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GASTRIC ULCERATION IN THE HORSE

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ABSTRACT OF THESIS (Regulation 3.5.10)

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A survey relating to the foal gastroduodenal ulcer syndrome was distributed to 55 equine practices in Ireland and Britain. Respondents had a annual responsibility for 6650 foals. The occurrence of the syndrome was confirmed but the incidence was low (0.58%).

Ulceration or erosion of the proventricular mucosa was noted in 173(28.2%) of 614 horses examined at an abattoir. Ulcers were less than 1cm^2 in 53(31%) of horses, between 1cm^2 and 5 cm^2 in 69(40%) of horses, and greater than 5 cm^2 in 51(29%) of horses. Lesions occurred close to the margo plicatus in the region of the lesser curvature in 89% of horses. In 135 (26%) of horses, verrucose proliferations of the margo plicatus at the lesser curvature were recorded. Of 271 Thoroughbred horses examined 90(33%) had ulcerations or erosions but only 43(24%) of 182 ponies examined had ulcers / erosions, a statistically significant difference ($p<0.05$).

The prevalence of bots larvae was 43%. Only 60 (37 %) of horses with ulceration had concurrent bot burdens and no statistical relationship between bots and the occurrence of ulceration existed.

Stomach tissue samples (largely from the proventricular mucosa) from 121 horses were examined histologically. Epithelial change was characterised by increased epithelial thickness, increased depth and irregularity of the rete pegs, hyperkeratosis and hydropic degeneration. Additionally in acute erosions, reticular degeneration and spongiosis was noted. Acute and subacute ulcers describe lesions where the deficit extended down to the lamina propria and in chronic ulcers and erosions fibrosis was a significant feature. Scarring was characterised by increased corrugations of the epithelium, fibrosis of the lamina propria and ectopic glands.

Sera from 71 horses, 41 of which had gastric pathology were examined using an ELISA, for antibody against *Helicobacter pylori*. A statistically significant difference ($p<0.05$) in the mean *H. pylori* optical density values (OD) between horses with gastric pathology (0.53) and horses with no pathology (0.375) was noted. There was a overlap between OD values in horses with gastric pathology (0.17-1.11) and normal horses (0.16-0.73).

Serum gastrin levels (n=151) ranged from <20 pg /ml to 96 pg /ml. There was a statistically significant relationship ($p<0.01$) between serum gastrin levels and the weight of gastric contents but no association occurred between serum gastrin levels and gastric pathology.

Serum pepsinogen levels (n= 85) were not associated with gastric pathology .

"Two roads diverged in a wood, and I-
I took the one less traveled by,
and that has made all the difference."

Robert Frost.

DEDICATION.

To my parents and my wife Anne,
Whose sacrifices at different stages
Allowed me travel this far.

DECLARATION.

The work contained in this thesis, with the exception of where otherwise acknowledged, is entirely my own work. Furthermore, this thesis has been written and compiled by myself.

Harry J. Sweeney.

12 August ,1993.

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CONTENTS

CHAPTER 1

GASTRIC ULCERATION: A REVIEW

Introduction.

Peptic ulcer disease is arguably the most important upper gastrointestinal disease in man with resultant high economic costs (Sipponen 1989). Gastric ulceration has also been described in many other species including the horse (Hammond et al. 1986), pig (O'Brien 1986), cattle (Jensen et al. 1976), calf (Welchman and Baust 1987), dog and cat (Twedt and Magne 1989), rabbit (Hinton 1980; George and Somvanshi 1987), fox (Shillinger 1929), seals (Schroeder and Wegeforth 1935), sheep (Jensen and Frederick 1939), ferret (Fox 1988) , mouse (Fox et al. 1984) and guinea pig , rat and mink (O'Brien 1969). Gastric ulcers, many of them fatal, have been recorded in diverse zoological animals including such species as hedgehog, monkey, potto, Bennett's wallaby, sloth, beaver, muskrat, porcupine, agouti, coypu, coyote, raccoon, mink, otter, hyena, tiger, ocelot, walrus, elephant, hyrax, tapir, forest hog, yak, gayal, antelope, and gazelle (O'Connor Halloran 1955). In birds ulceration has been recorded in the crop, gizzard and proventriculus (O'Connor Halloran 1955).

Comparative anatomy and physiology.

Animals are conventionally classified on the basis of their

eating habits into three categories; carnivores (meat eaters) have simple stomachs with enzymatic digestion and minimal microbial digestion; herbivores (forage eaters) in contrast depend predominantly on microbial fermentation and this group can be further divided into animals with compartmentalised pregastric specialised fermentation regions (rumen) such as cow and sheep and those with simple stomachs (horse) in which microbial fermentation takes place in the distal part of the digestive tract and finally omnivores which feed on both animals and plants but whose digestion is mainly alimentary enzymatic in nature (Argenzio 1984). The digestive tract of the dog is relatively short [ratio of body length to intestinal length (BL:IL) = 1 : 6] with a simple noncompartmentalised stomach and only a small caecum, a pattern common in many carnivores. The pig also has a simple stomach but has a caecum , sacculations of the colon and a longer small and large intestine (BL:IL 1:14). The equine stomach is similar to the pig but the equine caecum and colon are much more voluminous and the small intestine is shorter (BL:IL = 1:12). The rat, usually a herbivore, and the rabbit follow the same general pattern as in the horse with the exception that the rat's stomach is slightly compartmentalised. Cows , sheep and goats have a highly compartmentalised and voluminous stomach with a reticulorumen section, an omasum and an abomasum but the small and large intestine in these species are relatively simple. In many herbivores, there appears to be an inverse relationship between the relative complexity and capacity of the stomach versus the large intestine. The capacity of the equine stomach varies from 8-15 l and expressed as a percentage of the total capacity of the gastrointestinal tract is only 8.5% , considerably smaller than other

domestic species (ox 70.8%; sheep 67.9%; cat 69.5%; dog 62.3% and pig 29.9%, Argenzio 1984)

The major functions of the stomach include storage, maceration and physical breakdown of food. Additionally, in most vertebrates, protein digestion begins in the stomach through the action of pepsin and hydrochloric acid. In general, there are three different histologically recognisable areas within the mucosa of the stomach based on the predominant gland type but no gross distinct lines of demarcation exist. The area close to the gastroesophageal junction, the cardiac region, has a predominance of mucus secreting cardiac glands. The fundic glands, which in addition to containing mucus secreting cells have hydrochloric acid producing parietal cells and pepsinogen secreting chief cells are concentrated in the body mucosa (proper gastric glandular mucosa).The pyloric glands which largely consist of mucus secreting cells and some gastrin producing cells are located in the pylorus (pyloric mucosa). However, the stomachs of many species (belonging to ten of the mammalian orders) contain an additional region of nonglandular stratified squamous epithelium extending from the oesophageal sphincter. In the pig this area is small (5cm x 8cm) but in the horse, rat, mouse, hamster, guinea pig, gerbil, kangaroo, anteater and muskrat, 30% or more of the entire surface area of the stomach is involved (Stevens 1988; Ghoshal and Bal 1989). In ruminants, both the reticulorumen and the omasum are entirely lined by stratified squamous epithelium, whereas the abomasum contains proper gastric and pyloric glandular tissue. The stomach of insectivores also has a prominent stratified squamous epithelium and it is believed that this mucosa protects the stomach of insectivores from insects and that of herbivores from plant roughage (Stevens 1988).

In this respect, the stomachs of the rabbit and hare (strict herbivores) are curious because they are entirely lined by glandular mucosa. The equine gastric proventricular mucosa, unlike the ruminant forestomach is almost completely impermeable to volatile fatty acids (Argenzio 1990). The stomach of man, dog , cat and most carnivores does not contain a stratified squamous epithelial mucosa.

Classification of gastric ulceration:

Three distinct types of ulceration commonly occur in the stomachs of man and animals and are described below.

Stress ulceration (Synonyms: acute haemorrhagic gastritis, acute erosive gastritis). Stress ulceration is characterised by congestion, oedema of the gastric mucosa accompanied by petechial haemorrhages , numerous bleeding focal erosions and acute ulcers which arise 3-6 days after the onset of a severe stress such as widespread burns (Curling's ulcer) , severe intracranial or spinal trauma (Cushing's ulcer), therapeutic drugs or alcohol and usually heal without scarring (Sloan 1989). Stress ulceration is an acute condition and lesions are often superficial, without penetrating the muscularis mucosa and therefore correctly should be called erosions.

Peptic ulceration Peptic ulceration is a term used to refer to ulcerative disorders of the upper gastrointestinal tract, involving principally the duodenum and the stomach in which acid and pepsin are involved in the pathogenesis (McGuigan 1991). These ulcers are chronic , are usually solitary (Sipponen 1989) and penetrate the muscularis mucosa (Lee 1985). Peptic ulceration is about three or four times more common in the duodenum in man than the stomach

(Spiro 1983) but the subsequent discussion will be limited to aspects of gastric peptic ulceration.

Squamous mucosa ulceration. Ulceration occurs in isolation or with a much higher incidence in the squamous mucosa of some species including the pig (O' Brien 1986) and horse (Hammond et al. 1986) and it has been suggested that these lesion should be considered separately from other gastric lesions (Rothenbacher et al. 1963; Muggenburg et al. 1964).

Pathogenesis of ulceration.

Irrespective of the various causes of ulceration which will be discussed later, it is probable that the pathogenesis of ulceration in different species is similar. Irrespective of the specific cause, ulceration is likely to develop because of disruption of the gastric mucosal barrier which protects the gastric mucosa from the potentially harmful effects of gastric acid and pepsin. A delicate balance exists between mucosal protective factors and the aggressive effects of acid, pepsin and bile salts and disruption of this delicate interplay allows diffusion into the mucosa of hydrogen ions and pepsin resulting in auto digestion and ulcer formation (Crampton and Rees 1986). The major mucosal protection factors include the mucus layer into which bicarbonate is secreted and which neutralises acid diffusing towards the epithelium, provides a barrier against pepsin and protects against mechanical shearing damage by luminal contents; the rapid proliferation of epithelial cells which can result in extensive re-epithelisation within an hour of mucosal damage; tight epithelial intercellular junctions and a trans-mucosal potential difference discourage the back diffusion of acid; mucosal blood flow which in

addition to maintaining the integrity of the other mucosal protection components, disposes of any hydrogen ions which have diffused in from the lumen and thus maintains mucosal acid-base balance and finally, prostaglandins which have a cytoprotective action mediated by an increase in mucus and bicarbonate secretion and increasing gastric mucosal blood flow (Sloan 1989). A significant trophic effect is provided by other hormones including gastrin, glucagon and the epidermal growth factor which significantly stimulate cell renewal and repair (Sipponen 1989).

Although acid is involved in the pathogenesis of gastric stress ulceration, the major initiating cause is believed to be ischaemia of the gastric mucosa (which has a high metabolic rate and is highly energy dependent) caused by hypotension or hypovolemic shock with resulting compromised bicarbonate secretion, mucosal buffering , prostaglandin synthesis and the disposal of hydrogen ions. However, in addition to ischaemia of the mucosa, other adverse factors may be required such as duodenal reflux of bile, increased acid and gastrin secretion and increased gastric motility and tonicity secondary to central stimulation of the parasympathetic nervous system in severe central nervous system disease or injury (Sloan 1989). Furthermore, patches of gastric mucosa are supplied by end arteries with no collateral blood supply and such areas may be more vulnerable to decreased perfusion resulting in erosion (Piasecki 1979; Jones 1979). However, ischaemia is only one in a series of aetiological factors in stress ulceration but it is probably the most important one. It is interesting that studies on sepsis induced stress ulceration in pigs indicated increased rather than decreased mucosal blood flow (Sloan 1989). A combination of ischaemia, bile salts and acid is highly

ulcerogenic.

Chronic peptic ulceration has a more complicated aetiopathogenesis and as yet remains largely unexplained (Lee 1985). Although very few acute ulcers progress to chronic ulcers, it is impossible to conceive that a chronic ulcer does not start with a lesion resembling an acute ulcer (Piper 1984). The factors that determine chronicity are largely unexplained but the exposure to acid and pepsin is essential for ulcer formation (McGuigan 1991). However, most patients with gastric peptic ulcers have normal basal and maximal acid outputs (Baron 1984) and a portion have a reduced gastric acid secretion (Spiro 1983). Therefore it seems probable that reduced mucosal resistance is involved in the pathogenesis of peptic ulcers (Lee 1985) and it is interesting that the distribution of areas of mucosa supplied only by end arteries with no collateral blood supply is similar to the distribution of peptic ulcers (Sloan 1989). Conversely, increased acid production and increased serum pepsinogen is associated with duodenal ulceration in man (Wastell 1979).

Pathology.

Stress ulceration is characterised by congestion and oedema of the gastric mucosa accompanied by petechial haemorrhages, numerous bleeding focal round shallow erosions (1-3 mm), and acute ulcers containing fresh or altered blood, and in man, occur principally in the fundus and body of the stomach. Histologically, lesions are usually superficial with focal necrosis and haemorrhage into the crater and extravasation of blood into adjacent intact mucosa. Within 2-3 days an inflammatory response consisting largely of polymorphs , lymphocytes, plasma cells and

eosinophils occurs. Re-epithelisation and healing occurs usually without scarring. Occasionally the lesion penetrates the muscularis mucosa. Drug induced ulcers may be less numerous but histologically are similar and in the horse, mucosal atrophy (Collins and Tyler 1985) and microthrombosis of the pyloric mucosa have been recorded (Meschter et al. 1990).

Peptic ulcers are usually singular, rarely exceed 3 cm in diameter and are usually round or oval with sharply defined deeply shelving margins. Histologically, the wall of the ulcer has a layer of polymorphs on the surface covering an intensely eosinophilic layer of fibrinoid necrosis (Lee 1985). Underlying this is a zone of organising granulation tissue of variable thickness. Deeper still is a layer of mature fibrous scar tissue. In man, peptic ulcers usually occur on the lesser curvature (80%) and occur in the upper, middle and lower thirds of the stomach in 17%, 42% and 41% of cases respectively.

Ulceration of the squamous mucosa in pigs and horses have a number of common factors and are characterised by epithelial changes of parakeratosis, irregularity of the rete pegs, spongiosis and intracellular odema leading to erosion and ulceration (O'Brien 1986, Yamagishi et al. 1986).

Haemorrhage is common in association with acute stress lesions in man (Sloan 1989) and occurs in association with about 25% of peptic ulcers (McGuigan 1991). In the adult horse haemorrhage from gastric ulceration is not common but has been recorded in the foal (Becht and Byars 1986). In veal calves ulcers do not cause severe haemorrhage (Welchman 1986).

Ulcer location.

An interesting feature regarding the location of gastric ulcers is their frequent location in a number of species close to a mucosal junction. Stress ulcers in man occur in the fundus and body usually along the greater curvature, where as peptic ulcers are usually found on the lesser curvature immediately distal to the junction of the antral mucosa with the acid secreting mucosa of the body of the stomach (McGuigan 1991). Abomasal ulcers are predominantly pyloric in the calf but the location of perforations is close to the pyloric / fundic mucosal junction on the greater curvature (Welchman 1986). The location of 95% of 269 gastric ulcers in calves also coincided with a mucosal border (Welchman and Baust 1987). In the horse, the majority of ulcers occur in the proventricular mucosa close to the margo plicatus, the junction with the glandular mucosa (Hammond et al 1986; Murray et al. 1989).

Aetiology of gastric ulceration.

Pharmacological

Analgesics and anti-inflammatory drugs such as salicylates (aspirin) and nonsteroidal anti-inflammatory drugs (NSAID's) including phenylbutazone and flunixin have been shown to cause multiple stress ulcers in man, horse and the dog (Traub-Dargatz 1987; Sloan 1989; Meschter et al. 1990). Salicylates damage the mucous bicarbonate layer thus allowing back diffusion of acid while NSAID's interfere with prostaglandin synthesis (Sloan 1989). Corticosteroids are now not considered to cause ulceration (Lendrum 1984_b). In man, smoking and alcohol consumption have been associated with gastric ulceration

(Lendrum 1984_b; Jones 1979; Sipponen 1989).

Diet.

In man, diet may be of limited significance as a cause of ulceration but fresh vegetables and a high fibre diet may protect against ulceration (Sipponen 1989). Coffee, spices and Japanese pickles have been suggested as causes of gastric ulcers (Jones 1979, Lendrum 1984_b). In the horse, high fibre may also be protective (Coenen 1990; Hammond et al 1986) but in the calf access to straw was associated with an increased incidence of ulceration (Welchman and Baust 1987). Too much milk replacer has been suggested as a cause of ulceration in veal calves (Welchman 1986) and diet has also been indicated in feedlot cattle and lambs (Jensen et al. 1976). In feedlot cattle the prevalence of ulceration is 3.6% as compared to one per cent in normal adult animals (Van Kruiningen 1988).

The method of food processing is a further factor in the aetiology of gastric ulceration and in man, milled rice and refined wheat may cause ulceration (Jones 1979). Equally in the pig it has been demonstrated that heat and cold pelleting and the fineness of particle size in compounded diets are major contributing factors in the occurrence of gastric ulceration (Driesen et al. 1987).

Infectious agents.

The association between *Helicobacter pylori* (formerly *Campylobacter pylori*), gastritis and gastric ulceration is receiving enormous attention in man (Sloan 1989). Campylobacter like organisms have been described in association with gastric ulceration in the ferret (Fox 1988). Many infectious agents have been incriminated in the horse including rotavirus, *Helicobacter* sp.,

Campylobacter sp., *Clostridium* sp., *Salmonella*, *Escherichia coli* and *Candida* sp. but as yet none have been proven (Sweeney 1991). *Candida* sp. have also been considered in the aetiology of ulceration in the pig (Embaye 1987).

Parasites

In the horse, *Gasterophilus intestinalis* has often been incriminated as a cause of ulceration (Rooney 1964; Waddell 1972) but recent evidence would tend to contradict this (Sweeney 1990). The fish parasite *Eustomatum rotundum* causes chronic ulceration in people who eat raw fish (Lendrum 1984_b) and *Ascaris suum* has been incriminated in porcine gastric ulceration (Qureshi et al. 1978). Gastric nematodes may be important in the pathogenesis of ulceration in seals (Schroeder and Wegeforth 1935).

Acid.

In duodenal ulceration in man, acid hypersecretion occurs in 50 % of cases (Sipponen 1989) . However, most patients with gastric peptic ulcers have normal basal and maximal acid outputs (Baron 1984) and a portion have a reduced gastric acid secretion (Spiro 1983). Hammond (1990) has shown that the pH of the proventricular portion of the equine stomach is lower than the glandular portion and furthermore, the area where erosion and ulceration commonly occurs in the adult horse (the junction of the squamous and glandular mucosa at the margo plicatus in the region of the lesser curvature) had the lowest pH of all. In addition, horses with ulceration of the squamous mucosa close to the margo plicatus had a significantly lower pH at this site than normal horses. He suggested that gastric acidity was significantly related to gastric ulceration in the adult horse as did Murray and Grodinsky (1989).

Jensen et al. (1976) suspected that lactic acidosis from overeating fermentable concentrates by unadapted ruminants in which the ruminal lactic acid increases to 320mm/l and histamine to 70$_{u}$g/ml could cause abomasal ulceration.

Psychopathology.

There is a widely held belief that personality traits and mental stress make important contributions to the milieu in which peptic ulcers thrive. However, stressful events such as illness, family bereavement or financial difficulty occur no more frequently prior to exacerbations of gastric or duodenal ulceration than in matched control patients (Lendrum 1984$_{b}$). It is known that in man, anxiety, resentment, hostility and aggression (not leading to action) cause increased gastric acid secretion and motor function and that dejection and despair have the opposite effects (Dotevall 1985) but these conclusions are largely based on research carried out over 160 years ago. More recent research has shown that in general, personality features of hostility, irritability, hypersensitivity and impaired coping ability (low ego strength) each correlate significantly with serum pepsinogen concentrations in ulcer patients and it was postulated that emotional stress may predispose to ulcers by producing gastric hypersecretion as manifested by hyperpepsinogenaemia (Walker et al. 1988). Psychosomatic factors such as aggression, transport, mixing and being housed in groups have been discussed as causes of ulceration in veal calves (Welchman 1986) and Dammrich (1983) proposed that abomasal ulcers in calves were indicators of animal welfare. However, Welchman and Baust (1987) concluded that abomasal ulcers were

not deleterious to growth rate or welfare. Jensen et al. (1976) concluded that the stress of shipping may cause gastric ulceration in feedlot cattle and sheep and transport has also been incriminated in the horse (Acland et al. 1983). Weather factors were incriminated in ulceration in the foal (Dwyer and Powell 1989) and a seasonal variation in incidence has been reported in the pig (O'Brien 1969). The stress of parturition , weaning and low plasma antibody have been discussed in the foal in connection with gastric ulceration (Wilson 1986).The occurrence of stress ulceration in the trap death syndrome is well recognised and ulcers can be induced in laboratory animals by restraint and other social factors (Jubb, Kennedy and Palmer 1985_a). In pigs, stresses such as increased stocking density, transport and mixing of unfamiliar pigs resulted in increased prevalence of ulceration (Muggenburg et al. 1967).

Epidemiological aspects of gastric ulceration.

In man, there is a strong genetic component to increased susceptibility to peptic ulcer and an association with inherited characteristics such as blood group O and non secretion of A, B and H substances have been demonstrated (Lendrum 1984_b). Gastric ulcers are more common in men (three times)and peak incidence occurs between the ages of 60- 64 years (Lendrum 1984_b, Sipponen 1989). Other aspects include geographical factors, gastric ulcers being rare in India, common in England and less common in the United States of America; social class, an increased incidence occurring in the lower social classes and urbanization (Watkinson 1979). No breed or sex difference occurred in ulceration in veal calves (Welchman 1987) but an increased incidence of

ulceration was recorded in female dogs (Murray et al. 1972). In rabbits, ulceration is more common in does and the incidence increases with age. A seasonal factor in the prevalence of gastric ulcers in pigs and cattle and in several other domestic animals has been described (Jensen and Frederick 1939; Van Kruiningen 1988). Although no breed difference has been demonstrated, a familial effect in the occurrence of ulceration in pigs and a higher incidence in barows has been recorded (Moran 1982; Driesen et al. 1987).

Incidence of gastric ulceration.

In man, about 10 % of the population are affected by peptic ulcer disease during their lifetime and in the United States of America four million people simultaneously have active peptic ulcer disease but duodenal ulcers outnumber gastric ulcers clinically by approximately 3 : 1 (Sipponen 1989). It is important to note that at necropsy , gastric ulceration is as common as duodenal ulceration (McGuigan 1991). In horses, about 50% of foals and 30 % of adult horses have gastric ulceration, predominantly in the proventricular mucosa but these lesions are usually asymptomatic (Murray et al. 1990; Sweeney 1990). In cattle, 3 -3.5% of fattened cattle have abomasal ulcers (Jensen et al. 1976) but 87% of veal calves have ulceration (Welchman and Baust 1987). The prevalence of ulceration in the pig varies greatly and reports vary from 2.5% to 100% (O'Brien 1986).In a study of 1289 rabbit autopsies, ulceration occurred in 33 (2.6%) (George and Somvanshi 1987) and a similar survey of 1000 autopsies revealed a 7.3 % incidence of ulceration but no ulceration in 137 controls (Hinton 1980). Gastric ulcerations were recorded in all of 47 seals examined by Schroeder and

Wegeforth (1935). Stress ulcerations are common in dogs, especially in the intensive care patient or dogs with severe neurological disease and have a significant associated mortality but peptic ulceration is less common (Twedt and Magne 1989) and peptic ulcers are rare in the cat.

Clinical syndromes

In man, typical peptic ulcer symptoms include a nagging cramp-like epigastric or left hypochondrium pain which is often nocturnal and may be accompanied by flatulence, abdominal distension, nausea and belching (Morgan 1984). However, up to 75% of gastric peptic ulcers may be asymptomatic (Sipponen 1989; McGuigan 1991). In the foal, the majority of ulcers are asymptomatic but in clinical cases salivation, mild abdominal pain and teeth grinding are noted (Becht, Hendricks and Merritt 1983). In the adult horse the majority of ulcers are asymptomatic but recently ulcers have been indicated as causing ill thrift, colic and poor appetite (Murray 1988) but as yet such conclusions have not been definitively confirmed. Blood tinged vomitus is the most significant sign in dogs (Twedt and Magne 1989). Pale, weak, anaemic and unthrifty pigs are suggestive of gastric ulceration (O'Brien 1969). In cattle and calves no clinical signs associated with ulceration are usually seen unless ulcer perforation has occurred (Jensen et al. 1976; Welchman 1986).

Diagnosis.

In man and most species a tentative diagnosis of ulceration is usually made on clinical signs alone. In man, as in the horse and dog, endoscopy is the method of choice to confirm the

presence of ulceration (Adamson and Murray 1990) but in man and the dog, double contrast barium radiographic examination offers another acceptable alternative.

Treatment of gastric ulceration.

The aim of ulcer treatment is to hasten ulcer healing by reducing gastric acidity and protecting the ulcer from further insult by pepsin or gastric acid. A number of possible options to achieve this end are available . The stimulus for effective secretion of hydrochloric acid by the parietal cells in the gastric mucosa requires three separate chemical mediators (secretagogues), namely gastrin, histamine and acetylcholine (Argenzio 1984). The blocking of any of these receptor sites on the parietal cell results in effective control of acid secretion and it is in this way that the histamine type 2 (H_2) receptor antagonist drugs (cimetidine, ranitidine, fomotidine and nizatidine) mediate their effect (Lewis 1983). Histamine type 2 antagonists have been used extensively in horses (Campbell-Thompson andMerritt 1987_a; MacAllister, Sangiah and Amouzadeh 1987; Furr and Murray 1989) and in the dog and cat (Twedt and Magne 1989).

Anticholinergic agents have been recommended in the treatment of gastric ulceration in man but because of associated side effects including delayed gastric emptying and lack of evidence as to their effect their use does not appear justified (McGuigan 1991). In the dog the use of anticholinergics in association with H_2 receptor antagonists is more potent than either drug used in isolation (Moreland 1988).

Active secretion of hydrochloric acid is accomplished by the proton pump (acid pump) located in the apical secretory membrane of the parietal cell and actively transports hydrogen ions into the gastric lumen in exchange for potassium ions (Carlsson and Wallmark 1986) . Since the hydrogen ion concentration in the gastric mucosa is some three to four million times greater than that of plasma, a large amount of energy (circa 1500 cal per liter of gastric juice) is required to transport the hydrogen ion into the lumen (Argenzio 1984). Hydrogen potassium adenosine-triphosphatase (H+,K+ ATPase) is the enzyme responsible for hydrolysing adenosine-triphosphate (ATP) to provide energy for the proton pump and the development of a proton pump inhibitor, omeprazole, which binds directly to the H+,K+ ATPase enzyme offers a new and precise approach to the control of gastric acid secretion (Carlsson and Wallmark 1986). Omeprazole (Losec ®), a substituted benzimidazole, has proved to be a powerful inhibitor of gastric acid secretion in man and several animal models (dog, rat, mouse and guinea pig) and higher ulcer healing rates, more effective pain relief, and lower recurrence rates were recorded when omeprazole was compared with ranitidine and cimetidine in the treatment of gastric and duodenal ulcer in man (Anon. 1988). Omeprazole targets very precisely the proton pump of the parietal cell (pH 1) and is biologically inactive at the physiological pH (7.1) prevailing in other organs and consequently as may be expected, adverse reactions are uncommon and occur with similar frequency to that recorded with placebo or ranitidine treatment (Anon. 1988). Omeprazole has also been investigated in the horse (Sangiah, MacAllister and Amouzadeh 1989).

Carbenoxolone , a hydrolytic product of glycyrrhizic acid

(derived from licorice root), has been shown to decrease symptoms and increase the rate of ulcer healing in man by increasing the life span of gastric mucosal epithelium by 50% and increasing the secretion and viscosity of mucus but aldosterone-like side effects including sodium and water retention have reduced it's popularity (McGuigan 1991).

Antacids such as magnesium hydroxide and aluminium hydroxide (Aludrox ®; Maalox ®) neutralise gastric acid when administered orally but require frequent administration and have the further disadvantage of diarrhoea and constipation as side effects (Nappert, Vrins and Larybyere 1989) and have little role to play in the therapy of equine, canine and feline gastric ulceration (Twedt and Magne 1989). However, in man antacids have an equal efficacy to H_2 receptor antagonists (McGuigan 1991).

Synthetic analogues of the prostaglandin E group (PGE) have cytoprotective properties mediated by an increased viscosity of gastric mucus, increased mucosal blood flow, increased bicarbonate secretion and reduced stimulated gastric acid secretion (Misiewicz 1988) and have been indicated in the therapy of equine gastric ulceration (Becht and Byars 1986). However, the results of clinical trials in man using five different PGE derivatives (arbaprostil, trimoprostil, misoprostol, endprostil and rioprostil) revealed clinical results similar to ranitidine but poorer pain relief and diarrhoea as a side effect (Misiewicz 1988). In the horse, the administration of prostaglandin E^2 reduced the development of phenylbutazone induced gastric ulcers (Collins and Tyler 1985) and the administration of misoprostol to healthy adult horses resulted in an increase of pH to greater than 3.5 for a period of two hours and a

concomitant 65 to 99 per cent reduction in basal free acid content for a period of eight hours (Sangiah et al. 1989).

Sucralfate (Antepsin ®) is a complex of aluminium hydroxide and a sulphated sucrose and when exposed to acid forms a viscous, adherent chemical complex which binds to the site of ulceration, protecting it from gastric insult (Lewis 1983). It's cytoprotective effects are derived from it's ability to inhibit the action of pepsin, deplete bile salts, increase luminal release of cytoprotective prostaglandin E2 and neutralise small amounts of acid (Lewis 1983). It is well tolerated in the foal and two to four grams three or four times daily have been recommended (Becht and Byars 1986). In man an efficacy equal to that of cimetidine has been demonstrated when sucralfate alone is used in the treatment of peptic ulcer (Lewis 1983). Other cytoprotectants used in man include tri-potassium di-citratobismuthate (De-Nol ®) which additionally has a bactericidal-like action against *Helicobacter pylori*

Clenbuterol, a beta2-adrenoceptor agonist has been reported to reduce the incidence of ulceration in veal calves from 68% to 17%, presumably by increasing mucosal blood flow (Marcato et al. 1986) but less impressive results were obtained by Breukink et al. (1989). Vissar and Gruys (1989) reported increased growth rate and decreased adipose tissue when clenbuterol was used in veal calves but it has also been associated with increased condemnation of livers and kidneys at meat inspection.

Cimetidine has been used with apparent success in the treatment of suspected abomasal ulceration in zoological ruminants including moose, musk-ox, wapiti and white tailed deer (Haigh 1982).

Complications of gastric ulcers.

Perforation has been recorded in many species including the horse (Valdez 1979), pig (O'Brien 1986), cattle and sheep (Jensen et al. 1976) dog and cat and was particularly common in young women at the turn of the century who wore the then fashionable Victorian corset. Haemorrhage occurs in about 25% of peptic ulcer cases in man (McGuigan 1991) and commonly in ulceration in the pig (O'Brien 1986) but is not a feature of ulceration in the horse. In man, healing of peptic ulcers resulting in scarring may cause pyloric stenosis (hour glass stomach). Anaemia has been described in man in association with gastric ulcers (Lee 1985) and is a common finding in the pig.

Ulceration in association with other diseases.

Peptic ulceration in man may occur in association with and further complicate chronic renal failure, hyperparathyroidism, cirrhosis, cardiovascular disease and chronic respiratory disease (Lendrum 1984_a). Gastric ulceration is recorded in association with the Zollinger- Ellison syndrome in the dog, cat and man (Lee 1985; Twedt and Magne 1989). Also in the cat and dog, renal disease (decreased renal metabolism of gastrin resulting in elevated serum gastrin levels), liver disease, adrenocortical insufficiency , systemic mastocytosis and inflammatory bowel disease can cause ulceration (Twedt and Magne 1989; Jergens et al. 1990). In calves and cattle, ulcers and erosions are known to be an accompaniment to a variety of infectious diseases including bovine viral diarrhoea, bovine malignant catarrh, infectious bovine rhinotracheitis, salmonellosis and fungal infections (Welchman 1986). In adult cattle, 76% of

animals with confirmed abomasal ulceration had concurrent disease conditions, abomasal displacement occurring in the majority of these animals (Smith et al. 1983). Erosions and ulcers of the glandular region in pigs have been associated with hog cholera, erysipelas and necrotic enteritis (Muggenburg et al. 1964). In the horse, Japanese encephalitis has been reported to cause gastric ulceration (Yamagiwa et al. 1959). In rabbits, major lesions in other organs were found in association with ulceration in 91% and 60% of cases in two individual studies (Hinton 1980; George and Somvanshi 1987).

Economic significance.

About 10 % of the population of developed countries are affected by peptic ulcer disease at some stage during their life time and in the United Sates of America 4 million people simultaneously have active peptic ulcer disease (Sipponen 1989). Therefore in terms of therapy and lost work time the economic cost of peptic ulceration is enormous.

In veal calves, gastric ulceration does not reduce growth rate and is not deleterious to welfare (Welchman and Baust 1987). However, in adult feedlot cattle, about 1.6% of all mortalities are due to fatal perforating abomasal ulcers (Jensen et al. 1976).

In pigs, most recent research suggests that subacute and chronic ulcers do not cause a reduced growth rate and efficiency (O'Brien, 1986; Driesen et al. 1987) and therefore, with the exception of mortalities from acute haemorrhage due to ulceration, the economic significance of ulceration may be small. However, this is in contradiction of earlier work (Blackshaw and Kelly 1980) and in Japan, the Ministry of Agriculture reported in 1971 production loses

of between $15-20 million due to porcine pars oesophageal ulceration.

Direct losses from foal ulceration are small but recently there is a trend towards prophylactic treatment of foals considered at risk and this may be of greater economic significance (Dwyer and Powell 1989). Clinical significance has only recently been attributed to gastric ulceration in the adult horse and if the association of colic, poor appetite and ill thrift and the presence of ulceration is confirmed, equine ulceration could have great economic significance as therapeutic recommendations at current prices amount to about st£700 per individual horse (Furr and Murray 1989).

Gastric ulceration in companion animals is of negligible economic significance.

CHAPTER 2

GASTRODUODENAL ULCERATION IN FOALS

A REVIEW

Introduction.

In 1982 a new clinical syndrome in foals, the foal gastroduodenal ulcer syndrome, the cause of which is attributed to the presence of gastric and / or duodenal ulceration, was first described in the United States of America (Rebhun, Dill and Power 1982) and it has subsequently been recorded in Japan (Yoshihara et al. 1986) and France (Collobert et al. 1987). However, the incidence of the syndrome is low and a recent survey of 531 foal post-mortem examinations in the U.S.A. indicated that ulcer related deaths accounted for only 4.5 per cent of all foal mortalities and ranked approximately equal to central nervous system disease as a cause of foal mortality (Dwyer and Powell 1989). Although only recently recognised as a clinical syndrome, cases of fatal perforating foal gastroduodenal ulceration have been reported in Britain (Orr 1972) and Ireland (O'Reilly 1973).

Clinical signs.

The major clinical signs of the foal ulcer syndrome include teeth grinding (bruxism), salivation (ptyalism), depression, intermittent mild signs of abdominal colic and a preference to lie for prolonged periods in dorsal recumbency (Becht and Byars 1986) . Occasionally, gastric reflux can be elicited by nasogastric intubation and clinical signs are often more pronounced after nursing or feeding. If ulcer perforation occurs, signs of circulatory collapse and a rapidly fatal fulminating peritonitis will be observed. Affected foals are usually under six months of age and clinical signs have been recorded in a one day old foal (Becht and Byars 1986). Affected foals frequently have a history of a recent episode of diarrhoea.

It is important to note that many normal foals and adult horses have gastric ulceration without exhibiting any clinical signs (Hammond, Mason and Watkins 1986) and a recent video endoscopic survey of foals in Ireland and England revealed a 51 per cent prevalence of such asymptomatic ulcers (Murray et al. 1990).

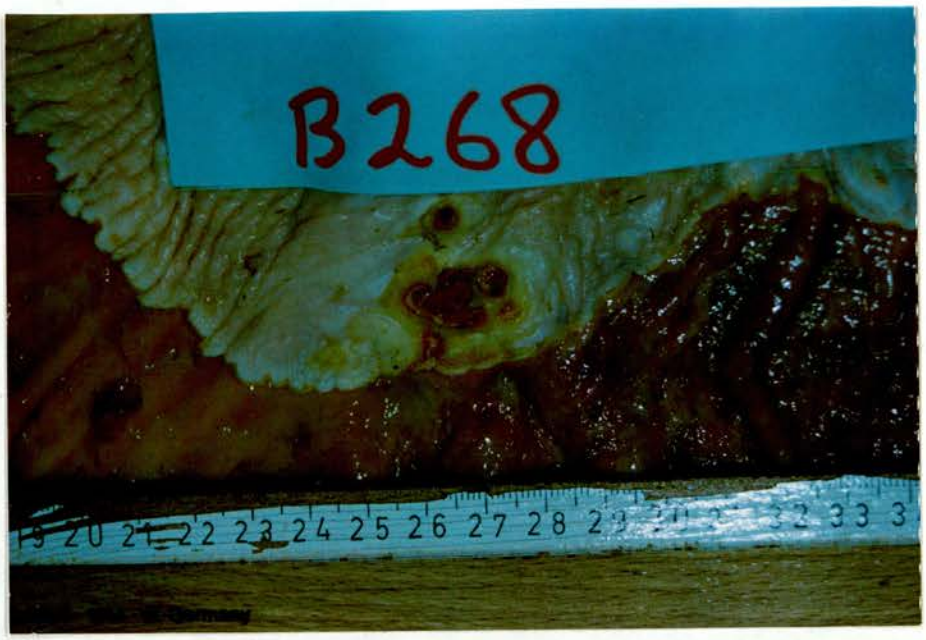
Pathology.

The equine stomach, like that of other herbivores, has a grossly clearly demarcated, non-glandular, stratified squamous epithelium lined area, which covers about one third of the total gastric surface area . The majority of perforating ulcers (53 per cent) and asymptomatic ulcers (81 per cent) occur in this non-glandular area especially in the region of the margo plicatus which is the junction between the glandular and non-glandular mucosa (Wilson 1986; Dwyer and Powell 1989). Ulcers may be

singular or multiple, craterform or irregular in shape and usually have obvious slightly raised edges often surrounded by a zone of brown-staining keratin (figure 2:1). Ulcers vary in size from small circumscribed lesions less than one centimetre in diameter to lesions which involve the majority of the squamous mucosa but containing islands of normal or thickened squamous mucosa (figure 2:2). The glandular mucosa like that of man and the dog, contains chief, parietal and mucus secreting cells and although ulceration is recorded with less frequency in this area (perforating 27 per cent; non-perforating 32 per cent), some authors believe that such ulcers are rarely asymptomatic and are usually clinically significant (Murray 1990). Glandular ulcers are usually solitary, oval in shape and the mucosal excavation is surrounded by an intense zone of inflammation. Occasionally, an hour glass-like stenosis of the proximal duodenum associated with fibrosis and granulation tissue is observed at post mortem examination (figure 2:3) and it is believed that this represents a healed or healing duodenal ulcer. Twenty per cent of all ulcers are found in the proximal duodenum and an equal proportion of perforating ulcers occur in this area (Wilson 1986).

Diagnosis and differential diagnosis.

Diagnosis of gastroduodenal ulceration is largely based on clinical signs but definitive diagnosis is problematical. The use of a two meter flexible or video endoscope (figure 2:4) permits the direct examination of the majority of the stomach



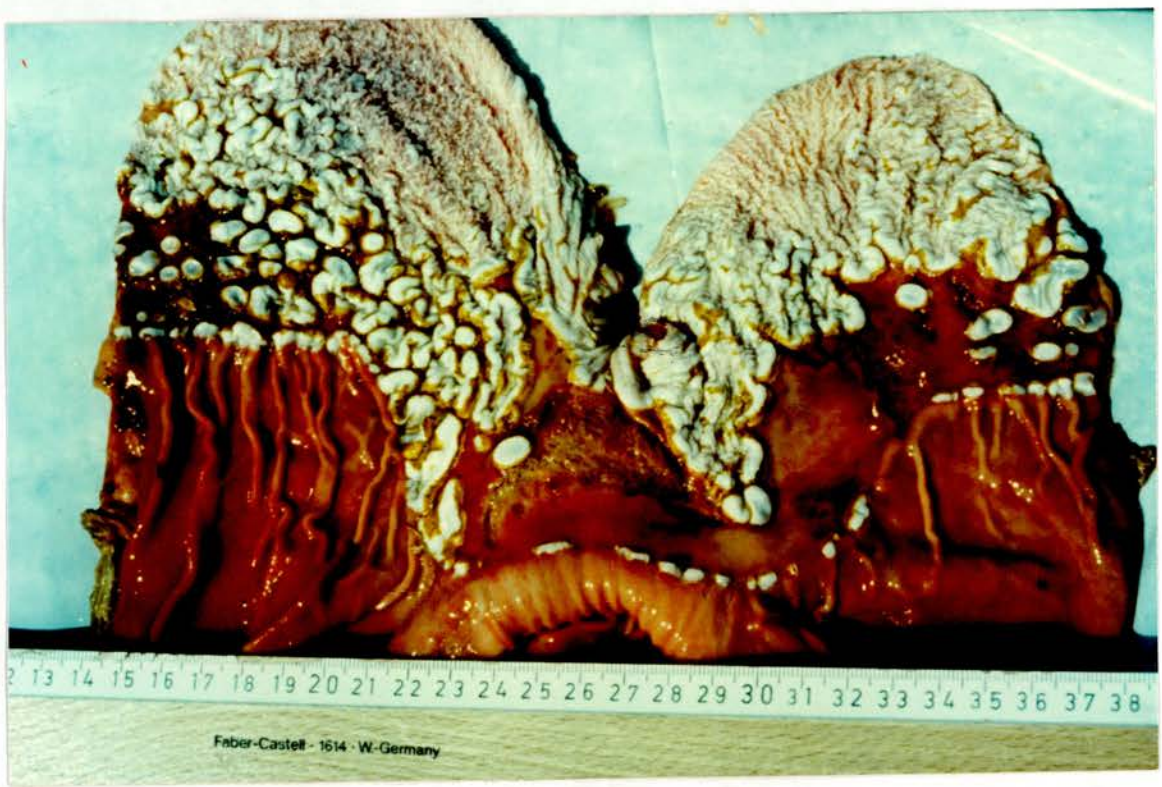
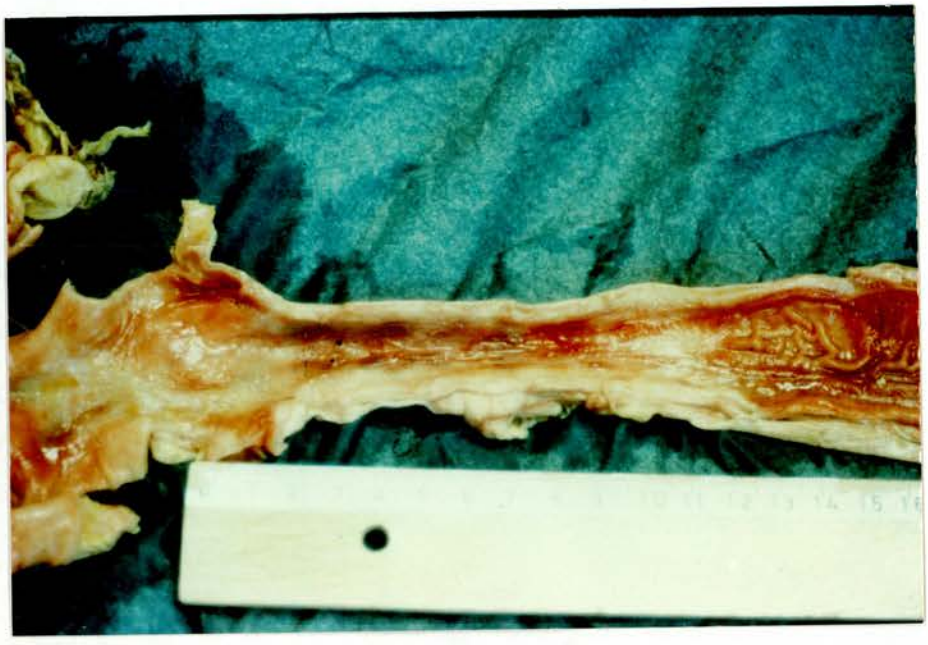


Figure2:1 Ulceration of the squamous mucosa of a clinically normal foal.

Figure 2:2 Extensive ulceration of the squamous mucosa in a foal that died showing typical gastric ulceration signs.



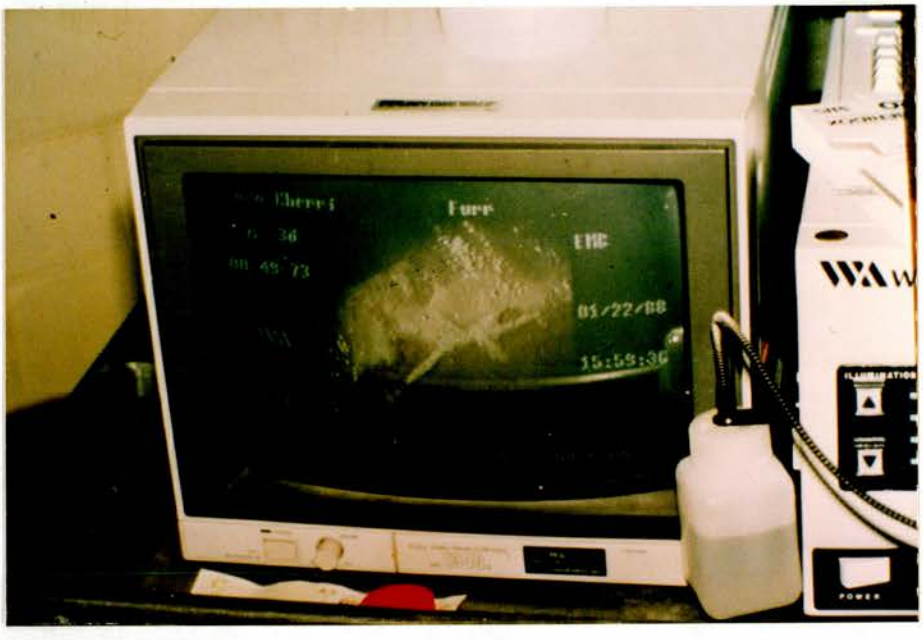


Figure 2:3. Hour glass stenosis of the duodenum of the foal represented in figure 2:2.

Figure 2:4. Monitor of video endoscope used to examine equine stomachs.

(Murray et al. 1990) but even after 18 hours fasting, over one third of the mucosa is obscured by ingesta in foals over two months of age ona hay diet (Wilson 1989) . In addition, because of the acute angle between the cardia and pylorus, it is extremely difficult to introduce the endoscope into the duodenum (Brown, Slocombe and Derksen 1985).

Serum pepsinogen levels have been investigated in foals with ulceration and although many foals had significantly higher serum levels when compared to normal foals, considerable overlap existed precluding its use for definitive diagnosis (Wilson and Pearson 1986).

The presence of blood in gastric reflux or a positive faecal occult blood test occurs occasionally in association with foal ulceration but should be interpreted very cautiously because it is an inconsistent finding and gastrointestinal bleeding occurs in more than 30 other equine diseases (Pearson, Smith andMcKim 1987).

Radiographic techniques for upper gastrointestinal tract examination in the foal have been described (Campbell, Ackerman and Peyton 1984) and mucosal lesions, delayed gastric emptying and duodenal obstruction can be determined using barium contrast studies but requires considerable radiographical expertise and accurate interpretation.

Although the clinical signs of ulceration are almost pathognomonic, a number of uncommon conditions should be considered in the differential diagnosis and include hypoplasia of the stomach (Pearson and Murfitt 1988), choke (Freeman 1982), megaoesophagus (Freeman 1982), pyloric stenosis (Church, Baker and May 1986), gastric dilatation (Robertson 1982) and

gastric stenosis (Robertson 1982).

Aetiology and Pathogenesis.

Many factors have been proposed as causes of equine gastric ulceration (table 2:5) but with the exception of non- steroidal anti-inflammatory drugs (N.S.A.I.D.) (Traub et al.1983), few have been proven. The occasional clustering of cases in certain studs has prompted the suggestion of an infectious cause (Becht and Byars 1986) but investigations have failed to demonstrate the presence of pathogens (Acland, Gunson and Gillette 1983; Collobert-Laugier et al. 1989). Weather related stress has been indicated as a possible cause of gastric ulceration by Dwyer and Powell (1989) but requires further investigation.

In the pig, diet is considered the major cause of ulceration in the non glandular mucosa. There is evidence that in the horse this is also true. Coenen (1990) indicated the need to feed roughage and to distribute concentrate feed over three feeds to avoid ulceration. A dietary factor was also suggested by Hammond and others (1980). A post mortem survey of grass sickness, a condition predominanting in pastured horses, revealed ulceration in only 2% of animals (Pogson 1989, personal communication) as compared to 28% in the general population (Sweeney 1990).

The equine gastric parasite *Gasterophilus intestinalis* causes deep ulcerative pits in the squamous mucosa yet it is generally considered to be of limited clinical significance.

Irrespective of the specific cause, ulceration is likely to develop because of disruption of the gastric mucosal barrier which protects the gastric mucosa from the potentially harmful effects of

gastric acid and pepsin. A delicate balance exists between mucosal protective factors and the aggressive effects of acid, pepsin and bile salts (figure 2:6) and disruption of this delicate interplay allows diffusion into the mucosa of hydrogen ions and pepsin resulting in auto digestion and ulcer formation (Crampton and Rees 1986).

Treatment.

The aim of ulcer treatment in the foal, like that in man, is to hasten ulcer healing by reducing gastric acidity and protecting the ulcer from further insult by pepsin or gastric acid. The principles of ulcer therapy have been discussed in chapter 1.

The intravenous administration of 0.5 mg/kg of ranitidine (Zantac®) to young horses (6 to 12 months) caused a significant decrease in total hourly acid output but surprisingly did not significantly change the pH (Campbell-Thompson and Merritt 1987_a). Other workers demonstrated an increase in pH from 2.2 to >3.6 for a period eight hours following the oral administration of cimetidine (Tagamet®; Dyspamet®) at a dose of 8.8 mg/kg or ranitidine at a dose of 2.2 mg/kg orally (MacAllister,Sangiah and Amouzadeh 1987). However, twice daily administration to nursing foals of 4.4 mg/kg ranitidine did not prevent the development of phenylbutazone induced ulcers (Smith et al. 1987) and clinical experience indicated that a dose rate of 6.6 mg/kg of ranitidine three times daily was required for ulcer healing in adult horses (Furr and Murray 1989).

An increase in basal gastric pH to 3.5 for two hours and a 65 to 90 per cent reduction in the basal free acid content for seven hours was recorded in a small trial in horses (n=5) following

	Proposed aetiologies.	Reference.
N.S.A.I.D.	(Phenylbutazone	Traub-Dargatz 1988.
Stress.	(Temperament	Valdez 1979.
	(Weather	Dwyer et al. 1989.
	(Parturition	Wilson 1985.
	(Weaning	Wilson 1985.
	(Low plasma antibody	Wilson 1985.
	(Orthopaedic surgery	Rebhun et al. 1982.
	(Exsanguination	Traub-Dargatz 1985.
Infectious	(Rota virus	Becht et al. 1983.
	(Campylobacter sp.	Atherton et al. 1980.
	(Helicobacter pylori	Nappert et al. 1989.
	(Escherichia coli	Becht et al. 1986.
	(Clostridium perfringens	Acland et al. 1983.
	(Clostridium botulinum	Swerczek 1980.
	(Candida sp	Lee Gross et al. 1983.
	(Salmonella	Nappert et al. 1989.
Diet	(High corn	Hammond et al. 1986.
	(Low fibre	Hammond et al. 1986.
	(High fibre	O'Reilly 1973.
	(Depraved appetite	O'Reilly 1973.
	(Alfalfa	Swerczek 1980.
Parasitic	(Migrating parasites	Orr 1972.
	(Gasterophilus larvae	Waddell 1972.
	(Habronema megastoma	Robertson 1982.
Miscellaneous	(Foreign body	Rooney 1964.
	(Corticosteroid (endogenous)	Swerczek 1980.
	(Corticosteroid (exogenous)	Swerczek 1980.
	(Sex hormones	Wilson 1985.
	(Delayed gastric emptying	Orsini et al. 1986.
	(Congenital	Wagner et al. 1979.
	(Corrosive substances	Barth et al. 1980.
	(Excercise	Murray et al. 1989.

Figure 2.5. Factors proposed in the aetiology of gastroduodenal ulceration.

Agressive Factors.

Acid.
Pepsin.
Bile.
Pancreatic enzymes.
Mucosal ischaemia.

Protective Factors.

Mucosal repair.
Prostaglandins
Epithelial cells.
Intra-cellular junctions.
Alkali secretions.
Mucosal circulation.
Mucus.

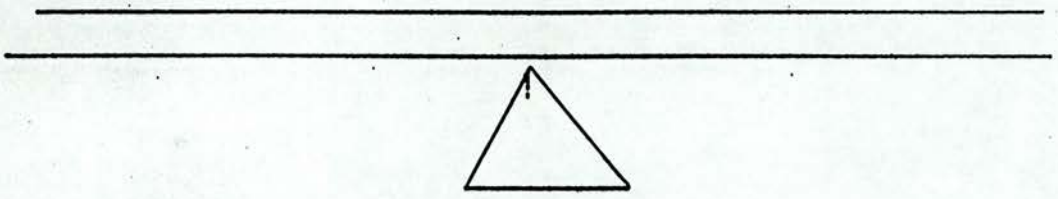


Figure 2:6 The integrity of the mucosal barrier relies on a delicate balance between protective and aggresive factors.

H_2 receptor antagonists.	(Ranitidine. (Cimetidine. (Famotidine. (Nitazidine.
Proton pump inhibitor.	(Omeprazole.
Cytoprotectants.	(Sucralfate. (Carbenoxolone. (Tri-potassium di-citrato bismuthate.
Prostaglandin analogues.	(Misoprostol.
Antacids.	(Aluminium hydroxide. (Magnesium hydroxide.
Surgery.	(Gastroduodenostomy. (Gastrojejunostomy. (Duodenojejunostomy. (Jejunojejunostomy.
Supportive therapy.	(Fluid therapy. (Analgesics. (Probiotics.

Figure 2:7 Approaches to the management of the foal gastroduodenal ulcer syndrome.

omeprazole administration intravenously at 0.5 mg kg (Sangiah, MacAllister and Amouzadeh1989). However, in man, oral administration of omeprazole is more effective (Sangiah et al 1989) and more research in the horse is required.

If duodenal stenosis is diagnosed, surgical intervention may be required but only limited success has been recorded following gastroduodenostomy, duodenojejunostomy, jejunojejunostomy and gastrojejunostomy (Orsini and Donawick1986; Campbell-Thompson et al. 1986). Supportive treatment, including fluid therapy and analgesics should be used as required. Total ileus lasting for 30 to 45 minutes has been reported in normal horses following a single recommended dosage of xylazine (Owen 1983) and its use in foals with gastroduodenal ulceration is contraindicated as it may aggravate primary or even precipitate a secondary gastric dilatation. A decrease in the normal population of lactobacilli has been noted in association with ulceration of the squamous mucosa in pigs (Embaye 1987) and the administration of probiotics to foals with gastric ulceration should be considered.

