An Investigation into the Pathology of Gastritis and Gastric Ulceration in the Horse

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I declare that this dissertation has been composed by myself and the work described is entirely my own.

Henny Martineau

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LIST OF ABBREVIATIONS

APE	Aminopropyltriethoxysilane slide
API	Analytical Profile Index system
BEVA	British Equine Veterinary Association
CNS	Central nervous system
CPC	Cetylpyridinium chloride
DAKO	DakoCytomation
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGUC	Equine Gastric Ulcer Council
FDC	20% formalin, 79% distilled water and 1% cetylpyridinium chloride
FISH	Fluorescent in situ hybridisation
G cell	Gastrin cell
GID	Gastrointestinal disease
Gl no	Glandular number

Gl sev	Glandular severity
GORD	Gastroesophageal Reflux Disease
H and E	Haematoxylin and Eosin
HCl	Hydrochloric acid
HLO	*Helicobacter*-like organism
HP	*Helicobacter pylori*
IL8	Interleukin 8
L1	First stage larva
L2	Second stage larva
MALT	Mucosa-associated lymphoid tissue
NSAID	Non-steroidal-anti-inflammatory drug
OCT	10.24% w/w polyvinyl alcohol, 4.26% w/w polyethylene glycol, 85.50% w/w nonreactive ingredients
PAS	Periodic Acid Schiff
PBS	Phosphate buffered saline (pH 7.3)

PCR	Polymerase chain reaction
rRNA	ribosomal Ribonucleic acid
Sq no	Squamous number
Sq sev	Squamous severity
TB	Thoroughbred
VFA	Volatile fatty acid

PHOTOCOPY DECLARATION

THIS DISSERTATION OR ANY PART THEREOF MAY BE PHOTOCOPIED SUBJECT TO THE PERMISSION OF THE AUTHOR.

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ABSTRACT

The last century has seen an increase in the prevalence of gastritis and gastric ulceration in the horse with both clinical and post mortem studies presently identifying over 80% of high performance horses to be affected. Lesions are most commonly found within the squamous region of the stomach where the pathogenesis is thought to be physiological. Glandular injury is less frequent and its pathogenesis is poorly understood in the absence of non steroidal anti-inflammatory drug treatment.

This study was designed to investigate the true extent and variety of gastric lesions in a mixed population of 21 adult horses at post mortem. These horses were presented to Glasgow University Veterinary School over a period of nine months for euthanasia for reasons unrelated to this study. Immediately following euthanasia, each stomach was photographed and visible lesions recorded using the MacAllister lesion classification system. Full thickness gastric samples were taken from six predesignated sites incorporating all squamous and glandular types within the stomach. A representative selection of samples from gross lesions was also obtained. The presence of any *Helicobacter* like organisms (HLOs) was determined by sending tissue samples directly for culture on *Helicobacter* medium in microaerobic conditions, and fixing them in formalin for histological evaluation. Special staining methods including Warthin-Starry, immunohistochemistry and modified Giemsa stains were used to highlight the presence of any HLOs.

One of the 21 stomachs had no evidence of ulceration at all, seven had lesions in the squamous region only, two in the glandular region only and eleven had lesions in both. Gross lesions in the squamous region were classified as hyperkeratosis, punctate erosions/ulcerations, diffuse erosions/ulcerations and injury at the margo plicatus (margo injuria). Within the glandular region hyperaemia, erosions and ulcerations were identified. Histological evaluation corresponded with some of these findings and included the identification of glandular metaplasia. However, distinction between erosions and ulcerations made on macroscopic grounds particularly within the glandular region were not always found to be accurate. This was confirmed by histology.

Prompted by the significant role that gastritis plays in the aetiology of peptic ulceration in humans, the type and degree of gastritis in these stomachs was also assessed. A modification of the gastritis classification system in humans was used, (the Updated Sydney System), and the cellular infiltrate and reactive changes were recorded in a systematic fashion. Given the lack of existing data regarding gastritis in the horse, and the small numbers examined in this study, no specific patterns of inflammation were observed. However it was apparent that different types of inflammatory infiltrate were present (chronic and active) and some were accompanied by reactive changes (eg. hyperkeratosis, glandular atrophy), within the surrounding mucosa. No *Helicobacter* like organisms were identified through culture or in formalin fixed sections.

In conclusion, gastritis and gastric ulceration were observed in the majority of a mixed population of adult horses examined. Lesions within the squamous region

were consistent with a physiological aetiology (eg. increased gastric acidity). The Updated Sydney System was applied to the histology of the equine stomach for the first time. The inflammatory picture found within the glandular region was varied and could still be consistent with an infectious cause to this disease but no specific aetiology was determined. Further work is required to obtain more post mortem samples to assess the true variety and extent of gastritis in adult animals and use more sensitive methods for HLO detection. The basic histological investigations in this study have highlighted the subjectivity and inaccuracy of gross lesion assessment, the wide variety of lesions present and the need for further gastric sample evaluation from a larger group of horses.

CHAPTER ONE – LITERATURE REVIEW

1.1 INTRODUCTION

Gastritis, literally meaning an inflammation of the gastric mucosa, is a result of the stomach's response to injury. In humans it is now known to be a precursor to the formation of gastric ulcers (Price 1991). One of the first reports of gastritis in man was by G. E. Stahl in 1728, where he refers to "inflammation of the inner lining of the stomach and the intestine". The true prevalence of the disease was subsequently highlighted by observations made at post mortem by surgeons during the Napoleonic and First World Wars (Schindler 1947).

In the animal kingdom, gastritis was first recognised as an important disease in pigs, where gastric erosions and ulcers were identified as early as 1926. By the 1960s, ulcer prevalence was reported to range between 4–54% (Bivin et al 1974) with more recent reports recording a 10–17% prevalence of ulceration (Cole et al 2002). In horses, the incidence of gastritis / gastric ulceration has increased over the last 60 years according to a Swedish post mortem survey on a mixed population of horses (Sandin et al 2000). Recent studies on racehorses report over 80% to have gastric lesions, with those in training being the most frequently affected (Hammond et al 1986, Johnson et al 1994). This has both economical and welfare implications.

The clinical signs described in horses vary from a mild, non-specific decline in performance or loss of weight, to the development of fatal peritonitis following gastric rupture. In both pig and horse, gastritis and gastric ulceration are thought to

be the result of a combination of stressful management conditions and strict dietary requirements, instigated through intensive farming or racing procedures, respectively.

In humans, the accuracy of *in vivo* diagnosis of gastritis and gastric ulceration was improved by the development of endoscopy and biopsy sampling techniques. Histopathological examination aided the discovery of *H. pylori* in 1984 by Warren and Marshall. Confirmation of its aetiological role in the pathogenesis of active chronic gastritis was achieved through an heroic act by Marshall. He succeeded in fulfilling Koch's postulates by self-administration of the organism, and in doing so transformed our understanding of the disease (Marshall 2002). This discovery prompted further experimental work to investigate the true incidence of gastritis and gastric ulceration in other species. The possible role of *Helicobacter*-like organisms (HLOs) in the aetiology of this disease was also sought. Identification of good animal models for the human disease is an ongoing task and studies have examined naturally infected cats (Esteves et al. 2000), Mongolian gerbils (Ikeno et al. 1999), Beagle dogs (Rossi et al 1999), nonhuman primates (Reindel et al 1999) and other rodents (Ross et al 1992).

The development of various classification systems has contributed to the knowledge of gastritis and gastric ulceration. These enable an effective and repeatable method of recording findings, in turn leading to accurate ways of comparing and recognising different patterns of inflammation. The Updated Sydney Classification System is the most recent system for humans, which combines topographical, morphological and aetiological information from each sample (Dixon et al 1996). Each feature is then

scored using a visual analogue scale, from which an overall diagnosis, prognosis and finally treatment can be made.

This system has been modified for use in dog, cat and pig (Happonen et al 1998, Queiroz et al 1996). Several species of *Helicobacter* have been isolated from these animals, although their exact role in the pathogenesis of gastritis is still being determined (Jalava 1999). Evidence of cross species transmission includes the isolation of *H. pylori* from a colony of cats (Esteves et al 2000) and cynomolgus monkeys (Reindel et al 1999), and similar strains of *H. heilmannii* being isolated from two cats and their owner (Dieterich et al 1998). The goat and horse remain the only two common domestic animals where a species of *Helicobacter* has yet to be identified (Gueneau et al 2002)

This study has been prompted by the paucity of literature concerning the gross and histopathological appearance of gastritis and gastric ulceration in the horse. It reviews information specifically relating to gastritis and gastric ulceration in the horse, along with relevant material generated from human medicine and experimental science. Detailed post mortem accounts of the gross and histological appearances of 21 horse stomachs from a mixed population then follow, with a description of the special stains and cultures used to identify any HLOs through histological methods. It is hoped that analysis of the findings will contribute to fresh ideas about the pathogenesis of gastritis and gastric ulceration in the horse.

1.2 ANATOMY OF THE EQUINE STOMACH

"Its figure is round and somewhat long, resembling a Bag-pipe; though on the left side and at the bottom it is bunching and more capacious than on the right side; for there is little and little narrowed that it might give place to the liver." (The anatomy of an horse, Andrew Snape, 1683).

1.2.1 Gross Anatomy

A thorough understanding of gastric anatomy is essential if the pathogenesis of gastritis is to be examined accurately. Anatomical differences between species and the associated link to susceptibility of gastritis or ulcer formation provided a starting point for this study. The following section gives a general overview of gastric anatomy with a more detailed account specifically given on equine gastric anatomy.

The anatomy of the stomach is defined by its function, which includes storage and digestion of the bolus passed from the oesophagus. This passage is achieved by secretion of gastric acid from the glandular area within the stomach, and the subsequent digesta is then propelled into the duodenum by contraction of the thick muscular wall. The pacemaker responsible for subsequent peristaltic movements within the distal intestine lies in the wall of the pylorus of the stomach.

Stomachs are defined as either simple (one compartment e.g. carnivores, pigs, horses) or complex (four compartments e.g. ruminants), with a variation in mucosal type between species. Humans and carnivores have entirely glandular stomachs, whereas the pig, horse and rat have composite stomachs with varying

proportions of squamous and glandular components. Although the gastric squamous epithelium is similar in structure to the squamous mucosa of the oesophagus, both organs develop separately during embryogenesis.

Thorough and classical accounts of the gross anatomy of the equine stomach have been given by Sisson and Grossman (1976), Rooney (1977), and Nickel et al (1979). The horse stomach is simple (one compartment) and composite (glandular and non-glandular epithelial lining), with a surprisingly small capacity (8–15 litres) for the size of animal. In life, gastric contents are capped by a large air-filled fundus (saccus caecus) that rises above the level of the cardia.

The stomach is found on the left hand side of the abdomen in a dorsal location, caudal to the diaphragm and liver. Both inspiratory efforts and degree of gastric fullness affect its position within the rib cage, though it is always contained by it to some degree. Removal of the stomach from the carcass involves careful dissection of the greater omentum from the greater curvature and the diaphragm at the cardia, followed by sectioning of the gastrophrenic, hepatogastric and gastrosplenic ligaments. The last is adherent to the greater curvature towards the right side of the stomach, and runs along the ventral plane of the pylorus. After attaching to various loops of the intestine, it links the greater curvature of the stomach to the spleen.

Once the stomach is opened, the distinction between the "whitish, smooth and firm" non-glandular region and pink glandular region becomes obvious. The former has a lining of stratified squamous epithelium, which extends approximately half way into the body of the stomach from the cardia. Its distal margin is bordered by the margo

plicatus, a linear junction between the two regions, which acts as a stricture when the stomach is very full. The glandular area contains three distinct types of gland. Cardiac glands are present in a narrow strip running parallel to the margo plicatus but not extending as far as the lesser curvature. The proper gastric or fundic glands cover the body of the stomach, and the pyloric glands extend from the margo plicatus on the lesser curvature, through the antrum to the pylorus.

The stomach wall has the expected structure of any tubular organ with serous, muscular, submucous and mucous layers. However, the musculature is unusual in that along with the customary longitudinal and circular fibres, it contains internal oblique fibres. These occur in the area of the fundus and the corpus along the greater curvature, and can be related to the expansion of the stomach during development. They are responsible for the formation of the cardiac loop along the lesser curvature, which forms a vestigial gastric groove that can only be seen on removal of the mucous membrane.

Gastric blood supply is by way of the coeliac artery, which branches into the left gastric and splenic artery, and the right and left gastroepiploic arteries. These all supply various areas of the stomach (Pasquini 1983). The microvascularisation of the individual connective tissue papillae within the non-glandular region of the stomach differs between the pars oesophagea and the margo plicatus. The thicker epithelium and lamina propria of the margo plicatus is supplied by fewer but longer intrapapillary blood vessel systems than that of the non-glandular area (Staszyk et al 2001). Another study on blood flow (Manohar et al 1995) showed that the glandular area of the stomach has a higher rate of blood flow than the squamous portion at rest,

and that this is reduced due to vasoconstriction during exercise.

The innervation is from the vagus and sympathetic nerves and includes intramural and subserosal plexuses. Lymphatic drainage of the stomach is to the gastric, splenic, coeliac, and pancreatico-duodenal lymph nodes.

1.2.2 Histopathology

General details of the histological appearance of the equine stomach can be found in The Colour Atlas of Veterinary Histology (Bacha and Wood 1990) and The Textbook of Veterinary Histology (Dellmann and Eurell 1998). A more specific and detailed description of the non-glandular region of the equine stomach is described by Argenzio (1999).

The stratified squamous epithelium is composed of four layers. The most superficial is the stratum corneum, which as the name suggests is a cornified layer. It is several cells thick, and these are either anucleate or contain pyknotic nuclei. Beneath this the stratum transitionale is composed of cells with large round nuclei. Then comes the stratum spinosum, where cells are smaller and oblong in shape. Finally, 2–4 layers of cuboidal shaped cells with large central nuclei make up the stratum basale, which borders the lamina propria. The thickness of these layers varies according to their proximity to the margo plicatus (Murray et al 2001a).

The glandular region of the stomach consists of three types of gland. The cardiac gland region is very narrow and lies adjacent to the margo plicatus towards the greater curvature. It contains glands that are short, simple, branched, coiled and

tubular, all of which excrete mucus. The cells lining these glands are cuboidal with basal nuclei and they empty into relatively shallow gastric pits that communicate with the gastric lumen. Parietal cells occur at the junction of the cardiac and fundic gland region.

The fundic gland region occupies more than one third of the stomach. The glands are straight, branched and tubular, and extend into the lamina muscularis. Each has a short neck, long body and slightly dilated blind end or fundus. Mucous neck cells, chief cells, parietal cells and endocrine cells comprise the secretory components of this region. Mucous neck cells are found in the neck region or isthmus of the proper gastric gland. They have a basophilic cytoplasm with a squashed flat nucleus situated towards the base of the cell. When stained with Periodic Acid Schiff (PAS), the entire cell stains dark pink (PAS positive), in contrast to the surface epithelial cells where only two thirds of the cell contains PAS positive material. The most abundant cell in this region is the chief cell, which is cuboidal in shape, with a round nucleus towards the base of the cell. It has a basophilic staining cytoplasm and secretes pepsinogen, which is transformed to pepsin by hydrochloric acid (HCl). This acid is produced by the parietal cells, which mostly appear singly, with only a narrow apex of the cell bordering the gland lumen. They stain deeply with eosin due to the presence of numerous mitochondria, and have a prominent round nucleus. The population of parietal cells dwindles towards the fundus of the gland where the chief cells predominate. Endocrine cells are also present, and produce the various gastrointestinal hormones e.g. gastrin, secretin, cholecystokinin and gastric inhibitory peptide. They stain poorly on routine Haematoxylin and Eosin (H and E), but are found between the basement membrane and chief cells where some

excrete hormones into the gland lumen in response to changes in the contents.

The pyloric gland region covers the antrum and pylorus of the stomach, and contains branched coiled tubular glands, which are relatively short compared to those of the other regions. This is in contrast to the gastric pits, which are much deeper.

In the growing foal, the majority of epithelial differentiation occurs during the last month of gestation and first 2 weeks post partum. There is thickening of the squamous epithelium from 2–20 cells in the first 2 weeks of life, with secretion of the mucus layer occurring in the glandular region as early as 2 days old (Murray 1991a). This may be the result of increased exposure to an acidic environment, in combination with stimuli from local- and milk-derived growth factor effects (Murray 1999).

1.3 GASTRIC DISEASES IN THE HORSE

In the horse the incidence of gastrointestinal disease is second in importance only to musculoskeletal disease (BEVA 1965). Although the stomach accounts for a mere 4% of the entire gastrointestinal capacity, any disturbance throughout the tract will generally induce changes in gastric physiology and pathology. Gastric disorders can be categorised into the following: functional disturbances, parasitic infection, neoplasia, and gastritis and gastric ulceration (Colahan 1999).

1.3.1 Functional disturbances

Acute gastric dilatation and rupture may be the result of excessive overload or distal obstruction. The former may occur in situations involving fermentable carbohydrate overload, sudden access to lush pasture, or an excessive intake of water. The condition is generally fatal with the stomach tearing along the greater curvature and causing peritonitis. Gastric rupture may also be the consequence of a distal bowel obstruction or acute grass sickness and occurs in 5% of colic cases submitted to veterinary hospitals (Barker et al 1993). Pyloric stenosis also obstructs gastric outflow and either occurs as a congenital problem due to hypertrophic pyloric stenosis or secondary to a local chronic inflammatory condition (Colahan 1999). Clinical signs associated with this may include abdominal pain and gastric reflux, with gastric rupture being a possible but unlikely consequence.

1.3.2 Parasitic disease

There are six parasites commonly found in the equine stomach: arthropod larvae *Gasterophilus intestinalis* and *G. nasalis;* and nematodes *Trichostrongylus axei, Habronema muscae, H. majus* and *Draschia megastoma* (Blagburn et al 1991). Of these, the two *Gasterophilus* species (Bots) are the most prevalent and pathogenic in the UK. Equine parasite control programmes are generally targeted at their demise and the discussion here will concentrate on their larvae.

The life cycle of *Gasterophilus sp.* involves deposition of pale ellipsoid eggs by the adult female fly on horse hair, where self-grooming stimulates the first stage larvae (L1) to hatch spontaneously. The horse then ingests these and over a period of 4 weeks, development to third stage larvae (L3) occurs in the buccal or pharyngeal tissues. The larvae then migrate to the stomach and attach to the mucosa in the squamous (*G. intestinalis*) or glandular (*G. nasalis*) region. Ten months later they are passed in the faeces and develop into pupae in the soil where they remain for 5 weeks.

Much work has been done to investigate the prevalence of these parasites in countries across the world (Lyons et al 1987, 2000, Lyon et al 2000, Höglund et al 1997, Coles and Pearson 2000). More horses are reported to be infected with *G. intestinalis* (33–55%) than G. *nasalis* (5%), although multiple inconsistent parameters within these studies (age, location, anthelmintic therapy) prevent accurate direct comparisons. Seasonal influences on infection have been found in Kentucky and Texas (Price and Stromberg 1987, Lyons et al 2000), while recent information

on the actual burden of parasites comes from an Irish abattoir survey (Sweeney 1990), in which only 2% of infected stomachs harboured more than 100 bots. While the increased use of anthelmintics may have led to reduced infection rates over the last 20 years (Lyons et al 2000), the fact that a single female *G. intestinalis* lays over 900 eggs in her lifetime may explain the continued persistence of the species.

The literature remains uncertain as to whether or not infection with *Gasterophilus sp.* causes ulceration in the horse stomach. While early reports of gastric rupture in foals are blamed on the mechanical trauma caused by *Gasterophilus* larvae (Rooney 1964), other causes such as stress, *Candida sp.* infection and non-steroidal-anti-inflammatory drug (NSAID) administration are now more frequently implicated (Gross and Mayhew 1983, Wilson 1985, Becht and Douglas Byars 1986).

In the adult horse, discrepancies exist between sites of larval attachment and location of ulceration, and there is a lack of correlation between parasitic burden and lesion prevalence (Waddel 1972, Sweeney 1990). Although the designated area of attachment for *G. intestinalis* is the saccus caecus in the non-glandular region of the stomach, depending on the season and burden, they can be found scattered throughout the pars oesophagea forming small pit-like lesions (Principato 1988). The number of epithelial defects has been found to exceed the number of larvae present, indicating that the parasites are mobile (Price and Stromberg 1987). Gross descriptions report lesions to vary in size from 0.5mm to 12cm, and they can be accompanied by oedematous swelling of the surrounding tissues. Ulcer edges are irregular, with evidence of lesion coalescence from multiple sites of larval attachment, although it is seldom that parasites are attached to the base of the lesion.

Attempts to recognise and classify specific gastric parasitic infections through observations of gross lesions alone have failed (Principato 1988).

Histological studies report a difference in the host's reactivity to the two species of *Gasterophilus* larvae with increased fibrosis accompanying *G. intestinalis* infection. However, it is more likely to be the differing location (pars oesophagea vs. proximal duodenum) that is responsible for the varying reaction to insult. Histology shows sites of erosion or ulceration to arise adjacent to larval attachment. Mouthparts are seen in contact with the lamina propria, and surrounding epithelium can be separated from the lamina propria by areas of necrosis and bacterial infection. The lesion is seen to heal by fibrosis when the larvae move on. Sub-larval abscesses have been found in two stomachs, one of which eroded the subserosa and set up adhesions between the stomach and spleen (Waddell 1972). Other consequences of *Gasterophilus* parasitic infection include reports of liver cirrhosis (Tadmor et al 1981) and damage to ovaries subsequent to parasite migration (Drudge et al 1956).

The few articles on gastric nematode prevalence and the pathogenesis of ulcers probably reflect the reduced pathogenic impact these parasites have. Infection with *T. axei is* most frequent when the horse has been housed with infected cattle or sheep. Development of L3 to adult stage occurs in the glandular region of the stomach. It may result in localised gland metaplasia resembling pale circular 1cm plaques grossly, and/or the formation of hyperplastic nodules in the region of the pylorus and proximal duodenum. Heavy infections can raise plasma pepsinogen levels and are frequently accompanied with other gastrointestinal parasites. Adult worms of *D. megastoma* burrow into the submucosa of the glandular mucosa at the

margo plicatus producing lesions 0 - 290mm from the margo plicatus with a median size of 1–25mm. They produce a granulomatous reaction with large numbers of eosinophils and have rarely been known to lead to abscessation. *Habronema* worms lie on the mucosal surface and only cause a mild gastritis or erosion (Lyons et al 1984, Blagburn et al 1991, Barker et al 1993).

1.3.3 Neoplasia

In general gastric neoplasms are uncommon. In the horse, the most frequently encountered gastric neoplasm is the squamous cell carcinoma (Barker et al 1993,Tennant et al 1982, McKenzie et al 1997). It occurs in middle-aged horses and arises from the squamous area of the stomach, occasionally involving the cardiac sphincter. Its appearance is that of an irregular cauliflower-like mass ranging in size from 10 – 40 cm in diameter and it frequently metastasises to the abdominal or thoracic cavity. Clinical signs include weight loss, dysphagia and respiratory embarrassment depending on the degree of metastatic involvement. Other reported neoplasms include adenocarcinoma of the glandular mucosa and leiomyoma, although these are rare.

1.3.4 Gastritis and gastric ulceration

Incidence: In foals, gastroduodenal ulceration is a term used to encompass all degrees of inflammation and ulceration involving the stomach or duodenum (Murray 1991a). It has been found in over 50% of a healthy population in foals, with an increased prevalence in those less than 10 days old (Murray et al 1990). Most frequently lesions lie adjacent to the margo plicatus along the greater curvature,

although they can occur throughout the stomach and proximal duodenum. Secondary complications include gastric perforation, or stricture whilst healing, particularly in the duodenum (Becht and Douglas Byars, 1986). Clinically, the foal may appear to be asymptomatic, or show signs of tooth grinding, gastric reflux and dorsal recumbency.

Specific predisposing factors include an already existing disease – especially diarrhoea (Murray et al 1990) – while other stressful situations including peri-natal adjustment, weaning and even hot weather have also been blamed. Infectious agents including *Salmonella, Clostridium perfringens* and *Escherichia coli* have been isolated from affected foals (Wilson 1985), and *Candida* has been found in association with lesions (Gross and Mayhew 1983), but their precise role in the pathogenesis is uncertain. *H. pylori* has been searched for, but not isolated from stomachs displaying gastritis/ulcer disease in asymptomatic foals (Murray 1999) and adult horses (Johnson et al 1994) Parasitic migration (Rooney 1964) and excessive dosage with NSAIDs are recognised ulcerogenic agents (Traub et al 1983) in both the foal and the adult horse.

The reported prevalence of gastric ulceration in the adult horse is greatest in the Thoroughbred in training (82%). Other populations examined have found 58% of show horses and 52% of retired Thoroughbreds to be affected (Johnson et al 1994). A more generalised Swedish study examined a mixed population of adult horses from 1924–1990, and found a 17% prevalence. In this study Thoroughbreds and Standardbred trotters were three times more likely to be affected than cold-blooded horses. Clinical signs of colic and concurrent disorders involving the bowel, liver

and oesophagus have also been associated with a higher ulceration score (Murray 1992b, Sandin et al 2000, Vatistas et al 1999a). The sex of the horse has also been found to be important, with studies finding stallions to have the greatest number of ulcerations, although there is no distinction in mucosa type (Sandin et al 2000). In contrast however, when the age and sex of horses were compared, the relative risk for ulceration increased with age in geldings, but decreased in mares and stallions (Rabuffo et al 2002). It was proposed that testosterone release would affect the production of epidermal growth factor (EGF) by the salivary glands. In rats and mice, increased levels of EGF have been found in basal cells at the margin of an ulcer, and are assumed to be important with respect to ulcer healing (Jeffrey et al 2001).

Pathophysiology: The pathophysiology of gastric ulcer formation must involve an imbalance between aggressive and protective factors of the gastric epithelium. HCl and pepsin act as aggressive factors throughout the stomach with protective mechanisms varying between regions. The non-glandular region relies on intercellular tight junctions and intracellular buffering systems, while the glandular region possesses a 200 μm thick protective mucus layer into which bicarbonate ions are secreted (Murray 1992a). EGF has also been found to contribute to the healthy maintenance and repair of equine gastric epithelium. To allow all these mechanisms to occur, adequate mucosal blood flow and mucus secretion is essential, and is achieved through appropriate prostaglandin release. Any disruption in the chain of these events may act to promote damage and ulceration (Jeffrey et al 2001).

Non-steroidal anti-inflammatory drugs and ulceration: A well-known cause of gastroduodenal ulceration in domestic animals is that of excessive NSAID administration. Almost all these drugs are weak carboxylic or enolic acids and they act as analgesics and antipyretics with a peripheral anti-inflammatory activity. Their primary mechanism of action is through cyclo-oxygenase inhibition (Bishop 2001), which reduces the synthesis of prostaglandin and related compounds inhibiting gastric acid secretion. When given in excessive quantities, or to an already compromised patient, side effects including gastro-intestinal irritation and ulceration may occur. Vasoconstriction via the prostaglandin pathway and local topical toxic effects of the drug are the proposed pathways for lesion formation (Higgins and Lees 1984).

The NSAID phenylbutazone is commonly used in the horse as a short- or long-term analgesic at a recommended dose of 8.8mg/kg (Meschter et al 1990). Doses of 8 – 30mg/kg day given to adult horses for up to 3 weeks caused gastric mucosal damage in 6 out of the 8 horses involved. Damage was present in the glandular region of the stomach only, and ranged in size from microscopic erosions to 4cm ulcerations. Varying degrees of large and small intestinal inflammation, renal papillary necrosis and vascular thrombi were also noted (Mackay et al 1983). Attempts to understand the pathogenesis of this toxicity concentrated on identifying early lesions and their progression (Meschter et al 1990). Multifocal pinpoint lesions in the pyloric region coalesced to form spoke-like rays around the circumference of the pylorus. Histologically, there was a separation of foveolar crest epithelium and microthrombosis of superficial lamina propria. Erosions were accompanied by foci of necrosis in the lamina propria and thrombosed capillaries with dense

eosinophilic walls. The clefts progressed to breaks in the mucosal epithelium, with the epithelium of the gland neck first lifting off, then peeling back, to expose the lamina propria. There were mitotic figures in the mucosal epithelial cells, which were flattened in places in an attempt to cover eroded areas. In the lamina propria subadjacent to lesions, there were engorged capillary loops and small thrombi.

Stress and ulceration: Stress, being defined as the non-specific response of the body to any excessive demand on it (Yabana and Yachi 1988), has long been recognised as a possible causative factor of acute gastric ulceration in all species. In humans, stress ulcers are said to occur as a result of "physical or thermal trauma, shock, sepsis or head injury" (Moody et al 1976). However, clinical evidence is only found in a small proportion of those patients at risk, illustrating the importance of variable individual susceptibility to this condition.

The numerous biochemical and physiological disruptions that go hand in hand with lesion formation prevent identification of a single specific pathway for stress ulceration. It is certain that the influence of a multitude of stress factors, both physical and/or psychological, act to trigger a common pathway that is recognised by the central nervous system (CNS) (Lee 2000). The CNS then acts via the limbic system to affect gastrointestinal motility and secretion, endogenous substance release (e.g. histamine), and cardiovascular parameters. In turn, this induces hypotension, so disturbing the microcirculation within the gastric mucosa and leading to ischaemia. Oxygen-derived free radicals released during reperfusion are capable of further damage. The resistance of the gastric mucosal barrier is then reduced and capillary integrity compromised resulting in necrosis, erosions, haemorrhage and/or

ulceration.

Rats have been the target of much of the experimental work on stress-related gastritis. Induction has been shown to be possible through exposure to adverse conditions such as cold temperatures and water submersion (Basso et al 1988). Even extra handling on one of two similar groups was sufficient to increase the severity of gastric ulceration, illustrating that stress is an important factor to measure, define and quantify. Serum cortisol is an effective indicator of stress in adult horses, pigs and cattle (Furr et al 1992) but not foals (Yabana and Yachi 1988). Reports on the location of stress ulceration in equidae vary, from adjacent to the margo plicatus (racing thoroughbreds on feed deprivation) to the pyloric region of the glandular mucosa. Both steroid release and local ischemia have been implicated as possible factors involved in stress ulceration (Barker et al 1993).

Diet and ulceration: Diets can affect gastric ulceration in different ways. The frequency of feeding has been identified as an important factor in the formation of ulcers in the horse. A feed deprivation programme involving sequential 24 hour periods of starvation and hay *ad libitum* (Murray and Eichorn 1996), resulted in the formation of erosions and ulcerations in all individuals. These were found in the non-glandular region, adjacent to the margo plicatus on the lesser curvature. It was thought that the pathogenesis of ulceration in this situation was due to excess acidity, the implications of which suggest that regular feeding of stabled horses may be beneficial. Another study measuring the variation of gastric pH throughout the squamous mucosa found areas of increased acidity to correspond with those more prone to ulceration (Hammond 1990). Stomachs with more severe ulceration had

significantly lower pH values compared to those with mild or no lesions.

Physical and chemical properties of the food also influence gastric ulceration. Assessment of the carbohydrate composition and ulceration potential of diets was first investigated in pigs (Potkins and Lawrence 1989, 1992). Diets containing readily fermentable carbohydrates were found to produce increased levels of volatile fatty acids (VFAs) through bacterial fermentation in the stomach. These VFAs acted as weak acids both penetrating and acidifying the underlying non-glandular tissue. This disrupted sodium transport and cell volume regulation resulting in cell swelling and necrosis (Lang et al 1998, Argenzio and Eisemann 1996). Similar experiments in horses failed to find a positive correlation between the quantity of VFAs produced and the number and severity of non-glandular ulceration, perhaps due to the buffering capability of the diet was suggested as a reason for this discrepancy (Nadeau et al 2000).

In pigs, the particle size of the diet has an influence on non-glandular ulceration (Lang et al 1998), with finely ground diets affecting gastric emptying rates, which increases fluidity and mixing of contents within the stomach. This would disturb the finely tuned pH gradient that normally exists within the stomach, so exposing parts of the gastric mucosa to foreign acidity, initiating ulceration. This has not been investigated in the horse.

Bile reflux and ulceration: Damage to stratified squamous epithelium by gastric contents, has been found to increase when existing HCl combines with refluxing bile acids. In humans, research has shown that patients suffering from oesophageal reflux

disease have increased levels of bile acids in their postprandial gastric fluid (Schweitzer et al 1986). In horses, *in vitro* work found HCl combined with bile acids to be more injurious than HCl alone, sufficiently altering the electrolyte transport of the stratified squamous epithelium (Berschneider et al 1999). Similar gastric conditions can be found in the horse after a 14 hour feed deprivation protocol (Lang et al 1998).

Physical causes of ulceration: The relationship between exercise and intragastric pressure in the Thoroughbred horse was discussed at the 7th International Equine Colic Research Symposium in Manchester (2002). Preliminary and unpublished results found an increase in exercise intensity to correspond with increased intra-abdominal pressure and hence gastric compression. It was proposed that this compression might act to push acidic gastric contents into the non-glandular region of the stomach. Increased acid exposure would therefore occur during periods of continuous training, giving an explanation for the greater number of horses in active race training that are affected by this disease (Lorenzo-Figueras et al 2002).

1.4 CLASSIFYING GASTRITIS AND GASTRIC ULCERATION

Finding ways to classify gastric lesions is essential if the full range and variety of appearances present is to be realised and recorded accurately. It allows the correlation between clinical signs and severity of lesions to be tabulated, and helps in assessing the effectiveness of any treatments.

1.4.1 Gross classification of gastric lesions in animals

Before the invention of the endoscope, methods of gross observation relied on post mortem examination alone. In pigs, attempts at classification were made as early as 1926. However, it was not until the 1960s, when gastric ulceration was recognised as an important welfare and economical problem, that detailed recording began (Curtin et al 1963, Muggenburg et al 1964, Bivin et al 1974). In the stratified squamous region, gross lesions were defined according to their age: epithelial changes, acute erosions, subacute ulcers, chronic ulcers and scars. Only acute erosions in the fundic area were described in the glandular region. In a later study, epithelial changes were redefined into four degrees of hyperkeratosis and glandular lesions included pyloric and cardiac ulcers along with fundic erosions and ulcers. In 1997, a post mortem survey of finishing pigs added the presence of bile staining of the pars oesophagea and of the stomach contents as extra criteria to be recorded in the classification of gastritis (Guise et al 1997).

In the horse, endoscopy made it possible to develop a wealth of classification systems that detailed various combinations of ulcer severity, number, location and

depth. One of the earlier systems was similar to that of pig, recording lesions according to their age (Johnson et al 1994). Others allocated scores for lesion number and severity (Murray and Eichorn 1996), number and location (Vatistas et al 1999b), or severity and location (Hammond et al 1986), often scoring just lesions in the non-glandular mucosa, or the most severe lesion present. More complicated systems also existed, scoring squamous and glandular regions separately, but still combining lesion number and severity using a 10 point scale (Murray et al 1996).

In 1999, The Equine Gastric Ulcer Council realised the confusion being caused by these conflicting systems and the resulting difficulty in comparing results from different studies. To combat this they devised a 5 point scoring system combining information on the number, depth and severity of gastric lesions into a single score. It could be applied to lesions in both the squamous and glandular regions, and was considered user-friendly and detailed enough for use by practitioners and researchers alike.

For the purposes of this study, it was thought to be more accurate to record the lesion location, number and severity separately. Given that the squamous and glandular regions differ anatomically and physiologically, altering both the appearance and rate of lesion formation/regression, combining the scores of these two areas might lead to major inaccuracies. Similarly, a single score for lesion number, severity and location might lead to over/underestimation of lesions. As a result, a modification of an already existing system designed by MacAllister et al (1997) using a 4 and 5 point scale was chosen. This had separate categories for ulcer number, severity and location (squamous versus glandular), so recording the maximum amount of

information most accurately, using a numerical scoring system. It was designed by a group of five researchers already familiar with gastric disease in the horse for use in future studies. The grading system was established using slides of lesions as examples of the disease. Each person was then required to grade 16 stomachs using a video of the endoscope, and score variations between the different observers were recorded. The only parameter to vary significantly between observers was the number of non-glandular lesions, but it was thought that post mortem observation would be more accurate and so prevent this from happening.

1.4.2 Histopathological classification

In humans: Human medical research led the way in the development of histological classification systems for gastritis. Schindler (1947) was one of the first to correlate gross and microscopic appearances of gastritis using a gastroscope and intraoperative biopsy techniques. As fibre optics and target biopsies became more available, he developed his system to identify both acute and chronic forms of the disease. The latter he divided into superficial (with inflammation limited to the level of the gastric pits and surrounding lamina propria) and atrophic (loss of specialised glands) forms of gastritis. In 1972, a morphological classification recording four parameters from each case was published: mucosal type (antrum or corpus); grade of gastritis; activity (degree of neutrophil invasion); and presence of metaplasia (Whitehead et al 1972). A year later a topographical classification was attempted separating samples into A (corpus) or B (antrum) with AB being used to record a diffuse gastritis. Further modification of these models was frequent but acted to confuse rather than enhance diagnoses.

