BUFFERED ASPIRIN, CARPROFEN, AND  
ETODOLAC IN HEALTHY DOGS**

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## Abstract

Twenty-four healthy, mixed breed dogs were divided into 4 groups. Group 1 received a placebo PO q12h, group 2 received an average of 16.5 (range, 15.1-17.8 ) mg/kg buffered aspirin PO q12h, group 3 received an average of 2.2 (range, 2.0-2.4) mg/kg carprofen PO q12h, and group 4 received an average of 12.8 (range, 11.7-13.8) mg/kg etodolac PO q24h (with a placebo in the P.M.). All treatments continued for 28 consecutive days. Gastroduodenal endoscopy was performed on days -9, 0, 5, 14 and 28. Multiple gastric biopsies were obtained endoscopically on day -9 to determine each dog's *Helicobacter spp.* status.

Four regions in the stomach and one region in the proximal duodenum were evaluated endoscopically, and each was assigned a score from 1 to 11. Scores for each region then were summed to give a total score for each endoscopic evaluation.

Erosions and submucosal hemorrhages were seen in all dogs receiving aspirin. Only minor gastric lesions were observed in the carprofen, etodolac, and control groups. No adverse clinical signs were noted in any dog given any treatment. Median total score on days 0, 5, 14, and 28 were as follows: group 1, 5.0, 5.0, 5.0, 5.0; group 2, 5.0, 27.0, 26.0, 27.5; group 3, 5.0, 5.0, 6.0, 5.0; group 4, 5.0, 7.0, 5.0, 5.0, respectively.

There was no significant difference among dogs receiving carprofen, etodolac, or placebo. The administration of carprofen, etodolac, or placebo to healthy dogs resulted in significantly less gastroduodenal lesion development than in dogs receiving buffered aspirin.

Non-steroidal anti-inflammatory drugs (NSAIDs) frequently are used for the treatment of musculoskeletal disease in veterinary medicine and are the most common medications used by humans worldwide.<sup>1</sup> They generally are recognized for their analgesic, anti-pyretic, and anti-inflammatory properties. In humans, NSAID therapy is associated with upper gastrointestinal (GI) symptoms in 25% of patients, and ulcers and erosions in 40% of patients.<sup>1</sup> The prevalence of NSAID-induced GI lesions in veterinary medicine is unknown. Adverse effects of NSAID therapy recognized in dogs include gastrointestinal bleeding, ulceration, hepatopathy, and possible nephrotoxicity.<sup>2-5</sup> NSAID-induced GI lesions primarily are thought to be due to the inhibition of prostaglandin synthesis via inhibition of cyclooxygenase.

The purpose of the present study was to investigate the adverse GI effects of 2 NSAIDs that recently have been introduced to veterinary medicine, carprofen and etodolac, and compare them to aspirin (positive control) and placebo (negative control). The main purpose of this research was to provide information regarding adverse GI effects of drugs that are commonly used in dogs suffering from osteoarthritis.

## Material and Methods

Acclimation of animals. The study was approved by and met all criteria of the Animal Care Committee, Virginia Polytechnic Institute and State University. Twenty-four random source dogs (14 males, 10 females) were acclimated for 4 weeks. Dogs were treated with ivermectin (Ivomec 1%, MSD-Ag Vet, Rahway, NJ; 200  $\mu$ g/kg PO once), fenbendazole (Panacur Suspension, Hoeschst, Kansas City, MO; 50 mg/kg PO q24h x 3 days), sulfadimethoxine (Albon, Roche, Nutley, NJ; 50 mg/kg PO once, then 25 mg/kg PO q24h x 14 days), and metronidazole (Flagyl, Searle, Chicago, IL; 50 mg/kg PO q24h x 7 days) for at least 2 weeks before the start of the study. Zinc sulfate fecal flotation was negative for intestinal parasites in each dog within one week of the beginning of the study. The diet consisted of a cereal based dry food (Hill's Science Diet Canine Maintenance, Topeka, KS). Dogs were entered into the study on the basis of normal physical examination, normal values for hematocrit, total solids, BUN by dipstrip test (Azostick, Bayer Corporation, Elkhart, IN), a negative zinc sulfate fecal flotation, and normal gastroduodenoscopic examination.

Helicobacter determination. Endoscopy was performed 9 days before administration of medications for the purpose of determining *Helicobacter spp.* status and assessing the presence of a grossly normal gastric and duodenal mucosal surface. Gastric mucosal biopsies in each of 4 gastric regions (cardia, pylorus, greater curvature, and angularis incisura) were obtained for use in the 'Campylobacter Like Organism' test (CLOtest®, Tri-Med Specialties, Inc., Lenexa, KS). Positive results of this test were interpreted as being suggestive of urease production by *Helicobacter* organisms.<sup>6</sup>

CLOtest®: Gastric mucosal biopsy samples from each region were pushed into the CLOtest® gel. The tissue was completely immersed in order that maximal contact with the urea and bacteriostat in the gel was achieved. The CLOtest® was kept at room temperature, and a final determination was made after 24 hours. If the test from one gastric region was positive, the dog was considered to be *Helicobacter spp.* positive.

Dogs were stratified into treatment groups based on the results of the CLOtest®; an equal number of CLOtest® negative dogs were randomly placed in each group and all other dogs then were randomly assigned. Endoscopy on day 0 was performed before drug administration to determine that all biopsy sites had healed.

Group assignments. Four groups of 6 dogs were randomly assigned a treatment. Dogs received either placebo, buffered aspirin (Bufferin®), carprofen (Rimadyl®), or etodolac (Lodine®) as follows: Group 1, placebo, received a #2 gel capsule with methylcellulose, orally q12h; Group 2, buffered aspirin, received an average of 16.5 mg/kg (range 15.1-17.8 mg/kg), orally q12h; Group 3, carprofen, received an average of 2.2 mg/kg (range 2.0-2.4 mg/kg), orally q12h; Group 4, etodolac, received an average of 12.8 mg/kg (range 11.7-13.8 mg/kg), orally q24h. Group 4 also received a placebo (same as group 1) at the second treatment time so that all dogs were treated twice daily. Drug administration was begun 12 hours after the endoscopy performed on day 0 and was continued for 28 consecutive days.