Conclusions.

A direct relationship between the presence of ulceration and the occurrence of clinical signs has not yet been demonstrated and in over 50 per cent of foals dying from ulcer perforation no premonitory illness was observed (Dwyer and Powell 1989). Furthermore, foals with experimentally induced gastric ulceration rarely exhibit "typical" signs of gastric ulceration (Smith et al. 1987). Diagnosis may be further confused by the fact that 51 per cent of

clinically normal foals have endoscopic evidence of gastric ulceration (Murray et al. 1990). Furthermore, teeth grinding and profuse salivation have been recorded in other gastrointestinal conditions (Church, Baker and May 1986).

If gastroduodenal ulceration in the horse parallels that in man and the pig, it is likely that gastric squamous, gastric glandular and duodenal ulceration should be considered heterologous conditions with different aetiologies, clinical signs and significance (Rothenbacher, Nelson and Ellis 1963; Lee 1985).

A number of authors (Wilson 1986; Dwyer and Powell 1989) in post mortem surveys described non perforating ulcers as the primary problem or cause of death in a number of cases. The justification for such a post mortem diagnosis should be rigorously defined. In man, post mortem surveys usually reveal a higher prevalence of gastric ulceration than do clinical surveys. Curiously, in the foal the reverse is true (Murray and others 1990; Dwyer and Powell 1989). This may partly be explained by inconsistency in the scoring and interpretation of lesions.

Many questions regarding the aetiology, pathogenesis and significance of the foal ulcer syndrome remain unanswered and it is possible that other factors in addition to ulceration, such as gastric distension, may be important in the clinical syndrome. In pigs, with the exception of those that die from acute haemorrhaging squamous ulcers, the presence of subacute and chronic ulcers are considered to be of no economic significance (O'Brien 1986). Gastric ulceration has recently been incriminated in ill thrift, poor appetite and colic in adult horses (Murray et al. 1989).

CHAPTER 3

A SURVEY BY MAIL QUESTIONNAIRE OF THE INCIDENCE OF THE FOAL GASTRODUODENAL ULCER SYNDROME IN IRELAND AND BRITAIN

Summary.

A mail questionnaire survey relating to the foal gastroduodenal ulceration syndrome was distributed to 55 equine practices in Ireland and Britain. Forty two per cent usable replies were received and respondents had a cumulative annual responsibility for 6650 foals. Forty two per cent of respondents at the time of the survey were not aware of the described clinical syndrome in foals associated with gastric and duodenal ulceration. The occurrence of gastric and duodenal ulceration in foals in Ireland and Britain was confirmed but even at its highest level, the incidence was low (0.58 per cent). Clinical cases were sporadic. Fifty two per cent of respondents had recalled seeing foals with clinical signs compatible with the syndrome for a mean of 10 years (range 2-17 years) prior to 1982 when the syndrome was first described. The use of histamine type two receptor antagonists in the therapy of clinical cases was common.

Introduction.

In 1982 a new clinical syndrome in foals under six months of age associated with gastric and duodenal ulceration was first described in the United States of America (Rebhun, Dill and Power 1982). Subsequent clinical reports have helped to clarify the syndrome and typical presenting signs include depression, mild intermittent abdominal pain, teeth grinding, excessive salivation and a preference to lie in dorsal recumbency for prolonged periods (Jones 1983; Gross and Mayhew 1983: Becht, Hendricks and Merritt 1983). If ulcer perforation occurs, signs of fulminating peritonitis such as acute abdominal pain, endotoxic shock, peripheral vasculature collapse and death will be exhibited.

The foal gastroduodenal syndrome has also subsequently been described in Japan (Yoshihara *et al.* 1986), France (Collobert-Laugier *et al.* 1989), and Canada (Barth, Barber and McKenzie 1980). The primary purpose of this study was to investigate by mail questionnaire the occurrence and incidence of this syndrome in Ireland and Britain. In addition, a small number of questions relating to the epidemiology and therapy of the syndrome were included.

Materials and methods.

In July 1986, a two-page 10-question questionnaire was distributed by mail to exclusively equine practices, predominantly equine practices or resident stud veterinarians in Ireland (n=35) and in Britain (n=20). A brief description of the clinical signs, epidemiology and gross pathology was included. Additionally, a short covering letter explaining the aims and purpose of the

survey and a prepaid first class addressed white envelope was included. The questions and format of the questionnaire are outlined in Figure 3:1. In particular, information was requested on 1) familiarity of veterinary practices with the syndrome; 2) number of foals under their care; 3) numbers of foals showing clinical sings compatible with a diagnosis of gastroduodenal ulceration; 4) numbers of cases in which a definitive diagnosis of gastroduodenal ulceration was made; 5) the occurrence of the gastroduodenal ulcer syndrome prior to 1982; 6) possible clustering or seasonal occurrence of cases and 7) therapeutic regimes and their success.

Results.

From the total of 55 practices contacted, replies were received from 28 (51%) but 4 replies were discarded because of incomplete, contradictory or confusing replies (Figure 3:2). The 24 cooperating practices in the survey included the majority of "key" equine stud practices and had a cumulative responsibility for 6,650 foals yearly. At the time of the survey 10 (42%) of respondents were not aware of the existence of a described clinical syndrome in foals associated with gastric or duodenal ulceration. However, 52% of respondents had recalled seeing foals exhibiting clinical signs compatible with gastric or duodenal ulceration for a mean of 10 years (range 2-17 years) prior to 1982 when the syndrome was first described. The numbers of Thoroughbred foals under 7 months of age showing depression, colic, frequent teeth grinding or obvious salivation is outlined in Figure 3:3. A total of 117 clinical cases of the gastroduodenal ulceration syndrome were reported in the survey population in the

Strictly Confidential.

FOAL ULCER SYNDROME.

1. Name of Veterinary Surgeon _____ Practice Address _____

2.(a) The foal ulcer syndrome was first described in 1982 and the few publications on this subject have all been in American journals. to date, little attention has been paid to this condition in Europe. were you previously aware of this condition ?.

Yes / No

(b) If yes, for how long? _____

3.(a) How many foals showing depression and at least two of the following clinical signs have you seen in the last five years?. Obvious salivation, frequent teeth grinding, colic. Please indicate for each year.
1982____ 1983_____ 1984_____ 1985_____ 1986_____

(b) In how many of these foals, if any, was a definitive diagnosis of ulceration made using endoscopy, contrast radiography studies or postmortem examination ?.

_____

4.(a) How many foals showing inappetance and occasional grinding of teeth (possibly in association with diarrhoea) have you seen in the last five years?. Please indicate for each year.
1982____ 1983_____ 1984_____ 1985_____ 1986_____

(b) In how many of these foals, if any, was a definitive diagnosis of ulceration made using endoscopy, contrast radiography studies or postmortem examination ?.

_____

5.(a) Have you seen cases prior to 1982 which would fit the description in question 3 and 4 above?.

Yes / No

(b) If yes, for how long?. _____

6. Do you consider the incidence of this condition may be

i) increasing
ii) decreasing
iii) static
iv) non-existent (please tick)

7. Have you observed a higher incidence of the foal ulcer syndrome on certain studs compared to others ?.

Yes / No

8. During which month of the year do you most commonly observe this condition ?.

_____

9.(a) How many foals in the last number of years have you treated with H2 receptor antagonists (Tagamet / Zantac) or other anti-ulcer medicants ?. Please indicate for each year.

1982____ 1983_____ 1984_____ 1985_____ 1986_____

(b) In the five years combined what percentage of these foals appeared to respond to treatment ?.

_____%

10. Approximately how many foals are primarily under your care during an average stud season ?.

_____

11. Comments.

Thank you for your co-operation.

Please return to - Harry J. Sweeney,
Royal (Dick) School of Veterinary Studies,
Easter Bush,
near Roslin,
Midlothian EH 25 9RG,
Scotland.

Figure 3:1. Format of the mailed foal ulcer syndrome questionnaire.

	Ireland	Britain	Total
Questionnaires distributed	35	20	55
Total replies	14(40%)	14(70%)	28(51%)
Usuable replies	11(32%)	13(65%)	24(44%)
Foal sample size	3300	3350	6650
Respondents not aware of the F.U.S.	6(55%)	4(31%)	10(42%)

FIGURE 3:2. Questionnaire sample frame, response rate and sample size.

YEAR	1982	1983	1984	1985	1986
IREALND	3	4	4	4	20
BRITAIN	11	12	18	23	18
TOTAL	14	16	22	27	38

FIGURE 3:3. Number of foals showing signs of depression, colic, frequent teeth grinding or obvious salivation.

YEAR	1982	1983	1984	1985	1986
IREALND	3	3	7	18	27
BRITAIN	10	3	37	24	14
TOTAL	13	6	44	43	41

FIGURE 3:4. Numbers of foals recorded with mild inappetance and occasional grinding of teeth.

years 1982-1986 inclusive but in only 35 (29%) of these cases was a definitive diagnosis made on the basis of endoscopic, radiographic or postmortem examinations. There was an apparent increase in the incidence of this syndrome between 1982 when 14 cases were reported and 1986 when 38 cases were reported. The numbers of recorded cases of mild inappetance and occasional grinding of the teeth in foals for the years 1982-1986 inclusive is outlined in Figure 3:4. A total of 147 such cases were recorded in Ireland and Britain during the five years under survey but in only 2 of these foals was the presence of gastric or duodenal ulceration confirmed by endoscopic, radiographic or postmortem examinations. A trend similar to that of the more severe clinical syndrome was noted with an increase from 13 cases in 1982 to 41 cases in 1986 occurring. The highest total number of foals affected with either the severe or mild manifestations of the gastroduodenal ulcer syndrome occurred in 1986, the last year of the survey when a total of 79 foals were affected but this only represented 1.2% of the population under survey. Only 25% of respondents believed that the incidence of the foal ulcer syndrome was increasing. A large proportion of respondents (43.8%) considered that its incidence was static and equal numbers of participants (15.6%) believed that the gastroduodenal ulcer syndrome did not exist at all or felt unable to respond to the question ("don't know").

There was an overwhelming belief among respondents that the syndrome was sporadic without clustering of cases and only one practice reported the occurrence of 2 cases on the same premises. Clinical cases of the foal ulcer syndrome occurred in every month between March to October inclusive but the vast

majority occurred between April and July inclusive. Seventeen practices reported the use of histamine type 2 receptor antagonists (ranitidine / cimetidine) in the therapy of suspected clinical cases of foal ulceration and during the years of the survey 110 foals were treated in this manner. There was an increase in the use of this therapy from 0 in 1982 to 37 cases in 1986 (1983, 19; 1984, 20; 1985, 34;). Of the practices with clinical experience of H_2 receptor antagonist therapy in the foal ulcer syndrome, 9 (53%) considered clinical cases to be 100% responsive; 3 practices believed it to be between 50 and 100% effective; one practice found it beneficial in 30% of cases and one practice recorded no success with its use. Three practices were unable to evaluate its efficacy in therapy.

Discussion.

The results of this survey confirm for the first time the occurrence in Ireland and Britain of a clinical syndrome in foals attributed to gastric or duodenal ulceration. The incidence of foals showing obvious salivation and frequent teeth grinding in association with colic and depression was very low (0.58%) and assuming an associated mortality rate of probably less than 25% (Becht *et al.* 1983) it was estimated that only 0.15% of the total foal population died from this condition. In a survey of 2222 Thoroughbred foals under 2 months of age in England, a mortality rate of 3% for all conditions including twins was recorded (Platt 1975). Assuming an equal mortality rate during the years of this survey, gastroduodenal ulceration / perforation probably accounted for 5% of foal mortalities which is

approximately equal to the numbers of foal deaths attributed to the foal neonatal maladjustment syndrome.

A 1.3% incidence of fatal perforating gastric ulcers was recorded in a survey of 600 foal postmortem examinations in the United States of America (Rooney 1964). However, a similar retrospective examination of foal postmortem examinations between 1980 and 1984 revealed a significantly higher incidence (7.8%) of perforating ulcers (Wilson 1986). The incidence of gastroduodenal ulcer associated deaths in foals in the Kentucky area in 1982 and 1983 was reported by Becht *et al.* (1983) to be 0.74% and 0.47% respectively in an extensive survey involving 71 stud farms and a total of 8,419 foals. The total foal mortality in the above farms in 1982 and 1983 was 2.47% and 2.14% respectively and of these, the cause of death was diagnosed as gastroduodenal ulceration in 29.9% and 21.9% of deaths respectively (Becht *et al.* 1983). However, subsequent surveys in the Lexington area based on foal postmortem examinations revealed a decreasing incidence of perforated gastric / duodenal ulcers from 6% of all foal mortalities in 1984 to 3% in 1988 and attributed this decline to the use of prophylactic anti-ulcer medication in addition to greater awareness of the condition with consequent earlier diagnosis and more prompt therapy (Dwyer and Powell 1988; Dwyer and Powell 1989). Furthermore, these surveys attributed the cause of death in an additional 1.5% of foal mortalities to non perforating gastric ulcers but the criteria for such a diagnosis were not described.

Many practitioners reported the frequent observation that many foals with infectious or foal heat diarrhoea occasionally grind their teeth, and their belief that this is associated with the

diarrhoea and not with gastric ulceration. Therefore, foals showing only mild inappetance and occasional grinding of teeth (question 4a) have not been included in computing the foal ulcer syndrome incidence. Furthermore, as the majority of cases reported in this study were diagnosed on clinical signs alone, the use of clearly defined strict clinical criteria is essential.

The not infrequent incidental postmortem finding of gastric ulceration in foals dying from another obvious cause has been recognized for some time (Jubb, Kennedy and Palmer 1985_a; Rooney 1964). An endoscopic survey of normal foals in Ireland and Britain revealed a 51% prevalence of asymptomatic ulcers (Murray *et al.* 1990).

Although a clinical syndrome associated with gastroduodenal ulceration was only first described in 1982, cases of perforating gastric / duodenal ulceration have been described considerably earlier in the United States of America (Rooney 1964; Valdez 1979); Scotland (Orr 1972) and Ireland (O'Reilly 1973). This is in agreement with the findings of the survey in respect to the opinion of 52% of respondents who had recalled seeing foals with typical ulcer clinical signs prior to 1982.

Reported cases of foal gastric ulceration were sporadic and only 2 cases occurred on the same farm. Previous reports confirm the sporadic nature of this condition (Becht *et al.* 1983). The higher incidence of ulceration during April and July is probably due to the popularity of early spring foaling in thoroughbred studs rather than a true seasonal factor in the occurrence of disease.

The survey confirmed the widespread use of histamine type2 receptor antagonists in the therapy of foal gastric ulceration

and offered subjective assessment of its efficacy. The treatment of foal gastric ulceration has recently been reviewed (Sweeney, 1991)

It is difficult to assess the significance of the revelation that 42% of respondents were not previously aware of the foal gastric ulceration syndrome. It is likely that the clinical signs of depression, teeth grinding and salivation would be attributed to some vague colic by clinicians unaware of the ulcer syndrome. However, in view of this the survey did not ask how many foals had been diagnosed as having the foal ulcer syndrome but instead requested information on the number of foals with major clinical signs of teeth grinding, salivation and depression which are considered pathognomonic for the syndrome. Therefore, unfamiliarity with the clinical syndrome may not have adversely influenced the resulting incidence.

The response rate of 51% (42% usable replies) falls within the range often recorded for veterinary mailed surveys (40-50%) (Martin, Meek and Willeberg 1987_a) but response rates up to 89.4% have been recorded where two mailed reminders were sent (Newland, Waters, Standford and Batchelor 1977; Simpson and Wright 1980). A higher response rate would have increased the validity of the findings because collaborators are self selected volunteers and could very well have different opinions, management styles and levels of disease than those who refuse to collaborate (Martin, Meek and Willberg 1987_a). Recent veterinary postal surveys in Britain have resulted in various response rates including 24.7%(Gettinby, Armour, Bairden and Plenderleith 1987), 42%(Morgan, Nicholas, Glover and Hall 1990) and 44% (Bennett 1991). From 14 resident stud veterinarians contacted only, one

replied and this group accounted for approximately half of the non returns (non return rate 49%). It is possible that if the survey had been totally anonymous a higher response rate would have resulted but this would have sacrificed the opportunity to obtain follow up information. Non respondents may have felt that they had nothing to contribute because they had not seen any foals showing signs compatible with gastric ulceration. In this case the foal ulceration incidence reported here would be over estimated. Nevertheless, the large sample fraction , 6650 foals out of 9728 thoroughbred foals born in 1986 (Anon. 1986) would be expected to yield a result with a 95% level of confidence with an absolute precision of 1%(99% sure that the estimated prevalence level is within 1% of the true prevalence) .

Ruppanner (1972), discussed the problem of under reporting in surveys of veterinary surgeons because modern production practices allow the veterinarian to see only a portion of the total number of cases of animal disease. However, this is unlikely to be a significant factor in relation to thoroughbred stud farms.

The two main criteria for the success of a questionnaire are reliability and validity (Thrusfield 1986). A questionnaire, like a diagnostic test is reliable if it produces consistent results. As this survey spanned five years its reliability depends on accurate record keeping by respondents. On a number of occasions the author had the opportunity to interview some of the respondents after they had returned the questionnaire and consistent answers were received confirming the reliability of the survey. Simpson and Wright (1980) demonstrated a high degree of repeatability when comparing veterinary postal and interview questionnaires. Validity is a measure

of the degree to which answers, on average, reflect the truth (Thrusfield 1986). The validity of this survey is supported by the fact that the Irish Equine Center and Animal Health Trust pathology units only very occasionally record a foal death attributed to gastric ulceration or perforation thus confirming at least a very low mortality from this syndrome. However,concern over the validity of the survey remains as there is no convenient diagnostic test for this syndrome and the clinical syndrome has not been definitively described.

The survey was designed as an economic rather than an investigation type of survey, the emphasis being applied to the incidence and importance of foal ulceration rather than attempting to investigate its natural history (aetiopathogenesis). Requirements for a valid investigational survey include an objective method of study based on factual observation and a firm basis of measurement in addition to the availability of some hundreds of cases (Leech 1971). The low incidence and absence of a definitive diagnostic test for foal ulceration exclude the possibility of a detailed investigational survey at this time (Selby, Edmonds and Hyde 1976).

An informal pretest of the questionnaire was made by allowing colleagues to read each question followed by an explanation of the purpose of that question. In this way helpful comments on the structure and clarity of questions were received and these alterations were incorporated in the survey. A formal pretest of the survey was not considered possible because of the limited extent of the available sampling frame.

As the target population in this survey was largely concentrated in a small number of geographical areas a cluster sampling technique was used because of its convenience and

economy. However, the information obtained in this way is less precise than if either a systematic or random sample containing the same number of animals were selected because disease incidence tends to be more variable between groups than within them (Thrusfield 1986). It is likely that the studs represented in this study were largely intensively managed public or private studs with a high level of husbandry and management and that the smaller rural "hobby" breeder where different husbandry practices may be employed is under represented and is a possible source of bias. Equally, the high non-response rate by stud veterinarians is a potential source of bias but as management in these studs is unlikely to have been significantly different from cooperating studs, similar results could have been anticipated.

A postal survey was chosen because of the wide geographical scatter of potential interviewees, low cost involved , convenience and speed. Further advantages are absence of interviewer bias and the opportunity for a highly motivated respondent to research the facts over a period of time (Thrusfield 1986). Disadvantages of the mailed self completed questionnaire include the absence of an opportunity to clarify a question if not fully understood, the opportunity for the respondent to review and modify previously answered questions and a lower response rate than if direct interviews were used .

The questionnaire design incorporates a number of different features. In total there were 16 questions included in the survey and 9 of these requested quantitative answers relating to continuous variables and in these cases open ended questions were used (example 2b, 3a and 3b). Three questions were closed, dichotomous and mutually exclusive (2a, 5a, 7) and question six

was a multiple choice closed question. Question 11 was a free story question and although not always answered useful information was obtained. The advantage of the type of questions used in this survey (with the exception of question 11) is speed of completion for the respondent and ease of analysis of results. The main disadvantage is that the respondent is not allowed freedom to pass opinion or comment and discuss related events which may be significant.

As the foal ulceration syndrome was only first described in 1982 and as yet not clearly defined or widely recognized it was felt necessary to rigorously define the clinical signs , those of teeth grinding and salivation being almost pathognomonic for the syndrome.This was considered preferable to inquiring as to numbers of diagnosed cases of ulceration. However, it is also recognized that many foals, frequently in association with diarrohea occasionally grind their teeth (probably for reasons other than gastric ulceration) and to clarify the question the adjectives obvious and frequent were used. Ideally questions should be brief and clear and the the inclusion of ambiguous imprecise vocabulary such as frequent and obvious should be avoided but in this situation their use was considered unavoidable.

A number of improvements could have been made to the questionnaire. In general the questions are best ordered from general to specific with the intention that the initial questions put the respondent at ease, serves to focus the attendants thinking and enhances recall. (Waltner-Toews 1983). In this regard, questions 6,7,8 and 10 may have been best introduced at the beginning of the survey.

It is debatable if there was any need to inquire from

respondents if they were previously aware of the the existence of a described clinical syndrome attributed to foal gastric ulceration (question 2). As the questions asked about foals exhibiting clinical signs of ulceration irrespective of what the diagnosis was, awareness of the clinical syndrome would not have altered the survey results. Even though question 2 points out that the syndrome was described only five years ago and that all publications till that time had been in North American Veterinary Journals the question is emotive, sensitive and potentially embarrassing and may have resulted in 'prestige' bias (Waltner-Toews 1983). In any respect this question would have been best placed towards the end of the questionnaire.

Although a clear statement of confidentiality was included, total anonymity may have reduced the non return rate especially in the resident stud veterinarian group who may have been concerned about divulging the health records of a particular stud farm.

Conclusions.

This survey confirms for the first time the occurrence of a clinical syndrome in foals in Ireland and Britain attributed to gastric ulceration. The incidence of the syndrome is low and even at its highest level, less than 0.6 % of that years foal population was affected. The apparent increase in the incidence of ulceration over the period of the survey may indeed be a true increase but may be affected by such factors as increased awareness, fashion and the memory or quality of records of respondents (Martin *et al.* 1987_b). The mortality rate is low so the economic significance of this condition is marginal.

Further definition of the clinical syndrome and a convenient diagnostic test would produce a more valid result.

CHAPTER 4

THE PREVALENCE OF GASTRIC ULCERS AND EROSIONS IN ADULT HORSES: AN ABATTOIR SURVEY

Summary.

The stomachs of 614 adult horses were examined at a horse abattoir over one calendar year. Ulceration or erosion of the proventricular mucosa was noted in 173 (28.2%) of horses. Ulcers were less than 1cm^2 in 53 (31%) of horses, greater than 1 cm^2 and less than 5 cm^2 in 69 (40%), and greater than 5 cm^2 in 51(29%) of horses. Lesions occurred close to the margo plicatus in the region of the lesser curvature in 89% of affected horses. In 135 (26%) of horses, papilloma-like proliferations of the margo plicatus at the lesser curvature were recorded. There was no association between the occurrence of ulceration / erosion and the presence of *Gasterophilus intestinalis* larvae. Of the 271 Thoroughbred horses examined 90(33%) had ulcerations or erosions but only 43(24%) of 182 ponies examined had ulcers / erosions, a statistically significant difference ($p<0.05$).

Introduction.

The incidental observation at post mortem examination of gastric ulcers in the equine stomach has been recognised for some time (Rooney 1964; Jubb, Kennedy and Palmer 1985$_a$). In 1982, a

new clinical syndrome in foals, the foal gastroduodenal ulcer syndrome, the cause of which is attributed to the presence of gastric and / or duodenal ulceration, was first described in the United States of America (Rebhun, Dill and Power 1982). Lesions largely occur in the nonglandular mucosa and typical clinical signs include mild intermittent abdominal pain, teeth grinding and salivation.

Hammond , Mason and Watkins (1986) reported a 66% prevalence of asymptomatic ulceration in a survey of 195 thoroughbred race horses in Hong Kong. Lesions occurred in the non glandular proventricular mucosa and were significantly more prevalent in horses in training than those retired for two or more months. Adult gastric ulceration in the proventricular mucosa has also been described in Japan (Yamagishi *et al.* 1986; Yamagiwa *et al.* 1959). Murray (1988) reported a 52% prevalence of ulceration in the proventricular region of the stomach in clinically normal horses more than one year old. Recently, clinical signs of poor body condition, decreased appetite, recurrent colic, acute colic and diarrhoea have been attributed to gastric ulceration in adult horses (Murray 1988; Murray *et al.* 1989).

The purpose of this research was to investigate the prevalence, location, extent and gross pathology of gastric ulcers in adult horses in Ireland and to consider epidemiological factors associated with such lesions.

Anatomy:

The equine stomach is a small "J " shaped sac which intervenes between the oesophagus and the duodenum and is located caudal to the diaphragm in the dorsal abdominal cavity

mainly to the left of the median plane. The capacity of the equine stomach varies from 8-15 l and expressed as a percentage of the total capacity of the gastrointestinal tract constitutes only 8.5% , considerably less than that found in other domestic species (ox, 70.8%; sheep, 67.9%; cat, 69.5%; dog, 62.3% and pig, 29.9%).The cranial visceral surface of the stomach nestles against the diaphragm and the left lobe of the liver while laterally the stomach is adjacent to the diaphragm and spleen (de Boom 1975). To the right, liver and large colon adjoin the stomach. Caudally the visceral face of the stomach is related dorsally to the relatively firmly fixed transverse colon and more ventrally to the coils of small colon. The borders between the visceral and parietal surfaces are termed the curvatures. The lesser curvature is very short extending from the termination of the oesophagus to the junction with the small intestine. The greater curvature is very extensive and normally rests upon the dorsal colon; when it is empty coils of small intestine are interposed; when it is distended it may push the left dorsal colon aside.

The stomach when viewed from the outside has a few note-worthy sections. The dorsally placed rounded extensive sac is called the saccus caecus while the pyloric part is much smaller and is continuous with the duodenum, the junction being indicated by a marked constriction which internally presents as a circular ridge caused by a ring of muscular tissue, the pyloric sphincter. About 10 cm from the pylorus is a constriction which marks the pyloric antrum. The oesophagus enters the stomach very obliquely at the cardia forming the powerful cardiac sphincter, which appears not as a typical sphincter but as two semilunar folds, one dorsal and one ventral, which are slightly staggered in position. The more

proximal, ventral one is formed by a loop of the circular fibre coat, the more distal, dorsal one by a loop of the internal oblique fibre coat. This arrangement provides better occlusion than a typical sphincter. In additionally, numerous folds of mucous membrane further occlude the opening.

The stomach is held in position mainly by the pressure of surrounding viscera and by the oesophagus (Sisson 1975). The following peritoneal folds connect it with the adjacent parts. The gastrophrenic ligament connects the greater curvature with the crura of the diaphragm. The lesser omentum (the hepatogastric ligament) connects the lesser curvature with the liver. The gastrosplenic ligament passes from the left part of the greater curvature to the hilus of the spleen. The greater omentum loosely connects the ventral part of the greater curvature with the terminal part of the great colon and the initial part of the small colon. The gastropancreatic fold extends from the sac dorsal to the cardia and is attached to the liver, vena cava and pancreas.

The wall is composed of four coats; serous, muscular, submucous and mucous. The serous coat covers the greater part of the organ and is closely adherent to the muscular coat. In the region of the lesser curvature, extensive elastic tissue is located beneath the serous coat and this assists in maintaining the curved form of the stomach. The muscular coat consists of three incomplete layers. The external longitudinal fibre layer is very thin, only exists along the curvatures and is not present in the saccus caecus. The middle circular layer is present only in the glandular part. The internal oblique layer is arranged in coarse bundles in two layers. The external oblique fibres cover the left sac. The internal oblique layer is also found on the left sac and is continuous with the circular layer

of the stomach and exchange fibres with the external oblique layer. They form a remarkable loop around the cardiac orifice constituting the powerful cardiac sphincter.

The submucous coat is a layer of loose connective tissue which connects the muscular and mucous coats. In this layer vessels and nerves ramify before entering the mucosa. The stomach receives blood from all branches of the celiac artery and the gastric veins drain into the portal vein. The lymph vessels go chiefly to the gastric lymph nodes, thence to the cisterna chyli. The nerves are derived from the vagus and the sympathetic nerves.

The stomach mucosa is clearly divided into two parts. The proventricular part is glistening white in colour , composed of thick stratified squamous epithelium and is entirely without glands. It covers approximately one third of the mucosa area (corresponding approximately to the saccus caecus) and ends abruptly at the margo plicatus, a slightly raised irregular serrated sinuous border with the glandular mucosa. The glandular mucosa has a velvet like texture and is usually covered by a thick layer of viscus mucus. This mucosa contains three main gland types; mucus secreting cardiac glands, fundic glands which in addition to containing mucous secreting cells have hydrochloric acid producing parietal cells and pepsinogen secreting chief cells and pyloric glands which largely consist of mucus secreting cells. The glandular part is divided into three parts depending on the predominant type of gland but no gross distinct lines of demarcation exist. A narrow zone along the margo plicatus, but not extending to the greater curvature has a yellowish grey colour containing cardiac glands and is known as the cardiac gland region.Since the majority of glands here are not typical cardiac glands(like those of the pig and other species) , but

are intermediate in type between these and pyloric glands, the term intermediate zone might well be used. In any case, this zone is very narrow (0.5 to 1.0mm) towards the greater curvature but becomes 2.5 cm wide toward the pyloric part (Sisson 1975).

Materials and methods.

A total of 614 adult horse and pony stomachs were examined over a one year period between April 1988 and March 1989 at a horse abattoir . Both sexes were represented and age ranged from one to 25 years, but the majority were over 10 years old. Many horses had mild clinical ailments such as chronic lameness, poor reproductive history, laryngeal paralysis, exercise induced pulmonary haemorrhage or were culled because of poor race track performance. All horses used in this study subsequently passed meat hygiene standards required for meat destined for human consumption.

Stomachs were removed immediately after death by severing the gastrophrenic ligament, the lesser omentum (the hepatogastric ligament) , the gastrosplenic ligament , the greater omentum and the gastropancreatic fold. All stomachs were examined within five hours of slaughter. Stomachs were incised along the greater curvature from the duodenum to the cardia and gently washed with running water. The serosa, subserosal elastic tissue and external longitudinal muscle which all help to keep the stomach in its normal curved position were incised in the region of the lesser curvature so that the organ could be laid flat without puckering of the tissues (Figure 4:1).

The stomach wall tone in addition to the nature and fluidity of gastric contents were noted. The presence or absence of gastric

ulcers or erosions (other than the typical craterform pit or coalescing pit lesions normally associated with the attachment of bot larvae), hyperkeratinisation and scarring were recorded. The precise location of lesions was recorded and the location of lesions occurring close to the margo plicatus (within 5 cm of the junction with glandular tissue) were categorised as follows; left greater curvature, left junction of greater and lesser curvature, left lesser curvature, central lesser curvature, right lesser curvature, right junction of lesser and greater curvature and right greater curvature. Lesions occurring more than five cm from the margo plicatus were categorised as squamous fundus and lesions occurring in the glandular mucosa were categorised as glandular fundus. The size of ulcers or erosions were approximately measured and expressed in cm^2. The shape of ulcerative lesions was also noted.

The margo plicatus along the lesser curvature was examined and the degree of serration was evaluated and scored (Figure 4:2 - 4:5). Where the margo plicatus at the lesser curvature under slight tension was an almost straight line with only mild curvations is was classified as grade 1. If however, obvious serration of the margo plicatus remained it was classified as grade 2. If one to three projecting verrucose proliferations were present at the margo plicatus it was classified as grade 3. Where there were more than three verrucose proliferations a classification of grade 4 was made and grade 5 denoted stomachs where the margo plicatus was ulcerated to an extent where it was not possible to properly evaluate it.

The presence, location and number of *Gasterophilus intestinalis* larvae were identified (according to Zumpt, 1965) and recorded. Photographic records and histopathological samples

were obtained where considered appropriate.

In addition, breed, age, sex, source and reason for slaughter were recorded where possible.

Results

Between 27 and 101 stomachs were examined in each calendar month during the study period (Figure 4.7). A total of 173 (28.2%) horses of the 614 examined had ulceration or erosion in the squamous mucosa. Bot larvae were not detected in or within 4 cm distance of these ulcers /erosions and the pathological nature of these lesions were other than that described in the classification of macroscopic lesions produced by *G. intestinalis* as reported by Principato (1988). Grossly, lesions were singular or multiple, linear, circular or irregular and craterform in shape with obvious thickened and hardened lipped edges (Figures 4:9 - 4:11). Usually there was a brown staining ring of hyperkeratinisation surrounding ulcers (Figure 4:6). Brown staining necrotic material was often recorded in the base of ulcers. Surrounding tissues were palpably thickened, more corrugated and less glistening and white in colour than normal . Occasionally, yellow bile staining was noted. Haemorrhagic lesions were rarely observed.

The total ulcerated area was less than 1 cm^2 in 53 (31%) of affected horses and between 1 and 5 cm^2 in an additional 69 (40%) of horses. Fifty one (29%) of affected horses had ulcers /erosions larger than 5 cm^2. The single biggest ulcer was 60 cm^2.

The precise location was recorded for a total of 353 ulcers or erosions. The majority of ulcers (n=314, 89%) occurred close to the margo plicatus in the region of the lesser curvature and were distributed as follows; left lesser curvature , 67 (19%) ; central

lesser curvature, 101 (29%) and right lesser curvature, 65 (18%). An additional 34(10%) and 47 (13%) of lesions were located at the left and right junctions of the lesser and greater curvatures. In sixteen (5%) and 21 (6%) cases, lesions were located at the right and left greater curvatures respectively but usually in association with lesions near the lesser curvature. Only two horses(1%) had lesions in the squamous fundus. Two horses had glandular ulcers (Figure 4:12).

Brownish staining flaking hyperkeratisation was recorded in 86 horses, usually in the proventricular mucosa in the region of the lesser curvature (Figure 4:6). In 66 (77%) of horses there was concurrent ulceration or erosion.

The margo plicatus scores were recorded for 538 horses and results were as follows; grade 1, n=161(30%); grade 2, n= 229 (43%); grade 3, n=105 (20%); grade 4, n=30(6%) and grade 5, n=13(2%).

Of the 173 horses with gastric ulceration / erosion, 60 (37%) had concurrent bot infestation (Figure 4.7). Clustering of bot larvae and macroscopic bot lesions were confined to the squamous mucosa adjoining the margo plicatus on the cranial aspect of the stomach (Figure 4.8). An association between the occurrence of ulceration / erosion and the presence of bots was investigated on a combined annual aggregate basis using the Chi square test but no association was demonstrated ($Chi^2 = 0.03$, $p>0.05$). Equally, there was no association between the presence of bots and erosions / ulcers when examined on a monthly basis (April, 0.16; May, 0.86; June, 0.03; July, 2.3; August, numbers too small for computation; September, 2.79; October, 0.56; November, 0.07; December, 0.00; January , 0.03; February , 0.11 and March

0.04).

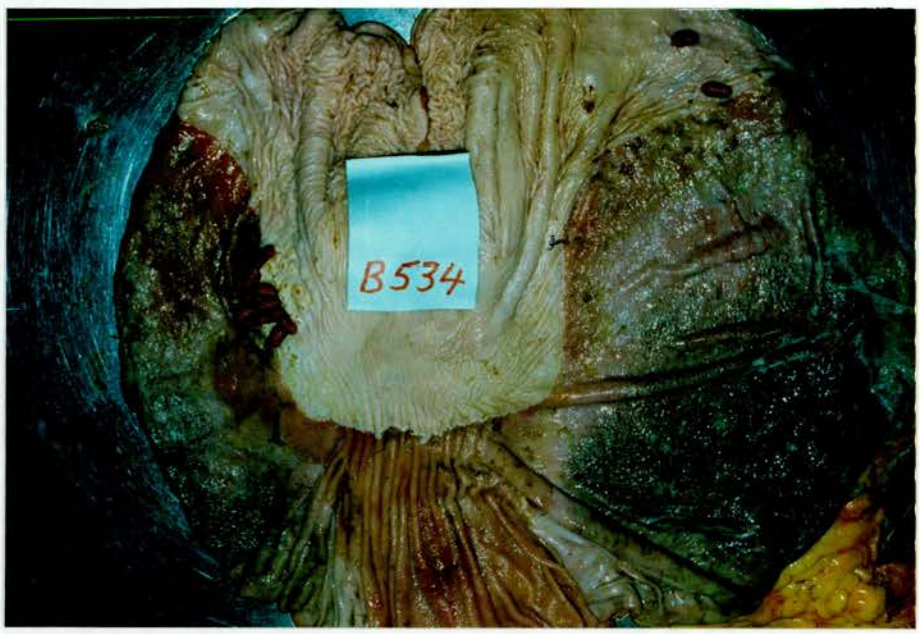
The breeds of horses used in the study were Thoroughbred 273 (44%); pony, 185 (30%) ; work horses, 83(14%); halfbred, 31(5%) and others/ unknown 42(7%). The prevalence of ulceration in each breed was as follows; Thoroughbred, 90(33%); pony, 43(24%); halfbred, 5(16%); work horses, 17(20%); others, 6(15%). There was a statistically significant difference between the presence of ulcers in Thoroughbred horses and ponies ($0.01<p< 0.05$) but numbers in other breeds were too small to permit meaningful statistics.

The source of 496 horses in this study was determined. Three hundred and four (61%) horses were sold privately, directly to the abattoir ; 168 (34%) were sold to the abattoir by horse dealers and 23 (5%) horses were fattened at the abattoir for a period greater than one month before slaughter. The prevalence of ulceration in these groups was not statistically different and was 28%, 31% and 22% respectively.

The gastric contents of stomachs in this study were usually dry in consistency. No association between tone of stomach, consistency of contents or odour of contents and the presence or absence of ulcers or erosions was noted.

Discussion.

This report confirms the common occurrence of ulcers and erosions in the proventricular part of the stomach in apparently clinically normal horses in Ireland. The prevalence in this study (28 %) is considerably lower than that recorded in Hong Kong (66%) in a study of 195 thoroughbred horses (Hammond *et al.* 1986) and that recorded by Murray *et al.* (1989) (52%) in a study of 100



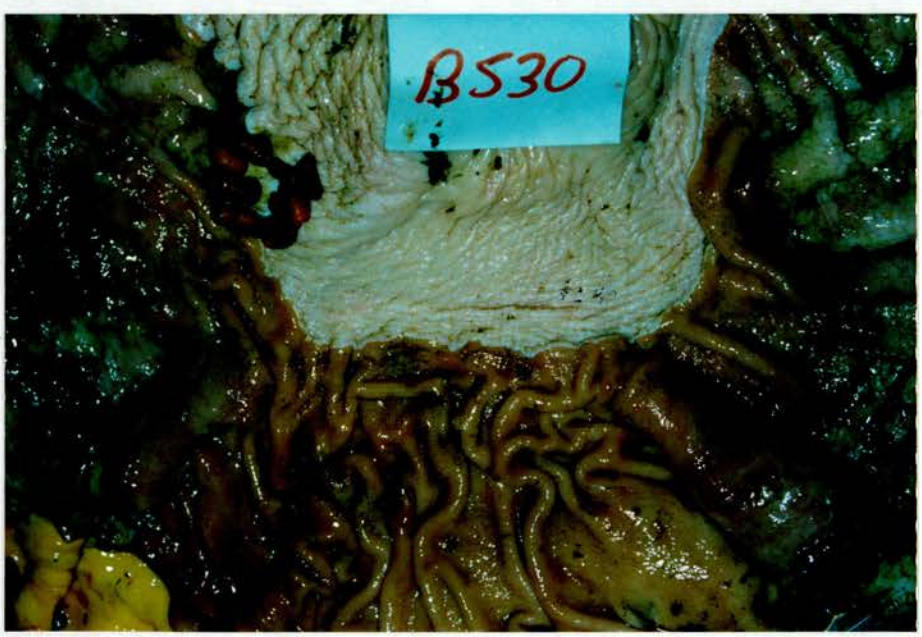
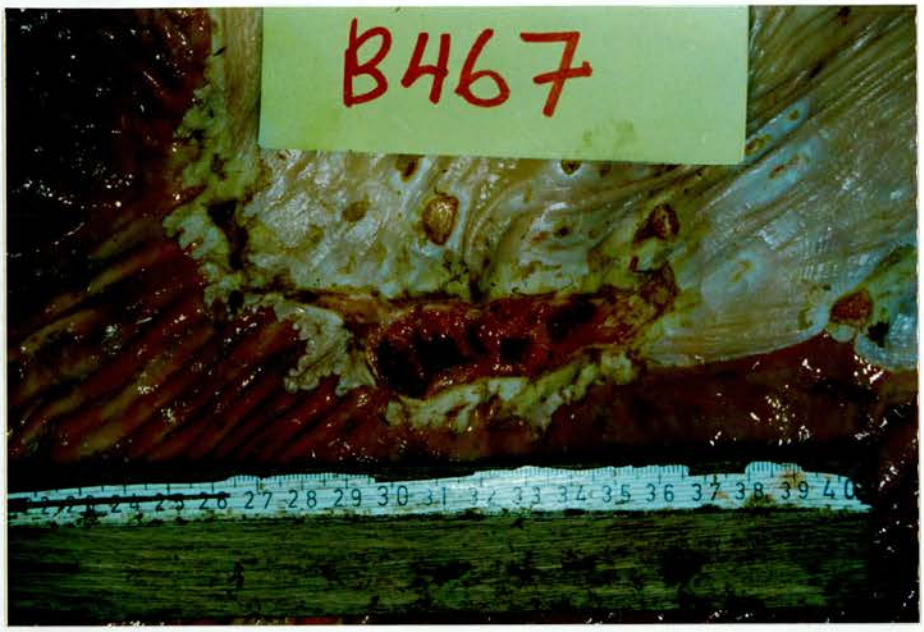


Figure 4:1 Stomach incised along the greater curvature with the mucosa visible.

Figure 4:2 Margo plicatus score grade 1.



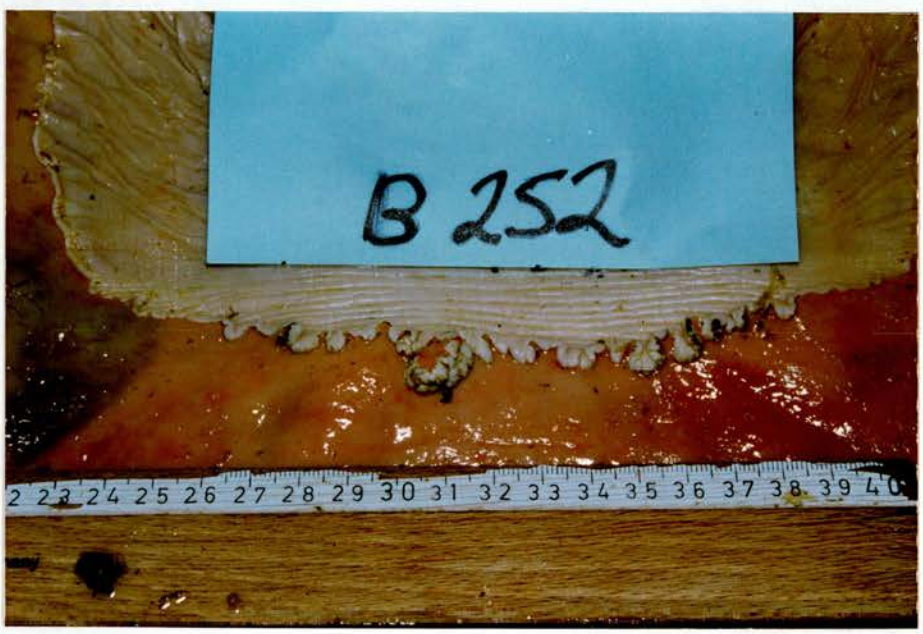


Figure 4:3 Margo plicatus score grade 2 and haemorrhagic ulcer.

Figure 4:4 Margo plicatus score grade 3.

Figure 4:5 Margo plicatus score grade 4.

Figure 4:6 Hyperkeratinisation of the proventricular mucosa close to the margo plicatus.

Month	Number examined	No. with ulcers/erosions	Percentage with ulcers/erosions	No. with ulcers/ erosions and bots	
April	65	26	40	9	(35%)
May	31	7	23	4	(57%)
June	69	20	29	9	(45%)
July	29	9	31	1	(11%)
August	35	12	34	0	(0%)
September	92	18	20	1	(6%)
October	101	21	21	7	(33%)
November	27	9	33	5	(56%)
December	56	20	36	9	(45%)
January	30	8	27	5	(63%)
February	30	5	17	3	(60%)
March	49	18	37	7	(39%)
Total	614	173	28	60	(37%)

Figure 4:7 Monthly prevalence of ulceration / erosion in equine stomachs.

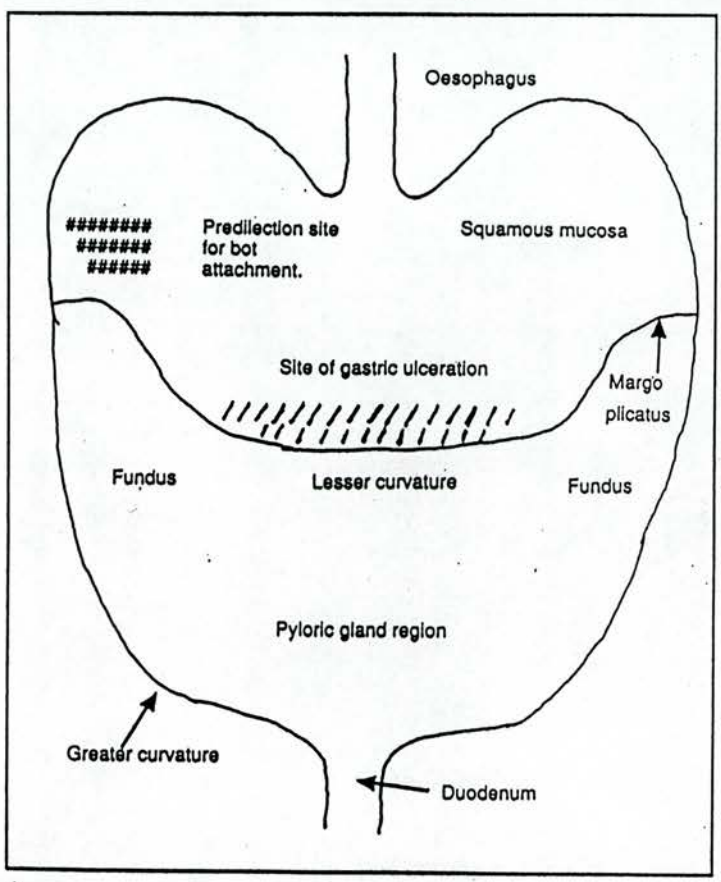
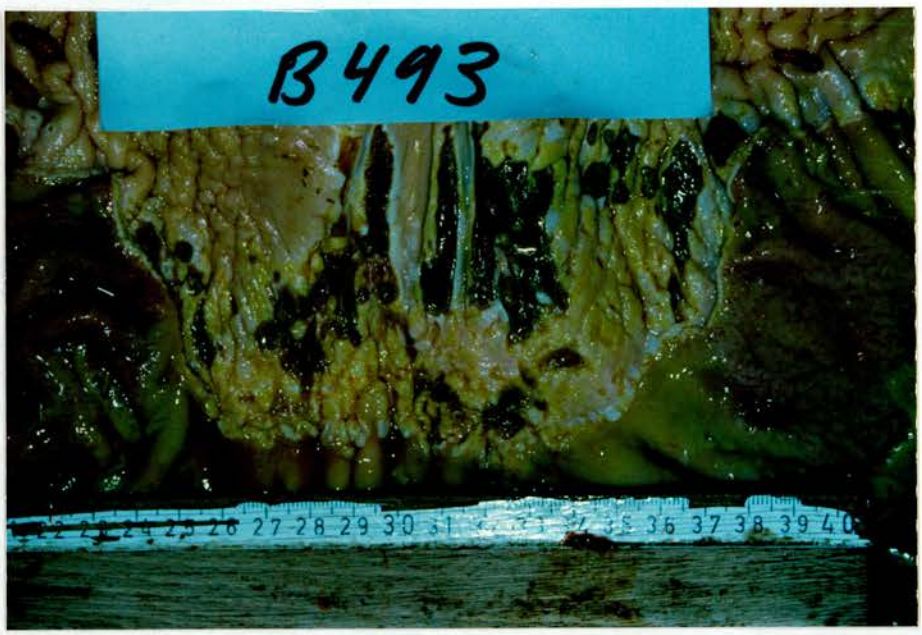


Figure 4:8 Diagram of an equine stomach incised along the greater curvature with the lumen visible to outline the anatomy, site of gastric ulceration and predilection site for bot attachment.



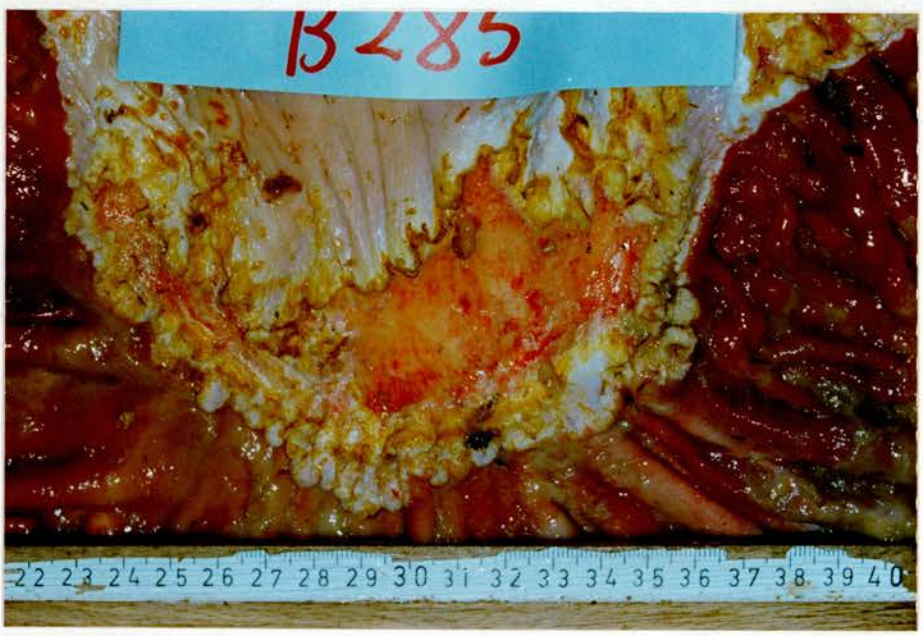
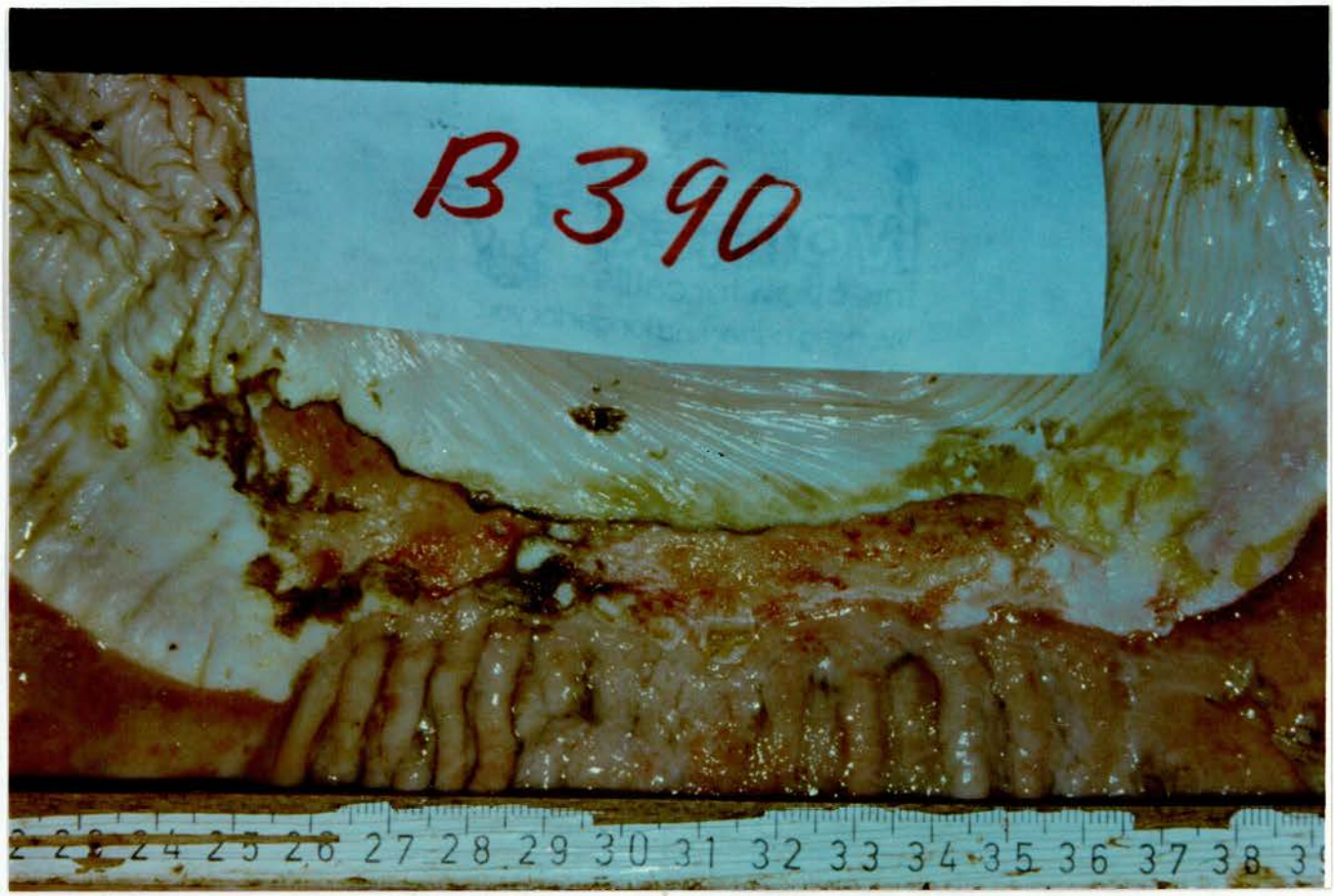


Figure 4:9 Acute linear erosions with necrosis in the proventricular mucosa.

Figure 4:10 Extensive ulcer in the proventricular mucosa.