In 1991 Price reviewed the literature and concluded that while the ideal classification would relate to the aetiology of gastritis alone, this would not be practically possible. He went on to develop the Sydney System, which represented an amalgamation of already existing systems described by an Advisory Group of Pathologists in Sydney Australia (Price 1991). The new system managed to combine aetiology, topography and morphology by drawing details from aspects of previous classifications. Users were still not content and complained about the loss of popular descriptive terms for gastritis e.g. diffuse antral gastritis. Further modifications this time from Houston, Texas in September 1994, led to the development of The Updated Sydney Classification (Dixon et al 1996). This fulfilled all the required criteria and is now used worldwide as a reference for histological cases of gastritis. It has aided the discovery of other distinctive forms of gastritis that were previously unclassified. The information gleaned from each case allows accurate prognostic information due to the close link between patterns of inflammation and their disease associations.

It is recommended that five biopsies be taken from each patient for histopathological examination (two from the corpus, two from the antrum, and one from the incisura angularis). The degree of *H. pylori* density, neutrophil infiltration, chronic inflammation, atrophy (antrum or corpus separately or combined) and metaplasia are then assessed according to a visual analogue. Quantitative definitions are normal, mild, moderate or marked, and the use of special stains for *H. pylori* and the identification of intestinal metaplasia are recommended. Other non-graded variables to note include erosion or surface epithelial damage, presence of lymphoid follicles (100% of *H. pylori* infected individuals show evidence of follicle formation), foveolar hyperplasia, pseudopyloric metaplasia, pancreatic metaplasia and

endocrine cell hyperplasia. The pattern of gastritis should then be assessed and categorised as antral predominant/corpus predominant, if intestinal metaplasia is present whether it is multifocal or diffuse. It may be possible to predict a precise aetiology due to the close association of epidemiology with different topographical patterns. For example, chronic *H. pylori* infection produces a predominantly antral gastritis, or that a subset of those patients who develop a marked chronic inflammation within the antrum will go on to develop a duodenal ulcer. Finally, the classification should be given, listing an aetiological, topographical and morphological component e.g. active, antral predominant or chronic *H. pylori* gastritis.

This classification does not cover several types of special gastritis (acute, chemical, lymphocytic, granulomatous, eosinophilic), each of which has its own specific recognisable characteristics.

In animals: Attempts at histopathological classification of gastritis within the animal kingdom have also been made. These were first developed for pigs, where they took the form of written descriptions without specifically scoring any criteria (Bivin et al 1974, Muggenburg et al 1964, Rothenbacher 1965). The importance of histological examination of gross lesions in pigs was recognised by Embaye et al in 1990. It was found that reported changes in the gross appearance of the squamous epithelium related directly to the degree of hyperkeratosis histologically.

Since then, modifications of the Updated Sydney Classification System have been successfully adapted for use in dogs and cats (Hermanns et al 1995, Happonen et al

1998). In these animals it has been used to establish the normal histological appearance of the gastric mucosa, and to detect any influence HLOs might have on the cellular infiltrate or gastric epithelium. It has also been used to match cellular reactions in the ongoing search for a good experimental animal model for human disease.

In horses little is known about gastric histological changes in either healthy or diseased animals. The limits of normal histological variation within the non-glandular and glandular regions have not been tabulated. There is one paper (Murray et al 2001a) that attempts to illustrate epithelial changes associated with artificially induced acute ulceration in the non-glandular region. However, it only records measurements of thickness, omitting type and degree of cellular infiltrate present. The histopathology of chronic ulceration and any accompanying underlying inflammatory reaction has yet to be determined. This information could help significantly in the understanding of the pathogenesis of the disease.

1.5 INFECTIOUS CAUSES OF GASTRITIS AND GASTRIC ULCERATION

1.5.1 *Helicobacter sp.* and ulceration

In humans, Marshall chose the centenary of Koch's postulates to convince fellow research sceptics of the importance of *H. pylori* in causing chronic gastritis in humans. By resorting to self-infection with a heavily laden *H. pylori* broth, he rapidly experienced the tell-tale signs of nausea, vomiting and halitosis. Gastric biopsies confirmed the association of spiral bacteria with a primarily active inflammatory infiltrate, even prompting him to let his long suffering wife in on the nature of this dangerous experiment and the reason for his recent illness.

One of the earliest descriptions of spiral organisms in the stomach of mammals was given by Giulio Bizzozero on the 18th March, 1892. In a lecture at Turin's Medical Academy he "*found in all of the six canine stomachs I have examined a great number of cells of the glandular neck which contained 1–4 and even more spirilli in their protoplasm*". His own accurate illustrations show this, along with his observation that the spiral organisms in the glandular epithelium were associated with vacuolation of these cells. It was later considered that they were most likely to be *H. heilmannii* or *H. felis* (Figura and Oderda 1996). It was the first time that the presence of bacteria within a cell did not appear to be affecting its functioning, and there was an absence of clinical signs in the affected animal.

Extensive ongoing research in humans has shown that only a small percentage of

those infected with *H. pylori* display clinical signs of disease. *Helicobacter pylori*, originally *Campylobacter pyloridis,* is now regarded as the most common cause of chronic gastritis in humans and is also associated with gastric and duodenal ulceration, mucosa-associated lymphoid tissue (MALT) lymphoma, chronic inflammation and gastric cancer (Blom et al 2000). As a result of this it has been typed as a class 1 carcinogen by the World Health Organisation.

Microscopy of Gram-stained smears reveals a Gram-negative rod, which in humans is found between the mucus layer and surface epithelium of the stomach. It requires microaerobic conditions in which to grow and has several features enabling it to survive the adverse conditions in the human stomach. To aid colonisation and adhesion it has flagellae, which, with screw-like movements enable penetration of the mucin layer. It creates a neutral environment around itself by producing urease, enabling it to survive the acidic conditions. Outer membrane proteins and phospholipids aid adhesion to mucin and gastric epithelial cells. Tissue damage is achieved by the production of proteases (e.g. glycosulfatase), which degrade gastric mucin, and toxins (e.g. vacuolating cytotoxin A), which produce vacuoles in gastric epithelial cells, eventually destroying them. Its ability to transform into coccoid forms under adverse conditions is thought to allow extended survival e.g. in river water, so influencing future transmission of the organism (Mitchell 2001).

In developing countries infection of *H. pylori* mainly occurs during childhood and its prevalence is greatly influenced by socio-economic status i.e. increased levels of infection in areas with a high density of living and an absence of running water. There are three suggested routes for transmission. The oral-oral route is supported by

isolation of *H. pylori* from dental plaque and saliva in patients suffering from *H. pylori*-induced dyspepsia. There is also a high density of infection occurring in areas of Africa where mothers premasticate their children's food. Contradictory to this, dentists do not display increased levels of infection. The faecal-oral route is supported by the fact that faecal isolation of *H. pylori* DNA (Deoxyribonucleic acid) has been successful, though this does not necessarily mean that the *H. pylori* found is viable. Adaptation of the bacteria to a journey through the changing environments of the intestines is unlikely. Finally, the gastro-oral route of transmission is supported by isolation of *H. pylori* from vomitus.

Much work has been done in the attempt to label water sources as a potential source of *H. pylori.* Conflicting results ensue due to the unknown relevance of finding *H. pylori* DNA and the specificity of the Polymerase Chain Reaction (PCR) techniques used in new environments. No attempt to culture the bacteria from water sources has been successful (Mitchell 2001).

The zoonotic potential of *H. pylori* and the possibility of reservoirs of infection being present within the animal kingdom are also causes for concern. The first positive isolation of *H. pylori* in animals was from a colony of domestic cats obtained from a commercial vendor in 1994 (Handt et al 1994). A positive culture from six cats and histological recognition with fatty acid analysis and 16S rRNA (ribosomal ribonucleic acid) sequence analysis in 15 more was enough to confirm any suspicions of its presence. The location of the bacteria (proximal to mucosal epithelial cells and in mucus layers of glandular or surface epithelium) and its pathology (a mild to severe lymphoplasmacytic infiltrate) was similar to human

cases and further supported the possible use of the cat as a model for human disease (Esteves et al. 2000).

Other investigations include work by Dore et al (2001) who proposed that the high percentage (98%) of *H. pylori*-positive Sardinian shepherds was attributable to continual contact with their sheep and dogs (family members were only 73% positive). Identification of *H. pylori* using PCR (Polymerase chain reaction) and culturing, convinced him that sheep milk might be an intermediate transmission medium and that sheep could be the original host of *H. pylori.* (Mitchell 2001).

Reports of increased *H. pylori* infection in abattoir workers prompted a full scale investigation by Dimola and Caruso (1999) into its prevalence in cattle, horses, pigs, and rabbits in an abattoir. Chicken carcases were chilled for 24 hours before inspection. One sample was taken from the prepyloric region immediately after slaughter in the antral area of each animal's stomach. The density of HLOs present was scored on a scale of 1–5. A Warthin–Starry stain showed that 68% of the large animals had "rod shaped organisms similar to that commonly reported for *H. pylori*".The density was highest in the calves and pigs and immunohistochemical detection using polyclonal rabbit anti-HP was positive in these animals. Bacilli were only detected in 40% of the horses, and were found next to partially digested material and not in the mucus stream. No further diagnostic testing was done on these organisms. This is the only claim so far to have identified *H.pylori* histologically in the horse. A recent abstract using indirect methods has identified the presence of *Helicobacter* antibodies in serum samples of foals over 2 days old. This indicates the transmission of maternal antibodies, which change as the animal ages

and natural immunity takes over (Murray et al 2003).

1.5.2 The pathology of *H. pylori* gastritis

Following initial infection with *H. pylori*, a short-lived often asymptomatic acute phase reaction occurs in both the antrum and corpus of the stomach, which is seldom reported (Dixon 2001). After passing through the mucus layer, replication of the organism takes place next to the gastric epithelial layer. The release of lipopolysaccharides contained within the Gram negative cell envelope initiates polymorphonuclear infiltration and oedema of the lamina propria. The normal cascade of degranulating mast cells and further activation of inflammatory mediators then proceeds along with the *H pylori* specific production of IL8 (interleukin 8, a potent neutrophil chemokine interleukin). This is accompanied by a period of hypochlorhydria and the failure of secretion of ascorbic acid into the gastric acid produced.

Occasionally in children and a few adults, the host's immune response is capable of eliminating the infection at this point, with a resultant loss of inflammatory cells and a return to the normal histology. Generally, however, chronic inflammatory cells persist, neutrophils are lost, and the disease progresses to an active chronic gastritis.

Further amplification of the inflammatory reaction with cytokine and anti *H. pylori* antibody production leads to the release of mucosally protective Ig A antibodies. This follows B-cell lymphoid follicle formation encouraged by the remaining *H. pylori,* which acts as an antigenic stimulant. The histological picture at this point is said to be "active chronic". In children, the inflammatory response is minimal,

leading to a recognisable nodularity of the mucosa due to predominant follicle formation. This may be due to differing adhesion methods between *H. pylori* and gastric epithelial cells in children compared to adults (Blom et al 2000). Differences in the inflammatory picture post infection are also seen in various animal models. This may be due to both the strain of bacteria and the individual's response to infection, all of which must therefore be taken into account when examining the specimen.

There are two other main histological findings associated with *H. pylori* infection in humans: atrophy and metaplasia. Atrophy can be caused by repeated injury and indicates a loss of glandular tissue or specialised cells, which may be replaced by fibrosis. The injury may be the result of a physical insult e.g. erosion or ulceration extending to the glandular region of the stomach, or due to chronic inflammation, possibly induced by bacterial infection. With *H. pylori* infection, a preatrophic stage is possible where there is loss of parietal /chief cells without glandular destruction. Proposed mechanisms include *H. pylori* induced production of antibodies to react with antigens on foveolar cells and secretory canaliculi of the parietal cells, creating a self-perpetuating autoimmune reaction. The degree of atrophy increases over time but is not associated with the change in age of the patient.

Atrophy may be followed by metaplasia of the cells, where one fully differentiated cell is replaced by another more suitable to the existing environment. Intestinal metaplasia refers specifically to the change of gastric epithelial cells being replaced by small /large intestinal cells. It is commonly found in cases of chronic gastritis and parallels atrophy development. It is more frequently found in patients infected with

H. pylori and increases in severity with age. It is found more commonly in the antrum than the corpus of the stomach and is associated with gastric rather than pyloric or duodenal ulceration.

1.5.3 Animal models of human disease

The search for an animal model to illustrate and further explain the pathogenesis of *H. pylori* induced gastritis in humans has resulted in several trials. Ideally the model should mimic the pathogenesis in humans as closely as possible, with an early acute phase gastritis followed by a chronic active phase with atrophy and gland metaplasia. Other more practical considerations include size and ease of manipulation of the animal (for endoscopy), expense, temperament and successful colonisation after introduction of human strain of *H. pylori.* The most recent model uses conventional piglets (Poutahidis et al 2001) previously disregarded due to difficulties in infecting them with *H. pylori.* This was overcome by administering cimetidine (to reduce acid secretion), and dexamethasone (to delay immune response and increase urea production via protein synthesis promotion) prior to inoculation. The timing of inoculation second day post weaning coincided with the disappearance of *Lactobacillus sp.*, which can inhibit growth of *H. pylori.* Existing microflora (*Lactobacillus*) made cultural isolation of the organism impossible due to overgrowth.

1.5.4 *Helicobacter* species in other animals

In contrast to *H. pylori, H. heilmannii* has a very wide host range having already been detected in dogs, cats, monkeys, pigs and humans. In a study on 60 pigs from

six farms in Western France (Cantet et al 1999) there was an 86% infection rate with *H. heilmannii.* Detection was by a combination of PCR and histological techniques. Out of those infected, 10% showed ulcerous lesions of the pars oesophagea and there was an increased frequency of lymphoid aggregations present. Roosendaal et al (2000) attempted to classify the species of *Helicobacter* present in 122 pig stomachs through microscopy, culture and PCR. They found *H. heilmannii* to be the main species present. Its predominance in the glandular region of the stomach (as opposed to the pars oesophagea), led to suggestions of an *H. heilmannii* induced G (gastrin) cell hyperfunction, or direct parietal cell influence to explain the presence of ulceration in the non-glandular area. The bacterial species is extremely acid-resistant enabling parietal cells to be colonised. In attempts to investigate its pathogenesis, one group of gnotobiotic piglets was fed a diet containing commensal bacteria and a high-carbohydrate diet. The second group was fed the same diet with *H. heilmannii* and failed to develop gastric ulcers, suggesting that this *Helicobacter* species does not have a primary role in ulcer pathogenesis (Krakowka et al 1998).

In humans the incidence of infection with *H. heilmannii* is low (0.1% of 7,926 symptomatic patients examined in Italy, 0.3% overall average) and in the event of infection a lower grade of gastritis is produced with less evidence of glandular atrophy and metaplasia than with *H. pylori.* Mixed infections are uncommon suggesting competitive colonisation (de Groote et al 2000). In this study a distinctive picture involving lymphocyte exudation in gastric foveolae was also observed. Its association with low-grade MALT lymphoma is higher than *H. pylori*, but antimicrobial therapy can lead to remission.

It is likely that many of the first *Helicobacter* species to be observed (Bizzozero 1892 cited by Figura and Ordera 1996) were in fact *H. heilmannii,* but in those days there was no definite way of determining this. A thorough investigation into species found in healthy and affected dogs was undertaken in 1998 (Happonen 1998), when *Helicobacter* species were detected in 100% of the healthy and 95% of the affected dogs. They were found least frequently in the antral area and were assumed to be part of the normal gastric flora. Those isolated were *H. bizzozeronii, H. felis* and *H. salomonis,* all of which are indistinguishable from *H. heilmannii* when 16s rRNA sequencing is compared (de Groote et al 2000). There was no significance given to the degree of bacterial colonisation and gastritis score. An earlier investigation (Hermanns et al 1995) observing colonisation of HLOs in dogs and cats found infection present in 82% of dogs and 76% of cats. It was also discovered that in these cats only, there was a relationship between degree of HLO colonisation and histopathological changes.

H. felis was isolated in pure culture for the first time in 1988 (Lee et al 1988) and is very sensitive to acid, so restricting colonisation to the antrum and cardia. In its natural hosts (dog and cat) it can induce a mild gastritis with lymphofollicular hyperplasia. A recent report of gastroduodenal ulceration in eight cats (Liptak et al 2002) mentions HLO in only one case with these organisms being found in crypts surrounding the duodenal ulcers. Due to the lack of inflammation adjacent to the ulcers a non-infectious process was assumed. The histopathology in the rest of the stomach was not mentioned.

1.5.5 Humans and the zoonotic potential of *Helicobacter* species

This issue is addressed in a review by de Groote et al (2000) where they highlight numerous occasions that animal helicobacters have been recovered from human stomachs. A link between human infection and animal contact has been established by Stolte et al (1994) where a survey of 125 people infected with *H. heilmannii* showed 70.3% had contact with one or more animals (compared to 37% in the normal population). Dieterich et al (1998) reported that a 38 year old man suffering from multiple antral ulcers was infected with a *H. heilmannii* strain identical to one found in his cat. It is uncertain whether man or cat acquired the infection first, but it does illustrate that *H. heilmannii* is capable of cross species transmission.

This thesis explores the association between equine gastric ulceration and gastritis, while the search for an HLO is prompted by the central role *H. pylori* plays in the pathogenesis of gastritis and related diseases in humans.

CHAPTER 2 – GROSS FINDINGS

2.1 INTRODUCTION

This chapter describes the occurrence and location of gastritis and gastric ulceration in a mixed population of horses in the UK. Using post mortem material, the lesion number, severity and location was systematically recorded for each stomach. Associations between breed, sex and existing disease of the horse were also investigated. All the horses were euthanased for reasons unrelated to this study, and the investigation was sanctioned by the ethics and welfare committee of the Veterinary Faculty.

A Swedish post mortem study on a mixed population of 3715 horses (Sandin et al 2000), found 10.3% to have evidence of gastric ulceration/ erosion. A majority of these lesions were located along the margo plicatus and measured less than 1cm. Animals with disease involving the digestive organs had a higher incidence of gastric ulceration/erosion. Much of the existing literature on gastritis/gastric ulceration concentrates on the high performance horse, probably because in these animals, both clinical and economical implications warrant further research. A thorough knowledge of the true incidence and nature of the disease in other horse populations is not available, and could add to the understanding of its pathogenesis.

In the live horse, endoscopy is the primary method available for identification of gastric lesions. It has practical limitations including impaired visibility due to excess ingesta and poor accessibility to some areas of the stomach (Murray et al 2001b). A

recent experiment found endoscopy to underestimate both the presence of glandular, and the severity of non-glandular gastric ulcers as determined pathologically (Andrews 2002). Much of the published data on gastric ulceration refers to endoscopic findings, and may therefore be inaccurate. The use of post mortem material facilitates detailed inspection of all areas of gastric mucosa and was therefore deemed appropriate for this investigation.

The results of the gross post mortem examination were recorded using photographs and hand drawn stomach maps depicting lesion types and location. From these, detailed written descriptions were made of each stomach and a scoring system applied to both the squamous and glandular regions of the stomach. Scoring systems for gastric ulceration were first developed in man as a method for recording endoscopic results numerically. The aim was to minimise individual variability between recorders and allow accurate and consistent documentation. The application of such a system to the horse would enable efficient comparison of results between cases and establish the presence of any trends or rarities that were present. Several such systems have been developed for use in the live horse, and a modification of one of these is used in this study.

2.2 MATERIALS AND METHODS

All the horses used in this study were presented to Glasgow University Veterinary School between October 2001 and May 2002. Euthanasia was chosen due to the hopeless prognoses given for their survival or return to use. Horses that had been dead for over two hours before samples were taken were not included in this study

due to the possibility of post mortem artefact. All animals included in this study were over 9 months of age so avoiding the analysis of unpredictable age related histological changes in the gastric epithelium (Murray and Mahaffey 1993).

2.2.1 General Post Mortem Procedure

Each horse that was submitted for post mortem examination was necropsied in a similar fashion by the author. Individual clinical histories were recorded when available. Euthanasia was achieved through the use of a free bullet or the intravenous administration of a lethal dose of barbiturate. The latter was performed in the case of a suspected CNS lesion (144439). A minority of the horses arrived already dead due to euthanasia immediately following surgery (143791, 145216, 143863).

If possible, immediately after death the carcass was suspended from its hind legs and bled out via an incision made into the internal carotid artery at the thoracic inlet. The carcass was then lowered and a thorough inspection made of the ears, eyes, nares, buccal cavity, external genitalia and the integument. It was then re-hung from the fore and hind left legs and a subcutaneous incision made from the angle of the mandible along the ventral midline to the anus. The hyoid apparatus was transected and the trachea and oesophagus exteriorised. A cleaver was used to split both the sternum and the pelvic symphysis and all internal organs were examined in situ. The rectum was freed from surrounding musculature and the diaphragm and the root of mesentery transected, allowing exteriorisation of all the organs.

The stomach was separated from its surrounding viscera by cutting its attachments to the greater omentum along the greater curvature, the diaphragm at the cardia, the

gastrophrenic and gastrosplenic ligaments and the hepatogastric ligament, which runs from the lesser curvature to the liver. The oesophagus was stripped from the trachea and transection of the duodenum occurred a foot from the pylorus. Detailed procedures used in inspection of the stomach are given in **2.2.3.**

The oesophagus was opened and the mucosal surface inspected. Examination of the respiratory tract involved exposure of the tracheal and bronchi lumen, and incision into several areas of the lung parenchyma. The pericardium was removed and cuts made into both left and right atrial and ventricular chambers of the heart following the blood supply. Both thyroids were also examined.

The liver was detached and the diaphragmatic and visceral surfaces were inspected both visually and manually. A similar procedure was adopted with the spleen, kidneys, adrenals and coeliacomesenteric ganglia, with full thickness incisions being made into all tissues.

All mesenteric attachments along the length of remaining gut and the bands connecting areas of caecum, colon and small intestines were sectioned. The intestines (from proximal duodenum to rectum) were laid out in a linear fashion and routine samples for histology taken from the jejunum (4m distal to the pylorus), ileum (0.75m proximal to the caecum), ventral colon, dorsal colon, small colon and rectum. The entire mucosal surface was inspected closely for evidence of parasitism, ulceration or any other areas of irregularity.

All lymph nodes were inspected visually with incision only if enlarged or abnormal in appearance. The brain and pituitary gland were only inspected if there was

reason to believe clinically that a lesion might be present.

Dissection of limbs was only undertaken if there was any clinical evidence of disease, or something grossly abnormal was detected during external inspection of the carcass.

The stomach was always removed from the carcass as soon as possible to keep post mortem change to a minimum. The average time taken for the entire necropsy varied between 1 and 3 hours depending on the degree and location of pathology present.

2.2.2 Gross Inspection of the Stomach

The stomach was removed from the carcass as described in 2.2.1. The tip of a sharp knife was inserted at the pylorus and used to cut through the stomach wall along the greater curvature. The contents were rinsed gently away with cold water and any fat or tissue tags adherent to the serosal surface trimmed with scissors. It was then placed mucosal surface up, as flat as possible onto a blue board for photography. All handling of the stomach was kept to a minimum in order to prevent unnecessary trauma to the epithelium for future histological observation.

One photograph recorded the stomach as a whole, while some erosions/ ulcers were captured individually in more detail. The locations and description of appearance of all areas of hyperaemia/ erosions/ ulcers / were also noted down manually on a cartoon map of the stomach. All this information was used to compile a detailed written description of each stomach (Appendix 1).

2.2.3 Analysis of Results

The Scoring system

In order to facilitate result analysis and allow case comparisons, a number/severity scoring system was used to give each stomach a numerical gastric ulcer score, Tables 1 and 2. The system used was based on that designed by MacAllister et al. 1997, as it was considered to be the scoring system which would lead to the least experimental error.

The number of lesions in each stomach was scored on a scale of 0-4, and assessed separately for non-glandular and glandular regions. The score was dependent on both the number and individual extent of each lesion.

The severity of the lesions was scored on a scale of 0-5, again with separate assessment for non-glandular and glandular regions. A more detailed written description by the same author of how to actually assess the severity score for each stomach was consulted when necessary (Table 3).

Table1:The MacAllister gastric lesion number score

Number score	Description
0	0 lesions
1	1-2 localised lesions
2	3-5 localised lesions
3	6-10 lesions
4	> 10 lesions or diffuse (very large) lesions

Table 2:The MacAllister gastric lesion severity score.

Severity score	Description
0	No lesion
1	Appears superficial (only mucosa missing)
2	Deeper than 1 and includes deeper structures
3	Multiple lesions and variable severity (1,2 and/or 4)
4	Same as 2 and has active appearance (active = hyperaemic and/or darkened lesion crater)
5	Same as 4 plus active haemorrhage or adherent blood clot

Table 3:MacAllister's guide for detailed severity score assessment

Severity Score	Description
1	Superficial lesions with the possible involvement only of the mucosa. These lesions do not have raised edges or dark and hyperaemic ulcer craters. Typically the interior of the lesion has a pink appearance. These lesions may have evidence of mild haemorrhage without increasing the severity score.
2	Lesions appear deeper than number 1 severity and probably include structures deeper to the mucosa. These lesions typically have raised edges and the ulcer crater usually has a pink granulation tissue – like appearance.
3	Used for those stomachs with multiple lesions that are of different severity. To receive a severity score of three there must be at least one lesion present that would receive a score of four.
4	Is for those stomachs having primarily lesions that obviously have involvement of structures deep to the mucosa and have an active appearance. Active appearance is typically a hyperaemic or darkened, necrotic appearing lesion crater
5	Recorded least often but must have the severity of lesion score 4 but also have active haemorrhage or adherent blood clots.

Statistical Analysis

A Spearman Correlation Coefficient was used to assess the relationships between gross number and severity of lesions in the squamous and glandular regions. These values were also compared with the breed (Thoroughbred vs. non-Thoroughbred) and disease (gastrointestinal disease and other) of each animal using a Wilcoxon rank-sum test. A two sample t test was used and the significance level was set at 0.05% for both procedures. The Kolmogorov–Smirnov test was applied to compare the distribution of the data between the groups.

2.3 RESULTS

2.3.1 Clinical details

21 horses over the age of nine months were examined for this study, the clinical diagnoses of which are shown in Table 4.

Further details of the clinical history are given in Appendix 1 under the case number of each individual horse. Accurate records of medical treatment prior to death were not always available. In particular information about the exact dose and frequency of administration of known gastric irritant drugs (e.g. phenylbutazone and flunixin meglumine) was often absent. Such details as are available are listed in Appendix 1.

Out of the 21 horses examined, the most common breed was Thoroughbred/Arab (nine horses). There were five riding horses (possible TB crosses), five ponies (one of which was a Welsh Mountain) one Clydesdale and one Dutch Warmblood. Eight of the horses were mares, eleven were geldings and two were stallions. All

horses were adult and ranged in age from 9 months to 26 years and had a variety of uses. These included hacking, rescue ponies, competition horses and Riding for the Disabled.

There was a wide range of clinical diagnoses, none of which included gastric ulceration as a primary problem. They were put into the following categories: lameness (7), gastrointestinal disease (GID) (6), cardiac (3), central nervous system (CNS) (1), dental (2) and other (2) (haemangiosarcoma and head shaker) Table 4. More detail regarding clinical histories is given in Table 5.

Table 4:Summary of Disease Categories for the 21 horses examined.

Disease	No.Horses
Lameness	7
GID	6
Cardiac	3
CNS	1
Dental	2
Other	2

Table 5:Individual Case Details

Case Number	Breed	Age	Sex	Usage	Clinical diagnosis
133191	Pony	Aged	F	Riding for Disabled	Haemangiosarcoma
138643	Thoroughbred	7	MN	Hacking	Suspensory Ligament Desmitis
139923	Thoroughbred	5	MN	Competition	Tricuspid regurgitation
140373	Riding horse	14	F	Hacking	P1 fracture
140459	Riding horse	5	MN	Hacking	Head Shaker
143696	Welsh mountain pony	9 months	M	Breeding	Ventricular septal defect
143791	Riding horse	13	F	Hacking	Caecal rupture
143818	Pony	3	MN	Rescue pony	Tooth abnormality
143819	Pony	26	F	Rescue pony	Worn teeth
143863	Thoroughbred	17	F	Brood mare	Strangulating lipoma
143989	Thoroughbred	19	F	Hacking	Navicular bursitis
144056	Clydesdale	1yr 9mth	M	Showing	Cardiac failure
144095	Riding horse	6	MN	Hacking	P1 fracture
144241	Dutch Warmblood			Breeding	Complex tenosynovitis
144292	Thoroughbred	10	MN	Competition	Navicular syndrome
144439	Arab	15	F	Hacking	Meningioma
144468	Riding horse	8	MN	Hacking	Eosinophilic enteritis
144476	Thoroughbred	11	MN	Eventer	Grass sickness
145037	Pony	3	MN	Riding pony	Grass sickness
145216	Thoroughbred	5	F	Competition	Colonic torsion
145235	Arab	10	MN	Hacking	Navicular bursitis

2.3.2 Pathological details

The post mortem findings mostly confirmed the clinical diagnoses and in general, the decision for euthanasia was justified by the pathology found. However in the case of 140459, the head shaker, there were no explanatory lesions apart from the gastric ulceration on which to blame this abnormal behaviour.

No parasitic larvae were visible in any of the stomachs, although 144292 showed two raised plaque like lesions within the cardiac gland region, possibly consistent with a nematode infection. The stomach content of those horses which had not been suffering from gastrointestinal disease was sweet smelling (fresh tobacco), moist and firm (it held its shape when the stomach wall was incised and peeled back). In contrast, some of those which had undergone intestinal surgery or were affected with grass sickness had sloppy foul smelling gastric contents.

2.3.3 The gastric lesions present.

The normal appearance of the stratified squamous region of the stomach was assumed to be a smooth, white glistening surface, occasionally thickened and rolled over in the region of the margo plicatus at the lesser curvature. The glandular region varied in hue according to glandular type, but within these regions a uniform colour was considered normal (Fig 1).

A full detailed written description of each stomach can be found in Appendix 1. Several specific abnormal appearances of the gastric mucosa were seen to

occur in more than one stomach. They were categorised according to their location and appearance and are described below.

Hyperkeratosis: This lesion was found in seven stomachs and took the form of patches of exaggerated corrugation, thickening and yellow staining of the non-glandular epithelium (Fig.2). In more severe cases, the surface layer was friable and easily removed with light friction. These sometimes diffuse areas were generally located towards the greater curvature of the stomach close to the margo plicatus , although there was extension towards the cardiac sphincter in one case. There was no scope to record these lesions numerically according to the MacAllister system.

Punctate scars: Twelve stomachs displayed scatterings of punctate dark brown/yellowed scars in the squamous region (Figs.3 and 4). These measured 1-3mm in diameter and varied in depth from almost flat to shallow depressions. In general, they were located away from the margo plicatus towards the cardiac sphincter and the greater and lesser curvatures. Rarely, these scars were surrounded by a shallow ring of pale keratinised epithelium but there was no evidence of central granulation tissue. These lesions were given a severity score of 1 according to the MacAllister scoring system.

Diffuse erosions : These were only present in two stomachs. Here there were diffuse areas of erosion in the squamous area, primarily on the lesser curvature towards the cardia (Fig.5). This eroded squamous epithelium resembled glandular epithelium due to the smooth pink appearance but contained proud

islets of squamous tissue (Fig.6). In one horse the epithelium was markedly hyperaemic and almost scalded in comparison to the other which was paler with more extensive islets of normal squamous epithelium (Fig.7). These lesions were given a MacAllister severity score of 1 as only superficial structures appeared to be involved, but a high numerical score due to the extensive area covered.

Margo injuria: The margo plicatus had an abnormal appearance in 13/21 of the horse stomachs examined. The lesser curvature was affected in 11 of these. The margo plicatus was often uneven and wavy with a moth eaten appearance (Fig. 8). This was due to small tongues of non-glandular and glandular epithelium interdigitating with each other.

Lesions ranged in severity from raised bumps of squamous mucosa with little evidence of erosion, to profuse nodular squamous proliferations containing central cores of erosion and granulation tissue/ possible glandular epithelium (Fig.9). In 143696, a 2cm round yellowed polyp was attached at this location (Fig.10). In some stomachs, foci of erosion/ulceration lay in the adjacent glandular and non-glandular epithelium. They took the form of punched out lesions, some being raw shallow erosions with hyperaemic craters, while others appeared deeper and contained central necrotic debris with a ring of pale keratinised epithelium (Fig.11). Severity scores for these lesions were variable depending on the individual depths of the lesions.

Glandular region

Glandular lesions ranged in severity from areas of localised hyperaemia, to

large ulcers with active haemorrhage. The pyloric gland region at the pylorus was the most commonly affected area.

Hyperaemia: Hyperaemia of the glandular area was found in 10 of the stomachs examined. It occurred on its own and in conjunction with erosions and ulcers, most frequently seen as a blushing of the rugal folds in the vicinity of the pylorus (Fig.12).

Focal Erosions: 9 stomachs showed points of shallow erosion in the cardiac, fundic and most frequently the pyloric gland regions. They were seen as shallow circular coalescing depressions, hyperaemic in colour generally lying along the crest of the rugal folds (Fig.13) occasionally perpendicular to them (Fig.14). In the cardiac gland region they took the form of focal blanched 0.5cm circular lesions dotted along the middle of the cardiac gland area at the greater curvature (Fig.15). Pale blistering lesions were also noted in the pyloric gland region (Fig.16).

Ulceration: Four stomachs showed evidence of active ulceration. One displayed obvious damage in each of the glandular regions (Fig 17). In the cardiac and fundic gland areas, they took the form of multiple, deep, ellipsoid ulcers, varying in length from 1-3cm (Fig. 18). Their depth may have appeared exaggerated due to the apparent pale thickening around the circumference of some lesions (they were accompanied by shallow erosions towards the pylorus). Three displayed ulceration in the pyloric gland region only, varying in size from 1–6cm long, some involving an entire rugal fold which was thickened and oedematous along

with diffuse ulceration along the crest (Figs.19,20 and 21). Another showed evidence of recent haemorrhage in the fundic gland area (Fig.22), but this masked any underlying lesions preventing a detailed description.

The gross findings are summarised in Table 6.

Table 6: Summary of gross gastric lesions

Case Number	Hyperkeratosis	Punctate lesions	Diffuse erosions	Margo injuria	Glandular hyperaemia	Glandular erosion	Glandular ulceration
133191	yes	yes	no	yes	no	no	no
138643	yes	yes	no	yes	yes	yes	no
139923	no	yes	no	yes	yes	yes	no
140373	no	no	no	no	no	yes	yes
140459	no	yes	no	yes	no	yes	yes
143696	no	no	no	yes	no	no	no
143791	no	no	yes	no	yes	yes	no
143818	no	yes	no	yes	yes	yes	no
143819	no	yes	no	no	yes	yes	no
143863	yes	yes	no	yes	no	no	no
143989	no	no	no	yes	no	yes	yes
144056	no	no	no	yes	yes	yes	no
144095	no	no	no	yes	no	no	no
144241	yes	yes	no	yes	no	no	no
144292	no	no	no	no	no	yes	no
144439	no	no	no	yes	yes	yes	no
144468	yes	yes	no	no	no	no	no
144476	no	yes	no	yes	no	no	no
145037	yes	yes	yes	no	yes	yes	no
145216	yes	yes	no	no	yes	no	yes
145235	no	no	no	no	yes	no	no
Total	7	12	2	13	10	8	4

2.3.4 Application of gross scoring system

The MacAllister scoring system was applied to both the squamous and glandular regions of each stomach and the following scores were given (Table 7). Specific categories (Thoroughbreds only and those with Gastrointestinal disease) were then analysed and compared to the scores of the remaining horses. (Table 8 and 9).

Table 7 : Individual gastric ulcer scores

Case Number	Sq No.	Sq sev	GI No.	GI sev.
145235	0	0	0	0
144468	4	1	0	0
140373	0	0	1	5
144292	0	0	3	1
133191	1	1	0	0
143696	2	1	0	0
144095	1	1	0	0
139923	2	1	1	1
144439	1	1	2	1
140459	1	1	4	5
143989	3	4	4	5
143863	2	1	0	0
143819	4	1	2	1
143818	2	1	2	2
138643	3	2	1	1
144241	3	1	0	0
144476	2	4	0	0
143791	4	2	1	1
144056	4	1	1	1
145216	4	1	4	5
145037	4	1	1	1
Total	47	26	27	30

According to this system, only one out of the 21 stomachs examined had no evidence of gross ulceration at all. Seven had evidence of squamous injury only, and two glandular only. The overall score for the number of ulcers was greater in the squamous area (47 total) than the glandular area (27 total). The severity of ulceration was quite similar in the glandular (30 total) and non-glandular (26 total) region. The mean gross squamous number was 2.24, almost double that of the mean gross

glandular number (1.29). The mean gross glandular and squamous severity were similar (1.43 and 1.23 respectively).

Breed

Table 8 : Gross scores for Thoroughbreds

Case Number	Squamous number	Squamous severity	Glandular number	Glandular severity
144292	0	0	3	1
139923	2	1	1	1
143989	3	4	4	5
143863	2	1	0	0
138643	3	2	1	1
144476	2	4	0	0
145216	4	1	4	5
Total	16	13	13	13

All Thoroughbreds (TB) had evidence of gastric ulceration, two in the squamous and one only in the glandular region. Both the squamous and glandular score for numbers of ulcers ranged from 0–4 .The range in severity score of the squamous ulcers was 0–4, but reached 0–5 in the glandular region. Comparison of TB mean values found the squamous and glandular scores for number and severity of ulceration to be almost the same. The remaining breeds had similar scores for the number of squamous ulcers (2.29TB vs.2.21) but there was a reduction in squamous severity, glandular number and glandular severity (1.9TB vs. 0.93, 2 TB vs. 1, 2TB vs. 1.2) compared to the Thoroughbreds. This data is summarised in Table 8.