*Endoscopic Examination.* Nine days before administration of medication, as well as on days 0, 5, 14, and 28 of the study, dogs were premedicated with 0.1 mg/kg acepromazine and 0.05 mg/kg atropine sulfate SQ. Anesthesia was induced with sodium thiopental to effect (10 - 15 mg/kg); dogs were intubated and maintained with halothane in oxygen. Gastroduodenoscopy (Fujinon E67-FPZ Video Endoscope, Fujinon Inc. Wayne, NJ) was performed, and an endoscopic score assigned by an endoscopist (MSL) unaware of the treatment groups. Endoscopic images were recorded (Sony Promavica Still Video Recorder, Model MVR5300, Sony Corporation, Japan).

The stomach and duodenum were divided endoscopically into 5 regions as described previously<sup>7</sup>: (1) pylorus and pyloric antrum, (2) angularis incisura, extending along the lesser curvature, (3) greater curvature, from the cardia to the pyloric antrum, (4) cardia, extending from the greater curvature region to the lesser curvature that was not included with the angularis incisura, and (5) proximal duodenum to the major duodenal papilla. The endoscope was placed into the body of the stomach, and the stomach insufflated to distend the rugal folds in order to evaluate the body. The angularis then was visualized, followed by the antrum. The endoscope then was retroflexed to view the cardia and lesser curvature. Finally, the duodenum was evaluated. If there was mucus, bile, hair, or other debris obscuring the gastric or duodenal mucosa, tap water was infused through the operating channel of the endoscope to clear the material and allow visualization.

Each region was systematically evaluated and scored before iatrogenic trauma from the endoscope could be induced. Lesions that bordered two regions were assigned to the most appropriate region and not counted twice when assessing the adjacent region. Each of the 5 regions was assessed individually and assigned a numerical value based on an 11 point scale similar to that previously described<sup>7</sup> (**Table 1**). Submucosal hemorrhage was defined as an area of hemorrhage judged to be 1-3 mm diameter covered by an intact mucosa (Figure 1). Erosion was defined as a superficial discontinuation of the mucosal epithelium (Figure 2). An ulcer was defined as a lesion producing wide discontinuation of the mucosa with a central defect and a raised margin. Each score was assigned based on the most severe lesion(s) present in each region. Scores for each region were summed and a total score was given to each dog at each endoscopy.

*Animal monitoring.* Dogs were observed 3 times daily to assess appetite, vomiting, and to assign a fecal score. Fecal scores were assigned based on a scale of 1 to 5; 1 being watery diarrhea and 5 being defined as a dry, well-formed stool. Dogs were assigned a positive or negative daily value based on vomiting or incomplete consumption of food for the entire day. The number of positive dog days was used for comparison among groups.

*Statistical Analysis.* Median scores for each group at each time period were determined. The Kruskal - Wallis test<sup>8</sup> was used to determine effects of treatments at each time period and effects within a treatment group at different time periods. Fisher's exact test<sup>9</sup> was used to compare scores assigned to each of the 5 gastroduodenal regions within a treatment group and over time periods. For all statistical tests,  $p \leq 0.05$  was considered significant.

## Results

All dogs, with the exception of 4, tested positive for *Helicobacter spp.* by CLOtest®. One of the 4 negative dogs was randomly assigned to each treatment group. All dogs had grossly healed biopsy sites on day 0 when endoscopy was performed.

Figure 3 presents the median total gastroduodenal lesion scores for each treatment group. Dogs receiving aspirin had significantly ( $P<0.0001$ ) higher median scores (27.0, 26.0, 27.5) on days 5, 14, and 28, respectively than dogs receiving placebo (5.0, 5.0, 5.0), carprofen (5.0, 6.0, 5.0), and etodolac (7.0, 5.0, 5.0).

There was no significant difference in median total gastroduodenal lesion scores among dogs receiving placebo, carprofen, or etodolac. There was no significant difference among any of the 5 regions evaluated in the placebo, carprofen, or etodolac groups. Duodenal lesion scores were significantly ( $P<0.0001$ ) lower than the 4 individual stomach regions in the group treated with aspirin.

Figure 4 shows the range of lesion scores for each treatment group at each time period. Most dogs in the placebo, carprofen, and etodolac groups received individual scores of 5 on all treatment days. There were some dogs in each of these groups that exhibited gastric lesions on different days. Most of these represented submucosal hemorrhages and increased the total lesion scores to  $<10$ . On day 28, 1 dog in the placebo group had a large trichobezor in the gastric body and received a score of 13. The dog with the highest individual score (18) in the carprofen group on day 28 had 2 regions with 2-5 and  $>5$  erosions, and one region with 2-5 submucosal hemorrhages. On day 5, 1 dog receiving etodolac had a total lesion score of 16, with two regions of 1 and 2-5 erosions and one region with 2-5 submucosal hemorrhages. The dog in the etodolac group on day 14 with a score of 17 had 2 regions of 1 and  $>5$  erosion(s) and one region of 2-5 submucosal hemorrhages. Individual scores in the dogs receiving buffered aspirin ranged between 21-29 on days 5, 14, and 28. Most dogs in this treatment group had individual stomach region scores of 7. No ulcers were seen.

There were only 2 days on which vomiting occurred in any dog in the aspirin group, and one day each for the carprofen and etodolac groups. There were 15 dog days of diarrhea (fecal score  $\leq 2$ ), 14 dog days in the etodolac group, and 1 dog day in the carprofen group. Twelve of the 14 dog days in the etodolac group occurred in a single dog. The diarrhea in that dog started approximately one week after the initiation of drug administration. Increased frequency and hematechezia characterized the diarrhea. Multiple zinc sulfate fecal flotations for intestinal parasites were negative. The dog did not have an increase in clostridial spores on multiple rectal cytology specimens, however, the dog was positive for clostridial enterotoxin, using the Reverse Latex Agglutination Test (RPLA Kit, Microbio, Denver, CO). Diarrhea resolved within 1 week of discontinuation of the etodolac.

## Discussion

The clinical relevance of *Helicobacter spp.* in healthy animals has been questioned. In one report, 100% of healthy laboratory and shelter dogs and 67% of pet dogs (without gastric disease) had gastric spiral bacteria similar to *Helicobacter felis* or *H. heilmannii*.<sup>10</sup> At least 13 species of *Helicobacter* have been identified in animals, however, not all the species are pathogenic.<sup>11</sup> The 2 most common species in dogs and cats are *Helicobacter felis* and *Helicobacter heilmannii*.<sup>10-13</sup> This study found a high prevalence of *Helicobacter spp.* in clinically normal animals.