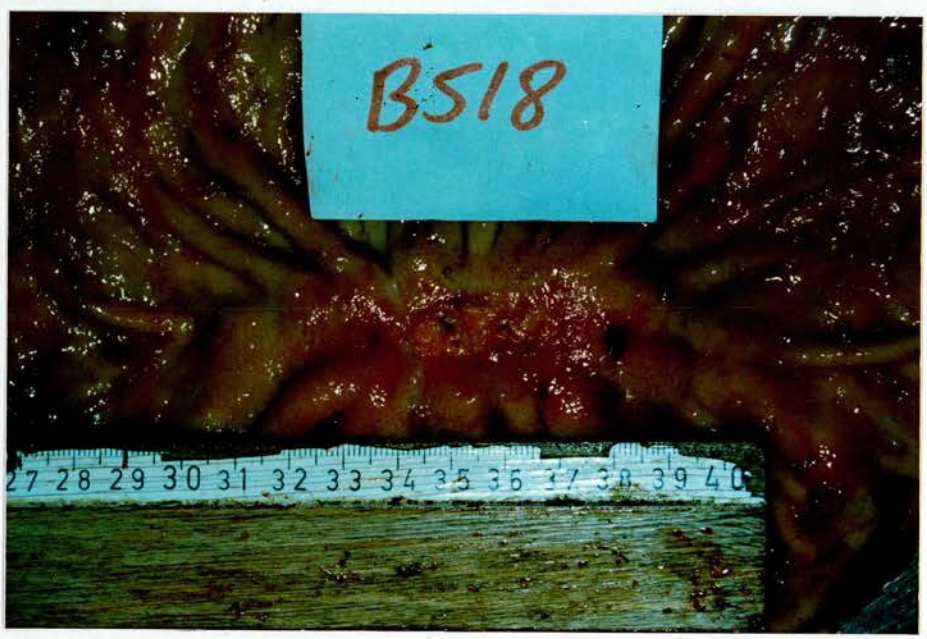


Figure 4:11 Extensive ulcer in the proventricular mucosa.

Figure 4:12 Ulcer in the glandular mucosa.

normal yearling and adult horses. Lesions in this study were concentrated in the region of the lesser curvature close to the margo plicatus (89%) and a further 11% of lesions were close to the margo plicatus in the region of the greater curvature. Less than one per cent of lesions occured in either the squamous or glandular fundus. The location of lesions in this study is in agreement with the work of Hammond *et al* (1986) who reported that the majority of lesions occurred in the proventricular mucosa close to the margo plicatus in the region of the lesser curvature and only 2.5% of ulcers occured in the glandular mucosa. However, Murray *et al* (1989) in an endoscopic survey of 187 horses (87 of which had clinical signs) reported that 39% of lesions occured along the greater curvature, 35% in the squamous fundus , 23% in the lesser curvature and 4% in the glandular fundus. The reason for the apparent different location of lesions in the the study of Murray *et al* (1989) may be due to difficulties in spatial orientation in endoscopic examination or loose anatomical nomenclature. It is interesting to note that in the pig, where ulcers of the proventricular stomach also occur, that lesions predominantly occur close to the junction with the glandular mucosa (Muggenburg, McNutt and Kowalczyk 1964).

Murray (1988) described old dark haemorrhagic or actively bleeding ulcers in 13 of 42 horses (31%) with colic, a feature in contradiction with the results of this work. This difference may be explained by the fact that the ulcers in Murray's study were considered by him to be the major cause of abdominal discomfort in these horses and that clinical signs were reduced or abolished after healing of the ulcer with histamine type 2 receptor antagonist therapy. In a further study , Murray *et al* (1989) reported haemorrhage associated with gastric lesions in 29 (33%) of 87

horses with clinical signs. In the study of Coenen (1990) involving 10 horses with proventricular ulceration, haemorrhage was not noted.

Scarring of the proventricular mucosa as a result of healing of an ulcerated area has not been described in the horse although it has been well described in the pig (Embaye, Thomlinson and Lawrence 1990). Grade 1 and 2 margo plicatus scores in this study (mild to obvious serrations) were considered normal but the presence of verrucose proliferations were considered to signify healed ulceration with resulting contraction, puckering and distortion of the squamous-glandular junction at the margo plicatus. This hypothesis is supported by histological evidence illustrating the presence of fibrosis and ectopic glands in association with the verrucose projections (Sweeney 1992, chapter 5). Further circumstantial evidence is that the verrucose projections were located only along the lesser curvature and their distribution paralleled that of ulcers and erosions. Similar lesions have been described by Becker (1966) who termed them papillomatous proliferations of the mucosa (precancerous processes) and attributed their cause to trauma of the gastric wall caused by roughage.

A seasonal incidence of ulceration (heat stress) has been described in the pig (O'Brien 1986) but no seasonal incidence of ulceration was apparent in this study. Furthermore, horses which passed through the hands of dealers and who may have been subjected to stress did not have a higher prevalence of ulcers or erosions.

Many grossly diagnosed ulcers were subsequently on histopathological examination found to be erosions (Sweeney 1992, chapter 5) . The greatly increased thickness of the epithelium

in the vicinity of the margo plicatus (2 to 3 fold) gave the false impression of a substantial mucosal deficit but on histopathological examination it was found that the entire thickness of the epithelium had not been penetrated and consequently were not ulcers but erosions. For this reason, no attempt is made to separate ulcerations and erosions in this paper. In the study of Hammond *et al.* (1986), photographic records were made of the stomachs and from these, lesions were subsequently classified. It is likely therefore, that many erosions were falsely described as ulcers.

Ulceration of the glandular mucosa only occurred in two horses in this study, one of which was receiving high doses of phenylbutazone. Ulceration of the equine stomach has been experimentally induced by toxic doses of non-sterodial anti-inflammatory drugs (phenylbutazone and flunixin meglumine) in foals and adult horse (Snow *et al.* 1979; MacKay *et al.* 1983; Traub *et al.* 1983; Collins and Tyler 1985;Traub-Dargatz 1987 and Meschter *et al.* 1990). Gastric lesions (ulceration and erosions) were confined to the glandular mucosa and no NSAID-induced pathological changes were described in the proventricular mucosa. Typical clinical signs of toxicity included depression, oral ulceration, decreased appetite, abdominal and ventral oedema, diarrhoea and death. Clinical signs of teeth grinding or abdominal pain were not features of NSAID induced ulceration and only in one study did salivation occur in 2 out of ten foals (Traub *et al.* 1983).

Rooney (1964) believed that ulceration of the oesophageal region with or without perforation in foals is directly related to mechanical trauma by *Gasterophilus intestinalis* larvae and Pandey *et al.* (1980) reported that bots cause gastric ulcers, erosions, and nodules or polpypous growths. Waddell (1972)

incriminated *G. intestinalis* in the aetiology of gastric ulcers and erosions affecting the squamous mucosa close to the margo plicatus along the lesser curvature. He supported this conclusion on the grounds that 92% of ulcerated stomachs had concurrent bot infestations (between 18-340 *G. intestinalis* larvae) and histopathological evidence of under-running of the epithelium near bot larvae by necrotic tissue containing bacteria which he considered important in the initiation of gastric ulcers. However, he did not consider bots the sole cause of gastric ulcers and erosions. The findings and conclusions of this study are almost in direct contradiction to those of Waddell (1972), (Sweeney 1990). In this study, only 34.7% of horses with gastric ulceration /erosion had concurrent bot infestation or evidence of recent bot infestation and no statistical correlation was present between the presence of bot larvae and the occurrence of ulceration /erosion when examined both on a monthly basis and on a combined annual aggregate basis. Furthermore, despite the seasonal absence of bot larvae from the equine stomach (July-September), no monthly or seasonal variation occurred in the prevalence of gastric ulceration /erosion. Additionally, a distance usually well in excess of 10 cm was present between the site of ulceration and the predilection site for bot attachment and therefore, it seems unlikely that under- running of the epithelium by necrotic material in the immediate vicinity of bot larvae could be important in the initiation of gastric ulcers /erosions in the area of the lesser curvature. Furthermore, no evidence for this was found on histopathological examination (Sweeney 1992, chapter 5). Support for this conclusion is obtained from the work of Hammond, Mason and Watkins (1986)

who, in a survey of 195 Thoroughbreds in Hong Kong where *Gasterophilus* larvae have never been reported, recorded a 66% prevalence of gastric ulceration. Conversely, in a survey of 70 horses in New Zealand, 97% of which had *G. intestinalis* larvae (mean=63), Kettle (1974) did not observe the presence of any gastric ulcers /erosions.

Diet has been incriminated as causing gastric ulceration in the horse (Swerczek 1980) and Hammond *et al.* (1986) suggested that a high fibre / high protein diet reduced the prevalence of ulceration. In a study by Coenen (1990), no ulcers developed in 21 ponies fed a hay diet but occurred in the squamous mucosa at the margo plicatus in 10 of 27 ponies fed a mixed diet including a pelleted ground ration. He suggested that diet and particularly fineness of grinding may be a factor in the aetiology of equine gastric ulceration and suggested that prevention of ulceration could be achieved by feeding high roughage diets and high energy feed over three or more meals per day. In the pig, fineness of grinding of feed influences the occurrence of ulceration (Embaye *et al.* 1990; O'Brien 1986) and this is thought to be mediated by increased fluidity of gastric contents and greater exposure of the oesophageal zone to gastric secretions. However, increased fluidity of gastric contents in association with gastric ulceration was not noted in this study or the study of Coenen (1990).

Irrespective of the specific cause of ulceration, the pathogenesis is likely to be similar and involves a disruption of the delicate balance between the mucosal aggressive factors (acid, pepsin, bile) and protective factors (mucus, alkali secretion, tight intercellular junctions,prostaglandins and epithelial cell turnover)(Sweeney, 1991). Mucosal ischaemia is often thought to

be the central lesion in the pathogenesis of ulceration and in this context it is interesting to examine the blood supply to the proventricular mucosa where the majority of ulcers occur. The stomach receives blood from all branches of the celiac artery and the gastric veins drain into the portal vein. Reedy *et al* (1977) by investigating regional blood flow using injected carbonised microspheres labeled with ^{85}Sr demonstrated that perfusion of the stomach's non-glandular wall and mucosal / submucosal layers was considerably lower than the glandular stomach, the duodenum, the jejunum or ileum. Yoshihara *et al* (1986) supported this finding by showing, using an angiogram, sparser arterial blood supply in the nonglandular stomach than in the glandular stomach .

Anatomical reasons for ulceration close to the lesser curvature were considered by Hammond (1990) who proposed a direct ulcerogenic effect of acid on the proventricular mucosa because this mucosa was most ventral in the standing horse and consequently most frequently bathed in gastric juices.

Exercise as a cause of ulceration was suggested by Murray (1990). Other possible causes of ulceration in the adult horse including *Candida spp* (Sweeney 1992, chapter 5) and *Campylobacter pylori* (Sweeney 1992, chapter 7) have been discussed elsewhere.

This work and that of Murray *et al* (1989) and Hammond *et al* (1986) confirm the predominance of ulceration in the proventricular mucosa. As this mucosa is also different anatomically from the glandular mucosa, it seems reasonable to consider ulceration of the squamous and glandular gastric mucosa as different entities with different aetiologies.

Furr and Murray (1989) reported the successful treatment

of gastric ulceration in adult horses using ranitidine (a histamine type 2 receptor antagonist) at a dose rate of 6.6mg/kg body weight *t.i.d.* for periods of up to three weeks. Regrettably, untreated controls were not used in this study and as clinical signs of poor appetite , colic and failure to thrive are vague, blind assessment of clinical response and blind endoscopic examination would add further weight to their work. At current prices, a 3 weeks treatment regime for an adult 550 kg horse at the above doses would cost about £700. It is noteworthy that gastric ulceration in the pig, with the exception of pigs that die from haemorrhage, is now generally considered to be without clinical or subclinical significance (O'Brien 1986). In a survey of 304 veal calves, no decrease in growth rate was observed in association with abomasal ulceration and it was concluded that most lesions are innocuous (Welchman 1987). In man, only 25% of gastric ulcers are associated with clinical signs (Spiro 1983)

The occurrence of gastric erosions and ulcers in adult horses in the proventricular mucosa have been described in association with Japanese encephalitis (Yamagiwa *et al.* 1959) and as the histopathological findings in their study were at variance with this study (Sweeney 1992, chapter 5), it may be that there was a direct casual relationship with the virus of Japanese encephalitis. In a study of gastric impaction in horses, Milne *et al.* (1990) noted proventricular and gastric mucosa ulcers in some cases. Robertson (1982) suggested that gastric ulceration may predispose to gastric dilation and rupture.Todhunter *et al.* (1986) reported that 90 % of cases gastric rupture occurred along the greater curvature and postulated that gastric ulceration, by weakening the gastric wall, may predispose to gastric rupture. However, in a different study

ulcers were present in only three out of 50 cases of gastric rupture in the horse and it is interesting that only 7% of gastric ruptures occurred along the lesser curvature, the predeliction site for gastric ulcers (Kiper, Traub-Dargatz and Curtis 1990).

Further research is required to assess the clinical and subclinical significance of gastric ulceration in the adult horse.

CHAPTER 5

THE HISTOPATHOLOGY OF GASTRIC PROVENTRICULAR ULCERS AND EROSIONS IN ADULT HORSES

Summary.

Tissue specimens (largely from the proventricular mucosa) were collected from the stomachs of 121 horses at an abattoir and processed by the H &E method. Sixty five of the above horses had gross ulceration or erosion and a further 29 horses had scarring. The least severe pathological change observed was described as epithelial change and was characterised by increased epithelial thickness, increased depth and irregularity of the rete pegs, hyperkeratosis and hydropic degeneration. More severe lesions were classified as acute erosion and in addition to the changes described for epithelial change, erosion, reticular degeneration and spongiosis was noted. Acute and subacute ulcers described lesions where the deficit extended down to the lamina propria and in chronic ulcers and erosions fibrosis was a significant feature. Scarring was characterised by increased corrugations of the epithelium, fibrosis of the lamina propria and ectopic glands.

Introduction.

The histopathological characteristics of proventricular erosion and ulceration in the horse have received only scant attention (Yamagiwa *et al.* 1959; Rooney 1964; Rebhun, Dill and Power 1982; Gross and Mayhew 1983; Buergelt 1984; Yoshihara *et al.* 1986 and Yamagishi *et al.* 1986). The histology of the normal equine proventricular region has been described by Wille, Schnorr and Merl (1973).

The aim of this study was to describe the histopathology encountered in asymptomatic proventricular ulceration in the adult horse and to ascertain the sequence of development of lesions.

Materials and methods.

A total of 614 healthy adult horse and pony stomachs were examined over a one year period as previously described (Sweeney, chapter 4). Representative samples were obtained from normal and pathological tissues. Tissue samples were fixed in 10% buffered formal saline and processed by the paraffin method. Sections were cut at 5 um and stained with haematoxylin and eosin. Tissue sections were examined blindly (without knowing what the gross diagnosis was) and pathological lesions were scored on a scale of 1(mild) to 4 (severe).

A total of 318 tissues were examined from 121 horses. The margo plicatus was represented in 138 tissue slides collected from 89 horses; squamous tissue in 51 slides from 30 individual horses and glandular tissue in 129 slides from 71 horses. A range of 1-8 pieces of stomach wall per horse were collected. In 15 horses only glandular tissue was examined.

A scoring system for the degree of scarring of the margo plicatus has been described (Sweeney 1992, chapter 4). A Kruscal-Wallis ranking test was used to investigate the relationship between fibrosis and the scarring scores, and between ectopic glands and scarring scores.

Results.

Of the 121 horses examined histopathologically the major findings on macroscopic examination were normal squamous mucosa (n=20); normal glandular mucosa (n=10); proventricular ulceration (n=50); proventricular erosion (n=15); scarring (n=29) and gastritis (n=8).

Normal proventricular mucosa

The stratified squamous epithelium of the proventricular part of the equine stomach was usually 15-30 cells in depth and consisted of many layers. The upper layer of this epithelium, the stratum corneum was thin and minimally keratinised , pyknotic nuclei being visible at the outer border (Figure 5:1). This layer was composed of closely packed dead cells and the most superficial cells which were being sloughed sometimes formed a separate layer, the stratum disjunctum. Directly beneath the stratum corneum lay a well defined pale staining layer, 4-10 cells thick, the stratum lucidum, composed of dead or dying cells with agranular cytoplasm. Directly beneath this, a very narrow stratum granulosum was sometimes observed. The deepest layer of the epithelial covering is the stratum germinativum which was composed of a single layer of basophilic cubodial cells (stratum basale) and a thick more dorsal layer of pale staining cells which became progressively more flattened towards the luminal surface (stratum

spinosum). Downward finger like projections of the epithelium, the rete pegs, alternated with upward papillae projections of the lamina propria. In normal epithelium the papillae of the lamina propria extended upwards for approximately 40% of the entire thickness of the epithelium. There were no glands in the squamous epithelium and the junction with the glandular mucosa at the margo plicatus was very abrupt and lymphoid follicles were a common feature at this junction (Figure 5:2).

Epithelial changes.

Epithelial changes were the least severe pathological changes observed and were recorded in 10 % of sections which were macroscopically considered normal. This diagnosis was made in 9 horses. In these cases the epithelium was thicker than normal and the rete pegs extended deep into the lamina propria and were more numerous and irregular in shape. Lamina propria papillae extended up 67 % of the total thickness of the epithelium. Hyperkeratosis (parakeratosis) was present often involving many layers of cells and a stratum disjunctum was more commonly observed (Figure 5:3). Pyknosis, karyorrhexis and karyolysis were common in the parakeratoic layer. Hydropic degeneration (intracellular oedema) was the major lesion in this classification and was characterised by increased cell size, cytoplasmic pallor and a displacement of the nucleus to one side. Extravasation of polymorphonuclear leucocytes was noted in 5 of the cases. Gram positive cocci and bacilli were recorded on the surface in 4 out of 9 cases. Mitotic figures were noted in the stratum spinosum (Figure 5:4). The lamina propria was often infiltrated with lymphocytes, eosinophils and polymorphonuclear leucocytes.The above lesions were often diffuse. Erosion or ulceration was not present at this

stage.

Acute erosion.

Acute erosions were diagnosed in 24 horses and were characterised by a deficit in the epithelium which did not penetrate to the lamina propria. A dark staining basophilic necrotic zone containing bacteria and fungi was often present on the surface of the lesion (Figure 5:5). In addition to the epithelial changes of parakeratosis and hydropic degeneration already described, reticular degeneration was observed in acute erosions (Figure 5:6). The cellular infiltrate was predominately polymorphonuclear leucocytes but although a constant feature, this reaction was less intense than might be anticipated. Seven out of the 24 horses had microscopic abscesses in the hyperkeratotic zone with an associated intense reaction (Figure 5:7). Spongiosis (intercellular oedema) characterised by widening of the intercellular spaces was a prominent feature in the stratum basale and the stratum germinativum (Figure 5:8). A focal inflammatory infiltrate in the lamina propria and congestion of blood vessels was a constant feature and some cases had small amounts of immature fibrous tissue. Hyperplastic lymphoid tissue was common at the junction of the glandular mucosa and the proventricular mucosa (Figure 5:9).

Acute and subacute ulcers.

Acute and subacute ulcers had the same characteristics as acute erosions except that the epithelium deficit was more severe and extended down to the lamina propria (Figure 5:10). Some fibrous tissue was also present in these cases.Thirteen horses were diagnosed as having acute ulcers.

Chronic ulcers and erosions.

Chronic ulceration was the histopathological diagnosis in

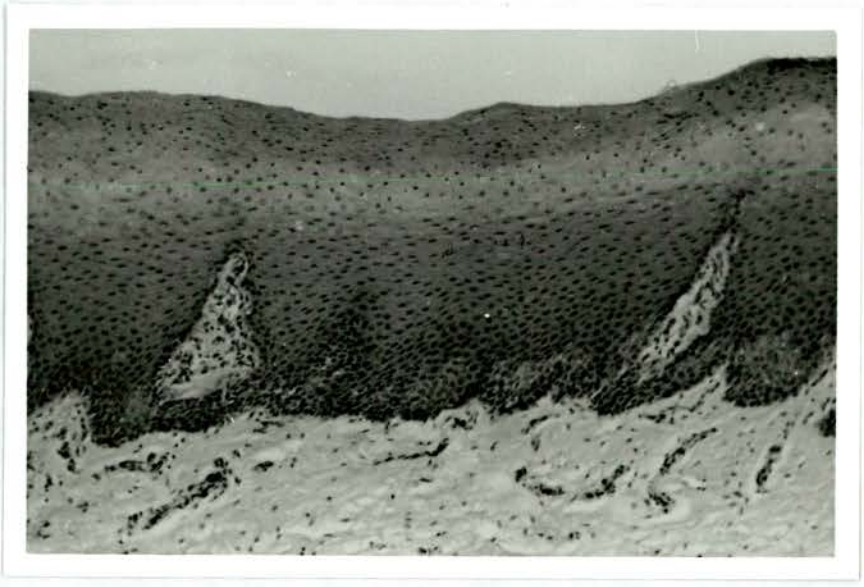
11 horses and chronic erosion in 3 horses. In addition to the features of acute ulcers and erosions these lesions had significant amounts of mature fibrous tissue in the lamina propria (Figure 5:11) and increased numbers of blood vessels (Figure 5:12). Spongiosis was a less important feature of chronic lesions. Most chronic ulcers contained areas in which an acute process was also occurring. A wide variety of micro organisms including cocci , bacilli and fungi were commonly present in the surface of the lesions.

Scars.

Scars were diagnosed in 29 horses. Lesions were always close to the margo plicatus and resulted in increased corrugations of the epithelium. There was a significant amount of fibrous tissue in the lamina propria underlying the squamous mucosa often in association with ectopic gastric glands (Figure 5:13). Presumably this is due to contraction of scar tissue and dragging of glandular tissue under the squamous mucosa resulting in distortion of the normal anatomy in that area. Hyperkeratosis was also often recorded. Rete pegs were prominent and lamina propria papillae extended up about 60% the entire depth of the epithelium. Thirty per cent of horses with scars had an associated mixed cellular infiltrate.

Glandular Tissue.

One glandular ulcer was recorded. Despite the gross diagnosis of gastritis in 8 horses, this diagnosis was only substantiated on histopathological examination in three cases , five of the other cases being diagnosed as congestion and the remaining case as normal. Eosinophils and lymphoid cells were prominent in normal glandular epithelium.



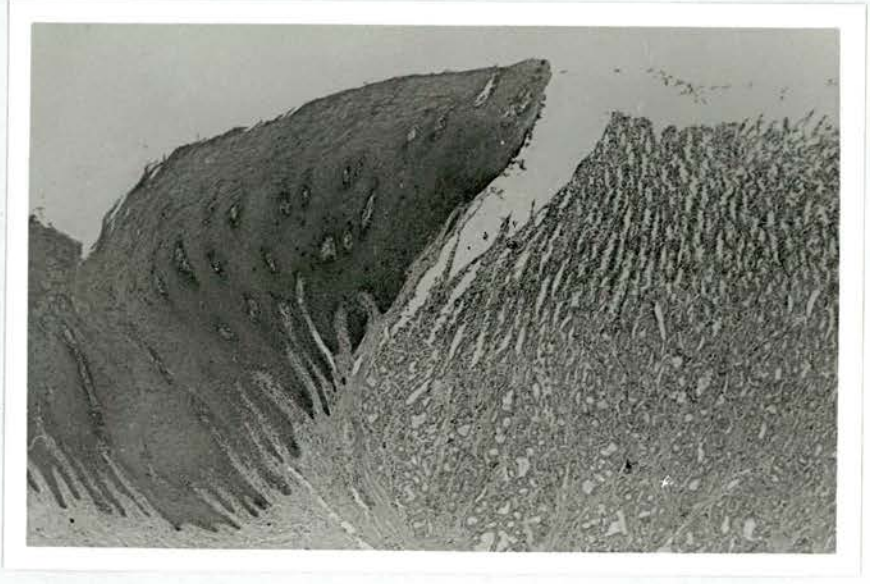
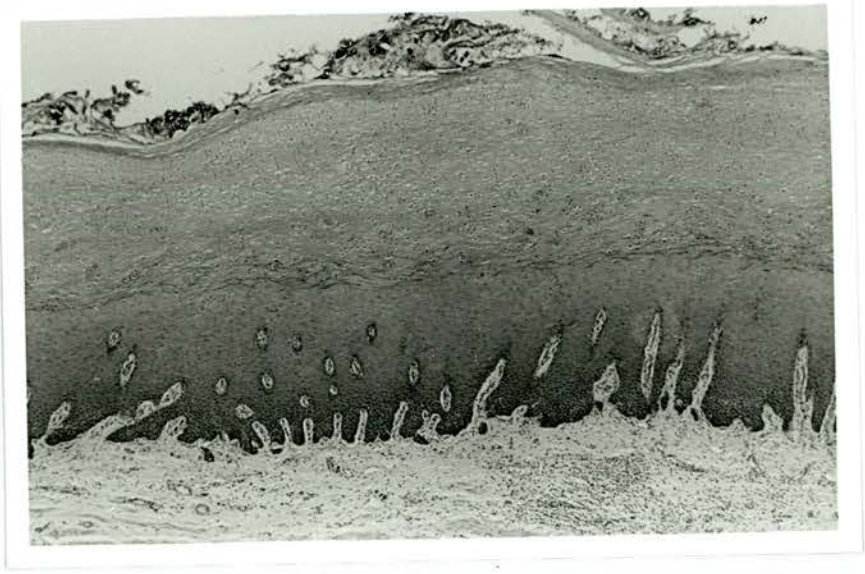


Figure 5:1 Normal gastric proventricular epithelium.

Figure 5:2 Normal gastric glandular-proventricular epithelium junction.



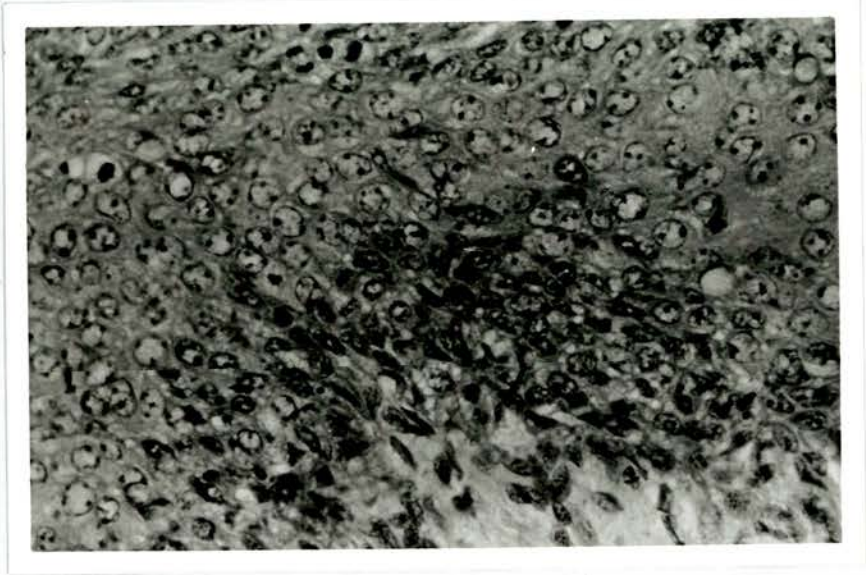
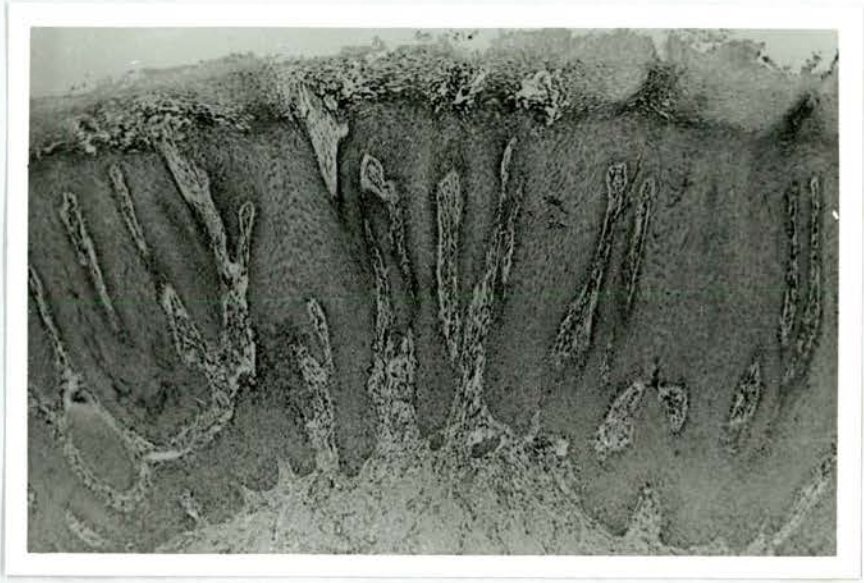


Figure 5:3 Epithelial change: hyperkeratosis, hydropic degeneration and infiltration of the lamina propria.

Figure 5:4 Numerous mitotic figures in the stratum spinosum.



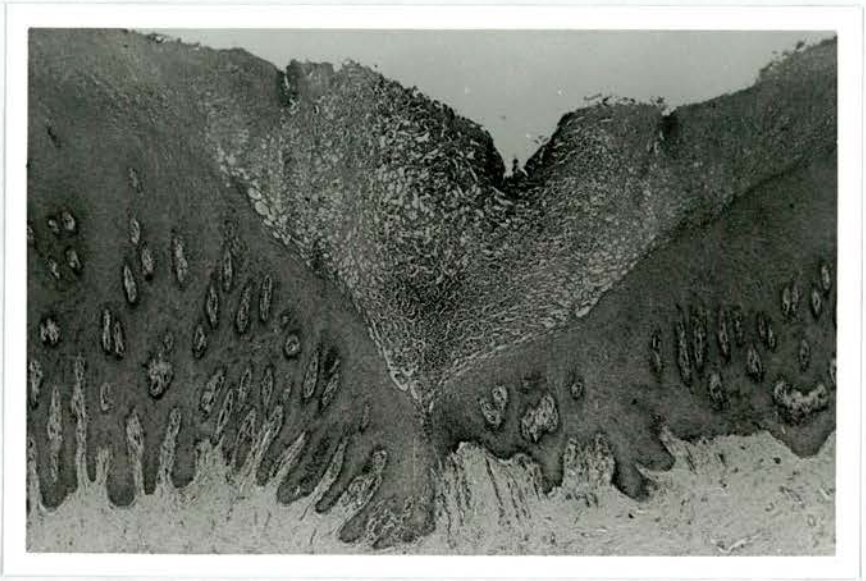


Figure 5:5 A dark staining basophilic necrotic zone containing bacteria and fungi was often present on the surface of lesions.

Figure 5:6 Acute erosion: In addition to the epithelial changes of parakeratosis and hydropic degeneration , reticular degeneration was observed in acute erosions.

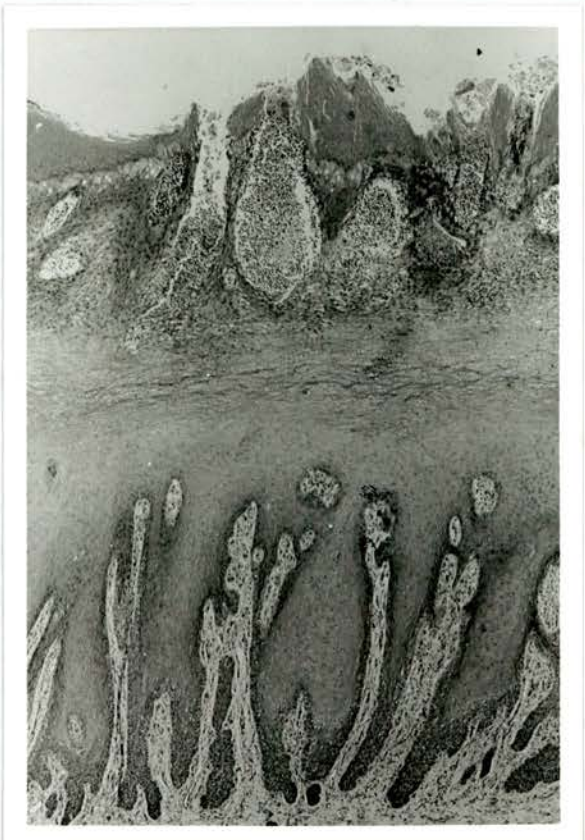


Figure 5:7 Microscopic abscesses in the hyperkeratoic zone with an associated intense inflammatory reaction.

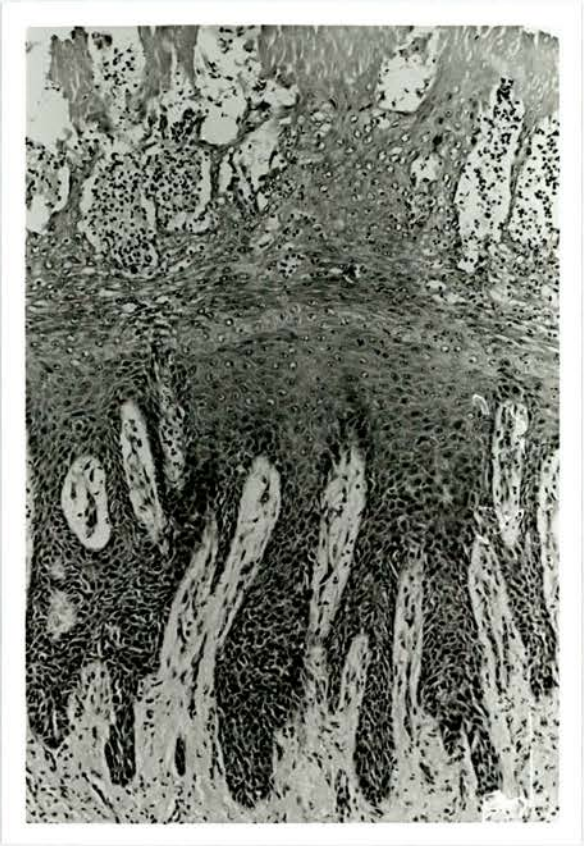
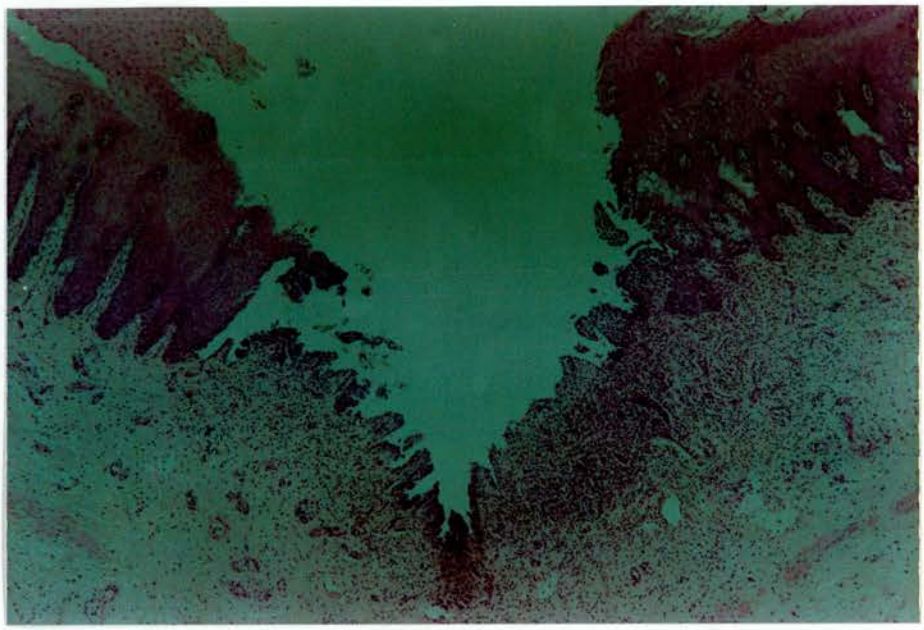


Figure 5:8 Spongiosis (intercellular oedema) characterised by widening of the intercellular spaces was a prominent feature in the stratum basale and the stratum germinativum in acute erosions.

Figure 5:9 Hyperplastic lymphoid tissue at the junction of the glandular mucosa and the proventricular mucosa



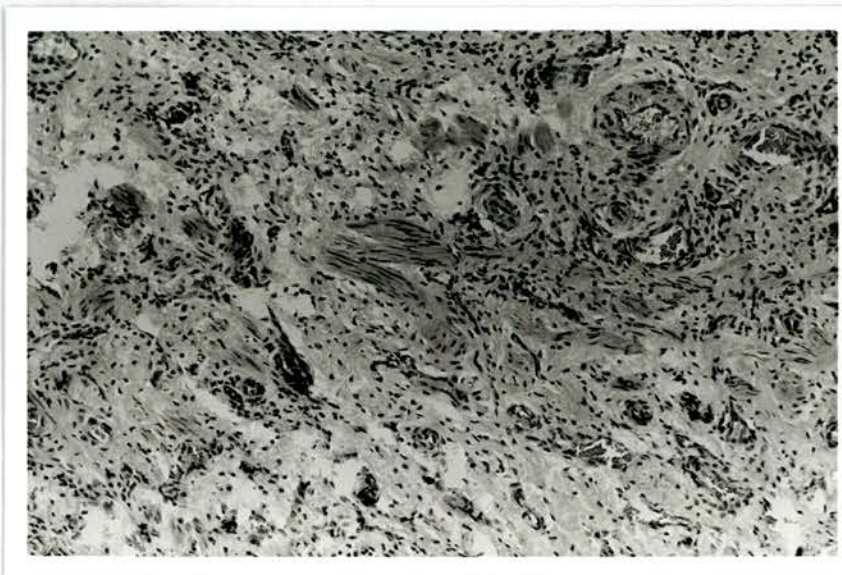
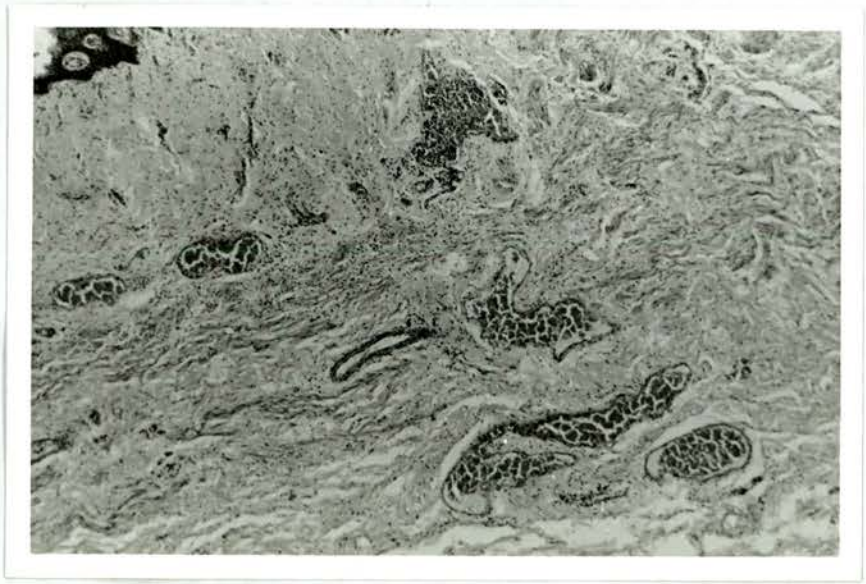


Figure 5:10 Acute ulcer.

Figure 5:11 Mature fibrous tissue in the lamina propria.



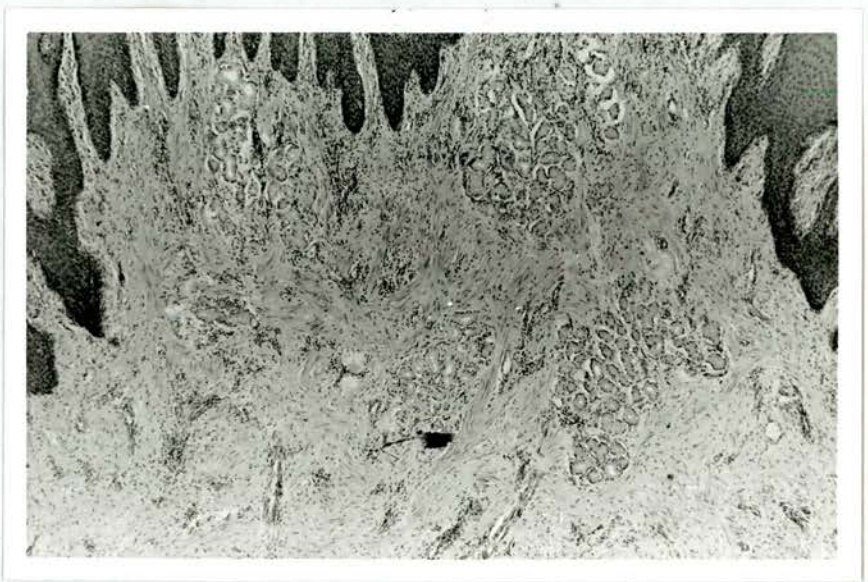


Figure 5:12 Congestion and increased numbers of blood vessels in the lamina propria.

Figure 5:13 Fibrous tissue in the lamina propria underlying the squamous mucosa in association with ectopic gastric glands.

Correlation between gross and histopathological diagnosis.

Of 21 tissues grossly classified as normal , 16 (76%) were also considered normal on histopathological examination, two were noted to have epithelial change, two had scarring and one had a microscopic acute erosion. Of fifty tissues grossly diagnosed as ulceration only 19 (38%) were confirmed as ulceration histologically but 24 (48%) were shown to have acute or chronic erosions. Of 15 horses with a gross classification of erosion 7 (47%) were confirmed histologically but 5 (33%) were histologically classified as epithelial change. Of 22 horses with a gross diagnoses of scarring graded either grade 3 or grade 4, 18 (82%) were confirmed as scarring histologically and four (18%) were considered normal. Of 10 horses with grade 2 gross scarring, 5 (50%) were confirmed histologically as scarring, 3 (30%) were normal and 2 (20%)had epithelial changes. As numbers in these groups were small, scarring scores grade 1 and 2 were combined as were grades 3 and 4. Using a Kruscal-Wallis ranking test , the presence or absence of fibrosis was associated with the degree of scarring ($p<0.05$). Additionally, the Kruscal-Wallis ranking test demonstrated an association between the presence or absence of ectopic glands and the scarring scores ($p<0.05$). Of eight glandular stomach sections classified grossly as gastritis only 2 (25%) were histologically confirmed, 50 % of tissues being shown to be congested only and the remaining samples were histologically normal.

The incidence of the main histopathological diagnosis are outlined below. Acute erosions occurred in 24 horses, acute ulcers occurred in 13 horses; one horse had a glandular ulcer; chronic ulcers occurred in 11 horses and chronic erosions in 3 individuals.

In total erosions / ulcers were recorded in 52 horses all, with the exception of one occurring in the proventricular mucosa. Epithelial change was the main lesion in 9 horses and scarring was recorded in 29 horses. Congested glandular mucosa occurred in 6 horses and glandular mucosa gastritis was recorded in 3 horses. Twenty seven horses had normal squamous mucosa and an additional 10 horses had normal glandular mucosa.

Discussion.

The histopathological lesions described here are in broad agreement with the description of foal ulceration by Gross and Mayhew (1983) who in addition described frequent thrombosis of submucosal vessels, a feature also recorded by Buergelt (1984). Yamagishi *et al.* (1986) described a series of 11 cases of ulceration in adult horses and classified them into 4 stages; parakeratosis, acute erosion, acute ulcer and chronic ulcer. Yamagiwa *et al.* (1959) in a series of 14 cases of erosion or ulceration in the proventricular mucosa of horses with Japanese encephalitis described necrobiosis, eosinophilic infiltration, thrombosis of large and small vessels, fibrinoid swelling of the walls of blood vessels and oedema of the mucosa as the characteristic histopathological changes. The authors attribute these lesions to the virus of Japanese encephalitis and emphasise the association of thrombosis with lesions. Yoshihara *et al.* (1986) described parakeratosis in the squamous mucosa of 12 foals with ulceration but in addition described oedema of the epithelium, disappearance of the basal layer and lamellation of layers, features not encountered in this study. In a foal that died as a result of stomach rupture, Johnson, Hultine, Cook and Leipold (1980) described

marked acanthosis in the squamous mucosa, a condition they termed leukoplakia. Nevertheless, photographs of the gross lesions resembled extensive ulceration with islands of normal epithelium within the ulcerated area as described by Sweeney (1990).

The classification described in this paper is a pathological one and does not in any way indicate the clinical significance, if any, of the lesions. Many microscopic erosions were incorrectly classified as ulcers on gross pathological examination . The greatly increased thickness of the epithelium at the margo plicatus and in association with lesions (2 to 3 fold) gave the false impression of a substantial mucosal deficit but on histopathological examination it was found that the entire thickness of the epithelium had not been penetrated and consequently they were not ulcers but erosions. Changes in the depth of the mucosa in association with gastric lesions have been reported in veal calves (Pearson *et al.* 1987) and pigs (Embaye, Thomlinson and Lawrence 1990).

With the exception of lesions grossly classified as ulceration there was a reasonable correlation between the gross and histopathological classification of tissues considering that in some cases the gross lesion may not have been adequately represented in the histopathological slide. The occurrence of focal areas of brown staining hyperkeratosis with pronounced flaking caused some lesions to be grossly interpreted as erosions when on histological examination these changes were confined to epithelial changes. Of eight stomachs grossly diagnosed as glandular gastritis on the basis of deep red colour and increased amounts of mucus, only two were histologically confirmed. This emphasises the instruction of Rooney (1970) that acute gastritis is very rare in the horse and is characterised by excessive mucus production,

severe hyperaemia and petechiation. In addition in chronic gastritis there is a marked increase in the cobblestone appearance of the gastric mucosa.

Many of the pathological changes described here such as spongiosis, hydropic degeneration, exocytosis, hyperkeratosis, fibrosis and scarring are common non-diagnostic features of many acute and chronic inflammatory conditions (Yager and Scott 1985). However, the description of each category suggests a possible path by which ulcers and erosions of the gastric squamous mucosa may develop. Epithelial changes of hyperkeratosis, hydropic degeneration, exocytosis and spongiosis were present in all cases of ulceration / erosion and were also recorded in the absence of ulcers and erosions in a number of horses and therefore it is proposed that epithelial changes are the first step in the development of ulcerative lesions. Damaged epithelium may be gradually eroded away resulting in an erosion or ulcer. Infiltration of inflammatory cells and fibrosis may result in healing of the ulcer, or if ulcerogenic factors persist, it may enlarge and become a chronic ulcer / erosion. Perforation appears to be an uncommon sequel. Healing and contraction of fibrous tissue results in distortion of the squamous-glandular mucosa junction and the dragging of gastric glands under the squamous tissue.

There are remarkable similarities between the pathology of erosions and ulcers described here and those described in the pars oesophagea of the pig (Curtin, Goetsch and Hollandbeck 1963; Muggenburg *et al.* 1964; O'Brien 1986 and Embaye, Thomlinson and Lawrence 1990). However, in the pig lesions are deeper, involving the lamina propria, submucosa and the muscularis mucosa and numerous thrombosed vessels with

periarteritis are usually prominent. Thrombosis of blood vessels is a feature of ulceration in many species but it is not regarded as aetiologic (Van Kruiningen 1988). In equine ulceration and erosion there is minimal vasculature damage.

The pathogenesis or aetiology of equine erosion / ulceration is not known but is likely to involve a disruption of the gastric mucosal barrier (Sweeney 1990). Hyperacidity has been described as a cause of gastric ulceration and Hammond (1990) has shown that the pH of the proventricular portion of the equine stomach is lower than the glandular portion. Furthermore, the area where erosion and ulceration commonly occur in the adult horse (the junction of the squamous and glandular mucosa at the margo plicatus in the region of the lesser curvature) had the lowest pH of all. In addition, horses with ulceration of the squamous mucosa close to the margo plicatus had a significantly lower pH at this site than normal horses. He suggested that gastric acidity was significantly related to gastric ulceration in the adult horse as did Murray and Grodinsky (1989), although regional gastric mucosal pH measurements from both studies were not in total agreement. In man, reflux oesophagitis is commonly due to reflux of acidic gastroduodenal contents causing gross lesions of erythema, streaking and linear erosions and may be the condition most analogous to gastric ulceration in the adult horse (Hammond 1990). Further support for the hypothesis of Hammond would be gained if the histopathological lesions in the horse stomach and those from clinical cases of reflux oesophagitis in man were similar. However, in man the major histopathological lesion is controversially considered to be basal cell hyperplasia (acanthosis)(Day and Husain 1986; Thompson 1989), a feature not

recorded in the horse in this study. Chronic oesophageal epithelial damage or ulceration in man may lead to Barret's oesophagus, a condition characterised by the overgrowth and upward extension of gastric columnar epithelium to areas previously occupied by squamous mucosa (Thompson 1989). Barret's oesophagus-like lesions were not observed in this study.

A common infectious cause of oesophagitis in man is the opportunist pathogen, the yeast *Candida albicans* (candidiasis) which causes lesions presenting grossly as discrete adherent white plaques which overlie a friable, erythematous mucosa (Day 1989). Gross and Mayhew (1983) suggested that candidiasis may be a factor in the aetiopathogenises of ulceration in the foal but in this study the invasion of lesions by yeast-like structures was uncommon. Buergelt 1984 isolated *Candida* species from the hyperkeratoic mucosa in 7 out of 33 cases of foal gastric ulceration. Rooney (1964) failed to demonstrate fungal elements in eight cases of perforating gastric ulceration. In porcine proventricular ulceration *Candida albicans* has been observed frequently (Ito and Miura 1974) and it has been suggested that the fungus might play a role as a secondary factor in accelerating the occurrence of erosive and ulcerous changes. More recent studies have shown that in pars oesophagea pathology in the pig, the normal population of lactobacilli is reduced in number and that *Candida albicans* and *C. glabrata* were the species most commonly associated with stomach lesions (Embaye 1987).

CHAPTER 6

THE PREVALENCE AND PATHOGENICITY OF *GASTEROPHILUS INTESTINALIS* LARVAE IN HORSES IN IRELAND

Summary.

At an Irish horse abattoir 614 horse stomachs were examined over a twelve month period (between 27 and 101 per month) . Excluding the months of July to September inclusive when the stomach is largely free of bots, 458 stomachs were examined, 197 (43 per cent) of which had *Gasterophilus intestinalis* larvae. The majority of horses (90 per cent) had less than 50 bot larvae and only 1.4 per cent harboured more than 100 bots. Ulceration or erosion of the gastric stratified squamous mucosa close to the margo plicatus was recorded in 173 (28.2 per cent) of the 614 stomachs and lesions varied in size from 0.5 cm^2 to over 60cm^2. Only 60 (37 per cent) of horses with ulceration had concurrent bot burdens. Analysis of meteorological data revealed that the corresponding periods of larval development and fly activity were colder , duller, wetter and more windy than normal. By comparison with a similar survey in Ireland published in 1976 in which a 90 per cent prevalence of *G. intestinalis* was reported, it is concluded that a dramatic reduction in the prevalence and extent of bot infestations had occurred and this is largely attributed to the introduction of

ivermectin and to a lesser extent, the climatic conditions which may have adversely affected fly activity and larval development. Furthermore, it is concluded that bot larvae are not important in the aetiopathogenesis of gastric ulceration or erosion.

Introduction.

The larval stages of *Gasterophilus* species of flies (bots) are common obligate parasites in the gastrointestinal tract of horse in many parts of the world (Zumpt 1965). The commonest species occurring in northern temperate zones is *Gasterophilus intestinalis* which completes one life cycle per year, approximately 10 months of which are spent in the parasitic larval form, largely as second and third stage instars, in the equine stomach (Figure 6:1). During the month of July in Ireland and Britain, the mature third stage larvae are expelled from the stomach via the faeces. Pupation occurs in the soil after an approximate 35 day temperature dependent period and egg laying activity on horses legs by adult flies is usually observed during the months of August to October inclusive (Edwards 1982). The minimum period between egg deposition and first appearance in the stomach of a new generation of second stage larvae is 5 weeks (Wells and Knipling 1938) and attachment of second stage larvae to their predilection site on the gastric squamous mucosa is first observed in mid September. Consequently, as the equine stomach is largely free of bot larvae from July to September inclusive, it is necessary to exclude these months when evaluating the annual prevalence of bot infestations.

Hatch, McCaughey and O'Brien (1976) reported a prevalence of 90% and 65.8% of equine stomach bots in two individual Irish horse abattoirs during the months October to June

inclusive. Similarly, Edwards (1982) reported a 69% prevalence of *G. intestinalis* larvae during a similar period in a population of horses in Northern England and Wales.

The pathogenic effect of bot larvae within the stomach is unclear (Soulsby 1982) and many horses carrying large numbers of bot larvae show no overt clinical signs. Nevertheless, bots have been incriminated in causing ulcers and subserosal abscesses (Waddell 1972), gastric rupture and suppurative peritonitis (Dart, Hutchins and Begg 1987) and subclinical effects on gastric function and consequently overall performance (Edwards 1982).

This paper records the prevalence and magnitude of *G. intestinalis* infestations in 614 horses and investigates their role in gastric ulceration.

Materials and methods.

A total of 614 horse and pony stomachs were examined between April 1988 and March 1989 inclusive at a horse abattoir in Straffan, Co. Kildare. No donkey stomachs were examined. Between 27 and 101 stomachs were examined in each calendar month during this period (Figure 6:2). Each stomach was incised along the greater curvature from the duodenum to the cardia, and the presence, location and number of *G. intestinalis* larvae identified (according to Zumpt, 1965) and recorded. The duodenum was not examined for the presence of *Gasterophilus nasalis* larvae and second and third stage larvae were not always differentiated. The previous anthelmintic management of these horses was not known. In addition, the presence or absence of gastric ulcers or erosions (other than the typical craterform pit or coalescing pit lesions normally associated with the attachment of

bot larvae) were recorded as well as precise location, severity and extent. Photographic records were obtained where considered necessary.

Meteorological data from six meterologicial stations (Mullingar, Claremorris, Shannon Airport, Kilkenny, Casement Aerodrome and Birr) which encompased approximately the areas of origin of horses in this study were obtained from the Meteorological Service and the monthly mean temperature, rainfall, duration of sunshine and wind speed computed for the months of June to November 1987 / 1988 inclusive.

A Chi square test was used to test for an association between the presence of bots and the presence of ulcers / erosions.

Results.

A total of 614 horse and pony stomachs were examined in the twelve month period and 216 (36%) were infested with second or third stage *G. intestinalis* larvae. Excluding the months of July to September inclusive when the equine stomach is largely devoid of bot larvae, 458 stomachs were examined and 197 (43%) had bot larvae (Figure 6:2). Only one stomach out of 29 examined in July had bots and none of 35 stomachs examined in August had either second or third stage bot instars. The monthly mean larvae burden in infested horses ranged between 11 and 33 and the highest individual bot burden was 184 second stage larvae recorded in October .

Of 32 stomachs examined on June 29, 1988, 10 (31.3%) were infested with bots which is not statistically significantly different ($x^2 = 1.44$, $p>0.05$) from the 133 stomachs examined up till June 9 of which 57 (43%) were similarly infested.

The percentage of horses harbouring 1-50, 51-100, and >100 *G. intestinalis* larvae each were 89.8, 8.8 and 1.4 per cent respectively (Figure 6:3). The total aggregate number of bots recorded in the study was 4367 *G. intestinalis* larvae.

The site of attachment of the bot larvae was very consistent and typical craterform pit lesions or larger coalescing multiple pit lesions were only observed in the squamous mucosa close to the margo plicatus in the more dorsal aspects of the parietal (cranial) border of the stomach (Figure 6:4 and Figure 6:5). In very heavy bot infestations a small number of bots were observed attached to the glandular mucosa adjoining this area. Occasionally, a number of bots were recorded in a variety of different locations but when removed, no gross bot-induced pathological change was visible at these sites and invariably an almost precise number of vacant typical craterform attachment sites were recorded in the normal predilection site.