Disease

The mean score values of horses with a clinical diagnosis of gastrointestinal disease (GID) were compared with the rest of the population (Table 9). There was a higher score for the number of squamous ulcers (3.3 GID vs. 1.8) but the other values were

similar (sq sev. 1.7 GID vs.1,gland no. 1.2 GID vs. 1.4, gland sev. 1.3 GID vs. 1.5).

Table 9: Gross scores for Gastrointestinal disease

Case Number	Squamous number	Squamous severity	Glandular number	Glandular severity
144468	4	1	0	0
143863	2	1	0	0
144476	2	4	0	0
143791	4	2	1	1
145216	4	1	4	5
145037	4	1	1	1
Total	20	10	6	7

Statistical analysis

On application of the Spearman Correlation Coefficient, positive correlations were found between gross squamous number and gross squamous severity (probability = 0.0134) and gross glandular number and gross glandular severity (probability = <0.0001). In order to compare the effect of various physical attributes (breed, disease) on ulcer number and severity between horses, a Wilcoxon two sample test was applied. There were no significant correlations found between the breed of the animal (TB / not TB) and gross lesions although gross squamous severity and gross glandular number were found to have an almost significant positive correlation with being Thoroughbred (0.0912 and 0.0945 respectively). Horses with gastrointestinal disease were found to have a positive correlation between GI disease and the gross squamous number of lesions and almost with the gross squamous severity (prob = 0.0963) but no positive correlation with the gross glandular number or severity.

The Kolmogorov-Smirnov test showed there to be no difference in the spread of data between the two groups.

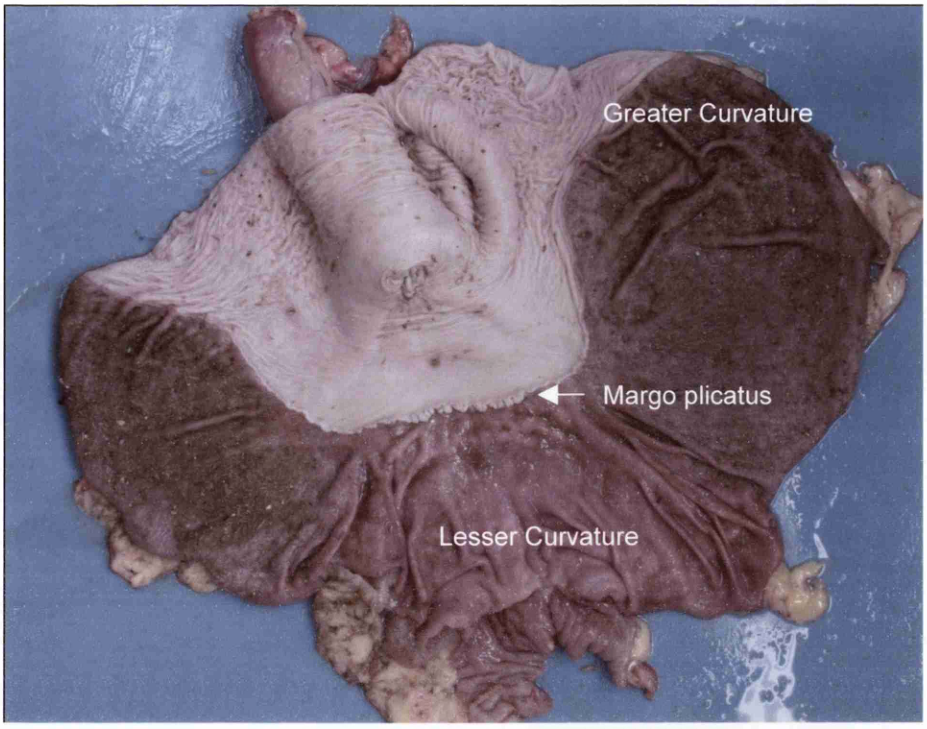


Figure 1. Case number 145235 : a normal stomach.

Figure 2. Case number 133191: hyperkeratosis (arrows) in the region of the greater curvature

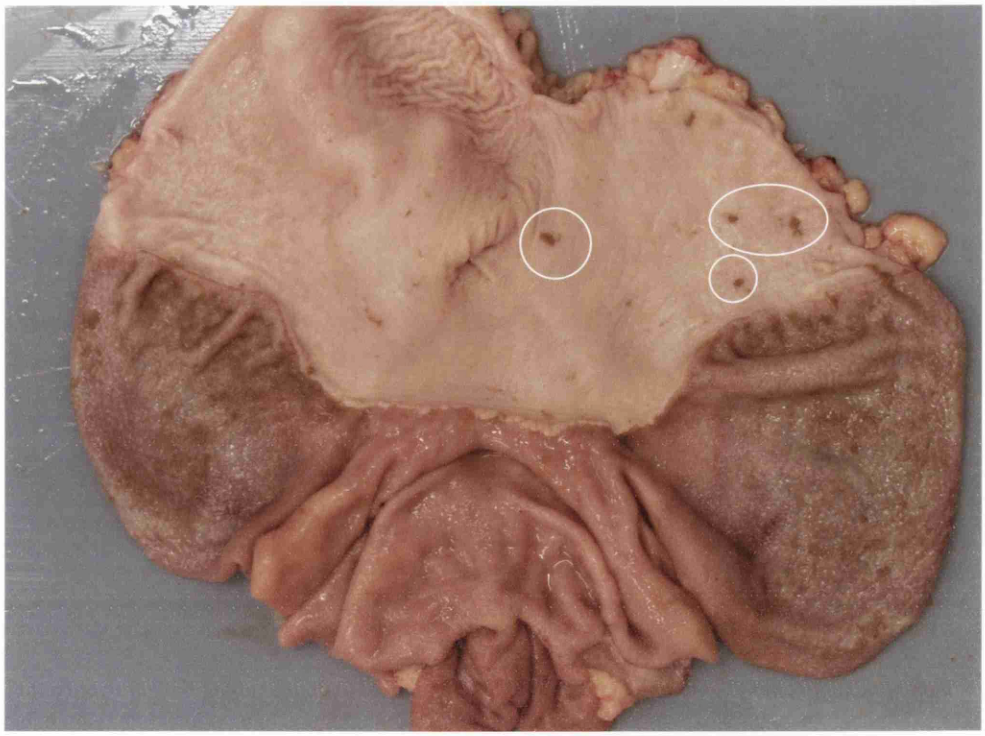


Figure 3. Case number 143819: scattered punctate scars within the squamous region (white circles).

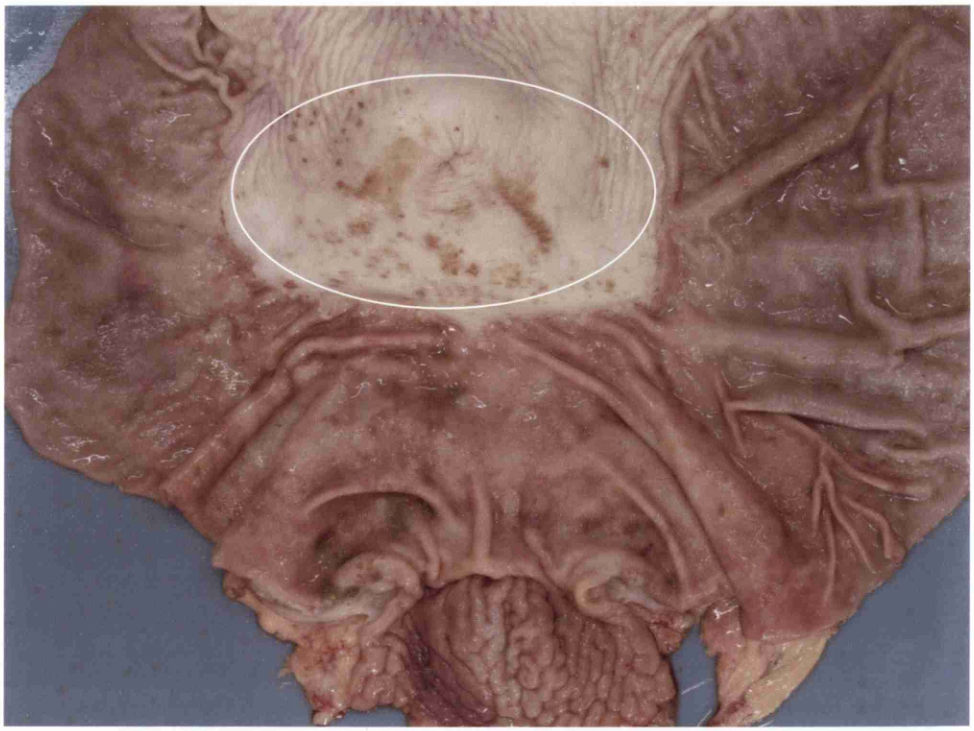


Figure 4. Case number 144056: marked punctate scars within the squamous region (white circle).

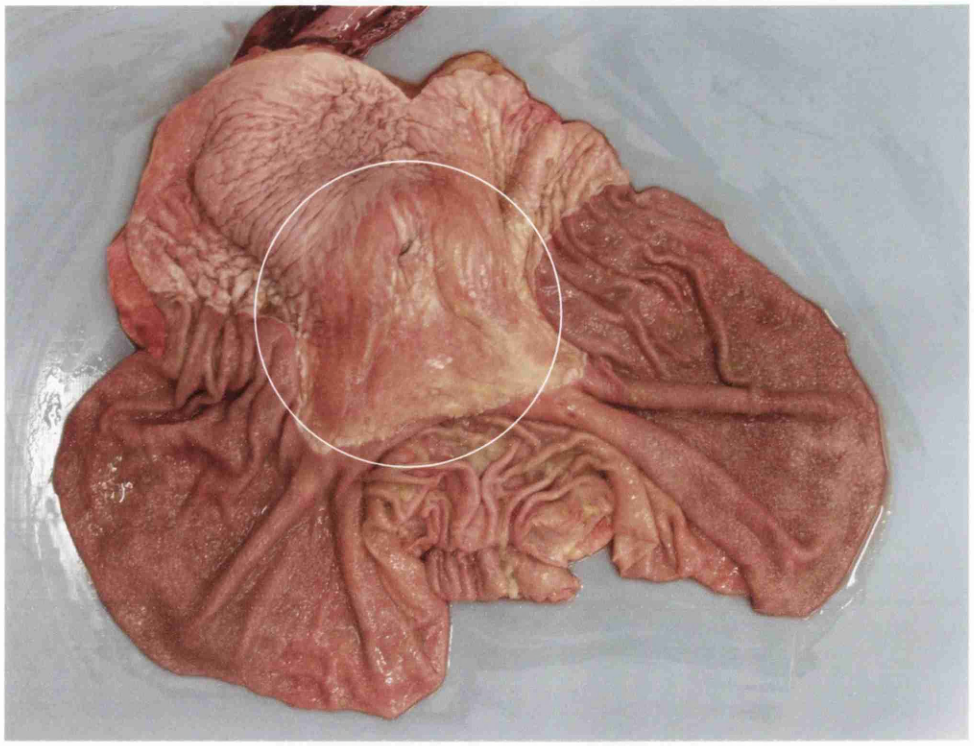


Figure 5. Case number 143791: note the diffuse area of hyperaemic scalding and erosion involving the lesser curvature of the stratified squamous region.

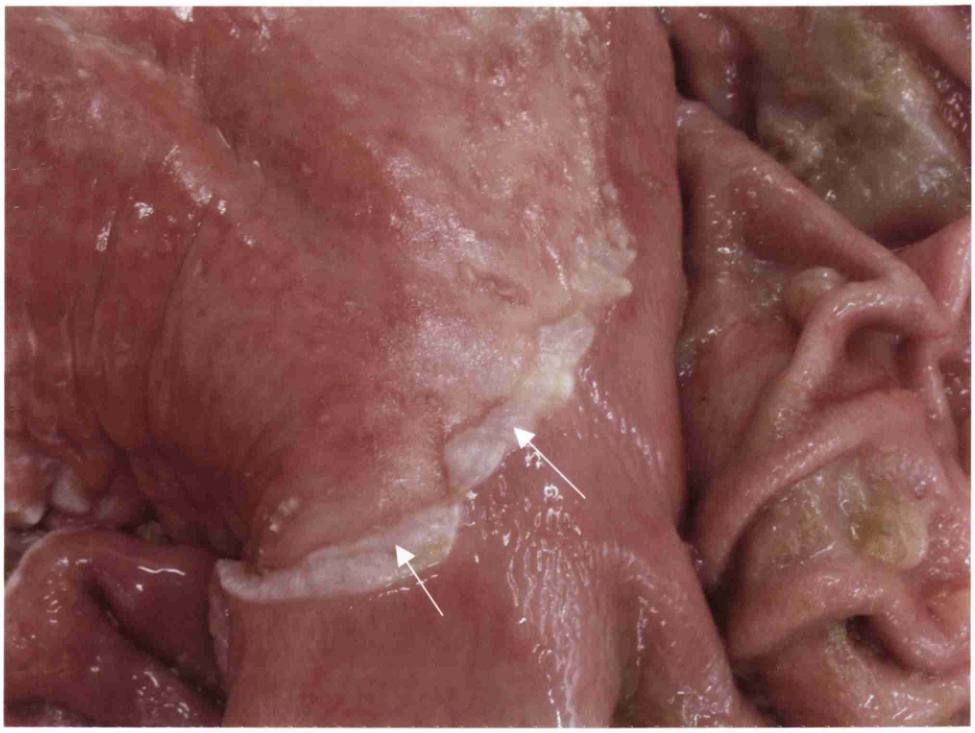


Figure 6. Case number 143791: detail of diffuse erosion, note intact squamous islands (arrows).

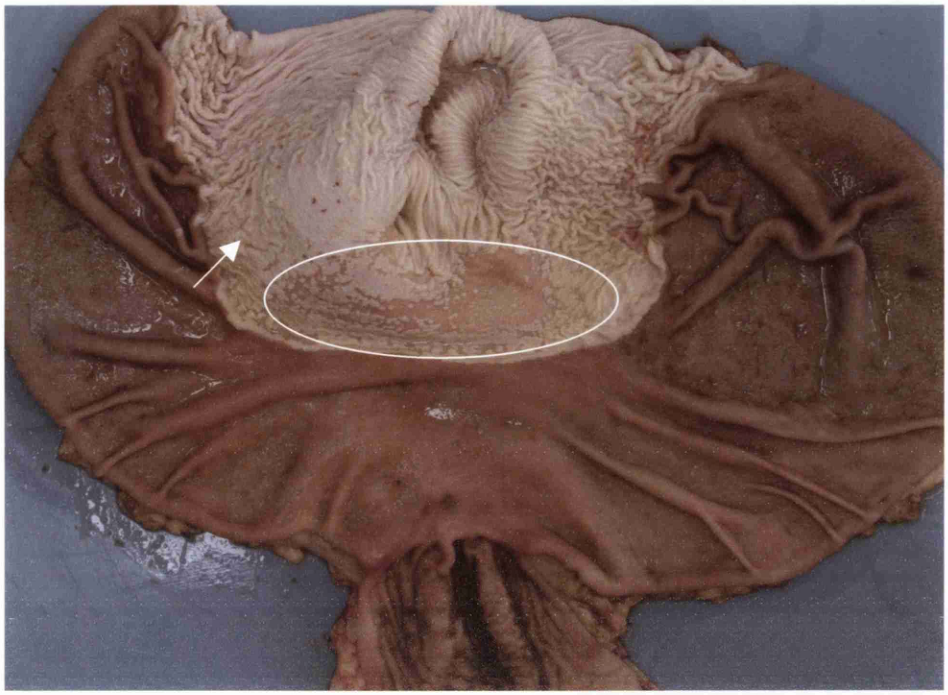


Figure 7. Case number 145037: diffuse erosion on lesser curvature (circle). Note yellow bile stained hyperkeratotic area (arrow).

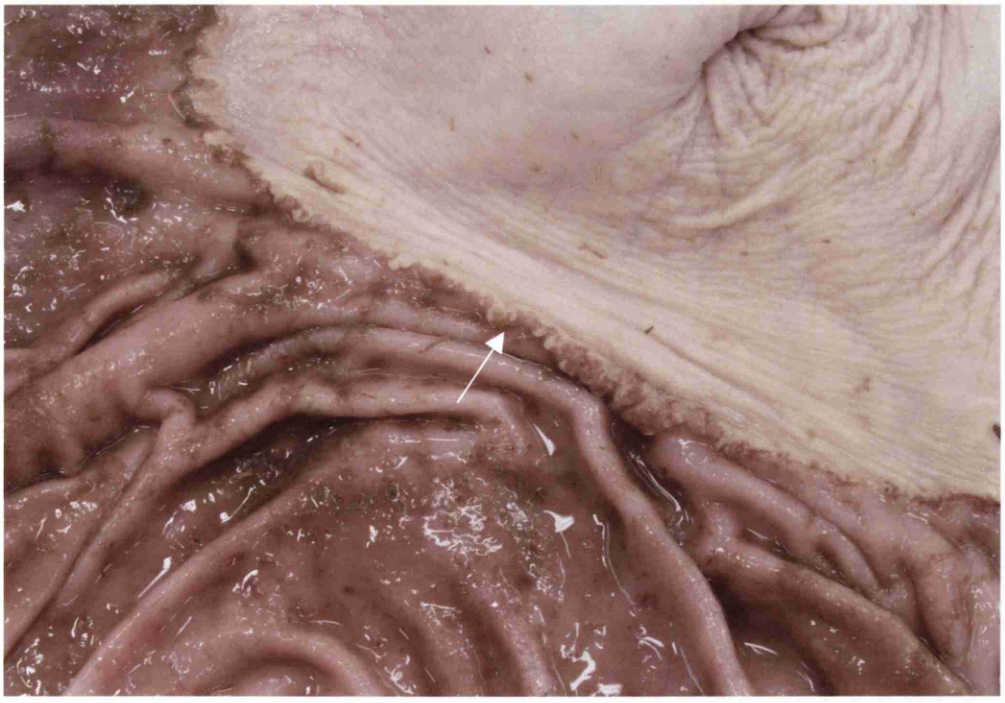


Figure 8. Case number 140459: interdigitating squamous and glandular areas at the margo plicatus along the lesser curvature with erosion/ulceration.

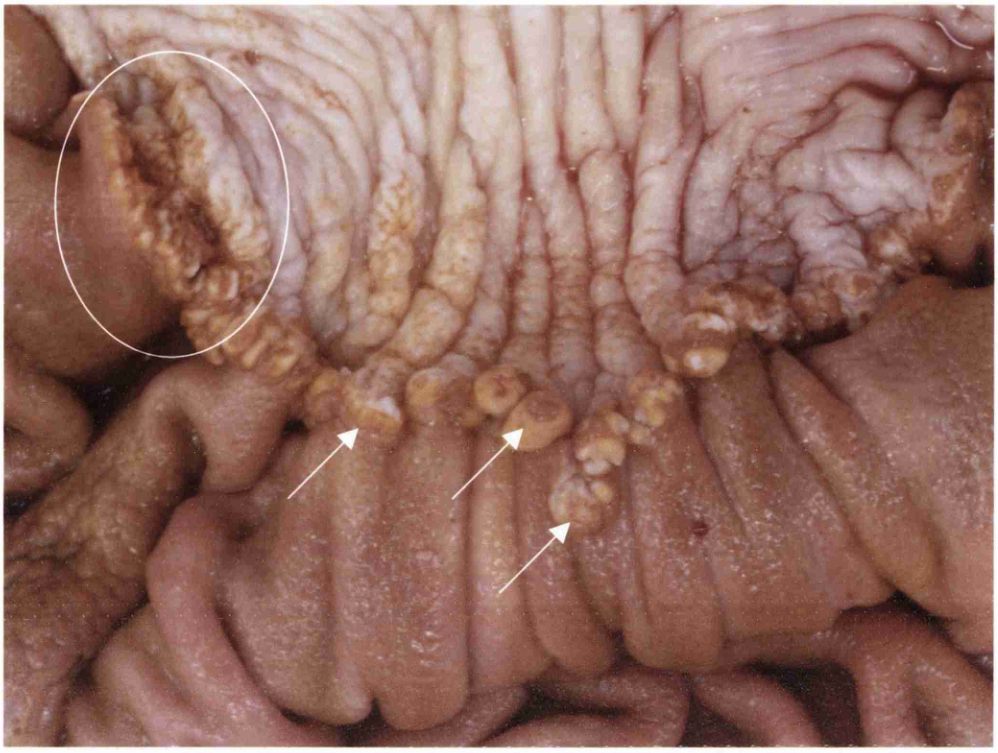


Figure 9. Case number 144476: nodular squamous proliferations with central core of glandular tissue along the margo plicatus (arrows). Punched out ulcerative lesions are visible to the left of the picture (circle).

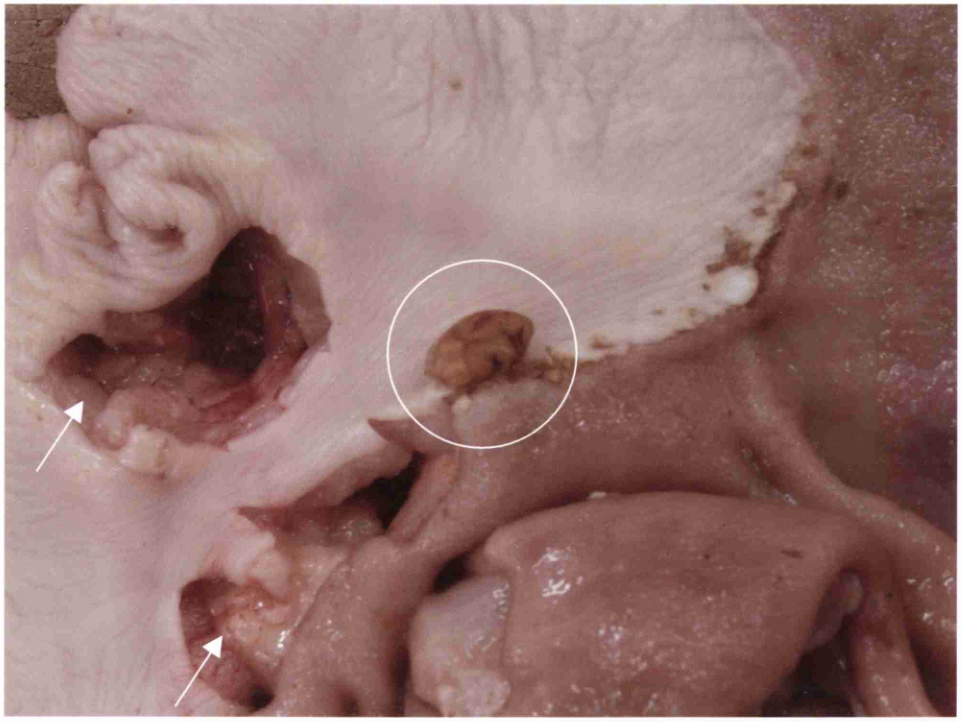


Figure 10. Case number 143696: a polyp at the margo plicatus. (circle) Samples have already been taken from the marked areas (arrows).

Figure 11. Case number 133191: punched out lesions along the margo plicatus, with central necrotic debris ringed by thickened pale keratinised epithelium (circles).

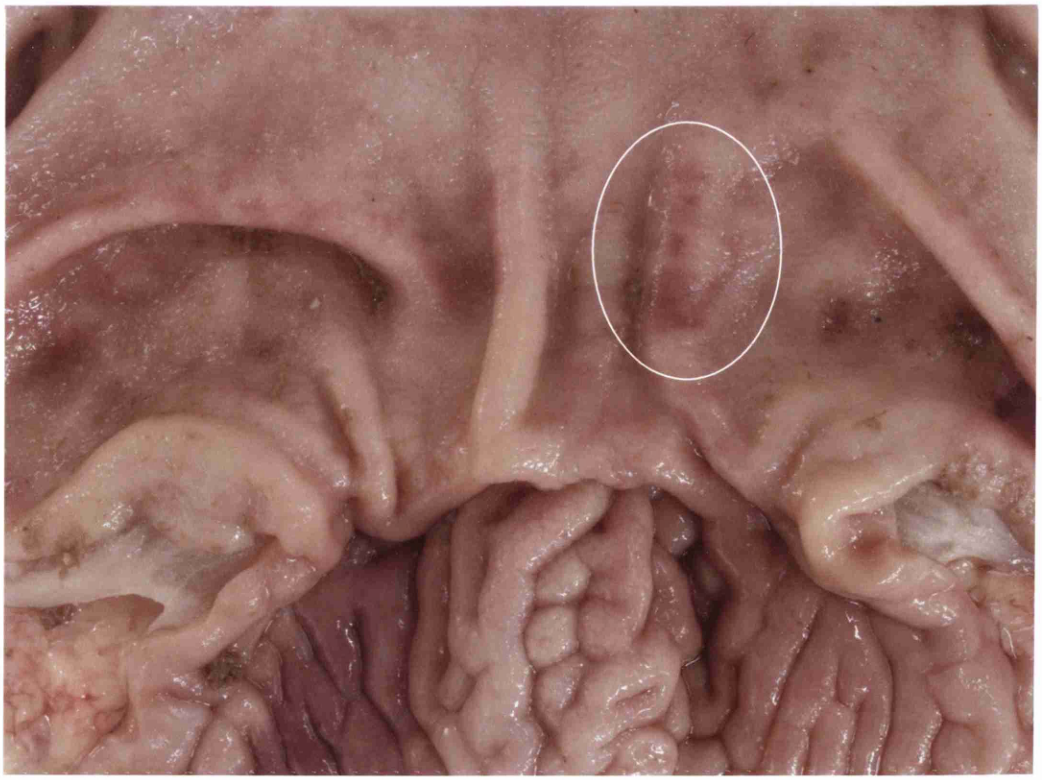


Figure 12. Case number 144056: hyperaemia seen as blushing of the rugal folds in the pyloric region (circle).

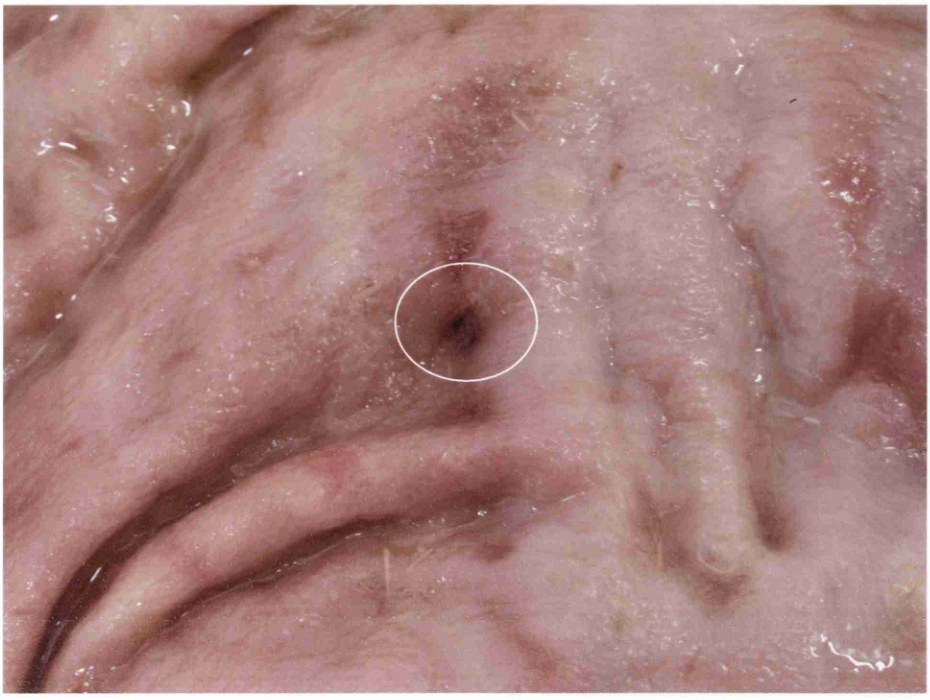


Figure 13. Case number 143818: a focal erosion in the pyloric gland region.

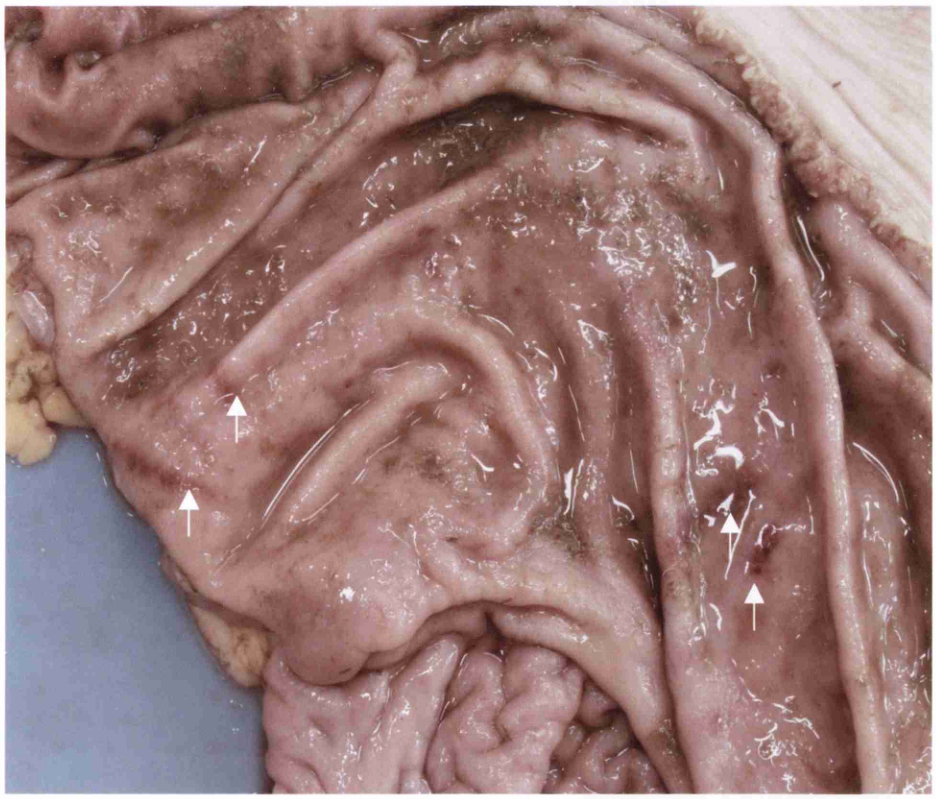


Figure 14. Case number 140459: focal erosions, some lying perpendicular to the rugal folds (arrows).

Figure 15. Case number 144292: focal blanched circular lesions in the cardiac gland region (circle).

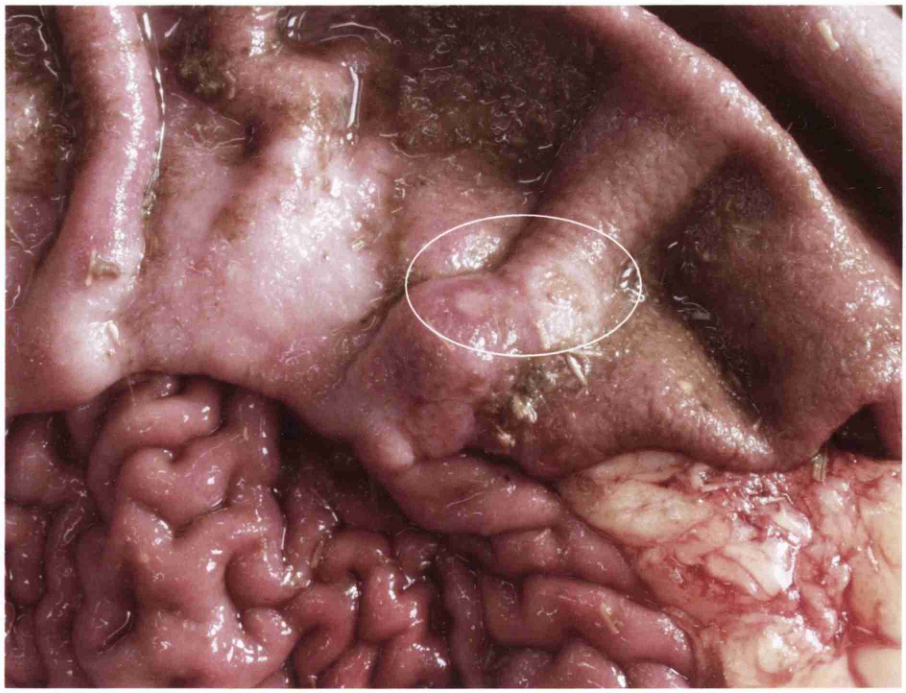


Figure 16. Case number 144439: blistering erosions on the apex of the rugal fold in the pyloric gland region (circle).

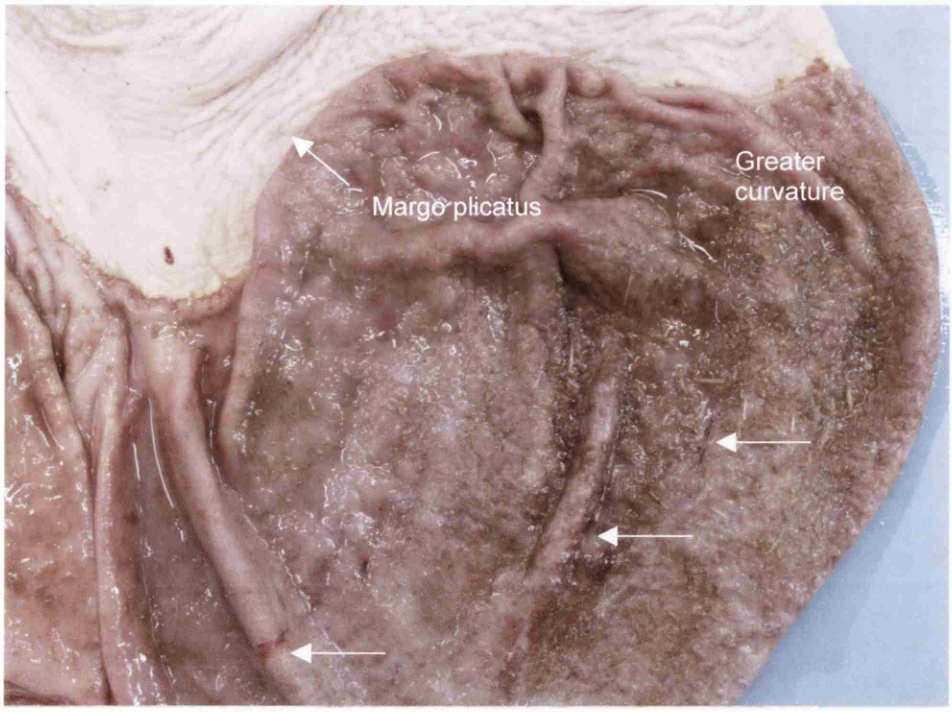


Figure 17. Case number 140459: multiple lesions within the cardiac and fundic gland regions (arrows).

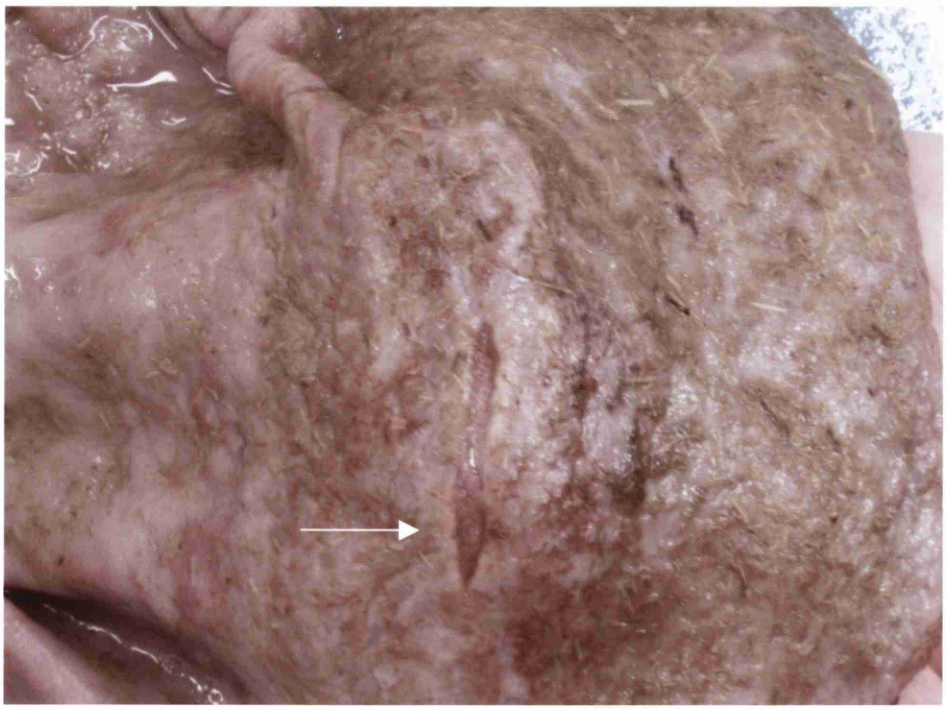


Figure 18. Case number 140459: deep ellipsoid erosion/ulcer in the fundic gland region (arrow).