In humans, *H. pylori* is the only organism that expresses urease in the stomach.<sup>14</sup> Canine and feline gastric *Helicobacter spp.* are ultrastructurally and antigenically similar to *H. pylori*.<sup>15</sup> In veterinary medicine, it has been assumed that *Helicobacter spp.* are the only organisms to produce urease in the stomach, and therefore the only organisms to cause a color change in the CLOtest®. False positive tests are rare, but can occur with overgrowth of urease-producing commensal organisms, such as *Proteus spp.*, in an acid-free stomach.<sup>16</sup> False negative CLOtest® results can be due to a low density of *Helicobacter spp.* organisms in the histologic specimen.<sup>16</sup>

There are only 4 NSAIDs approved for use in dogs in the United States: phenylbutazone, meclufenamic acid, carprofen, and etodolac. Carprofen is a member of the propionic class of NSAIDs that includes ibuprofen, naproxen, and ketoprofen, and is a reversible inhibitor of cyclooxygenase. In animal models, carprofen is approximately 35 times less likely to cause gastric ulceration than aspirin.<sup>17</sup> Additionally, the potency of carprofen as both an anti-inflammatory agent and analgesic is comparable to indomethacin, and is greater than aspirin and phenylbutazone.<sup>18</sup> Carprofen is 90% absorbed after oral administration and reaches peak plasma concentrations after 1-3 hours in dogs.<sup>19</sup> The mean half-life for elimination in dogs is approximately 8 hours (range, 4.5-9.8 hours) after a single oral administration.<sup>17</sup> Reported adverse reactions to carprofen are vomiting, diarrhea, lethargy, and change in appetite.<sup>17</sup> Acute hepatocellular necrosis also has been reported with carprofen administration.<sup>20</sup>

Etodolac is a NSAID of the pyranocarboxylic class and is characterized by potent analgesic activity. Peak plasma concentrations in dogs after oral administration occur between 30-60 minutes with an elimination half-life of 10-14 hours.<sup>21-23</sup> In vitro studies suggest that etodolac preferentially inhibits cyclooxygenase - 2.<sup>24,25</sup> Consequently, fewer adverse effects are expected. Endoscopic studies on human subjects demonstrated etodolac to be indistinguishable from the placebo with respect to GI adverse effects.<sup>26,27</sup> The prevalence of severe GI adverse effects reported for etodolac ranges between 0.005% and 0.1% in humans.<sup>27</sup> Few adverse effects were noted in an 8 day evaluation of etodolac for the treatment of hip dysplasia in dogs.<sup>28</sup>

Aspirin probably is the anti-inflammatory drug used most commonly in companion animals<sup>29</sup>, but it is not approved for use in the dog. Severe blood loss, gastric ulceration, and

perforation are recognized complications of aspirin use and occur in a dose related fashion.<sup>7,30-32</sup> Endoscopically, aspirin has been shown to cause GI bleeding at a dosage of 25 to 35 mg/kg PO q8h.<sup>7,30-33</sup>

In the present study, carprofen and etodolac caused significantly fewer GI lesions than buffered aspirin in healthy dogs. Gastrointestinal lesion scores in dogs treated with carprofen and etodolac were no different than those of the placebo treatment group. No dogs, including those in the aspirin group, showed severe GI signs. Lesions observed in dogs receiving carprofen and etodolac were very mild and most likely of no clinical relevance.

Gastric and duodenal mucosa is rich in prostaglandins.<sup>34</sup> Prostaglandins are important for maintenance of natural gastric mucosal defenses including gastric blood flow, bicarbonate secretion, and mucus secretion.<sup>34</sup> The NSAIDs inhibit production of endogenous prostaglandins, and gastric mucosal damage is attributed in part to prostaglandin deficiency. Other mechanisms of action such as neutrophil adherence, decreased cytokine production, direct chemical damage, and lipooxygenase inhibition also may play roles with some NSAIDs.<sup>29</sup>

Cyclooxygenase is an enzyme needed for the synthesis of prostaglandins from arachidonic acid. There are 2 isoforms of this enzyme. Cyclooxygenase-1 (COX-1), which is present in most cells and tissues including platelets, kidneys, synovium, and gastric mucosa, functions to produce physiologically protective prostaglandins.<sup>1,35,36</sup> Cyclooxygenase-2 (COX-2) is an induced enzyme and functions to produce proinflammatory prostaglandins and other mediators of inflammation.<sup>1,35,36</sup> According to current theory, agents that selectively inhibit COX-2 without affecting the production of COX-1 may reduce the adverse effects of NSAIDs.<sup>36</sup> The cyclooxygenase selectivity of an NSAID often is expressed as a COX-2 : COX-1 ratio.<sup>36</sup> A ratio of greater than one signifies that COX-1 is inhibited more than COX-2, suggesting that the GI adverse effects will be greater. Both etodolac and carprofen have low COX 2: COX 1 ratios based on *in vitro* studies. Etodolac has a COX-2 : COX-1 ratio of less than one, whereas carprofen has a ratio of approximately one.<sup>29,37,38</sup> Aspirin has a high COX-2: COX-1 ratio.<sup>37,38</sup> When the activities of carprofen and etodolac were evaluated against isoenzymes of canine COX-1 and COX-2, carprofen potently inhibited canine COX-2.<sup>39</sup> Etodolac did not have selectivity for canine COX-2, and was only marginally selective for canine COX-1.<sup>39</sup> These findings may reflect a difference in the structure of canine COX, compared to COX of other species.<sup>39</sup> The significance of *in vitro* COX-1 and COX-2 testing has not been well defined *in vivo*.

Aspirin, and other acidic NSAIDs such as indomethacin and ketoprofen, are thought to have topical effects on the gastric mucosa in addition to the systemic effects of prostaglandin inhibition.<sup>40</sup> These NSAIDs can freely diffuse across the gastric mucosal barrier and become ionized. In the ionized form, they become sequestered or trapped within the mucosal cells and cause damage.<sup>41</sup> The topical effects of these NSAIDs however are not thought to play a major role in the development of GI injury. When NSAIDs are given parenterally or rectally, GI lesions still occur.<sup>41</sup>

The drug dosages used in this study are the dosages currently recommended by the manufacturer (carprofen, etodolac) or commonly recommended in the veterinary literature (aspirin).<sup>42</sup> It has been found that a dosage of 15 mg/kg PO q12h of aspirin provides symptomatic relief for many dogs with osteoarthritis.<sup>42</sup> The clinical relevance of the lesions seen in the dogs receiving aspirin in this study is unknown. None of the dogs showed any clinically relevant adverse effects from the administration of the medications, however, subtle signs were not evaluated by the study design. Perhaps, a longer treatment period would have led to clinical signs.