A total of 173 (28.2%) horses out of the 614 examined had ulceration or erosions in the squamous mucosa close to the margo plicatus in that region of the lesser curvature which abuts the pyloric glandular mucosa (Figure 6:4, Figure 6:6 and Figure 6:7). Bot larvae were not detected in or within 4 cm distance of these ulcers/erosions and the pathological nature of these lesions were other than that described in the classification of macroscopic lesions produced by *G. intestinalis* as reported by Principato (1988). Grossly, lesions were singular or multiple, irregular and craterform in shape with obvious thickened edges. Occasionally, necrotic material was recorded in the base of the ulcer. The total ulcerated area was less than 1 cm^2 in 31% of affected horse and between 1 and 5 cm^2 in an additional 40% of horses. Twenty nine

per cent of recorded ulcers /erosions were larger than $5cm^2$. The single biggest ulcer was 60 cm^2.

Of the 173 horses with gastic ulceration/erosion, only 60 (37%) had concurrent bot infestation (Figure 6:6). An association between the occurrence of ulceration/erosion and the presence of bots was investigated on a combined annual aggregate basis using the Chi square test but no association was demonstrated (x^2= 0.03, p>0.05). Equally, there was no association between the presence of bots and erosions / ulcers when examined on a monthly basis (April, x^2 = 0.16: May,x^2=0.86: June, x^2=0.03: July, x^2= 2.3: August, numbers too small for computation: September, x^2= 2.79: October,x^2 = 0.56: November,x^2= 0.07: December, x^2= 0.00: January,x^2 = 0.03: February, x^2= 0.11 and March,x^2= 0.04).

The meteorological data is summarised in Figure 6:8 and in general the months of June to November inclusive in 1987 and 1988 were colder, duller and wetter than the corresponding mean values for the previous 25 years. This was particularly true in 1988 when in addition the months of July to October were more windy than normal. In July 1988, the mean monthly temperature was 1.3 ^oC colder than normal, there was 1 hour less sunshine daily, 70% more rain and wind speeds were 19% stronger than normal.

Discussion.

The survey published by Hatch *et al.* (1976) was in part, like this current study, carried out in the Irish Horse Abattoir, Straffan, Co. Kildare and a comparison of these findings indicates a substantial reduction in the prevalence of *G. intestinalis* larvae. At that time, 90% of horses during the months October to June inclusive had stomach bot infestations compared to only 43% in this

Stage	Location	Development time	Months	References
Pupae	Ground	5 weeks (temperature dependent). (viable for 3 months)	July-Sept.	Zumpt (1965) Soulsby (1982) Wehr (1933)
Adults	Air	Live, 3 weeks	Aug.-Oct.	Wells *et al.* (1938)
Eggs	Hair on fore legs	5-10 days (viable for 4 months)	Aug.-Dec.	Edwards (1982) Soulsby (1982) Drudge *et al.* (1975)
First instar	Tongue, Gingiva	21-28 days	Aug.-?Jan.	Soulsby (1982) Edwards (1982)
Second instar	Stomach	5-7 weeks	Sept.-Feb.	Drudge *et al.* (1975) Edwards (1982)
Third instar	Stomach	9 months	Oct.-June.	Edwards (1982)

Month	Number examined	Number infested	Percentage	Mean no. of larvae	Range of larvae
April	65	25	39	29	3-75
May	31	13	42	22	3-85
June	69	29	42	20	1-90
July	29	1	3	1	0- 1
August	35	0	0	0	-
September	92	18	20	9	2-19
October	101	37	37	26	1-184
November	27	14	52	11	1-40
December	56	25	45	13	1-88
January	30	18	60	26	1-93
February	30	16	53	1	2-24
March	49	20	41	24	1- 93
Total	614	216	36	18	1-184

Figure 6:1 Summary of *Gasterophilus intestinalis* life cycle in Ireland.

Figure 6:2 Monthly prevalence of *Gasterophilus intestinalis* in equine stomachs.

No. of larvae per infestation	Number of horses	Percentage of horses	Hatch *et al.* (1976) Straffan	Hatch *et al.* (1976) Saintfield	Edwards (1982) England
1 - 50	194	89.8%	72.7%	53.2%	75.5%
51 - 100	19	8.8%	20.1%	24.8%	16.5%
> 100	3	1.4%	7.2%	22.0%	8.1%

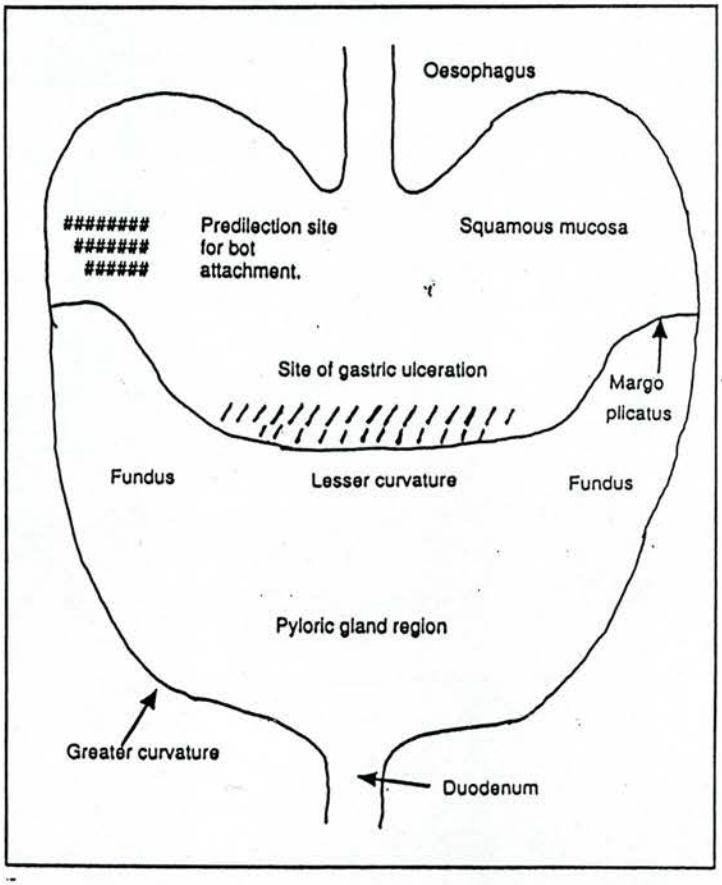
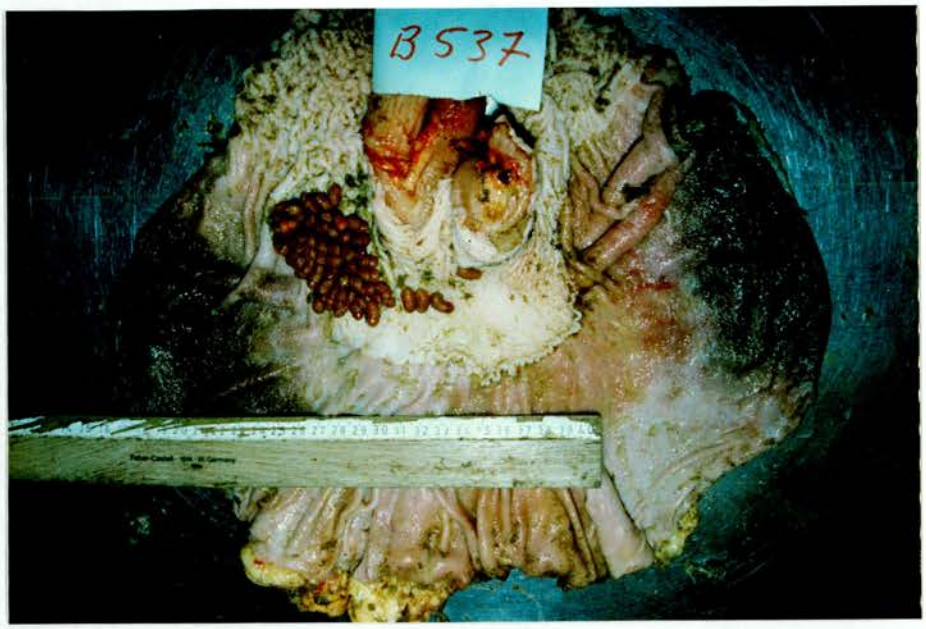


Figure 6:3 *Gasterophilus intestinalis* frequency distribution in infested horses: Comparison with two surveys.

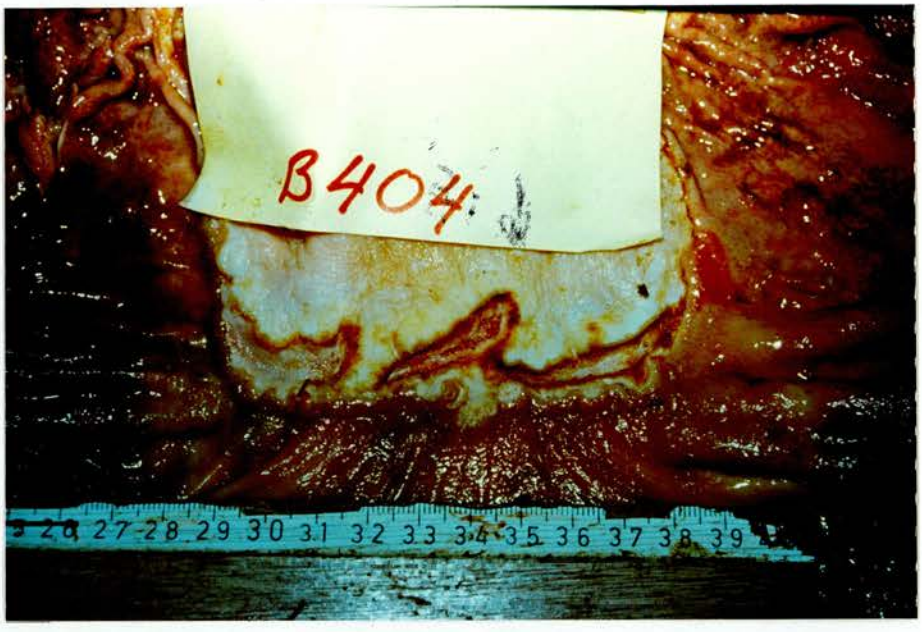
Figure 6:4 Diagram of equine stomach incised along the greater curvature with the lumen visible to outline the anatomy, site of gastric ulceration and predilection site for bot attachment.



Month	Number examined	No. with ulcers/erosions	Percentage with ulcers/erosions	No. with ulcers/ erosions and bots	
April	65	26	40	9	(35%)
May	31	7	23	4	(57%)
June	69	20	29	9	(45%)
July	29	9	31	1	(11%)
August	35	12	34	0	(0%)
September	92	18	20	1	(6%)
October	101	21	21	7	(33%)
November	27	9	33	5	(56%)
December	56	20	36	9	(45%)
January	30	8	27	5	(63%)
February	30	5	17	3	(60%)
March	49	18	37	7	(39%)
Total	614	173	28	60	(37%)

Figure 6:5 Predilection site for bot attachment.

Figure 6:6 Monthly prevalence of ulceration / erosion in equine stomachs.



	Mean temperature (°C)			Sunshine (hrs)			Rain fall (mm)			Wind speed (knots)		
	1987	1988	25 year mean	1987	1988	25 year mean	1987	1988	25 year mean	1987	1988	25 year mean
June	12.3	14.6	13.4	3.02	5.52	5.55	106	43	59	6.4	5.2	7.6
July	15.5	13.6	14.9	3.77	3.45	4.44	33	116	68	6.9	8.8	7.4
Aug	15.0	13.9	14.7	3.91	3.89	4.63	65	93	78	6.2	8.5	7.2
Sept	12.2	12.3	12.9	4.52	3.19	3.72	103	78	87	7.7	8.5	8.0
Oct	8.3	10.1	10.5	3.08	3.16	2.74	106	102	88	7.2	9.1	8.4
Nov	6.9	6.4	6.7	1.92	2.46	2.07	68	43	88	7.3	5.9	8.9

Figure 6:7 Medium sized ulcer (circa 1cm x 5 cm) in the squamous mucosa close to the margo plicatus.

Figure 6:8 Mean meterological data from six meterological stations with months which were colder, duller, wetter or more windy underlined.

study. A similar study carried out between September 1971 and August 1972 inclusive in an abattoir in Saintfield, Co. Down revealed a 65.8% prevalence of *G. intestinalis* larvae during the months October to June inclusive (Hatch *et al.* 1976). A more recent study by Edwards (1982), carried out in northern England and Wales revealed a 69% prevalence of *G. intestinalis* during these months.

In addition to the decreased prevalence of stomach bots, a remarkable reduction in the extent of such infestations was noted. The single largest stomach bot burdens recorded in Straffan and Saintfield by Hatch *et al.* (1976) were 414 and 513 respectively as compared to 184 in this study. The largest *G. intestinalis* burden recorded by Edwards (1982) was 295 third stage larvae. Furthermore, only 10.2% of all bot infestations in this study harboured more than 50 *G. intestinalis* bots as compared to 27.2% (Straffan) and 46.7% (Saintfield) as reported by Hatch *et al.*, (1976) and 24.6% by Edwards (1982), (Figure 6:3).

The reason for the reduction in the prevalence of *G. intestinalis* in the 17 year period spanning both Straffan surveys may in part be due to increased owner awareness of the life cycle of *G. intestinalis* and consequent improved horse husbandry. It must also be considered that both populations may not be strictly comparable as Thoroughbreds which usually are managed more intensively, comprised 45% of the animals examined in this survey, where as farm and work animals are likely to have been more important in the survey of Hatch *et al.* (1976). Nevertheless, it is the opinion of the author, that the introduction of and widespread use of effective new generation, wide spectrum equine anthelmintics with concurrent potent ecto /endoparasitic and boticidal actions is the single most important factor in mediating this reduction. Foremost

among such drugs are the ivermectins (Eqvalan Paste®; MSD AGVET) which enjoys a 100% efficacy against first, second and third stage *Gasterophilus* instars in addition to efficacy against large and small strongyles, lungworm, oxyuris, strongyloides and parascaris (Yazwinski, Hamm and Greenway and Tilley, 1982; Evans, 1988). Equine oral ivermectin preparations were introduced in Ireland in 1983 and in excess of 50,000 doses suitable for 500 kg horses of Eqvalan® (ivermectin) were sold in the Republic of Ireland in 1988 and Eqvalan paste® now accounts for half the market world-wide for equine anthelmintic oral pastes (Bloomfield, 1988). Prior to the era of ivermectins, carbon disulphide and a number of organophosphorous compounds (dichlorvos and trichlorphon) were the main boticidal agents in use but disadvantages included narrow therapeutic spectrum, narrow safety range, digestive disturbances, a requirement to withhold food and water for a period before and after treatment and variable efficacy (70 to 100%), (Drudge and Lyons, 1983).

Beesley (1966) reported that in general terms, a cold wet summer tends to be followed by a spring when the number of warbled animals is lower than normal and this was clearly demonstrated by Tarry (1980) who demonstrated a strong correlation between average air temperature /hours of bright sunshine and the percentage of hides damaged by cattle warble fly. The climatic conditions in July to October 1987 / 1988 were not optimal for the pupal and adult stages of *G. intestinalis* and the colder, duller, wetter and more windy weather, especially in 1988 is likely to have been partly responsible for the observed decreased prevalence of stomach bots from September 1988 to March 1989.

The possible effects of climate are as follows:

1. A temperature increase of about 2.8°C (5°F) results in doubling of fly activity (Williams 1940, cited by Tarry 1978) and with the exception of August 1987, the months of August to October 1987 / 1988 (the period of adult fly activity) were colder than normal and the October 1987 mean daily temperature was 2.2°C colder than normal. As a minimum temperature of 12.8°C is apparently required before *G. intestinalis* is capable of flying and temperatures of >15.5°C required before oviposition can occur (Wells and Knipling, 1938), the colder autumns during this study may have had serious implications for the bot prevalence rates recorded in the following winters and springs.

2. Wind speeds influence the daily flight of flies and the sheep head fly, *Hydrotaea irritans* is generally not active in wind speeds of 6m s^{-1} (Nielsen *et al*, 1972, cited by Tarry 1978). As mating by *G. intestinalis* is generally begun during flight with the male engaging the female in flight (Wells and Knipling 1938), the stronger winds of August, September and October 1988 may have been significant.

3. As bright sunshine is said to be important for successful mating, poor egg viability may be related to overcast conditions (Ganser 1956, cited by Tarry 1980). The summers of 1987 and 1988 were duller than normal and in July 1988 there was on average 1 hour less sunshine per day than the 25 year mean.

4. The period required for pupae development is temperature dependent (Zumpt 1965, Edwards 1982) and the months of July to September 1988 inclusive were significantly colder than normal.

5. Under very dry conditions the successful emergence rate of flies from pupae in the ground will be low because surface soil layers will be difficult to penetrate (Marr 1957, cited by Tarry 1980). Less than

half the normal amount of precipation was recorded in July 1987.
6. Excessive rainfall can destroy pupae (Walton 1928, cited by Tarry 1980) and in July 1988, 71% more precipation than normal was recorded.

Gasterophilus intestinalis is a cosmopolitan parasite and its prevalence in many parts of the world has been documented (figure 6:9). A substantial variation in the prevalence and severity of *G. intestinalis* infestations is apparent and is illustrated by a 100 % prevalence of bot larvae in donkeys in Morocco, 60% of which harboured more than 100 bots (Pandey, Ouhelli and Elkhalfane, 1980) and a 0% prevalence of bots in Hong Kong (Hammond, Mason and Watkins 1986). These variations in prevalence may be explained by differences in geographical location and associated differences in the suitability of the local ecology for the extra-host stages; climatic conditions; age and breed; husbandry method; the frequency of grooming and housing during the periods of *Gasterophilus* oviposition; the numbers of horses and donkeys in an ecological area; probably the strain of the parasite and the availability and use of effective boticidal agents.

It is also likely that varying local seasonal factors cause local fluctuations in bot prevalence from year to year. Ribbeck, Heide, Schicht and Hiepe (1983) reported an increased prevalence of *G. intestinalis* larvae from 26% in 1977/78 to 59% in 1979/80. Similarly, Hilali, Derhalli and Baraka (1987) reported a 98.3% prevalence of *G. intestinalis* larvae in Egypt in 1983 compared to a 60% prevalence in 1942. Varying local climatic conditions are advanced as the explanation why 62.5% and 10.5% respectively of horses examined in July and August by Hatch *et al.* (1976) had stomach burdens of *G. intestinalis* as compared to only 3.4% and

Country	Year	Number examined	Prevalence %	Mean no. larvae	Max. no. larvae	Reference
U.S.A.	1951-73	476	68	168	1046	Drudge *et al.* (1975)
Mexico	1983	650	44	78	-	Alanis Tafolla (1983)
Venezuela	1958	-	98	-	-	Quintero (1958)
Burkina Faso	1986	30	100	155	449	Kaboret *et al.* (1986)
Germany	1977-80	2539	9	33	500	Ribbeck *et al.* (1983)
Italy	1978-80	285	7	-	-	Guizzardi (1982)
Yugoslavia	1978-80	88	83	-	-	Guizzardi (1982)
U.S.S.R.	1983	866	100	-	4525	Rastegaev (1983)
Egypt	1982-83	118	98	-	547	Hilali *et al.* (1987)
Morocco	1977-78	94	100	219	838	Pandey *et al.* (1980)
India	1969	12	100	-	-	Singh *et al.* (1974)
China	1986	-	100	-	714	Yang (1986)
Hong Kong	1983-84	195	0	0	0	Hammond *et al.* (1986)
Australia	1966-71	331	64	32	610	Waddell (1972)
New Zealand	1972-73	70	97	63	406	Kettle (1974)

Figure 6:9 The prevalence of *Gasterophilus intestinalis* in various countries worldwide.

0% respectively for similar months of this study which was carried out in the same abattoir as that of Hatch *et al.* (1976).

Further useful information on the dynamics of the *Gasterophilus intestinalis* life cycle in Ireland may have been obtained had second and third stage larvae constantly been differentiated during the study. Nevertheless, some important observations have been made. There was no significant difference in the prevalence of bot infestations in stomachs examined before June 9th and stomachs examined at the end of June.Therefore, it appears that significant numbers of bot larvae do not leave the equine stomach in late June as suggested by Hatch *et al.* (1976) and the exclusion of June results in addition to those of July, August and September in computing the annual prevalence rate is unjustified. Support for the belief of an extensive evacuation of third stage larvae from the stomach in early July is provided by the examination of 29 stomachs on July 20 from which only one solitary bot larva was recovered.

The examination of 37 stomachs in early September (8-9-88) failed to demonstrate the presence of second stage *G. intestinalis* larvae whereas 13 (40.6%) of 32 stomachs examined on September 21st had second stage instars. As the duration between egg deposition and the first arrival of second stage larvae in the stomach is 5-6 weeks (Figure 6:1), it is unlikely that any adult egg laying activity occurred before August. Edwards, (1982) proposed that oviposition commences in late July in Wales and northern England despite the absence of bot eggs on the limbs of 66 horses examined by him in July. From this work and the work of other authors a composite outline of the probable life cycle of *G. intestinalis* in Ireland is provided (Figure 6:1). It is important to note

that in other climates such as Kentucky in the United States of America, egg laying activity of the female extends over much longer periods and second and third stage larvae are found simultaneously in the stomach during every month of the year (Drudge, Lyons, Wyant and Tolliver 1975).

Panitz (1978) proposed an overwintering phase of dormant pupae which hatch in the subsequent spring and considered the overwintering of adults possible but less likely. The finding of only one solitary *G. intestinalis* larvae in 101 stomachs examined between July and mid September does not support the occurrence of an overwintering phase in Ireland.

Waddell (1972) observed from his survey of 331 stomachs that larvae were attached to all parts of the oesophageal region from the margo plicatus to within 1 cm of the cardia and that the majority of the larvae were attached to the mucosa of the saccus caecus. Hilali *et al.* (1987) reported that in the stomachs of donkeys, *G. intestinalis* larvae were mainly clustered in groups near the boundary of the glandular and the non glandular mucosa. The selection of the attachment sites of *G. intestinalis* larvae within the stomach was investigated by Price and Stromberg (1987) who reported a pronounced preference for locations in the squamous mucosa along the margo plicatus on the cranial surface of the stomach and a secondary preference for an area slightly cranial to the most dorsal aspect of the saccus caecus.

In this study, major clustering of bot larvae and typical macroscopic bot larvae lesions were only found in the squamous mucosa adjoining the margo plicatus on the cranial aspect of the stomach (Figure 6:4). In very heavy bot burdens a small proportion of larvae were attached to the glandular mucosa

adjoining this predilection site. Occasionally, bot larvae were recorded either free in the digesta or attached elsewhere to the stomach mucosa but macroscopic bot lesions were not evident when detached and almost invariably an appropriate number of vacant typical craterform bot lesions were simultaneously recorded in the normal predilection site. It has been suggested that bot larvae move around the mucosa (Jubb, Kennnedy and Palmer 1985_b) but as some horses in this study were dead for up to 6 hours before examination, it is likely that post-mortem induced changes in the stomach microenvironment provoked migration by some of the larvae.

Unlike the findings of Price and Stromberg (1987) clustering of *G. intestinalis* larvae in the most dorsal aspect of the saccus caecus was not recorded in this study. The much heavier bot burdens (up to 950 bot larvae) in the study of Price and Stromberg (1987) does not adequately explain their observation of a secondary predilection site because they reported that attachment sites for *G. intestinalis* larvae were not influenced by season or intensity of infestation.

Despite the dramatic appearance of heavy bot infestations, their true pathogenic effect is obscure (Urquhart, Armour, Duncan, Dunn and Jennings 1987). Soulsby (1982) concluded that it was unreasonable to assume that extensive ulceration of the stomach by large numbers of parasites is without general effect. Subclinical effects on stomach function associated with the presence of bots and consequently on overall performance was considered probable by Edwards (1982), and Nolan (1963) considered bots a cause of debility. Waddell (1972) associated bots

with the occurrence of subserosal abscesses and Pandey *et al.* (1980) reported that bots cause gastric ulcers, erosions, and nodulesopolypous growths. Rooney (1964) reported that ulceration of the oesophageal region with or without perforation in foals is directly related to mechanical trauma by *Gasterophilus intestinalis* larvae. Great annoyance is caused to horses by the darting behaviour of adult flies during oviposition (Urquhart *et al.* 1987) and migration in the tongue and gingiva by the first and second stage larvae causes severe disruption of the tissues and the formation of microabscesses (Cogley 1989).

Other exceptional untoward sequelae to bot infestation include gastric rupture and fatal peritonitis (Rainey 1948; Dart *et al.* 1987), paralysis of the oesophagus (Horton 1925), impaction (Nolan 1963) and fatal hepatitis (Tadmor, Perl and Weinberg 1981). Rarely, *G. intestinalis* larvae have been recorded in such ectopic locations as the ovary (Drudge, Leland and Behlow 1956), diaphragm and large omentum (Belli and Gevrey 1981), liver (Tadmor *et al.* 1981) and the brain (Poynter 1963).

Waddell (1972) incriminated *G. intestinalis* in the aetiology of gastric ulcers and erosions affecting the squamous mucosa between the cardia and that part of the margo plicatus which borders the pyloric glandular region along the lesser curvature (Figure 6:4). He supported this conclusion on the grounds that 92% of ulcerated stomachs had concurrent bot infestations (between 18-340 *G. intestinalis* larvae) and histopathological evidence of under-running of the epithelium near bot larvae by necrotic tissue containing bacteria which he considered important in the initiation of gastric ulcers. However, he did not consider bots the sole cause of gastric ulcers and erosions.

With regard to gastric ulceration /erosion, the findings and conclusions of this study are almost in direct contradiction to those of Waddell (1972). In this study, only 34.7% of horses with gastric ulceration /erosion had concurrent bot infestation or evidence of recent bot infestation and no statistical correlation was present between the presence of bot larvae and the occurrence of ulceration /erosion when examined both on a monthly basis and on a combined annual aggregate basis. Furthermore, despite the seasonal absence of bot larvae from the equine stomach (July-September), no monthly or seasonal variation occurred in the prevalence of gastric ulceration /erosion. In addition, a distance usually well in excess of 10 cm was present between the site of ulceration and the predilection site for bot attachment and therefore, it seems unlikely that under-running of the epithelium by necrotic material in the immediate vicinity of bot larvae could be important in the initiation of gastric ulcers /erosions in the area of the lesser curvature.

It is concluded from the above observations that *G. intestinalis* larvae have no role to play in the aetiopathogenesis of equine gastric ulceration and erosion. Support for this conclusion is obtained from the work of Hammond, Mason and Watkins (1986) who, in a survey of 195 Thoroughbreds in Hong Kong, where *Gasterophilus* larvae have never been reported, recorded a 66% prevalence of gastric ulceration. Conversely, in a survey of 70 horses in New Zealand, 97% of which had *G. intestinalis* larvae (mean=63), Kettle (1974) did not observe the presence of any gastric ulcers /erosions.

The dynamics of the *G. intestinalis* life cycle in northern temperate climates such as Ireland is now reasonably well

established (Figure 6:1). *Gasterophilus intestinalis* has a discrete annual life cycle with one generation yearly and no overlap of successive generations. Edwards (1982), noted the occurrence of second stage *G. intestinalis* as late as February and therefore a second treatment with an effective drug in January in addition to the traditional treatment for bots in early winter would be prudent.

CHAPTER 7

AN EVALUATION OF THE ROLE OF *HELICOBACTER PYLORI* (*CAMPYLOBACTER PYLORI*) IN EQUINE GASTRIC ULCERATION

Summary.

Sera from 71 adult horses, 41 of which had gastric pathology (ulceration, erosion, keratinisation or gastritis) were examined using an ELISA, for antibody against *H. pylori.* A statistically significant difference ($p<0.05$) in the mean *H. pylori* optical density values (OD) between abnormal horses (0.530) and horses with no gastric pathology (0.375) was recorded and the mean optical density in horses with gastric pathology was 41.3% higher than in normal horses. In normal horses the highest optical density recorded was 0.734 OD and only 8 horses (27%) had OD values over 0.400.There was a very substantial overlap between OD values in horses with gastric pathology (0.170-1.106) and normal horses (0.160-0.734). The highest mean OD value (0.553, range: 0.281-0.928) was recorded in the 18 horses with gross keratinisation of the squamous mucosa. In nine horses with gross evidence of glandular gastritis the mean OD value was 0.437

(0.206-0.635).

Helicobacter pylori were not noted on histopathological examination.

Introduction.

The aetiology of equine gastric ulceration is not fully understood and although many factors have been incriminated, none with the exception of NSAIDs have been proven (Sweeney 1992, chapter 2).

In man the role of *Helicobacter pylori* {formerly *Campylobacter pylori* (Anon. 1989; Goodwin, Gordon and Burke 1990)} in upper gastrointestinal disease has received enormous attention in the last decade and has been reviewed (Blaser 1990; Graham 1989). A strong association between *H. pylori* and duodenal ulceration (almost 100% of cases; O'Connor and Axon 1989), greater than 99% of cases of human superficial / chronic atrophic gastritis and gastric ulceration (70% of cases) has been reported (Dixon 1989) but there is a weaker link with non-ulcer dyspepsia (Shallcross, Rathbone and Heatley 1989). The evidence supporting *H. pylori* as a primary pathogen in normal gastric mucosa has been strengthened by ingestion studies which provoked clinical and histopathological gastritis with seroconversion (Morris and Nicholson 1989). In addition, therapeutic trials in normal healthy individuals that demonstrated a therapeutic efficacy equal to that of histamine type 2 (H_2) receptor antagonists when various *H. pylori* active antibacterials with no direct ulcer healing properties were used alone or in

combination with colloidal bismuth subcitrate (CBS) in the management of gastritis (Hirschl and Pletschette1989) and peptic ulceration (Coghlan,Tobin and O'Morain 1989). Furthermore, the above trials also demonstrated the additional advantage of a significantly reduced relapse rate in peptic ulcer and gastritis cases treated with antimicrobials as compared to those treated with conventional H_2 receptor antagonists. The prevalence of *H. pylori* infection correlates well with the intensity of histologically observed antral mucosal inflammation and furthermore, the elimination of bacteria correlates well with an improvement in symptoms, histology and appearance at endoscopy (Iserhard *et al.* 1990). However, there is not universal agreement on the significance of *H. pylori* and its role in the pathogenesis of gastric disease remains somewhat controversial (Bartlett, 1988).

The high prevalence of *Helicobacter pylori* in human populations (15-50%, Dwyer, Kaldor, Tee and Raios 1989) has prompted a search for this organism in other species and *Helicobacter pylori* -like organisms (HPLO) have been cultured from the stomachs of the rhesus monkey (*Macaca mulatta*) both at necropsy and from pinch biopsies obtained at endoscopy (Newell 1989) and similar culture characteristics, biochemistry, morphology, total protein profiles and antigenic profiles to *H. pylori* but significant differences from *Campylobacter jejuni* have been demonstrated . *Helicobacter pylori* -like organisms have also been recovered from the pig tailed monkey (*Macaca nemestrina*) and baboon and in all non-human primates in which HPLO were isolated there was an associated gastritis similar to that in man (Newell 1989).

Additionally, HPLO have been recovered from the stomachs of ferrets but reports of its pathogenicity need clarification (Tompkins 1989; Fox and Lee 1989). Although sharing many characteristics with *H. pylori*, the ferret gastric HPLO differ in many respects including different protein profiles, differences in protein antigens and total polar lipid and phospholipid profiles (Tompkins 1989).

Experimental infection with *Helicobacter pylori* in gnotobiotic pigs has been successfully established and *H. pylori* recovered from the fundus, antrum, distal oesophagus and proximal duodenum (Lambert and Borromeo 1989). Gastritis and seroconversion occurred in the experimental piglets but no pathological changes were observed in the oesophagus and the authors considered it likely that the bacteria recovered from the oesophagus were due to colonisation or contamination during the post-mortem period. *H. pylori* infection persisted in gnotobiotic infected pigs after they were transferred to conventional housing (Eaton, Morgan and Krakowka 1990). A spiral micro-organism morphologically similar to *H. pylori* (*Gasterospirillum suis*) that may cause antral gastritis has been recovered from the pig gastric mucosa (Mendes *et al.* 1990).

Helicobacter pylori colonises the stomachs of gnotobiotic dogs resulting in lesions similar to those seen in humans (Radin *et al.* 1990). The occurrence of large numbers of *H. pylori*-like spiral shaped bacteria in dog and cat stomachs has been documented for some time (Salomon 1896, cited by Lee 1989) but although sometimes associated with mild gastritis it has been proposed that they be considered part of the normal canine flora (Henry, Long, Burns and Charbonneau 1987). However, the possibility that some

of these spiral organisms in animals could be pathogenic in man was raised by Dent, Mc Nulty, Uff, Wilkinson and Gear (1987) who reported spiral organisms that were neither *Campylobacters, Helicobacters* or *Spirochetes* in the gastric mucosa of three patients, two of which had severe oesophagitis and none of which had endoscopic evidence of gastric or duodenal disease. Subsequent serological investigation of these three patients revealed high levels of specific antibody to the cat spiral organism and a low level to *H. pylori* and it was concluded that these organisms were of animal origin (Lee, Dent, Hazell and McNulty 1988).

The possibility that *H. pylori* might be a zoonosis has been raised by by a serological survey of 98 abattoir workers in Italy which demonstrated a significantly higher IgG titre to *H. pylori* in workers with direct contact with freshly cut meat and viscera compared to clerical workers in the same factory with no such contact (Vaira, D'Anastasio, Holton, Dowsett, Londei, Bertoni, Beltrandi, Granuenfels, Salmon and Gandolfi 1988). Of 28 abattoir workers with high *H. pylori* titres that agreed to endoscopy and biopsy all had *H. pylori* associated histologically proven gastritis and all showed a decrease in *H. pylori* IgG titres after a four week course of colloidal bismuth subcitrate (240mg twice daily).

In common with the horse, the pig has a stratified squamous epithelial area in the stomach, the pars oesophagea, in which ulceration like that which occurs in the horse is commonly found (O'Brien 1986). A study of these oesophagastric lesions in pigs by Embaye (1987) revealed a decreased number of the normal population of *Lactobacilli* in parakeratoic lesions and ulcers but an increase in the number of yeasts (*Candida albicans* and

Candida glabrata) with the age and severity of lesions. Equally, Griffing (1963), recovered *C. albicans* from 86% of pig stomachs with ulcers and denuded stratified squamous epithelium but in only 14% of normal stomachs.

Bacteriological examination of the stomachs of 172 slaughter pigs yielded *Campylobacter* strains (*C. jejuni*, 21%; *C. coli*, 74% and *C. fetus*, 4%) in 95 (55%) of stomachs (Schreiner 1985). Localised gastritis was found in five stomachs but there was no clear relationship between the bacteriological or histological detection of *Campylobacter* in the stomachs and the pathological changes.

A high prevalence of abomasal ulcers (87%) largely occurring in the pylorus has been reported in veal calves (Welchman and Baust 1987). The worst lesions occurred in the heaviest calves and were not deleterious to their welfare. An association between ulceration and straw feeding was demonstrated and an infectious aetiology was generally discounted. However, from calves dying from perforating abomasal ulcers Johnson, Hudson and Bohlender (1981) isolated *Clostridium perfringens* and a reduced incidence of abomasal tympanitis and ulceration was recorded when cows and / or calves had been vaccinated against *Clostridium perfringens* C and D.

Jensen and Frederick (1939) isolated *Streptococci* from stomach ulcers in the pig, sheep, calf, cow and dog and concluded that these organisms were primary pathogens rather than secondary agonal invaders after they successfully induced gastric ulcers in rabbits and dogs by the intravenous or root canal injection of *Streptococci*.

Swerczek (1980) reported the isolation of *Clostridium*

botulinum type B from stomach ulcers occurring near the margo plicatus in 5 out of 8 horses (4 foals) which died from what he considered to be toxicoinfectious botulism. No role in the pathogenesis of the ulcer was proposed for *Clostridium botulinum* and it was considered that the necrotic area of the ulcer produced an anaerobic environment for the proliferation and toxin production by the organism.

Stress ulcers have been described in a wide variety of laboratory animals including rats, guinea pigs and monkeys (Van Kruinnigen 1988); ferrets (Fox 1988) and mice (Fox, Cohen and Loew 1984). Gross ulceration in the pylorus / fundus was observed in 7.3% of 1000 rabbit stomachs examined by Hinton (1980) but no fungi or bacterial pathogens were demonstrated.

The common finding that occurrences of the foal gastroduodenal ulcer syndrome have frequently been preceded by episodes of infectious diarrhoea and the occasional clustering of affected foals on certain studs has suggested an infectious aetiology (Becht and Byars 1986) but attempts to identify a causal organism in a series of foals with perforating duodenal ulcers was not successful (Acland, Gunson and Gillette 1983). However, in a report of five clinical isolates of oxidase and catalase positive *campylobacter* organisms from foals, three of the five foals involved showed clinical or pathological signs compatible with the foal gastroduodenal ulcer syndrome (Atherton and Ricketts 1980).

The isolation of *Campylobacter* organisms from foals with the gastroduodenal ulcer syndrome (Atherton and Ricketts 1980), the possibility that *H. pylori* may be a zoonosis (Vaira *et al.* 1988) and the isolation of spiral bacteria from stratified squamous epithelium in the upper gastrointestinal tract in the pig and in man

(Dent *et al.* 1987; Lambert and Borromeo 1989) has prompted this study to evaluate the possible role of *H. pylori* in gastric ulceration in the adult horse.

Materials and methods.

Horses

Seventy-one adult horses from a larger study on equine gastric ulceration were used in the study. Both sexes were represented (35M,36F) and the horses were of varying ages and breeds (Thoroughbred 41, pony 21, halfbred 5 and draught 4). All horses were presented for slaughter at a commercial abattoir and the carcasses were subsequently sold for human consumption. Clotted blood samples were collected antemortem by jugular venopuncture and after recovery the sera were stored at-20^{o}C until required.

The stomachs of all horses were incised along the greater curvature, gently washed with running water and examined for the presence of gastric ulcers, erosions, gastritis and parasitism. Appropriate biopsy specimens were collected from pathological and suspect pathological tissues within the stomach but biopsies were also collected from normal stratified squamous and glandular gastric mucosae. Gastric biopsies were stored in 10% buffered formal saline and subsequently stained with haematoxylin and eosin. In addition, biopsies from 32 horses were stained with the Warthin Starry silver stain as described by Stevens (1982) with the modification that slides were developed at room temperature instead of 60^{o}C.

Serology.

Antigen preparation.

A clinical isolate of *H. pylori* from a human stomach obtained in St. James Hospital, Dublin, was grown on chocolate agar (columbia base) for 4 days at 37^{o}C in a jar flushed with CO_2 and containing moistened wads of filter paper. An acid glycine extract of this strain was prepared by the method described by Goodwin, Blincow, Peterson, Sanderson, Cheng, Marshall, Warren and McCulloch (1987) using 0.2M glycine hydrochloride buffer, pH 2.2.

ELISA.

ELISA plates (Nunc) were coated overnight at room temperature with 100 ul of antigen (5 ug protein / ml) in carbonate buffer, pH 9.6. After washing, the plates were incubated with 1:100 dilutions of horse sera in phosphate buffered saline containing 0.5% bovine serum albumen, for sixty minutes. The plates were washed again and inoculated with peroxidase labelled anti-horse IgG (Sigma) for 30 minutes. After washing, orthophenylene diamine was used as the chromogenic substrate. The reaction was stopped after 10 minutes at room temperature with 1M H_2SO_4 and read at 492 nm in aTitertek-Multiskan Plus Elisa reader and the results expressed as optical densities (OD).

The tests were done in duplicate in two sittings. Two sera from the first batch were included in the second sitting. The OD of these two sera were corrected to obtain the values obtained in the first sitting and the correction factor applied to the remaining sera in the second batch for compatibility as described by McLaren, Lillywhite and Au (1981).

No attempt was made to culture *H. pylori*

Results.

Gross pathology.

Thirty seven horses had gastric ulcers / erosions affecting the stratified squamous epithelium close to the margo plicatus in that area which joins the pyloric glandular mucosa and the results are summarised in Figure7:1. The ulcers were singular or multiple, craterform, circular, irregular or linear in shape, had obvious slightly raised edges often surrounded by a zone of brown staining keratin,and the bases contained brown necrotic material and occasionally were haemorrhagic. The size of the ulcerated area varied from about 0.25 cm^2 to 60 cm^2 and the mean ulcerated area was 7.87 cm^2. The mucosa in the region of the ulcer was often thickened, roughened and sometimes corrugated. Bile staining was not observed. On no occasion was blood, blood clots or fibrinous exudate found in the stomach or in the environs of the ulcer. In addition, 18 horses had extensive gross keratinisation (cornification) of the gastric squamous mucosa evidenced by the layering of a brown stained slightly elevated superficial scale that could easily be removed by scraping and in 16 of these horses there was concurrent ulceration or erosion.

In addition to ulceration or erosion six stomachs had reddening or discolouration of the glandular mucosa, a more pronounced cobblestone appearance and an apparent increase in the volume of mucus and were grossly diagnosed as glandular gastritis. Three stomachs had the above features in the glandular mucosa without ulceration in the squamous mucosa.

Thirty horses had normal gastric glandular and non

glandular tissues.

Histopathology.

The least severe pathological change observed was described as epithelial change and was characterised by increased epithelial thickness, increased depth and irregularity of the rete pegs, hyperkeratosis and hydropic degeneration. More severe lesions were classified as acute erosion and in addition to the changes described for epithelial change, erosion, reticular degeneration and spongiosis was noted. Acute and subacute ulcers described lesions where the deficit extended down to the lamina propria and in chronic ulcers and erosions fibrosis was a significant feature. Scarring was characterised by increased corrugations of the epithelium, fibrosis of the lamina propria and ectopic glands. In a number of ulcers various bacteria and branching fungal hyphae were observed near the surface of the lesion.

Despite the gross diagnosis of gastritis in 9 horses, this diagnosis was only substantiated on histopathological examination in three cases , five of the other cases being diagnosed as congestion and the remaining case as normal. Eosinophils and lymphoid cells were prominent in normal glandular epithelium.

Helicobacter pylori were not noted on the Warthin-Starry silver stained slides.

Serology.

A statistically significant difference ($p<0.05$) in the mean *H. pylori* optical density between abnormal horses (0.530) and horses with no gastric pathology (0.375) was recorded and the mean optical density in abnormal horses was 41.3% higher than in normal horses. Five ulcerated horses had optical density over

0.800. In normal horses the highest optical density recorded was 0.734 OD and only 8 horses (27%) had OD values over 0.400. There was a very substantial overlap between OD values in horses with gastric pathology (0.170-1.106) and normal horses (0.160-0.734) and this is illustrated in Figure 7:2. It is interesting that the highest mean OD value (0.553, range: 0.281-0.928) was recorded in the 18 horses with gross keratinisation of the squamous mucosa. In the nine horses with gross evidence of glandular gastritis the mean OD value was 0.437 (0.206-0.635).

Discussion.

In man, most patients colonised with *H. pylori* elicit a measurable systemic antibody response but this is complex and highly variable and no single protein antigen has yet been investigated which consistently reacts with all positive sera investigated (Newell and Stacey 1989). A number of serological assays to detect *H. pylori* have been described including complement fixation, haemagglutination, bacterial agglutination and immunoflorescence but currently enzyme linked immunosorbent assay (ELISA) is considered to be the method of choice because of its speed, low cost, simplicity and reproducibility. In this study, ELISA was performed using an acid glycine extract of *H. pylori*. This antigen is partically purified and has minimal cross reactivity with *Campylobacter* spp..

A statistically significant difference ($p<0.05$) in the *H. pylori* OD values between horses with gastric pathology and horses with no gastric pathology was recorded and the mean optical density in ulcer horses was 41.3% higher than in normal horses. It is

Number	Ulcer size (cm)	Erosion	Keratinisation	Gastritis	OD values
432	6.00				1.11
238	4.00				1.01
199	0.60		**		0.93
267	0.75		****		0.87
229	0.50		****		0.87
186	1.60		***		0.65
142		****	**	***	0.63
270		****	****		0.62
285	36.00			**	0.61
185	2.00			**	0.59
89	1.00		**		0.58
273	1.25		****	***	0.57
135	9.00		****		0.57
237	60.00				0.57
111	15.00				0.55
225	4.00		****		0.53
179	7.50		*		0.52
273	9.00		****		0.51
99	3.00				0.50
203	38.00				0.48
436	4.00	**	**		0.45
66	3.00				0.44
344	6.50		***		0.43
231	2.00				0.42
438	4.00				0.42
247	11.00				0.41
80	4.50				0.40
73	6.00				0.39
78	1.13				0.35
100	2.00		**		0.34
433	2.00	*			0.34
103	5.00		**	**	0.33
104	5.00				0.33
150	9.00				0.33
329	0.25				0.30
281	3.00			***	0.17
205				***	0.45
218				***	0.38
181			**		0.29
330			**		0.28
76				***	0.21

Figure 7:1 Description of the major pathology and *H. pylori* OD values in the 41 horses with gastric pathology.

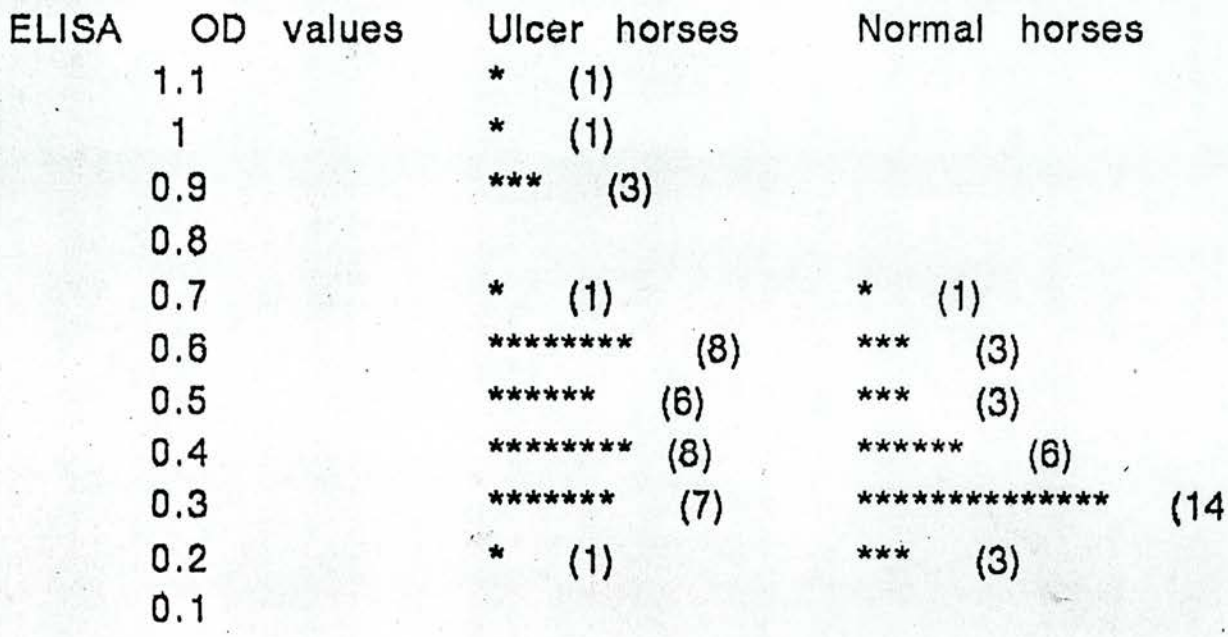


Figure 7:2 *Helicobacter pylori* ELISA OD values in horses with ulceration or erosion and in normal horses.

interesting that the highest mean OD value was recorded in the 18 horses with gross keratinisation of the squamous mucosa and it is tempting but very premature to speculate that the keratin forms a protective layer over the bacteria attached to the epithelial cells much in the same way as mucus does in human gastric mucosa.

It is relevant to note the findings of Newell (1989) who reported low levels of specific antibody to *H. pylori* in Rhesus monkeys with HPLO associated gastritis when compared to antibody levels in infected humans. Nevertheless, significantly higher antibody levels were observed in monkeys with HPLO associated gastritis than in those monkeys which had no evidence of HPLO infection.

However, the significance of all OD values in the horse must be tempered by the consideration of cross reactivity. Originally, crude antigens such as whole cell sonicates were used in man but gave high backgrounds, a high proportion of false positives and poor sensitivity. However the use of partly purified antigens such as a glycine acid extract, as used in this experiment, has significantly improved specificity and greatly reduced cross reactivity with other *Campylobacter* spp. and ELISAs using these complex antigens have been reported to detect approximately 95% of patients with *H. pylori* infection with a specificity of 90% (Newell and Rathbone 1989; Newell and Stacey 1989). In the horse *Campylobacter jejuni / coli* infections are not considered important pathogens (Garcia, Eaglesome and Rigby 1983) and out of 304 diarrhoeic samples examined specifically for *Campylobacter* spp. in a foal diarrhoea survey only in one sample was *Campylobacter* spp. isolated (Browning *et al.* 1991).

The absence of *H. pylori* -like organisms in the ulcer

biopsy samples is not entirely surprising because of the location of the ulcer in a stratified squamous epithelium without a mucus covering and in man *H. pylori* have not been identified histologically in such an epithelium. However in patients with oesophageal inflammation or gastritis, Walker, Birch, Stewart, Stoddart, Hart and Day (1989) were successful in culturing *H. pylori* from the oesophagus of 27% of such patients but failed to demonstrate it by light or electron microscopy. Equally in gastroesophageal reflux disease in man *H. pylori* have been identified in 65% of patients (Hendenbro, Schalen, Wadstrom, Willen 1989) and anti-*H. pylori* IgG antibodies have been described in 46% of patients with oesophagitis (Anderson 1989). However, whether *H. pylori* is important in oesophageal pathology or merely represents a contaminant is a matter of some dispute (Fallingborg, Agnholt, Moller-Peterson, Christensen, Lomborg, Sondergaard,Teglbjaerg and Rasmussen 1989: Lambert and Borromeo 1989). Furthermore, in the successful experimental infection of gnotobiotic pigs with *H. pylori* described by Lambert and Borromeo (1989), the organism was cultured from the oesophagus but oesophageal biopsies did not exhibit histopathological changes characteristic of gastric epithelium colonised by the microbe.

It is of interest that all biopsies from inflamed and normal equine mucosa were negative for *H. pylori* on histopathological examination but as only a maximum of two glandular biopsies (usually from the fundic glandular region) were collected from horses with ulceration and only rarely from the pyloric glandular region (antrum) it is impossible to exclude the presence of *H. pylori* in equine gastric mucosa.

In man, *H. pylori* is identified histologically in tissue sections by virtue of its characteristic morphology, diffuse distribution, high population density and its position closely applied to the surface of epithelial cells (Wyatt and Gray 1989). The distribution of *H. pylori* in the gastric mucosa of man is patchy and evidence for a negative finding can only be accepted if sufficient biopsy specimens have been collected from different areas of the stomach and at least two biopsies from the antral mucosa (Goodwin, Armstrong and Marshall (1986): Aase *et al.* 1989).

The histopathological finding of fungi, bacteria and large spiral organisms in some of the biopsy samples is considered unremarkable because of their low density and the possibility that they may represent post-mortem invasion. Gross and Mayhew (1983) suggested that *Candida* infection may have predisposed five foals to fatal ulceration of the gastric stratified squamous mucosa. As *Candida* spp. which are not considered primary pathogens, are obligate saprophytes of animals, occur frequently as normal inhabitants in the upper gastrointestinal tract and only become opportunist pathogens when the resistance of the host has been lowered by systemic disease, immunosuppression, neoplasia or prolonged antibiotic therapy (Gross and Mayhew 1983; Van Kruiningen 1988), the histopathological finding of *Candida* in equine gastric squamous epithelial ulcers especially in the absence of typical candidiasis lesions of whitish raised rose-like clusters on the epithelial surface is unconvincing.

The results of this study suggest a possible association between *H. pylori* and equine gastric ulceration but we could not confirm these findings with histopathological examination and culture was not undertaken. Serological investigations using better,

more refined *H. pylori* antigens, gastric culture and a more comprehensive histological examination of the gastric mucosa are needed to clarify the position. It would be of particular interest to investigate the role of *H. pylori* in the foal gastroduodenal ulcer syndrome because, in addition to ulceration of the stratified squamous mucosa, 32% of clinical cases have ulceration of the glandular mucosa (Wilson 1986). Research is required to determine why horses with no gastric pathology also had antibodies to *H. pylori.*

CHAPTER 8

THE EVALUATION OF SERUM GASTRIN LEVELS IN RELATION TO EQUINE GASTRIC PATHOLOGY

Summary.

Serum samples were collected from 151 horses prior to slaughter and serum gastrin levels measured using a human radioimmunoassay kit. Stomachs were examined and gastric pathology recorded. Recorded serum gastrin levels ranged from <20 pg /ml to 96 pg /ml. There was a statistically highly significant relationship between serum gastrin levels and the weight of gastric contents but no association occurred between serum gastrin levels and the presence or absence of ulceration / erosions, size of ulcerated or eroded area, margo plicatus hyperplasia or the presence or number of *Gasterophilus intestinalis* larvae.

Introduction.

Gastrin is a peptide hormone that is produced and secreted by G cells, the location of which have been demonstrated immunocytochemically, and in the horse are numerous in the pyloric mucosa, scarce in the duodenum and rare in the jejunum (Kitamura *et al.* 1984; Olowo-Okorun 1975). Following production, gastrin is secreted into the circulation and acts as a major mediator

of gastric acid secretion (Kovacs *et al.* 1989). The stimulus for effective secretion of hydrochloric acid by the parietal cells in the gastric mucosa requires three separate chemical mediators (secretagogues), namely gastrin, histamine and acetylcholine (Argenzio 1984). When circulating gastrin concentrations rise, the gastric parietal cells are stimulated to secrete acid. The blocking of any of these receptor sites on the parietal cell results in effective control of acid secretion and it is in this way that the histamine type2 (H_2) receptor antagonist drugs (cimetidine, ranitidine, fomotidine and nizatidine) mediate their effect (Lewis 1983).

Under physiological conditions gastrin is released from G cells as a result of distension of the stomach and stimulation by amino acids and partially digested food. The release of gastrin may be mediated by vagal mechanisms and may also be influenced by a peptide in the gastric mucosa having gastric releasing properties (Nilsson 1980).

Gastrin release is inhibited by acidification of the antral mucosa of the stomach and this is the major inhibitory factor in the release of gastrin. However, secretin, glucagon, vasoactive intestinal peptide, gastric inhibitory peptide, calcitonin and somatostatin have been shown to suppress gastrin release after parentral administration but is not clear if they have such an effect under physiological conditions (Nilsson 1980).

Gastrin also has a trophic effect on the fundic mucosa and in various species including the dog, stimulates DNA, RNA and protein synthesis in the acid-secreting mucosa and increases the number of parietal cells. Gastrin also stimulates antral motility and induces secretion of pancreatic enzymes, water and electrolytes

by the stomach (Nilsson 1980).

Gastrin catabolism occurs as a non specific process in capillary beds in as diverse tissues as the kidney, head and hind leg (Thompson *et al.* 1979).

Basal and postprandial serum gastrin values have been investigated in the horse using commercially available radioimmunoassay kits and basal levels have been reported from a small experiment (n=5) to be between 7.0 and 13.8 pg/ml and to be between 17.4 and 19.8 pg/ml within 75 minutes post prandially (Brown *et al.* 1987). However, another study using a different commercial gastrin radioimmunoassay kit which had been validated for use in the horse reported basal serum gastrin levels ranging from < 8 pg/ml to 17.5 pg/ml and a mean peak postprandial value of 70 pg/ml three hours after feeding (Young and Smyth 1988). Brown *et al.* (1987) concluded that gastrin levels in the horse may be lower than in other species but this may reflect methodology, the type and potency of the standard and specificity of the antibody used (Jaffe and Walsh 1979).