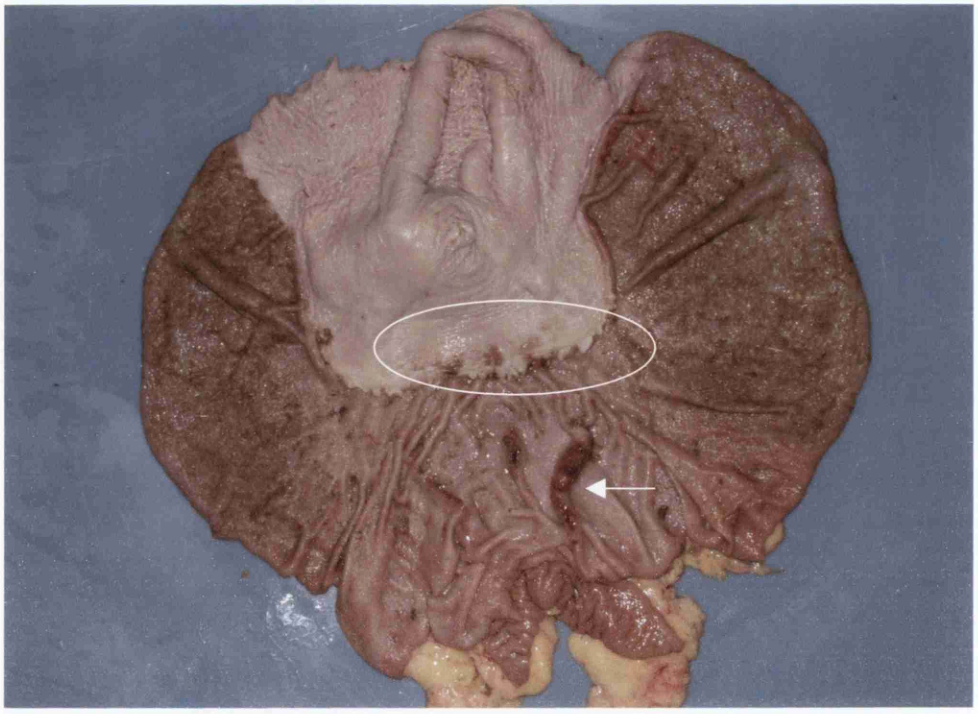


Figure 19 : Case number 143989: note ulcerations in the pyloric gland region, one involving a rugal fold (arrow). Also note squamous lesions along the lesser curvature (circle).

Figure 20. Case number 143989: detail of Figure 19, ulceration in the pyloric gland region.

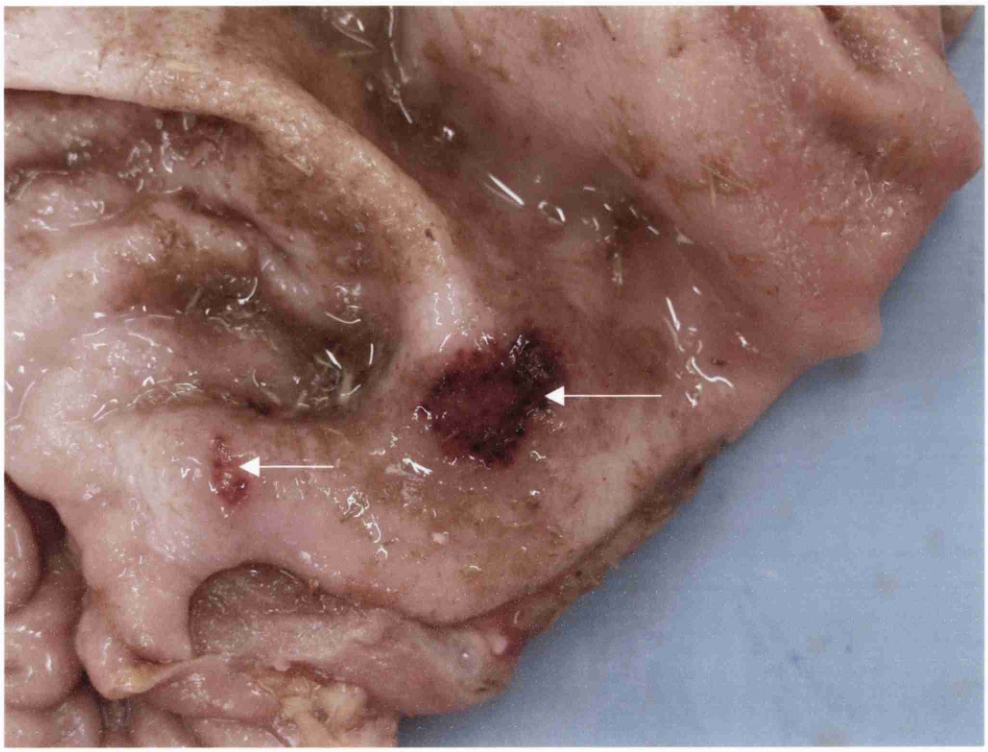


Figure 21. Case number 140459: note ulceration/erosion in pyloric gland region with recent haemorrhage (arrows).

Figure 22. Case number 145216: recent haemorrhage in the fundic gland region (arrow). Also note bile staining (yellow) at the lesser curvature (circle).

2.4 DISCUSSION

2.4.1 Limitations of method:

Population examined: Selection of subjects for this study required that they be euthanased at GUVS between October 2001 and May 2002, and that they were over 9 months old. As a consequence of this there were marked variations in age, breed, sex, usage and disease, with scant information available on previous medication, feed and transportation (i.e. stress). All these variables including the fact that only 21 horses were examined, prevented the formation of any kind of control group turning the investigation into more of an observational study as opposed to proving an hypothesis.

Practical difficulties: Practically, there were few problems encountered in stomach removal and preparation for photography. Rinsing of contents was not always complete depending on the consistency, and may have led to some inaccuracies in photographic interpretation. Another area of concern was the unpredictable nature of the stomach itself. Once incised, some stomachs had a thinned flaccid muscular wall and lay flat for photography and sample collection. Others contracted rapidly, and photographs only recorded a concertinaed appearance of the stomach. This acted to conceal erosions/ulcers and made well-orientated sampling for histopathology difficult to achieve. Some errors occurred during the actual photography, with photographs being out of focus or lost on the disc if the camera was not working properly. The hand drawn mapping was a useful adjunct to the photographs for lesion description, although the information recorded was not always adequate due to

time restrictions.

Written: Initially, establishment of the normal appearance and variation of the equine stomach, particularly at the margo plicatus, was probably the biggest challenge. Subtle colour changes of the mucosa were affected by the time after death and how well the horse had been bled out leading to errors in interpretation of hyperaemia within the glandular region. Food remnants adhering to the mucosa seen in photographs were similar to the appearance of scars, and the distinction between erosions and ulcers was also difficult to interpret, especially in the glandular area. All these factors may have led to the over / under interpretation of lesions in the written descriptions.

Scoring: The short time allotted for sample selection prohibited immediate scoring of each stomach. Photographs and the hand drawn map were used as an adjunct for erosion / ulcer assessment after all samples had been processed. The whole scoring process was very subjective and determining the number of lesions was especially difficult given that each was sometimes confluent with the next. The definition of a 'very large lesion' was confusing given that there were no measurement guidelines (which would probably be impractical during endoscopy). As mentioned previously any irregularity in the mucosa may also have caused misinterpretation of lesions. In the paper where this scoring system is first suggested (MacAllister et al 1997), the only parameter not to be consistent between recorders was the assessment of the number of lesions.

2.4.2 Effectiveness of Gross scoring methods

The numerical results of this study were dependant on the effectiveness of the scoring system used. Several scoring systems exist for use in the horse (see 1.4.1). They allocate scores to assess varying combinations of ulcer number, depth and severity. The fact that there is more than one system, let alone 5, highlights two points. Firstly, that none of them can be particularly effective (due to poor representation of what is actually there or difficulty in use) otherwise why develop another? Secondly that the initial aim of manufacturing a single scoring system to allow consistent case comparison between different recorders has been completely defeated. It was because of this latter point that an already existing system was chosen to record data, rather then adding to the list and making up yet another. It was also thought that if the same system was used for clinical and post mortem studies then it would aid case comparison within the literature and encourage a much needed collaboration between clinical and pathological findings in years to come. At the start of the project, the scoring system developed by MacAllister et al (1997) was thought to be the most thorough. Lesions were recorded according to their location and different values given according to number and severity. Other systems combined this information, so preventing as much information to be gleaned from a single score. However, the MacAllister system did not include scores for areas of hyperaemia and hyperkeratinisation, both of which were common and important in relation to the pathogenesis of ulcer initiation. It would be useful therefore to develop a separate scoring system for pathological use only. In clinical trials, it is the most severe lesions which pose most threat to the overall health of the horse and scoring systems have developed accordingly. It is effective during

treatment trials where it is necessary to record whether the lesion is improving or deteriorating. The EGUC in 1999 devised a concise system including scores for hyperaemia and hyperkeratinisation. However, it fails to distinguish between number and severity of lesion.

The scoring method used restricted the description of the location of the ulcers to squamous or glandular regions, and there was no way of recording their exact whereabouts within these areas. It was difficult to determine in which region lesions occurring at the margo plicatus fell, especially as islets of glandular tissue were found within the non-glandular border.

The interpretation of severity of ulceration was apparently designed for live animals, and euthanasia and exsanguination of the animal affected epithelial colour and areas of haemorrhage. Again the descriptions were too vague with the changes recorded relating more to the age of ulcer rather than the severity. A bleeding ulcer may be recent and superficial whereas a deeper more chronic ulcer may not actually be bleeding but be deeper, and pose more of a threat of perforation or indicate disease chronicity.

There was debate as to whether scarring should be scored as ulceration given that it is not so recent. It was decided that these lesions indicated the past disease status of the animal which was also important, and the ability to assess age of lesion grossly was not reliable enough to allow dismissal. In this study these small scars were given a severity score of 1. There was no scope for recording areas of hyperaemia or hyperkeratinisation according to the scoring system used, so these features were just

described (Appendix 3).

2.4.3 Gastric disease

Incidence: Approximately 95% of the horse population examined in this study had evidence of gastric ulceration. This was generally higher than other post mortem studies where 17% of a mixed population of horses in Sweden (Sandin et al 2000) were found to have ulcers, 80% of Thoroughbreds in training and 52% of retired Thoroughbreds (Johnson et al 1994). The higher incidence recorded in this study could be attributable to recent periods of transportation to the veterinary school, dietary changes, periods of inappetance due to other illnesses and various drug treatments, all of which are suggested aetiologies for gastric ulceration (EGUC 1999). There is a similar variability between clinical investigations which report a 58% prevalence of ulceration in show horses (McClure et al 1999), 66-80% Thoroughbreds in training and 66% of retired Thoroughbreds (Hammond et al 1986). In a study examining horses with signs of abdominal discomfort, 82% were found to have gastric ulceration (Murray 1992b). Discrepancies within similar populations probably reflect different methods of observation (post mortem vs. endoscope), sample sizes (20-3715 horses) and personal observational differences due to a lack of standardised criteria.

Location of ulceration: The percentage of horses in this study with ulceration in both squamous and glandular areas was 52%. From those remaining, 33% had squamous ulceration only and 14% had glandular ulceration only. Given this lack of correlation between the two areas, theories suggesting different aetiologies of ulceration in squamous and glandular regions (Murray et al 2001b) may be

accepted. In contrast to this, a previous study on 162 horses from a mixed population, found an identical prevalence of ulceration in the squamous and antrum/pylorus regions of the stomach (Murray et al 2001b). In this study, the total score for the number of ulcers in the squamous region was almost double that of the glandular. Squamous ulcers have been found to be more prevalent than glandular in several other studies (McClure et al 1999, Hammond et al 1986, EGUC 1999). Factors found to increase squamous ulceration but not glandular include recent racing (Murray et al 1996) and feed deprivation. A recent communication highlights the difficulty of detecting gross glandular lesions with the use of an endoscope, so some may have been overlooked.

Influencing factors: Existing studies have reported that various factors influence the incidence of gastric disease. These include the breed and sex of the horse, and any already existing gastrointestinal disease, including gastric parasitism. 100% of the Thoroughbreds in this study had evidence of gastric ulceration with an equal number and severity of ulceration in the squamous and glandular mucosa. However statistical analysis failed to find a significant correlation between the degree of ulceration and whether the animal was Thoroughbred or not. One other mixed breed study has found a higher prevalence of ulceration in Thoroughbreds (Sandin et al 2000) while others concentrate on Thoroughbreds alone so preventing comparison.

Statistical comparisons between horses both with and without GID found a positive correlation between the gross squamous number and GID, and an almost significant correlation with gross squamous severity, but none with the glandular lesions. This could be an example of secondary squamous disease, suggested to be the result of

mechanical disturbances further down the tract, affecting gastric emptying and normal content mixing (A. Merritt, personal communication). Other reports have also found the highest number of ulcers to be present in conjunction with other severe lesions of the digestive tract as opposed to lesions in other organs (Sandin et al 2000). This may indicate that some types of gastric ulcer are just one presentation of a more complex gastrointestinal malfunction. The fact that the glandular region remains unaffected could be due to the more efficient mechanisms of epithelial protection in this region.

Age comparisons in this study were not identified as a majority of the horses were over 2 years old and in other studies it was in horses younger than this that significant changes were found. Stallions were under-represented in the group examined so sex influences were also not examined.

Parasitic infection: No parasitic larvae were found in any of the stomachs with only one showing blanched lesions in the cardiac gland region possibly consistent with a nematode infection. In contrast, recent surveys have found bot infestations in up to 53% of horses examined at abbatoirs (Lyon et al 2000) in the South-West of England. This discrepancy probably reflects the difference in ownership diligence between horses referred to the vet school and those reaching an abattoir.

2.4.4 Interpretation of results

Squamous lesions: Descriptive assessment of each stomach, found different areas to be associated with specific lesions.

Focal hyperkeratosis (areas of thickened epithelium with a characteristic yellow staining) has been described in several studies (Hammond et al 1986, Murray et al 1996, Murray 2001b). In Thoroughbreds in training, 82% were found to be affected (Murray et al 1996), but only 19% of a mixed population of horses studied by Vatistas. In another survey (Vatistas et al 1994), the same author notes the location of these "areas of hyperkeratotic ridges" to occur primarily toward the greater curvature of the stomach, as was found in this study. However he also found it in those stomachs with the most severe ulceration scores which might suggest that it played an important role in ulcer pathogenesis, (not so in this study). Similar appearances have been described in several other species, but most frequently in pig stomachs. Here, areas of 'abnormal cornification' are reported to take over the entire squamous region of the stomach (Bivin et al 1974). Photographs show this epithelium to be thrown into thicker, more yellowed rugae than seen in the horse, with suggested causes including vitamin A deficiency (Embaye et al 1990). Causative factors in other species (foals, man, pigs, kangaroos and dolphins) include immunosupression and secondary infection with *Candida* (Gross and Mayhew 1983).

Scattered yellowed shallow scars were found throughout the non-glandular region of 8 of the 21 stomachs examined. These were individual discrete lesions which occurred in conjunction with all different types and scores of ulceration. Detailed observations by Principato (1988) attributed lesions such as these (pit-like and non prominent with very small diameters 0.5-1mm) to the clasping or partial penetration of 2nd instar larvae of *Gasterophilus intestinalis*. Despite the predilection site for these larvae being at the greater curvature adjacent to the margo plicatus

and in the saccus caecus, seasonal dispersion within the stomach has been reported (Price and Stromberg 1987). Using detailed image analysis, he located some larvae attaching throughout the squamous region with even a few in the glandular region. In this study, although no actual larvae were seen at the time of post mortem, it is possible that recent anthelmintic administration would have rid the stomach of infection just leaving these healing scars visible. However, their high prevalence may indicate another cause.

In the two horses affected, diffuse areas of erosion were found to extend primarily from the margo plicatus at the lesser curvature towards the cardia, with irregular focal patches stretching towards the greater curvature. These horses suffered from chronic grass sickness and caecal impaction, both conditions which would act to alter the time and type of gastric content exposure of the squamous epithelium.

The margo plicatus has been found to be the most common site for gastric squamous epithelial damage, and this study was no exception with 20 out of the 21 horses examined being affected to some degree. The types of lesions found can be arranged in order of increasing chronicity. Initially, what is considered to be normal must be defined, which in this case was the stomach with a completely flat and straight margo plicatus with no evidence of any erosions (145235). The most acute lesion showed areas of fresh erosion at the junction, with some interdigitation of squamous and glandular mucosa, but no step like gradient between the two. When the latter occurred, it was restricted to the lesser curvature, and appeared both with and without areas of fresh erosion/ulceration. Some stomachs showed further proliferation of squamous tissue, containing central islets of what appeared grossly

like glandular tissue. This would sometimes be raw and ulcerated.

From these observations, it could be assumed that this represents the way in which the stomach responds to acute injury and then heals. To assess this further, it is helpful to look at the condition of Gastroesophageal reflux disease (GORD) in humans, which affects 5-10% of the entire population. This occurs at the junction of glandular and squamous tissue (distal oesophagus) – or the Z line as it is sometimes called, due to its wavy appearance. In humans this is said to become more exaggerated with an increase in age. As the name suggests, the cause is due to acid reflux and exposure of the squamous epithelium to the acidic gastric contents. It causes ulceration and replacement of squamous epithelium by glandular epithelium with tall columnar cells. This epithelium in turn is capable of intestinal metaplasia and can eventually develop into adenocarcinoma of the distal oesophagus. Although tempting, it is important not to extrapolate all these findings directly to horses. Certainly it is interesting to find that the Z line appearance is normal (a fact which may suggest that what was originally assumed to be an abnormal finding of the interdigitating of the squamous and glandular epithelium is actually not so). The reaction of the squamous epithelium in responding to unaccustomed acid exposure by metaplasia into glandular tissue might help explain what is happening in the centre of the proliferative squamous polyp-like lesions but this must be confirmed histologically. A report on gastric ulceration in pigs (Curtin et al 1963) also noted that a large area of ulceration in the squamous area, healed through glandular advancement containing islets of squamous tissue. There was no evidence of scar tissue formation across the healed ulcer. As yet there have been no reports of glandular metaplasia or adenocarcinomas occurring at this site in horses or pigs.

That the condition is caused / exacerbated through exposure to acid is in no doubt, the most convincing evidence for which is illustrated by the use of a histamine type 2 receptor antagonist (ranitidine) in horses with induced gastric ulceration. It acts to reduce the amount of acid released, and corresponded with regression of the ulceration (Murray and Eichorn 1996). Other reports claim that the ulceration will begin to heal on its own, but the reason for the increased susceptibility of the squamous epithelium over the glandular epithelium to acid damage is not clear.

Undoubtedly the difference in physiological capabilities between the two areas must play a role. It is common knowledge that the squamous defence mechanisms against injury by acidic exposure are limited to tight junctions in the *stratum corneum* acting as weak acid barriers, and intracellular buffering. In contrast the glandular epithelium is protected by a bicarbonate laden mucus layer on the surface, restitution (a process by which denuded mucosa is resealed by epithelial migration from adjacent mucosa) and local prostaglandin production (which can increase blood flow, mucus and bicarbonate secretion if required). None of these protection mechanisms have been found to occur in the stratified squamous epithelium as yet (Argenzio 1999).

Statistical analysis of the results from this study support this theory that the pathogenesis of gastric damage differs between the two areas. This was shown by the fact that there was no correlation between the number or severity of gross lesions between the squamous and glandular regions.

Murray reports the margo plicatus to be the most mobile region of the stomach

(MacAllister et al 1997). Additional muscle contraction could affect blood flow to the area leaving it more susceptible to ischaemic damage. Hammond (1990) found this area along the margo plicatus to have the lowest pH in comparison to the rest of the stomach, and he also correlates those horses with the most severe gastric ulceration with those with the lowest pH. Whether the latter is a result of the low acidity causing increased damage, or existing damage affecting bicarbonate excretion and so lowering pH, is unknown. In a normal standing horse, the contents of the stomach will generally cover the margo plicatus at the lesser curvature, but the greater curvature is situated dorsal to this and may therefore have a reduced exposure to acid contents, so explaining the reduction in ulceration of this area.

It must be remembered that the gastric contents, with regards to their ability to damage squamous epithelium are hugely affected by diet type, frequency of feeding, and other conditions affecting gastric motility overall.

The fluidity of the diet and its content of fermentable carbohydrates, will directly affect the production of VFAs within the stomach (1.3.4).

The effect of bile salts on stratified squamous epithelium has been the subject of much work in rabbits, pigs, humans and horses (Schweitzer et al 1986,Lang et al 1998, Dixon et al 2001). Various studies have proven that there is a synergistic reaction between bile salts and HCl (Lang et al 1998). In acidic conditions, bile salts become de-ionised and lipid soluble and develop the ability to accumulate in the stratified squamous epithelium so altering its permeability to hydrogen ions (Argenzio 1999) and degree of erosion/ulcerations. Therefore any condition which

alters the bile content of the stomach, so predisposes it to gastric ulceration e.g. conditions causing gastroeosphageal reflux (GI blockage, grass sickness). In man violent passion, fat or oily food, irritation of the pyloric extremity of the stomach, and kneading over the hepatic area have all been incriminated as possible causes of increased bile reflux (Reed and Kidder 1970). In horses, feed deprivation is also found to cause ulceration (Murray and Eichorn 1996) by affecting the gastric emptying of solids and the mixing of stomach contents. This acts to destroy the proximal to distal pH gradient within the stomach and increases the amount of duodenal reflux into the stomach.

The yellow staining which was frequently observed in combination with hyperkeratosis of the stratified squamous region was assumed to be due to bile staining from duodenal reflux. Darker yellow/brown staining was also seen involving many of the punctate scars and the base of some erosions. Confirmation that this colour change was indeed due to bile staining has been proven in pigs where similar lesions have given a clear positive reaction for bilirubin with both Ehrlich's diazo reagent and Fouchet's reagent. Here they found that the darkening of the yellow to green/brown happened over a period of time, and indicated an increase in the age of lesion (Reed and Kidder 1970). The fact that the bile staining coincided with areas of abnormal epithelium suggests that this damaged epithelium was more susceptible to staining. In pigs as the percentage of normal oesophageal epithelium decreased so the bile staining increased, so supporting this theory.

Other more mechanical explanations have again been extrapolated from humans. It is well known that race horses in training have more ulceration, as do humans who run

extensively have increased levels of GORD. Proposed theories include changes in intragastric pressure compressing stomach contents and exposing susceptible epithelia to hostile conditions.

Finally infectious causes have been found to be a factor in pigs with gastric ulceration , and the degree of ulceration in the pars oesophagea is related to the presence and load of *H heilmannii* in the rest of the stomach (Barbosa et al 1995). To the contrary, humans infected with *Helicobacter sp.* have been found to have a lower incidence of GORD. This is thought to be a consequence of reduced acid production secondary to inflammatory damage from this bacteria.

Glandular Lesions

The appearance of lesions within the glandular region of the stomach were not as distinctive as those recorded in the squamous region. They ranged from foci of hyperaemia, to shallow erosions, to active ulcers with haemorrhagic areas and chronic ulcers with associated scarring. As previously described, there are several effective mechanisms available for mucosal protection within the glandular epithelium (bicarbonate mucus, restitution, prostaglandin). Damage in this area is therefore thought to result from a defect in one or more of these mechanisms leading to injury, rather than excessive exposure to acid, as is the case in the squamous region

The pyloric gland region at the pylorus was the most frequently affected area. In two stomachs this was accompanied by focal erosions and in one other there were large chronic ulcers in the pyloric antrum along with fresh erosions at the

pyloric/fundic gland region. The stomach with the most severely affected fundic gland region (140459) only displayed mild erosions at the pylorus. There did not appear to be any direct relationship between the degree/location of damage in the glandular area and that of the squamous region. Equally, there was no pattern found linking severity/location of lesions within the glandular region alone.

These are similar to the findings in a study by Murray et al (2001b) who also fails to pinpoint a connection between squamous and glandular lesions and those within the glandular region alone. This study was executed in live horses with the use of an endoscope, and the author mentions its limitations with regards to visibility and accurate interpretation of the lesions themselves. Other studies have found 12/111 stomachs of horses with signs of abdominal discomfort to have glandular ulceration (Murray 1992b) and 51% of racing thoroughbreds to have glandular ulcerations. There was an increased prevalence in horses that were over 4 years old but otherwise the lesions were unaffected by sex, recent racing, frusemide and corticosteroid administration and NSAIDs.

It is well known that NSAIDs have been found to be a cause of pyloric ulceration in the horse, with other toxic side effects including renal papillary necrosis and ulceration of the dorsal colon (MacKay et al 1983). In this study, the four horses with maximum severity score numbers for pyloric ulceration had all received some kind of NSAID within the last month of life. As the dosages given did not exceed the recommended amount, and as other horses received similar levels of treatment there was no clear evidence for this view. There were also no other signs of NSAID toxicity in these individuals. The literature places too much emphasis on this

correlation, seeing as the drug trials generally prove that ulceration only occurs when over 8.8mg/kg (brand dependant) is given. However there is a known individual variability in susceptibility, and some of these horses may have been medically compromised making them more susceptible. Certainly other studies where there is detailed knowledge of NSAID use and good glandular observance, they have failed to find a link between the two (Vatistas et al 1994).

The pyloric region is also a site for stress ulceration (humans, cattle). The lesions reported appear as fresh punched out lesions and not large chronic ulcers. Due to the recent history of some of these horses it is certain that they would have been stressed prior to euthanasia.

Bile reflux has also been suggested as a causative factor in glandular lesions (Murray et al 2001b) and this has been proven in humans. However it is likely that mucosal protective properties would have to be impaired before this could happen. There was no gross evidence of bile staining within the glandular region, although it is not ruled out as the natural pink colour of the glandular epithelium would most likely mask any lighter yellow staining.

The disparity in presence and severity of lesions in the pyloric and fundic and cardiac gland regions of the stomach suggests separate aetiologies for these areas. Lesions in the pyloric region were much more frequent, and in the one stomach (140459) where lesions were more widespread and severe in the fundic and cardiac gland region, they were milder in the pyloric region. Anatomical differences between the areas may account for it. It is suggested (Murray et al 2001b) that the presence of

mucin-containing submucosal glands in the pyloric antrum maybe a differentiating factor. One might expect the fundic gland region to be the most severely affected as this is the gastric region responsible for HCl production but this does not appear to be the case.

A recent paper (Nicol et al 2002) suggests an association between crib biting and gastric ulceration in foals. The horse with the most severe fundic ulceration in this study was a renowned head shaker. It could be possible that this combination of disorders was not a coincidence, and either that the pain of the gastric ulceration is reflected by the head shaking, or the excessive amounts of stress the horse must have been in, in order to head shake could have induced these lesions.

A form of pyloric ulcer disease that manifests as a primary ulceration of the pyloric glandular mucosa has also been recognised (A.Merrit, personal communication) where the squamous mucosa is normally free of lesions. Lesions in the glandular region without any in the squamous was noted in two horses in this study (140373,144292). If lesions around the pylorus are left untreated, they can induce sufficient scarring to interfere with gastric emptying, so causing severe post prandial distress . Primary ulceration of the pyloric glandular mucosa is the most likely form to be caused by an HLO, but this has yet to be proven. *H. pylori* is the most common cause of gastritis and gastric ulceration in humans, but similar organisms have yet to be detected in equidae. As well as identifiying the actual organism, specific patterns of gastritis in humans have been associated with *Helicobacter* infection. The only way to identify these would be through a detailed histological study sampling several areas of the stomach. This is carried out in the next chapter.

2.5 CONCLUSION

In this study 20 out of 21 horse stomachs examined were found to have some degree of gross abnormality (erosion/ ulceration). Application of the scoring system chosen (that of MacAllister et al 1997) succeeded in recording all types of lesions except for areas of hyperaemia and hyperkeratosis. Parameters which were found to correlate positively included the gross glandular number and gross glandular severity of ulceration i.e. the more lesions present, the deeper they were. Also the number and severity of lesions in the squamous region, but not the glandular, were increased in horses with gastrointestinal disease. The fact that the number and severity of lesions within the squamous and glandular regions did not appear to correlate positively suggests that the aetiology of the lesions in these two areas is distinct.

CHAPTER 3 – HISTOLOGICAL FINDINGS

3.1 INTRODUCTION

To date, a majority of the detailed studies investigating gastritis/gastric ulceration in the horse only focus on the gross appearance of lesions. Those few that do incorporate histopathology use animals that have undergone specific ulcer induction programmes where the only type of lesion examined is acute. They also provide limited information regarding cellular infiltrates at the lesion site and the rest of the stomach.

The purpose of this detailed histological examination was to identify the microscopic appearance of all the gastric lesions observed on gross examination from a mixed population of 21 horses. In addition to this, both the type and quantity of inflammatory cell infiltrate present in the remaining stomach would be established.

Methods used to record and compare histological findings of gastritis in other species include written descriptions (Hermanns et al 1995) and scoring systems. Many of the latter are developed through modifications of the Updated Sydney System (Dixon et al 1996), which is used in humans to provide a consistent method of recording and comparing cases. It assesses the degree and type of cellular infiltrate in biopsy specimens by matching the appearance on each slide to a graded visual analogue scale. Specific details regarding reactive changes of the glands e.g. metaplasia, dysplasia, atrophy are also recorded and this enables the recognition of different inflammatory patterns. In some cases it is then possible to allocate a

specific aetiology for the disease, so improving early treatment and accurate prognosis.

Due to the lack of existing data in the horse, this study used both written descriptions and classification of the gastritis via a modification of the Updated Sydney System to assess the pathology present.

It was important that all regions of the squamous and glandular stomach were examined. Ideally, the entire organ would be cut into strips and analysed, however this is only practical in smaller species e.g. Mongolian gerbil (Ikeno et al 1999). In humans it is recommended that four biopsies be taken, two from the antrum and two from the corpus (Dixon et al 1996). In the dog, studies examine the fundic gland area only (Hermanns et al 1995), or take two or more random samples from anywhere (Happonen et al 1998). For this horse study six fixed samples were required, two from the stratified squamous region, three from the glandular region and one from the proximal duodenum. In addition to this, representative samples were taken from any areas of gross abnormality (e.g. erosion/ulceration). This was considered sufficient to give a good representation of the histological picture within each stomach.

Assessment of the inflammatory infiltrate involved scoring cell types (lymphocytes, eosinophils, neutrophils) according to their density (absent, mild, moderate, marked). Parameters used to indicate reactivity within the non-glandular epithelium were extrapolated from other studies on stratified squamous epithelium in humans (Ismail–Beigi et al 1970) and horses (Murray et al 2001a). These included measuring

the thickness of keratinised and non-keratinised epithelium and the length of the lamina papillae. Reactive changes noted within the glandular region of the stomach included evidence of gland atrophy, dysplasia, gland metaplasia and the presence of lymphoid follicles.

It must be realised that conclusions which may be drawn from these results refer to a small population of horses, most of which have previously had unknown quantities of antibiotics and other treatment along with debilitating diseases. Therefore this should be regarded as a preliminary series of detailed observations rather than a definitive study of equine gastritis.

3.2 MATERIALS AND METHODS

3.2.1 Histopathological samples

General sample selection: Euthanasia and gross post mortem examinations were carried out as described in Chapter 2. Routine samples were taken for histopathology from the lung, liver, kidney, spleen, adrenal gland, coeliacomesenteric ganglia and the gastrointestinal tract. If clinical signs inferred, or the presence of particular lesions or changes in other organs was noted at post mortem examination, further appropriate samples were taken.

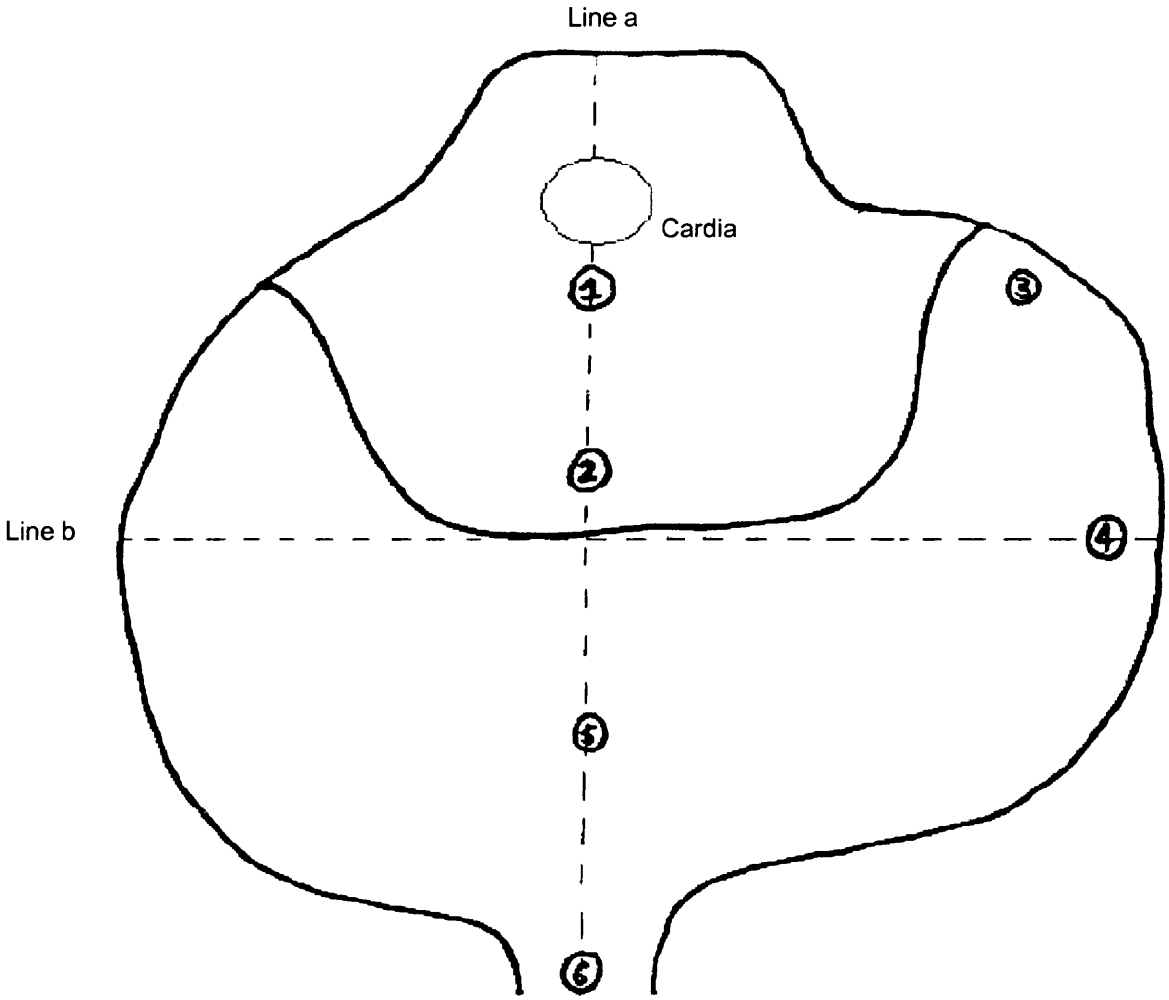
Gastric sample selection: Within the stomach two sampling techniques were used. One assessed the histological appearance of the gross lesions described in Chapter 2, and the other aimed to collect information regarding the picture of gastritis in all regions of the stomach.

For the latter technique, a pre-drawn map of the stomach (Fig. 23) determined six primary areas from which full thickness samples of the wall were taken. Two imaginary lines were drawn: Line **a** ran along the lesser curvature from the centre of the cardiac sphincter, through the non-glandular and glandular regions, to the centre of the proximal duodenum. Line **b** was at right angles to this at the level of the margo plicatus. Sample sites **1** and **2** were within the non-glandular area and lay on line **a,** 2 cm distal to the cardiac sphincter and 2cm proximal to the margo plicatus respectively. Site **3** lay 2cm distal to the margo plicatus on the greater curvature within what was assumed to be the cardiac gland area. Site **4** was on line **b** on the greater curvature in the fundic gland region of the stomach. Sites **5** and **6** lay on line **a,** the former 6 cm distal to the margo plicatus within the pyloric gland region and the latter in the proximal duodenum.

Following this procedure, tissue from a representative selection of any further areas of gross abnormality was also taken. Due to time restrictions, it was not always possible to include all types of lesion observed grossly in each stomach.

Gastric sample collection: Blocks of tissue measuring approximately $2cm^2$ were dissected from each site using forceps and a fresh scalpel blade. An effort was made to cut them perpendicular to the epithelium as angled sections where the epithelial surface is missing can be mistaken for areas of erosion/ulceration histologically. The extreme thickness of the longitudinal and circular muscles did lead to some complications during sampling as the force required to cut through the tissue sometimes resulted in crushing injury to the epithelium, but the aim was to include all layers of the stomach wall from the epithelial surface to the peritoneal surface.

Figure 23: A hand drawn map of the horse stomach detailing the location of the six fixed samples which were taken for histopathology.



These blocks of tissue were then divided into two; one was placed in 10% neutral buffered formalin for 24 – 96 hours, while the other went straight for bacteriological analysis (see Chapter 4). Those in formalin underwent routine processing and orientation with embedment in paraffin wax. Following microtomy they were fixed onto glass slides at a thickness of 2μm and stained with H and E. Fixing the fresh tissue in formalin frequently led to contraction of the muscle and subsequent separation from the epithelium. Some slides therefore only presented with the epithelium and lamina propria to examine. Where intact, the toughness of the muscle layer made it difficult to section thinly, resulting in shattering and incomplete sections. When samples were required for snap freezing a small block of tissue measuring 0.5 x 0.2mm was removed from one of these two and immediately frozen.

3.2.2 Special stains

Within the non-glandular region, special stains were primarily used to highlight evidence of any infectious agents. PAS stain (Cook 1996) was used to indicate the presence of tissue carbohydrates in yeasts and fungi, which stained pink. However as it also stained certain mucins positive, a Grocott stain (Stevens and Francis 1996) was also required for identifying some fungi.