One dog in the etodolac group developed large bowel diarrhea within one week of initiation of drug treatment. The diarrhea resolved within one week of discontinuation of the drug. The dog tested positive for clostridial enterotoxin and negative for clostridial spores on rectal cytology, but it is unknown what role clostridium plays in animals with diarrhea. The organism usually is found in the feces of normal dogs.<sup>43</sup> No relationship was found between the number of spore-forming rods on cytology and the presence of enterotoxin in feces and there was poor correlation between the number of spore-forming rods and the presence of diarrhea in a recent study.<sup>43</sup> The diarrhea noted in this animal may have been due to the etodolac.

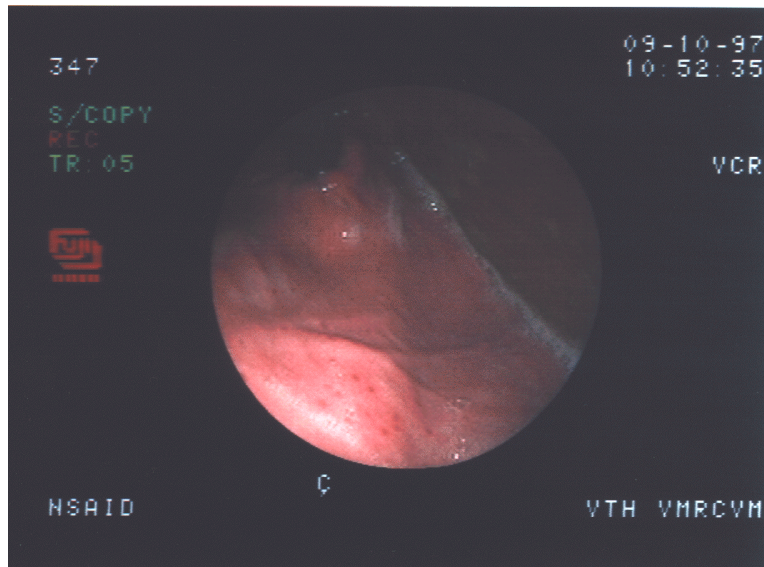
To the authors' knowledge, there are no reports in the veterinary literature of large bowel diarrhea associated with NSAID use in the dog. In humans, there have been several clinical reports of NSAIDs causing colonic ulceration and inflammation as well as strictures and perforations.<sup>44-47</sup> Approximately 10-15% of human NSAID users develop diarrhea.<sup>1</sup> In several instances, quiescent inflammatory bowel disease has been exacerbated after NSAID use.<sup>48</sup> NSAIDs may increase colonic permeability and inhibit prostaglandin synthesis in the large intestine.<sup>47</sup>

In the present study, administration of carprofen and etodolac to healthy dogs resulted in significantly fewer GI lesions than in dogs receiving aspirin. There were no significant differences in the GI lesions in dogs receiving carprofen, etodolac, or placebo. The study lasted only 28 days, however and most animals with osteoarthritis require treatment for a longer period of time. Gastrointestinal lesions may occur after longterm treatment with these drugs.

**Table 1**

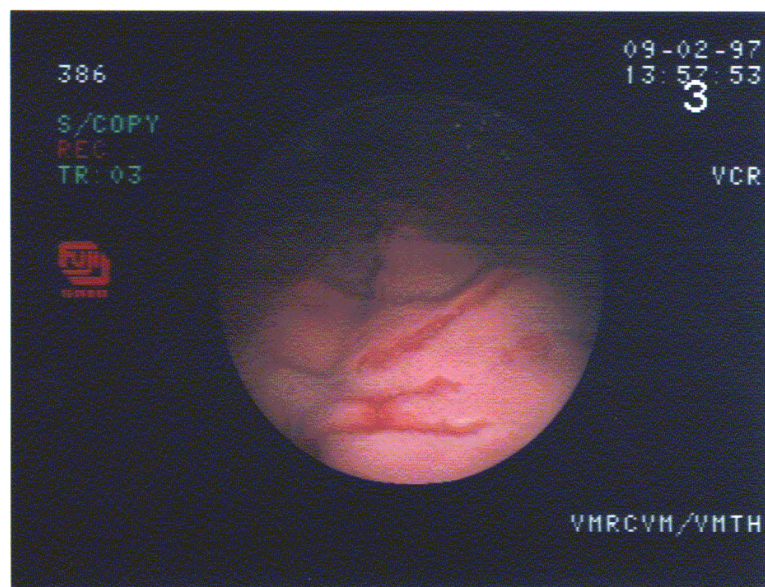
**Numerical Value Assigned to Each Region Based on the Following Criteria as Recognized from Gastroduodenoscopy (modified from Johnston, et al<sup>6</sup>)**

| <u>Score</u> | <u>Description</u>         |
|--------------|----------------------------|
| 1            | Normal                     |
| 2            | 1 submucosal hemorrhage    |
| 3            | 2-5 submucosal hemorrhages |
| 4            | >5 submucosal hemorrhages  |
| 5            | 1 erosion                  |
| 6            | 2-5 erosions               |
| 7            | >5 erosions                |
| 8            | 1 ulcer                    |
| 9            | 2 ulcers                   |
| 10           | $\geq 3$ ulcers            |
| 11           | perforating ulcer          |



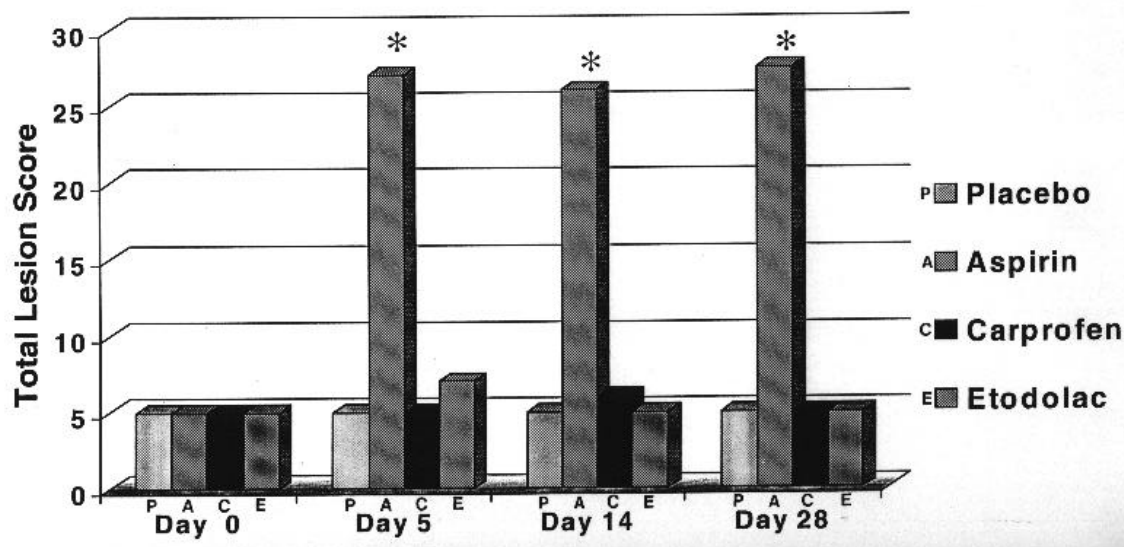
**Figure 1**

Multiple submucosal petechial hemorrhages are present near the cardia. This region received a lesion score of 4.