In the foal, basal , peak postprandial (sixty minutes) and 180 minutes postprandial serum gastrin values in the one-day old foal (n=6) were reported to be 25.2 ± 4.3 pg/ml , 47.4 ± 15.2 pg/ml and 19.6 ± 5.6 pg/ml (Smyth *et al.* 1989). Postprandial gastrin concentrations did not increase to the same extent in one-week and one-month old foals, and in three -month old foals there was no increase in postprandial gastrin concentrations. Murray and Luba (1989) reported plasma gastrin values shortly after birth to be 53.8 ± 20.6 pg/ml and 38.3 ± 14.6 pg/ml at 28 days of life (n=9).

Pathophysiology.

Hypergastrinaemia has been described in man in

association with the Zollinger-Ellison syndrome which is characterised by a non-beta islet cell tumor of the pancreas (gastrinoma), strikingly increased gastric acid secretion and severe ulcer disease of the upper gastrointestinal tract which is clinically indistinguishable from common peptic ulcer disease (McGuigan 1983). Additionally, patients have increased serum gastrin values and vary from 150-450,000 pg/ml. A number of provocative tests are available, including intravenous secretin injection, intravenous calcium infusion and ingestion of a standard test meal, each performed with multiple measurements of serum gastrin concentrations. A Zollinger -Ellison like syndrome has rarely been described in the dog and cat (Breitschwerdt *et al.* 1986; Twedt and Magne 1989) and basal fasting serum levels may be increased 10 fold (Straus *et al.* 1977).

Gastrin has been extensively studied in relation to abomasal parasitism in sheep and cattle. Hypergastrinaemia (a 4-10 fold increase over controls) occurred in lambs after experimental *Haemonchus contortus* infestation and serum gastrin levels were highly significantly correlated with serum pepsinogen levels (Fox *et al.* 1988_b; Nicholls *et al.* 1988). In parasite naive sheep fed infective *Ostertagia circumcincta* larvae, plasma gastrin levels increased at least 10 fold (Anderson *et al.* 1981; Anderson *et al.* 1988). It is believed that the parasite is initially responsible for the hypergastrinaemia and that the increase in pH associated with parasitism would result in a further stimulus to gastrin secretion (Anderson *et al.* 1981; Nicholls *et al.* 1988; Anderson *et al.* 1988).

Similarly in calves, a significant increase (7.4 fold) in

plasma gastrin occurred after infestation with *Ostertagia ostertagi* (Fox *et al.* 1987) and less dramatic but significant increases in serum gastrin levels were recorded after infestation of calves with *Ostertagia ostertagi* and /or *Trichostrongylus axei* (Snider *et al.* 1988). However, in adult dairy cows, while pepsinogen increased in response to subclinical infestation with *Ostertagia ostertagi* no significant increase in plasma gastrin level occurred and therefore gastrin may be of diagnostic value in adult cattle for indicating when elevated pepsinogen levels are merely associated with a rise in larval intake and not with the establishment of large adult worm burdens (Pitt *et al.* 1988). It has been suggested that the decreased appetite associated with ostertagiasis is mediated through the increased blood gastrin concentrations which result from infection (Fox *et al.* 1989).

Ovine and bovine gastric parasitism result in hyperplastic gastritis with increased abomasal mucosal mass, increased mucosal thickness and increased mucosal cross-sectional area (Anderson *et al.* 1988; Snider *et al.* 1988) and as gastrin has trophic effects on some gastric tissues it was considered at least in part, a probable cause of the hyperplasia.

Controversy exists regarding the association of gastrin with gastric ulceration in man. In duodenal ulceration in man acid hypersecretion occurs in 50 % of cases (Sipponen 1989) . However, most patients with gastric peptic ulcers have normal basal and maximal acid outputs (Baron 1984) and a portion have a reduced gastric acid secretion (Spiro 1983). No increased G cell density or total G cell mass was recorded in association with gastric ulceration in man although large individual variations occurred (Nilsson 1980). However, plasma gastrin concentrations are often elevated to

various extents in patients with gastric ulcer disease and the level of basal gastrin in plasma seems to be inversely related to the the basal rate of gastric acid secretion (Nilsson 1980). No increase in basal serum gastrin has been recorded in association with duodenal ulcer disease but after stimulation with a protein meal a greater gastrin response was recorded in duodenal ulcer patients than in controls (Nilsson 1980). Patients with duodenal ulcer have an increased parietal cell mass and since gastrin appears to be the only agent known to induce parietal cell hyperplasia, a relationship between gastrin and duodenal ulceration must be suspected (Nilsson 1980). Recently, it has been demonstrated that the eradication of *Campylobacter pylori (Helicobacter pylori)* in duodenal ulcer patients resulted in decreased basal serum gastrin levels which preceeded reductions in parietal cell mass (McColl *et al.* 1989; Levi *et al.* 1989).

In the pig no association between plasma gastrin and the occurrence of epithelial hyperplasia and ulceration of the pars oesophagea could be found and it was concluded that if hypersecretion of gastric acid was involved in the pathogenesis of these lesions, that factors other than gastrin appear to be involved (Bunn *et al.* 1981).

Hammond (1990) has shown that the pH of the proventricular portion of the equine stomach is lower than the glandular portion and furthermore, the area where erosion and ulceration commonly occur in the adult horse (the junction of the squamous and glandular mucosa at the margo plicatus in the region of the lesser curvature) had the lowest pH of all. In addition, horses with ulceration of the squamous mucosa close to the margo plicatus had a significantly lower pH at this site than normal horses.

He suggested that gastric acidity was significantly related to gastric ulceration in the adult horse as did Murray and Grodinsky (1989).

Only one report deals with equine gastrin levels in association with disease. A comparison of normal (n=9) and diarrhoeic foals (n=9) revealed plasma gastrin levels greater than the mean + 1 sd in 3 diarrhoeic foals Murray and Luba 1989).

A three fold increase in serum gastrin levels was recorded in horses after an 80 km endurance ride but no increase occurred in horses in a 42 km race (Hall *et al.* 1982) but these results need to be interpreted in relation to feeding times.

Hypergastrinaemia in association with canine gastric distension has been reported (Leib *et al.* 1985).

The purpose of the current study was to further define normal gastrin levels in the horse and to investigate serum gastrin levels in relation to the occurrence of gastric ulceration / erosion, *Gasterophilus intestinalis* infestation and the weight of gastric contents.

Material and methods.

Blood samples were collected by jugular venopuncture from 151 adult horses immediately prior to slaughter at a commercial horse abattoir. Samples were identified, allowed to clot and the serum, after harvesting, was stored at -20^{o}C until examined.

Horse stomachs were examined as described previously by Sweeney 1992 (chapter 4). Stomachs were removed immediately after death but the time of the last previous meal was not known. Stomachs together with gastric contents were weighed and afterwards stomachs were incised along the greater curvature

from the duodenum to the cardia and gently washed with running water. Stomachs were again weighed and the weight of gastric contents computed. The presence or absence of gastric ulcers or erosions hyperkeratinisation and scarring were recorded. The size of ulcers or erosions were approximately measured and expressed in cm^2. The margo plicatus along the lesser curvature was examined and the degree of serration was evaluated and scored as described in chapter 4.

The presence, location and number of *Gasterophilus intestinalis* larvae were identified (according to Zumpt 1965) and recorded.

Serum gastrin levels were assayed using a commercially available human radioimmunoassay kit (Cambridge Medical Diagnostics, Billerica, MA 01865, USA). This was a double antibody radioimmunoassay kit employing rabbit anti-gastrin antiserum, ^{125}I labeled human synthetic gastrin and goat anti-rabbit gamma globulin and polyethylene glycol as a precipitating agent. Synthetic human gastrin was used as a gastrin standard and gastrin control in this kit. Results were expressed in pg /ml.The performance data of this assay according to the manufacturer,was a 95.5 to 99.5% recovery of known amounts of gastrin added to a human serum pool and intra and inter-assay coefficient of variation of between 5.2 and 10%.

The relationship between serum gastrin levels and the presence or absence of ulcers / erosions, size of ulcers and erosions, the presence and number of bot larvae, the weight of gastric contents and margo plicatus scores were evaluated using the Mann- Whitney test and regression analysis.

Results.

Serum gastrin values recorded in this study ranged from <20 pg /ml to 96 pg / ml. A highly significant correlation ($p<0.01$) was demonstrated between serum gastrin levels and the weight of gastric contents. However, no association occurred between serum gastrin levels and the presence or absence of ulceration / erosions, size of ulcerated or eroded area, margo plicatus scores (hyperplasia) or the presence or number of *Gasterophilus intestinalis* larvae.

Discussion.

A significant correlation between serum gastrin levels and the weight of gastric contents was demonstrated and this finding is in keeping with the belief that basal and postprandial gastrin values differ. Basal and postprandial values have been described in many species including the dog (Gabbert *et al.* 1984), foal and adult horse (Brown *et al.* 1987; Smyth *et al.* 1989), cattle (Yasuda *et al.* 1986) and man. In general, postprandial values are about twice basal resting values but the results of this study in part contradict the work of Smyth *et al.* 1989 who were unable to demonstrate a postprandial rise in gastrin in 3 month old foals. The postprandial serum gastrin increase is influenced by the nature of the diet and in man, protein and amino acids produce up to a five fold increase in serum gastrin levels, followed in potency by alcohol, fat and glucose (Korman *et al.* 1971). A dietary influence on serum gastrin levels was also demonstrated in the calf by Fox *et al.* (1988_a) who reported peak values in calves fed hay alone which were almost double that of calves fed on a largely concentrate diet and this

difference was explained by the effect that these diets are likely to have on abomasal pH. The role of diet in influencing equine postprandial gastrin levels has not been studied. Furthermore, basal and postprandial serum gastrin levels are age dependent , the highest levels occurring in the neonate and young and this trend has been shown in the horse (Smyth *et al.* 1989), cattle (Yasuda *et al.* 1986), pig (Bunn *et al.* 1981), sheep(Shulkes *et al.* 1982) and in man.

Many antisera used in radioimmunoassay are of sufficient affinity to eliminate the problem of sensitivity from the gastrin assay and are capable of detecting 1pg (or less) of added gastrin. The only hormone in serum known to pose any significant cross-reactivity problem is cholecystokinin-pancreozymin (CCK-PZ) which possesses the same C-terminal five amino acid residue as gastrin. The degree of cross reactivity varies considerably among antibodies, and antibodies prepared against C-terminal fragments show a high degree of cross reactivity (Jaffe and Walsh 1979). Gastrin heptadecapeptides have been isolated from the pig, dog, cat, sheep, cow and man and have been found to differ by only one or two amino acid substitutions in the middle of the linear peptide chain. Consequently, there is also considerable cross reactivity between other species of gastrin and since these substituted gastrins react in a parallel fashion with human (or porcine) gastrin it is acceptable to express results as human or porcine equivalents (Jaffe and Walsh 1979). With this technique, absolute values may be artificially low, but reflect relative serum gastrin concentrations.

A further problem in evaluating serum gastrin levels is the variety of molecular forms of circulating gastrin and varying

specificity of antibodies to bind to big and small gastrin. Gastrin is found in serum in a variety of molecular forms, the most abundant in serum being a 34 amino acid peptide designated big gastrin (G-34). However, a smaller form of gastrin, a 17 amino acid chain peptide (heptadecapeptide) designated small gastrin is the predominant form in gastric mucosa (>90%) (Nilsson 1980) and is six times more potent in stimulating gastric acid secretion than big gastrin. Both big and small gastrin may also be present in the sulphated form (Gabbert *et al.* 1984). Several other forms of gastrin have been recorded in tumours and serum from human patients with Zollinger-Ellison syndrome including Big Big gastrin and Gastrin 14. In total over 20 different gastrins may exist (Nilsson 1980). However, antibody 1296 recognises equally all forms of gastrin with intact carboxyl-terminal fragments and thus measures total gastrin activity (Jaffe and Walsh 1979).

Increased serum pepsinogen levels in association with *Trichostrongylus axei* infestation in ponies have been recorded (Herd 1986), and although not reported, it would seem probable that serum gastrin levels would also be increased correspondingly.

It should be remembered that at least in man, conditions other than the Zollinger-Ellison syndrome and peptic ulcer disease can cause an increased serum gastrin value including pernicious anaemia, gastric atrophy, chronic gastritis, renal insufficiency and some patients with massive resection of small intestine (McGuigan 1983). Also, in the absence of a gastrinoma, hypergastrinaemia may occur due to G cell hyperplasia (Nilsson 1980). Therapy of gastric ulceration also influences plasma gastrin and long term treatment studies with cimetidine indicate elevations of basal as well as meal stimulated plasma gastrin levels that decrease when

therapy is discontinued (Hansky *et al.* 1979; Nilsson 1980).

Until the physiology of gastrin in the horse is further explained it is difficult to envisage a role for gastrin assay as a diagnostic aid. The role of diet, age and the specificity of antibodies used also need to be further clarified.

CHAPTER 9

EVALUATION OF SERUM PEPSINOGEN LEVELS IN RELATION TO GASTRIC ULCERATION, EROSION AND *GASTEROPHILUS INTESTINALIS* LARVAE IN THE HORSE

Summary.

Serum pepsinogen levels from 85 adult horses, 37 (43%) of which had bots (range 1-69 bot larvae) and 59 (64%) of which had either ulceration or erosion of the proventricular mucosa were measured using a modification of the method of Hirschowitz (1955). The mean serum pepsinogen values for all horses was 0.21 IU/l (SD ± 0.18) but no association between serum pepsinogen levels and the presence or absence of ulceration / erosion, the extent of ulceration or erosion, the presence or absence of bot larvae or the number of bot larvae present was demonstrated.

Introduction.

The typical clinical signs of the foal ulcer syndrome or adult ulceration are not pathognomonic and definitive diagnosis can usually only be made on direct visualisation of the lesion by endoscope or alternatively by barium double contrast radiography

(Campbell, Ackerman and Peyton 1982 ; Campbell, Ackerman and Peyton 1984; Brown, Slocombe and Derksen 1985; Dik and Kalsbeek 1985; Adamson and Murray 1990). However, these techniques require special equipment not commonly available in equine practices and considerable expertise. Therefore, a blood test that might aid diagnosis would be extremely convenient and it was in this connection that serum pepsinogen was investigated in the horse.

Pepsinogen is secreted into the gastric lumen by chief cells located in the fundic glands which have in addition hydrochloric acid producing parietal cells. The stimuli for pepsinogen secretion are similar to those for acid secretion, however, the strongest stimulation is through cholinergic mediation (Argenzio 1990). Gastric pepsin output varies widely but generally parallels acid output (Campbell-Thompson and Merritt 1987_b). Pepsinogen is converted into the proteolytic enzyme pepsin in the presence of acid (pH <5.0).

In man, elevated blood pepsin has been recorded in association with gastric and duodenal ulcer disease (Niederman, Spiro and Sheldon 1964; Ichinose *et al.* 1982; Samloff, Stemmermann, Heilbrun and Nomura 1986;) . Recent research has shown that in general, personality features of hostility, irritability, hypersensitivity and impaired coping ability (low ego strength) each correlate significantly with serum pepsinogen concentrations in ulcer patients and it was postulated that emotional stress may predispose to ulcers by producing gastric hypersecretion as manifested by hyperpepsinogenemia (Walker *et al.* 1988). The presence of two immunologically distinct groups of

pepsinogens in the gastric mucosa of man is recognised and these are pepsinogen I (Pg I) which consists of five components (Pg 1-Pg5) and pepsinogen II (Pg II) which consists of two components (Pg 6 and Pg 7) (Samloff 1982). Specific radioimmunoassays have been developed for each group and it has been shown that duodenal ulcer patients have high levels of Pg I and it appears to be inherited as an autosomal dominant trait in first -degree relatives of duodenal ulcer patients (Samloff 1982). An elevated Pg I level is a major risk factor for duodenal ulcer, whereas an elevated serum Pg II level and a low Pg I /Pg II ratio are major factors for gastric ulcer (Samloff *et al.* 1986). In patients with pernicious anaemia there is a marked reduction in the level of Pg I. It has been demonstrated that the pepsinogen I to pepsinogen II ratio in combination with the absolute level of serum pepsinogen is predictive of the histologic status of the gastric mucosa in 70% of cases (Samloff, Varis, Ihamaki, Siurala and Rotter 1982).

In cattle and sheep elevated serum pepsinogen levels have been recorded in association with *Ostertagia ostertagi*, *Trichostrongylus axei*, *Ostertagia circumcinta* and *Haemonchus contortus* infections (Ross *et al.* 1967; Armour *et al.* 1979; Pitt *et al.* 1988; Mostofa *et al.* 1990_a). Non-parasitic abomasitis is also associated with elevated serum pepsinogen levels (Voros, Meyer and Stober 1984) as is haemorrhagic abomasal ulcers in cows (Welchman 1986). Different types and subtypes of pepsinogens have recently been identified in cattle (Eckersall, Macaskill, McKellar and Bryce 1987) and sheep and goats (Mostofa, McKellar and Eckersall 1990_b).

In the pig, serum pepsinogen levels are unrelated to the

presence or severity of gastric lesions (including ulceration) in the pars oesophagea but elevated levels are recorded in association with cold weather (Bunn *et al.* 1981; Zamora *et al.* 1975).

In the horse, serum pepsinogen levels have been investigated in relation to gastric ulceration in the foal and although levels were about 30% higher in foals with ulceration as compared to controls a wide scatter of results severely limits the clinical usefulness of this test (Wilson and Pearson 1986). Regrettably, this study did not record the location or the extent of gastric lesions or the method in which diagnosed.

The aim of this project was to investigate serum pepsinogen levels in adult horses in relation to the presence and extent of ulceration / erosin of the proventricular mucosa and the presence and number of *Gasterophilus intestinalis* larvae.

Materials and methods.

Blood samples were collected by jugular venopuncture from 85 adult horses immediately prior to slaughter at a commercial horse abattoir. Samples were identified, allowed to clot and the serum after harvesting was stored at -20^{o}C until examined.

Horse stomachs were examined as described previously by Sweeney 1992 (chapter 4).

Serum pepsinogen levels were determined using the technique of Hirschowitz (1955) with the modification that haemoglobin was not added, but allowing the serum proteins to act as substrate as in the method of Korot'ko and Islyamova (1963) (cited by Ross, Purcell, Dow and Todd 1967). To 0.5 ml of serum were added 2.5 ml of 0.06N hydrochloric acid and the mixture incubated at 37^{o}C for three hours. The reaction was then stopped

by adding 2 ml of 10% trichloracetic acid. A parallel control was prepared for each test by precipitating the mixture as above without incubation. The tubes were allowed to stand for 10 minutes and, after centrifuging (at 2,000 r.p.m.) for 10 minutes, 2 ml of the supernatant was made alkaline with 4 ml of 0.5N sodium hydroxide and 1 ml of freshly diluted (1 in 3) Folin and Ciocalteu's reagent was added. Colours of test and control samples were read within 20 minutes at 560 mu and tyrosine-like products estimated by reference to standard tyrosine (0.2µmol) and blank controls (water) treated similarly. Results were expressed as international units /liter at 37°C.

The relationship between serum pepsinogen levels and the presence or absence of ulcers / erosions, the extent of the ulcerated/ eroded area and the presence and number of bot larvae were evaluated statistically using the Mann-Whitney and Kruscal-Wallis tests.

Results.

Of the 85 horses, 37 (43%) had bots (range 1-69 bot larvae) and 59 (64%) of horses had either ulceration or erosion of the proventricular mucosa, 21 (39%) of these having pathology involving greater than 5 cm^2 of mucosa.

The mean serum pepsinogen values for all horses was 0.21 IU/l (SD ± 0.18) but no association between serum pepsinogen levels and the presence or absence of ulceration / erosion, the extent of ulceration or erosion, the presence or absence of bot larvae or the number of bot larvae present was demonstrated.

Discussion.

The elevated serum pepsinogen associated with bovine abomasal parasitism is largely considered to be due to the elevated abomasal pH associated with reduced hydrochloric acid production due to the replacement of parietal cells in parasitised glands by rapidly dividing and undifferentiated cells and resulting in a reduced parietal cell mass (Armour *et al.* 1979). Consequently, pepsinogen is not converted to pepsin and relatively large amounts enter the circulation and this back diffusion is exacerbated by disruption of the normal intercellular junctions (Armour *et al.* 1979). In the horse, *Gasterophilus intestinalis* larvae are largely confined to the non-glandular proventricular mucosa with minimal pathological affect on the glandular mucosa (Sweeney 1990; Sweeney 1992, Chapter 6) and consequently it is not surprising that serum pepsinogen levels were not influenced by the presence of bot larvae.

In the horse, *Trichostrongylus axei* is an invasive parasite that burrows deeply within the glandular mucosa and large numbers cause a chronic proliferative gastritis with formation of nodules, ulcers and plaques. However, the effect of *T. axei* on equine serum pepsinogen levels is not entirely clear. Waddell and McCoskar (1969) reported elevated serum pepsinogen levels in association with *T. axei* in the horse but results from naturally infected ponies (rotational grazing with cattle and sheep) yielded equivocal results (Herd 1986). In the latter work, stabled thoroughbred race horses considered unlikely to be at risk had mean serum pepsinogen levels of 0.36 iu/l (SD $\pm$ 0.26) whereas 38 at risk ponies had a mean value of 0.21$\pm$ 0.071 iu/l. However, 14 additional ponies had values higher than 1.0 iu/l on at least one

occasion during the grazing season and had a mean value of 0.54 iu/l (SD ± 0.128).

In duodenal ulceration in man acid hypersecretion occurs in 50 % of cases (Sipponen 1989) . However, most patients with gastric peptic ulcers have normal basal and maximal acid outputs (Baron 1984) and a portion have a reduced gastric acid secretion (Spiro, 1983). Hammond (1990) has shown that the pH of the proventricular portion of the equine stomach is lower than the glandular portion and furthermore, the area where erosion and ulceration commonly occur in the adult horse (the junction of the squamous and glandular mucosa at the margo plicatus in the region of the lesser curvature) had the lowest pH of all. In addition, horses with ulceration of the squamous mucosa close to the margo plicatus had a significantly lower pH at this site than normal horses. He suggested that gastric acidity was significantly related to gastric ulceration in the adult horse as did Murray and Grodinsky (1989). How this influences serum pepsinogen levels is unclear. As pepsin output parallels acid secretion (Campbell-Thompson and Merritt 1987_a) it may be that there is increased secretion of pepsinogen in association with gastric ulceration and erosion but the lower pH may result in the more rapid conversion of pepsinogen to pepsin before back diffusion of pepsinogen through the ulcerated mucosa can occur.

Further research is required before serum pepsinogen levels can be adequately interpreted in relation to equine gastric pathology. The development of a more precise assay method such as radioimmunoassay or enzyme-linked immunosorbent assay would be advantageous as would the investigation of the existence

and nature of sub populations of pepsinogen in the horse. In addition, the influence of *T. axei, Draschia* and *Habronema* species on serum pepsinogen levels requires clarification.

CHAPTER 10

FINAL CONCLUSIONS

Equine gastric ulceration: fact or fancy?.

The occurrence of non-perforating gastric ulcers in horses of all ages has been recognised for some time and such ulcers were previously considered to be insignificant incidental post mortem findings. However, within the last decade a clinical syndrome in horses especially in foals, attributed to gastric ulceration has been described and has been reported in the United States of America, Canada, Australia, France, Ireland, Britain and Japan. Within the last decade progress has been made in the understanding of equine gastric physiology and the treatment of gastric ulceration. However, there is still little known about the aetiology and pathogenesis of this condition and definitive diagnosis of clinical gastric ulceration in horses remains problematical and is usually diagnosed on clinical grounds alone. Many questions regarding the cause, pathogenesis, significance and even the existence of the condition remain unanswered and some of these are addressed below. More work is required to clearly define the clinical signs and aetiology.

1. A direct relationship between the clinical signs of teeth grinding, salivation and colic with the presence of gastric ulceration has not yet been definitively demonstrated. The visualization of gastric

ulcers alone is insufficient evidence as over 50 % of normal foals have asymptomatic ulcers (Murray and others 1990). A video endoscopic survey in Ireland and Britain involving 75 clinically normal foals revealed gastric ulceration in 38 (56%) of foals. A number of surveys in America have revealed a similar prevalence of gastric ulcerations in normal foals. Such foals do not exhibit clinical signs compatible with gastric ulceration and as yet there are no reports of any such foals subsequently developing the ulcer syndrome.

2. Over 50% of foals dying from perforated gastric ulcers are recorded as sudden deaths and show no clinical signs at all (Dwyer and Powell 1989).

3. NSAID's toxicity resulting in ulceration of the gastric mucosa does not cause the clinical signs associated with the gastric ulceration syndrome (Smith and others 1987).

4. Most workers in equine gastric ulceration have drawn extensively from human research but man may not be the most appropriate model. Equine gastric ulceration mostly occurs in the non glandular squamous mucosa, a mucosa not present in man. The pig and rat stomach have well demarcated squamous mucosa sections .

5. If gastric ulceration in the horse parallels that in other species it is likely that gastric squamous, gastric glandular and duodenal ulceration are heterologous conditions with different clinical signs and significance.

6. It is possible that other factors in addition to ulceration, such as gastric distension, may be important in the clinical syndrome.

7. The equine gastric parasite *Gasterophilus intestinalis* causes deep ulcerative pits in the squamous mucosa yet it is generally considered to be of limited clinical significance.

8. In pigs, with the exception of those that die from acute haemorrhaging squamous ulcers, the presence of subacute and chronic ulcers are considered to be of no economic significance (O'Brien 1986).

9. A number of authors (Wilson 1985, Dwyer and Powell 1989) in post mortem surveys described non perforating ulcers as the primary problem or cause of death in a number of cases. The justification for such a post mortem diagnosis should be rigorously defined.

10. Melaena is a feature of gastric ulceration in other species but is not common in the horse.

11. In man, post mortem surveys usually reveal a higher prevalence of gastric ulceration than do clinical surveys. Curiously, in the foal the reverse is true (Murray and others 1990; Dwyer and Powell 1989). This may partly be explained by inconsistency in the scoring and interpretation of lesions when viewed endoscopically.

12. Whether the presence of ulcers in clinically normal foals could be responsible for subclinical disease such as decreased growth rate is entirely open to conjecture. In a survey of 304 veal calves, no decrease in growth rate was observed in association with abomasal ulceration and it was concluded that most lesions are innocuous (Welchman and Baust 1987). In man, only 25% of gastric ulcers are associated with clinical signs (Spiro 1983)

13. Furr and Murray (1989) reported the successful treatment of gastric ulceration in adult horses using ranitidine (a histamine type 2 receptor antagonist). Regrettably, untreated controls were not used in this study and as clinical signs are vague, blind assessment of clinical response and blind endoscopic examination would add further weight to their work.

There remains many basic questions even regarding the existence of the condition and a strong case could be advanced for the belief that the condition did not exist at all. However, there is no easy option to resolving these questions and this difficulty is primarily caused by the paucity of clinical cases.

Incidence.

Foal.

Chapter 3 outlined the results of a postal survey of equine practitioners with a total cumulative responsibility for 6,650 foals yearly which revealed a 0.58% incidence of clinical gastric ulceration, the majority of which were diagnosed on clinical grounds alone . The apparent increase in the incidence of ulceration over the period of the survey may indeed be a true increase but may be affected by such factors as increased awareness, fashion and the memory or quality of records of respondents .

The survey was designed as an economic rather than an investigation type of survey, the emphasis being applied to the incidence and importance of foal ulceration rather than attempting to investigate its natural history (aetiopathogenesis). Requirements for a valid investigational survey include an objective method of study based on factual observation and a firm basis of measurement in addition to the availability of some hundreds of cases (Leech 1971). The low incidence and absence of a definitive diagnostic test for foal ulceration exclude the possibility of a detailed investigational survey at this time.

The survey is believed to have given a reasonably

accurate guide to the incidence of the condition. However,concern over the validity of the survey remains as there is no convenient diagnostic test for this syndrome and the clinical syndrome has not been definitively described.

The result of this survey confirms the futuility of a research project into the foal ulcer syndrome in Ireland or Britain based on clinical cases. Furthermore, even if large numbers of cases were available, the nature of the horse breeding business and the high values of some individual animals would place extreme restrictions on the investigational techniques that could be employed. This confirms the wisdom of North American researchers in concentrating on aspects of foal gastric physiology that may give a better understanding of the possible pathogenesis of gastric ulceration.

It is interesting to examine the trend in North America in regard to the incidence of foal gastric ulceration. A Kentucky farm survey in the years 1982 and 1983 involving approximately 4,000 foals yearly revealed that ulcer associated deaths accounted for 30% and 22% respectively of all foals deaths. A post mortem survey (also Kentucky based) spanning the years 1984-1988 revealed a steady decrease in the rate of perforated gastric ulcers over the survey years. In the final year of the survey 3% of all foal deaths were due to gastric perforation and an additional 1.5% of foals were diagnosed as dying from non - perforating ulcers. However, the justification for a diagnosis of non - perforating ulcers as a cause of death have not been published and may be difficult to substantiate. The large decrease in the incidence of gastric ulcer related deaths from 30% in 1982 to 5% in 1988 is remarkable and may be due to increased awareness of the condition, earlier diagnosis, more

effective treatment and the prophylactic treatment of foals considered at risk. However, the apparent reduction in the incidence of the syndrome may in some degree be caused by a decrease in the "fashion" of this condition. Furthermore, one decade ago a diagnosis of gastric ulceration was a tempting refuge when diagnostically destitute but as the mystery of this syndrome gradually evaporates this temptation is likely to be increasingly resisted.

Adult Horse.

The incidental observation at post mortem examination of gastric ulcers in the adult equine stomach has been recognised for some time . Hammond *et al.* (1986) reported a 66% prevalence of asymptomatic ulceration in a survey of 195 thoroughbred race horses in Hong Kong. Murray (1988) reported a 52% prevalence of ulceration in the proventricular region of the stomach in clinically normal horses more than one year old. Recently, clinical signs of poor body condition, decreased appetite, recurrent colic, acute colic and diarrhoea have been attributed to gastric ulceration in adult horses (Murray 1988; Murray *et al.* 1989).

In this study , the prevalence, location, extent and gross pathology of gastric ulcers in adult horses in Ireland and epidemiological factors associated with such lesions were investigated.

Between 27 and 101 stomachs were examined in each calendar month during the study period . A total of 173 (28.2%) horses of the 614 examined had ulceration or erosion in the squamous mucosa. The majority of ulcers (n=314, 89%) occurred close to the margo plicatus in the region of the lesser curvature.

Two horses had glandular ulcers .

The source of 495 horses in this study was determined. Three hundred and four (61%) horses were sold privately, directly to the abattoir ; 168 (34%) were sold to the abattoir by horse dealers and 23 (5%) horses were fattened at the abattoir for a period greater than one month before slaughter. The prevalence of ulceration in these groups was not statistically different and was 28%, 31% and 22% respectively. Horses which passed through the hands of dealers and who may have been subjected to stress did not have a higher prevalence of ulcers or erosions.

A seasonal incidence of ulceration (heat stress) has been described in the pig (O'Brien 1986) but no seasonal incidence of ulceration was apparent in this study.

The prevalence of ulceration in each of the various breeds used in this study was as follows; Thoroughbred, 90(33%); pony, 43(24%); halfbred, 5(16%); work horses, 17(20%); others, 6(15%). There was a statistically significant difference between the presence of ulcers in Thoroughbred horses and ponies ($0.01>p<0.05$) but numbers in other breeds were too small to permit meaningful statistics. The reason for the higher prevalence of ulceration in Thoroughbreds is not clear. An association with stress has been proposed by other authors but this work is insufficiently detailed to comment on this matter. It is my opinion that it is equally probable that the higher prevalence in Thoroughbreds could have a genetic basis or be related to Thoroughbred management practices.

This report confirms the common occurrence of ulcers and erosions in the proventricular part of the stomach in apparently clinically normal horses in Ireland. The prevalence in this study (28

%) is considerably lower than that recorded in Hong Kong (66%) and that recorded by Murray *et al.* (1989) (52%) in a study of 100 normal yearling and adult horses.

Many grossly diagnosed ulcers were subsequently on histopathological examination found to be erosions . The greatly increased thickness of the epithelium in the vicinity of the margo plicatus (2 to 3 fold) gave the false impression of a substantial mucosal deficit but on histopathological examination it was found that the entire thickness of the epithelium had not been penetrated and consequently were not ulcers but erosions.

This work and that of Murray *et al.* (1989) and Hammond *et al.* (1986) confirm the predominance of ulceration in the proventricular mucosa. As this mucosa is also different anatomically from the glandular mucosa, it seems reasonable to consider ulceration of the squamous and glandular gastric mucosa as different entities with different aetiologies.

A more detailed epidemiological investigation of ulceration in the adult horse could well yield further useful information. However, in the setting of the current study (an abattoir) this was not possible as the sale of a horse to an abattoir was for many owners an emotive and secretive issue and did not lend itself in many circumstances to further epidemiological investigation.

Histopathology.

The histopathological characteristics of proventricular erosion and ulceration in the horse have received only scant attention (Yamagiwa *et al.* 1959; Rooney 1964; Rebhun *et al.* 1982; Gross and Mayhew 1983; Buergelt 1984; Yoshihara *et al.* 1986 and Yamagishi *et al.* 1986).

The aim of this study was to describe the histopathology encountered in asymptomatic proventricular ulceration in the adult horse and to ascertain the sequence of lesion development.

Tissue specimens (largely from the proventricular mucosa) were collected from the stomachs of 121 horses at an abattoir. Sixty five of the above horses had gross ulceration or erosion and a further 29 horses had scarring. The least severe pathological change observed was described as epithelial change and was characterised by increased epithelial thickness, increased depth and irregularity of the rete pegs, hyperkeratosis and hydropic degeneration. More severe lesions were classified as acute erosion and in addition to the changes described for epithelial change, erosion, reticular degeneration and spongiosis was noted. Acute and subacute ulcers described lesions where the deficit extended down to the lamina propria and in chronic ulcers and erosions fibrosis was a significant feature. Scarring was characterised by increased corrugations of the epithelium, fibrosis of the lamina propria and ectopic glands. It is suggested that the different classes represent sequential morphological stages in the development of gastric ulceration in the horse.

The classification described is a pathological one and does not in any way indicate the clinical significance, if any, of the lesions. However, the description of each category suggests a possible path by which ulcers and erosions of the gastric squamous mucosa may develop. Epithelial changes of hyperkeratosis, hydropic degeneration, exocytosis and spongiosis were present in all cases of ulceration / erosion and were also recorded in the absence of ulcers and erosions in a number of horses and therefore it is proposed that epithelial changes are the first step in

the development of ulcerative lesions. Damaged epithelium may be gradually eroded away resulting in an erosion or ulcer. Infiltration of inflammatory cells and fibrosis may result in healing of the ulcer, or if ulcerogenic factors persist, it may enlarge and become a chronic ulcer / erosion. Perforation appears to be an uncommon sequel. Healing and contraction of fibrous tissue results in distortion of the squamous-glandular mucosa junction and the dragging of gastric glands under the squamous tissue.

There are remarkable similarities between the pathology of erosions and ulcers described here and those described in the pars oesophagea of the pig (Curtin *et al.* 1963; Muggenburg *et al.* 1964; O'Brien 1986 and Embaye *et al.* 1990). For this reason if would be of great interest to investigate the role of diet and in particular particle size in the aetiology of equine gastric ulceration.

The pathogenesis or aetiology of equine erosion / ulceration is not known but is likely to involve a disruption of the gastric mucosal barrier (Sweeney 1990). Hyperacidity has been described as a cause of gastric ulceration. In man, reflux oesophagitis is commonly due to reflux of acidic gastroduodenal contents causing gross lesions of erythema, streaking and linear erosions and may be the condition most analogous to gastric ulceration in the adult horse (Hammond 1990). Further support for the hypothesis of Hammond would be gained if the histopathological lesions in the horse stomach and those from clinical cases of reflux oesophagitis in man were similar. However, in man the major histopathological lesion is controversially considered to be basal cell hyperplasia (acanthosis)(Day and Husain 1986; Thompson 1989), a feature not recorded in the horse in this study. Chronic oesophageal epithelial damage or ulceration

in man may lead to Barrett's oesophagus, a condition characterised by the overgrowth and upward extension of gastric columnar epithelium to areas previously occupied by squamous mucosa (Thompson 1989). Barrett's oesophagus like lesions were not observed in this study.

The histopathological lesions described in this paper are those occurring in asymtomatic ulceration and erosion in the adult horse. It would be interesting to examine histopathologically mucosa from clinical ulceration in adults and foals.

Aetiology and Pathogenesis.

Many factors have been proposed as causes of equine gastric ulceration but with the exception of non- steroidal anti-inflammatory drugs (N.S.A.I.D.) (Traub et al. 1983), few have been proven. Weather related stress has been indicated as a possible cause of gastric ulceration by Dwyer and Powell (1989) but requires further investigation.

This study concentrated on two possible causes of ulceration, namely *Gasterophilus intestinalis* and *Campylobacter pylori.*

Despite the dramatic appearance of heavy bot infestations, their true pathogenic effect is obscure (Urquhart, Armour, Duncan, Dunn and Jennings 1987). Soulsby (1982) concluded that it was unreasonable to assume that extensive ulceration of the stomach by large numbers of parasites is without general effect. Subclinical effects on stomach function associated with the presence of bots and consequently on overall performance was considered probable by Edwards (1982). Waddell (1972) associated bots with the occurrence of gastric ulcers and subserosal abscesses and Pandey *et al.* (1980) reported that bots

cause gastric ulcers, erosions, and nodulesopolypous growths. Rooney (1964) reported that ulceration of the oesophageal region with or without perforation in foals is directly related to mechanical trauma by *Gasterophilus intestinalis* larvae.

With regard to gastric ulceration /erosion, the findings and conclusions of this study are almost in direct contradiction to those of Rooney (1964) and Waddell (1972). In this study, only 34.7% of horses with gastric ulceration /erosion had concurrent bot infestation or evidence of recent bot infestation and no statistical correlation was present between the presence of bot larvae and the occurrence of ulceration /erosion when examined both on a monthly basis and on a combined annual aggregate basis. Furthermore, despite the seasonal absence of bot larvae from the equine stomach (July-September), no monthly or seasonal variation occurred in the prevalence of gastric ulceration /erosion. In addition, a distance usually well in excess of 10 cm was present between the site of ulceration and the predilection site for bot attachment and therefore, it seems unlikely that under-running of the epithelium by necrotic material in the immediate vicinity of bot larvae could be important in the initiation of gastric ulcers /erosions in the area of the lesser curvature.

It is concluded from the above observations that *G. intestinalis* larvae have no role to play in the aetiopathogenesis of equine gastric ulceration and erosion. Support for this conclusion is obtained from the work of Hammond, Mason and Watkins (1986) who, in a survey of 195 Thoroughbreds in Hong Kong, where *Gasterophilus* larvae have never been reported, recorded a 66% prevalence of gastric ulceration. Conversely, in a survey of 70 horses in New Zealand, 97% of which had *G. intestinalis* larvae

(mean=63), Kettle (1974) did not observe the presence of any gastric ulcers /erosions.

In man the role of *Helicobacter pylori* {formerly *Campylobacter pylori* (Goodwin *et al.* 1990)} in upper gastrointestinal disease has received enormous attention in the last decade. A strong association between *H. pylori* and duodenal ulceration (almost 100% of cases; O'Connor and Axon 1989) and gastric ulceration (70% of cases) has been reported (Dixon 1989).The evidence supporting *H. pylori* as a primary pathogen in normal gastric mucosa has been strengthened by ingestion studies which provoked clinical and histopathological gastritis with seroconversion (Morris and Nicholson 1989). Additionally, therapeutic trials demonstrated a therapeutic efficacy equal to that of histamine type 2 (H_2) receptor antagonists when various *H. pylori* active antibacterials with no direct ulcer healing properties were used alone or in combination with colloidal bismuth subcitrate (CBS) in the management of gastritis (Hirschl and Pletschette 1989) and peptic ulceration (Coghlan *et al.* 1989). However, there is not universal agreement on the significance of *H. pylori* and its role in the pathogenesis of gastric disease remains somewhat controversial (Bartlett, 1988).

Helicobacter pylori -like organisms (HPLO) have been cultured from the stomachs of the rhesus monkey , the pig tailed monkey and baboon and in all non-human primates in which HPLO were isolated there was an associated gastritis similar to that in man (Newell 1989). Additionally, HPLO have been recovered from the stomachs of ferrets (Tompkins 1989).

The common finding that occurrences of the foal

gastroduodenal ulcer syndrome have frequently been preceded by episodes of infectious diarrhoea and the occasional clustering of affected foals on certain studs has suggested an infectious aetiology (Becht and Byars 1986) but attempts to identify a causal organism in a series of foals with perforating duodenal ulcers was not successful (Acland *et al.* 1983). However, in a report of five clinical isolates of oxidase and catalase positive *Campylobacter* organisms from foals, three of the five foals involved showed clinical or pathological signs compatible with the foal gastroduodenal ulcer syndrome (Atherton and Rickets 1980).

Chapter 7 evaluates the possible role of this organism in equine gastric ulceration. A statistically significant difference ($p<0.05$) in the *H. pylori* OD values between horses with gastric pathology and horses with no gastric pathology was recorded . The absence of *H. pylori* -like organisms in the tissue samples is not entirely surprising because of the location of ulcers in the stratified squamous epithelium and in man *H. pylori* have not been identified histologically in such an epithelium. It is of interest that all tissues from inflamed and normal equine gastric glandular mucosa were also negative for *H. pylori* on histopathological examination but as only a maximum of two glandular biopsies (usually from the fundic glandular region) were collected from horses with ulceration and only rarely from the pyloric glandular region (antrum) it is impossible to exclude the presence of *H. pylori* in equine gastric mucosa. The distribution of *H. pylori* in the gastric mucosa of man is patchy and evidence for a negative finding can only be accepted if sufficient biopsy specimens have been collected from different areas of the stomach and at least two biopsies from the antral mucosa (Goodwin *et al.* 1986; Aase *et al.*

1989).

In this study, ELISA was performed using an acid glycine extract of *H. pylori*. This antigen is partially purified and has minimal cross reactivity with *Campylobacter spp*. Such antigens have been reported to detect approximately 95% of patients with *H. pylori* infection with a specificity of 90% (Newell and Rathbone 1989; Newell and Stacey 1989). In the horse *Campylobacter jejuni / coli* are not considered important pathogens (Garcia *et al.* 1983) and out of 304 diarrhoeic samples examined specifically for *Campylobacter spp* in a foal diarrhoea survey only in one sample was *Campylobacter spp* isolated (Browning *et al.* 1991).

The results of this study suggest a possible association between *H. pylori* and equine gastric ulceration but we could not confirm these findings with histopathological examination and culture was not undertaken (because all gastric tissue samples were obtained post mortem). Serological investigations using better more refined *H. pylori* antigens, gastric culture and a more comprehensive histological examination of the gastric mucosa are needed to clarify the position. It would be of particular interest to investigate the role of *H. pylori* in the foal gastroduodenal ulcer syndrome because in addition to ulceration of the stratified squamous mucosa 32% of clinical cases have ulceration of the glandular mucosa (Wilson 1986).

This study offers some circumstantial evidence to incriminate diet as a cause of equine gastric ulceration. In the pig, diet is considered the major cause of ulceration in the non glandular mucosa and the remarkable similarity in location and histopathological appearance of equine and porcine gastric ulceration call for further investigation of the possible role of diet in

equine ulceration. Other workers have also hinted at diet as a cause of equine ulceration. Coenen (1990) indicated the need to feed roughage and to distribute concentrate feed over three feeds to avoid ulceration. A dietary factor was also suggested by Hammond and others (1980). A post mortem survey of grass sickness, a condition predominating in pastured horses, revealed ulceration in only 2% of animals (Pogson 1989, personal communication) as compared to 28% in the general population (Sweeney 1990).

Diagnosis.

The typical clinical signs of the foal ulcer syndrome or adult ulceration are not pathognomonic and definitive diagnosis can usually only be made on direct visualisation of the lesion by endoscope or alternatively by barium double contrast radiography (Adamson and Murray 1990; Brown, Slocombe and Derkensen 1985; Dik and Kalsbeek 1985; Campbell, Ackerman and Peyton 1984; Campbell, Ackerman and Peyton 1982). However, these techniques require special equipment not commonly available in equine practices and considerable expertise. Therefore, a blood test that might aid diagnosis would be extremely convenient and it was in this connection that serum pepsinogen was investigated in the horse.

Serum pepsinogen levels have been investigated in foals with ulceration and although many foals had significantly higher serum levels when compared to normal foals, considerable overlap existed precluding it's use for definitive diagnosis (Wilson and Pearson 1986).

In this study serum pepsinogen levels from 85 adult horses, 37 (43%) of which had bots (range 1-69 bot larvae) and

64% (n=59) of which had either ulceration or erosion of the proventricular mucosa were measured using a modification of the method of Hirschowitz. No association between serum pepsinogen levels and the presence or absence of ulceration / erosion, the extent of ulceration or erosion, the presence or absence of bot larvae or the number of bot larvae present was demonstrated.

Further research is required before serum pepsinogen levels can be adequately interpreted in relation to equine gastric pathology. The development of a more precise assay method such as radioimmunoassay or enzyme-linked immunosorbent assay would be advantageous as would the investigation of the existence and nature of sub populations of pepsinogen in the horse. Additionally, the influence of *T. axei, Draschia* and *Habronema* species on serum pepsinogen levels requires clarification.

Gastrin is a major mediator of gastric acid secretion and hypergastrinaemia has been described in man in association with the Zollinger-Ellison syndrome and plasma gastrin concentrations are often elevated to various extents in patients with gastric ulcer disease (Nilsson 1980).

Hypergastrinaemia occurred in lambs after experimental *Haemonchus contortus* infestation and in calves, a significant increase in plasma gastrin occurred after infestation with *Ostertagia ostertagi* .

Basal and postprandial serum gastrin values have been investigated in the horse using commercially available radioimmunoassay kits but reported basal levels have varied greatly between reports.

Only one report deals with equine gastrin levels in association with disease. A comparison of normal (n=9) and

diarrhoeic foals (n=9) revealed plasma gastrin levels greater than the mean + 1 sd in 3 diarrhoeic foals (Murray and Luba 1989).

The purpose of the current study was to further define normal gastrin levels in the horse and to investigate serum gastrin levels in relation to the occurrence of gastric ulceration / erosion, *Gasterophilus intestinalis* infestation and the weight of gastric contents.

Serum gastrin levels were assayed using a commercially available human radioimmunoassay kit and serum gastrin values ranged from <20 pg /ml to 96 pg / ml (38 $\pm$ 16). A highly significant correlation ($p<0.01$) was demonstrated between serum gastrin levels and the weight of gastric contents. However, no association occurred between serum gastrin levels and the presence or absence of ulceration / erosions, size of ulcerated or eroded area or the presence or number of *Gasterophilus intestinalis* larvae.

The correlation between serum gastrin levels and the weight of gastric contents is in keeping with the belief that basal and postprandial gastrin values differ but the role of diet in influencing equine postprandial gastrin levels has not been studied. Furthermore, basal and postprandial gastrin serum levels are age dependent , the highest levels occurring in the neonate and young and this trend has been shown in the horse and in man.

Until the physiology of gastrin in the horse is further explained it is difficult to envisage a role for gastrin assay as a diagnostic aid. The role of diet, parasitism, age and the specificity of antibodies used also need to be further clarified.

Major conclusions of this study.

1. The foal gastroduodenal ulceration syndrome occurs in Ireland and Britain but the incidence is very low.
2. Gastric ulceration occurs frequently (28%) in healthy adult slaughter horses.
3. The prevalence of ulceration in thoroughbred horses is higher than in ponies.
4. There is no seasonal factor in the occurrence of ulceration.
5. In the adult horse erosions and ulcers occur in the nonglandular squamous gastric mucosa and only very rarely in the glandular mucosa.
6. Histopathologically, gastric ulceration in the adult horse is very similar to porcine gastric ulceration.
7. *Gasterophilus intestinalis* larvae do not cause gastric ulceration.
8. *Helicobacter pylori* may be involved in the pathogenesis of equine gastric ulceration but require more research.
9. Serum pepsinogen evaluations is not a useful diagnostic test for equine gastric ulceration.
10. Serum gastrin assay is not a useful diagnostic test for equine gastric ulceration.

Possible further avenues of research resulting from this study.

1. It would be interesting to observe endoscopically the behaviour of naturally occurring ulceration in adult horse.
2. The result of feeding ulcerogenic diets (fine particle size) to a number of horses and controls could shed valuable information on the aetiology of gastric ulcers.
3. The behaviour of ulceration in treated horses as compared to controls needs to be described.

4. A detailed search for *Helicobacter pylori* in equine glandular mucosa is needed.

5. Ingestion studies using *Helicobacter pylori* in the horse would be easy to complete and would help to clarify the role of this organism in equine gastric ulceration.