General bacteria were identified using Gram stain (Stevens and Francis 1996) while Warthin and Starry method (Stevens and Francis 1996), modified Giemsa method (Stevens and Francis 1996) and immunohistochemistry were used to locate any spiral bacteria (e.g. *Helicobacter spp).* Immunohistochemistry for T and B lymphocytes (CD3 DAKO M7254 and CD79 DAKO M7051) and blood vessels

(Von Willebrands DAKO A0082) was also implemented for some sections.

Any evidence of intestinal metaplasia within the glandular region was sought using a PAS/Alcian Blue stain (Cook 1996). This stains metaplastic cells blue or purple as they contain acid glycoproteins, in contrast to the neutral mucins present in normal epithelium which stains pink (Dixon et al 1996). A Sirius Red stain (Francis 1996) was used to highlight areas of fibrosis and scar tissue and aid the detection of atrophy. Orange G/PAS (Wilson and Chalk 1996) helped to identify the location and quantity of parietal cells present within the fundic gland region.

3.2.3 Histological assessment of all slides

Each slide was screened in order to obtain a general impression of normality, so enabling subtle changes to be realised later. Following that, they were examined in detail under low and high magnification and a general description written (Appendix 3).

In order to be able to make accurate comparisons between slides and to tabulate changes in a systematic fashion, a histological scoring system was devised using the Updated Sydney System as a template. For each slide, both the type and density of cellular infiltrate were assessed in three separate fields. Specific cell types analysed were mononuclear cells (lymphocytes, plasma cells), neutrophils and eosinophils. An estimate of the average infiltrate from these three fields was then matched with the appropriate grading panel of the visual analogue scale

The number of neutrophils/mononuclear cells/eosinophils was considered to be

normal when none or only a few cells were seen per high power field. A mild infiltration was recorded when there were several cells per high power field. A moderate infiltration was recorded when there were many cells per high power field, and a marked infiltration when there were numerous cells per high power field. Where the cellular infiltrate was limited to one specific area of the slide e.g. neutrophils at the site of an ulcer, the density was not averaged and scoring was taken for this cell type at the point of maximum intensity. Lymphoid follicles were also recorded as being present or absent. The individual results of cellular infiltrate and reactivity for each slide can be found in Appendix 2, and an example of the three grades of cellular infiltrate for mononuclear cells is illustrated in Fig. 24 (a–c).

All this information was combined to give a written diagnosis of the type of gastritis present within the non-glandular and glandular region of each stomach, as is done in the Sydney system. Initially the average severity of the gastritis was calculated by looking at the degree of cellular infiltrate. A score of 0–3 was assigned to the cellular infiltrate (normal = 0, mild = 1, moderate = 2, marked = 3) for all samples from each stomach. The average number for the squamous and glandular region of each stomach was then calculated, and the corresponding degree of infiltrate recorded for each cell type. Gastritis was recorded if inflammatory cells were present. It was termed chronic if the cellular infiltrate consisted of mononuclear cells alone and chronic active if neutrophils were also present. Within squamous and glandular regions, if all sites (1 and 2 for squamous, 3–5 for glandular) were involved no mention was made of inflammatory cell location. If only one site was affected then the infiltrate was said to be focal, and a topographical qualifier (e.g. cardia/margo plicatus or cardiac/fundic/pyloric predominance) added. If more than two

samples had been taken and more than one site but not all were involved, then the infiltrate was said to be regional and no topographical indicators were given. If all were involved it was recorded as diffuse.

Anatomical tissue changes within each section were also incorporated into the diagnosis of gastritis. This included identifying a reactive epithelium and the presence of glandular metaplasia. Within the non-glandular region (sites 1 and 2), variations in the thickness of the keratinised and non-keratinised epithelial layers, and the degree of rete pegging were recorded. Average thickness values were calculated from the stomach which had been perceived to be grossly normal (145235), and any measurements exceeding these were assumed to represent a reactive epithelium i.e. hyperkeratosis, acanthosis or rete peg lengthening. These parameters were measured by counting cell numbers along an imaginary straight line drawn perpendicular to the epithelial surface and efforts were made to assess only full thickness areas that had not been cut at an angle. An average thickness was taken from three measurements in each section, avoiding epithelium directly adjacent to or involving areas of erosion/ulceration. Any evidence of glandular metaplasia was also incorporated into the diagnosis of gastritis. Its definition included nests of glandular material found anywhere within the lamina propria of the stratified squamous epithelium, including sections from the margo plicatus where glandular tissue was seen to under run stratified squamous epithelium.

Sections from sample sites 3–6 (glandular region) were inspected for evidence of glandular atrophy, dysplasia and metaplasia. Atrophy was defined as a reduction in the number of glands within the gastric mucosa, which could be accompanied by a

thinning of the mucosa. Dysplasia was taken to represent areas where glands appeared irregular and tortuous in shape and were accompanied by an inflammatory cell infiltrate. Metaplasia was recorded when there was replacement of normal cells with ones generally found elsewhere. These parameters were only recorded as being present or not and were not graded according to their severity.

Infectious agents (e.g. parasites, bacteria, yeasts) were recorded and searched for using the specific staining methods already mentioned.

Statistical analysis: A Spearman Correlation Coefficient was used to assess the relationship between the histological findings in the squamous and glandular regions, and also to compare these results with those of the gross results. In order to do this, numerical values were assigned to each stomach by translating each Sydney Classification diagnosis into a number which would then be comparable with the gross scores i.e. create four scores per stomach. Both the distribution of the inflammation (absent=0, focal=1, regional =2, diffuse = 3, comparable to numbers of lesions grossly) and the intensity of the inflammation (absent = 0, mild=1, moderate =2, marked = 3, comparable to the severity of lesions grossly), were scored for the squamous and glandular regions. The values for lymphocytes (chronic) and neutrophils (active) were multiplied together in order to obtain a single score for the gastritis.

3.3 RESULTS

Several methods have been used to present and analyse the histopathological findings from this study. Initially, histological interpretation of the types of

lesion identified grossly is described, with full written description of each slide recorded in Appendix 3. These findings are also summarised in this Chapter, and the important features highlighted. A modification of the Updated Sydney System is then used to classify the presence and type of gastritis within the non-glandular and glandular regions of each stomach.

General histopathological samples have also been taken from each carcass to identify the cause of death/reason for euthanasia and these are also recorded in Appendix 2.

3.3.1 Summary of Written results

For the purpose of these written descriptions, thickness was estimated by eye. Within the stratified squamous epithelium, obvious diffuse or irregular reductions in thickness involving the stratum corneum and/or the stratum spinosum, granulosum, basale were termed erosions. If there was exposure of the underlying lamina propria without remnants of epithelial covering then this was termed ulceration. Within the glandular region, an erosion represented any areas of glandular tissue loss, with ulceration only being recorded if there was complete destruction of glandular tissue and exposure of underlying lamina muscularis.

Erosions and ulcerative lesions taken from grossly abnormal epithelium

Stratified squamous epithelium: Histological examination of samples taken from grossly abnormal areas found examples of intracorneal pustules, erosions, ulceration and glandular metaplasia. The erosions varied in depth from little destruction of surrounding stratum corneum to complete loss of stratum spinosum leaving only rete

peg remnants formed from basal epithelial cells. The histological interpretation for each of the gross categories as defined in Chapter 2 is as follows.

Hyperkeratosis: These lesions were represented both by irregular parakeratotic/ orthokeratotic hyperkeratosis and focal intracorneal pustules (Figs.25, 26). These latter areas of microabcessation were also accompanied by underlying capillary congestion and an increase in lymphocytes within the surrounding lamina propria.

Punctate scars: These were represented by erosions varying in depth and ulcerations, and were located both adjacent to and away from the margo plicatus. Many erosions occurred in conjunction with adherence of bacteria to damaged surface tissue and elongation of surrounding lamina papillae, which were seen to extend to the stratum corneum or luminal surface . Deeper erosions showed basal epithelial cells to be capped with necrotic epithelium and interspersed with vascular lamina propria. (Figs.27, 28, 29) There was generally marked hydropic change of the basal epithelial cells and a neutrophilic infiltrate underunning adjacent stratum corneum. In one section this was accompanied by a thrombus in an underlying vessel, and an inflammatory reaction extending deep into the lamina muscularis.

Abrupt areas of ulceration extended to the lamina muscularis where there was a bed of granulation tissue, capped with eosinophilic necrotic debris and an intense gathering of neutrophils. There was balloon degeneration, karyolysis and karyorrhexis of surrounding keratinocytes in the bordering epithelium. Inflammatory infiltrates varied between localised mixed inflammatory cells in the superficial lamina papillae and lamina propria, to deep intense infiltrates extending to the

muscularis mucosa. Marked oedema of the lamina propria, lymphatic dilatation and congestion of localised vessels were also evident. Bacteria and PAS positive hyphae and cocci (*Candida spp*) were often seen bordering areas of ulceration and in the superficial layers of the surrounding epithelium (Figs. 30, 31, 32).

Diffuse erosions: These were represented by erosions and ulceration (Figs. 33,34, 35). Diffuse areas of ulceration consisted of a wider area of damage without a bed of granulation tissue and marked hydropic change of bordering basal epithelial cells. Here, there was complete loss of the stratified squamous layer, leaving only lamina propria exposed. This was covered by a layer of necrotic tissue with marked congestion of underlying capillaries. The cellular infiltration was mild, showing an increase in lymphocytes and neutrophils scattered within the lamina propria.

Margo injuria: As well as the punctate ulcerations already mentioned, some areas of grossly abnormal epithelium in this region, were found to represent glandular metaplasia. They took the form of PAS positive nests of glandular tissue in the mid lamina propria beneath deep erosions in two stomachs (Figs. 36, 37). These were situated away from the margo plicatus beneath shallow rete peg remnants with a localised plasma cell and lymphocytic inflammatory cell infiltrate and capillary congestion in the lamina propria. Other foci of glandular metaplasia were seen in the region of the margo plicatus where breaks in the stratified squamous epithelium were seamlessly interspersed with islands of glandular epithelium (Figs. 38, 39).

Glandular region: Within the glandular region no actual areas of ulceration were identified contrary to the gross observations.

Hyperaemia: Some of these areas appeared normal histologically, while others were represented by congestion of superficial capillaries with or without a mild inflammatory infiltrate (Fig. 40).

Focal erosion: The erosions found varied in depth from superficial single epithelial cell necrosis, to loss of half the depth of the glandular tissue (Figs. 41, 42). Most frequently surface epithelial cell necrosis was accompanied by exudation of fibrin, neutrophils and variable amounts of haemorrhage. This was sometimes found in conjunction with surrounding gland dysplasia and thickening of the lamina propria about the pyloric glands, with a mild to moderate mononuclear cell infiltrate (Figs 43,44). In one section (143989), a dilated cystic glandular structure lined with cuboidal PAS positive epithelial cells was seen within the lamina muscularis (Fig. 45). This lay beneath a deep erosion (half the height of the epithelium) and marked glandular dysplasia with a moderate mononuclear cell infiltrate. Isolated clumps of glandular tissue were occasionally seen to infiltrate the underlying lamina muscularis (140373). Another section shows an abrupt area of erosion overlying a foreign body present in an underlying blood vessel (144056) with infiltration of the lamina propria with neutrophils and lymphocytes and congestion of capillaries. Focal formation of crypt abscesses (144439) was found once, with infiltration of neutrophils towards the pyloric glands from a surface erosion. Isolated areas of glandular hyperplasia were also noted in some sections (Fig 46).

Ulceration: Despite the gross appearance of these stomachs, there did not appear to be any areas of ulceration within the glandular region.

3.3.2 Summary of the findings from the fixed sites

Site 1: Variations in the appearance of the stratified squamous epithelium at site 1 included changes in the thickness of individual anatomical layers within the epithelium, and alterations in the appearance of specific cell types.

Despite the absence of an inflammatory cell infiltrate or evidence of erosion/ulceration in 14 stomachs, there were still a number of cellular changes to be noted. The stratum corneum varied in thickness and nucleation (143819). An increase in the thickness of the stratum corneum was termed hyperkeratosis. Most frequently this was found to be fully nucleated (parakeratotic, 144095) but anucleate (orthokeratosis, 143819) and a combination of the two were also seen. A disruption in the natural keratinisation of the stratum corneum was termed dyskeratosis, and was accompanied by karyorrhexis and karyolysis of cells within the stratum corneum. Clumps of bacteria were also seen adherent to surface keratin in some stomachs (144241). Oedema was detected in the stratum transitionale where the cells were swollen and pale and it appeared as a pale narrow band. It also affected the stratum basale in the form of clear cytoplasmic vacuolations within the basal epithelial cells (hydropic degeneration). Variations in the length of the lamina papillae were also apparent between cases. Three stomachs had a mild infiltrate of mononuclear cells but showed similar changes to those already mentioned.

Erosions were apparent in four stomachs and were either focal or diffuse. In 143791 the stratum corneum and stratum spinosum were diffusely thinned and all basal cells exhibited severe hydropic degeneration but there was little inflammatory cell

infiltrate. In contrast, diffuse erosion in 144056 was accompanied by a primarily neutrophilic infiltrate underunning a necrotic stratum corneum. Again the basal epithelium showed severe hydropic degeneration. A focal erosion in 139923 showed vacuolation of surrounding keratinocytes and bacterial adherence, spongiosis and hydropic degeneration of the stratum basale and congestion and increased rete pegging in the underlying epithelium.

The appearance of the acini resembling oesophageal glands (PAS positive, mucus producing) within the lamina propria was apparent in two cases. In one (140459) they were found in the mid lamina propria, accompanied by a local infiltrate of plasma cells, lymphocytes, and two lymphoid follicles underlying an erosion. Here, the stratum corneum was thinned and nucleated, and difficult to distinguish from the underling stratum transitionale. The other occurred beneath two erosions where the stratum corneum was absent and only the tips of the rete pegs were left intact. Here, there was marked hydropic degeneration of the basal epithelium and oedema of the adjacent lamina propria, elongated lamina papillae, and infiltration of neutrophils and lymphocytes into all layers. This latter appearance also occurred in another section without acini in the lamina propria. There was no evidence of ulceration at site 1.

Site 2: In this area there were nine stomachs which showed no evidence of erosion or ulceration. A majority of these displayed parakeratotic and orthokeratotic hyperkeratosis along with lengthening of rete pegs and extension of the lamina papillae further towards the epithelial surface. These appearances were frequently exaggerated towards the region of the margo plicatus. Here, they were also

accompanied by an increase in the intensity of lymphocytic infiltrate or lymphoid follicles (133191, 144292) within the lamina propria. At times, clumps of glandular tissue extending from the adjacent pyloric gland area were seen to underun stratified squamous epithelium (144439). Hydropic change, an increase in the number of mitotic figures in the stratum basale and oedema of the stratum spinosum were also seen.

Shallow erosions were frequently seen in parakeratotic epithelium and took the form of flaking of the stratum corneum with bacterial adherence and fungal involvement (Fig 47) (133191, 145235). Intracorneal microabscesses (144241) consisted of degenerate neutrophils enclosed within the stratum corneum with neutrophils infiltrating the surrounding epithelium. The lamina papillae were increased in length and the rete pegs appeared more dendritic. The lamina propria contained increased numbers of lymphocytes with follicle formation at the margo plicatus.

Other erosions (145037) were more severe and diffuse and just left bacteria-covered rete peg remnants capping hyperplastic basal cells above a lymphocytic infiltrate within the lamina propria.

Areas of ulceration were either focal (133191) with a bed of granulation tissue and a capping of neutrophils and necrotic debris with little effect on surrounding epithelium, or diffuse (143791, 143989). Here large areas of epithelium were lost and inflammatory cells seen to underun the stratum corneum in surrounding areas. Occasionally PAS staining highlighted fungal species infiltrating the stratum corneum of these sections both adjacent to and away from the area of ulceration.

In one section (143696) the ulceration affected a polyp, and neutrophils were seen to underun the surrounding stratum corneum. Throughout the section there were elongated rete pegs and spongiosis of the stratum basale and stratum spinosum.

Site 3: Many sections taken from this area were found to represent fundic glands rather than cardiac gland tissue. A mild lymphocytic infiltrate was seen within the lamina propria in several sections (140373, 140459, 143818), and an epithelial covering of surface mucus containing sloughed epithelial cells was also a common finding. These were assumed to be normal changes. A lymphocytic infiltrate of the lamina propria was also found in association with capillary congestion of the surface epithelium and a lymphoid follicle at the level of the gastric glands in one section. Gastric gland dilatation, apoptosis of cells at the isthmus and oedema of the submucosa were all seen without evidence of an inflammatory cell infiltrate. One section showed an erosion capped with necrotic tissue and adherent bacteria. Beneath this were a focal lymphocytic infiltrate, capillary congestion, and a lymphoid follicle at the level of the gastric glands with oedema of the submucosa (143791). There was no evidence of ulceration at this site.

Site 4: The most common finding at this site was varying degrees of lymphocytic infiltrate in the lamina propria. Mild capillary congestion and oedema of the submucosa were also noted. There was no evidence of erosion or ulceration in any of the sections.

Site 5: There were few slides from this location which did not have an inflammatory infiltrate of some description (140373, 144468). The most frequent finding was

varying degrees of lymphocytic infiltrate within the lamina propria extending up between the glands from the pits towards the superficial epithelium. Sometimes this also involved a thickened lamina muscularis. Frequently the surrounding glands appeared hyperplastic, tortuous and distorted (dysplasia), although hyperplasia was seen to occur alone also (Fig. 46). There were two erosions in 133191, which were shallow and accompanied by neutrophils, lymphocytes and surface capillary congestion. The pyloric glands resembled shallower cardiac glands in focal areas of two sections, and lymphoid follicles were present in a few sections (144292, 140459). Another unusual appearance was that of hyperplasia of the surface mucus cell epithelium. This was seen in 144476 within the antral and pyloric regions. Special stains showed the epithelial cells to be hyperplastic and producing a thicker layer of PAS positive mucous substance.

Site 6: A majority of the sections exhibited a diffuse lymphoplasmacytic infiltration of the lamina propria of the villi. This was sometimes accompanied by eosinophils and multiple lymphoid follicles. A mild scattering of neutrophils was visible in one section but there was no evidence of erosion or ulceration.

Table 10 . The Modified Sydney System classification for the glandular and squamous regions of each horse stomach

Number	Squamous	Glandular
133191	Mild chronic gastritis, marked regional activity with mild focal eosinophillia	Mild chronic gastritis, mild focal activity (pyloric) Mild focal dysplasia (pyloric)
138643	Moderate chronic gastritis, marked regional activity with mild eosinophillia	Mild chronic gastritis
139923	Mild chronic gastritis, mild focal activity (margo plicatus) Reactive	Mild chronic gastritis, mild focal activity (pyloric) Mild focal dysplasia (pyloric)
140373	Mild, focal, chronic gastritis (margo plicatus) Reactive MP	Mild chronic gastritis, marked focal activity (pyloric) Mild focal dysplasia (pyloric)
140459	Moderate chronic gastritis, moderate regional activity with mild focal eosinophilia Gland metaplasia	Mild chronic gastritis, mild regional activity and moderate focal eosinophilia (cardiac) Mild focal dysplasia (pyloric)
143696	Mild focal chronic gastritis with marked activity (margo plicatus) Reactive MP	Normal
143791	Mild chronic gastritis with mild regional activity and mild focal eosinophilia (margo plicatus)	Mild chronic gastritis, mild focal activity (pyloric)
143818	Normal	Marked focal chronic gastritis (pyloric) mild focal eosinophilia (cardiac) Mild focal dysplasia (pyloric)
143819	Normal Reactive cardia	Mild focal chronic gastritis and eosinophilia (pyloric)
143863	Moderate focal chronic gastritis with moderate activity and mild eosinophilia (margo plicatus)	Mild chronic gastritis with mild regional eosinophillia Mild focal dysplasia (pyloric)
143989	Moderate regional chronic gastritis, with moderate regional activity Reactive cardia	Moderate regional chronic gastritis with marked focal activity (pyloric) Moderate dysplasia (pyloric)
144056	Moderate chronic gastritis with moderate activity Reactive cardia	Mild chronic gastritis, marked focal activity (pyloric) Dysplasia

Number	Squamous	Glandular
144095	Normal Reactive cardia	Mild chronic gastritis, mild focal eosinphilia (pyloric) Dysplasia
144241	Mild regional chronic gastritis, marked regional activity Reactive MP	Mild focal chronic gastritis, mild focal activity (pyloric) Dysplasia
144292	Mild focal chronic gastritis, mild focal activity and eosinophilia (margo plicatus) *Reactive MP*	Mild focal chronic gastritis (pyloric) Dysplasia
144439	Mild focal chronic gastritis (margo plicatus) Gland metaplasia	Mild regional chronic gastritis, moderate regional activity (pyloric)
144468	Moderate chronic gastritis	Normal
144476	Mild regional chronic gastritis Gland metaplasia	Mild focal chronic gastritis, mild activity (pyloric)
145037	Mild chronic gastritis, mild activity Reactive cardia	Moderate regional chronic gastritis Dysplasia
145216	Moderate chronic gastritis	Moderate regional chronic gastritis
145235	Mild focal chronic gastritis (margo plicatus) Glandular metaplasia	Moderate focal chronic gastritis, mild focal acitivity (pyloric)

3.3.3 Summary of Sydney System diagnoses

Within the stratified squamous region, one stomach received a normal diagnosis (143818) and two showed reactivity with no cellular infiltrate (143819 and 144095). Nine stomachs showed a generalised chronic gastritis. Four of these (133191, 139923, 143791, 145037) were mild, and were accompanied by mild/marked regional activity, mild focal activity and mild generalised activity. The other five (138643, 140459, 144056, 144468, 145216) either showed moderate/marked activity (regional/generalised) or no activity. Six stomachs showed focal chronic gastritis all in the region of the margo plicatus (145235, 143863, 143696, 140373, 144292), and three of these were associated with activity. Three stomachs showed regional chronic gastritis (144476, 144241, 143989) two of which were accompanied by regional activity. Glandular metaplasia was recorded in four stomachs with all types of gastritis. Five stomachs showed reactive changes at the cardia, and four at the margo plicatus.

Within the glandular region two stomachs were classified as normal (144468, 143696). Nine stomachs showed a mild generalised gastritis (133191, 138643, 139923, 140373, 140459, 143791, 143863, 144056, 144095) five with pyloric activity (mild/moderate), three with regional activity and one with no activity. Seven stomachs showed focal chronic gastritis (six were mild and one moderate) in the pyloric region. Three of these also had an active component, and two showed focal mild eosinophilia in the cardiac and pyloric regions. Four stomachs showed regional gastritis (145216, 145037, 144439, 143989), which was moderate/mild and accompanied by regional/focal activity in two cases. Dysplasia was seen in twelve

stomachs within the pyloric region.

Looking at each stomach as a whole, six stomachs displayed generalised gastritis in both the squamous and glandular regions (133191, 138643, 139923, 140459, 143791, 144056). Two stomachs which had in the squamous region showed focal gastritis in the glandular, while the other showed a generalised chronic gastritis in the glandular region (143819, 144292, 144095).

Once each stomach had received its histological classification using the modified Sydney classification system, the diagnosis was converted back to a numerical score in order to allow direct comparison with the gross result scores and apply statistical tests. The gross 'number' of lesions were represented histologically by the diffuseness i.e. absent=0, focal =1, regional = 2 diffuse = 3. The severity of the gastritis was calculated by multiplying the infiltrate of neutrophils and lymphocytes together.

3.3.4 The statistical results

A Spearman correlation test was used to compare all these values to see if there was any positive correlation between the gross and histological findings. The squamous gastritis number and glandular gastritis number were the only traits that were correlated, but this was not quite significant ($r_s = 0.42$; $p = 0.061$).

Table 11: Numerical values of gross score for use in statistical evaluation

	GROSS			
	Squamous		glandular	
	Number	Severity	Number	Severity
133191	1	1	0	0
138643	3	2	1	1
139923	2	1	1	1
140373	0	0	1	5
140459	1	1	4	5
143696	2	1	0	0
143791	4	2	1	1
143818	2	1	2	2
143819	4	1	2	1
143863	2	1	1	1
143989	3	4	4	5
145037	4	1	1	1
133191	1	1	0	0
144241	3	1	0	0
144292	0	0	3	1
144439	1	1	2	1
144468	4	1	0	0
144476	2	4	0	0
144056	4	1	1	1
145216	4	1	4	5
145235	0	0	0	0

Table 12: Numerical scores for cellular infiltrates calculated from Sydney System classification to aid statistical evaluation

Case Number	Squamous Number	HISTO Lymph Severity	Squamous Number	Neut Severity	Glandular Number	Lymph Severity	Glandular Number	Neut Severity
133191	3	1	2	3	3	1	1	1
138643	3	2	2	3	3	1	0	0
139923	3	1	1	1	3	1	1	1
140373	1	1	0	0	3	1	1	3
140459	3	2	2	2	3	1	2	1
143696	1	1	1	3	0	0	0	0
143791	3	1	2	1	3	1	1	1
143818	0	0	0	0	1	3	0	0
143819	0	0	0	0	1	1	0	0
143863	1	2	0	0	3	1	0	0
143989	2	2	2	2	2	2	1	3
144056	3	2	2	2	3	1	1	3
144095	0	0	0	0	3	1	0	0
144241	2	1	2	3	1	1	1	1
144292	1	1	1	1	1	1	0	0
144439	1	1	0	0	2	1	2	2
144468	3	2	0	0	0	0	0	0
144476	2	1	0	0	1	1	1	1
145037	3	1	1	2	2	2	0	0
145216	3	2	0	0	2	2	0	0
145235	1	1	0	0	1	2	1	1

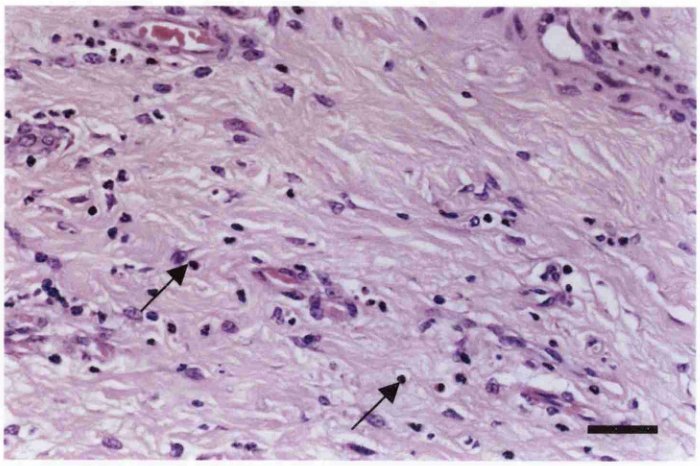
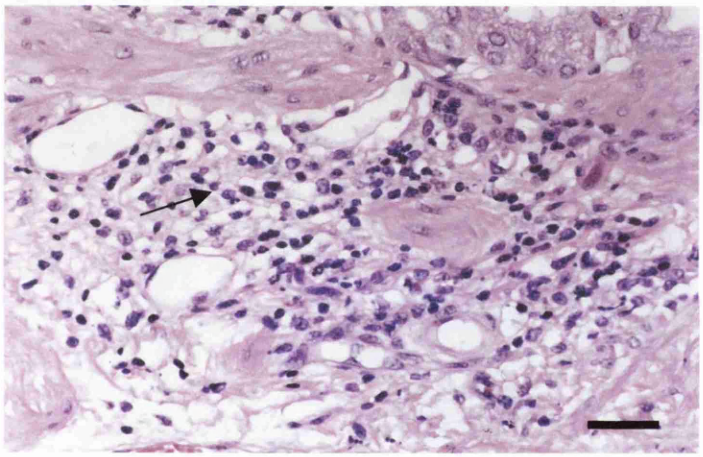
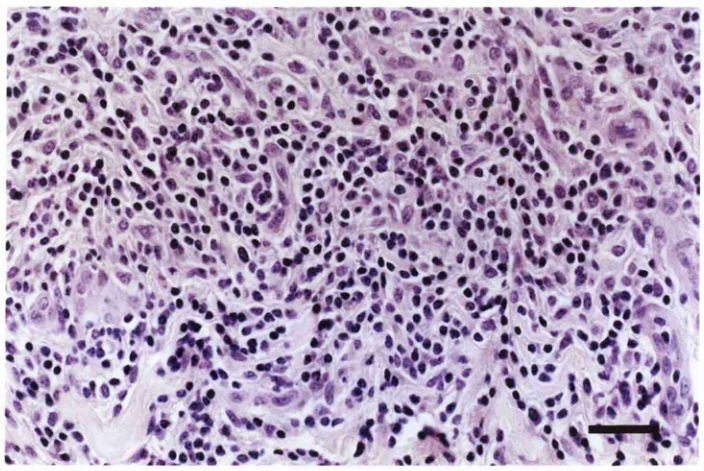


Figure 24 (a-c) : The histological scoring system for gastritis in the horse. Cell types scored were mononuclear cells, neutrophils and eosinophils.

a : A mild infiltrate of mononuclear cells (arrow): several cells per high power field. Bar = 100µm



b : A moderate infiltrate of mononuclear cells (arrow): many cells per high power field Bar = 60µm



c : A marked infiltrate of mononuclear cells : numerous cells per high power field. Bar = 60µm

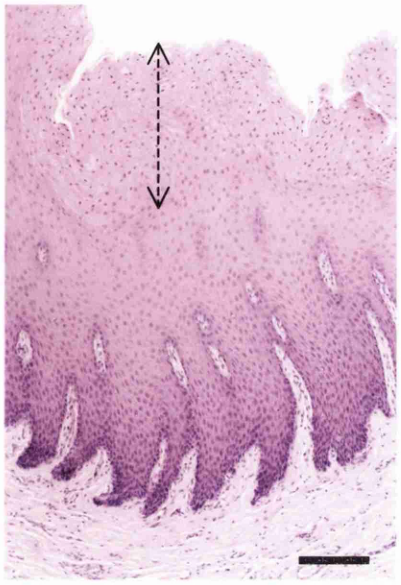


Figure 25 (left): Stratified squamous epithelium showing parakeratotic hyperkeratosis. Note the irregular thickening of the *stratum corneum.*(dashed line) due to the accumulation of nucleated keratinocytes. Bar = 400µm

Figure 26 (below) : Stratified squamous epithelium showing a pocket of neutrophils and necrotic debris within the *stratum corneum.* This is an intracorneal pustule (arrow). Bar = 1000µm

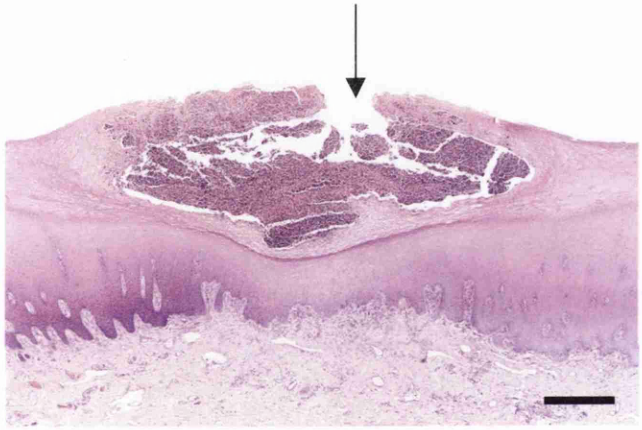
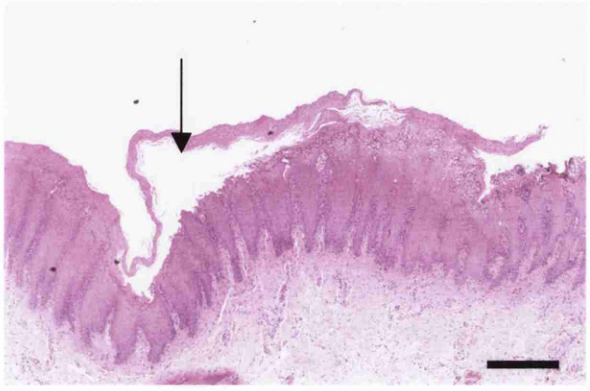
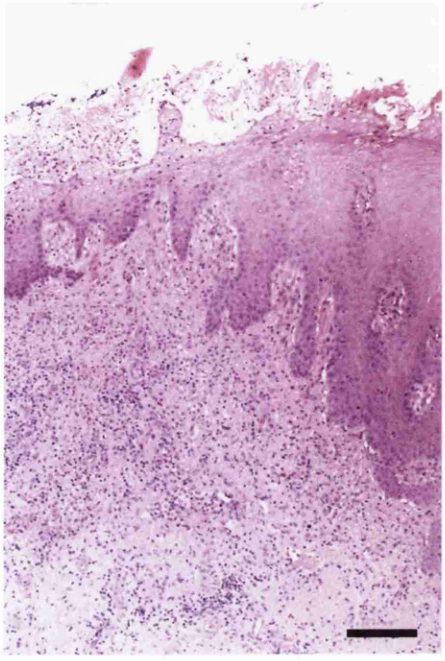


Figure 27 (left) : Erosion involving entire depth of epithelium Bar = 1000µm

Figures 28 (below) and 29 (right) :
Stratified squamous epithelium showing erosion with lifting of *stratum corneum* (arrow) and underunning with neutrophils. Bar = 1000µm Bar = 500µm respectively.