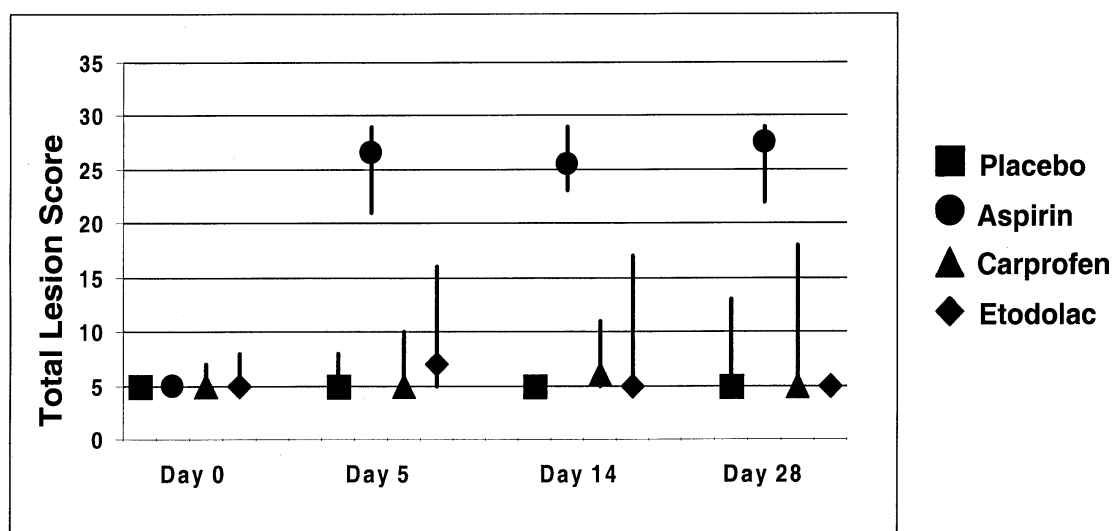
**Figure 2**

Multiple erosions are present on the greater curvature. This region received a lesion score of 7.



**Figure 3**

Median total gastroduodenal lesion scores over time for dogs in 4 treatment groups. (\*  $p < 0.05$ )



**Figure 4**

Median and range of total gastroduodenal lesion scores over time for dogs in 4 treatment groups.

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**Comparison of the Clotest® to Histopathologic Evaluation in Identifying the Presence of *Helicobacter Spp.* in Healthy Dogs**

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Diagnosis of *Helicobacter spp.*

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## Abstract

Thirty healthy, random source, dogs were evaluated to determine the prevalence of *Helicobacter spp.*, and to compare the 'Campylobacter-like organism' test (CLOtest®) to histopathologic identification of *Helicobacter spp.* organisms. Gastric mucosal biopsies from each of four gastric regions (cardia, pyloric antrum, greater curvature, and angularis incisura) were obtained endoscopically for use in the CLOtest® and for histopathologic evaluation. Twenty-seven of 30 dogs (90%) were positive for spiral bacteria suspected to be *Helicobacter spp.* by histopathologic evaluation in at least one of the four gastric regions. Three dogs (10%) were negative for *Helicobacter spp.* in all gastric regions by histopathologic evaluation. The CLOtest® was found to have a sensitivity, specificity, and positive predictive value of 84%, 81%, and 92%, respectively, when compared to histopathologic evaluation. When only the angularis incisura was evaluated, the sensitivity, specificity, and positive predictive value increased to 92%, 94%, and 96%, respectively. The angularis incisura had the highest, whereas the pyloric antrum had the lowest, prevalence of positive test results when compared to dogs determined to be overall *Helicobacter spp.* positive (histopathologic positive in at least one gastric region). The results of this study suggest the prevalence of *Helicobacter spp.* in apparently healthy dogs is high. For accurate and economical detection of *Helicobacter spp.* in a dog undergoing upper gastrointestinal endoscopy, a tissue sample should be taken from the angularis incisura for CLOtest® sampling.

## Introduction

In humans, the two most common causes of gastrointestinal ulcerative disease are non-steroidal anti-inflammatory drugs (NSAIDs) administration and *Helicobacter pylori* (formerly *Capmylobacter pylori*).<sup>1,2</sup> The presence of *H. pylori* in people is associated with a wide variety of clinical entities ranging from asymptomatic chronic gastritis to gastric carcinomas.<sup>3,4</sup> *Helicobacter spp.* are thought to naturally inhabit the stomach of many mammalian species including man<sup>5</sup>, dogs,<sup>6,7</sup> cats,<sup>7,8</sup> cheetahs,<sup>9</sup> ferrets,<sup>9</sup> and non-human primates.<sup>9</sup> *Helicobacter spp.* have been found to inhabit between 20-90% of people, with the majority of cases being subclinical.<sup>5,9</sup> Ninety to 100% of human patients with duodenal ulcers and 70-90% of patients with gastric ulcers have *H. pylori* infections.<sup>9-15</sup>

The clinical significance of *Helicobacter spp.* in clinically normal animals is unknown. However, previous studies suggest *Helicobacter spp.* are present in a large percentage of the canine and feline population. In one report, 100% of laboratory and shelter dogs and 67% of pet dogs (with non-gastric diseases) were colonized with gastric spiral bacteria similar to *Helicobacter felis* or *Helicobacter heilmannii*.<sup>16</sup> In a study in which tissue samples were endoscopically obtained, 82% of dogs and 76% of cats had *Helicobacter*-like organisms present.<sup>17</sup> A post mortem study showed that 100% of dogs and 60% of cats were infected with *Helicobacter*-like organisms.<sup>7</sup> Another post mortem study of 55 random source cats revealed *Helicobacter*-like organisms in 70% of juvenile cats and 97% of adult cats.<sup>5,9</sup>