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APPENDIX 1

RESULTS

Abbreviation	Explanation
Date	060488; April 6, 1988
Ref number	Reference number
Ulcer cm sq.	Ulcer area in cm square
Erosion cm sq.	Erosion area in cm square
Margo plicatus score	1-5. See chapter 4.
Bots	Number of bot larvae in stomach
Breed	TB = thoroughbred; P = pony; H = halfbred; D = draught; U = unknown.
Source	Source of horses. D = dealer; P = private. U = unknown.
H.Pylori	Helicobacter pylori serology expressed in optical densities (OD).
Full stomach gms.	Weight of full stomach in grams.
Empty stomach gms.	Weight of empty stomach in grams.
Gastric contents gms.	Weight of gastric contents in grams.
Gastrin ng/ml	Serum gastrin values in ng/ml
Pepsin IU/L	Serum pepsinogen levels in international units/l
Ref	Reference number
H+E stain	Number of tissues stained with haematoxylin and eosin
W.S stain	Number of tissues stained with the Warthin

Abbreviation	Explanation
	Starry silver stain
Tissue	Type of tissue examined gl. = glandular Sq. f. =squamous fundus m.pl. = margo plicatus
Lesion site	gl. = glandular Sq. f. =squamous fundus m.pl. = margo plicatus
Les. Dist.	Lesion distribution. foc. = focal diff. = diffuse Y = yes
Hyperkeratosis	parak. = parakeratosis p.e.h. = pseudoepithelimatous hyperplasia ie., 70 = lamina propria papillae extended up 70% of the total thickness of the epithelium. mit.f. = mitotic figures; scored from * to ****.
Ref	Reference number.
Degeneration	sp.= spongiosis hy.de. = hydropic degeneration ret. de. = reticular degeneration ul. = ulcer er. = erosion
Exocytosis	crust p.m.n. = neutrophils eos = eosinophils lym. = lymphocytes mic. ab. = micro abscess
Exud.	exudate

Abbreviation	Explanation
R.P.Sp.	rete peg spongiosis
Lamina propria	p.m.n. = neutrophils eos = eosinophils lym. = lymphocytes foc. = focal diff. = diffuse
Fibrosis	imm. = immature mat. = mature
Vasculature	incr.# bvs = increased number of blood vessels cong. = congestion
Margo plicatus	ect. g.= ectopic glands ass. fib. = associated fibrosis (with ectopic glands) ass.er. = associated erosion (with ectopic glands) corr. = corrugations of epithelium
Glandular	p.m.n. = neutrophils eos = eosinophils lym. = lymphocytes

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
060488	B1	0.00	0.50	-	0	TB			7500	2000	5500	0.023	
060488	B2	0.00	0.25	-	0	TB			4500	1600	2900	<0.02	
060488	B3	0.00	0.00	-	3	TB			2100	1750	350	<0.02	
060488	B4	0.00	0.00	-	0	TB			1900	1450	450	0.020	
060488	B5	0.00	0.00	-	3	TB			2100	1500	600	0.020	
060488	B6	1.90	0.00	-	0	TB			4000	1650	2350	0.023	
060488	B7	0.38	0.00	-	0	P			2500	1200	1300	0.023	
060488	B8	0.32	0.00	-	0	TB			4000	1550	2450	0.022	
060488	B9	0.00	0.00	-	0	TB			2000	1400	600	<0.02	
060488	B10	0.00	0.00	-	0	P			5500	1450	4050	0.025	
060488	B11	0.00	0.00	-	0	TB			4700	1900	2800	<0.02	
060488	B12	0.00	0.00	-	30	TB			4000	1600	2400	0.022	
060488	B13	0.00	0.00	-	0	H			6400	1450	4950	0.052	
060488	B14	0.00	0.00	-	28	H			5000	1800	3200	0.025	
060488	B15	0.00	0.00	-	0	P			1900	1750	150	<0.02	
060488	B16	0.00	0.00	-	0	P			5000	1250	3750	<0.02	
070488	B17	0.00	0.00	-	0	P			2500	1950	550	0.026	
070488	B18	0.00	0.00	-	12	P			1750	1350	400	0.029	
070488	B19	0.00	0.24	-	12	TB			1250	950	300	<0,02	
070488	B20	0.00	0.00	-	0	P			5500	1800	3700	<0.02	
070488	B21	0.00	0.00	-	0	TB			3200	1500	1700	0.026	
070488	B22	0.00	0.00	-	10	TB			4600	2000	2600	0.033	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
070488	B23	0.00	0.00	-	0	D			2600	1600	1000	<0.02	
070488	B24	0.00	0.00	-	0	P			2600	900	1700	0.020	
070488	B25	0.00	0.00	-	0	TB			3800	1150	2650	<0.020	
070488	B26	0.20	0.00	-	40	P			4200	1100	3100	0.038	
070488	B27	0.00	0.00	-	30	P			3000	1700	1300	0.022	
070488	B28	0.00	0.00	-	0	H			7500	2000	5500	0.022	
070488	B29	0.00	0.00	-	23	P			2800	1850	950	0.022	
070488	B30	1.00	0.00	-	50	P			2500	1500	1000	<0.02	
070488	B31	0.00	0.00	-	0	P			3000	1500	1500	0.025	
070488	B32	0.00	0.00	-	37	P			7100	1800	5300	0.500	
070488	B33	0.16	0.00	-	60	P			6800	1600	5200	0.620	
070488	B34	0.00	0.05	-	18	P			3800	850	2950	<0.02	
070488	B35	0.28	0.00	-	0	TB			4000	1400	2600	0.040	
070488	B36	0.00	0.00	-	12	TB			5000	2100	2900	0.020	
270488	B37	0.00	0.00	2	17	TB			6500	1750	4750	0.030	
270488	B38	36.00	0.00	-	0	H			2000	1750	250	0.025	0.24
270488	B39	0.40	0.00	1	37	P			2400	1850	550	0.029	
270488	B40	0.00	0.00	3	0	H			3200	1950	1250	0.020	
270488	B41	0.32	0.00	-	0	TB			7000	1900	5100	0.035	
270488	B42	0.30	0.00	-	0	TB			6500	1900	4600	0.027	
270488	B43	0.00	0.00	-	0	P			2400	1850	550	0.027	0.07
270488	B44	0.06	0.00	1	52	TB			5200	1400	3800	0.047	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
270488	B45	0.02	0.00	2	0	TB			4000	1450	2550	0.033	
270488	B46	1.60	-	1	0	H			3400	1350	2050	0.047	
270488	B47	0.32	0.00	1	67	TB			2500	1500	1000	0.052	
270488	B48	0.00	0.00	1	39	TB			11000	2100	8900	0.043	
270488	B49	0.74	0.00	1	0	TB			3300	1450	1850	0.060	
270488	B50	0.00	0.00	3	0	TB			1900	1450	450	0.038	
270488	B51	0.00	0.00	2	25	P			2500	1200	1300	0.060	
270488	B52	0.00	0.00	2	3	P			2600	900	1700	0.049	
270488	B53	0.00	0.00	2	0	P			3500	1150	2350	0.079	
270488	B54	0.00	0.30	1	0	P			3500	1000	2500	0.023	
280488	B55	0.00	4.00	2	0	P			1450	1250	200	0.020	
280488	B56	6.00	0.00	1	0	P			3300	1800	1500	0.023	
280488	B57	0.00	0.00	2	0	TB			850	800	50	0.023	
280488	B58	0.00	0.00	3	0	P			3800	1800	2000	0.060	
280488	B59	0.00	0.00	1	41	P			1650	1000	650	0.030	
280488	B60	0.00	11.00	3	0	P			4000	1350	2650	0.045	
280488	B61	0.75	0.00	2	0	H			1550	1100	450	0.038	
280488	B62	0.00	0.18	2	0	P			2550	1000	1550	0.033	
280488	B63	0.00	0.00	1	0	P			6500	2000	4500	0.040	
280488	B64	0.00	0.00	2	10	U			2150	650	1500	0.022	
280488	B65	9.00	0.00	-	75	U			1250	850	400	0.040	
250588	B66	3.00	0.00	-	12	TB	P	0.437	4500	1800	2700	0.062	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
250588	B67	0.00	0.00	1	0	TB			3500	1350	2150	0.025	
250588	B68	0.00	0.00	2	0	TB	P		1950	1250	700	0.033	
250588	B69	0.00	0.00	2	0	TB	D		2750	1250	1500	0.026	
250588	B70	0.00	0.00	3	70	P	P		2750	1450	1300	0.029	
250588	B71	0.00	0.00	1	3	H	P		12500	1900	10600	0.022	
250588	B72	0.00	0.00	1	0	TB	D		2000	1550	450	0.025	
250588	B73	6.00	0.00	3	19	H		0.387	2500	1950	550	0.020	
250588	B74	0.00	0.00	4	12	TB	P		4000	1450	2550	-	
250588	B75	0.00	0.00	3	3	TB	D		3000	1750	1250	0.029	
250588	B76	0.00	0.00	2	0	H	P	0.206	6000	1400	4600		
250588	B77	20.00	0.00	1	12	TB	P		7750	1550	6200		0.13
250588	B78	1.13	0.00	-	0	TB	D	0.345	1650	1200	450		
250588	B79	0.00	0.00	2	0	H	P		10000	2200	7800		
250588	B80	4.50	0.00	-	0	P	D	0.400	2750	1100	1650	0.052	
250588	B81	0.00	0.00	1	11	TB	U		3250	1100	2150		
250588	B82	0.00	0.00	2	2	P	U		1650	650	1000		
250588	B83	0.00	1.89	3	0	P	P		6250	1350	4900		
260588	B84	0.00	0.00	2	0	P	D		1000	800	200		
260588	B85	0.00	0.00	3	30	P	D		14000	1400	12600		
260588	B86	-	-	-	-	P	P		1950	1250	700		
260588	B87	0.00	0.00	2	0	P	D		2750	1000	1750		
260588	B88	0.00	0.00	2	0	P	P		3500	900	2600		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
260588	B89	1.00	0.00	1	30	TB	D	0.575	5000	1250	3750	0.047	
260588	B90	0.00	0.00	2	3	D	U		3600	1825	1775		
260588	B91	0.00	0.00	1	0	H	P		3750	1600	2150		
260588	B92	0.00	0.00	3	0	TB	P		2050	1700	350		
260588	B93	0.00	0.00	3	0	P	U		3750	2150	1600		0.05
260588	B94	0.00	0.00	1	0	H	P		4500	2100	2400		
260588	B95	0.00	0.00	2	85	P	U		1650	1250	400		
260588	B96	0.00	0.00	1	0	TB	D		2750	1250	1500		
070688	B97	0.00	0.00	3	4	U	D		2850	1700	1150		
070688	B98	0.00	0.00	4	1	U	D		4500	2050	2450		
070688	B99	3.00	0.00	2	24	P	D	0.500	1500	1100	400		0.2
070688	B100	2.00	0.00	1	85	U	U	0.342	4750	1500	3250		
080688	B101	0.00	0.00	3	0	H	D		2500				
080688	B102	0.00	0.00	1	0	TB	D		4750	1650	3100		
080688	B103	5.00	4.00	5	0	TB	D	0.331	5500	1750	3750		0.29
080688	B104	5.00	0.00	1	0	TB	P	0.331	2500	1100	1400		
080688	B105	0.00	0.00	2	0	TB	P		1500	1000	500		
080688	B106	0.20	0.00	1	3	TB	U		2000	1200	800		
080688	B107	0.00	0.00	2	0	TB	D		2000	1300	700		
080688	B108	0.00	0.00	2	3	TB	D		4500	1550	2950		
080688	B109	0.00	0.00	1	0	TB	P		3000	900	2100		
080688	B110	0.00	0.00	2	3	D	P		5750	1800	3950		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
080688	B111	15.00	0.00	-	0	TB	P	0.547	1450	1250	200		0.12
080688	B112	0.00	0.00	3	1	TB	U		8000	1600	6400		
080688	B113	0.60	0.00	1	0	P	P		3000	1600	1400		
090688	B114	0.00	0.00	1	8	P	D	0.540	1000	900	100		
090688	B115	5.00	0.00	5	3	P	D		3000	1100	1900		
090688	B116	0.00	0.00	1	0	TB	D		4250	1600	2650		
090688	B117	0.00	0.00	3	0	D	D	0.580	5750	2750	3000		
090688	B118	0.00	0.00	1	46	P	D		3500	1300	2200		0.9
090688	B119	0.00	0.00	1	10	P	D		2500	1100	1400		
090688	B120	0.00	0.00	1	6	P	D		1500	900	600		
090688	B121	3.00	0.00	1	65	U	D		4000	1150	2850		0.36
090688	B122	0.00	0.00	1	0	P	D		7500	1700	5800		0.17
090688	B123	4.50	2.00	2	3	U	D		1400	1050	350		0.31
090688	B124	2.25	0.00	1	19	TB	D		1550	1500	50		0.01
090688	B125	0.00	0.00	2	0	P	D		2750	800	1950		
090688	B126	0.00	0.00	2	45	U	U		2750	950	1800		0.31
090688	B127	2.50	0.00	1	1	P	D		4250	1250	3000		0.2
090688	B128	0.00	0.00	1	0	D	D		1300	1050	250		
090688	B129	0.40	0.00	1	0	P	D		5000	1600	3400		
090688	B130	0.00	0.00	2	0	TB	D		3250	1300	1950		
090688	B131	0.00	0.00	2	0	TB	P		2750	1100	1650		
090688	B132	0.00	0.00	1	0	P	D		3500	1150	2350		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
090688	B133	0.00	0.00	2	29	TB	D		3000	1250	1750		
290688	B134	0.00	0.00	1	0	U	D		2750	1950	800		
290688	B135	9.00	0.00	5	0	U	D	0.569	2000	1550	450		0.1
290688	B136	0.00	0.00	1	0	TB	D		5500	1250	4250		
290688	B137	0.00	0.00	1	0	TB	D		2100	1200	900		
290688	B138	1.00	0.00	1	4	TB	D		2000	1250	750		
290688	B139	0.00	0.00	1	1	TB	P		1100	1000	100		
290688	B140	0.00	0.00	3	0	TB	D		1400	1200	200		
290688	B141	0.00	0.00	3	0	TB	P		6000	1800	4200		
290688	B142	0.00	2.00	3	0	TB	P	0.633	1750	1750	0		0.07
290688	B143	0.00	0.00	2	0	TB	P		4250	1750	2500		
290688	B144	0.00	0.00	1	0	H	U		2100	1450	650		
290688	B145	0.00	0.00	1	3	TB	D		4000	1500	2500		
290688	B146	0.00	0.00	2	35	D	D		5000	1900	3100		
290688	B147	0.00	0.00	3	35	P	U		4500	850	3650		
290688	B148	0.00	0.00	1	35	P	D		6500	1100	5400		
290688	B149	0.00	0.00	1	8	P	U		7250	1100	6150		
300688	B150	9.00	0.00	2	0	P	D	0.330	1200	750	450		0.18
300688	B151	0.00	0.00	1	0	TB	P		1750	1200	550		
300688	B152	0.00	0.00	1	90	P	U		1300	800	500		
300688	B153	0.00	0.00	1	6	D	D		6250	1500	4750		
300688	B154	0.00	0.00	2	7	TB	D		2500	1500	1000		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
300688	B155	0.00	10.00	1	0	P	D		10750	1500	9250	0.078	
300688	B156	0.00	0.00	3	0	U	D		3250	1200	2050		
300688	B157	0.00	0.00	1	0	P	D		7500	1300	6200		
300688	B158	0.00	0.00	1	0	U	D		2500	1600	900		
300688	B159	0.00	0.00	1	0	TB	P		3500	1800	1700		
300688	B160	0.00	0.00	2	0	P	D		1500	750	750		
300688	B161	3.00	0.00	1	0	P	P		1850	1100	750		
300688	B162	0.00	0.00	3	0	P	D		1000	600	400		
300688	B163	0.00	0.00	1	0	P	D		2250	1100	1150		
300688	B164	4.00	0.00	1	0	TB	D		1450	1200	250		
300688	B165	0.00	0.00	1	0	TB	P		6000	1700	4300		
200788	B166	0.00	0.00	1	0	P	U		1350	800	550		
200788	B167	3.50	1.00	2	0	TB	P		2500	2150	350	0.038	0.85
200788	B168	0.00	0.00	1	0	TB	D		5250	1300	3950		
200788	B169	0.00	0.00	1	0	TB	P		4000	1150	2850		
200788	B170	0.00	0.00	2	0	TB	D		6750	1100	5650		
200788	B171	0.00	0.00	-	0	P	D		4750	900	3850		
200788	B172	0.00	0.00	2	0	P	D		2750	650	2100		
200788	B173	0.00	0.00	2	0	TB	P		3000	1700	1300		
200788	B174	0.00	0.00	1	0	TB	P		6000	1600	4400		
200788	B175	0.00	0.00	2	0	TB	P		3000	1650	1350		
200788	B176	0.30	0.30	1	0	TB	D		2100	900	1200		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
200788	B177	0.00	0.00	1	0	TB	D		3500	750	2750		
200788	B178	1.50	1.50	2	0	P	D		4250	1500	2750		0.17
200788	B179	7.50	0.00	-	1	TB	P	0.520	3750	1250	2500		0.02
210788	B180	0.00	0.00	2	0	H	D	0.587	14500	2650	11850		
210788	B181	0.00	4.80	3	0	TB	D	0.290	5500	2600	2900	0.050	
210788	B182	0.00	0.00	2	0	P	D		600	600	0		
210788	B183	0.00	0.00	1	0	D	D		5000	2500	2500		
210788	B184	0.00	0.00	1	0	P	D		2500	1100	1400		
210788	B185	2.00	0.00	2	0	TB	D		3250	1350	1900	0.023	
210788	B186	0.64	0.50	3	0	TB	D	0.650	2750	2100	650	0.062	
210788	B187	0.00	0.00	2	0	P	D		1600	1200	400		
210788	B188	0.00	0.00	4	0	TB	D		9000	1800	7200		
210788	B189	0.00	0.00	1	0	P			9750	1000	8750		
210788	B190	0.60	1.20	2	0	TB	P		2150	1700	450	0.031	
210788	B191	0.00	0.00	2	0	TB	P		3250	1350	1900		
210788	B192	0.00	0.00	3	0	TB	P		10750	1950	8800		
210788	B193	0.00	0.00	3	0	H	P		3750	1700	2050		
210788	B194	3.00	0.00	2	0	P	D		2750	800	1950		
240888	B195	0.00	0.00	2	0	P	P		1000	800	200		
240888	B196	0.00	0.00	1	0	D	D	0.398	3750	3050	700		
240888	B197	0.00	0.00	4	0	TB	D		1450	1400	50		
240888	B198	0.00	0.00	1	0	P	D	0.367	900	850	50		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
240888	B199	0.60	0.00	2	0	P	U	0.928	3000	1850	1150		0.07
240888	B200	0.50	0.00	1	0	TB	P		2450	400	2050		
240888	B201	0.00	0.00	4	0	U			11750	1750	10000		
240888	B202	0.00	0.00	1	0	TB	P		1900	1200	700		
240888	B203	37.00	0.00	4	0	TB	D	0.475	1950	1900	50		
240888	B204	0.00	0.00	1	0	H	P	0.523	4000	2450	1550		
240888	B205	0.00	0.00	1	0	TB	D	0.450	4500	1750	2750		
240888	B206	0.00	0.00	1	0	H	P	0.300	10500	2400	8100		
240888	B207	1.80	0.00	4	0	P	D		1800	1200	600	<0.02	
240888	B208	0.00	0.00	2	0	D	D		4500	1700	2800		
240888	B209	0.00	0.00	2	0	TB	P		2550	1500	1050		
240888	B210	4.40	0.00	5	0	TB	P		2150	1400	750	<0.02	0.01
240888	B211	0.00	0.00	2	0	TB	P		3500	1600	1900		
240888	B212	2.00	2.00	5	0	TB	D		1500	1350	150	0.047	
240888	B213	0.00	0.50	3	0	TB	D		2100	1750	350		
250888	B214	0.00	0.00	3	0	P	D		1500	1100	400		
250888	B215	11.00	0.00	5	0	TB	P		1600	1350	250		0.4
250888	B216	0.00	0.00	4	0	TB	P		4250	1750	2500		
250888	B217	0.00	0.00	2	0	D	D		1400	1300	100		
250888	B218	0.00	0.00	2	0	P	D	0.357	3250	1000	2250		
250888	B219	0.00	7.00	-	0	D	D		2250	1450	800	0.098	
250888	B220	0.00	0.00	2	0	TB	D		2750	1700	1050		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
250888	B221	0.00	0.00	1	0	P	D		6500	1750	4750		
250888	B222	0.00	12.50	2	0	P	D		4000	900	3100		
250888	B223	0.00	0.00	1	0	TB	D	0.599	3750	950	2800		
250888	B225	4.00	6.00	2	0	P	P	0.527	5000	1500	3500		0.21
250888	B226	0.00	0.00	2	0	P	P		3250	1100	2150		
250888	B227	0.00	0.00	1	0	TB	D	0.395	2750	1300	1450		
250888	B228	0.00	0.00	3	0	TB	D		2250	2100	150		
250888	B229	0.50	4.00	-	0	TB		0.868	2000	1050	950	0.056	
250888	B230	0.00	0.00	1	0	TB	P		4750	2050	2700		
070988	B231	2.00	0.00	2	0	TB	D	0.420	2300	1300	1000	0.039	
070988	B232	0.00	0.00	3	0	TB	D		2500	2000	500	0.026	
070988	B233	0.00	0.00	1	0	TB	D	0.160	3250	1800	1450	-	
070988	B234	0.00	0.00	3	0	TB	P		5000	1800	3200	0.043	
070988	B235	0.00	0.00	2	0	D	D		6000	1750	4250	0.027	
070988	B236	0.00	0.00	2	0	TB	D		1600	1250	350	0.054	
070988	B237	0.00	24.00	1	0	P	D	0.522	2100	1900	200	0.031	0.01
070988	B238	4.00	0.00	2	0	TB	P	1.013	4500	1300	3200	0.070	
070988	B239	0.00	0.00	2	0	TB	P		7500	2250	5250	0.043	
070988	B240	0.00	0.00	2	0	TB	P		1600	1250	350	0.048	
070988	B241	0.00	0.00	3	0	TB	D		5500	1600	3900	0.043	
070988	B242	0.00	0.00	2	0	TB	P		2800	2000	800	0.033	
070988	B243	0.00	0.00	2	0	TB	D		1650	1500	150	0.038	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
070988	B244	0.00	0.00	3	0	TB	P		3750	1800	1950	0.052	
070988	B245	2.00	0.00	5	0	TB	D		1900	1100	800	-	
070988	B246	0.00	0.00	1	0	TB	D		17000	3000	14000	0.063	
070988	B247	11.00	0.00	3	0	TB	D	0.406	2200	1600	600	0.038	0.19
070988	B248	0.00	0.00	2	0	TB	P		3250	1100	2150	0.039	
080988	B249	-	-	2	-	P	D		1800			0.066	
080988	B250	0.00	0.00	2	0	P	D		1750	1300	450	0.050	
080988	B251	0.00	0.00	3	0	TB	D		6250	1500	4750	-	
080988	B252	0.00	0.00	4	0	P	D		7000	1350	5650	0.050	
080988	B253	0.00	0.00	3	0	P	D		3250	1750	1500	0.052	
080988	B254	0.00	0.00	2	0	P	D		2250	900	1350	0.028	
080988	B255	0.00	0.00	3	0	TB	P		3500	1100	2400	0.052	
080988	B256	0.00	0.00	2	0	P	D		5500	1150	4350	0.043	
080988	B257	0.00	0.00	2	0	P	D		2750	1100	1650	0.043	
080988	B258	0.00	0.00	1	0	P	D	0.305	6000			0.072	
080988	B259	0.00	0.00	3	0	TB	D		3000	2200	800	0.056	
080988	B260	0.00	0.00	3	0	P	U		2750	1300	1450	-	
080988	B261	0.00	0.00	3	0	P	U		6250	1300	4950	0.040	
080988	B262	0.00	0.00	2	0	TB	D		4750	2000	2750	0.039	
080988	B263	0.00	0.00	2	0	TB	P		2250	950	1300	-	
080988	B264	0.00	3.70	-	0	TB	P		3750	1000	2750	0.033	
080988	B265	0.00	0.00	1	0	TB	P	0.284	5250	1800	3450	0.040	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
080988	B266	0.00	0.00	1	0	TB	D	0.424	7750	1250	6500	0.031	
080988	B267	0.75	0.00	1	0	H	D	0.871	3000	2550	450	0.027	
210988	B268	2.25	8.00	-	0	TB	D		4000	1850	2150		0.21
210988	B269	0.50	0.00	3	0	TB	P		3000	1100	1900	<0.02	
210988	B270	0.00	60.00	2	0	TB	P	0.620	10250	1500	8750		
210988	B271	0.00	0.00	3	0	TB	D		4750	1600	3150		
210988	B272	0.00	0.00	2	0	TB	D		4500	1350	3150		
210988	B273	1.25	0.00	-	0	TB	D	0.570	4750	1450	3300	<0.02	0.07
210988	B274	0.00	60.00	1	0	TB	P		4750	1550	3200	0.040	
210988	B275	0.00	0.00	2	0	H	P		3500	1700	1800		
210988	B276	0.00	0.00	2	0	D	P		2100	2100	0		
210988	B277	9.00	0.00	1	15	TB	D	0.511	2250	1550	700		0.1
210988	B278	0.00	0.00	1	0	P	D	0.281	4250	1550	2700		
210988	B279	0.00	0.00	3	5	TB	P	0.328	5500	1350	4150		
210988	B280	0.00	0.00	3	5	TB	P		9750	1950	7800		
210988	B281	3.00	0.00	2	0	TB	D	0.170	3750	1850	1900		
210988	B282	0.00	0.00	1	0	D	D	0.324	6250	2100	4150		
210988	B283	0.00	0.00	3	3	TB	D		6250	1500	4750		
220988	B284	0.00	0.00	4	0	TB	D		3250	1150	2100		
220988	B285	36.00	3.00	4	0	TB	D	0.612	2000	1650	350		0.1
220988	B286	0.00	0.00	3	0	P	D		1250	650	600		
220988	B287	0.00	0.00	3	6	P	D		4500	950	3550		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
220988	B288	0.00	0.00	2	2	P	D	0.312	2750	1200	1550		
220988	B289	0.00	0.00	3	4	P	D		2000	750	1250		
220988	B290	0.00	0.00	3	16	TB	P		2400	1200	1200		
220988	B291	0.00	0.00	3	2	TB	P		4500	1900	2600		
220988	B292	0.00	0.00	1	0	P	P		4000	1000	3000		
220988	B293	0.00	0.00	2	9	P	D		4500	1250	3250		
220988	B294	0.00	0.00	2	0	P	D		2250	1100	1150		
220988	B295	0.00	0.00	2	12	TB	D		1350	1250	100		
220988	B296	0.00	0.00	3	9	TB	P		3000	1600	1400		
220988	B297	0.00	0.00	1	11	P	P	0.328	3250	1650	1600		
220988	B298	0.00	0.00	1	0	TB	P	0.250	13750	2250	11500		
220988	B299	0.00	0.00	1	0	TB			4500	1100	3400		
220988	B300	0.00	0.00	4	0	P	D		3700	1000	2700		
280988	B301	0.00	0.00	2	0	TB	D		4750	1750	3000		
280988	B302	0.00	0.00	2	25	D	D		4000	1750	2250		
290988	B303	0.00	0.00	2	10	TB	D		3250	1500	1750		
290988	B304	0.00	24.00	2	0	P	U		1200	800	400		
290988	B305	0.00	0.00	2	0	D	D		3000	1750	1250		
290988	B306	0.00	0.00	2	13	P	D		5000	1700	3300		
290988	B307	0.00	0.00	2	0	P	D		1400	800	600		
290988	B308	0.00	0.00	2	0	TB	P		12750	1750	11000		
290988	B309	0.00	0.00	3	0	TB	P		3250	1400	1850		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
290988	B310	0.00	0.00	2	9	P	D		3250	1100	2150		
290988	B311	0.00	0.00	2	0	TB	D		4000	1350	2650		
290988	B312	0.00	0.00	2	0	D	D		4500	1750	2750		
290988	B313	0.00	0.00	2	0	TB	P		5000	1400	3600		
290988	B314	6.50	0.00	5	0	TB	P		2150	1350	800		
290988	B315	0.00	0.00	4	19	P	D		1800	1300	500		
290988	B316	0.00	0.00	2	3	P	D		4000	700	3300		
290988	B317	0.00	0.00	2	0	P	P		1500	1250	250		
290988	B318	0.40	0.50	2	0	P	D		4750	1100	3650		
290988	B319	0.00	0.00	2	0	TB	P		7500	1550	5950		
290988	B320	0.00	0.00	1	0	P	D		2100	1200	900		
290988	B321	0.00	0.00	2	0	P	P		5750	1150	4600		
290988	B322	0.00	0.00	1	0	P	U		650	500	150	0.052	
051088	B323	0.00	0.00	1	0	P	D	0.609	3000	1450	1550	-	
051088	B324	0.00	0.00	2	0	P	D		5500	2000	3500	0.049	
051088	B325	0.20	0.00	2	0	P	P		4000	1350	2650	0.033	
051088	B326	0.00	0.00	2	0	TB	P		1650	1600	50	0.045	
051088	B327	0.00	1.00	2	12	P	D		5250	1000	4250	0.044	0.14
061088	B328	0.00	0.00	3	0	TB	P		7000	1800	5200	0.032	0.02
061088	B329	0.25	0.00	1	0	TB	D	0.303	5750	1350	4400	0.062	
061088	B330	0.00	0.00	1	0	TB	D	0.281	3200	1200	2000	-	
061088	B331	0.00	0.00	1	0	TB	D		3500	1100	2400	0.038	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
061088	B332	0.10	0.00	2	0	TB	P		4000	1500	2500	0.045	
061088	B333	0.00	0.00	1	0	P	D	0.316	2050	1700	350	0.039	
061088	B334	0.00	0.00	2	6	P	D		3000	1200	1800	0.036	
061088	B335	0.00	0.00	2	21	P	D		3500	1250	2250	-	0.17
061088	B336	0.00	0.00	4	0	TB	P		3000	1700	1300	0.066	
061088	B337	0.00	0.00	2	8	H	P		5500	2150	3350	-	0.34
061088	B338	0.00	0.00	1	48	P	D	0.348	1550			-	
061088	B339	0.00	0.00	4	0	TB	D	0.398	13750	3250	10500	0.086	
061088	B340	0.00	0.00	-	0	D	D		3000	2650	350	-	0.19
061088	B341	0.00	0.00	2	17	TB	D		5250	2350	2900	0.049	
061088	B342	0.90	0.00	2	0	TB	D		7750	1450	6300	0.049	0.33
061088	B343	0.00	0.00	1	0	P	D	0.270	2750	550	2200	-	
061088	B344	1.50	0.00	3	0	P	P	0.344	4250	1350	2900	0.049	0.12
061088	B345	0.00	0.00	3	0	TB	P		5000	2200	2800	0.039	
061088	B346	0.00	0.00	2	0	P	D		8000	1600	6400	-	0.34
061088	B347	0.00	0.00	2	0	P	D		550	550	0	0.039	
121088	B348	1.75	0.00	4	0	P	P			1000			
121088	B349	0.00	0.00	2	4	H	P		11000	2150	8850		
121088	B350	0.00	0.00	1	4	TB	P		6000	2150	3850		
121088	B351	0.00	0.00	3	8	TB	P		4750	1950	2800		
121088	B352	0.00	0.00	1	0	P	P		3250	1500	1750		
121088	B353	0.00	0.00	1	2	U	D		2050	1100	950		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
131088	B354	0.00	0.00	2	9	P	P		9000	1700	7300		
131088	B355	0.00	0.00	2	0	P	P		1650	1100	550		
131088	B356	0.00	0.00	1	0	P	D		1050	1050	0		
131088	B357	0.00	0.00	2	0	P	D		1300	1250	50		
131088	B358	0.00	0.00	3	0	P	D		4000	1550	2450		
131088	B359	5.10	0.00	5	0	TB	D		3750	1300	2450		
131088	B360	0.00	0.00	3	0	TB	P		1850	1550	300		
131088	B361	0.00	0.00	2	44	P	D		1550	1550	0		
131088	B362	0.00	0.00	3	0	TB	D		4000	1700	2300		
131088	B363	0.00	0.00	4	0	TB	D		6500	1650	4850		
131088	B364	0.00	0.00	2	0	TB	D		5000	1850	3150		
131088	B365	0.00	0.00	2	0	TB			3500	1600	1900		
131088	B366	0.00	0.00	2	0	D	D		3250	2100	1150		
131088	B367	0.00	0.00	3	20	P	D		2100	1200	900		
131088	B368	1.00	0.50	2	0	TB	D		2500	1450	1050		
131088	B369	0.90	1.00	-	0	TB	D		4500	1850	2650		
131088	B370	0.00	0.00	2	22	P	D		2900	900	2000		
131088	B371	0.00	0.00	2	0	P	D		3200	950	2250		
131088	B372	0.00	0.00	1	0	N	U		2000	1750	250		
191088	B373	-	-	-	-	P	P		2000			0.033	
191088	B374	-	-	-	0	TB	P		2050	1100	950	0.034	
191088	B375	0.00	0.00	1	0	P	D	0.211	11750	2250	9500	0.043	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
191088	B377	0.00	0.00	2	56	TB	P		4000	1450	2550	0.027	0.62
191088	B378	0.00	0.00	2	69	H	P		7500	1500	6000	0.043	0.31
201088	B379	0.00	0.00	4	0	D	D		4250	2050	2200	0.076	
201088	B380	0.00	0.00	3	22	TB	P		3000	1650	1350	0.039	
201088	B381	0.00	0.00	2	2	TB	U		1650	1450	200	0.030	
201088	B382	0.00	0.00	2	2	TB	U		1300	1300	0	0.033	0.51
201088	B383	0.00	0.00	2	24	TB	D		3500	2100	1400	0.037	
201088	B384	0.00	0.00	2	42	TB	D		2300	1450	850	0.034	
201088	B385	0.00	0.00	1	0	P	D	0.461	3750	1150	2600	0.036	
201088	B386	0.00	0.00	2	0	P	D		2500	1150	1350	0.096	
201088	B387	0.00	0.00	2	0	P	D		2750	1550	1200	0.031	
201088	B388	0.00	0.00	1	27	P	D		5750	1450	4300	0.031	
201088	B389	0.00	3.40	3	0	TB			1900	1550	350	0.043	
201088	B390	18.00	0.00	5	0	U	U		6000	1550	4450	0.045	0.33
201088	B391	2.50	1.00	2	17	U	D		2750	2000	750	0.043	0.47
201088	B392	0.00	0.00	1	0	TB	P	0.734	5750	1550	4200	0.070	
201088	B393	0.00	0.00	2	0	P	D		3000	1350	1650	0.030	
201088	B394	0.00	0.00	2	0	TB	P		6000	2100	3900	0.058	
201088	B395	0.00	0.00	4	0	TB	P		4000	1850	2150	0.034	
201088	B396	0.00	0.00	1	0	P	P	0.266	3250	1500	1750	0.031	
201088	B397	0.00	0.00	4	7	TB	P		5500	1700	3800	0.039	
201088	B398	0.00	0.00	1	2	U	D	0.352	1300	1250	50	0.058	

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
261088	B399	0.00	1.00	2	4	TB			2550	2200	350		
261088	B400	0.00	0.00	2	0	TB	P		8000	2000	6000		
261088	B401	0.00	0.00	3	7	H			4750	2000	2750		
261088	B402	0.00	0.00	2	180	H			3000	1250	1750		
261088	B403	0.00	0.00	2	0	D			1600	1400	200		
261088	B404	10.00	0.00	1	0	TB	P		2350	1100	1250		
271088	B405	0.00	0.00	2	0	TB	P		3500	1450	2050		
271088	B406	0.00	0.00	1	0	TB	D		2450	1700	750		
271088	B407	0.00	0.00	1	8	TB	P		9250	2150	7100		
271088	B408	0.00	0.75	3	3	H	D		1750	1550	200		
271088	B409	1.20	2.00	2	0	TB	D		1800	1200	600		
271088	B410	0.00	1.50	1	184	TB	D		3000	1250	1750		
271088	B411	0.00	0.00	3	0	TB	D		1700	1400	300		
271088	B412	0.00	0.00	1	25	TB	D		4500	2350	2150		
271088	B413	0.00	0.00	3	0	P	P		4000	2000	2000		
271088	B414	0.00	0.00	2	13	U			4250	3100	1150		
271088	B415	0.00	0.00	2	19	P	D		1300	1250	50		
271088	B416	0.00	0.00	1	0	P	D		5500	2400	3100		
271088	B417	0.00	0.00	1	0	TB	P		2750	1150	1600		
271088	B418	0.00	0.00	1	0	D			6000	2500	3500		
271088	B419	0.00	0.00	2	0	TB	P		5000	1750	3250		
271088	B420	19.00	0.00	5	3	TB			2150	1600	550		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
271088	B421	0.00	0.00	3	0	P	D		1250	1100	150		
271088	B422	0.00	0.00	1	1	TB	D		5000	2100	2900		
271088	B423	0.70	0.00	1	24	TB	D		7500	1900	5600		
271088	B424	0.00	0.50	2	0	D			2150	850	1300		
171188	B425	0.00	0.00	2	11	P	D		1950	1500	450		
171188	B426	0.00	0.00	3	0	D	D		5000	1650	3350		
171188	B427	0.00	0.00	2	0	TB	P		4250	1950	2300		
171188	B428	0.00	0.50	3	0	P	P		1650	1600	50		
171188	B429	3.00	0.00	4	17	TB	P		2250	1400	850		0.15
171188	B430	0.00	0.00	2	7	P	D		1650	1500	150		
171188	B431	0.00	0.00	3	0	P	D		2050	1500	550		
171188	B432	5.50	0.00	1	17	TB	P	1.106	2250	1700	550		
171188	B433	0.00	1.60	1	1	TB	P	0.340	3400	1300	2100		
171188	B434	0.00	0.00	1	0	TB	P	0.273	1800	1750	50		
171188	B435	0.00	0.00	2	0	P	D		1650	1600	50		
171188	B436	0.00	4.00	1	0	TB	P	0.445	5000	1700	3300		
171188	B437	0.00	0.00	1	0	P	P	0.312	4250	2450	1800		
171188	B438	2.75	0.00	1	3	P	D	0.420	2400	1650	750		
171188	B439	0.00	0.00	2	3	TB	P		3500	1500	2000		
171188	B440	0.00	0.00	1	7	P	D		3250	2100	1150		
171188	B441	0.00	0.00	1	19	TB	P		6500	1400	5100		
171188	B442	5.60	0.00	1	0	U	D		5500	1500	4000		0.35

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
171188	B443	0.00	0.00	3	8	H	P		2100	2000	100		
171188	B444	0.00	0.00	3	19	P	D		2750	1500	1250		
171188	B445	2.00	0.00	5	40	TB			3050	1650	1400		0.02
171188	B446	0.00	0.00	2	5	TB	D		6500	1500	5000		
171188	B447	0.00	0.00	3	0	TB	P		6500	1800	4700		
171188	B448	0.00	0.10	1	0	TB	P		2400	850	1550		
171188	B449	0.00	0.00	2	0	P	D		750	650	100		
171188	B450	0.00	0.00	2	0	P			1450	1250	200		
171188	B451	0.00	0.00	2	2	P	D		1750	800	950		
081288	B452	0.00	0.00	3	3	8			1300	800	500		
081288	B453	0.00	0.00	2	4	8			1450	900	550		
081288	B454	0.00	0.00	2	1	8			1550	1000	550		
081288	B455	0.00	0.00	1	88	8	P		3650	2200	1450		
081288	B456	0.00	0.00	3	5	8			2200	1100	1100		
081288	B457	5.00	6.00	2	0	8			4500	2700	1800		0.07
081288	B458	0.40	6.00	2	0	8			3250	1100	2150		
081288	B459	0.00	0.00	1	0	8			6250	1750	4500		
081288	B460	0.00	0.00	2	0	8			2400	1700	700		
081288	B461	0.00	0.00	2	0	8			1000	900	100		
081288	B462	0.00	0.00	4	1	8			3500	2350	1150		
081288	B463	0.00	1.50	2	0	8			6500	1700	4800		
081288	B464	0.50	1.20	4	1	8			3450	2100	1350		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
081288	B465	4.40	0.00	2	6	8			6250	2500	3750		
081288	B466	2.00	1.50	1	0	8			2900	1850	1050		
081288	B467	15.00	2.00	2	0	8			3300	1600	1700		0.11
081288	B468	1.00	0.00	2	0	8			1050	850	200		
081288	B469	0.00	0.00	1	0	8			2100	1000	1100		
081288	B470	0.00	0.00	1	20	8			7500	1650	5850		
081288	B471	0.00	0.00	2	6	8			2400	1750	650		
081288	B472	24.00	0.00	2	1	8			2900	1750	1150		
081288	B473	0.00	0.00	2	28	8			2000	1950	50		
081288	B474	0.00	0.40	2	0	8			5250	2650	2600		
081288	B475	0.00	0.00	3	0	8			1750	1600	150		
081288	B476	0.00	0.00	1	0	8			1250	750	500		
081288	B477	0.00	0.00	3	0	8			1700	1000	700		
081288	B478	0.00	0.40	2	72	8			2800	1350	1450		
081288	B479	0.00	0.00	2	0	8			2100	1750	350		
151288	B480	0.00	0.00	2	1	TB	D		11500	1600	9900		0.05
151288	B481	0.00	0.20	2	0	TB	D		3650	1750	1900		0.07
151288	B482	0.00	0.00	2	4	TB	P		4500	1500	3000		0.19
151288	B483	0.00	0.00	2	5	TB	D			1500			0.09
151288	B484	0.00	1.10	2	0	TB	D		3500	1200	2300		0.49
151288	B485	0.00	0.00	2	0	TB	D		1550	1150	400		0.26
151288	B486	0.00	0.00	1	3	TB	D		1650	1650	0		0.16

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
151288	B487	0.00	0.00	1	0	P	D		3250	1150	2100		0.63
151288	B488	0.00	0.00	4	0	P	D		1000	900	100		0.28
151288	B489	0.00	0.00	3	15	TB	P		3750	2250	1500		0.12
151288	B490	0.00	0.00	2	1	P	D		2900	1400	1500		0.28
151288	B491	1.50	0.00	2	0	U	D			1600			0.09
151288	B492	0.00	0.25	2	3	TB	P		4000	1800	2200		0.09
151288	B493	17.00	0.00	2	7	TB	P		2450	1700	750		0.23
151288	B494	0.00	0.00	1	0	TB	D		2000	1450	550		0.19
151288	B495	2.80	0.00	2	0	P	D		2500	1100	1400		0.05
151288	B496	0.00	1.00	1	0	TB	D		3800	1550	2250		0.1
151288	B497	0.00	0.00	2	3	TB	P		4500	1950	2550		0.19
151288	B498	0.20	0.00	2	8	D	P		3250	1700	1550		0.02
151288	B499	0.00	0.00	2	0	D	D		3100	2100	1000		0.3
151288	B500	0.00	0.00	1	0	P	D		1400	1000	400		0.6
151288	B501	16.00	0.00	2	0	H	D		3500	1500	2000		0.41
151288	B502	0.00	0.00	3	1	U	D		6250	1500	4750		0.19
151288	B503	0.00	0.00	2	27	P	D		2950	1750	1200		0.28
151288	B504	0.00	0.60	2	0	P	D		2550	1100	1450		0.23
151288	B505	0.00	0.00	3	0	TB	D		3750	1750	2000		0.21
151288	B506	0.00	0.00	2	0	U	D		1850	1100	750		
151288	B507	0.00	0.00	1	0	TB	D		2450	1950	500		
120189	B508	17.00	0.00	1	0	U			4750	1250	3500		0.22

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
120189	B509	0.00	0.00	1	0	U	P		7500	2450	5050		1
120189	B510	0.00	0.00	4	0	P	P		2150	1600	550		
120189	B511	0.00	0.00	1	2	TB	P		4500	1650	2850		
120189	B512	0.00	0.00	2	0	TB	P		6000	1550	4450		
120189	B513	0.00	0.00	1	0	TB	P		9000	2000	7000		
120189	B514	0.00	0.00	1	22	TB	D		1600	1550	50		
120189	B515	0.00	0.00	3	93	U	D		2550	1250	1300		
120189	B516	0.00	0.00	1	0	TB	P		4350	1350	3000		
120189	B517	0.00	0.00	1	45	H	D		4100	1500	2600		
120189	B518	0.00	0.50	1	10	D	D		2800	1850	950		0.02
120189	B519	0.00	0.40	1	32	TB	D		3500	1500	2000		
120189	B520	0.00	0.00	3	1	H	P		4000	1700	2300		
120189	B521	0.00	0.00	1	0	TB	P		3100	1450	1650		
120189	B522	18.00	0.00	1	11	TB	D		11250	1550	9700		0.05
120189	B523	0.00	0.00	3	1	TB	D		2900	1450	1450		
120189	B524	0.00	3.50	1	0	D	D		2550	1900	650		
120189	B525	0.00	0.00	3	47	TB	P		2100	1950	150		0.13
120189	B526	0.00	1.00	2	9	U	D		4000	2350	1650		
120189	B527	2.00	3.00	2	3	P	D		3350	1300	2050		0.05
120189	B528	0.00	0.00	1	0	P	D		2050	1650	400		
120189	B529	0.00	0.00	1	0	P	D		2250	1000	1250		
120189	B530	0.00	0.00	1	15	P	D		1900	1600	300		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
120189	B531	0.00	0.00	1	0	TB	D		2900	1300	1600		
120189	B532	18.00	0.00	2	0	TB	D		2000	1700	300		0.05
120189	B533	0.00	0.00	2	10	H	P		3000	1900	1100		
120189	B534	0.00	0.00	1	53	P	D		7000	1500	5500		
120189	B535	0.00	0.00	3	6	TB	D		1950	1300	650		
120189	B536	0.00	0.00	2	46	U	D		2750	1300	1450		
120189	B537	0.00	0.00	2	79	U	D		3200	1050	2150		
150289	B538	0.00	0.00	1	0	H	D		2850	1950	900		
150289	B539	0.00	0.00	2	0	H	P		4350	1650	2700		
150289	B540	0.00	0.00	1	20	P	D		1750	1250	500		
150289	B541	0.00	0.00	3	0	TB	D		3350	1900	1450		
150289	B542	0.00	0.00	1	11	TB	D		3250	1250	2000		
150289	B543	0.00	0.00	3	2	H	P		6000	2000	4000		
150289	B544	0.00	0.00	1	0	TB	P		2100	1250	850		
160289	B545	0.00	0.00	3	24	D	D		7000	1750	5250		
160289	B546	0.00	0.00	2	2	D	D		6000	1600	4400		
160289	B547	0.00	0.00	2	22	U	D		5250	1950	3300		
160289	B548	0.00	0.00	2	21	P	D		6000	2250	3750		
160289	B549	17.00	0.00	3	22	H	D		4500	2200	2300		
160289	B550	0.00	0.00	3	2	P	D		4250	1600	2650		
160289	B551	0.50	0.00	1	0	D	D		4500	2300	2200		
160289	B552	0.00	0.00	2	0	TB	D		1750	1750	0		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
160289	B553	0.00	0.00	2	0	P	D		1400	1350	50		
160289	B554	0.00	0.00	1	18	TB	D		8250	1300	6950		
160289	B555	3.50	0.00	2	0	TB			5000	1800	3200		
160289	B556	0.00	0.00	1	0	TB	D		2700	1600	1100		
160289	B557	0.00	0.00	4	8	TB	P		4250	1900	2350		
160289	B558	0.00	0.00	1	0	TB	P		4000	200	3800		
160289	B559	0.00	0.00	3	24	H	D		4750	2100	2650		
160289	B560	0.00	0.00	3	0	TB	D		2300	1550	750		
160289	B561	0.00	0.00	2	0	TB	P		3150	2000	1150		
160289	B562	0.00	0.00	3	2	TB	D		3350	1400	1950		
160289	B563	0.00	0.00	3	16	P	D		2750	1325	1425		
160289	B564	2.50	0.00	2	8	TB	D		3600	2250	1350		
160289	B565	0.00	0.00	1	0	P	D		1600	1450	150		
160289	B566	0.00	0.00	1	14	H	D		3250	1700	1550		
160289	B567	1.00	0.00	5	0	U	D		4400	1800	2600		
080389	B568	0.00	0.00	4	58	U	D		2700	1800	900		
080389	B569	0.00	12.00	2	0	TB	D		1600	1450	150		
080389	B570	0.00	0.00	3	4	TB	D		2900	1450	1450		
080389	B571	0.00	0.00	2	0	H	P		7750	1650	6100		
080389	B572	0.00	13.00	2	0	TB	P		1100	1100	0		0.05
080389	B573	0.00	1.20	3	6	H	D		7000	1850	5150		
080389	B574	0.50	0.50	2	0	H	P		7500	1500	6000		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
090389	B575	2.00	0.00	3	0	TB	P		2600	1900	700		
090389	B576	8.50	0.00	2	0	P	D		3250	1500	1750		
090389	B577	0.00	0.00	2	127	P	D		4200	1300	2900		
090389	B578	0.00	0.00	2	0	P	D		2000	1400	600		0.05
090389	B579	0.50	0.00	2	0	TB	P		2150	1500	650		
090389	B580	2.00	0.00	2	23	H	D		3500	1600	1900		0.02
090389	B581	7.00	0.00	3	26	TB	D		3000	1550	1450		0.26
090389	B582	5.00	0.00	3	2	TB	D		2550	1500	1050		
090389	B583	0.00	0.00	3	0	TB	P		5500	1600	3900		
090389	B584	0.00	0.00	3	60	U	D		2200	1600	600		
090389	B585	0.00	0.00	2	0	TB	P		5000	1950	3050		
090389	B586	0.00	0.00	1	0	TB	P		3600	1650	1950		
090389	B587	0.00	0.00	3	0	P	D		1800	1500	300		
090389	B588	0.00	0.00	2	0	TB	P		6000	1600	4400		
090389	B589	0.00	0.00	1	0	TB	P		5000	1300	3700		
090389	B590	0.00	0.00	2	0	TB	D		4500	1550	2950		
090389	B591	0.00	0.00	2	0	P	D		8000	2200	5800		
300389	B592	0.00	0.00	2	0	D	P		4500	1500	3000		
300389	B593	0.00	1.00	4	6	P	P		1700	1150	550		
300389	B594	0.00	0.00	4	17	P	P		1950	1100	850		
300389	B595	0.00	0.75	1	0	TB	P		4000	1500	2500		
300389	B596	0.00	0.00	1	2	TB	D		2100	2100	0		

Date	Ref number	Ulcer cm sq.	Erosion cm sq.	Margo plicatus score.	Bots	Breed	Source	H.Pylori (OD)	Full stomach gms.	Empty stomach gms.	Gastric contents gms.	Gastrin ng/ml	Pepsin IU/L
300389	B597	0.00	1.50	2	0	TB			5500	1700	3800		
300389	B598	0.00	0.00	3	0	P	D		6000	1600	4400		
300389	B599	0.00	0.00	2	0	TB	D		6500	1850	4650		
300389	B600	0.00	0.00	1	0	TB	D		2150	2100	50		
300389	B601	0.00	1.00	2	3	P	D		1600	1300	300		
300389	B602	0.00	0.00	1	0	TB	D		2600	1500	1100		
300389	B603	1.40	0.00	3	14	TB	D		4250	2000	2250		
300389	B604	0.00	0.00	3	4	TB	P		4500	1550	2950		
300389	B605	0.00	0.00	2	3	TB	P		2800	1550	1250		
300389	B606	0.00	0.00	4	0	TB	P		8000	2050	5950		
300389	B607	0.00	0.00	2	1	P	D		7000	1100	5900		
300389	B608	0.00	0.00	2	12	P	D		2800	1400	1400		
300389	B609	1.00	0.00	3	7	P	D		1500	1350	150		
300389	B610	1.25	0.00	2	0	TB	D		1800	1350	450		0.15
300389	B611	2.20	0.00	2	8	TB	P		4000	1650	2350		
300389	B612	0.00	0.00	1	0	U	P		6250	1850	4400		
300389	B613	0.00	0.00	2	0	P	D		1750	950	800		
300389	B614	0.00	0.00	1	0	TB	D		10000	1750	8250		
300389	B615	0.00	0.00	1	0	U	D		2050	1000	1050		
300389	B616	0.00	0.00	1	93	U	D		12000	1350	10650		

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyperkeratosis		
	stain	stain	gl	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B7	4	-	2	-	2	Y	Y	-	Y	-	-	30	_
B24	4	-	2	1	1	-	-	-	-	Y	-	30	-
B66	3	3	3	1	-	-	Y	-	Y	-	**	70	-
B80	1	1	2	2	2	-	-	-	-	-	-	25	-
B89	2	2	2	-	2	-	Y	-	Y	-	****	40	**
B93	1	-	-	-	1	-	-	-	-	-	-	40	-
B100	1	1	-	4	-	-	Y	-	Y	-	-	25	*
B104	6	4	4	2	2	-	Y	-	Y	-	-	35	-
B111	6	6	3	3	-	-	Y	-	Y	-	-	35	-
B113	3	-	2	1	1	-	-	-	-	-	-	50	-
B114	1	1	1	1	1	-	Y	-	Y	-	-	80	**
B121	2	-	-	-	2	-	-	Y	Y	-	-	80	**
B127	1	-	-	-	1	-	Y	-	Y	-	**	40	***
B129	2	-	1	-	1	-	Y	-	Y	-	*	80	-
B136	2		1	1	-	-	-	-	-	-	-	30	-
B138	2	-	1	-	1	-	-	Y	Y	-	****	95	**
B139	2	-	-	1	-	-	-	-	-	-	-	40	-
B141	2	-	1	-	1	-	-	-	-	-	-	80	-
B143	4	-	1	1	2	-	Y	-	Y	-	-	80	-
B144	2	-	1	-	1	-	-	-	-	-	-	20	-
B145	2	-	-	-	2	-	-	-	-	-	-	50	-
B146	2	-	2	-	-	-	-	-	-	-	-	-	-

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyper kerato sis		
	stain	stain	gl.	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B150	3	1	1	2	-	-	Y	-	Y	-	-	70	**
B155	2	-	-	1	1	-	-	-	-	-	-	30	-
B156	1	-	-	-	1	-	-	-	-	-	-	80	-
B157	1	-	-	-	1	-	-	-	-	-	-	80	-
B158	1	-	-	1	-	-	Y	-	-	Y	*	20	*
B159	1	-	-	-	1	-	-	-	-	-	-	40	-
B160	1	-	-	-	1	-	-	-	-	-	-	40	-
B162	1	-	-	-	1	-	-	Y	-	-	-	50	*
B163	2	-	1	-	1	-	-	-	-	-	*	60	-
B164	2	1	1	-	1	-	Y	-	Y	-	*	90	*
B165	2	-	1	-	1	-	-	-	-	-	-	50	-
B176	2	-	1	-	1	-	Y	Y	-	-	**	80	***
B179	1	-	-	-	1	-	Y	-	Y	-	***	80	****
B181	2	-	-	-	2	-	-	-	-	-	-	-	-
B185	4	1	2	1	2	-	-	Y	Y	-	**	90	***
B187	1	-	-	-	1	-	-	-	-	-	-	-	-
B188	2	-	1	-	1	-	-	-	-	-	-	55	-
B189	3	-	1	-	2	-	-	-	-	-	-	55	-
B194	2	-	-	-	2	-	-	Y	Y	-	-	60	-
B199	5	3	-	8	5	-	Y	-	Y	-	-	50	-
B200	1	-	-	-	1	-	-	Y	Y	-	*	30	***
B201	1	-	-	-	1	-	Y	Y	Y	-	-	50	-

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyperkeratosis		
	stain	stain	gl.	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B203	1	-	-	-	1	-	Y	-	-	Y	**	100	-
B207	2	1	-	-	3	-	-	-	-	-	-	20	-
B213	1	-	-	-	1	-	-	-	-	-	-	?	-
B215	3	-	1	-	2	-	-	-	-	-	-	20	-
B216	1	-	-	-	1	-	-	-	-	-	*	50	-
B219	1	-	-	-	1	-	-	-	-	-	-	50	-
B225	2	1	-	-	2	-	Y	-	Y		***	80	-
B229	3	3	2	1	2	-	-	-	-	-	-	70	-
B231	4	-	2	2	-	-	Y	-	Y	-	-	70	-
B232	2	-	1	-	1	-	-	-	-	-	*	50	-
B234	2	-	1	-	1	-	-	-	-	-	-	-	-
B237	3	-	1	-	2	-	Y	-	Y	-	-	50	-
B238	5	2	4	-	7	-	Y	-	Y	-	*	90	*
B240	2	-	2	1	-	-	-	-	-	-	-	70	-
B241	2	-	2	-	-	Y	-	-	Y	-	-	-	-
B244	2	-	1	-	1	Y	-	-	-	Y	-	40	-
B245	3	-	2	-	1	-	-	-	-	-	-	-	-
B246	2	-	2	-	-	Y	-	-	-	Y	-	-	-
B247	3	-	-	-	3	-	Y	-	Y	-	*	30	**
B252	3	-	1	-	2	-	-	Y	Y	-	-	50	-
B253	1	-	-	-	1	-	-	Y	-	-	-	20	-
B255	3	-	2	-	1	Y	-	-	-	Y	-	-	-

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyper kerato sis		
	stain	stain	gl	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B257	2	-	2	-	1	-	-	-	Y	-	-	50	-
B258	3	-	1	-	2	-	-	Y	Y	-	-	70	-
B259	4	1	8	-	-	Y	-	-	-	Y	-	-	-
B260	2	-	2	-	-	-	Y	-	-	Y	-	-	-
B262	2	-	2	-	1	-	-	-	-	-	-	30	-
B263	3	-	3	-	-	-	-	-	-	-	-	-	-
B264	2	-	2	2	-	-	Y	-	-	Y	*	90	-
B266	3	-	3	-	-	Y	-	-	Y	Y	-	-	-
B267	5	3	3	-	4	-	-	Y	Y	-	***	50	-
B268	6	-	-	-	6	-	Y	-	-	Y	*	39	**
B270	1	-	-	-	1	-	Y	-	Y	-	**	50	-
B273	3	3	-	-	5	-	Y	-	Y	-	-	50	*
B277	3	2	-	3	-	-	Y	-	Y	-	*	90	**
B280	2	-	-	-	2	Y	-	-	Y	-	-	50	**
B283	1	-	-	-	1	-	-	Y	Y	-	-	50	**
B284	1	-	-	-	1	-	-	Y	Y	-	*	80	*
B285	3	-	2	-	1	Y	-	Y	Y	-	**	50	-
B300	1	-	-	-	1	-	-	-	-	-	-	30	-
B304	1	-	-	-	1	-	-	-	-	-	-	-	-
B309	1	-	-	-	1	-	-	-	-	-	-	-	-
B314	1	-	-	-	1	-	Y	-	-	Y	**	80	-
B315	1	-	-	-	1	-	-	Y	Y	-	-	50	-

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyper kerato sis		
	stain	stain	gl.	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B316	1	-	-	-	1	-	-	Y	Y	-	-	80	**
B339	2	-	-	-	2	-	Y	-	Y	-	***	90	-
B342	2	-	-	1	1	-	Y	Y	-	Y	-	70	-
B344	2	-	1	1	-	-	Y	-	-	Y	*	30	-
B348	4	-	1	-	3	-	-	Y	Y	-	***	80	-
B390	2	-	1	-	1	-	Y	-	-	Y	-	-	-
B404	2	-	-	-	2	-	-	Y	Y	-	***	80	-
B457	2	-	-	2	-	-	Y	-	-	Y	-	50	-
B467	1	-	-	-	-	-	-	-	-	-	-	-	-
B488	1	-	1	-	-	-	-	-	-	-	-	-	-
B491	3	-	2	-	1	-	-	Y	Y	-	****	80	-
B493	1	-	1	-	-	Y	-	-	-	-	-	-	-
B495	3	-	2	1	-	-	Y	-	-	Y	-	50	-
B501	3	-	4	-	-	-	-	-	-	-	-	-	-
B518	2	-	-	1	1	-	Y	-	-	Y	-	-	-
B522	4	-	2	1	1	-	Y	-	-	Y	-	80	-
B524	1	-	-	-	Y	-	-	-	-	-	-	-	-
B525	1	-	1	-	-	Y	-	-	-	-	-	-	-
B526	5	1	4	1	-	-	Y	-	-	Y	-	50	-
B527	3	1	1	-	2	-	Y	-	Y	-	-	50	-
B532	5	1	3	-	2	-	Y	-	-	Y	***	80	-
B541	2	2	2	-	-	-	-	-	-	-	-	-	-

Ref	H+E	W.S	Tissue			Lesion site			Les. Dist.		Hyperkeratosis		
	stain	stain	gl	sq. f.	m.pl.	gl.	sq.f.	m.pl.	foc.	diff.	parak	p.e.h.	mit.f.
B549	3	3	2	-	1	-	-	Y	-	Y	**	50	-
B559	1	-	-	-	1	-	-	Y	-	-	-	-	-
B572	3	2	1	2	--	-	Y	-	Y	-	****	80	-
B578	3	3	3	-	-	Y	-	-	Y	-	-	-	-
B580	2	2	1	-	1	-	Y	-	Y	-	-	50	-
B581	2	-	1	-	1	Y	-	-	-	Y	-	50	***
B584	1	1	1	-	-	Y	-	-	-	Y	-	-	-
B600	1	-	1	-	-	Y	-	-	-	Y	-	-	-
B610	3	2	2	-	1	-	-	Y	Y	-	***	50	*