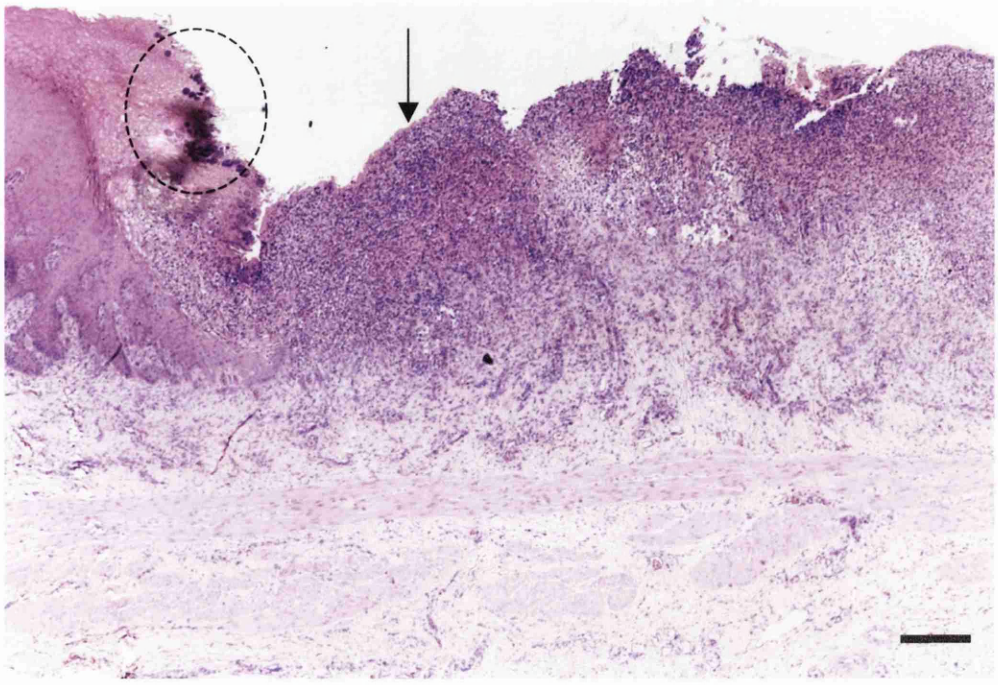


Figure 30 (above) : Stratified squamous epithelium showing an area of ulceration (arrow) consisting of a bed of granulation tissue capped with a marked infiltration of neutrophils and necrotic debris. Bar = 400µm

Figure 31 (right, detail of fig.30) : Note the clusters of bacteria (arrow) invading the fraying stratum corneum at the edge of the lesion. Bar = 150µm

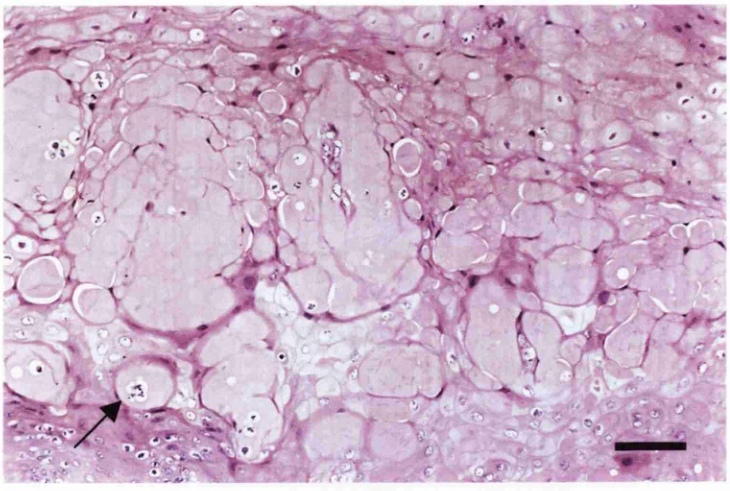


Figure 32 (right): Detail of balloon degeneration of keratinocytes (arrow), often seen adjacent to ulceration. Bar = 150µm

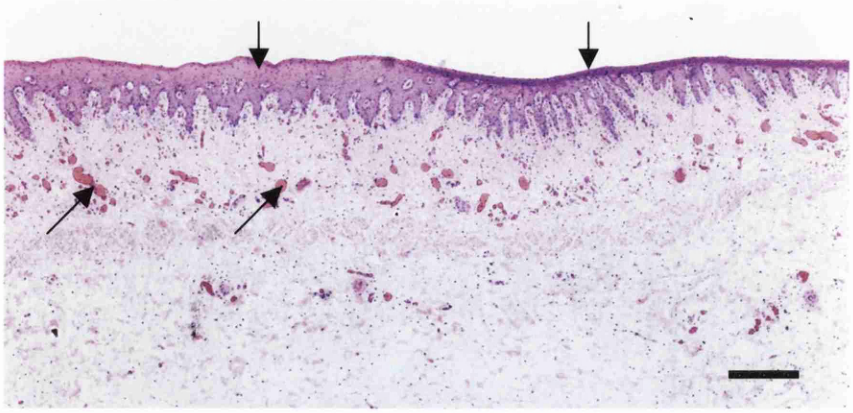


Figure 33 (above) : Stratified squamous epithelium, diffuse erosion with thinned epithelium and congested underlying capillaries (arrows). Bar =1000µm

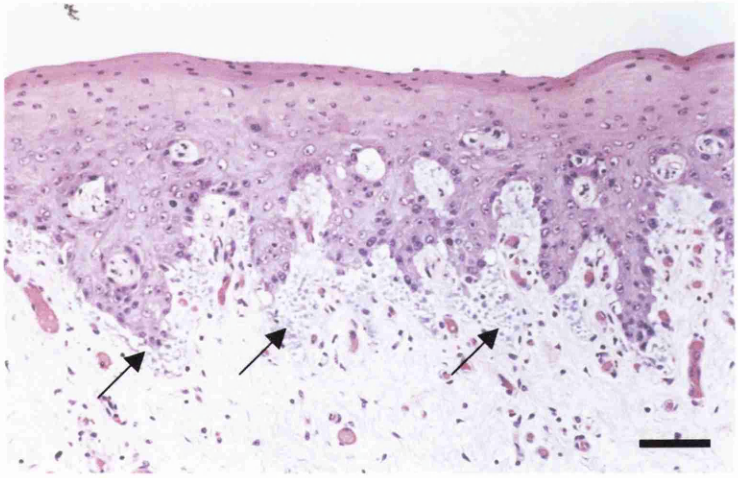


Figure 34 (above) : Detail of Fig 33, note marked hydropic degeneration of basal epithelial cells (arrows). Bar = 200µm

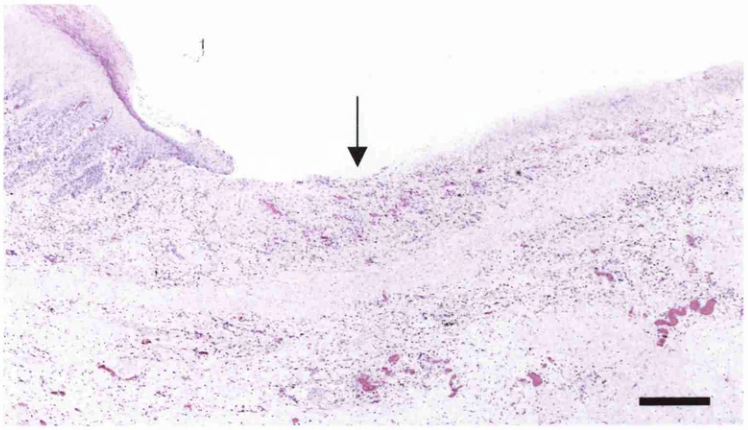


Figure 35 (above) : Diffuse ulceration.Note exposed lamina propria with covering of necrotic tissue (arrow). Bar =1000µm

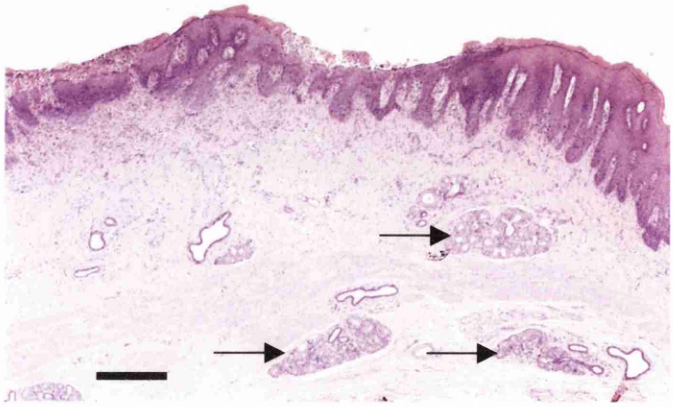


Figure 36 : Glandular metaplasia. The section shows nests of mucus producing glands (arrows) scattered throughout the lamina propria beneath an area of erosion.
Bar = 1000µm

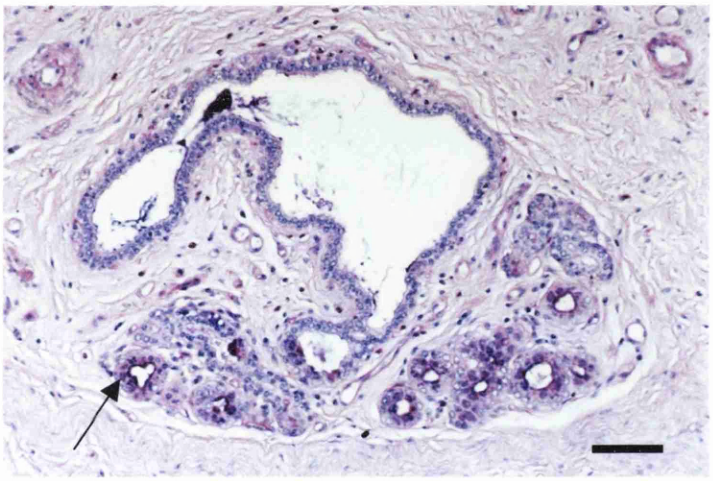
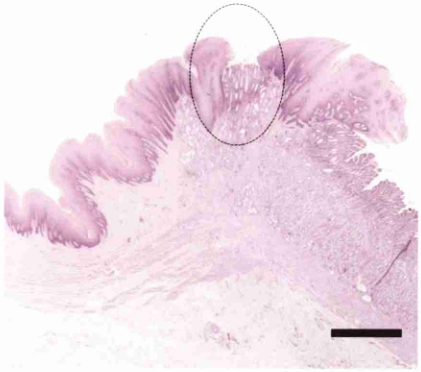


Figure 37 : Detail of glands with PAS positive material in the cytoplasm of the glandular epithelium (arrow).
PAS/Alcian blue stain.
Bar =200µm

Figure 38 (below) : Glandular metaplasia adjacent to the margo plicatus. Bar =2200µm

Figure 39 (right): Glandular tissue underuns stratified squamous epithelium creating an island of central glandular tissue (arrow).
Bar =500µm



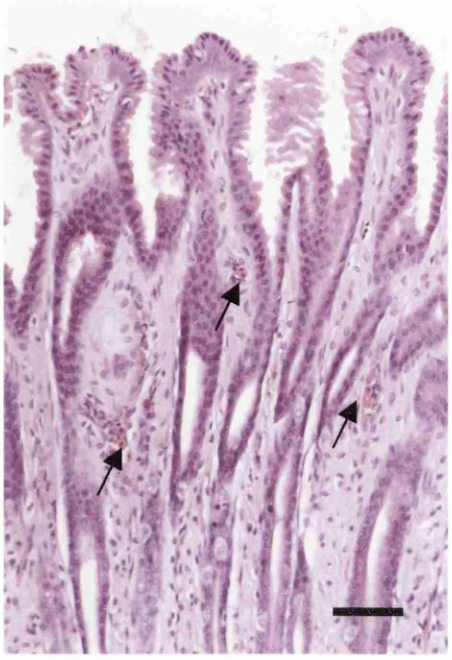


Figure 40 (left) : Pyloric gland region with congestion of superficial capillaries (arrows) and a mild infiltrate of neutrophils visible at high power. This is taken from a grossly hyperaemic area of tissue. Bar = 300µm

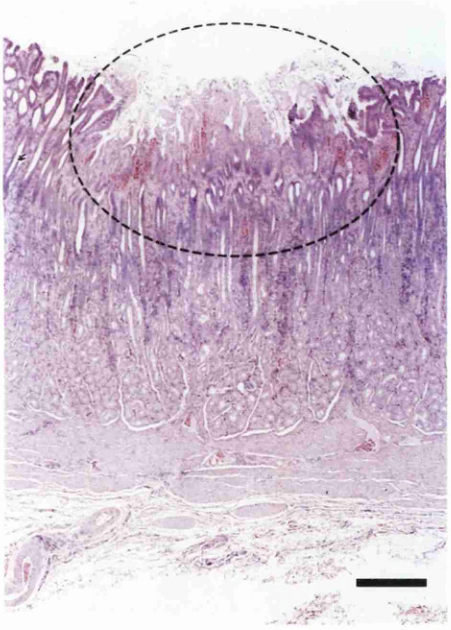
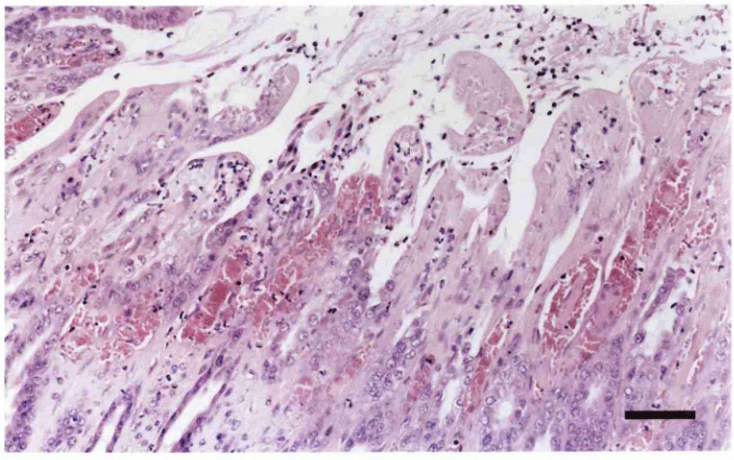


Figure 41 (left). Pyloric gland region. Note central area of erosion with superficial haemorrhage and exudation of fibrin. Bar = 1500µm

Figure 42 (right): Pyloric gland region detail. Note capillary congestion, neutrophil accumulation and marked fibrin exudation. Bar = 200µm



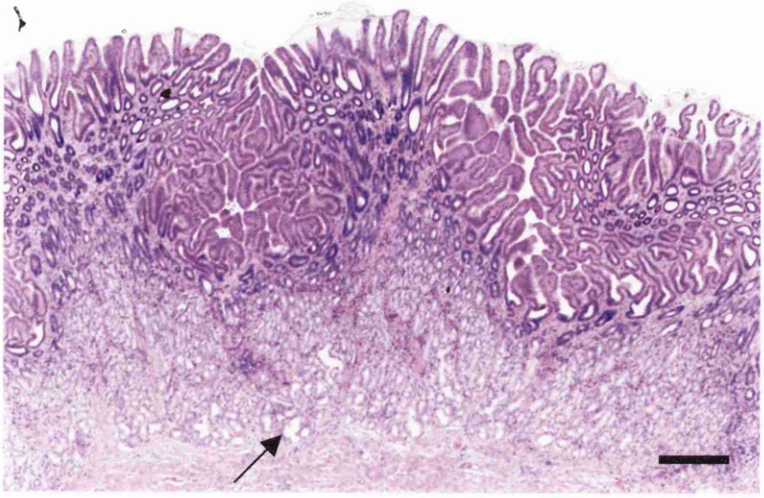


Figure 43 : Glandular dysplasia. Section of pyloric gland region showing dilation and distortion of glands towards the lamina muscularis (arrow). Bar = 1000µm

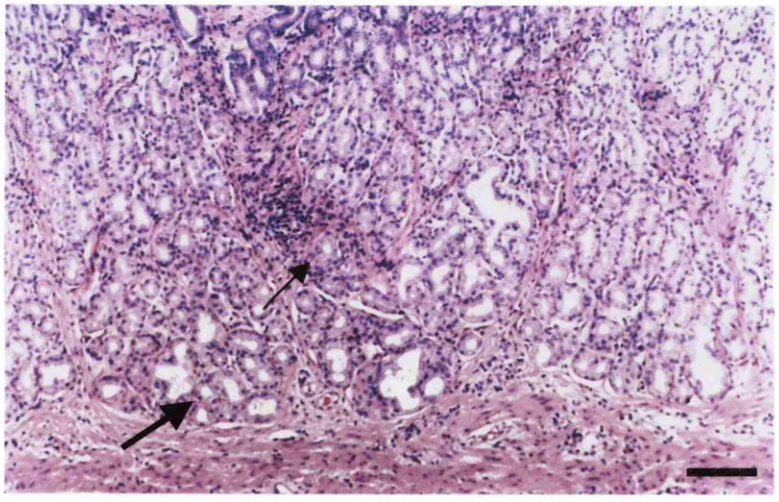
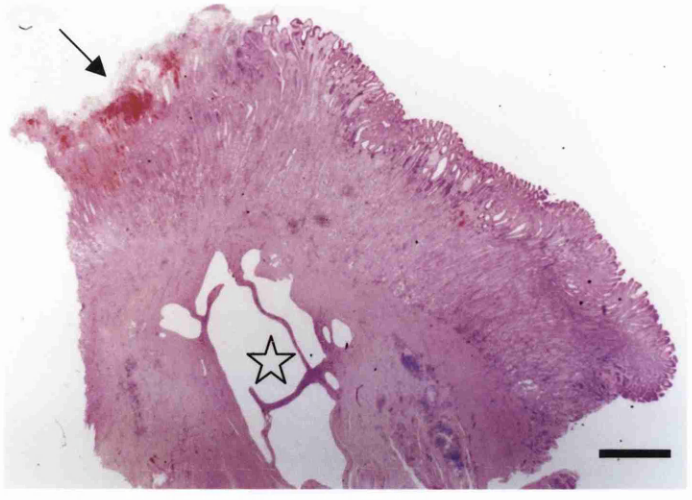


Figure 44 : Detail showing a mononuclear inflammatory cell infiltrate accompanying the glandular irregularities (arrow). Bar = 500µm

Figure 45 : Pyloric gland region. Note deep erosion (arrow), and cystic structure (star) within the lamina muscularis. Bar = 2000µm



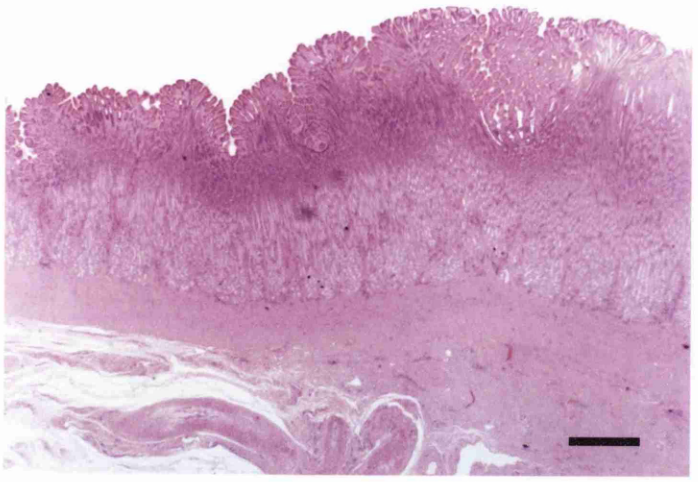


Figure 46 : Pyloric gland region. Note the thickening of the glandular region to the right of the picture (dotted arrow). This is due to glandular hyperplasia. Bar = 1500µm

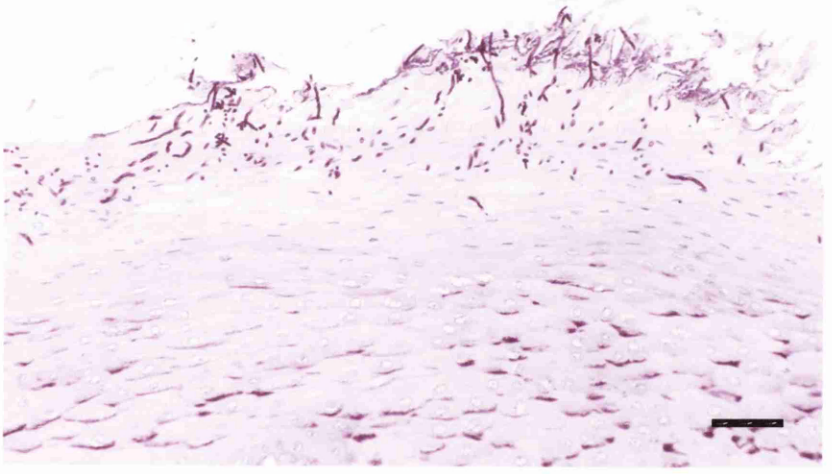


Figure 47 : Fungi visible as thick purple rods colonising the superficial layer of the stratified squamous epithelium . PAS stain. Bar = 100µm

3.4 DISCUSSION

In this study, full written descriptions succeeded in recording all the pathology effectively, but did not provide a succinct means of labelling the type of injury present in each stomach to facilitate case comparison. This was achieved through application of a modified version of the Updated Sydney System used to classify gastritis in humans. Each stomach was given two diagnoses referring to the squamous and glandular region of the stomach. Both the findings and value of the method used to record the pathology present will now be discussed.

The histological interpretation of samples taken from areas of grossly abnormal equine gastric epithelium provided evidence of erosion, ulceration and glandular metaplasia. Bacteria and fungi were also involved with these lesions. There are few existing studies in horses with which to compare these results as only artificially induced acute lesions in the non-glandular region have been examined histologically (Murray et al 2001a). In this study both erosions and ulcerations were identified, but there was no mention of additional infective agents or glandular metaplasia.

Extensive work in pigs however, provides a large bank of comparable material (Embaye et al 1990, Bivin et al 1974, Muggenburg et al 1964). Similarities with this animal include not only the most frequent lesion location (squamous/glandular junction) but also the lesion appearance. Some authors describe erosions as forming from gradual sloughing of surface epithelium, finally exposing denuded apexes of lamina papillae, with the impetus for damage coming from the luminal edge. Associated reactive changes are similar to those recorded in this study and

include parakeratosis, acanthosis, lengthening of rete pegs, balloon keratinocytes, inflammatory cell infiltration, vessel congestion and oedema.

The patterns of lesions observed allow certain ideas about the age of lesion to be developed. Diffuse lesions involving epithelial thinning or ulceration of entire sections appeared to have limited cellular infiltrate, reactive basal epithelial cells or surface necrosis of exposed lamina propria. There is little reactivity in the way of hyperkeratosis, acanthosis and rete peg lengthening. The pathogenesis of these lesions is likely to be scalding (as both horses were suffering from diseases causing gastric reflux) and therefore acute. The punctate lesions recorded where the base of the ulcer consists of granulation tissue and the surrounding epithelium is reactive and contains a marked inflammatory infiltrate are more chronic. Their small size may be due to lesion contraction whilst healing, or they may have been punctate originally. Interestingly, in pigs the ranges of lesions observed are classified on a scale of acute to chronic. The acute lesions appear similar to those described in this study as diffuse (i.e. complete loss of surface epithelium with a layer of necrotic tissue overlying a lamina propria with markedly congested vessels and only a mild superficial mixed inflammatory cell infiltrate). The chronic lesions are similar to those described as punctate, beds of granulation tissue supporting a fibrinopurulent exudate. Classifying lesions on these terms could be a possibility for further studies.

Proposed pathogeneses for gastric ulceration in the pig include the formation of parakeratosis and its subsequent flaking and progression to erosion. However, it does seem that the extent and degree of parakeratosis present in pig stomachs is greater than that found in the horse stomachs in this study, so reducing the importance of its

role in the pathogenesis of erosion and ulceration in the horse.

The involvement of infectious agents has also been mentioned in pigs, with records of yeast infections seen in older pigs with severe lesions (Embaye et al 1990). Although no specific identification was made of the species of yeast involved in these cases, it is likely that they have the morphology of *Candida spp* (oval or round blastospores, measuring 3–6nm. Budding mixed with pseudohyphae comprising chains of elongate yeast like cells or with tubular septate hyphae). These yeasts are capable of adhering to stratified squamous epithelium in the presence of carbohydrates, which are necessary for keratinolyis and hyphae formation. In pigs, they are reported to invade parakeratotic epithelium, promoting the formation of a spongy epithelium containing yeasts, hyphae and pockets of neutrophils and bacteria within the stratum corneum. Subsequent desquamation of this epithelium may then produce small ulcers. In this study the yeasts observed were generally associated with existing erosions/ulcerations, suggesting that they were secondary invaders. The presence of microabscessation within the stratum corneum seen in this study was also likely to be caused by *Candida spp.* Previous reports from equidae include their isolation from five foals (Gross and Mayhew 1983) where they were seen involved with severe ulceration of the margo plicatus. Infection is said to be associated with antibiotic therapy, environmental and social stress and the administration of anti-inflammatory drugs many of which the horses in this study were exposed to (Hammond et al 1986, Barker et al 1993).

In a majority of the sections, bacteria were seen to be adherent to surface keratinocytes both in normal and reactive epithelium. The specific identity of these

bacteria was unknown although they were not *Helicobacter spp* (Chapter 4). In pigs the Gram positive rods resembling *Lactobacillus spp* have been found most frequently on normal but also parakeratotic epithelium. These bacteria are normal inhabitants of the gastrointestinal tract and pars oesophagea in pigs. Their relevance in this disease lies in their ability to release lactic and acetic acids (potentially epithelium damaging) as by-products of anaerobic glycolysis, so contributing to the overall gastric acid concentration. Experiments using gnotobiotic piglets (Krakowka et al 1998) found gastroesophageal erosion and ulceration to be produced when a carbohydrate enriched diet was fed in combination with mono-infection with *Lactobacillus* and *Bacillus.* Synergism was assumed as infection with *Helicobacter spp* did not appear to be ulcerogenic. However, another study has revealed that *Lactobacillus* is not always adherent to the epithelium and can also be found in sloughed epithelial debris and gastric contents, so detection on epithelium is not a true representation of its presence.

There were two types of glandular metaplasia observed within the stomachs in this study. The first showed the formation of PAS positive mucous glands within the mid lamina propria away from the margo plicatus in two separate stomachs. Erosions and ulcerations were present in both sections, and there was a mild scattering of mononuclear cells about these glandular structures. There are no reports of similar findings in the horse, pig or composite stomached animals. In the horse, oesophageal glands (which these resembled) are said to be present at the level of the trachea within the submucosa and not to extend towards the thoracic inlet. This is an example of stem cell metaplasia. Stem cells are responsible for tissue repopulation either during normal circumstances or post injury. They are defined by two

functions, the first is their ability to reproduce themselves throughout the lifespan of the animal, and the second their potential to give rise to differentiated cells. However, subsequent to tissue damage and regeneration, stem cell metaplasia can occur. Metaplasia is the formation of one differentiated cell type from another in postnatal life, which occurs as a result of stem cells changing their state of developmental commitment. During embryogenic development, adjacent cells which develop in common cell sheets will have similar transcription factors and may just differ in one transcription factor gene. (Slack 2000) In the horse embryo, the squamous and glandular regions of the stomach develop together separately from the oesophagus. This might indicate that the stem cells within the non-glandular and glandular region differ by only one gene, so enabling glandular metaplasia in this region.

As previously mentioned, glandular gastric epithelium is protected from the acidic gastric contents by a mucous layer that allows a pH gradient to exist between the surface epithelium and gastric lumen. The vulnerability of the squamous region to damage is partly blamed on this lack of mucus protection. However, there is one report detailing the presence of surface mucus over the non-glandular region (Bullimore et al 2001). In this study they mention the lack of mucus producing glands visible within the squamous region, and suggest that it might originate from the glandular region, nasal, salivary or respiratory mucin. These glands present a likely origin for the mucus, and provide an example of how tissue metaplasia might occur as a protective mechanism. It is possible that the development as metaplastic tissue occurred in order to protect the relatively vulnerable stratified squamous epithelium from an over acidic environment. Their association with

already eroded/ulcerated epithelium suggests this theory and not that they are a normal feature of this type of epithelium which has gone undiscovered so far.

The second type of glandular metaplasia was not always associated with erosion/ulceration, and occurred at the margo plicatus as an extension of glandular tissue, in islet form, towards and within the non-glandular region. This could be a natural progressive development that occurs throughout the life of the horse due to continual exposure of the squamous epithelium to gastric contents adjacent to the margo plicatus. Extensive research involving any change in measurements of the two areas with respect to age or pH within individual stomachs would help to determine this hypothesis. In one stomach this appearance of glandular islands within normal stratified squamous epithelium was particularly pronounced (144476). Adjacent pyloric glandular tissue showed mucous hyperplasia, with PAS staining showing the mucous layer to be thickened and hence more protective. This, in combination with this marked glandular metaplasia at the margo plicatus could suggest that the gastric contents in this horse were particularly acidic/corrosive.

A similar feature of glandular metaplasia occurs in humans at the glandular squamous junction of the distal oesophagus and glandular cardia region. This is named the Z line due to the interdigitation of these two types of epithelium. It is said to be a normal occurrence if glandular metaplasia is present in the distal 2cm of the oesophagus (Dixon et al 1996). However, intestinal metaplasia of the stratified squamous epithelium is a common sequel (Barrett's oesophagus) and it is this condition in humans which is preneoplastic. There is no evidence of an increased incidence of intestinal metaplasia or gastric neoplasia of this region in the horse.

In pigs there is one report (Curtin et al 1963) where healing of an erosion at the squamous glandular junction was seen to occur by spread of glandular tissue towards the cardia without the formation of scar tissue. However histological evidence of this phenomenon is not given.

3.4.1 Glandular changes

Sections taken from areas of gross abnormality within the glandular region were found to represent erosions and reactive glandular changes but most likely to be from the pyloric gland region. Some were found to be normal, which was similar to one study in pigs, where it was found that more glandular lesions were identified grossly than confirmed histologically (Bivin et al 1974). Other areas were represented by superficial vessel congestion, erosion and glandular hyperplasia with or without dysplasia and a chronic inflammatory infiltrate. Contrary to the gross observations made in Chapter 2, no areas of ulceration were identified histologically.

There are fewer texts in the literature concerning the lesions in the glandular regions in both pigs and horses. The difference in glandular anatomy between the two species (pigs have a wider cardiac gland region, an extensive fundic gland region and a limited pyloric gland region) makes comparison of disease in this area less suitable. In pigs, lesions have been identified in the fundic gland region, where they appear as superficial necrosis of mucosa and capillary haemorrhage with a limited inflammatory reaction. Other studies have identified deeper erosions in the fundic gland area extending into the glands of the lamina propria. A thin layer of necrotic tissue and exudate covered the surface of the crater, with neutrophils, plasma cells

and lymphocytes accompanying it. (Muggenburg et al 1964). No specific causes were assigned to these lesions.

In this study only one horse (143791) showed evidence of erosion within the fundic gland region, and this was most likely due to severe duodenal reflux as the horse had suffered from a caecal impaction. Reasons for erosion within the pyloric gland region include inappropriate NSAID administration, prolonged bile reflux, stress or bacterial infection (Chapter 1). It is difficult to assign a specific cause for each case: whatever the cause, glandular mucosa tends to follow a common pathway to ulceration of the epithelium. However stress and recent NSAID administration are likely to induce acute lesions with erosion, superficial eosinophilic necrotic debris, neutrophils and haemorrhage. Bile reflux and bacterial infections are more prolonged processes and might be more likely to induce chronic glandular changes as well.

In this study, chronic changes found in association with erosions included glandular hyperplasia, glandular dysplasia, islets of glands infiltrating the lamina muscularis and a large mucoid cyst like structure within the submucosa variable mononuclear cell infiltrates. One stimulus of glandular hyperplasia is parasitic infections (e.g. *T. axei),* but in 140459 where glandular hyperplasia was most diffuse, no parasites were detected. A more likely cause is that these were areas of old erosion/ulceration where hyperplasia of glands next to the lesion has occurred and remained after the original lesion has healed.

Reports of glandular changes associated with alkaline reflux gastritis also include glandular hyperplasia (Dixon et al 1986). Other features are elongation, tortuosity

and hypercellularity of the gastric pits (foveolar hyperplasia), and vasodilation and congestion of capillaries in the superficial lamina propria. Increased smooth muscle fibres in lamina propria are also reported; a feature which might be responsible for the thickening of lamina propria between the gastric pits seen in this study. There is generally a paucity of inflammatory cells accompanying these reactive changes. It is therefore possible that some of the hyperplasia seen could have been due to reflux gastritis.

3.4.2 Gastritis

Analysis of the results using a modification of the Updated Sydney System specifically designed for this study found 18/21 stomachs to have evidence of some kind of gastritis within the stratified squamous region. One aim of applying this system was to enhance recognition of any specific patterns of gastritis that that might be present. In this region, mild/moderate generalised chronic gastritis or focal chronic gastritis both with areas of activity in the region of the margo plicatus were seen. Reactive epithelium was classified in ten stomachs and glandular metaplasia in four. Within the glandular region two stomachs were classified as being normal and nine had a generalised gastritis. Areas of activity were generally restricted to the pyloric region. Most glandular changes were recorded as being dysplastic with no evidence of glandular atrophy or intestinal metaplasia. In humans, atrophy is specifically related to *H. pylori* infection and can take the form of loss of specialised components e.g. parietal and chief cells being replaced by mucus producing cells, or overall loss of glandular structures secondary to inflammation and fibrous tissue formation. Intestinal metaplasia of glandular tissue which is also another feature of

H. pylori gastritis in humans was not found.

Using this classification system it was difficult to identify any specific patterns of inflammation. This was partly because there were so many variables in each diagnosis but also due to the small sample size which made trends difficult to identify. More stomachs than not appear to have some degree of gastritis, be it localised or generalised. An underestimation of the normal level of cellular infiltrate in the horse could explain this, as the visual analogue scale was based on the cellular infiltrate expected in human stomachs. Certainly initial attempts at classification of gastritis in dogs found all animals to have an infiltrate of inflammatory cells of some degree, so in the second study the criteria were modified (Happonen et al 1998). Further studies with more samples would be needed to confirm this in the horse.

Statistical tests were applied to scores translated from the gastritis classifications, in an attempt to identify any relationships present between the extent and severity of gastritis in the squamous and glandular regions. The weak correlation found between the squamous gastritis number and the glandular gastritis number could indicate that there was a shared stimulus for gastritis in both regions. The lack of correlation between gross lesion severity/number and gastritis severity suggests that the gross lesions of erosion and ulceration seen may not be the result of an underlying gastritis.

3.4.3 The Effectiveness of the Modified Sydney Classification

Originally for humans, the Sydney System was devised to provide a means of accurate diagnosis of gastritis within the living patient. In order to tailor it to the

needs of the horse stomach several modifications were made. Most importantly the composite nature of the equine stomach, and the complete absence of any information regarding its normal histological appearance, required that several full thickness samples be taken from each animal. This excluded the possibility of using this precise system in the live animal as biopsies are not full thickness and the number of samples required would be impractical.

However, as a post mortem means of analysis, the system did manage to relate several of the features recorded in the written descriptions e.g. the frequent activity recorded in the region of the margo plicatus reflected the higher number of erosions and ulcerations in this area. It also highlighted that reactive epithelium was not always related to an increase in the inflammatory cell infiltrate suggesting some external stimulus for this reactivity. It was not possible to distinguish between the types of glandular metaplasia present using this system, and the severity of erosion/ulceration was not reflected by the degree of activity, as this only measures the neutrophilic infiltrate, which was not so marked in some very acute lesions.

For the classification, the parameters chosen to indicate reactivity within the stratified squamous epithelium were extrapolated from other species and may not be as representative in the horse as these other species. In the horse other studies (Murray et al 2001a) have measured actual thicknesses of cell layers to locate reactive epithelium. However due to individual cell swelling this is not as accurate a representation of thickness as counting the actual cell number. The fact that there are no reference ranges for normal measurements also led to an estimation, using the only stomach with a normal gross appearance as a guideline as to what was normal.

This may have led to large inaccuracies regarding the reactivity of epithelium and hence classification of gastritis. It was interesting to note that even stomachs without an inflammatory infiltrate still had signs of reactive epithelium.

The guidelines to assess the degree of cellular infiltration were taken directly from humans, and may have resulted in an over diagnosis of gastritis in these animals. In this study it was impossible to have a control group as none of the animals were admitted with clinical signs specific to gastritis, and only one showed no evidence of gross ulceration. The actual location of the cellular infiltrate was also not recordable using this classification method.

Further changes that were not recordable using this classification method included oedema, vascular congestion, erosions and ulcerations. The latter two were assumed to be present if 'activity' was noted (i.e. neutrophils). However the severity/depth of the lesion did not always appear to be related to the degree of cellular infiltrate (e.g. acute lesions) making this an inaccurate assumption.

Although samples were taken from each glandular region, it would have been more beneficial to take two samples from the pyloric gland region, one in the antrum near the margo plicatus and the other the same as number 5 from the pylorus. The sample from the proximal duodenum did not enhance the overall diagnosis of gastritis in the horse's stomach.

3.5 CONCLUSION

Histological assessment of the stomachs of 21 horses reported the main findings as

being gastritis, erosions, ulcerations and glandular metaplasia in the stratified squamous regions, and gastritis, glandular dysplasia and erosions in the glandular region.

The application of a modified version of the Updated Sydney System to classify the type of gastritis in each horse was found to correlate well with the general written description. However some important parameters were non-recordable and the separate diagnoses for the glandular and non-glandular regions made it difficult to assess the overall diagnosis of gastritis in each stomach.

Application of Spearman statistical tests found no positive correlation between the gross and histological appearances of the stomachs. However, the distribution of gastritis within the squamous and glandular regions was found to weakly correlate.

Future work needs to concentrate on establishing what the normal inflammatory picture of an equine stomach is without previous illness/therapeutics. Then a more accurate modification of the Sydney system could be used and adapted to fulfil a role in diagnosing the disease in the live animal.

CHAPTER 4 – THE SEARCH FOR *HELICOBACTER*-LIKE-ORGANISMS

4.1 INTRODUCTION

The role of infectious causes, specifically *Helicobacter sp*, in the pathogenesis of equine gastritis/gastric ulceration has not yet been fully explored. In the previous chapter, the cellular infiltrates and reactive tissue changes recorded neither confirmed nor excluded the possibility of *Helicobacter sp* involvement.

Before the positive identification of *H. pylori* in humans, doctors were in a similar quandary as to the cause of chronic gastritis. Research since then has led to the identification of over 17 different species of *Helicobacter* (Fox and Lee 1997) that have been found to inhabit the gastrointestinal tract of various mammals and birds. While the exact pathogenic potential of each strain has yet to be determined, development of hepatic adenomas and adenocarcinomas in one inbred strain of mice has been linked to infection. Both the desire to find a comparable model for human disease and the unknown zoonotic potential of the bacteria have been the basis for extensive research.

The goat, horse and rabbit are the only remaining domestic animals from which the bacteria have not been isolated (Scanziani 2001). Recent work in foals has identified the presence of antibodies against *H. pylori* proteins in sera taken from post-colostral foals. These were identical to those identified in the dams, and new bands were seen to develop as the animals aged, presumably due to the formation of innate

antibodies. However, the bacteria have never been cultured or identified histologically in these animals.

Given the similarities in gastric anatomy, ulcer location and prevalence in the horse and pig, findings in the latter are particularly relevant and useful for comparison. Initially named *Gastrospirillum suis* (now *Helicobacter heilmannii)*, the most frequently identified species of *Helicobacter* in pigs was found in 62.5% of abattoir pigs and was established as a possible factor connected to the aetiopathogenesis of swine gastric ulcer (Barbosa et al 1995). More recent studies confirmed the association of this organism, present in all regions of the stomach, with lesions in the pars oesophagea (Queiroz et al 1996). However, it was also suggested that it played a secondary rather than primary role in lesion formation (Krakowka et al 1998) and Koch's postulates have yet to be fulfilled in determining a primary role in the pathogenesis of gastritis in this animal. All these findings prompted the search for an HLO in the horse.

Previous studies have shown the detection of the bacteria to vary greatly between isolation techniques. Methods of detection include isolation of the bacteria itself (culture, histology, PCR, FISH), its antibodies (serology, immunohistochemistry) or its products (urease detection from biopsy/breath testing). Further definitive verification of infection has been achieved through inoculation of infected gastric material to germ-free mice and the production of gastritis.

Culturing the organism is made difficult through its specific environmental requirements in which to grow (microaerophilic conditions, fresh moist agar, and

inhibition of growth by other organisms), and recognition of colonies (the morphology of which is species-specific). Some are reported to appear as a mucoid film across the surface of the agar that can easily be mistaken for water marks (Fox and Lee 1997).

Histological identification, a frequent method of detection in human gastric biopsies, is aided by the use of special stains to highlight the bacteria (Dixon et al 1996). Previous reports cite HLOs within the surface gastric mucus. Preservation of this layer is therefore paramount and special procedures involving snap freezing samples were adopted in order to achieve this (Bullimore et al 2001). When screening the stomach for specific bacterial colonisation it is important to include samples from all areas, as the infection can sometimes be region-specific. For example in humans *H. pylori* gastritis is generally more pronounced in the antrum than the corpus of the stomach.

In this study, both culturing techniques and histological identification were used to demonstrate any HLOs. Six samples were taken from each stomach, two from the stratified squamous region, one from the cardiac gland region, one from the fundic gland region, one from the pyloric gland region and one from the proximal duodenum. Obvious areas of erosion/ ulceration were also sampled as frequently as possible.