At least thirteen species of *Helicobacter* have been identified in animals, however not all the species are pathogenic.<sup>9</sup> The two most common species found in dogs and cats are *Helicobacter felis* and *Helicobacter heilmannii* (formerly *Gastrospirillum hominis*).<sup>7,9,16-18</sup> *Helicobacter pylori*, the most common organism in humans, are rarely found in domestic animals.<sup>3,16</sup>

The diagnosis of *Helicobacter spp.* can include invasive and noninvasive tests. Invasive tests use endoscopically obtained biopsy specimens and include histopathological examination, rapid urease tests, and culture.<sup>9</sup> Organisms can be visualized microscopically in the gastric mucosa; special staining techniques such as a silver impregnation can be used to enhance microscopic visualization and detection of the organisms.<sup>9,19</sup> This test in humans has high sensitivity (91-99%)<sup>17-19</sup> and specificity (95-100%)<sup>19-21</sup>, but false negatives are possible because of a patchy distribution of organisms within the stomach. It is recommended in humans that two specimens from each region in the stomach (antrum, cardia, angularis incisura, and fundus) be evaluated.<sup>9</sup> The use of histopathology also permits determination of the extent of the inflammatory changes.

Histochemical staining techniques used to visualize *Helicobacter*-like organisms have been studied in dogs and cats. In a limited study, hematoxylin and eosin (H & E) was compared to Warthin Starry silver impregnation technique, and in only one instance (of ten cases), did H&E staining fail to demonstrate organisms that were detected with the silver technique.<sup>22</sup> A

modified Diff-Quik stain is a reportedly easy staining technique to demonstrate these organisms as well.<sup>23</sup>

Rapid urease testing can be performed with an endoscopically obtained biopsy sample. *Helicobacter spp.* is a potent producer of urease and is thought to be the only organism in the stomach to produce significant amounts of urease.<sup>24,25</sup> A tissue sample is placed in a broth containing urea, bacteriostatic agents, and a pH indicator. A color change is seen as the urease breaks down the urea into ammonia and raises the pH. The 'Campylobacter-like organism test' (CLOtest®) is one example of a commercially available rapid urease test and was used in this study. The sensitivity of the CLOtest® in humans has been reported between 70-98%,<sup>9,19-21,26</sup> and the specificity between 93-100%.<sup>19-21,26</sup> Because this test is read within a 24-hour period (ideally at 20 minutes, 1 hour, 3 hours, and 24 hours), it enables a quick and convenient diagnosis. By one hour, 80-85% of positive human patients, and by 3 hours, 90% of positive human patients, are detected by the CLOtest®.<sup>21,27</sup> False negatives can occur when very low numbers of the organism are present.<sup>21,28</sup> At least 100,000 bacteria are required to produce a positive rapid urease test.<sup>29</sup> Therefore, it has been recommended that more than one biopsy specimen be evaluated. The CLOtest® is less sensitive after the patient has received antibiotics or bismuth, because the organism may be killed or be present in lower numbers. False positives are very rare and can occur when gastric acid is absent (patients taking H<sub>2</sub> antagonists), because commensal organisms such as *Proteus spp.* can grow in the stomach and produce the urease enzyme.<sup>21,28</sup>

Culture of the *Helicobacter spp.* organism is the least sensitive of the above diagnostic techniques. It has a sensitivity of 70%-77%<sup>19,21</sup> and a specificity of 100% in humans.<sup>19,21</sup> Many *Helicobacter* species have not yet been successfully cultured, such as *H. heilmannii*, the most common species in dogs and cats.<sup>9</sup>

Diagnosis and treatment of *H. pylori* in humans has been extensively investigated because of the risks associated with the development of gastric cancer. Many animals and humans are completely asymptomatic for the presence of *Helicobacter spp.*, despite the presence of *Helicobacter spp.* The clinical sign most commonly associated with *Helicobacter spp.* in dogs and cats is chronic intermittent vomiting. Less frequently, inappetance, pica, weight loss, and fever are associated with the presence of the organism.

The purpose of this study was 1) to determine the prevalence of *Helicobacter spp.* in a group of healthy dogs utilizing endoscopy, 2) to determine the overall sensitivity, specificity, positive and negative predictive value of the CLOtest® in a group of healthy dogs, and 3) to determine the individual sensitivity, specificity, positive and negative predictive value of the CLOtest® in four gastric regions.

## Material and Methods

Acclimation of animals. This study was approved by and met all criteria of the Animal Care Committee, Virginia Polytechnic Institute and State University. Thirty random source dogs (15 males, 15 females) were acclimated for 4 weeks. In preparation for another study, dogs were treated with ivermectin<sup>a</sup> (200ug/kg PO once), fenbendazole<sup>b</sup> (50 mg/kg PO QD x 3 days), sulfadimethoxine<sup>c</sup> (50 mg/kg PO once, then 25 mg/kg PO QD x 14 days), and metronidazole<sup>d</sup> (50 mg/kg PO QD x 7 days) at least two weeks prior to the start of the study. Zinc sulfate fecal flotation was performed for each dog within one week of the beginning of the study. The diet consisted of a cereal-based dry food<sup>e</sup>, and dogs were housed in individual runs. Dogs were entered into the study on the basis of normal physical examination and normal values for packed cell volume, total solids, azostick, and negative zinc sulfate fecal flotation.

Helicobacter determination. Gastroduodenoscopy<sup>f</sup> was performed for the purpose of determining *Helicobacter spp.* infection status. Dogs were premedicated with 0.1 mg/kg acepromazine and 0.05 mg/kg atropine sulfate subcutaneously. Anesthesia was induced with intravenous sodium thiopental to effect (10 - 15 mg/kg); dogs were intubated and maintained with halothane in oxygen.

The stomach was endoscopically divided into four regions for systematic evaluation: (1) pylorus and pyloric antrum, (2) angularis incisura, extending along the lesser curvature, (3) greater curvature, from the cardia to the pyloric antrum, and (4) cardia, extending from the greater curvature region to the lesser curvature that was not included with the angularis incisura. Two tissue samples were obtained from each dog at the following locations: (1) adjacent to the pylorus, (2) angularis incisura, (3) greater curvature at approximately 50% of the distance from the antrum to the cardia, and (4) cardia, adjacent to the gastroesophageal junction.

CLOtest®<sup>g</sup> A gastric mucosal biopsy sample from each region was pushed into a CLOtest® gel with a sterile 20g needle. The tissue was completely immersed in order that maximum contact with the urea and bacteriostat in the gel was achieved. The CLOtest® was kept at room temperature and a final determination was made at 24 hours. A sample was considered positive based on a color change from yellow to magenta.