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B7	**	**	*	-	**	*	**	-	-	**	-	-	-		*
B24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B66	*	-	-	-	**	-	*	-	-	-	-	-	-	-	-
B80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B89	***	-	***	-	**	*	***	-	-	**	***	*	-	-	**
B93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B100	**	**	***	-	***	-	***	-	-	**	***	***	-	-	**
B104	*	-	-	-	***	-	**	-	-	-	-	-	-	-	-
B111	-	-	-	*	***	-	*	-	-	-	-	-	-	-	**
B113	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B114	***	*	**	-	***	**	***	-	-	**	***	-	-	**	***
B121	**	**	-	-	-	-	**	-	-	**	-	-	-	*	-
B127	**	*	-	-	**	*	***	-	-	**	***	-	-	*	***
B129	**	*	*	***	-	*	****	-	**	*	-	-	-	-	-
B136	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B141	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B143	**	***	**	-	-	-	-	-	-	-	-	-	-	-	**
B144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B150	***	***	****	****	-	***	***	-	-	-	****	-	-	-	**

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B156	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B157	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B158	-	***	***	-	-	-	-	-	-	-	***	-	-	-	*
B159	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B163	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B164	-	*	-	-	-	-	-	**	-	-	-	-	-	-	-
B165	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B176	-	*	-	**	-	-	**	-	-	-	**	-	-	-	***
B179	*	**	-	-	**	-	-	-	-	-	-	-	-	-	****
B181	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B185	-	*	***	-	**	-	**	-	-	***	-	-	-	-	***
B187	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B188	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B189	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B194	-	**	-	-	-	-	-	-	-	-	-	****	-	-	-
B199	*	***	*	-	***	***	-	-	-	-	***	-	-	-	-
B200	-	***	-	-	**	-	***	-	-	-	-	-	-	-	-
B201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B203	-	-	-	****	-	-	*	-	-	-	-	-	-	-	-
B207	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B213	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B215	-	-	-	****	-	-	-	-	-	-	-	-	-	-	*
B216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B219	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B225	*	**	***	-	**	-	-	-	-	-	***	-	-	-	**
B229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B231	*	**	*	*	-	-	*	-	-	-	*	-	-	-	**
B232	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B234	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B237	-	*	-	**	-	-	*	-	-	-	-	-	-	-	-
B238	*	**	**	-	**	*	**	-	-	-	**	-	-	**	**
B240	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B241	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B244	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B245	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B246	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B247	*	*	*	-	-	-	*	*	-	-	*	-	-	-	**
B252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B255	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B258	-	-	-	*	-	-	***	-	-	-	***	-	-	-	-
B259	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B260	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B262	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B263	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B264	-	***	-	-	-	-	-	-	-	-	**	-	-	-	-
B266	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B267	*	**	*	-	*	*	-	-	-	-	**	*	-	-	****
B268	**	***	***	***	-	**	***	-	-	***	**	**	-	**	***
B270	-	**	-	-	-	-	-	-	-	-	**	-	-	-	**
B273	*	**	*	-	**	-	-	-	-	-	*	-	-	-	**
B277	-	*	-	****	-	-	*	-	-	-	*	-	-	-	*
B280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B283	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B284	-	*	**	-	-	-	**	-	-	-	-	-	-	-	***
B285	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B304	-	*	*	-	-	-	-	-	-	-	**	-	-	-	-
B309	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B314	-	*	-	*	-	*	***	-	-	-	-	**	-	-	-
B315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B316	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B339	**	**	-	-	***	-	**	-	-	-	**	-	-	**	*
B342	*	***	-	****	-	-	****	-	-	-	**	-	-	***	**
B344	***	**	**	*	**	*	***	-	-	-	-	-	-	**	**

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B348	**	*	-	-	***	*	*	-	-	-	-	-	-	-	**
B390	*	*	-	-	*	*	**	-	-	-	-	-	-	-	-
B404	-	*	**	*	-	-	-	-	-	-	-	-	-	-	-
B457	-	-	-	****	-	**	***	-	-	-	***	**	-	****	-
B467	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B488	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B491	-	**	****	-	**	-	****	-	-	-	-	****	-	-	-
B493	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B495	-	**	*	****	-	-	***	-	-	-	-	-	-	***	***
B501	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B518	-	-	-	****	-	-	-	-	-	-	-	-	-	-	-
B522	-	*	-	****	-	**	***	-	-	-	*	-	-	***	**
B524	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B525	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B526	-	*	-	****	-	**	**	-	-	-	***	-	-	***	-
B527	*	-	*	-	**	-	**	-	-	-	*	-	-	-	-
B532	**	**	**	-	***	-	**	-	-	-	-	-	-	-	***
B541	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B549	*	-	-	****	-	-	*	-	-	-	-	-	-	**	-
B559	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B572	-	**	-	-	-	-	*	-	-	-	-	-	-	-	-
B578	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B580	-	-	*	-	**	-	**	-	-	-	-	-	-	-	-

Ref	Degen eration					Exocy tosis					Bacteria			Exud.	R.P.Sp.
	sp.	hy. de.	ret. de.	ul.	er.	crust	p.m.n.	eos	lym.	mic.ab.	cocci	bacilli	fungi		
B581	-	-	-	-	***	**	***	-	-	-	-	-	-	***	***
B584	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B610	**	****	-	-	-	-	**	-	**	-	-	-	-	-	***

Ref	Lamina			Propria		Fibrosis		Vasculature		Margo Plicatus				Glandular		
	Infiltrate			foc.	diff.	imm.	mat.	incr.# bvs	cong.	ect.g.	ass.fib	ass.ero.	corr.	lym.	p.m.n.	eos.
	p.m.n.	eos.	lym.													
B7	-	-	*	Y	-	*	-	-	-	*	*	-	*	-	****	****
B24	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	****
B66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B89	-	-	**	Y	-	*	-	-	-	-	-	-	-	-	-	-
B93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B100	-	-	**	Y	-	*	**	-	-	-	-	-	-	-	-	-
B104	-	-	*	-	-	**	*	-	-	-	-	-	-	-	-	-
B111	-	-	*	Y	-	*	****	**	-	-	-	-	-	-	-	-
B113	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B114	*	-	*	-	-	-	-	-	-	*	-	-	-	**	-	**
B121	-	-	-	-	-	-	-	-	-	*	-	-	-	**	-	**
B127	*	-	**	Y	-	-	-	-	-	**	**	**	*	-	-	-
B129	***	**	**	-	-	-	-	-	-	-	-	-	-	-	-	-
B136	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B138	-	-	****	-	-	-	****	-	-	****	****	****	****	***	-	***
B139	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-
B141	-	-	-	-	-	-	-	-	-	***	***	***	***	***	-	**
B143	-	-	-	-	-	-	-	-	-	***	***	**	**	-	-	-
B144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B145	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-
B146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**
B150	*	-	*	-	-	-	****	***	-	-	-	-	-	**	-	*
B155	-	****	***	-	-	-	-	-	-	-	-	-	-	-	-	-

Ref	Lamina			Propria		Fibrosis		Vasculature		Margo Plicatus				Glandular		
	Infiltrate			foc.	diff.	imm.	mat.	incr.# bvs	cong.	ect.g.	ass.fib.	ass.ero.	corr.	lym.	p.m.n.	eos.
	p.m.n.	eos.	lym.													
B156	-	-	-	-	-	-	-	-	-	***	***	***	**	-	-	-
B157	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	****
B158	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-
B159	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-
B160	-	***	***	Y	-	-	-	-	-	***	***	-	-	-	-	-
B162	-	***	**	Y	-	-	-	-	-	****	****	***	***	-	-	-
B163	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-
B164	-	-	**	-	Y	-	**	**	-	-	-	-	-	-	-	-
B165	-	-	-	-	-	-	-	****	-	-	-	-	-	-	-	***
B176	*	-	**	Y	-	*	-	****	-	-	-	-	-	**	-	-
B179	-	-	*	-	-	*	***	***	-	*	*	-	*	-	**	-
B181	-	-	-	-	-	-	-	-	-	**	*	**	-	-	-	-
B185	-	**	***	Y	-	*	-	-	**	**	-	-	-	**	*	***
B187	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-
B188	-	-	-	-	-	-	-	-	-	-	-	-	***	-	-	-
B189	-	-	-	-	-	-	-	-	-	***	***	*	**	-	-	-
B194	-	-	-	-	-	-	-	-	-	****	****	-	***	-	-	***
B199	-	-	**	-	-	-	*	-	-	-	-	-	-	-	-	-
B200	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
B201	-	-	*	-	-	-	-	-	-	**	*	**	-	-	-	***
B203	****	-	**	-	Y	*	***	****	**	-	-	-	-	-	-	-
B207	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B213	-	-	*	-	-	-	-	-	-	****	*	-	-	-	-	****
B215	**	-	-	-	-	*	*	*	-	-	-	-	-	-	-	-

Ref	Lamina			Propria		Fibrosis		Vasculature		Margo Plicatus				Glandular		
	Infiltrate			foc.	diff.	imm.	mat.	incr.# bvs	cong.	ect.g.	ass.fib.	ass.ero.	corr.	lym.	p.m.n.	eos.
	p.m.n.	eos.	lym.													
B216	-	-	-	-	-	-	-	-	-	**	***	*	-	-	-	-
B219	-	-	-	-	-	-	-	-	***	-	-	-	-	-	-	-
B225	-	-	*	-	-	*	-	-	-	-	-	-	-	-	-	-
B229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**
B231	-	-	*	-	-	-	*	-	-	-	-	-	-	-	-	-
B232	-	-	-	-	-	-	-	-	-	****	***	***	-	-	-	-
B234	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B237	*	-	**	-	-	-	-	-	-	-	-	-	-	-	-	*
B238	-	-	**	-	Y	*	-	**	**	-	-	-	-	-	-	**
B240	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B241	-	-	**	-	-	-	-	-	-	-	-	-	-	*	-	-
B244	-	-	*	-	-	-	-	-	-	-	-	-	**	**	-	**
B245	-	-	-	-	-	*	*	-	-	-	-	-	-	-	-	-
B246	-	**	*	-	-	*	*	*	-	-	-	-	-	**	-	**
B247	*	-	**	-	*	*	***	***	**	-	-	-	-	-	-	-
B252	-	-	**	-	-	-	-	-	-	***	***	*	-	-	-	-
B253	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-
B255	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	***
B257	-	-	*	-	-	-	*	-	-	-	-	-	-	****	-	****
B258	-	-	**	Y	-	-	-	-	-	***	**	-	-	**	-	**
B259	-	-	-	-	-	-	-	-	-	-	-	-	-	****	-	***
B260	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
B262	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*
B263	-	-	-	-	-	-	-	-	-	-	-	-	-	**	*	*

Ref	Lamina Propria					Fibrosis		Vasculature		Margo Plicatus				Glandular		
	Infiltrate			foc.	diff.	imm.	mat.	incr.# bvs	cong.	ect.g.	ass.fib.	ass.ero.	corr.	lym.	p.m.n.	eos.
	p.m.n.	eos.	lym.													
B264	-	-	*	*	-	-	-	-	*	-	-	-	-	-	-	-
B266	-	-	-	-	-	-	-	-	-	**	*	**	-	*	-	*
B267	-	-	**	*	-	**	**	***	*	***	**	-	*	**	-	-
B268	*	-	**	-	*	**	***	*	-	**	**	-	-	*	-	-
B270	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-
B273	-	-	*	*	-	-	-	-	-	-	-	-	-	*	-	**
B277	***	-	*	-	**	*	****	-	-	-	-	-	-	-	-	-
B280	-	-	-	-	-	-	-	-	-	***	*	**	*	**	-	-
B283	-	-	-	-	-	-	-	-	-	***	*	**	*	**	-	-
B284	-	-	**	-	-	-	*	-	-	***	**	**	*	-	-	-
B285	-	-	-	-	-	-	-	-	-	**	**	-	-	**	-	**
B300	-	-	**	-	-	-	-	-	-	*	-	-	-	-	-	-
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B309	-	-	-	-	-	-	-	-	-	**	-	-	**	*	-	-
B314	****	-	-	-	Y	**	**	-	-	-	-	-	-	-	-	-
B315	-	-	**	Y	-	-	-	-	-	**	*	***	**	**	-	**
B316	-	-	**	-	-	-	-	-	-	***	**	*	**	-	-	-
B339	-	-	**	-	-	-	-	-	*	**	*	**	-	-	-	-
B342	**	-	-	-	-	***	-	-	-	**	**	**	-	-	-	-
B344	*	**	_	Y	-	**	-	*	-	-	-	-	-	-	-	-
B348	*	-	*	Y	-	**	-	-	-	**	*	-	-	-	-	-
B390	**	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-
B404	-	-	-	-	-	-	**	-	-	*	*	-	-	-	-	-
B457	****	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-

Ref	Lamina			Propria		Fibrosis		Vasculature		Margo Plicatus				Glandular		
	Infiltrate			foc.	diff.	imm.	mat.	incr.# bvs	cong.	ect.g.	ass.fib	ass.ero.	corr.	lym.	p.m.n.	eos.
	p.m.n.	eos.	lym.													
B467	***	-	-	-	-	**	***	**	-	-	-	-	-	-	-	-
B488	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
B491	-	-	*	-	-	-	-	-	-	-	-	-	**	*	-	-
B493	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B495	****	-	*	-	-	*	*	-	-	-	-	-	-	*	-	-
B501	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B518	-	-	***	-	Y	-	****	****	-	-	-	-	-	-	-	-
B522	***	*	**	-	Y	*	-	-	-	*	*	**	**	-	-	-
B524	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B525	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	**
B526	**	-	**	-	Y	**	**	**	***	-	-	-	-	*	-	***
B527	**	-	-	-	-	-	**	-	-	***	**	**	-	*	-	-
B532	**	-	*	Y	-	*	-	**	-	***	**	**	-	-	-	-
B541	**	*	*	-	Y	***	*	**	**	-	-	-	-	*	-	-
B549	**	-	-	-	-	*	**	-	-	**	**	-	-	-	-	-
B559	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B572	-	-	*	-	-	-	-	-	-	**	*	-	*	-	-	-
B578	-	-	*	-	-	-	-	-	-	-	-	-	-	**	**	-
B580	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-
B581	***	-	-	-	Y	**	**	-	-	-	-	-	-	*	**	**
B584	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B600	-	-	-	-	-	-	-	-	-	-	-	-	-	****	-	-
B610	*	-	**	Y	-	**	-	-	-	-	-	-	-	-	-	-

APPENDIX 2

PUBLICATIONS

CUMANN TREIDLIACHTA EIREANN/IRISH VETERINARY ASSOCIATION
53 Lansdowne Road, Ballsbridge, Dublin 4. Telephone 685263. Fax 604345

September 22, 1992

Mr. Harry J. Sweeney, MVB., MRCVS.,
Veterinary Surgeon,
Taiki Farm,
Hokkaido,
JAPAN.

Dear Harry,

On behalf of the Irish Veterinary Association, which holds the copyright of all of the material published in the Irish Veterinary Journal,I give you permission to reproduce in the publications appendix of your doctoral thesis a copy of the paper entitled: "Prevalence and Pathogenisity of Gasterophilus intestinalis larvae in horses in Ireland" which appeared in the third issue of Vol.43, pp 67-73, 1990.

Yours sincerely,

Dr. P.J. Hartigan,
EDITOR - IRISH VETERINARY JOURNAL

Irish Veterinary Journal 43: 67-73, 1990

The prevalence and pathogenicity of *Gasterophilus intestinalis* larvae in horses in Ireland

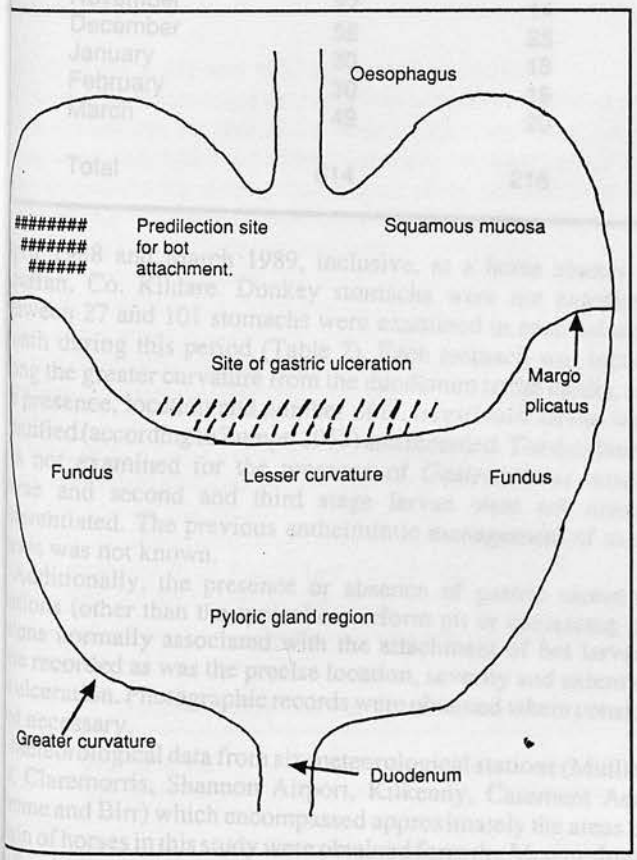
HARRY J. SWEENEY

At an Irish horse abattoir 614 horse stomachs were examined over a twelve-month period (between 27 and 101 per month). Excluding the months of July to September, inclusive, when the stomach is largely free of bots, 458 stomachs were examined, 197 (43 *per cent)* of which had *Gasterophilus intestinalis* larvae. The majority of horses (90 *per cent*) had less than 50 bot larvae and only 1.4 *per cent* harboured more than 100 bots. Ulceration or erosion of the gastric stratified squamous mucosa close to the margo plicatus was recorded in 173 (28.2 *per cent*) of the 614 stomachs and lesions varied in size from 0.5cm² to over 60cm². Only 60 (37 *per cent*) of horses with ulceration had concurrent bot burdens. Analysis of meteorological data revealed that the corresponding periods of larval development and fly activity were colder, duller, wetter and more windy than normal. By comparison with a similar survey in Ireland published in 1976 in which a 90 *per cent* prevalence of *G. intestinalis* was reported, it was concluded that a dramatic reduction in the prevalence and extent of bot infestations had occurred and this is largely attributed to the introduction of ivermectin and to a lesser extent, the climatic condition which may have adversely affected fly activity and larval development. Furthermore, it was concluded that bot larvae are not important in the aetiopathogenesis of gastric ulceration or erosion.

Key words: Horse, Stomach, *Gasterophilus intestinalis*, Gastric Ulcers, Abattoir Survey.

oduction

larval stages of *Gasterophilus* species of flies (bots) are mon obligate parasites in the gastrointestinal tract of horses any parts of the world (Zumpt, 1965). The commonest species rring in northern temperate zones is *Gasterophilus intestinalis* which completes one life cycle per year, approximately 10 months of which are spent in the parasitic larvae form, largely as second and third stage instars in the equine stomach (Table 1). During the month of July in Ireland and Britain, the mature third stage larvae are expelled from the stomach via the faeces. Pupation occurs in the soil after an approximate 35-day temperature-dependent period and egg laying activity on horses legs by adult flies is usually observed during the months of August to October, inclusive (Edwards, 1982). The minimum period between egg deposition and first appearance in the stomach of a new generation of second stage larvae is five weeks (Wells and Knipling, 1938) and attachment of second stage larvae to their predilection site on the gastric squamous mucosa is first observed in mid-September. Consequently, as the equine stomach is largely free of bot larvae from July to September inclusive, it is necessary to exclude these months when evaluating the annual prevalence of bot infestations.



re 1
gram of an equine stomach incised along the greater curvature with the lumen ble to outline the anatomy, site of gastric ulceration and predilection site for attachment.

Hatch, McCaughey and O'Bri (1976) reported a prevalence of 90 *per cent* and 65.8 *per cent* equine stomach bots in two individual Irish horse abattoirs during the months October to June, inclusive. Similarly, Edwards (1982) reported a 69 *per cent* prevalence of *G. intestinalis* larvae during a similar period in a population of horses in Northern England and Wales.

The pathogenic effect of bot larvae within the stomach is unclear (Soulsby, 1982) and many horses carry large numbers of bot larvae without showing overt clinical signs. Nevertheless, bots have been incriminated in causing ulcers and subserosal abscesses (Waddell, 1972), gastric rupture and suppurative peritonitis (Dart, Hutchins and Begg, 1987) and subclinical effects on gastric function and, consequently, on overall performance (Edwards, 1982).

This paper records the prevalence and magnitude of *G. intestinalis* infestations in 614 horses and investigates their role in gastric ulceration.

Materials and methods

A total of 614 horse and pony stomachs were examined between

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TABLE 1
Summary of *Gasterophilus intestinalis* life cycle in Ireland

Stage	Location	Development time	Months	References
Pupae	Ground	5 weeks (temperature dependent). (viable for 3 months)	July-Sept.	Zumpt (1965) Soulsby (1982) Wehr (1933)
Adults	Air	Live, 3 weeks	Aug.-Oct.	Wells *et al.* (1938)
Eggs	Hair on fore legs	5-10 days (viable for 4 months)	Aug.-Dec.	Edwards (1982) Soulsby (1982) Drudge *et al.* (1975)
First instar	Tongue, Gingiva	21-28 days	Aug.-?Jan.	Soulsby (1982) Edwards (1982)
Second instar	Stomach	5-7 weeks	Sept.-Feb.	Drudge *et al.* (1975) Edwards (1982)
Third instar	Stomach	9 months	Oct.-June.	Edwards (1982)

TABLE 2
Monthly prevalence of *Gasterophilus intestinalis* in equine stomachs

Month	Number examined	Number infested	Percentage	Mean no. of larvae	Range of larvae
April	65	25	39	29	3-75
May	31	13	42	22	3-85
June	69	29	42	20	1-90
July	29	1	3	1	0- 1
August	35	0	0	0	-
September	92	18	20	9	2-19
October	101	37	37	26	1-184
November	27	14	52	11	1-40
December	56	25	45	13	1-88
January	30	18	60	26	1-93
February	30	16	53	1	2-24
March	49	20	41	24	1- 93
Total	614	216	36	18	1-184

April 1988 and March 1989, inclusive, at a horse abattoir in Straffan, Co. Kildare. Donkey stomachs were not examined. Between 27 and 101 stomachs were examined in each calendar month during this period (Table 2). Each stomach was incised along the greater curvature from the duodenum to the cardia, and the presence, location and number of *G. intestinalis* larvae were identified (according to Zumpt, 1965) and recorded. The duodenum was not examined for the presence of *Gasterophilus nasalis* larvae and second and third stage larvae were not always differentiated. The previous anthelmintic management of these horses was not known.

Additionally, the presence or absence of gastric ulcers or erosions (other than the typical craterform pit or coalescing pit lesions normally associated with the attachment of bot larvae) were recorded as was the precise location, severity and extent of the ulceration. Photographic records were obtained where considered necessary.

Meteorological data from six meteorological stations (Mullingar, Claremorris, Shannon Airport, Kilkenny, Casement Aerodrome and Birr) which encompassed approximately the areas of origin of horses in this study were obtained form the Meteorological Service and the monthly mean temperature, rainfall, duration of sunshine and wind speed were computed for the months of June to November, inclusive, in 1987 and 1988.

A Chi-square test was used to test for an association between the presence of bots and the presence of ulcers / erosions.

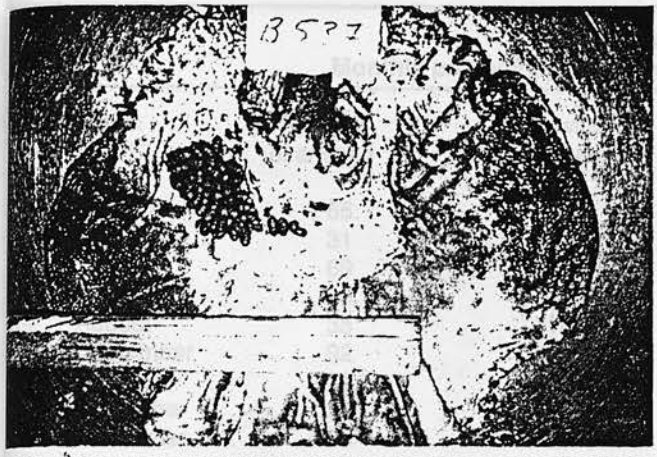
Results

The stomachs of 614 horses and ponies were examined in the twelve-month period and 216 (36 *per cent*) were infested with second or third stage *G. intestinalis* larvae. The equine stomach is largely void of bot larvae during the months of July, August and September and when the data for these months were excluded, it was found that 197 (43 *per cent*) of the remaining 458 stomachs had bot larvae (Table 2).

Only one stomach out of 29 examined in July had bots and none of 35 stomachs examined in August had either second or third stage bot instars. The monthly mean larvae burden in infested horses ranged between 11 and 33 and the highest individual bot burden was 184 second stage larvae recorded in October (Table 2).

Of 32 stomachs examined on June 29, 1988, 10 (31.3 *per cent*) were infested with bots which is not statistically significantly different ($Chi^2 = 1.44$, $p<0.05$) from the 133 stomachs examined before June 9, of which 57 (43 *per cent*) were similarly infested.

The percentages of horses that harboured less than 50 bots, 51 to 100 bots and more than 100 bots were 89.9, 8.8 and 1.4 *per cent*, respectively (Table 3). The total aggregate number of bots



ure 2. A large bot burden (79) attached to the squamous mucosa close to the rgo plicatus in the more dorsal aspects of the parietal (cranial) border of the mach.

gure 3. Medium sized ulcer (circa 1cm x 5cm) in the squamous mucosa close the margo plicatus.

corded in the study was 4367 *G. intestinalis* larvae.

The site of attachment of the bot larvae was very consistent and pical craterform pit lesions or larger coalescing multiple pit sions were only observed in the squamous mucosa close to the argo plicatus in the more dorsal aspects of the parietal (cranial) order of the stomach (Figures 1 and 2). In very heavy bot festations a small number of bots were observed attached to the andular mucosa adjoining this area. Occasionally, a number of ots were recorded in a variety of different locations but when moved, no gross bot-induced pathology was visible at these es and invariably an almost precise number of vacant typical raterform attachment sites were recorded in the normal predilec- on site.

A total of 173 (28.2 *per cent*) horses out of the 614 examined ad ulceration or erosions in the squamous mucosa close to the argo plicatus in that region of the lesser curvature which abuts e pyloric glandular mucosa (Table 4 and Figure 3). Bot larvae ere not detected in or within four cm distance of these ulcers/ rosions and the pathological nature of these lesions was other than that described in the classification of macroscopic lesions produced by *G. intestinalis* as reported by Principato (1988). Grossly, lesions were singular or multiple, irregular, and necrotic material was recorded in the base of the ulcer. The total ulcerated area was less than one cm^2 in 31 *per cent* of affected horse and between one and five cm^2 in an additional 40 *per cent* of the horses. Twenty nine *per cent* of recorded ulcers/erosions were larger than five cm^2. The single biggest ulcer was 60 cm^2.

Of the 173 horses with gastric ulceration/erosion, only 60 (37 *per cent*) had concurrent bot infestation (Table 4). An association between the occurrence of ulceration/erosion and the presence of bots was investigated on a combined annual aggregate basis using the Chi-square test but no association was demonstrated (Chi^2 = 0.03, $p>0.05$). Equally, there was no association between the presence of bots and erosions/ulcers when examined on a monthly basis (Chi^2 values on a monthly basis : April, 0.16; May, 0.86; June, 0.03; July, 2.3; August, numbers too small for computation; September, 2.79; October, 0.56; November, 0.07; December, 0.00; January, 0.03; February, 0.11, and March, 0.04).

The meteorological data are summarised in Table 5 and, in general, the months of June to November, inclusive, in 1987 and 1988 were colder, duller and wetter than the corresponding mean values for the previous 25 years. This was particularly true in 1988 when, additionally, the months of July to October were more windy than normal. In July 1988, the mean monthly temperature was 1.3°C colder than normal, there was one hour less sunshine daily, 70 *per cent* more rain and wind speeds were 19 *per cent* stronger than normal.

Discussion

The survey reported by Hatch *et al.* (1976) was carried out, like this current study, in the Irish Horse Abattoir, Straffan, Co. Kildare and a comparison of the relevant findings in the two surveys indicates a substantial reduction in the prevalence of *G. intestinalis* larvae. During the months October to June inclusive, Hatch *et al.* (1976) found that 90 *per cent* of horses had stomach bot infestations compared to only 43 *per cent* in this study. A similar study carried out between September 1971 and August 1972, inclusive, in an abattoir in Saintfield, Co. Down revealed a 65.8 *per cent* prevalence of *G. intestinalis* larvae during the months of October to June, inclusive (Hatch *et al.*, 1976). A more recent study by Edwards (1982), carried out in northern England and Wales, revealed a 69 *per cent* prevalence of *G. intestinalis* during these months.

In addition to the decreased prevalence of stomach bots, a remarkable reduction in the extent of such infestations was noted. The single largest stomach bot burdens recorded in Straffan and Saintfield by Hatch *et al.* (1976) were 414 and 513, respectively as compared to 184 in this study. The largest *G. intestinalis* burden recorded by Edwards (1982) was 295 third stage larvae. Furthermore, only 10.2 *per cent* of all bot infestations in this study harboured more than 50 *G. intestinalis* bots as compared to 27.2 *per cent* (Straffan) and 46.7 *per cent* (Saintfield) as reported by Hatch *et al.* (1976) and 24.6 *per cent* by Edwards (1982) (Table 3).

The reason for the reduction in the prevalence of *G. intestinalis* in the 17-year period between the two Straffan surveys may in part

TABLE 3

***Gasterophilus intestinalis* frequency distribution in infested horse: Comparison with two surveys**

No. of larvae per infestation	Number of horses	Percentage of horses	Hatch *et al.* (1976) Straffan	Hatch *et al.* (1976) Saintfield	Edwards (1982) England
1 - 50	194	89.8%	72.7%	53.2%	75.5%
51 - 100	19	8.8%	20.1%	24.8%	16.5%
> 100	3	1.4%	7.2%	22.0%	8.1%

TABLE 4
Monthly prevalence of ulceration/erosion in equine stomachs

Month	Number examined	No. with ulcers/erosions	Percentage with ulcers/erosions	No. with ulcers/ erosions and bots	
April	65	26	40	9	(35%)
May	31	7	23	4	(57%)
June	69	20	29	9	(45%)
July	29	9	31	1	(11%)
August	35	12	34	0	(0%)
September	92	18	20	1	(6%)
October	101	21	21	7	(33%)
November	27	9	33	5	(56%)
December	56	20	36	9	(45%)
January	30	8	27	5	(63%)
February	30	5	17	3	(60%)
March	49	18	37	7	(39%)
Total	614	173	28	60	(37%)

be due to increased owner awareness of the life cycle of *G. intestinalis* and consequent improved horse husbandry. It must also be considered that both populations may not be strictly comparable as Thoroughbreds, that usually are managed more intensively, comprised 55 *per cent* of the animals examined in this survey, whereas farm and work animals are likely to have been more important in the survey of Hatch *et al.* (1976). Nevertheless, it is the opinion of the author that the introduction of and widespread use of effective new generation wide spectrum equine anthelmintics with concurrent potent ectoparasitic/endoparasitic and boticidal actions is the single most important factor in mediating this reduction. Foremost among such drugs are the ivermectins (Eqvalan Paste; MSD AGVET) which enjoys a 100 *per cent* efficacy against first, second and third stage *Gasterophilus* instars in addition to efficacy against large and small strongyles, lungworm, oxyuris, strongyloides and parascaris (Yazwinski, Hamm, Greenway and Tilley, 1982; Evans, 1988). Oral preparations of ivermectin were introduced in Ireland in 1983 and in 1988 more than 50,000 doses suitable for 500kg horses were sold in the Republic of Ireland. Eqvalan paste now accounts for half the market world-wide for equine anthelmintic oral pastes (Bloomfield, 1988). Prior to the era of ivermectins, carbon disulphide and a number of organophosphorous compounds (dichlorvos and trichlorphon) were the main boticidal agents in use but disadvantages included narrow therapeutic spectrum, narrow safety range, digestive disturbances, a requirement to withhold food and water for a period before and after treatment and variable efficacy, 70 to 100 *per cent* (Drudge and Lyons, 1983).

Beesley (1966) reported that, in general terms, a cold wet summer tends to be followed by a spring when the number of warbled animals is lower than normal and this was clearly demonstrated by Tarry (1980), who demonstrated a strong correlation between average air temperature/hours of bright sunshine and the percentage of hides damaged by cattle warble fly. The climatic conditions in July to October 1987 and 1988 were not optimal for the pupal and adult stages of *G. intestinalis* and the colder, duller, wetter and more windy weather, especially in 1988 is likely to have been partly responsible for the observed decreased prevalence of stomach bots from September 1988 to March 1989. The possible effects of climate are as follows:

1. A temperature increase of about 2.8°C (5°F) results in doubling of fly activity (Williams 1940; cited by Tarry 1978) and with the exception of August 1987, the months of August to October 1987 and 1988 (the period of adult fly activity) were colder than normal and the October 1987 mean daily temperature was 2.2°C colder than normal. As a minimum temperature of 12.8°C is apparently required before *G. intestinalis* is capable of flying and temperatures greater than 15.5°C are required before oviposition can occur (Wells and Knipling, 1938), the colder autumns during this study may have had serious implications on

TABLE 5
Mean meterological data from six meterological stations with months which were colder, duller, wetter or more windy underlined

	Mean temperature (°C)			Sunshine (hrs)			Rain fall (mm)			Wind speed (knots)		
	1987	1988	25 year mean	1987	1988	25 year mean	1987	1988	25 year mean	1987	1988	25 year mean
June	12.3	14.6	13.4	3.02	5.52	5.55	106	43	59	6.4	5.2	7.6
July	15.5	13.6	14.9	3.77	3.45	4.44	33	116	68	6.9	8.8	7.4
Aug	15.0	13.9	14.7	3.91	3.89	4.63	65	93	78	6.2	8.5	7.2
Sept	12.2	12.3	12.9	4.52	3.19	3.72	103	78	87	7.7	8.5	8.0
Oct	8.3	10.1	10.5	3.08	3.16	2.74	106	102	88	7.2	9.1	8.4
Nov	6.9	6.4	6.7	1.92	2.46	2.07	68	43	88	7.3	5.9	8.9

TABLE 6
The prevalence of *G. intestinalis* in various countries worldwide

Country	Year	Number examined	Prevalence %	Mean no. larvae	Max. no. larvae	Reference
U.S.A.	1951-73	476	68	168	1046	Drudge *et al.* (1975)
Mexico	1983	650	44	78	-	Alanis Tafolla (1983)
Venezuela	1958	-	98	-	-	Quintero (1958)
Burkina Faso	1986	30	100	155	449	Kaboret *et al.* (1986)
Germany	1977-80	2539	9	33	500	Ribbeck *et al.* (1983)
Italy	1978-80	285	7	-	-	Guizzardi (1982)
Yugoslavia	1978-80	88	83	-	-	Guizzardi (1982)
U.S.S.R.	1983	866	100	-	4525	Rastegaev (1983)
Egypt	1982-83	118	98	-	547	Hilali *et al.* (1987)
Morocco	1977-78	94	100	219	838	Pandey *et al.* (1980)
India	1969	12	100	-	-	Singh *et al.* (1974)
China	1986	-	100	-	714	Yang (1986)
Hong Kong	1983-84	195	0	0	0	Hammond *et al.* (1986)
Australia	1966-71	331	64	32	610	Waddell (1972)
New Zealand	1972-73	70	97	63	406	Kettle (1974)

the bot prevalence rates recorded in the following winters and springs.

2. Wind speeds influence the daily flight of flies and the sheep head fly, *Hydrotaea irritans*, is generally not active in wind speeds of 6m per second (Nielsen *et al.*, 1972, cited by Tarry, 1978). As mating by *G. intestinalis* is generally begun during flight with the male engaging the female in flight (Wells and Knipling 1938), the stronger winds of August, September and October 1988 may have been significant.

3. As bright sunshine is said to be important for successful mating, poor egg viability may be related to overcast conditions (Ganser, 1956, cited by Tarry, 1980). The summers of 1987 and 1988 were duller than normal and in July 1988 there was, on average, one hour less sunshine per day than the 25-year mean.

4. The period required for pupae development is temperature-dependent (Zump, 1965; Edwards, 1982) and the months of July to September 1988, inclusive, were significantly colder than normal.

5. Under very dry conditions the successful emergence rate of flies from pupae in the ground will be low because surface soil layers will be difficult to penetrate (Marr 1957, cited by Tarry 1980). Less than half the normal amount of precipation was recorded in July 1987.

6. Excessive rainfall can destroy pupae (Walton, 1928, cited by Tarry, 1980) and in July 1988, 71 *per cent* more precipitation than normal was recorded.

Gasterophilus intestinalis is a cosmopolitan parasite and its prevalence in many parts of the world has been documented (Table 6). A substantial variation in the prevalence and severity of *G. intestinalis* infestations is apparent and is illustrated by a 100 *per cent* prevalence of bot larvae in donkeys in Morocco, 60 *per cent* of which harboured more than 100 bots (Pandey, Ouhelli and Elkhalfane, 1980) and a zero prevalence of bots in Hong Kong (Hammond, Mason and Watkins, 1986). These variations in prevalence may be explained by differences in geographical location and associated differences in the suitability of the local ecology for the extra-host stages; climatic conditions; age and breed; husbandry method; the frequency of grooming and housing during the periods of *Gasterophilus* oviposition; the numbers of horses and donkeys in an ecological area; probably, the strain of the parasite and the availability and use of effective boticidal agents.

It is also likely that varying local seasonal factors cause local fluctuations in bot prevalence from year to year. Ribbeck, Heide, Schicht and Heipe (1983) reported an increased prevalence of *G. intestinalis* larvae from 26 *per cent* in 1977/78 to 59 *per cent* in 1979/80. Similarly, Hilali, Derhalli and Baraka (1987) reported a 98.3 *per cent* prevalence of *G. intestinalis* larvae in Egypt in 1983 compared to a 60 *per cent* prevalence in 1942. Varying local climatic conditions are advanced as the explanation why 62.5 *per cent* and 10.5 *per cent*, respectively, of horses examined in July and August by Hatch *et al.* (1976) had stomach burdens of *G. intestinalis* as compared to only 3.4 *per cent* and 0 *per cent*, respectively, for similar months in this study, which was carried out in the same abattoir as that of Hatch *et al.* (1976).

Further useful information on the dynamics of the *Gasterophilus intestinalis* life cycle in Ireland may have been obtained had second and third stage larvae constantly been differentiated during the study. Nevertheless, some important observations have been made. There was no significant difference in the prevalence of bot infestations in stomachs examined before June 9th and stomachs examined at the end of June. Therefore, it appears that significant numbers of bot larvae do not leave the equine stomach in late June as suggested by Hatch *et al.* (1976) and the exclusion of June results in addition to those of July, August and September in computing the annual prevalence rate is unjustified. Support for the belief in an extensive evacuation of third stage larvae from the stomach in early July is provided by the examination of 29 stomachs on July 20 from which only one solitary bot larvae was recovered.

The examination of 37 stomachs in early September (8-9-88) failed to demonstrate the presence of second stage *G. intestinalis* larvae whereas 13 (40.6 *per cent*) of 32 stomachs examined on September 21 had second stage instars. As the duration between egg deposition and the first arrival of second stage larvae in the stomach is five to six weeks (Table 1), it is unlikely that any adult egg-laying activity occurred before August. Edwards (1982) proposed that oviposition commences in late July in Wales and northern England despite the absence of bot eggs on the limbs of 66 horses examined by him in July. From this work and the work of other authors a composite outline of the probable life cycle of *G. intestinalis* in Ireland is provided (Table 1). It is important to note that in other climates such as Kentucky in the United States of America, egg laying activity of the female extends over much longer periods and second and third stage larvae are found simultaneously in the stomach during every month of the year (Drudge, Lyons, Wyant and Tolliver, 1975).

Panitz (1978) proposed an overwintering phase of dormant pupae which hatch in the subsequent spring and considered the overwintering of adults possible but less likely. The finding of

only one solitary *G. intestinalis* larvae in 101 stomachs examined between July and mid September does not support the occurrence of an overwintering phase in Ireland.

Waddell (1972) observed from his survey of 331 stomachs that larvae were attached to all parts of the oesophageal region from the margo plicatus to within one cm of the cardia and that the majority of the larvae were attached to the mucosa of the saccus cecus. Hilali *et al.* (1987) reported that, in the stomachs of donkeys, *G. intestinalis* larvae were mainly clustered in groups near the boundary of the glandular and the non-glandular mucosa. The selection of the attachment sites of *G. intestinalis* larvae within the stomach was investigated by Price and Stromberg (1987) who reported a pronounced preference for locations in the squamous mucosa along the margo plicatus on the cranial surface of the stomach and a secondary preference for an area slightly cranial to the most dorsal aspect of the saccus cecus.

In this study, major clustering of bot larvae and typical macroscopic bot larvae lesions were only found in the squamous mucosa adjoining the margo plicatus on the cranial aspect of the stomach (Figure 1). In very heavy bot burdens a small proportion of larvae were attached to the glandular mucosa adjoining this predilection site. Occasionally, bot larvae were recorded either free in the digesta or attached elsewhere to the stomach mucosa but macroscopic bot lesions were not evident when detached and almost invariably an appropriate number of vacant typical craterform bot lesions were simultaneously recorded in the normal predilection site. It has been suggested that bot larvae move around the mucosa (Jubb, Kennedy and Palmar, 1985) but as many horses in this study were dead for up to 10 hours before examination, it is likely that postmortem-induced changes in the stomach microenvironment provoked migration by some of the larvae.

Unlike the findings of Price and Stromberg (1987), clustering of *G. intestinalis* larvae in the most dorsal aspect of the saccus cecus was not recorded in this study. The much heavier bot burdens (up to 950 bot larvae) in the study of Price and Stromberg (1987) does not adequately explain their observation of a secondary predilection site because they reported that attachment sites for *G. intestinalis* larvae were not influenced by season or intensity of infestation.

Despite the dramatic appearance of heavy bot infestation, their true pathogenic effect is obscure (Urquhart, Armour, Duncan, Dunn and Jennings, 1987). Soulsby (1982) concluded that it was unreasonable to assume that extensive ulceration of the stomach by large numbers of parasites is without general effect. Subclinical effects on stomach function associated with the presence of bots and, consequently, on overall performance was considered probable by Edwards (1982), and Nolan (1963) considered bots a cause of debility. Waddell (1972) associated bots with the occurrence of subserosal abscesses and Pandey *et al.* (1980) reported that bots cause gastric ulcers, erosions, and nodules or polypous growths. Rooney (1964) reported that ulceration of the oesophageal region, with or without perforation, in foals is directly related to mechanical trauma by *Gasterophilus intestinalis* larvae. Great annoyance is caused to horses by the darting behaviour of adult flies during oviposition (Urquhart *et al.* 1987) and migration in the tongue and gingiva by the first and second stage larvae causes severe disruption of the tissues and the formation of microabscesses (Cogley, 1989).

Other exceptional untoward sequelae to bot infestation include gastric rupture and fatal peritonitis (Rainey, 1948; Dart *et al.* 1987), paralysis of the oesophagus (Horton, 1925), impaction (Nolan, 1963) and fatal hepatitis (Tadmor, Perl and Weinberg, 1981). Rarely, *G. intestinalis* bot larvae have been recorded in such ectopic locations as the ovary (Drudge, Leland and Behlow, 1956), diaphragm and large omentum (Belli and Gevrey, 1981), liver (Tadmor *et al.* 1981) and the brain (Poynter, 1963).

Waddell (1972) incriminated *G. intestinalis* in the aetiology of gastric ulcers and erosions affecting the squamous mucosa between the cardia and that part of the margo plicatus which borders the pyloric glandular region along the lesser curvature (Figure 1). He supported this conclusion on the grounds that 92 *per cent* of ulcerated stomachs had concurrent bot infestations (between 18-340 *G. intestinalis* larvae) and histopathological evidence of under-running of the epithelium near bot larvae by necrotic tissue containing bacteria which he considered important in the initiation of gastric ulcers. However, he did not consider bots the sole cause of gastric ulcers and erosions.

In regard to gastric ulceration/erosion, the findings and conclusions of this study are almost in direct contradiction to those of Waddell (1972). In this study, only 34.7 *per cent* of horses with gastric ulceration/erosion had concurrent bot infestation or evidence of recent bot infestation and a statistical correlation between the presence of bot larvae and the occurrence of ulceration/erosion was not found when the data were examined either on a monthly basis or on a combined annual aggregate basis. Furthermore, despite the seasonal absence of bot larvae from the equine stomach (July/September), no monthly or seasonal variation occurred in the prevalence of gastric ulceration/erosion. Additionally, a distance usually well in excess of 10 cm was present between the site of ulceration and the predilection site for bot attachment and, therefore, it seems unlikely that under-running of the epithelium by necrotic material in the immediate vicinity of bot larvae could be important in the initiation of gastric ulcers/erosions in the area of the lesser curvature.

It is concluded from the above observations that *G. intestinalis* larvae have no role to play in the aetiopathogenesis of equine gastric ulceration and erosion. Support for this conclusion is obtained from the work of Hammond, Mason and Watkins (1986) who, in a survey of 195 Thoroughbreds in Hong Kong where *Gasterophilus* larvae have never been reported, recorded a 66 *per cent* prevalence of gastric ulceration. Conversely, in a survey of 70 horses in New Zealand, 97 *per cent* of which had *G. intestinalis* larvae (mean=63), Kettle (1974) did not observe the presence of any gastric ulcers/erosions.

The dynamics of *G. intestinalis* life cycle in northern temperate climates, such as Ireland, is now reasonably well established (Table 1). *Gasterophilus intestinalis* has a discrete annual life cycle with one generation yearly and no overlap of successive generations. Edwards (1982) noted the occurrence of second stage *G. intestinalis* as late as February and, therefore, it would be prudent to give a second treatment with an effective drug in January, in addition to the traditional treatment for bots in early winter.

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SATELLITE ARTICLE

Gastroduodenal ulceration in foals

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INTRODUCTION

In 1982, a new clinical syndrome in foals, the foal gastroduodenal ulcer syndrome, was first described in the U.S.A. (Rebhun, Dill and Power 1982). Subsequently, it has been recorded in Japan (Yoshihara *et al* 1986) and France (Collobert, Gillet, Vaissaire and Collobert 1987). However, the incidence of the syndrome is low and a recent survey of 531 foal post mortem examinations in the U.S.A. indicated that ulcer related deaths accounted for only 4.5 per cent of all foal mortalities and ranked approximately equal to central nervous system disease as a cause of foal mortality (Dwyer and Powell 1989). In Ireland and the U.K., a survey of equine practitioners with an annual cumulative responsibility for over 6,500 foals revealed the prevalence of the clinical syndrome to be 0.5 per cent (Sweeney 1987, unpublished data). Although only recently recognised as a clinical syndrome, cases of fatal perforating foal gastroduodenal ulceration have been reported in the U.K. (Orr 1972) and Ireland (O'Reilly 1973).

CLINICAL SIGNS

Major clinical signs of the foal ulcer syndrome include teeth grinding (bruxism), salivation (ptyalism), depression, intermittent mild signs of abdominal colic and a preference to lie for prolonged periods in dorsal recumbency (Becht and Byars 1986). Occasionally, gastric reflux can be elicited by nasogastric intubation and clinical signs often are more pronounced after nursing or feeding. If ulcer perforation occurs, signs of circulatory collapse and a rapidly fatal fulminating peritonitis will be observed. Affected foals are usually under six months of age and clinical signs have been recorded in a one-day-old foal (Becht and Byars 1986). Affected foals frequently have had a recent episode of diarrhoea.

It is important to note that many normal foals and adult horses have gastric ulceration without exhibiting any clinical signs (Hammond, Mason and Watkins 1986) and a recent video endoscopic survey of foals in Ireland and England revealed a 51 per cent prevalence of such asymptomatic ulcers (Murray *et al* 1990).

PATHOLOGY

The equine stomach, like that of other herbivores, has a clearly demarcated area of non-glandular, stratified squamous epithelium, and in the adult horse, it covers about one third of the total gastric surface area. Over half of the perforating ulcers (53 per cent) and most asymptomatic ulcers (81 per cent) occur in this non-glandular area especially in the region of the margo plicatus, which is the junction between the glandular and non-glandular mucosa (Wilson 1985; Dwyer and Powell 1989). Ulcers may be singular or multiple, craterform or irregular in shape and usually have obvious, slightly raised edges surrounded by a zone of brown staining keratin (Fig 1). Ulcers vary in size from small circumscribed lesions less than 1 cm in diameter to lesions that affect the majority of the squamous mucosa with 'islands' of normal or thickened squamous mucosa (Fig 2). The glandular mucosa, like that of man and the dog, contains chief, parietal and mucus secreting cells and although ulceration is recorded with less frequency in this area (perforating 27 per cent; non-perforating 32 per cent), some authors believe that such ulcers are rarely asymptomatic and are usually clinically significant (Murray 1990). Glandular ulcers usually are solitary, oval shaped and the mucosal excavation is surrounded by an intense zone of inflammation. Occasionally, an hour glass shaped stenosis of the proximal duodenum associated with fibrosis and granulation tissue is observed at post mortem examination (Fig 3), and it is believed that this represents a healed or healing duodenal ulcer. Twenty per cent of all ulcers are found in the proximal duodenum and an equal proportion of perforating ulcers occur in this area (Wilson 1985).

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Diagnosis of gastroduodenal ulceration is largely based on clinical signs, but definitive diagnosis is problematical. The use of a 2 metre flexible (video) endoscope permits direct examination of most of the stomach (Murray *et al* 1990). However, even after 18 h of fasting, over one third of the mucosa is obscured by ingesta in foals over two

DOD supplementation

If the DOD has arisen due to mineral deficiencies or imbalances, conventional inorganic mineral supplements may be inadequate and should be combined with organic mineral sources such as amino acid or other chelates and the gluconates.

The organic supplements are expensive compared to inorganic salts, although this must be considered in the light of the value of the animal.

CONCLUSION

Developmental orthopaedic disease probably has a multifactorial aetiology, but nutrition and feeding practices can be employed to reduce the likelihood that mineral imbalances or overfeeding are primary causes. Individual horses will respond differently to the same feeding regime; for example, some horses appear to use calcium from limestone less efficiently than others. Table 4 provides a checklist, compiled by Pagan (1989), for use in the field and in discussions with stud managers. This summarises some of the factors which may contribute to DOD.

Several research groups are now studying methods of assessing bone mineralisation, development and maturation. It is vital that this work is continued, together with further studies on the nutrient requirements of young, growing horses. This will allow veterinary surgeons and nutritionists to devise more accurate feeding programmes, with better assessment and monitoring techniques, to help reduce the incidence and severity of DOD and to improve the soundness and durability of competition and racehorses.

TABLE 4: Checklist of factors which may contribute to developmental orthopaedic disease in young horses (Pagan 1989)

Factor	Evaluation	Treatment	Corrected
Mineral deficiency	Ration analysis: Hay Pasture Grain (or other concentrate)	Balance ration	–
Mineral excess	Ration analysis: Hay Pasture Grain Supplements	Balance ration	–
Overfeeding			
Daily energy intake	Monitor growth rate (weight)	Regulate grain intake	–
Energy intake per meal	Measure amount fed per meal	Increase feeding frequency	–
Environment	Hardness of ground	Soften ground	–
	Amount of exercise	Regulate exercise	–
Genetics	Observe soundness and performance offspring	Introduce new bloodlines	–

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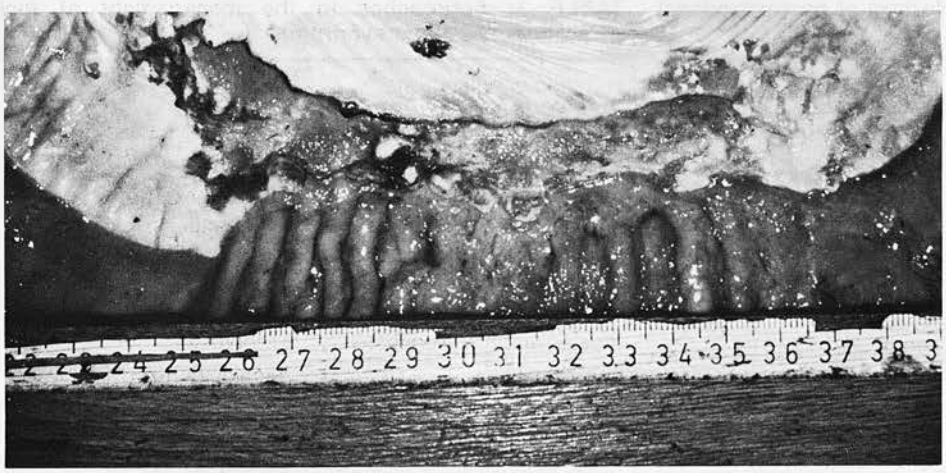

Fig 1: Asymptomatic ulceration in the squamous mucosa of a five-month-old foal that was destroyed because of a fracture involving the third metacarpal bone

months of age on a hay diet (Wilson 1989). Also, because of the acute angle between the cardia and pylorus, it is extremely difficult to introduce the endoscope into the duodenum (Brown, Slocombe and Derksen 1985).

Serum pepsinogen activities or concentrations have been investigated in foals with ulceration and, although many foals had significantly higher serum activities or concentrations compared with normal foals, considerable overlap existed precluding the use of this test for definitive diagnosis (Wilson and Pearson 1985).

The presence of blood in gastric reflux or a positive faecal occult blood test occurs occasionally with foal ulceration, but should be interpreted cautiously because it is an inconsistent finding and gastrointestinal bleeding occurs in more than 30 other equine diseases (Pearson, Smith and McKim 1987).

Radiographic techniques for upper gastrointestinal tract examination in the foal were described by Campbell, Ackerman and Peyton (1984). Mucosal lesions, delayed gastric emptying and duodenal obstruction can be determined using barium contrast studies, but these require considerable radiographical expertise and accurate interpretation.

Although the clinical signs of ulceration are almost pathognomonic, a number of uncommon conditions should be considered in the differential diagnosis and include hypoplasia of the stomach (Pearson and Murfitt 1988), choke (Freeman 1982), megaoesophagus (Freeman 1982), pyloric stenosis (Church, Baker and May 1986), gastric dilatation (Robertson 1982) and gastric stenosis (Robertson 1982).