4.2 MATERIALS AND METHODS

The presence of *Helicobacter spp* was assessed in samples of stomach tissue taken from six previously specified sites (detailed in Chapter 2) and any other

areas of ulceration within the stomach. Sterile forceps were used to handle the material at all times taking care not to disrupt the mucosal surface. These samples underwent routine and specialised histological and bacteriological processing to aid detection of this organism. Unfortunately samples for bacteriology had to be transported between buildings and this was not possible until a quick inspection had been made of the remaining carcass. This meant there was some delay before culturing could take place.

Samples for bacteriological culture were obtained by the application of a sterile wire loop to the mucosal surface of each specimen. It was scraped and twisted with some force along the stomach epithelium and the contents then inoculated onto various plates. These included MacConkey and Sheep Blood agar for aerobic incubation and *Campylobacter* medium and *H. pylori* medium (Columbia agar containing *H. pylori* selective supplement Dent.Oxoid) for microaerobic incubation. The loop and forceps were sterilised in a Bunsen burner flame between inoculations.

The plates were checked visually every 2 days and any suspicious colonies subcultured, Gram-stained and then examined under an oil immersion microscope lens. The incubation period for each plate was a maximum of 15 days.

In order to ensure that these environmental conditions were appropriate for the growth of *Helicobacter spp,* control material from human gastric biopsies was obtained. This took the form of colonies of *H. pylori* on Helicobacter medium, cultured from child gastric mucosa. This had been previously tested to be urease and catalase positive and had the correct morphological properties to be *H. pylori* (1mm

round, grey, shiny colonies). The API Campy system was used to confirm their identity and was found to be 98% positive for *Helicobacter pylori*. These colonies were successfully subcultured seven times and then stored at –70° C on beads (Microbank, Pro-lab diagnostics; Figs. 48–49).

Towards the end of the project some of these samples were thawed and reinoculated onto *H. pylori* specific agar. Once grown, they were subcultured several times until four plates were filled with pure viable colonies. Three 5ml vials each of PBS and saline solution were then warmed to 37° Celsius and poured onto the inoculated plates. These were then agitated to form a suspension of *H. pylori* which was poured back into the vials. Fresh samples of stomach tissue taken from sites 1 and 5 were immersed in these solutions and removed after 1, 2 and 3 hours. They were immediately placed into quantities of 10% formalin and fixed for 48 hours. They then underwent the processing mentioned at the beginning of this chapter.

Histological examination included routine staining with H and E and scoring of each section (Chapter 3). Special stains were used to identify the presence of *Helicobacter spp* or other bacteria. A Gram stain was used to identify any general bacteria (Gram-positive organisms blue, Gram-negative red). The Warthin–Starry silver stain was used to specifically identify argyrophilic organisms. Any positive organisms would show black with a gold background. The modified Giemsa stain highlighted any spiral organisms a dark pink, against a pink and orange (similar to H and E) background. Immunohistochemistry staining using *H. pylori* antibody supplied by DAKO, Cambridgeshire, UK, (BO471) would stain HLOs dark brown, along with connective tissue cell cytoplasms against a light yellow background. Positive

controls were used for each stain and consisted of *H. pylori* naturally infected human gastric mucosa and HLOs identified in canine gastric biopsies.

Extra samples were taken from two of the horses from all the same sites (Chapter 3). These were embedded onto OCT compound (Tissue Freezing medium, Jung) and snap frozen by a Cryo spray freezer spray gun (Cellpath, Powys, UK.). Cryostat sections were then cut at 7μm, mounted onto APE slides and stored at –70°C. Sections were air dried for 30 minutes before on slide fixation in FDC [(20 per cent formalin, 79% distilled water and 1% cetylpyridinium chloride (CPC)]. Routine and specialised staining then took place.

4.3 RESULTS

There was no positive identification of any HLOs through bacteriological culture or histological examination of formalin fixed tissue. Problems were encountered in both procedures. Interpretation of the bacteriological plates was made impossible when overgrowths of fungi, *Proteus spp* or other fast growing bacteria were present. This resulted in discarding plates before the end of the pre-designated 15 day incubation period. Gram staining of suspicious colonies was not possible for every new growth due to the sheer number, and the resultant subculture did not always grow on successfully preventing identification. This may have caused certain colonies to be overlooked or misidentified, so leading to errors of interpretation.

The samples which were suspended in a suspension of *H. pylori* medium were also negative for *H. pylori* on histological examination. All the special stains that have been previously mentioned were used for their detection.

In contrast to the results obtained with the horse samples, *Helicobacters* could be clearly identified in human gastric biopsies infected with *H. pylori,* and dog gastric samples with visible HLOs. These were initially identified with H and E stain (Figs. 50, 51), then modified Giemsa stain (Fig. 52) and Warthin-Starry (Fig. 53). The organism was readily and specifically identifiable using immunohistochemistry in controls (Figs 54 and 55).

Figure 48 and 49 : Small round grey translucent colonies of *Helicobacter pylori* growing on specific *H.pylori* medium.

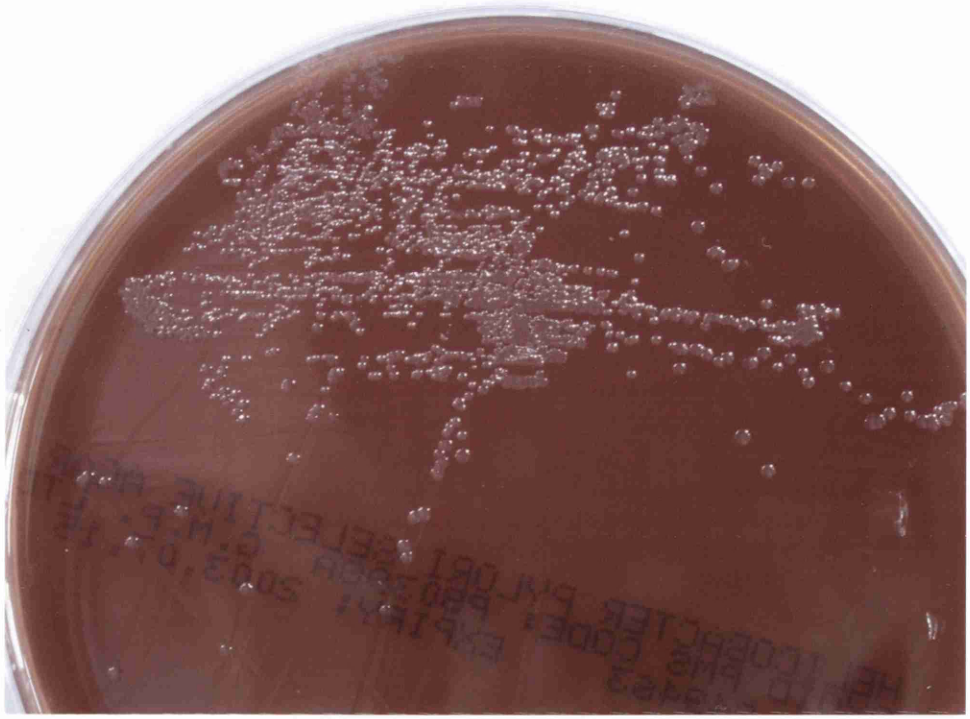


Figure 50: Canine gastric mucosa with spiral *Helicobacter* - like organisms visible in surface mucus. H and E stain .Bar = 10µm

Figure 51: Canine gastric mucosa with spiral *Helicobacter* – like organisms visible in the lumen of a gastric gland. H and E stain. Bar = 10µm

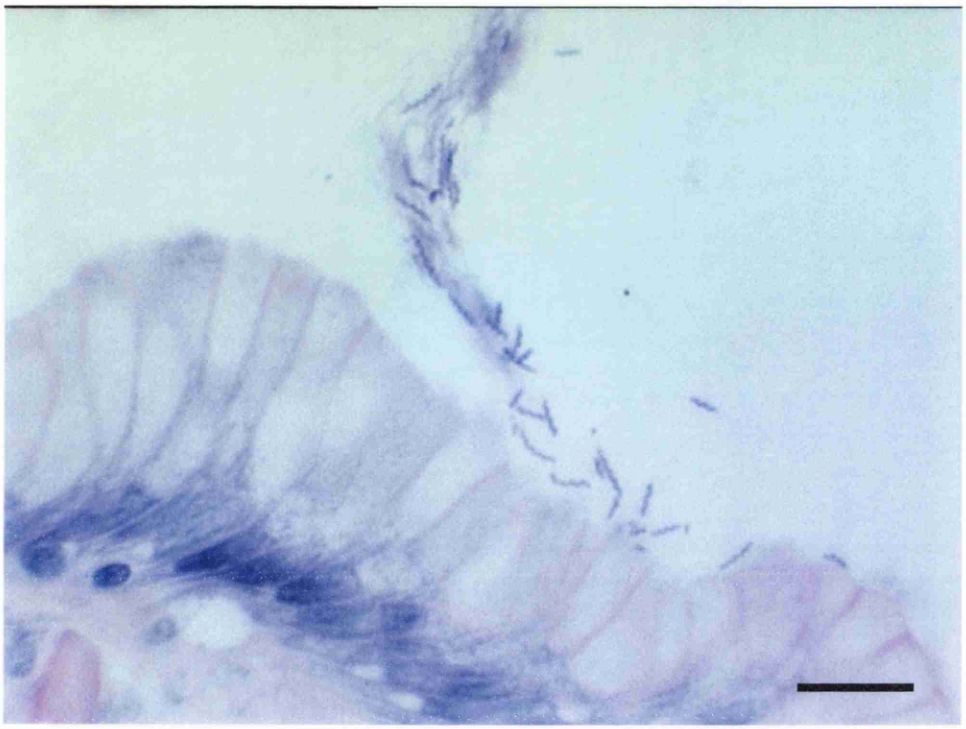


Figure 52 : Canine gastric mucosa with spiral *Helicobacter* –like organisms visible in stream of mucus. Modified Giemsa stain. Bar = 12µm

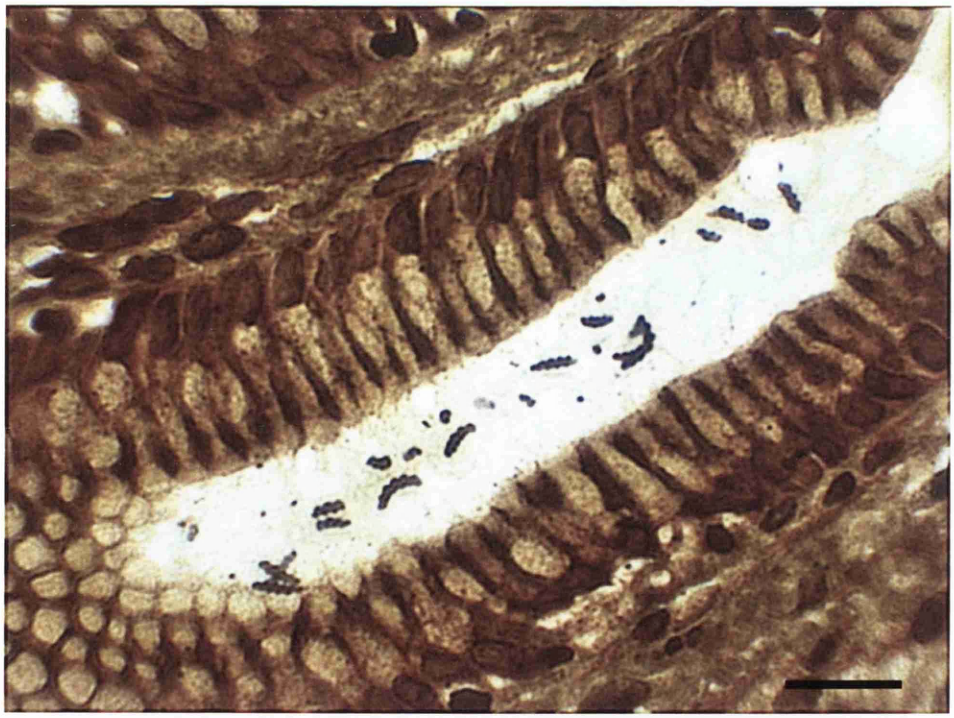


Figure 53: Canine gastric mucosa with black spiral *Helicobacter* – like organisms present in gastric gland lumen. Warthin Starry.stain Bar = 15µm

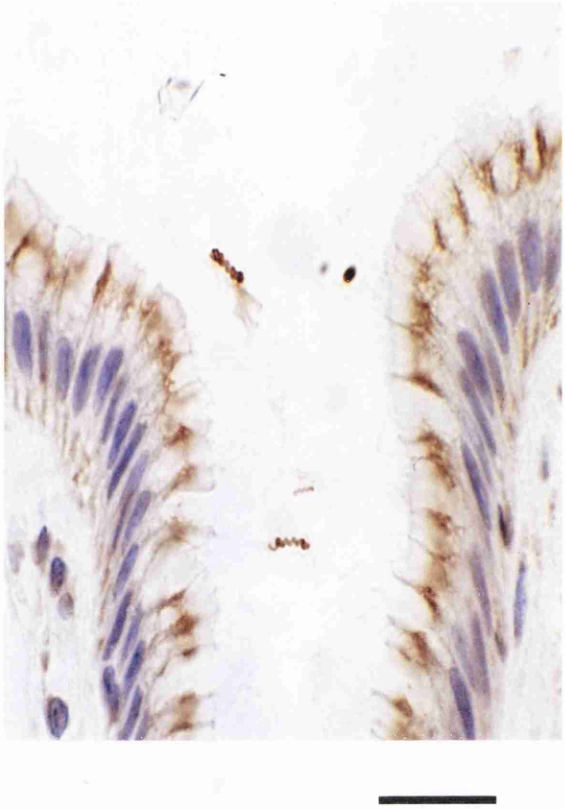


Figure 54: Canine gastric mucosa, brown spiral *Helicobacter* –like organisms visible in gastric gland lumen. Immunohistochemstry stain. Bar = 20µm

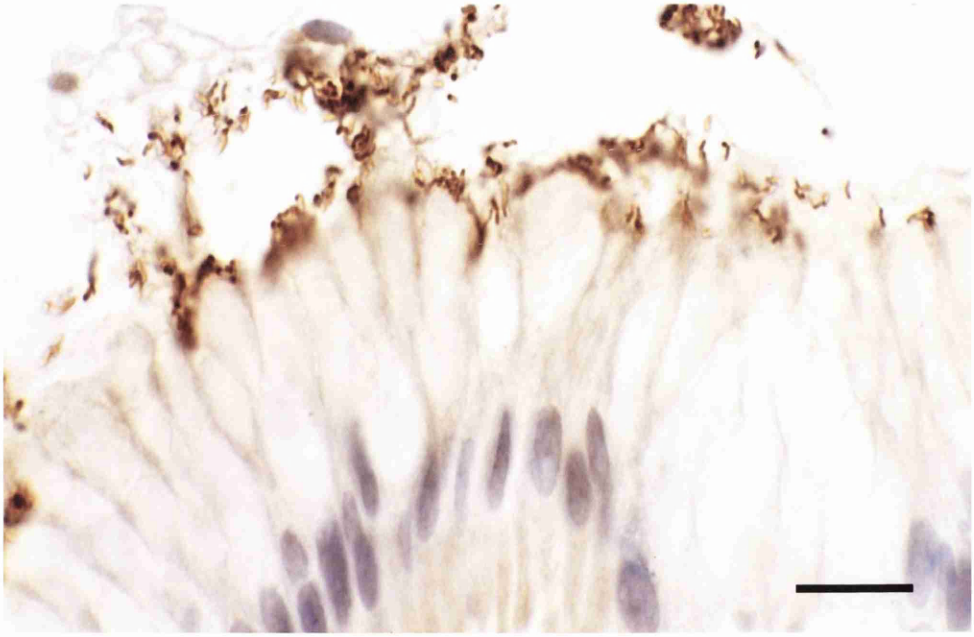


Figure 55: Human gastric mucosa with brown staining *Helicobacter pylori* present in surface mucus. Immunohistochemistry stain. Bar = 7µm

4.4 DISCUSSION

The failure to detect any HLOs may have been due to a number of reasons other than the obvious one that it actually is not present.

The first is that HLOs vary greatly in morphology, and it was therefore unknown what specific shape and size of organism to expect histologically or the appearance of the colonies on culture. Obviously details regarding similar species were used as guidelines but these may have been misleading and the bacteria overlooked.

Histological interpretation depended on the effectiveness of the special stains used, as routine H and E methods were sometimes difficult to interpret with the smaller sized organisms. The Warthin–Starry stain was not only tedious to perform (preparation time 3 hours) but very inconsistent in its outcome, with positive controls only working 50% of the time. In these sections the amount of background staining made distinguishing between gastric debris (exaggerated in sections with slight post mortem change) and possible HLOs an almost impossible task. The modified Giemsa method and immunohistochemistry were easier to interpret with a 100% positive control outcome (figs 50-55).

Processing the bacteriological samples was complicated by up to 20 minute delays occurring between sample selection and analysis due to general post mortem duties of the pathologist, and the 5 minute walk to the lab. These of course were kept to a minimum although it could be argued that the samples may have benefited from being placed in a transport medium (e.g. Fox and Lee 1997). The wire loop was

effective at removing mucus for inoculation from the glandular epithelium of the stomach. The non-glandular samples did not have such a viscous covering and so the sample may not have been representative of the microbiological flora present. Other studies recommend tissue homogenisation to ensure that all bacteria are sampled. However, it is widely found that the culture of HLOs is difficult and frequently fails despite positive identification using other techniques (Cantet et al 1999). Other successful methods of demonstration include PCR, inoculation into mice and urease detection.

The location of the organism, both topographically and within the mucosa could also have prevented identification. Although the predetermined sample sites attempted to include all epithelial and glandular types, large expanses were left unexplored specifically the pyloric antrum adjacent to the margo plicatus. In humans, once the active chronic stage of disease has been reached, organisms are more likely to be found in the antrum than the corpus of the stomach. Specific regions of the stomach reported to harbour the organisms in animals include the fundic gland region in dogs (Hermanns et al 1995) and multiple sites in pigs, including the antrum, fundus, cardia, and pars oesophagea (Roosendaal et al 2000). In horses a patchy distribution may have prevented identification from the six predetermined sample sites. Depending on the species, the organism is reported to reside between the mucus layer and the epithelial cells, or within the parietal cells. Snap freezing and FDC fixation were methods implemented to preserve the mucus layer to ensure no bacteria were missed. However, this technique was only applied to a few specimens. There may only be very few HLO present in the stomach, especially if chronic changes are marked and there is a hostile environment for the bacteria.

The precise history of previous antibiotic administration for these horses was unknown. In humans successful eradication involves the use of triple therapy techniques using amoxicillin or tetracycline with metronidazole, and omeprazole or bismuth subcitrate. These have also been found to be partially effective in reducing the bacterial load short term in dogs (Happonen et al 2000). It is therefore possible that previous therapy including some of these drugs may have already eliminated the organisms from the horse stomachs.

The age of the animal is certainly important with regards to the epidemiology of infection. In humans infection is acquired during childhood and is generally thought to persist for life, although there are some schools of thought that infection can be lost in childhood and adult infection is possible (Mitchell 2001). In dogs it has been suggested that puppies may acquire gastric *Helicobacter* infection from dams during the lactation period, and that puppies can infect each other during early life (Hänninen et al 1998). Very recent literature suggests that this maybe the same case in horses, as they have identified *Helicobacter* antibodies in foals over 2 days old. It may have been that the adult animals used in this study could have already rid themselves of infection attained earlier in life.

In humans, specific histological characteristics have been identified as hallmarks of infection with *H. pylori* including chronic active gastritis, glandular atrophy and metaplasia and multiple lymphoid follicle formation. Even in the absence of the organism itself eg. post-treatment or in areas of severe ulceration, the presence of these parameters should arouse suspicion of infection.

It was difficult to know what specific histological parameters to look out for in the horse as they would obviously vary according to the species of *Helicobacter* organism involved. Several species have been isolated from dogs, cats, rodents and pigs although, as already mentioned, their role in the pathogenesis of gastritis is yet to be established. In dogs for example, experimental infection of gnotobiotic beagle pups with *H. felis* caused large numbers of lymphoid nodules, mostly in the fundus and body, and a mild diffuse lymphocytic infiltrate with small numbers of plasma cells and eosinophils in the subglandular region (Lee et al 1992). Another investigation comparing the histological picture of gastritis in dogs with and without clinical signs isolated *Helicobacter spp* from over 95% of those individuals examined, with no significant difference in burden between the two groups. In addition to this, gastric inflammatory indices were not positively correlated with the colonisation density of *Helicobacter spp.* Parietal cell vacuolation was associated with intracellular presence of a helicobacter organism, but it was not clear whether they were inducing degeneration or colonising an already compromised cell. The histological picture showed a diffuse distribution of mononuclear cells, along with variable quantities of neutrophils, eosinophils and lymphocyte aggregates. Mild to moderate atrophy was recognised in three dogs and mild metaplasia in one dog. In cats gastric HLOs are also highly prevalent (86%) although histological findings in both infected and non infected animals include normal-mild-moderate chronic gastritis (Neiger and Simpson 2000). Both the lack of negative controls available and the presence of multiple species infections in these animals make it difficult to allocate certain gastritis patterns to specific infections.

In this study a majority of the stomachs were identified as having some degree of chronic gastritis in the squamous and glandular region with a positive correlation being found between the distribution or extent of inflammatory infiltrate in these two areas. This could indicate that there is a common cause between the two areas, although the severity of infiltrate did not appear to correlate.

Other findings included varying infiltrates of neutrophils in the pyloric gland region, occasional lymphoid follicles and eosinophils. Reactive changes did not find any evidence of intestinal metaplasia in the glandular region, and little atrophy, but there were large areas of glandular dysplasia accompanied by inflammatory infiltrates and erosions. There are no other reports detailing the normal findings in horse stomachs, so the relevance of these findings is hard to assess. However, they are as non-specific as those reported for gastritis in other animals, and do not follow the criteria for humans with infection with *H. pylori.* Infection however can therefore not be ruled in or out from these findings alone.

Individual host susceptibility has been found to be an important factor with regards to reaction to infection, with the production of variable types of gastritis in different strains of inbred mice (Sakagami et al 1996). In this small population of horses, it was difficult to pick up any specific trends as to the pattern of gastritis observed.

4.5 CONCLUSION

No HLOs were detected using histopathological and culturing techniques. However it is possible that the organisms are still present, as the methods used for

identification were not fool proof and histological picture of gastritis could still comply with infection.

CHAPTER 5 - FINAL CONCLUSION

This study is one of the first accounts to systematically detail both the gross and histological appearance of gastritis/gastric ulceration in the horse. The initial aim of the thesis was to increase the understanding of the pathogenesis of this disease, and it was hoped that specific initiating factors/ infectious causes might be identified. The conclusion that lesions in the squamous and glandular region were probably the result of a variety of insults agrees with much of the existing literature.

More important was the development of the ability to define the disease more accurately, through comparison of gross and histological appearances of specific lesions. The gross appearance of lesions was sometimes found to be misleading, as ulcers identified by eye were often revealed as erosions histologically. These discrepancies highlight the difficulty in gross lesion assessment especially within the glandular region, and raise suspicions as to the authenticity of already published material that rely on gross observations alone. As well as being inaccurate, this propensity to over diagnose the disease could have more serious consequences when unleashed on the general public. Animal welfare groups now cite gastric ulceration as an important 'illness' of racehorses. Without a concrete understanding of the lesions we actually see, the bucket term of gastric ulceration will be used to describe all types of gastric injury, carrying with it morbid connotations from human medicine and false ideas about the true severity of the disease seen down the endoscope.

So how important actually is this disease, does it warrant this recent amount of research and attention? There is no doubt that in the racehorse industry where animals are being pushed to their extremes, the clinical significance has been proven through treatment trials. In this mixed population of horses, the incidence of gastric injury was also high despite the lack of specific related clinical signs. This raises the possibility that some of the squamous lesions may not be pathogenic, especially after the discovery of glandular metaplasia in this study. This would appear to be the result of prolonged exposure to acid, and could be proven by future experiments measuring the advancement of glandular tissue to squamous relative to the age of horse. It might also be useful to look at the development of the foals' stomach in the first six months as the epithelium reacts to the acidity of the gastric contents and thickens. Ulceration of the *margo plicatus* is reported in a large proportion of growing foals, the majority of which show no clinical signs suggesting that this may not be pathogenic either.

Highlighting the incidence of disease in the glandular region has also been an important aspect of this study. Although no statistical significance was found between the severity of gross lesions and underlying gastritis, presence of the latter is important. The chronic histological changes observed, fuels the idea that *Helicobacter sp.* may still be involved in the pathogenesis of glandular gastric disease. There is always the danger though, that because this is the case in humans, it must also be so in animals. It was over 100 years ago that *Helicobacter heilmannii* was identified in dogs, and still its role in the pathogenesis of gastritis is still unclear. In horses, identification of antibodies to helicobacter urease proteins has recently

occurred, however the lack of concurrent histological observations prevents any conclusions to be drawn about related gastritis.

The techniques for gross and histological sampling used in this study pave the way for further similar studies in the horse. Once a bank of material has been collected and the appropriate classification systems applied, fine-tuning can be made to improve the classification system and a full knowledge of the true variety of gastric lesions which exist can be obtained. From this, specific patterns of gastritis and their aetiology could be observed, so introducing the idea of gastric biopsying for a more accurate diagnosis of gastric disease in the horse

In final conclusion, this study has highlighted the large variety of pathologies which exist in the adult horse stomach. Efficient means of recording these and future findings have been suggested. The use of these will allow the effective comparison of data collected from different sources so increasing the understanding of the disease.

APPENDIX 1 – GROSS POST MORTEM REPORTS

Unless otherwise stated each horse was euthanased using a free bullet. Clinical histories vary according to the amount of information given in the original written records and contain only what is deemed relevant for this study. The pathological diagnosis includes the primary disease (in bold) which was responsible for the death/euthanasia of the animal and then lists other findings in decreasing order of pathogenicity. The gross appearance of individual organs is only mentioned if abnormalities are present. This is also the case for histological findings throughout the carcass. A detailed histological description of each stomach can be found in Appendix 3.

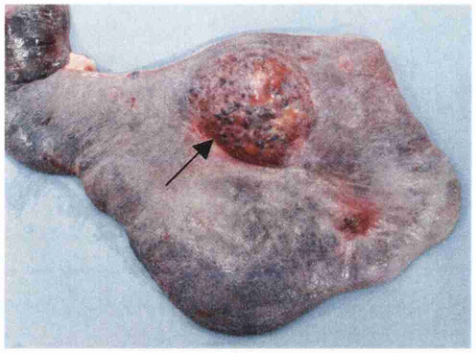
CASE NUMBER 133191 : 19 year old riding pony (mare).

Clinical history : Sudden onset haematuria for one weeks duration .

Pathological diagnosis : Haemangiosarcoma, nephrolithiasis, gastric ulceration.

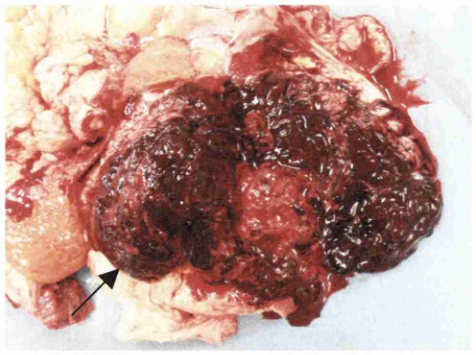
General post mortem : The carcass was in good condition. Dissection revealed numerous circumscribed fleshy dark red masses infiltrating the liver, spleen, right adrenal and omentum. These varied in size from 0.5cm – 3cm in diameter. The right kidney possessed a localised area of capsule that appeared pale and thickened. This covered an organising blood clot like structure between the capsule and the kidney itself. The left kidney contained a circular nephrolith, 7cm in diameter and weighing 2.33g. This was lodged in the renal pelvis so destroying the normal architecture and

leaving behind little viable tissue. There was extensive perirenal haemorrhage involving the surrounding fat and mesentery so preventing accurate identification of these individual structures. There was no evidence of tumour in the lung or the skeletal muscle.



Spleen : note tangerine sized nodular fleshy mass (arrow) on the parietal surface towards the base of the organ (haemangiosarcoma).

Nephrolith : located in pelvis of the left kidney, weighing 2. 33g.



Left kidney (arrow): note loss of normal renal architecture and replacement with friable, disorganised neoplastic tissue (nephrolith removed).

Stomach: The stomach was filled with sweet smelling partially digested fodder, which had a firm, moist texture. In the squamous region there were patchy areas of yellow bile stained epithelium in the region of the greater curvature, which extended from the margo plicatus to the cardia. This was situated near the greater curvature a few cm from the margo plicatus. An oval focus of proud granulation tissue, 0.5cm in diameter with no hyperplasia of the surrounding keratinised tissue was found adjacent to this. At the lesser curvature the margo plicatus was thrown into florrid cauliflower like proliferations, 0.5cm high. These formed a wavy junction with a step like gradient with the glandular tissue. In the centre of these squamous projections were islands of tissue resembling glandular epithelium, which were occasionally hyperaemic. The glandular region of the stomach was unremarkable.

General histology: Histological sections of masses from the mesentery show trabeculae of spindle cells lining cavernous haemorrhagic areas. The outer frameworks of cells appear plumper with increased numbers of mitotic figures and are interspersed with clusters of lymphocytes, neutrophils and macrophages. The right kidney shows an infiltration of thin pale staining spindle cells beneath the capsule. These are randomly orientated and surround a large area of haemorrhage and expanses of accelular fibrinous material. The kidney tissue itself remains uninfiltrated with these cells. What remains of the left kidney shows interstitial fibrosis and many protein filled dilated tubules. The pelvis is distended with blood and there is a stretch of spindle cells accompanying this. The spleen, adrenal and liver are also infiltrated with similar cells and in the latter there is bile duct proliferation within the tumour tissue. This neoplastic infiltrate is haemangiosarcoma

The development of the nephrolith may not have been associated with the tumour formation.

CASE NUMBER 138643: 7 year old Thoroughbred gelding

Clinical history: This horse had chronic bilateral hindlimb lameness with effusion of both femoropatellar joints. It had received phenylbutazone twice daily for the last month of its life.

Pathological diagnosis: Suspensory ligament desmitis, telangiectasis, gastric ulceration.

General post mortem: The carcass was in good condition. Dissection of the hind limbs of the horse revealed bilateral thickening and distinct swelling of the hind suspensory ligaments with gelatinous inflammation of the surrounding connective tissues. Transection of these tendons showed them to be pink and very friable, with multiple blood clots contained within a loose connective tissue framework. This was in sharp contrast to the regular pale, firm appearance of a normal healthy tendon.

Hind Suspensory ligaments: note difference in size and adhesion of surrounding connective tissues in a normal (left) and diseased (right) horse.

Stomach: Much of the squamous region of the stomach appeared yellow and thickened with a corrugated appearance, and was thrown into shallow folds towards the greater curvature. Punctate yellowed scars (1mm diameter), lay scattered about the cardiac sphincter. The margo plicatus remained flat although the squamous border was interrupted by punched out depressions eating into the squamous region. These were pale pink centrally with proliferation and some blistering of the surrounding off white squamous tissue. The widest of these measured 2cm and contained central necrotic debris. The glandular region had hyperaemic lines radiating out from the pyloric area towards the margo plicatus. There was a small erosion at the centre of one of these, but no bleeding was apparent.

Histopathology: Sections of the damaged tendons show separation of the fascicles of collagen with areas of haemorrhage and highly vascular granulation tissue between. There are clusters of neutrophils, lymphocytes and plasma cells within these areas. The liver contains a focus of hepatocytes with extensive clear vacuolation (telangiectasis) associated with a fibrous tagging on the capsule.

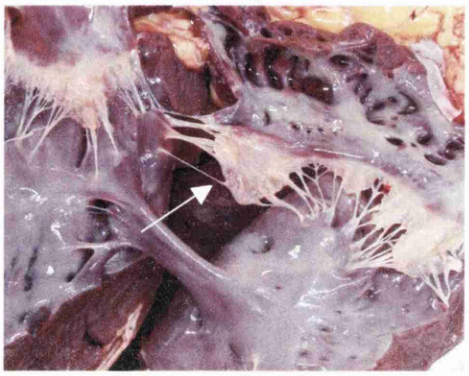
CASE NUMBER 139923: 5 year old Thoroughbred gelding.

Clinical history: A permanent grade 4-6 pansystolic cardiac murmur with a two year history of weight loss.

Pathological diagnosis: Tricuspid regurgitation, mild cyathostomiasis, gastric ulceration.

General post mortem: The carcass was in poor condition. Dissection of the heart

revealed mild stubbing of the tricuspid valves with an associated distension of the right ventricular wall. The lungs and liver were normal in appearance. Multiple scattered nodules a few mm in diameter and red/black in colour were visible on the caecal mucosal surface that were indicative of a cyathostomiasis infection.



Right ventricle: note mild stubbing of the tricuspid valves (arrow).

Stomach: The squamous region of the stomach is uniform in colour and texture apart from a few scattered punctate scars. The margo plicatus has a moth eaten appearance along the lesser curvature with tongues of squamous tissue measuring 2mm in length protruding into the glandular area. Some of these glandular edges appear raw and ulcerated. There is hyperaemia and a superficial erosion present in the pyloric region.

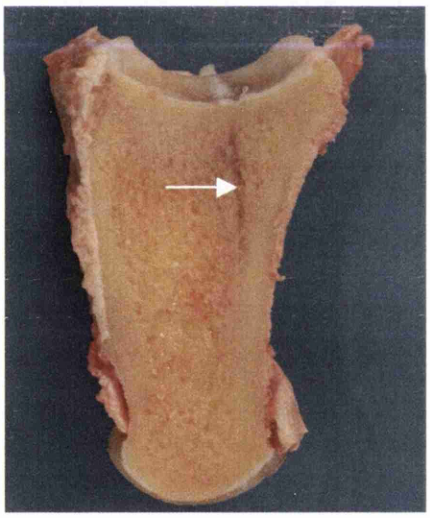
Histopathology: The entire length of gut has an increase in the number of eosinophils and lymphocytes distributed within the lamina propria. A few Cyathostome larvae were present in the lamina propria between the intestinal glands of the caecum where there were increased clusters of eosinophils surrounding them. The epithelial surface appeared intact in this area. One larva was present in the lumen of the dorsal colon.

CASE NUMBER 140373: 14 year old riding horse (mare).

Clinical history: Chronic fore limb lameness

Pathological diagnosis: P1 fracture

General post mortem: The carcass was in good condition with no gross abnormalities noted in the viscera. Dissection of the joint revealed a healing sagittal fracture at the proximal end of P1 towards the palmar aspect. It involved the entire thickness of bone and was marked by a protruding fibrous scar that extended approximately half way distally through the bone. There was no distortion of the bone through callus formation and no evidence of osteoarthritis in the fetlock joint.



First phalanx showing healing sagittal fracture.

Stomach: The squamous region appears normal.The glandular region of the stomach showed two areas of active erosion/ulceration in the pyloric area of the stomach. One was circular (1.5cm diameter) with a dark haemorrhagic ring with an irregular hyperaemic border and central core. The other was shallower with an irregular

outline and a pale centre measuring 0.5cm in diameter and showed no areas of acute haemorrhage.

CASE NUMBER 140459: 5 year old Riding horse (gelding).

Clinical History: Severe head shaker, chronic history of intermittent hind limb lameness.

Pathological diagnosis: Gastric ulceration.

Post mortem: The carcass was in good condition. Dissection of the gastrointestinal tract revealed profuse numbers of *Anaplocephala perfoliata* attached to the mucosal surface of the proximal caecum forming a fish scale pattern. The mucosal surface was puckered and oedematous with mild hyperaemia once the parasites were removed .

Anaplocephala perfoliata attached to oedematous caecal mucosa with a cobblestone appearance.

Stomach: Abnormalities observed in the squamous region of the stomach were scattered throughout in the form of small (1-2mm) brown punctate scars and also at the margo plicatus. It appeared as an irregular wavy line forming a rolled lip towards the lesser curvature, with islands of ulceration and squamous tissue stranded

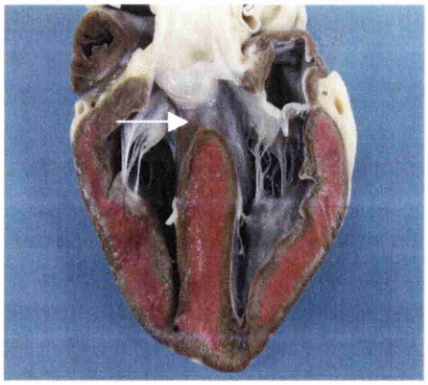
within the glandular. The glandular region displayed diffuse damage with ulceration found in the cardiac, fundic and pyloric regions of the stomach. Ulcers ranged from 1-3cm in length, were ellipsoid in shape and had thickened margins. Some of them were actively haemorrhagic while others appeared more superficial being square/rhomboid in shape. The lesions in the pyloric area appeared shallower than elsewhere (erosions) and ran perpendicular to the mucosal folds adjacent to the pyloric sphincter.

CASE NUMBER 143696 : 9 month old Welsh Mountain pony (colt).

Clinical History :. A persistent Grade 5 cardiac murmur was present with a palpable thrill over the left heart base. The pony was markedly cyanotic after exercise.