Histopathology. Samples from each area were fixed in 10% buffered neutral formalin, routinely processed and embedded in paraffin, sectioned at 5-6 µm, and stained with a modified Steiner silver impregnation technique. A single pathologist (RBD), who was unaware of the CLOtest® results, evaluated each tissue section in its entirety for the presence of spiral-shaped bacteria (Figure 1).

Statistical analysis. If one gastric region contained spiral bacteria (serpentine shaped; 0.25 µm x 6 µm), the dog was considered to be *Helicobacter spp.* positive. A dog negative for *Helicobacter spp.* did not have any spiral bacteria identified by histopathologic evaluation in the four gastric regions. The overall prevalence (presence of spiral bacteria in any one gastric region) of *Helicobacter spp.* was determined for the 30 dogs, as well as the prevalence of *Helicobacter spp.* at the individual gastric locations.

Statistical analysis consisted of determination of sensitivity, specificity, positive and negative predictive value of the CLOtest® correlated to the presence of organisms identified by histopathologic evaluation of paired samples derived from the same dog and location.<sup>30</sup> These parameters were evaluated for each gastric location and overall for all gastric samples (120).

The area of the stomach most likely to identify *Helicobacter* organisms by either CLOtest® or histopathologic evaluation were evaluated in dogs determined to be overall *Helicobacter spp.* positive. Fisher's exact testing<sup>31</sup> was used to determine if the proportions of dogs with positive CLOtest® results and positive histopathologic evaluation results in each of the four gastric regions were significant, compared to dogs determined to be overall *Helicobacter spp.* positive. The null hypothesis was that there was no agreement between the CLOtest® and the histopathological presence of spiral organisms. P values were obtained and  $p < 0.05$  was considered significant. Significant test results ( $p < 0.05$ ) concluded that the test (CLOtest® or histopathologic evaluation) was able to determine that *Helicobacter spp.* was detectable at that gastric location.

## Results

Twenty-seven of 30 dogs (90%) were found to be positive for *Helicobacter spp.* by histopathologic evaluation in at least one gastric region, and therefore given an overall positive status. The angularis incisura and the cardia had the highest prevalence of positive CLOtest® and histopathologic evaluation results (Table 1). One dog in the study was determined to be positive by histopathologic evaluation (in 3 of 4 regions) and negative by CLOtest® in all four regions. This was considered to be a false negative CLOtest® result.

One hundred seventeen samples (3 samples damaged in processing) were evaluated to determine the overall sensitivity, specificity, and positive and negative predictive values of the CLOtest®, which were 84%, 81%, 92%, and 64% respectively, when compared to histopathologic evaluation (Table 2). When the 30 samples from the angularis incisura were evaluated, the sensitivity, specificity, positive and negative predictive value increased to 92%, 94%, 96%, and 60%, respectively. The greater curvature had a specificity and positive predictive value of 100% because there were no false positive samples from that location in any of the 30 dogs.

The prevalence of positive CLOtest® and positive histopathologic evaluation in the four gastric regions was compared to dogs determined to be overall *Helicobacter spp.* positive (Table 3). The angularis incisura had the highest prevalence of positive test results, whereas the pyloric antrum had the lowest number of positive test results. Both tests in the angularis incisura, greater curvature, and cardia were able to determine that *Helicobacter spp.* were present ( $p < 0.05$ ). Neither test in the pyloric antrum was able to reliably determine that *Helicobacter spp.* were present.

## Discussion

In veterinary medicine, histopathologic evaluation of biopsy specimens has been used most extensively to detect the presence of *Helicobacter spp.* in gastric tissues. Topographic mapping at necropsy has shown that colonization with *Helicobacter spp.* has been highest in the fundus and corpus, and lower in the antrum.<sup>5-7</sup> Although it is difficult to compare studies because sampling regions were somewhat different, the angularis incisura, which was found to have the most consistently positive results in this study, may have been considered within the corpus of other studies. The results from the pyloric antrum, yielding the least sensitive results in this study, concur with the results of colonization in other studies. Neither CLOtest® nor histopathologic evaluation were able to reliably detect the organisms in the antrum in this study, as they may not have been present.

In humans, *H. pylori* is considered the only organism that significantly expresses the urease enzyme in the stomach.<sup>24,25</sup> Canine and feline gastric *Helicobacter spp.* are ultrastructurally and antigenically similar to *H. pylori*.<sup>2</sup> In veterinary medicine it has been extrapolated that *Helicobacter spp.* are the only organisms considered to produce significant quantities of urease enzyme in the stomach, and therefore, are the only organisms to cause a color change in the CLOtest®.

False positive tests are rare, but can occur with overgrowth of urease-producing commensal organisms, such as *Proteus spp.* or *Klebsiella spp.*, especially within an acid free stomach.<sup>32</sup> However, it is not likely that *Proteus spp.* or *Klebsiella spp.* would reach the concentration of colony forming units necessary to change the result of the urease test.<sup>25</sup> To avoid overgrowth of urease positive commensals or contaminants, bacteriostatic or bacteriocidal agents are included in the reaction media, such has been applied with the CLOtest®.<sup>25</sup>

False negative CLOtest® can be due to a low density or patchy distribution of *Helicobacter spp.* organisms in the histologic specimen.<sup>25,32</sup> Bismuth compound, antibiotic, or proton pump inhibitor use can also cause false negative test results as these drugs can suppress, but not eradicate, *H. pylori*.<sup>24,25</sup>

The CLOtest®, a commercially available urease test, has not been previously compared to histopathologic evaluation in dogs to the authors' knowledge. A non-commercial modified rapid urease test has previously been investigated using samples derived from 10 deceased dogs.<sup>22</sup> The results were determined at 30 and 60 minutes from samples obtained from the fundus, corpus, and antral regions of the stomach. Prevalence of positive samples at 60 minutes was 95%, 100%, and 62%, respectively.<sup>22</sup> At 60 minutes, the sensitivity was 87.5% and specificity was 100%.<sup>22</sup> In the current study, we found a high sensitivity and specificity for the CLOtest® compared to the modified Steiner silver impregnation technique. The overall CLOtest® results in this study yield similar sensitivity and specificity to those previously reported in the human literature.<sup>9,19-21,26</sup>

Upper gastrointestinal endoscopy is a good diagnostic test for dogs that are presented for chronic vomiting. Previous endoscopic evaluations for determination of *Helicobacter spp.* prevalence that have been reported have histopathologically evaluated the fundic gland region only<sup>17</sup> and the cardiac, antral and fundic regions only.<sup>16</sup> This is the first report in the literature that provides the sensitivity and specificity of a commercially available rapid urease test for veterinary medicine in live, healthy dogs. Identification of *Helicobacter spp.* by CLOtest® will yield an accurate early diagnosis before histopathologic findings are available, therefore treatment may be initiated earlier. When results of the CLOtest are positive, it has been suggested that there is no diagnostic benefit in performing additional tests to diagnose *Helicobacter pylori*.<sup>33</sup> Histopathologic evaluation, however, has the advantage of identifying *Helicobacter spp.* organisms in gastric glands and parietal cells, as well as evaluating for inflammatory changes.