AETIOLOGY AND PATHOGENESIS

Many causes of equine gastric ulceration have been proposed, but few have been proven except for non-steroidal anti-inflammatory drugs (NSAIDs) (Traub *et al* 1983). The occasional clustering of cases on certain studs has prompted the suggestion of an infectious cause (Becht and Byars 1986); however, investigations have failed to demonstrate the presence of pathogens (Acland, Gunson and Gillette 1983; Collobert-Laugier *et al* 1989). Weather related stress has been indicated as a possible cause of gastric ulceration by Dwyer and Powell (1989), but

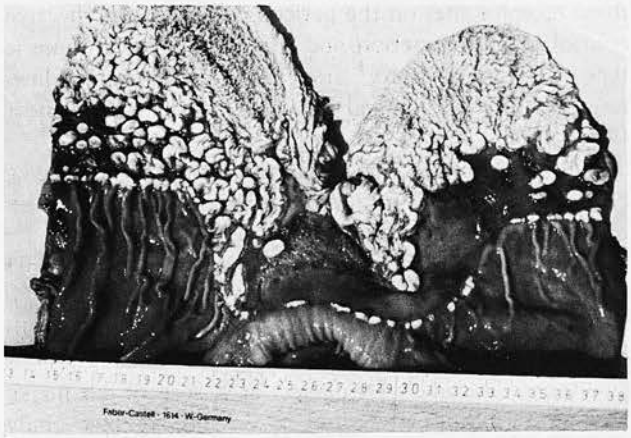

Fig 2: Extensive ulceration of the squamous mucosa of a three-month-old foal showing typical signs of gastroduodenal ulceration

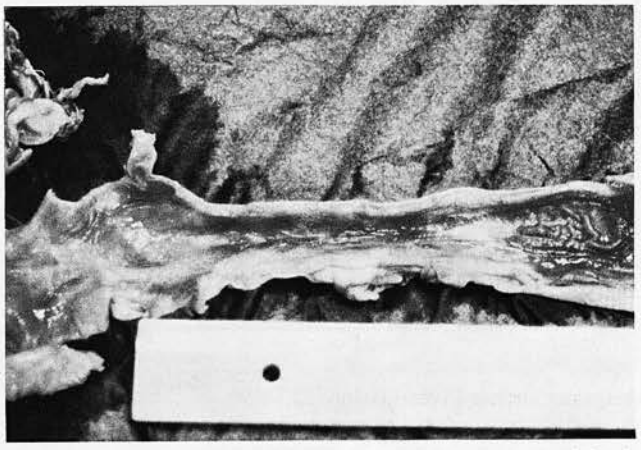

Fig 3: Stenosis of the proximal duodenum in a three-month-old foal that died from gastric perforation

TABLE 1: Proposed causative factors of gastroduodenal ulceration

	Factors	References
Drugs:	Phenylbutazone	Traub *et al* 1983
	Flunixin meglumine	Traub-Dagartz 1988
Stress:	Transport	Acland *et al* 1983
	Temperament	Valdez 1979
	Weather	Dwyer *et al* 1989
	Parturition	Wilson 1985
	Weaning	Wilson 1985
	Low plasma antibody	Wilson 1985
	Orthopaedic surgery	Rehbun, Dill and Power 1982
	Exsanguination	Traub-Dagartz 1985
Infections:	Rotavirus	Becht, Hendricks and Merritt 1983
	Campylobacter sp.	Atherton and Ricketts 1980
	Helicobacter pylori	Nappert *et al* 1989
	Escherichia coli	Becht *et al* 1986
	Clostridium perfringens	Acland *et al* 1983
	Clostridium botulinum	Swerczek 1980
	Candida sp	Gross and Mayhew 1983
	Salmonella	Nappert *et al* 1989
Diet:	High corn	Hammond *et al* 1986
	Low fibre	Hammond *et al* 1986
	High fibre	O'Reilly 1973
	Depraved appetite	O'Reilly 1973
	Alfalfa	Swerczek 1980
Parasites:	Migrating parasites	Orr 1972
	Gasterophilus larvae	Waddell 1972
	Habronema megastoma	Robertson 1982
Other:	Foreign body	Rooney 1964
	Corticosteroid (endogenous)	Swerczek 1980
	Corticosteroid (exogenous)	Swerczek 1980
	Sex hormones	Wilson 1985
	Delayed gastric emptying	Orsini *et al* 1986
	Congenital	Wagner *et al* 1979
	Corrosive substances	Barth, Barber and McKenzie 1980
	Exercise	Murray *et al* 1989

TABLE 2: Factors influencing the integrity of the mucosal barrier

Aggressive factors	Protective factors
Acid	Prostaglandins
Pepsin	Epithelial cell turnover
Bile	Intracellular junctions
Pancreatic enzymes	Alkali secretions
Mucosal ischaemia	Mucosal circulation
	Mucus

requires further investigation.

Irrespective of the specific cause, ulceration is likely to develop because of disruption of the gastric mucosal barrier that protects the gastric mucosa from the potentially harmful effects of gastric acid and pepsin. A delicate balance exists between mucosal protective factors and the aggressive effects of acid, pepsin and bile salts (Table 2). Disruption of this delicate interplay allows diffusion into the mucosa of hydrogen ions and pepsin resulting in auto digestion and ulcer formation (Crampton and Rees 1986).

TABLE 3: Approaches to the management of foal gastroduodenal ulcer syndrome

H_2 receptor antagonists:	Rantidine Cimetidine Famotidine Nitazidine
Proton pump inhibitor:	Omeprazole
Cytoprotectants:	Sucralphate Carbenoxolone Tri-potassium di-citrato bismuthate
Prostaglandin analogues:	Misoprostol
Antacids:	Aluminium hydroxide Magnesium hydroxide
Surgery:	Gastroduodenostomy Gastrojejunostomy Dudenojejunostomy Jejunojejunostomy
Supportive therapy:	Fluid therapy Analgesics Probiotics

TREATMENT

The aim of ulcer treatment in the foal, as in man, is to hasten ulcer healing by reducing gastric acidity and protecting the ulcer from further insult by pepsin or gastric acid. Treatment options are summarised in Table 3. The stimulus for effective secretion of hydrochloric acid by the parietal cells in the gastric mucosa requires three separate chemical mediators (secretagogues), ie gastrin, histamine and acetylcholine (Argenzio 1984). The blocking of any of these receptor sites on the parietal cell results in effective control of acid secretion and this is how the histamine type 2 (H_2) receptor antagonist drugs (cimetidine, ranitidine, fomotidine and nizatidine) mediate their effect (Lewis 1983).

The intravenous (iv) administration of 0.5 mg/kg bodyweight (bwt) of ranitidine to young horses (aged six to 12 months) caused a significant decrease in total hourly acid output but, surprisingly, did not change the pH significantly (Campbell-Thompson and Merritt 1987). Other workers demonstrated an increase in pH from 2.2 to over 3.6 for 8 h following the oral administration of cimetidine (Tagamet; Dyspamet) at a dose of 8.8 mg/kg bwt or ranitidine at a dose of 2.2 mg kg bwt orally (MacAllister, Sangiah and Amouzadeh 1987). However, twice daily administration of 4.4 mg/kg bwt ranitidine did

not prevent the development of phenylbutazone induced ulcers (Smith *et al* 1987), and clinical experience indicated that a dose rate of 6.6 mg/kg bwt of ranitidine three times daily was required for ulcer healing in adult horses (Furr and Murray 1989).

Active secretion of hydrochloric acid is accomplished by the proton pump (acid pump) located in the apical secretory membrane of the parietal cell and actively transports hydrogen ions into the gastric lumen in exchange for potassium ions (Carlsson and Wallmark 1986). Because the hydrogen ion concentration in the gastric mucosa is about three to four million times greater than that of plasma, a large amount of energy (circa 1,500 cal/litre of gastric juice) is required to transport the hydrogen ion into the lumen (Argenzio 1984). Hydrogen, potassium adenosine triphosphatase (H+, K+ ATPase), is the enzyme responsible for hydrolysing adenosine triphosphate (ATP) to provide energy for the proton pump and the development of a proton pump inhibitor, omeprazole, which binds directly to the H+, K+ ATPase enzyme, offers a new and precise approach to the control of gastric acid secretion (Carlsson and Wallmark 1986). Omeprazole, a substituted benzimidazole, has proved to be a powerful inhibitor of gastric acid secretion in man and animals (dog, rat, mouse and guinea pig). Higher ulcer healing rates, more effective pain relief and lower recurrence rates were recorded when omeprazole was compared with renitidine and cimetidine in the treatment of gastric and duodenal ulcer in man (Anon 1988). Omeprazole targets precisely the proton pump of the parietal cell (pH 1) and is biologically inactive at the physiological pH (7.1) prevailing in other organs. Consequently, adverse reactions are believed to be uncommon and occurred with similar frequency to that recorded with placebo or ranitidine treatment (Anon 1988). An increase in basal gastric pH to 3.5 for 2 h and a 64 to 90 per cent reduction in the basal free acid content for 7 h was recorded in a small trial in horses (n=5) following iv omeprezole administration at 0.5 mg/kg bwt (Sangiah, MacAllister and Amouzadeh 1989). However, oral administration of omeprazole in man is more effective (Sangiah *et al* 1989) and more research in the horse is required.

Antacids such as magnesium hydroxide and aluminium hydroxide (Aludrox; Maalox) neutralise gastric acid when administered orally, but require frequent administration. Also, they have the disadvantage of causing diarrhoea and constipation as side effects (Nappert, Vrins and Larybyere 1989) and add little to the therapy for equine gastric ulceration.

Synthetic analogues of the prostaglandin E group (PGE) have cytoprotective properties mediated by an increased viscosity of gastric mucus, increased mucosal blood flow, increased bicarbonate secretion and reduced stimulated gastric acid secretion (Misiewicz 1988) and have been indicated in the therapy of equine gastric ulceration (Becht and Byars 1986). However, the results of clinical trials in man using five different PGE derivatives (arbaprostil, trimoprostil, misoprostol, endoprostil and rioprostil) revealed a similar effectiveness to ranitidine but poorer pain relief and diarrhoea was a side effect (Misiewicz 1988). In the horse, the administration of prostaglandin E_2 reduced the development of phenylbutazone induced gastric ulcers (Collins and Tyler 1985) and the administration of misoprostol to healthy adult horses resulted in an increase of pH to over 3.5 for a period of 2 h and a concomitant 65 to 99 per cent reduction in basal free acid content for a period of 8 h (Sangiah *et al* 1989).

Sucralphate (Antepsin, Ayerst Laboratories Ltd, Dublin, Ireland) is a complex of aluminium hydroxide and a sulphated sucrose and when exposed to acid forms a viscous adherent chemical complex that binds to the site of ulceration, protecting it from gastric insult (Lewis 1983). Its cytoprotective effects are derived from its ability to inhibit the action of pepsin, deplete bile salts, increase luminal release of cytoprotective prostaglandin E_2 and neutralise small amounts of acid (Lewis 1983). It is well tolerated in the foal, and 2 to 4 g three or four times daily have been recommended (Becht and Byars 1986). In man, an efficacy equal to that of cimetidine has been demonstrated when sucralphate alone is used in the treatment of peptic ulcers (Lewis 1983). Other cytoprotectants used in man include carbenoxolone (Biogastrone, Winthrop, Dublin, Ireland) and tri-potassium di-citrato bismuthate (De-Nol, Gist Brocades, Surrey, England), which also has a bactericidal-like action against *Helicobacter pylori*.

If duodenal stenosis is diagnosed, surgical intervention may be required but only limited success has been recorded following gastroduodenostomy, duodenojejunostomy, jejunojejunostomy and gastrojejunostomy (Orsini and Donawick 1986; Campbell-Thompson *et al* 1986).

Supportive treatment, including fluid therapy and analgesics should be used as required. Total ileus lasting for 30 to 45 mins has been reported in normal horses following a single recommended dosage of xylazine (Owen 1983). Its use in foals with gastroduodenal ulceration is contraindicated because it may aggravate primary or even precipitate a secondary gastric dilatation. A decrease in the normal population of lactobacilli has been noted in association with ulceration of the squamous mucosa in pigs (Embaye 1987) and the administration of probiotics to foals with gastric ulceration should be considered.

CONCLUSIONS

A direct relationship between the presence of ulceration and the occurrence of clinical signs has not yet been demonstrated and, in over 50 per cent of foals dying from

ulcer perforation, no premonitory illness was observed (Dwyer and Powell 1989). Further, foals with experimentally induced gastric ulceration rarely exhibit 'typical' signs of gastric ulceration (Smith *et al* 1987). Diagnosis may be confused further by the fact that 51 per cent of clinically normal foals have endoscopic evidence of gastric ulceration (Murray *et al* 1990). Also, teeth grinding and profuse salivation have been recorded in other gastrointestinal conditions (Church *et al* 1986).

If gastroduodenal ulceration in the horse parallels that in man and the pig, it is likely that gastric squamous, gastric glandular and duodenal ulcerations should be considered heterologous conditions with different causes, clinical signs and significances (Rothenbacher, Nelson and Ellis 1963; Lee 1985).

Many questions regarding the cause, pathogenesis and significance of the foal ulcer syndrome remain unanswered and it is possible that factors beside ulceration, such as gastric distension, may be important in the clinical syndrome. Recently, gastric ulceration has been incriminated in ill thrift, poor appetite and colic in adult horses (Murray *et al* 1989).

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (1991) **3** (2) 86-93

TUTORIAL ARTICLE

Osteochondritis dissecans (OCD) of the femoropatellar joint

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DEFINITIONS

The most popular definition of osteochondrosis is one of defective endochondral ossification of immature cartilage (Olson 1978, 1982; Stromberg 1979; McIlwraith 1982; Glade *et al* 1983; Fessler 1984; Fischer and Barclay 1984). Osteochondritis dissecans occurs as proliferation of germinal cells near the articular surface continues, despite the defective ossification process, thus producing thickened articular cartilage in the affected areas. The deepest layers subsequently undergo degenerative change due to failure of capillary invasion from the subchondral bone; and fissures develop from the inability of the necrotic cartilage to withstand physiological joint pressures.

It should, however, be appreciated that this pathophysiological pathway differs radically from the proposals of other authors who favour primary involvement of subchondral bone with secondary cartilaginous pathology.

Because the primary lesion is not considered to be inflammatory, debate continues regarding use of the term 'osteochondritis'. However, once the affected joint exhibits signs of inflammation this terminology is justified.

ANATOMY

The femoropatellar joint encompasses the articulation between the asymmetrical trochlear ridges of the femur and the smaller quadrilateral articular surface of the patella with its medial fibrocartilaginous extension. The smoothly curved trochlear ridges are of similar dimensions in their distal thirds but the medial ridge becomes progressively wider and more prominent proximally. It also extends further proximally than the lateral ridge. Hyaline cartilage completely covers the medial trochlear ridge but extends only a short distance over the abaxial surface of the lateral ridge.

The joint capsule is thin. It is attached close to the articular margins of the patella but extends to the base of the trochlear ridges medially and laterally. Proximally a large suprapatellar pouch extends beneath the insertion of quadriceps femoris, and distally it contacts the femorotibial joint capsules. A slit-like opening at the base of the medial trochlear ridge usually indicates communication with the medial femorotibial joint.

SITES

The femoropatellar and tarsocrural joints are the principal predilection sites for osteochdritis dissecans in the horse (Stromberg 1979; Lindsell, Hillbert and McGill 1983; McIlwraith 1987). In the Thoroughbred, the former appears most common whereas, in Standardbreds and Warmblood breeds, the reverse is true.

Within the femoropatellar joint the lateral trochlear ridge of the femur is affected most frequently (Stromberg 1976, 1979; Rejno and Stromberg 1978; Trotter and McIlwraith 1981; Steenhaut, Verschooten and DeMoor 1982; Lindsell *et al* 1983; Fischer and Barclay 1984; McIlwraith and Martin 1984; McIlwraith 1985, 1987, 1990). Lesions may be localised (Fig 1) or widespread (Fig 2). Pathology is less commonly encountered on the medial trochlear ridge and is usually more focal. Osteochondritis dissecans of the articular surface of the patella is most frequent at the apex, but lesions may be seen elsewhere together with impingement pathology secondary to osteochondritis dissecans of the trochlear ridges of the femur.

Femoropatellar osteochondritis dissecans is frequently bilateral (Stromberg 1979; Lindsell *et al* 1983; McIlwraith and Martin 1984) and multiple sites may be encountered within a single joint.

INCIDENCE

An apparent increase in incidence of osteochondrosis has been recorded in the USA by Trotter and McIlwraith (1981). Stromberg (1979) agrees with the increased incidence in most European countries but does not believe osteochondritis dissecans to be a major problem in the UK and Ireland. Data from cases referred to the Animal Health Trust indicate that this is now a common problem in Great

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A survey by mail questionnaire on the incidence of the foal gastroduodenal ulcer syndrome in Ireland and Britain

HARRY J SWEENEY

A mail questionnaire survey relating to the foal gastroduodenal ulceration syndrome was distributed to 55 veterinarians engaged in equine practice in Ireland and Britain. Twenty four (42 *per cent*) usable replies were received; respondents had an estimated cumulative responsibility for 6,650 foals *per annum*. At the time of the survey 10 (42 *per cent*) of the 24 respondents were not aware of the described clinical syndrome in foals associated with gastric and duodenal ulceration. The survey confirmed that gastric and duodenal ulceration occurs in foals in Ireland and Britain but the incidence was low (0.58 *per cent*). Clinical cases were sporadic. Fifty two *per cent* of respondents recalled seeing foals with clinical signs compatible with the syndrome for a mean of 10 years (range two to 17 years) prior to 1982, when the syndrome was first described.

Key words: Horse, Foal, Gastroduodenal Ulceration, Questionnaire.

Introduction

In 1982, a new clinical syndrome in foals under six months of age associated with gastric and duodenal ulceration was first described in the United States of America (Rebhun, Dill and Power, 1982). Typical presenting signs include depression, mild intermittent abdominal pain, teeth grinding, excessive salivation and a preference to lie in dorsal recumbency for prolonged periods (Becht, Hendricks and Merritt, 1983; Jones, 1983; Lee Gross and Mayhew, 1983). If ulcer perforation occurs, signs of fulminating peritonitis such as acute abdominal pain, endotoxic shock, peripheral vascular collapse and death will be exhibited. The foal gastroduodenal syndrome has also been described in Japan (Yoshihara, Kaneko, Oikawa, Kanemaru, Hasegawa and Tomioka, 1986), France (Collobert-Laugier, Vaissaire, Jacquet, Dauguet and Plateau, 1989), and Canada (Barth, Barber and McKenzie, 1980). The purpose of this study was to investigate by mail questionnaire the occurrence and incidence of this syndrome in Ireland and Britain.

Materials and methods

In 1986, a two-page 10-question questionnaire was distributed by mail to 35 veterinarians in Ireland and 20 veterinarians in Britain, all of whom worked in equine practices or as resident stud veterinarians. A brief description of the clinical signs, epidemiology and gross pathology was included together with a short covering letter explaining the aims of the survey. The questions and format of the questionnaire are outlined in Figure 1.

Results and discussion

Twenty-eight (51 *per cent*) of the 55 questionnaires were returned but four replies were discarded because of incomplete, contradictory or confusing replies (Table 1). The 24 respondents in the survey included the majority of 'key' equine stud practices and had an estimated cumulative responsibility for 6,650 foals *per annum*. The response rate for 51 *per cent* (42 *per cent* usable replies) falls within the range often recorded for veterinary mailed surveys (40 to 50 *per cent*; Martin, Meek and Willberg, 1987a). The large sample fraction, 6,650 foals out of 9,728 Thoroughbred foals born in 1986 (Anon, 1986) would be expected to yield a result with a 95 *per cent* level of confidence with an absolute precision of one *per cent* (99 *per cent* that the estimated prevalence level is within one *per cent* of the true prevalence).

At the time of the survey 10 (42 *per cent*) of respondents were not aware of the existence of the described clinical syndrome in foals associated with gastric or duodenal ulceration. Nevertheless, when the questionnaire requested information on the number of foals with major clinical signs of teeth grinding, salivation and depression, which are considered almost pathonomonic for the syndrome, 52 *per cent* of respondents stated that they had seen foals exhibiting clinical signs compatible with gastric or duodenal ulceration for a mean of 10 years (range two to 17 years) prior to 1982, when the syndrome was first described. Fatal cases of perforating gastric ulceration have also been described in Scotland (Orr, 1972), Ireland (O'Reilly, 1973) and in the United States of America (Rooney, 1964; Valdez, 1979) prior to 1982.

The results of this survey confirm for the first time the occurrence in Ireland and Britain of a clinical syndrome in foals which may be associated with gastric or duodenal ulceration. The numbers of Thoroughbred foals under seven months of age that had shown depression, colic, frequent teeth grinding or obvious salivation are outlined in Table 2. A total of 117 clinical cases of gastroduodenal ulceration syndrome was reported in the survey population in the years 1982 to 1986, inclusive, but in only 35 (29 *per cent*) of these cases was a definitive diagnosis made on the basis of endoscopic, radiographic or necropsy

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TABLE 1
Questionnaire sample frame, response rate and sample size

	Ireland	Britain	Total
Questionnaires distributed	35	20	55
Total replies	14 (40%)	14 (70%)	28 (51%)
Usable replies	11 (32%)	13 (65%)	24 (44%)
Foal sample size	3300	3350	6650
Respondents not aware of Foal Ulceration Syndrome	6 (55%)	4 (31%)	10 (42%)

TABLE 2
Numbers of foals showing signs of depression, colic, frequent teeth grinding or obvious salivation

Year	1982	1983	1984	1985	1986
Ireland	3	4	4	4	20
Britain	11	12	18	23	18
Total	14	16	22	27	38

TABLE 3
Numbers of foals recorded with mild inappetance and occasional grinding of teeth

Year	1982	1983	1984	1985	1986
Ireland	3	3	7	18	27
Britain	10	3	37	25	14
Total	13	6	44	43	41

examinations. There was an apparent increase in the incidence of this syndrome between 1982 when 14 cases were reported and 1986 when 38 cases were reported. This may indeed be a true increase but it may be affected by such factors as increased awareness, and the memory or the quality of records of respondents (Martin, Meek and Willerberg, 1987b). Only 25 *per cent* of the respondents believed that the incidence of the foal ulcer syndrome was increasing. A large proportion of respondents (43.8 *per cent*) considered that the incidence was static while 15.6 *per cent* of participants believed that the gastroduodenal ulcer syndrome did not exist at all and another 15.6 *per cent* felt unable to respond to the question ("don't know").

The incidence of foals showing obvious salivation and frequent teeth grinding in association with colic and depression was very low (0.58 *per cent*) and, assuming an associated mortality rate of probably less than 25 *per cent* (Becht *et al.*, 1983), it was estimated that only 0.15 *per cent* of the total foal population died from this condition.

Several respondents reported that many foals with infectious or foal heat diarrhoea grind their teeth occasionally. They believe that this is associated with diarrhoea and not with gastric ulceration. Therefore, foals showing only mild inappetance and occasional grinding of teeth (question 4a) have not been included in computing the incidence of foal ulcer syndrome. Furthermore, as the majority of cases reported in this study were diagnosed on clinical signs alone, the use of clearly defined strict clinical criteria is essential.

The numbers of recorded cases of mild inappetance and occasional grinding of the teeth in foals for the years 1982 to 1986, inclusive, are outlined in Table 3. A total of 147 such cases was recorded by the respondents during the five years under survey but in only two of these foals was the presence of gastric or duodenal ulceration confirmed by either endoscopic, radiographic or necropsy examinations. There was an increase from 13 cases in 1982 to 41 cases in 1986. The highest total number of foals affected with either the severe or mild manifestations of the gastroduodenal ulcer syndrome occurred in 1986, the last year of the survey: 79 foals were affected but this only represented 1.2 *per cent* of the population under survey.

There was an overwhelming belief among respondents that the syndrome was sporadic without clustering of cases and only one practice reported the occurrence of two cases on the same premises. Previous reports have indicated the sporadic nature of this condition (Becht *et al.*, 1983).

Clinical cases of the foal ulcer syndrome occurred in every month between March to October, inclusive, but the vast majority occurred between April and July, inclusive. This is probably due to the popularity of early spring foaling in Thoroughbred studs rather than a true seasonal factor in the occurrence of disease.

Seventeen practices reported the use of histamine-type 2 (H_2) receptor antagonists (ranitidine/cimetidine) in the therapy of suspected clinical cases of foal ulceration. During the years of the survey 110 foals were treated in this manner. There was an increase in the use of this therapy from zero in 1982 to 37 cases in 1986 (1983:19; 1984:20; 1985:34). Of the respondents with clinical experience of H_2-receptor antagonist therapy in the foal ulcer syndrome, nine (53 *per cent*) considered clinical cases to be 100 *per cent* responsive; three believed that the therapy was effective in between 50 and 100 *per cent* of cases; one found it beneficial in 30 *per cent* of affected foals and one practice recorded no success with its use. Three practices were unable to evaluate the therapy. The treatment of foal gastric ulceration has recently been reviewed (Sweeney, 1991).

A 1.3 *per cent* incidence of fatal perforating gastric ulcers was recorded in a survey of *post-mortem* examinations on 600 foals in the United States of America (Rooney, 1964). However, a similar retrospective analysis of necropsies between 1980 and 1984 revealed a significantly higher prevalence (7.8 *per cent*) of perforating ulcers (Wilson, 1986). The prevalence of gastroduodenal ulcer-associated deaths in foals in the Kentucky area in 1982 and 1983 was reported by Becht *et al.* (1983) to be 0.74 *per cent* and 0.47 *per cent*, respectively, in an extensive survey involving 71 stud farms and a total of 8,419 foals. The total foal mortality in the above farms in 1982 and 1983 was 2.47 *per cent* and 2.14 *per cent*, respectively, and of these gastroduodenal ulceration was responsible for 29.9 *per cent* and 21.9 *per cent* of deaths, respectively (Becht *et al.*, 1983). However, subsequent surveys in the Lexington areas based on *post-mortem* examinations of foals revealed a decreasing incidence of perforated gastric/duodenal ulcers from 6 *per cent* of all foal mortalities in 1984 to 3 *per cent* in 1988; this decline was attributed to the use

of prophylactic anti-ulcer medication in addition to greater awareness of the condition with consequent earlier diagnosis and more prompt therapy (Dwyer and Powell, 1988, 1989). An additional 1.5 *per cent* of foal mortalities was attributed to non-perforating gastric ulcers but the criteria for such a diagnosis were not described.

The not infrequent incidental *post-mortem* finding of gastric ulceration in foals dying from another obvious cause has been recognized for some time (Rooney, 1964; Jubb, Kennedy and Palmer, 1985). An endoscopic survey of foals in Ireland and Britain revealed a 51 *per cent* prevalence of asymptomatic ulcers (Murray, Murray, Sweeney, Weld, Wingfield-Digby and Stoneham, 1990).

The two main criteria for the success of a questionnaire are reliability and validity (Thrusfield, 1986). A questionnaire, like a diagnostic test, is reliable if it produces consistent results. As this survey spanned five years, the reliability depends on accurate record keeping by respondents. The author interviewed some of the respondents after they had returned the questionnaire and consistent answers were received, confirming the reliability of the survey. Simpson and Wright (1980) demonstrated a high degree of repeatability when comparing veterinary postal and interview questionnaires. Validity is a measure of the degree to which answers, on average, reflect the truth (Thrusfield, 1986). The validity of this survey is supported by the fact that the pathology units at the Irish Equine Center and the Animal Health Trust only very occasionally record a foal death attributed to gastric ulceration or perforation thus confirming, at least, a very low mortality from this syndrome.

Conclusions

This survey confirms for the first time the occurrence of a clinical syndrome in foals in Ireland and Britain attributed to gastric ulceration. The incidence of the syndrome is low: in any of the years 1982 to 1986 inclusive, less than 0.6 *per cent* of the population of foals were affected. Further definition of the clinical syndrome and a convenient diagnostic test would be helpful.

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FIGURE 1.

STRICTLY CONFIDENTIAL

FOAL ULCER SYNDROME

PLEASE CIRCLE CORRECT ANSWER

1. Name of Veterinary Surgeon _____ _____

   Practice Address _____ _____ _____

2 (a) The foal ulcer syndrome was first described in 1982 and the few publications on this subject have all been in American journals. To date, little attention has been paid to this condition in Europe. Were you previously aware of this condition.
Yes/No

(b) If yes, for how long?

_____

3 (a) How many foals showing depression and at least two of the following clinical signs have you seen in the last five years? Obvious salivation, frequent grinding of teeth, colic. Please indicate for each year.
1982 _____1983_____1984_____1985_____1986_____

(b) In how many of these foals, if any, was a definitive diagnosis of ulceration made using endoscopy, contrast radiography studies or post-mortem examination?

_____

4 (a) How many foals showing inappetance and occasional grinding of teeth (possibly in association with diarrhoea) have you seen in the last five years? Please indicate for each year.

1982 _____1983_____ 1984_____1985_____1986_____

(b) In how many of these foals, if any, was a definitive diagnosis of ulceration made using endoscopy, contrast radiography studies or post-mortem examination?

_____

5 (a) Have you seen cases prior to 1982 which would fit the description in question 3 and 4 above?
Yes/No

(b) If yes, for how long?

_____

6. Do you consider the incidence of this condition may be

   i) increasing
   ii) decreasing
   iii) static
   iv) non-existent (Please tick)

7. Have you observed a higher incidence of the foal ulcer syndrome on certain studs compared to others?

   Yes/No

8. During which month of the year do you most commonly observe this condition?

_____

9 (a) How many foals in the last number of years have you treated with H_2 receptor antagonists (Tagamet/Zantac) or other anti-ulcer medicants? Please indicate for each year.

1982 ____1983 ____1984 ____1985____1986 ____

(b) In the five years combined what percentage of these foals appeared to respond to treatment?

%

10. Approximately how many foals are primarily under your care during an average stud season.

_____

11. Comments.

_____

_____

_____

_____

_____

Thank you for your co-operation.

Gastric ulceration syndrome in foals

Harry Sweeney

Harry Sweeney graduated from Dublin in 1983. He worked initially as a large animal houseman at the Royal (Dick) School of Veterinary Studies for one year and then for three years in the large animal practice at the 'Dick'. After a period in equine practice in Australia he spent two years in equine practice in the Curragh. For the past three years he has been managing a thoroughbred stud and training centre in Japan. Equine gastric ulceration is the topic of his DVM&S thesis which he has carried out as a part-time project.

THE occurrence of non-perforating gastric ulcers in horses of all ages has been recognised for some time. They were previously considered to be insignificant incidental post mortem findings. However, within the past decade a clinical syndrome attributed to gastric ulceration has been described in horses, particularly foals, and has been reported in the USA, Canada, Australia, France, Ireland, Britain and Japan. Within the past decade progress has been made in the understanding of equine gastric physiology and the treatment of gastric ulceration. However, there is still little known about the aetiology and pathogenesis of this condition and the definitive diagnosis of clinical gastric ulceration in horses remains problematical.

Incidence

The incidence of clinical gastric ulceration in foals in Ireland and Britain is low: a postal survey of equine practitioners with a total cumulative responsibility for 6650 foals annually revealed a 0·58 per cent incidence, the majority of which were diagnosed on clinical grounds alone (Sweeney, 1992). In the USA where the condition was first described there have been a number of attempts to quantify the problem:

- A Kentucky farm survey in the years 1982 and 1983 involving approximately 4000 foals yearly revealed that ulcer-associated deaths accounted for 30 per cent and 22 per cent, respectively, of all foal deaths.
- A post mortem survey (also Kentucky-based) spanning the years 1984-88 revealed a steady decrease in the rate of perforated gastric ulcers over the survey years (from 30 per cent in 1982 to 5 per cent in 1988). In the final year of the survey 3 per cent of all foal deaths were due to gastric perforation; an additional 1·5 per cent of foals were diagnosed as dying from non-perforating ulcers.

The justification for a diagnosis of non-perforating ulcers as a cause of death in the latter survey has not been published and may be difficult to substantiate. The large decrease in the incidence of gastric ulcer related deaths over the survey period is remarkable and may be due to increased awareness of the condition, earlier diagnosis, more effective treatment and the prophylactic treatment of foals considered at risk.

Clinical signs

Major clinical signs for gastric ulceration are listed in the box overleaf. The clinical syndrome occurs equally in colt and filly foals and is more common in foals under three months of age, although foals up to six months of age have been affected.

Teeth grinding (bruxism)

Salivation (ptyalism)

Mild intermittent colic

Moderate depression

Preference to lie in dorsal recumbency with legs flexed in the air for prolonged periods

Attempted retching in severe cases

Signs are often intermittent and can be exacerbated by nursing. The passage of a nasogastric tube may lead to gastric reflux and the subsequent alleviation of clinical signs. Pain when pressure is applied over the xiphoid process is uncommon. Affected foals usually continue to suck.

If ulcer perforation leads to a fulminating peritonitis signs of peripheral vasculature collapse (rapid weak heart beat, increased capillary refill time, injected mucous membranes and cold extremities), severe depression and rapid death occur.

The majority of clinical reports to date have involved thoroughbred foals but this is unlikely to reflect a true breed susceptibility.

Non-clinical ulceration

Many normal healthy foals if examined endoscopically are found to have gastric ulceration. A video endoscopic survey in Ireland and Britain involving 75 clinically normal foals revealed gastric ulceration in 38 (56 per cent). A number of surveys in the USA have revealed a similar prevalence of gastric ulcerations in normal foals. Such foals do not exhibit clinical signs compatible with gastric ulceration and, as yet, there have been no reports of any of them subsequently developing the ulcer syndrome.

Whether the presence of ulcers in clinically normal foals could be responsible for subclinical disease, manifesting in decreased growth rate, for instance, is entirely open to conjecture.

Diagnosis

The presence of gastric ulceration can only be confirmed by examination of the stomach with a flexible 2 m (video) endoscope. Because most endoscopes currently in use in equine practices have a functional length of less than 120 cm, the majority of cases of gastric ulceration are diagnosed on clinical grounds alone. The use of a long flexible endoscope has the additional disadvantage that, even after a foal has been fasted for 18 hours, one-third of the mucosa is still obscured by gastric contents.

In cases where duodenal stenosis is suspected, radiographic barium contrast studies may confirm delayed gastric emptying. If, in the normal foal, the administration of 5 mg/kg of barium sulphate after 12 hours fasting does not result in passage of barium into the small intestine after 60 minutes then gastric outflow obstruction is probable.

Serum pepsinogen activities, gastrin assays and fecal occult blood tests are not helpful in the diagnosis of this condition.

Differential diagnosis

Although the clinical signs of teeth grinding, salivation and abdominal pain are considered almost pathognomonic for equine gastric ulceration a number of rare conditions should be considered in the differential diagnosis. These include:

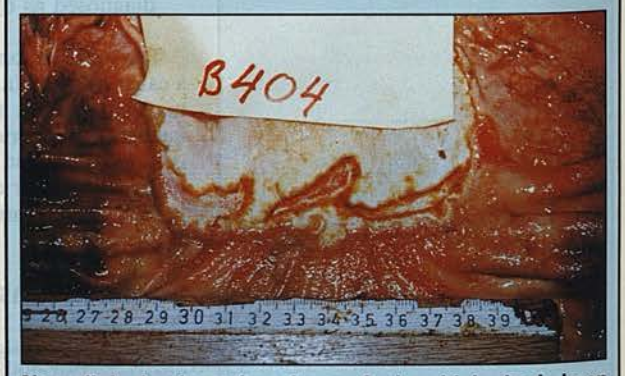
- Megaoesophagus
- Pyloric stenosis
- Gastric impaction
- Partial or complete obstruction of the small intestine
- Choke

Anatomy

The equine stomach is comparatively small in relation to the size of the gastrointestinal tract. It represents 8·5 per cent of the total capacity of the gastrointestinal tract, considerably less than the stomach of other domestic animals (dog, 62 per cent; cat, 69 per cent; pig, 29 per cent; ox, 70 per cent).

The stomach mucosa is clearly divided into two parts. The proventricular part is glistening white in colour, composed of thick stratified squamous epithelium and contains no glands. It covers approximately one-third of the mucosal area and ends abruptly at the margo plicatus, a slightly raised irregular serrated border with the glandular mucosa. The glandular mucosa has a velvet-like texture and is usually covered by a thick layer of viscus mucus. This mucosa contains three main gland types: mucus secreting cardiac glands; fundic glands which in addition to containing mucus secreting cells have hydrochloric acid producing parietal cells and pepsinogen secreting chief cells; and pyloric glands which consist largely of mucus secreting cells.

Extensive ulceration in the squamous mucosa in a foal showing typical clinical signs of the foal gastric ulceration syndrome



Non-clinical ulceration in a foal which had been euthanased because of a broken leg

Pathology

Ulcers may be singular or multiple and are most commonly located in the non-glandular squamous mucosa close to the margo plicatus along the lesser curvature of the stomach. They may be linear or irregular in shape and, with the exception of those in the glandular mucosa, are rarely circular in appearance. Ulcers in the squamous mucosa often have slightly raised brown-stained keratinised borders and contain small amounts of necrotic material at their base; frank blood is not common. They can vary in size from less than 0·5 cm^2 to over 30 cm^2. If very large, islands of normal epithelium may be present within the ulcer.

The majority of perforating and asymptomatic ulcers occur in the non-glandular mucosa. It should be noted that the stratified squamous epithelium close to the margo plicatus is many cell layers thick and what grossly appears to be a significant craterform ulcer in this area may, on histopathological examination, not involve the submucosa and therefore should correctly be described as an erosion. Ulcers in the glandular zone are less common and usually present as single circular or oval depressions surrounded by an intense zone of inflammation.

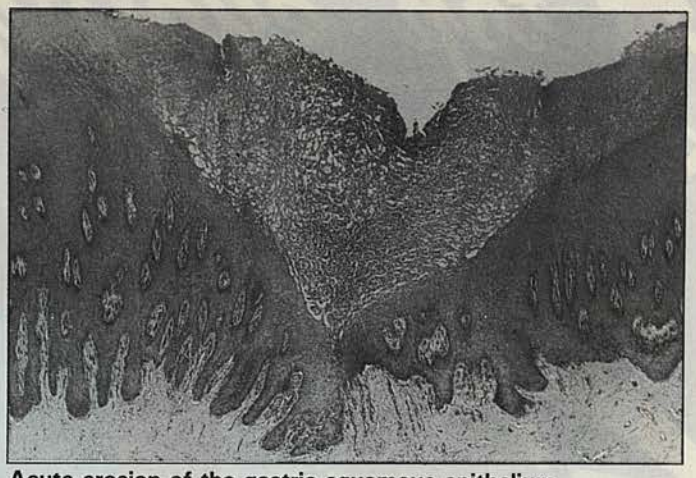
Occasionally, an hour glass shaped stenosis of the proximal duodenum associated with fibrosis and granulation tissue is observed at post mortem examination or exploratory laparotomy and it is believed that this represents a healed duodenal ulcer.

Histopathology

The least severe pathological changes observed histopathologically are described as epithelial changes and are characterised by increased epithelial thickness, increased depth and irregularity of the rete pegs, hyperkeratosis and hydropic degeneration. More severe lesions are classified as acute erosions and in addition to the changes described for epithelial change, erosion, reticular degeneration and spongiosis are noted. Acute and subacute ulcers describe lesions where the deficit extends down to the lamina propria and in chronic ulcers and erosions fibrosis is a significant feature.

Aetiology

The aetiology of equine gastric ulceration is unknown. Prolonged high doses of non-steroidal anti-inflammatory drugs (NSAIDs) are known to cause ulceration of the glandular mucosa but this is only a factor in a very few clinical cases. Many other factors have been suggested as causing gastric ulceration but, unlike NSAIDs, none have been proven.



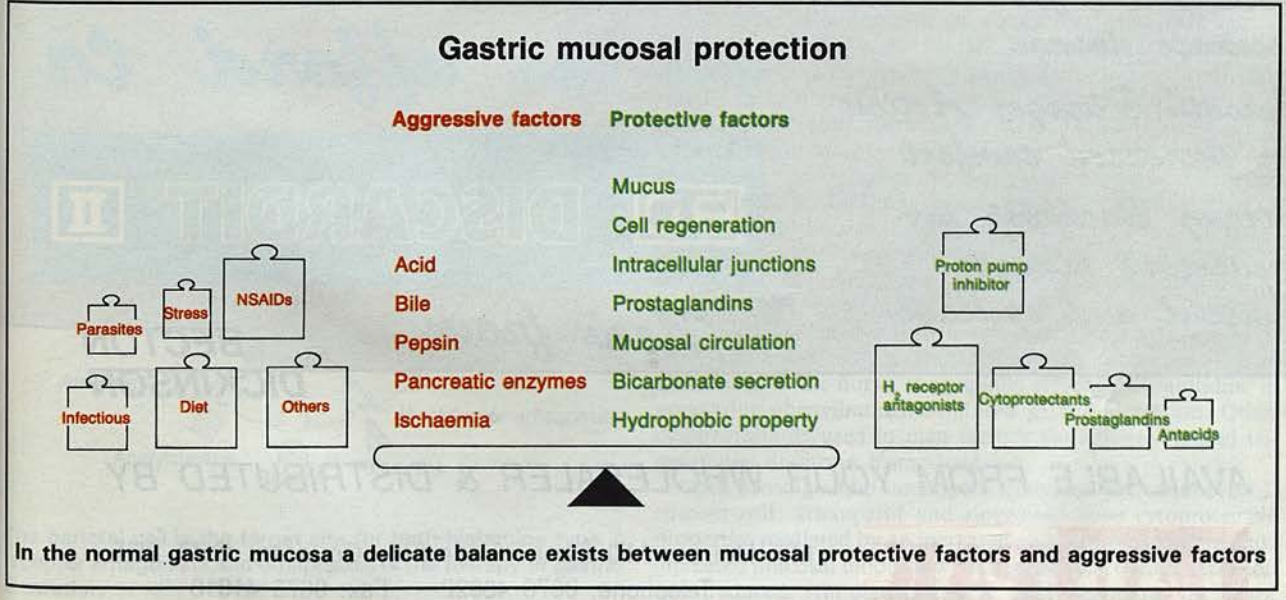
Acute erosion of the gastric squamous epithelium

A survey of veterinarians in Ireland and Britain identified 38 cases of gastric ulceration but only two cases occurred on the same premises, suggesting that infectious causes may not be important in the aetiology. The attempted isolation of an infectious organism from perforated gastric ulcers in the USA has been unsuccessful.

Stress is commonly believed to be a factor in gastric ulceration in man. Stress caused by weaning, transport, inclement weather, concurrent disease, anaesthesia and surgery has been suggested as contributing to gastric ulceration in foals but, as yet, has not been proven.

Gastric mucosal barrier

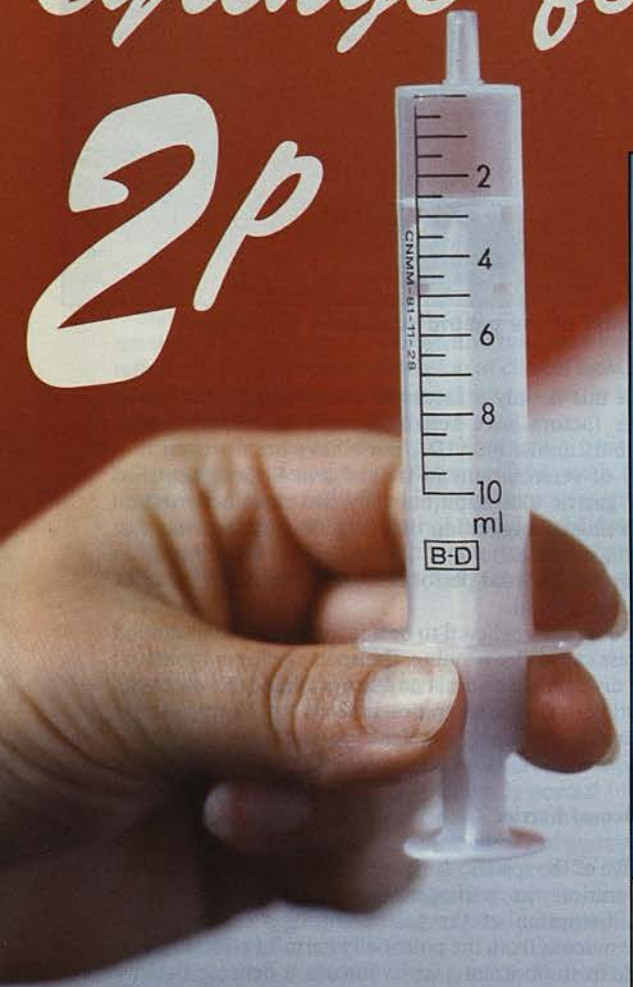
Irrespective of the specific causative factors involved in equine gastric ulceration the pathogenesis of ulceration probably involves a disruption of the gastric mucosal barrier which protects the mucosa from the potentially harmful effects of acid and pepsin. In the normal gastric mucosa a delicate balance exists between mucosal protective factors and aggressive factors (see below). The existence of an additional exogenous aggressive factor could tip the balance and overwhelm the endogenous protective factors allowing diffusion of hydrogen ions and pepsin into the mucosa resulting in autodigestion and ulcer formation.

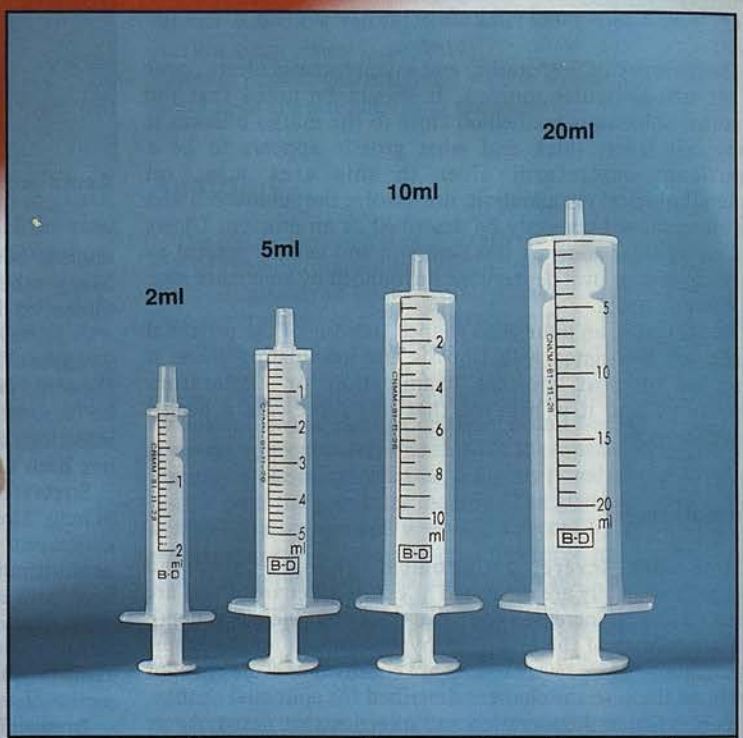


In the normal gastric mucosa a delicate balance exists between mucosal protective factors and aggressive factors

A top quality two-piece syringe for under

2p





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Treatment

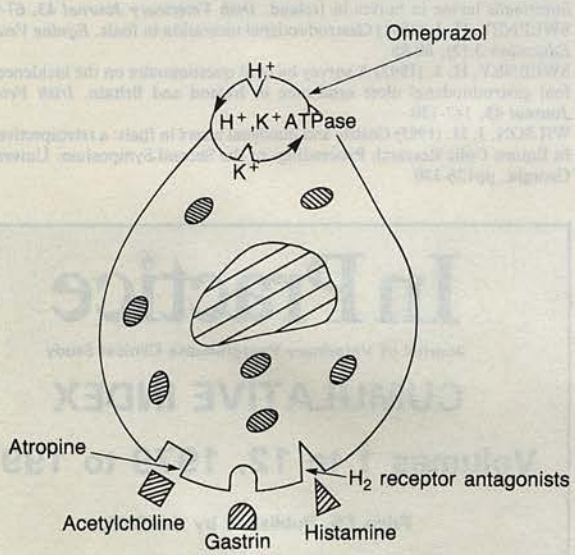
The aim of treatment is to restore the integrity of the mucosal epithelium by limiting the endogenous aggressive factors (increasing the PH), augmenting the endogenous protective factors (cytoprotectants) and removing the causative factor (if known). Using pharmacological means the balance between the gastric aggressive and protective factors can be restored. However, it is important to note that the majority of pharmacological agents described below have very limited or no clinical testing in the horse and are not yet licensed for use in this species.

Acid control

The parietal cells in the gastric glands secrete hydrochloric acid into the gastric lumen and this process is controlled by three different chemical mediators — histamine (the dominant secretagogue), acetylcholine and gastrin. The blocking of any of these receptor sites on the parietal cell results in effective control of acid secretion and it is in this way that histamine type 2 (H_2) receptor antagonists (cimetidine, ranitidine, famotidine and nitazidine) mediate their effect.

Ranitidine and cimetidine have been used commonly in foals and both are available in intravenous and oral formulations. Although both drugs are effective in the control of equine gastric acid secretion, ranitidine is preferred because it is six to 10 times more effective than cimetidine in reducing acid secretion in man and has fewer side effects. Additionally, the absorption of cimetidine after oral administration is slow and variable. However, very few clinical trials in the horse have been reported and the pharmokinetics of H_2 receptor antagonist drugs in the horse have not been fully described. Consequently, dosage recommendations are empirical and based largely on clinical impressions. The commonly used dosage rate in the foal for ranitidine is 4·4 mg/kg orally three times daily, and for cimetidine is 8·8 mg/kg orally three times daily. Treatment should continue until remission of clinical signs; this may take up to six weeks.

The hydrogen ion concentration in the gastric lumen is about four million times greater than that within the parietal cell and therefore an active transport mechanism is required to transport the hydrogen ion across this gradient. This is achieved by means of the proton pump (acid pump) located in the apical secretory membrane of the parietal cell. The energy for the proton pump is generated by the enzyme hydrogen, potassium adenosine triphosphatase (H+, K+ ATPase), and the development of omeprazole, a drug that binds to H+, K+ATPase and inactivates it, offers a new method for control of gastric acid secretion in the horse. The intravenous administration of 0·5 mg/kg in horses has caused up to a 90 per cent drop in basal free gastric acid content for up to seven hours. However, in man, oral administration of this drug is more effective and further clinical trials in the horse are required.



The parietal cell is the target site for both histamine type 2 receptor antagonists and omeprazole in the therapy of gastric ulceration

Agents commonly used in the treatment of equine gastric ulceration

Drug	Action	Dosage	Route
H_2 antagonists			
Cimetidine	Reduces acid secretion	4·4 mg/kg 4-6 times daily	Oral
Ranitidine	Reduces acid secretion	4·4 mg/kg 2-3 times daily	Oral
Cytoprotectants			
Sucralfate	Protects mucosa	1-2 g/100 kg 3-4 times daily	Oral
Antacids			
Aluminium hydroxide	Neutralises acid	1·5 g 8-10 times daily	Oral
Calcium hydroxide	Neutralises acid	1·5 g 8-10 times daily	Oral
Magnesium hydroxide	Neutralises acid	1·5 g 8-10 times daily	Oral
Prostaglandin			
Misoprostl	Protects mucosa	5 μg/kg twice daily	Oral
Proton pump inhibitor			
Omeprazole	Reduces acid	0·5 mg/kg 3-4 times daily	Intravenous

Antacids, such as magnesium hydroxide, calcium carbonate and aluminium hydroxide, when administered orally directly neutralise gastric acid in the stomach. However, because of the rapid emptying of the stomach and the dilution of its contents, frequent administration (eight to 10 times a day) is required. Additionally, antacids produce side effects of diarrhoea and constipation and are not used commonly in the therapy of foal gastric ulceration.

Mucosal protectants

Sucralfate is a complex of aluminium hydroxide and a sulphated sucrose. Despite the lack of clinical trials in the horse, it is popular in the treatment of gastric ulceration in this species, usually in combination with an H_2 receptor antagonist. It is available in tablet form and three to four times daily administration of up to 4 g per dose is recommended. Systemic absorption of sucralfate is minimal and within the acidic environment of the stomach it dissociates and polymerises to a viscous substance that selectively adheres to ulcerated tissue and forms a protective chemical 'bandage' preventing back diffusion of hydrogen ions. Sucralfate also inactivates pepsin, absorbs bile and increases the mucosal synthesis of prostaglandins, mucus and bicarbonate. In man, sucralfate is equally effective as H_2 receptor antagonists in the treatment of ulceration; in the horse, it is equally effective as ranitidine in preventing phenylbutazone-induced gastric ulceration. Other cytoprotectants used in man include carbenoxolone and tripotassium di-citrato bismuthate.

Oral prostaglandin analogues (misoprostol, endoprostil, trimoprostil, arbaprostil and rioprostil) have cytoprotective properties mediated by an increased viscosity of gastric mucus, increased mucosal blood flow, increased bicarbonate secretion and reduced stimulated gastric acid secretion. In man,

Gastric ulceration syndrome in foals: fact or fancy?

★ A direct relationship between the clinical signs of teeth grinding, salivation and colic with the presence of gastric ulceration has not yet been definitively demonstrated. The visualisation of gastric ulcers alone is insufficient evidence because over 50 per cent of normal foals have asymptomatic ulcers (Murray and others 1990).

★ Over 50 per cent of foals dying from perforated gastric ulcers are recorded as sudden deaths and show no clinical signs at all (Dwyer and Powell 1989). This suggests that factors other than ulceration may be required before typical clinical signs are exhibited.

★ NSAIDs' toxicity resulting in ulceration of the gastric mucosa does not cause the clinical signs associated with the gastric ulceration syndrome (Smith and others 1987).

★ Most workers in equine gastric ulceration have drawn extensively from human research, but man may not be the most appropriate model. Equine gastric ulceration usually occurs in the non-glandular squamous mucosa, a mucosa not present in man. The pig and rat stomach have well demarcated squamous mucosa sections.

★ If gastric ulceration in the horse parallels that in other species it is likely that gastric squamous, gastric glandular and duodenal ulceration are heterologous conditions with different clinical signs and significance.

★ In the pig, diet is considered the major cause of ulceration in the non-glandular mucosa. There is evidence that in the horse this is also true. Coenen (1990) indicated the need to feed roughage and to distribute concentrate feed over three feeds to avoid ulceration. A dietary factor was also suggested by Hammond and others (1986). A post mortem survey of grass sickness, a condition exclusive to pastured horses revealed ulceration in only 2 per cent of animals (D. Pogson, personal communication) as compared to 28 per cent in the general population (Sweeney 1990).

★ The equine gastric parasite *Gasterophilus intestinalis* causes deep ulcerative pits in the squamous mucosa yet it is generally considered to be of limited clinical significance.

★ In pigs, with the exception of those that die from acute haemorrhaging squamous ulcers, the presence of subacute and chronic ulcers is considered to be of no economic significance (O'Brien 1986).

★ A number of authors (Wilson 1985, Dwyer and Powell 1989) in post mortem surveys described non-perforating ulcers as the primary problem or cause of death in a number of cases. The justification for such a post mortem diagnosis should be rigorously defined.

★ Melaena is a feature of gastric ulceration in other species but is not common in the horse.

★ In man, post mortem surveys usually reveal a higher prevalence of gastric ulceration than do clinical surveys. Curiously, in the foal the reverse is true (Dywer and Powell 1989, Murray and others 1990). This may partly be explained by inconsistency in the scoring and interpretation of lesions.

prostaglandin analogues although equally effective as ranitidine in ulcer therapy are less effective in pain control and diarrhoea is a common side effect. Misoprostol can cause abortions in animals. Prostaglandin analogues offer no therapeutic advantage and consequently are not commonly employed in equine gastric ulcer therapy.

Surgery

If duodenal or pyloric stenosis is diagnosed, surgical treatment using gastroduodenostomy, duodenojejunostomy, gastrojejunostomy or jejunojejunostomy may be attempted. Access to the stomach is restricted making surgery difficult and reported success rates are less than 50 per cent.

Conclusion

Gastric ulceration is a new clinical syndrome in foals which is usually diagnosed on clinical grounds alone. Many questions regarding the cause, pathogenesis, significance and even the existence of the condition remain unanswered and some of these are addressed in the box. More work is required to clearly define the clinical signs and aetiology. A diagnosis of gastric ulceration is a tempting refuge when diagnostically destitute but this temptation should be resisted.

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