The results of this study suggest that the prevalence of *Helicobacter spp.* in apparently healthy dogs is high. The prevalence of positive histopathologic evaluation in this study was less than 100% in the individual gastric regions because, by definition, an animal was considered to be overall positive if histopathologic evaluation was positive in any *one* gastric region. Therefore, a dog may not have had organisms identified in one or more of the four gastric regions, yet may have been considered overall *Helicobacter spp.* positive if organisms were found in at least one of the gastric regions. When *Helicobacter spp.* was identified in any region of the stomach, a tissue sample from the angularis incisura was most likely to result in positive

identification of *Helicobacter spp.* organisms, while organisms were least likely to be identified by histopathologic evaluation in the pyloric antrum. Although the cardia and the angularis incisura both had similar prevalence of *Helicobacter spp.*, the sensitivity, although not significantly different from one another, was much lower.

The high prevalence of *Helicobacter spp.* organisms in this study may be related to the dogs housing environment. *H. pylori* has been shown to be transmissible by contact from infected to non infected dogs.<sup>34</sup> Although the dogs in this study were housed individually and separated by cinder block walls, each dog was exposed to the same common area while its run was being cleaned. Drainage from each individual run was not separate, although a 12 inch space separated an overlying grate from the drainage gutter.

The overall CLOtest® results in this study yield similar sensitivity and specificity to those previously reported in human literature, where the antrum and body of the stomach are most commonly evaluated for identification of *Helicobacter pylori*.<sup>28,35,36</sup> However, in our study the CLOtest® from the angularis incisura had the highest sensitivity and specificity when evaluated individually. We suggest that for a practical, quick, inexpensive, and effective method for detecting the presence of *Helicobacter spp.* in dogs undergoing upper gastrointestinal endoscopy, a tissue sample should be taken from the angularis incisura for CLOtest® sampling. Samples taken from this region will have the highest sensitivity, specificity, and positive predictive value with respect to CLOtest® results.

<sup>a</sup> Ivomec 1% ®, MSD-Ag Vet, Rahway, NJ

<sup>b</sup> Panacur Suspension ®, Hoeschst, Kansas City, MO

<sup>c</sup> Albon ®, Roche, Nutley, NJ

<sup>d</sup> Flagyl ®, Searle, Chicago, IL

<sup>e</sup> Hill's Science Diet Canine Maintenance, Topeka, KS

<sup>f</sup> Fujinon EG7-FP2 Video Endoscope, Fujinon Inc. Wayne, NJ

<sup>g</sup> Tri-Med Specialties, Inc., Lenexa, KS



**Figure 1. Numerous spiral shaped bacteria within a gastric pit. Canine gastric mucosa. Modified Steiner technique. Bar = 7.8  $\mu$ m.**

**Table 1. Prevalence of *Helicobacter spp.* by CLOtest® and histopathologic evaluation in four gastric regions.**

|                    | CLOtest®<br>(%) | Histopathologic Evaluation<br>(%) |
|--------------------|-----------------|-----------------------------------|
| Pyloric Antrum     | 40              | 34                                |
| Angularis Incisura | 83              | 87                                |
| Greater Curvature  | 67              | 86                                |
| Cardia             | 83              | 86                                |

**Table 2. The results of CLOtest® sensitivity, specificity, positive and negative predictive value compared to histopathologic evaluation.**

|                              | Sensitivity<br>(%) | Specificity<br>(%) | Positive Predictive<br>Value (%) | Negative Predictive<br>Value (%) |
|------------------------------|--------------------|--------------------|----------------------------------|----------------------------------|
| Total samples<br>(n=117)     | 84                 | 81                 | 92                               | 64                               |
| Pyloric Antrum<br>(n=29)     | 70                 | 79                 | 64                               | 83                               |
| Angularis Incisura<br>(n=30) | 92                 | 94                 | 96                               | 60                               |
| Greater Curvature<br>(n=29)  | 80                 | 100                | 100                              | 44                               |
| Cardia<br>(n=29)             | 84                 | 75                 | 95                               | 43                               |

**Table 3. Prevalence and p-values of CLOtest® and histopathologic positive samples in four gastric regions compared to dogs determined to be overall *Helicobacter spp.* positive (defined as histopathologic evaluation positive in any one gastric location; n=27; \* p<0.05 considered significant)**

|                       | CLOtest®<br>(%) | CLOtest®<br>p-value | Histopathology<br>(%) | Histopathology<br>p-value |
|-----------------------|-----------------|---------------------|-----------------------|---------------------------|
| Pyloric Antrum        | 44              | 0.2                 | 38                    | 0.63                      |
| Angularis<br>Incisura | 93              | 0.002*              | 96                    | 0.0009*                   |
| Greater<br>Curvature  | 74              | 0.03*               | 96                    | 0.001*                    |
| Cardia                | 85              | 0.008*              | 96                    | 0.001*                    |

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## VITAE

Michele Elan Reimer (formerly Allyn) was born on September 20, 1969 in Lakewood, New Jersey, daughter of Susan and John Allyn. Michele attended the Lacey Township High School before attending Cook College, Rutgers University in 1987. She graduated with honors obtaining her Bachelor of Science degree in Animal Science in 1991. Michele then attended the University of Tennessee College of Veterinary Medicine, where she graduated *summa cum laude* in 1995. She was awarded the American Animal Hospital Association Senior Student Award for excellence and proficiency in the practice of small animal clinical medicine and surgery.

Michele began her internship program at the Virginia-Maryland Regional College of Veterinary Medicine in July 1995. She received her Certificate of Internship in June 1996 and began a medicine residency the following month. Michele passed the qualifying portion of the boards examination for the American College of Veterinary Internal Medicine in May 1998. Michele married Dr. David Reimer, her best friend, in October 1998. After completing her case reports and publication requirements, Michele is eligible to sit for the certifying examination in June 1999. She plans on entering a private specialty practice after the birth of their first child in September 1999.