Pathological Diagnosis: Tetralogy of Fallot

General Post mortem: The carcass was in moderate condition. Detailed dissection of the heart showed the right side to be hugely enlarged and almost equal in size to the left. The heart weighed 0.8kg without the pericardium, and the base circumference measured 385mm. The width of the right ventricle was base 20mm, middle 25mm, apex 15mm. The base to apex length was 148mm. The diameter of the right AV valve was 50mm and left was 39mm. There was a VSD which measured 20mm in diameter and an overriding aorta. Significant pulmonary stenosis was present with ballooning of the pulmonary artery proximal to the valve. The lungs remained normal in appearance.



Heart: note VSD (arrow) and equal thicknesses of left and right ventricular walls.

The stomach: The squamous region of the stomach displayed scattered irregular brown 1mm foci of scarring along the margo plicatus on the lesser curvature. There was also a brown, 2cm polyp attached by a pedicle adjacent to a shallow fresh granulating erosion (1cm) parallel to the margo plicatus. The glandular region was normal in gross appearance.

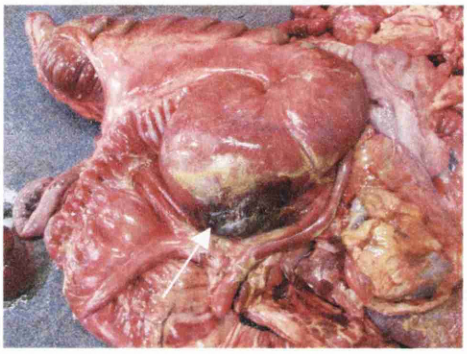
CASE NUMBER 143791: 13 year old riding horse (mare).

Clinical History : The horse had a three day history of acute onset colic with endotoxaemia. She was anaesthetised for one hour for surgical assessment before a hopeless prognosis was given and euthanased on the table with barbiturate.

Pathological Diagnosis: Caecal rupture , gastric ulceration

Post mortem: The carcass was in good condition with a sutured 50cm surgical incision in the ventral midline of the abdomen. Dissection released 2-3 gallons of seropurulent fluid from the abdominal cavity and showed quantities of yellow fibrinous material to be adherent to the serosal surfaces of the abdominal organs. There was diffuse thickening and oedema of the walls of the caecum, ventral and

dorsal colon. A black, necrotic diverticulum, measuring 30x30x8cm, bulged from the serosal surface of the base of the caecum. Externally this was seen to have a central 2cm translucent window through which caecal contents were visible. Incision into its lumen found a 10cm diameter circular depression underlying this, which was surrounded by a rim of black tissue (tear) The caecum was full of green dry content while the large and small colons were almost empty.



Caecum : note tear at base (arrow) with visible leakage of contents and localised peritonitis.

Dissection of the pericardial sac revealed echymotic haemorrhages around the base of the heart, the interventricular groove, and on the dorsal aspect of the lungs.

The stomach: The stomach was filled with green sloppy contents, which were foul smelling. The entire mucosal surface appeared diffusely hyperaemic. The non glandular area was salmon pink in colour with a freshly scalded appearance. These pink areas were patches of erosions which encompassed the mucosa between the margo plicatus and the cardia . There were a few scattered islands of slightly blanched, over white keratinised epithelium and two shallow ulcers with adherent digesta in the ulcer lumen at the margo plicatus on the lesser curvature. The glandular region was normal apart from some localised hyperaemia/erosions present in the pyloric area.

CASE NUMBER 143818 : 3 year old rescue pony (gelding).

Clinical History: Dental malocclusion preventing efficient use of the bit.

Pathological diagnosis: Brachygnathia superior

Post mortem: No abnormalities were noted in the carcass which was in good condition.

Stomach: The non-glandular mucosa had several 2mm punctate scars that were dark brown in colour. The margo plicatus displayed irregular tongues of glandular tissue overlapping into the non glandular area with two isolated areas of glandular tissue within the squamous area which did not appear inflamed. There were several areas of hyperaemia in the pyloric area, some of which contained central haemorrhage. There was one small erosion at the junction of the pyloric and fundic gland regions between the lesser and greater curvatures.

CASE NUMBER 143819 :26 year old rescue mare

Clinical history: Dental malocclusion

Pathological diagnosis: Dental malocclusion

General post mortem: No abnormalities were noted during post mortem examination.

The stomach: The non-glandular area of the stomach has scattered brown punctate scars ranging in size from 1mm to 5mm and concentrated towards the greater curvature. The margo plicatus had an irregular outline. There were four hyperaemic erosions (1-3mm) lying along the folds in the pyloric region and hyperaemia at the border of the fundic and pyloric gland region.

CASE NUMBER 143863: 17 year old Thoroughbred mare

Clinical history: The horse had an acute onset colic due to a strangulating lipoma. Surgery was attempted but due to a hopeless prognosis the horse was euthanased under anaesthesia.

Pathological diagnosis: Strangulating lipoma.

General post mortem: The carcass was in good condition and there was a ventral midline incision along the abdomen. Dissection found a necrotic length of gut in the mid jejunal area. This was one metre long, dark red/black in colour, and had a thickened oedematous wall which was very friable. There was no strangulating lipoma associated with this lesion as it had been removed at surgery. Other smaller lipomas were scattered throughout the mesentery. No further abnormalities were detected on gross post mortem.

The stomach: The stomach contained green foul smelling fluid and the walls were stretched and thinned with mucus adhering to the epithelium. 2mm scars were scattered across the non-glandular mucosa with corrugation (hyperkeratosis) and yellowing of the surface epithelium with circular blistered areas towards the greater

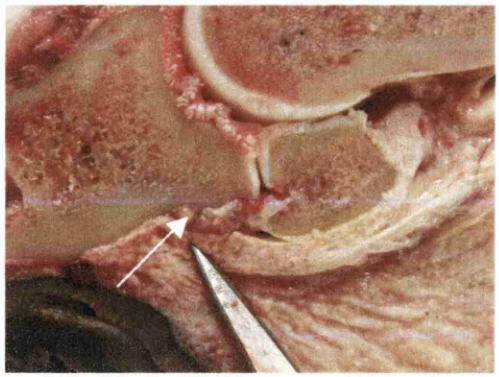
curvature. All along the margo plicatus at the lesser curvature there were florrid polyp like proliferative lesions. These had central glandular areas which were yellowed and eroded and were ringed by squamous tissue. Where areas of mucus were peeled off , some hyperaemic patches and erosions were visible. Moderate post mortem change and an adherent mucous layer made interpretation of the appearance of the glandular area difficult.

CASE NUMBER 143989 : 19 year old Thoroughbred mare.

Clinical History : Sudden onset right fore lameness. Had received phenylbutazone within the last month of life.

Pathological Diagnosis : Navicular bursitis

General post mortem: The carcass was in good condition. Dissection of the right fore revealed a thick off white exudate (pus) present between the distal ligament of the distal sesamoid bone and the third phalanx. A necrotic infection tract extended upwards and laterally into the navicular bursa. No evidence was found of a perforating wound originating from the sole but this remains the most likely explanation.



Coffin joint: note location of pus in navicular bursa (arrow).

Stomach: The non-glandular region of the stomach displayed a few shallow, hyperaemic coalescing lesions over the lesser curvature which increased in number and severity (some evidence of fresh bleeding) towards the margo plicatus. Three ellipsoid bleeding ulcerations were present in the mid pyloric region (antrum) and ran parallel to rugal folds. They had an active central granular hyperaemic appearance and measured 3-5 cm in length and 0.5-1cm width occupying the entire crest of the rugal fold. There was a small erosion at the pyloric fundic glandular border near the margo plicatus.

CASE NUMBER 144056 : 21 month old Clydesdale (Colt).

Clinical history: The horse had a history of condition loss and generalised oedema. There was a grade 4-5 heart murmur audible on the left side of the chest.

Pathological Diagnosis: Congestive cardiac failure

General post mortem: The carcass was in moderately poor condition. Massive subcutaneous oedema was present around the brisket, ventral abdomen and

the prepuce. There were 3 litres of clear fluid in the abdomen and massive oedema of the folds of the mesentery and around the wall of the small intestine. Dissection of the heart revealed white streaking present in both atria, while the ventricles were normal in appearance. The liver showed evidence of chronic venous congestion.

Liver: note the nutmeg appearance of hepatic chronic venous congestion.

The stomach: The non-glandular area of the stomach had diffuse patches of dark brown scarring radiating out from the cardiac sphincter. The margo plicatus had an irregular junction along the lesser curvature with islets of squamous tissue remaining. There was one locus of erosion at this point. Within the glandular region there were scattered areas of hyperaemia throughout the pyloric and fundic gland regions with a shallow erosion towards the pylorus.

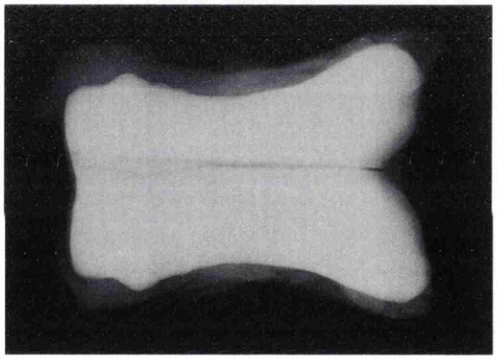
Histopathology: There is marked venous congestion of the liver with pulmonary oedema also. The ventricles of the heart appear normal but there is interstitial fibrosis in both atria. This change has been reported in severe cases of atrial fibrillation.

CASE NUMBER 144095: 6 year old riding horse (gelding).

Clinical History: Four-week history of acute onset right forelimb lameness.

Pathological diagnosis: P1 fracture

General post mortem: Dissection of the right fore revealed a complete sagittal fracture of P1. The two sides of bone were clearly unstable but some cartilage had formed in the middle of the fracture site. The ends of the fracture opened into both joints where the synovial membrane was thick and reddened.



X-ray of First Phalanx : shows the fracture extending the full length of P1.

Stomach: The stomach showed florid proliferation of the non-glandular mucosa at the margo plicatus towards the lesser curvature with no islet formation and scatterred shallow brown scars. The glandular mucosa appeared normal.

CASE NUMBER 144241: Dutch Warmblood

Clinical history: Chronic right hind limb lameness

Pathological diagnosis: Complex tenosynovitis

General post mortem: The carcass was in good condition. Dissection of the right hind limb found a thickening of the plantar annular ligament with synovial distension and adhesions within the digital sheath.

The stomach: The contents of the stomach were firm and sweet smelling. There were punctate brown scars throughout the non-glandular area with polyp formation at the margo plicatus. These were most abundant along the lesser curvature. There was some yellowing of the normal squamous epithelium towards the greater curvature. The glandular region appeared normal.

CASE NUMBER 144292: 10 year old Thoroughbred, gelding.

Clinical history: Bilateral forelimb lameness of seven months standing. Had received phenylbutazone within the last month of life.

Pathological diagnosis: Navicular disease

General post mortem: The carcass was in good condition. Radiography of the navicular bones revealed mushroom shaped radiolucencies within both navicular bones. There were small numbers of cyathostome larvae present in the caecum and ventral colon.

The stomach: There were no lesions visible in the non-glandular region of the stomach. Adjacent to the margo plicatus towards the greater curvature in the cardiac gland region there were several raised blanched foci measuring 1cm in

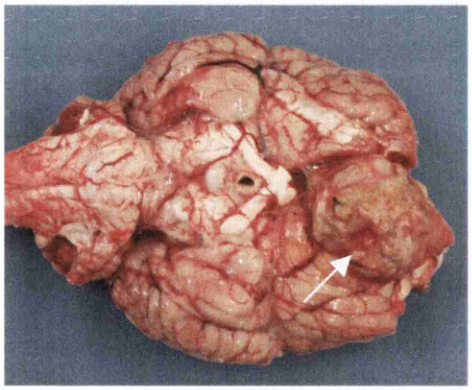
diameter. There was no evidence of ulceration or erosions.

CASE NUMBER 144439: 15 year old Arab mare.

Clinical history: The horse had been displaying behavioral changes with blindness, abnormal hindlimb gait, dullness, depression and nervousness and a head tilt to the right. The left eye had an absent menace reflex and pupillary light response but no pathology was visible grossly. The horse had been treated with prednisolone within the last month of its life.

Pathological diagnosis: Brain tumour

General post mortem : Tomography of the head post mortem revealed a hens egg sized lesion in the forebrain which was white and firm . This extended rostrally to the olfactory lobes and caudally to the thalamus. The right side of the brain was deviated from midline.



Ventral view of brain: note egg sized mass at rostral extremity (arrow).

Stomach: Lesions in the non – glandular region of the stomach were mainly restricted to the margo plicatus at the lesser curvature. Here, irregular tongues of squamous mucosa with central glandular islets extended into the glandular region.

There was one small area of scarring towards the cardia in the non-glandular region. There was some hyperaemia and pale blistering erosions of the apex of folds in the pyloric gland region.

CASE NUMBER 144468: 8 year old riding horse (gelding).

Clinical history: The horse had been suffering from recurrent colic signs post feeding and chronic weight loss for a three month period. Tests showed a reduction in SI malabsorption and a delayed gastric emptying. Also noted Gasterophilus larvae and hypertrophy of the non-glandular epithelium. Treatment included administration of prednisolone and phenylbutaz\one within the last month of life.

Pathological diagnosis: Equine granulomatous enteritis.

General post mortem: Abnormalities were detected in the gastrointestinal tract of the horse. The distal length of jejunum and entire ileum appeared stripy due to 5cm corrugated bands of rugal flattening interspersed with areas of mucosal hyperaemia and ulceration. The remaining intestines were normal in gross appearance. There was enlargement of some mediastinal lymph nodes which measured approximately 8cm by 3cm and had an external granular appearance. The surrounding lymphatics were prominent.

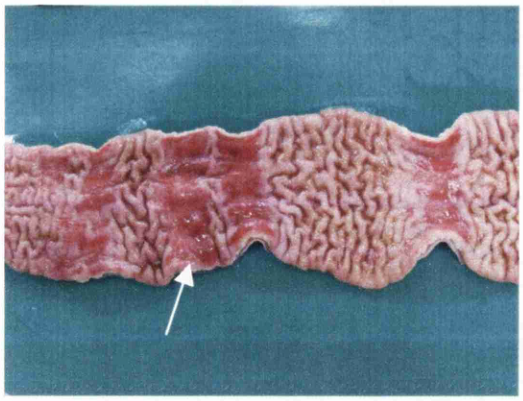


Fig. 15 Section of ileum: note hyperaemic bands of flattened mucosa (arrow).

Histopathology: Sections taken from a flattened area of ileum showed stunting and fusion of the villi with a diffuse infiltration of predominantly T lymphocytes. The submucosa was greatly thickened due to oedema and inflammatory cell infiltration. It contained multiple granulomatous lesions with a core of pink necrotic material and degenerate neutrophils, surrounded by a ring of macrophages. All the large intestine distal to this displayed marked submucosal oedema with huge distension of lymphatics. The mesenteric lymph nodes were also oedematous with distended subcapsular lymphatics. The liver showed a mild lymphocytic infiltration around the bile ducts with eosinophylic rich fibrin tags on the capsule (parasite migration). Equine granulomatous enteritis is rare in horses and has a predilection for standardbreds. As yet no aetiological agent has been identified and both ZN and PAS staining in this case failed to reveal anything further. Suggestions include that of Lindberg, that the lesions may represent an abnormal host inflammatory response to commonplace bacteria. Links with aluminium toxicity have also been reported although this is unconvincing.

Stomach: The non-glandular region shows scattered punctate brown scars about the cardia and towards the greater curvature. There is yellowing of a patch of epithelium

(hyperkeratosis) towards the greater curvature at the margo plicatus. The glandular region appears normal..

CASE NUMBER 144476: 11 year old Thoroughbred cross (gelding).

Clinical History: The horse had been suffering from a 6-week history of severe weight loss (100kg) accompanied by patchy sweating and muscle fasiculation. Treatment included daily Cisapride and phenylbutazone administration within the last month.

Pathological Diagnosis : Grass sickness

General post mortem: The carcass was emaciated with a starry coat . The entire gastrointestinal tract was shrunken with scanty contents. The rhinarial mucosa was covered in flaking yellow scabs (necrotic plaques) and there was a serous nasal discharge. Numerous thin fibrous tags were attached to the diaphragmatic surface of the liver capsule.

Histopathology: Sections of coeliacomesenteric ganglia showed an overall reduction in the number of neurones present with vacuolation and chromatolysis of the few remaining. The ileal ganglia were also scant and displayed contraction and pyknosis of several neurones.

Stomach: The stomach was distended and contained foul smelling fluid ingesta. The mucosa appeared normal except for the margo plicatus junction where there were

yellowed prominent 0.5cm cauliflower like protrusions proud to the non-glandular mucosa . Some of these contained central pink glandular areas. Towards the lesser curvature adjacent to the margo plicatus within the non glandular region, there were two oval ulcerations measuring approximately 1.5cm in length. They had pale hyperkeratotic rims and dark red/brown craters. There was no evidence of active haemorrhage.

CASE NUMBER 145037: 3 year old riding pony (gelding).

Clinical history: Sudden onset colic, referred in with muscle fasiculations, halitosis. It was anaesthetised to obtain ileal biopsy and inspirated gastric reflux during induction. The horse was stomach tubed every 3-4 hours after op.Treatment included flunixin meglumine (NSAID) every four hours before euthanasia.

Pathological diagnosis: Grass sickness.

General Post mortem: The carcass was in moderate condition. Dissection revealed areas of extensive linear oesophageal ulceration. The ileal mucosa was oedematous with punctate ulceration scattered throughout. The colon was impacted with dry green ingesta with a black tarry covering which adhered to the colonic epithelium. The liver was swollen and slightly orange in colour. The lungs had cranial areas of consolidation with patchy yellow stained areas (a bile pneumonia).

Oesophagus: note diffuse linear hyperaemic areas of ulceration.

Colon: note impacted dry contents with dark tarry covering typical of gut stasis secondary to grass sickness.

Stomach: The stomach was distended with foul smelling fluid contents. Within the squamous region there was diffuse erosion of the epithelium between the margo plicatus and the cardia along the lesser curvature accompanying scattered islets of white (normal) epithelium towards the periphery of the lesion. Much of the remaining epithelium was thickened, corrugated and yellowed (hyperkeratotic) predominantly located adjacent to the margo plicatus and extending to the greater curvature. There were a few punctate brown scars (1mm) towards the region of the cardia.. There was some hyperaemia of pyloric gland region with a punctate erosion towards the pylorus.

Histopathology: Sections of the coeliacomesenteric ganglia show a reactive astrocytosis with varying degrees of neurone dissolution: peripheral

vacuolation, pyknotic eccentric nuclei, condensation and loss of Nissl substance. Sections of the ileum show an actual reduction in number of neurones as well as the above changes.

CASE NUMBER 145216: 5 year old Thoroughbred (mare).

Clincial History: The horse suffered from an acute onset severe colic (five hours), non responsive to treatment. Surgery proved hopeless and the horse was euthanased under anaesthesia. It had received flunixin meglumine during this short disease onset.

Pathological diagnosis: Colonic torsion 1m distal to caecocolic junction.

General post mortem: There was a 180-degree torsion involving the dorsal and ventral colon. The pelvic flexure was at the apex of this , and the whole section of gut appeared congested and purple in colour with diffuse oedema of the involved colonic wall.

Colonic torsion: note location of torsion (arrow) and congestion of affected colon in comparison with normal intestine in background.

Stomach: There were small punctate brown scars scattered throughout the non-glandular region with some yellowing of the epithelium at the lesser curvature

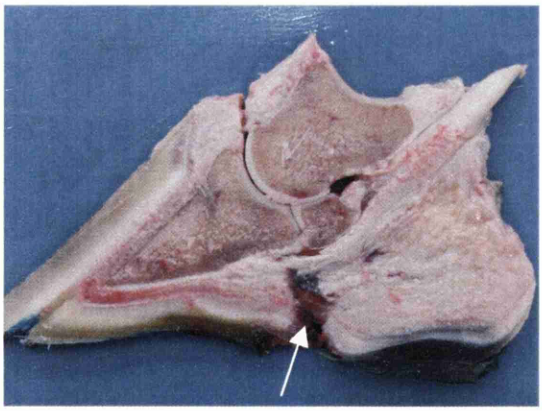
adjacent to the margo plicatus.There were fresh areas of haemorrhage in the cardiac gland region at the greater curvature and a small area of ulceration in the fundic gland region. There was hyperaemia of the pyloric gland region.

CASE NUMBER 145235: 19 year old Arab (gelding).

Clinical History: The horse had sufferred from a three week history of acute onset lameness of the right forelimb and had received phenylbutazone during this period.

Pathological diagnosis: Navicular bursitis.

General post mortem: There was a 2cm defect in the central region of the frog which led via a scar to the navicular bursa. There was a non-painful soft tissue swelling of forelimb. Coffin joint was not infected but the navicular bursa was. Had received phenylbutazone within the last month of life.



Note necrotic tract (arrow) leading to navicluar bursa from a foreign body penetration.

Stomach: There was an irregular junction at the margo plicatus at the lesser

curvature, but there was no scarring at this point. There was hyperaemia of the folds in the pyloric gland region.

APPENDIX 3 – HISTOLOGICAL DESCRIPTIONS OF EACH SLIDE

CASE NUMBER 133191

SITE 1 – slide 02/67-1

The stratum corneum is partially nucleated with an anucleate keratin layer at the luminal surface. This overlies shallow blunt rete pegs. There is a mild increase in lymphocytes within the lamina propria some of which show exocytosis into the stratum basale and stratum spinosum.

SITE 2 - slide 02/68-1

This section shows an area of erosion and a separate area of ulceration. Away from this there is diffuse irregular hyperkeratosis which is parakeratotic although the nuclei are pyknotic and reduced in number towards the luminal edge where small clusters of bacteria are adherent throughout. There is exaggerated elongated rete pegging throughout the section. In one area, the stratified squamous epithelium displays an abrupt area of full thickness ulceration extending to the lamina muscularis.This consists of necrotic debris and neutrophils covering a bed of granulation tissue with congestion of vessels ventral to this, deep within the lamina propria. There is bacterial colonisation of the wall of the lesion with the adjacent intact epithelium thickened, but free from cellular infiltrate. This is accompanied by fungal infiltration in some areas (PAS

stain). At the margo plicatus there is a shallow erosion with adherent bacteria above a well circumscribed lymphoid follicle lying in the superficial lamina propria.

SITE 2a - Slide 02/68-2

The section shows an area of stratified squamous epithelium which is interrupted by an erosion and a separate area of ulceration. Where intact, the stratum corneum is nucleated and thinned, and the rete pegs are shallow and blunt. There is a mild vacuolation of the cytoplasm of the basal epithelial cells, which have large pale nuclei and scattered mitoses. The lamina propria has a mild increase in the number of lymphocytes, which are concentrated within the lamina papillae. The erosion is half the depth of the total epithelial thickness with nucleated keratinocytes partially covering the base and clumps of adherent bacteria visible. There is no accompanying inflammatory cell infiltrate or vessel congestion.

The ulcer is full thickness extending to the muscularis mucosa. The central area is capped with necrotic eosinophilic debris, neutrophils and macrophages and is supported by a bed of granulation tissue (VWF shows up the capillaries in this). As the epithelium reappears adjacent to the ulcer it is increased in depth and there is balloon degeneration of surface keratinocytes. Where the keratin is seen to flake, fungal infiltration and bacterial adherence is also present (PAS staining) The stratum corneum appears oedematous, with karryohexis and karryloysis of the keratinocytes accompanying a neutrophillic infiltrate. The latter is intensified towards the ulceration , with exocytosis of the stratum spinosum with neutrophils

fed from capillaries within the lamina papillae. There is spongiosis of the basal epithelium and the nuclei appear swollen with prominent nucleoli. Here, the lamina propria contains a mixed inflammatory infiltrate limited to the superficial layer with dilation and congestion of some vessels deep to this.

SITE 3 - Slide 02/71-1

Fundic gland area. This is a section of normal mucosa with no evidence of epithelial damage or inflammatory cell infiltrate. Artefactual defects to surface epithelium are present in places.

SITE 4 – slide 02/70-1

Fundic gland area. Normal

SITE 5 – slide 02/69-1

Pyloric gland area. There are two areas of shallow erosion visible within the section. These involve loss of three or four cells of surface epithelium which is replaced by fibrin exudation and clusters of neutrophils. There is a mild focal superficial neutrophillic infiltrate bordering these lesions. The lamina propria appears thickened in places where it extends between the gastric pits, and here it is accompanied by a moderate mononuclear cell infiltrate. Some associated glands are dilated and others show increased tortuosity (dysplasia). Within the lamina muscularis there is a cellulose foreign body and a localised associated granulomatous reaction adjacent to a vessel.

SITE 6 – slide 02/72-1

Duodenum. The epithelium is intact. There is a diffuse lymphoplasmacytic infiltrate and some scattered neutrophils restricted to the lamina propria of the villi and several lymphoid follicles located between the Brunners glands within the submucosa.

CASE NUMBER 138643

SITE 1 – slide 01/4191-1

The stratum corneum appears to be regularin width and nucleated throughout the section. There is oedema of the stratum transitionale which appears as a pale band. The rete pegs are of moderate length and there is some exocytosis of lymphocytes into the stratum basale.

SITE 2 – slide 01/4192-1

The stratum corneum appears regular in width and diffusely nucleated. There is some adherence of bacteria to the stratum corneum. Otherwise the section appears normal.

SITE 2a – slide 01/4200-1

The section shows an area of erosion and an area of ulceration. The erosion shows a reduction in the width of the stratum corneum to two cells thick and there is some vacuolation of the cells with neutrophilic invasion. Beneath this

there is hydropic change and spongiosis of the basal epithelium with extension of some lamina papillae to the stratum corneum. These are also oedematous and filled with neutrophils. There is oedema of the underlying lamina propria and a mild infiltration of lymphocytes and neutrophils and scattered eosinophils. The area of ulceration is bordered by rete peg stubs with a necrotic surface. and marked spongiosis and hydropic change of the basal epithelium here. The ulcer itself is capped with eosinophilic necrotic debris over infiltrating neutrophils, eosinophils and granulation tissue. There is a thrombus in one of the underlying vessels and the inflammatory reaction extends deep into a very oedematous lamina propria with lymphatic dilation. One lymphoid follicle is present within the section.

SITE 2b – slide 4201-1

The section shows diffuse multiple areas of erosion. Away from these the stratum corneum appears oedematous with swollen pale keratinocytes. There is marked spongiosis of basal epithelial cells and a mild lymphocytic infiltrate within the lamina propria and some scattered eosinophils. The areas of erosion show almost complete loss of the stratum corneum (1-2 cells thick), extension of lamina papillae to the luminal surface and a moderate infiltration of neutrophils and mononuclear cells extending into the oedematous lamina propria and with a mild degree of exocytosis.

SITE 2c – slide 4202-1

This section shows multiple diffuse erosions, with much of the *stratum*

corneum that is present being oedematous and lifting off. In some areas it is intensely infiltrated with clusters of neutrophils beneath which there is marked spongiosis and hydropic change within the basal epithelial cells. Throughout the section the lamina propria is oedematous with prominent dilated lymphatics and has a dense infiltrate of mononuclear cells and neutrophils.

SITE 3 – slide 01/4194-1

Mild lymphocytic infiltrate.

SITE 4 – slide 01/4195

Fundic gland region. There is a mild lymphocytic infiltrate visible in this section.

SITE 5 – slide 01/4196

Pyloric gland region. There is an intact epithelium with scattered areas of mild fibrosis and lymphocytic infiltrate within the lamina propria at the level of the gastric pits. There is a focal area where the pits appear shallower and resemble cardiac mucosa.

SITE 6 – slide 01/4199-1

Duodenum. There is a diffuse lymphocytic infiltrate within the lamina propria of the villi. Large lymphoid follicles with obvious mitotic figures are found here and the Brunners glands in the submucosa.

CASE NUMBER 139923

SITE 1- slide 01/4075-1

The *stratum corneum* is thickened and appears oedematous with a loss of nucleation towards the lumen surface. Where present, the nuclei are pyknotic and surrounded by clear cytoplasmic vacuolation, while others displayed karyolysis and karyorrhexis. There is one shallow erosion extending half way into the keratin layer which is accompanied by adherent clumps of bacteria. The stratum spinosum appears thickened with prominent vascular lamina papillae within it reaching towards the stratum corneum. There is spongiosis and hydropic degeneration of some basal epithelial cells. The lamina propria is vascular with a mild increase in lymphocytic infiltrate.

SITE 2 – slide 01/4080-1

Margo plicatus. There is irregular hyperkeratosis of the stratum corneum throughout the section. Two areas of erosion involve the stratum corneum which appears flaky but has little inflammatory infiltrate associated with these lesions. At the glandulo-squamous junction pockets of keratinised epithelium are filled with degenerate neutrophils (microabscesses) and there is vacuolation of the cytoplasm of adjacent keratinocytes.The lamina papillae contain scattered neutrophils towards the erosions. Ventral to this there are aggregations of lymphocytes within the superficial layer of the lamina propria. The main erosion shows irregular destruction of keratinised epithelium encapturing pockets of

bacteria and neutrophils and extension of epithelial pegs into the keratin layer. The entire section displays thickened squamous (acanthosis) and keratinised (hyperkeratosis) layers of the epithelium and exaggerated rete peg formation

SITE 3 - slide 01/4078

Cardiac gland region. Normal.

SITE 4 – slide 01/4076-1

Fundic gland region. There is a mild lymphocytic infiltrate within the lamina propria at the level of the gastric glands.

SITE 5 – slide 01/4077

Pyloric gland region. The section shows a focal area of superficial vessel congestion accompanied by a mild neutrophilic and mononuclear infiltrate. There is a moderate lymphocytic infiltrate within the lamina propria, concentrated about the gastric glands.accompanied by mild gland atrophy. This extends into the lamina muscularis which is thickened and congested. There are focal areas of gland hyperplasia and disorganisation (dysplasia).

SITE 5a – slide 01/4081-1

Pyloric gland region – the section shows a mild increase in the number of lymphocytes within the lamina propria at all levels of the glands with

congestion of some surface capillaries. There is marked gland hyperplasia and disorganisation (dysplasia).

SITE 6 – slide 1/4079-1

Duodenum. There is a very mild lymphoplasmacytic infiltrate of the villi and lamina propria and prominent lymphoid follicles present within the submucosa.

CASE NUMBER 140373

SITE 1 – slide 02/328-1

The section shows regular parakeratosis of the *stratum corneum* overlying blunt rete pegs . There is some cytoplasmic vacuolation of the basal epithelium with an increase in the number of mitoses here. The lamina propria appears mildly oedematous.

SITE 2 – slide 02/329-1

The *stratum corneum* shows areas of irregular hyperkeratotic parakeratosis with oedema of keratinocytes and lengthening of the rete pegs. There is a mild lymphocytic infiltrate which is concentrated within the lamina papillae.

SITE 3 – slide 02/331-1

Cardiac gland region. There is a mild lymphocytic infiltrate about the cardiac glands in the lamina propria which extends towards the epithelium in one area.

This section is from the transitional zone as there is a cluster of parietal cells visible.

SITE 4 - slide 02/330-1

Fundic gland region . There is marked oedema of the submucosa and a mild infiltration of the lamina propria with chronic inflammatory cells. The rest of the section is normal.

SITE 5 – slide 2/332-1

Pyloric gland region has a mild mononuclear cell infiltrate.

SITE 5a – slide 02/334-1

Pyloric gland region. There is a focal area of erosion which shows fibrin exudation from surface epithelial cells, accompanied by neutrophils and haemorrhage. Beneath this the lamina propria contains scattered lymphocytes and neutrophils and is prominent between the gastric glands, some of which appear dilated and tortuous (dysplastic).The underlying lamina muscularis which has multiple areas of lymphocytic infiltrate also shows areas where isolated glandular tissue invades it.

SITE 6 – slide 02/333-1

Proximal duodenum. There is a very mild lymphoplasmacytic infiltrate within the lamina propria of the villi. No lymphoid follicles are visible.

CASE NUMBER 140459

SITE 1 – Slide 02/1751-1

The stratum corneum is nucleated and thinned, and there is a loss of distinction between this and the stratum transitionale. There is a moderate lymphocytic infiltrate limited to the superficial level of the lamina propria present in half the section. Here, two focal aggregates of lymphocytes are associated with a localised capillary proliferation in this area. There is one oesophageal gland like structure in mid lamina propria which is surrounded by a mild scattering of plasma cells and lymphocytes and remains distant from the inflammatory sites previously mentioned.

SITE 2 - slide 02/1752-3

The *stratum corneum* is nucleated and thickened but displays much karyorrhexis and karyolysis and acanthosis. The rete pegs are elongate and dendritic and there are several mitotic figures present in the basal epithelial layer. The adjacent pyloric glandular region has a diffuse mild lymphoplasmacytic lamina proprial infiltrate with no glandular atrophy. There are several prominent lymphoid follicles present throughout at the level of the gland isthmus in the pyloric gland region.

SITE 2a – slide 02/2770-1

There are two areas of erosion adjacent to the margo plicatus. Away from this the

stratum corneum oedematous and pale with mild vacuolation of keratinocytes towards the stratum transitionale. In this area there is lengthy rete pegging with spongiosis of the basal epithelium, and a mild infiltration of the oedematous lamina propria with mononuclear cells. The areas of erosion show underrunning of the stratum corneum with neutrophils which appears oedematous and anucleate adjacent to the stratum transitionale. The lamina papillae are prominent and some extend to the luminal surface infiltrated with neutrophils. Beneath these areas the lamina propria is densely packed with neutrophils and mononuclear cells and there is congestion of underlying vessels.

SITE 2b - slide 1760-1

This section shows a small erosion in the region of the margo plicatus. The rest of the section shows a parakeratotic stratum corneum which is pale and oedematous. This appearance is exaggerated towards the stratum transitionale in the form of a pale band. Beneath this the rete pegs are dendritic and elongate and there is a scattered infiltrate of mononuclear cells and neutrophils throughout the lamina propria. The erosion shows underunning of a necrotic stratum corneum with neutrophils and marked spongiosis of the underlying basal epithelial cells. The cellular infiltrate beneath this area is more intense.

SITE 2c – slide 02/1759-1

The section shows ulceration at the margo plicatus. There is a length of stratified squamous epithelium showing cytoplasmic vacuolation of the cells in the stratum corneum which appears thickened. This overlies elongated dendritic ret

pegs interdigitating with prominent lamina papillae. As the junction of the margo plicatus is approached, so these papillae increase in vascularity and fill up with neutrophils. At the area of erosion where only pegs of basal epithelium remain, there is exocytosis of the neutrophils into the stratum spinosum . Capping this is a layer of degenerate neutrophils and necrotic epithelium with some bacteria adherent at the lumen surface. Beneath this the lamina propria is narrowed and scarred with lymphocytes, plasma cells, eosinophils and neutrophils scattered through it. The adjacent cardiac gland mucosa shows congestion with neutrophils and lymphocytes at the apex of the glands. The remaining lamina propria contains scattered lymphocytes and eosinophils.

SITE 3 – slide 02/2770-1

Cardiac gland region. There is a moderate lymphoplasmacytic infiltrate in all levels of the lamina propria. One lymphoid follicle is present towards the gastric pits.

SITE 3a – slide 02/1759-1

This section from the cardiac gland region shows a diffuse infiltrate of mononuclear cells, neutrophils and eosinophils within the lamina propria between the glands. This is more marked towards the margo plicatus.

SITE 4 – slide 02/1753 –1

Fundic gland area. Normal.

SITE 4a – slide 02/1756-2

There is a mild superficial infiltrate of mononuclear cells seen throughout this section.

SITE 4b – slide 02/1756-3

The section shows marked hyperplasia and disorientation (dysplasia) of glandular tissue with a mild diffuse mononuclear cell infiltrate present throughout the section in the lamina propria at all levels.

SITE 4c – slide 02/1761-1

The section shows gland hyperplasia and a mild mononuclear cell infiltrate present throughout with a scattering of neutrophils towards the superficial layer of the epithelium. All vessels are markedly congested at all levels of the epithelium. There is a small area of superficial gland dysplasia.

SITE 4d – slide 02/1761-2

There is diffuse gland hyperplasia, with a mild lymphocytic infiltrate throughout and three lymphocyte aggregations at adjacent to the lamina muscularis within the lamina propria.

SITE 4e – slide 02/1761-3

There is a mild lymphocytic infiltrate throughout and marked vessel congestion.

SITE 5 – slide 02/1754-1

Pyloric gland region. There are areas of focal gland hyperplasia.There is thickening of the lamina muscularis with a mild infiltrate of mononuclear cells which extends between the glands in places.

SITE 5a – slide 02/1757-3 – Pyloric gland region.The section shows an area of superficial erosion involving the tips of the glands showing neutrophil migration, congestion and haemorrhage and necrosis of the tips of glands. Beneath this is a mild lymphocytic infiltrate within the lamina propria which extends throughout the section. Some areas show gland dilation disorientation (dysplasia).

SITE 6 – slide 02/1755-1

Proximal duodenum. There is a moderate lymphoplasmacytic infiltrate within the lamina propria of the villi and numerous prominent lymphoid follicles within the superficial Brunners glands.

CASE NUMBER 143696

SITE 1 – slide 02/382-1

The stratum corneum is nucleated but not thickened.

SITE 2 - slide 02/383-1

Margo plicatus. The section shows an ulcerated polyp with exposure of the

central core of granulation tissue and a neutrophilic infiltration beneath the stratum corneum (which was anucleate) of the keratin layer in the surrounding intact epithelium. Adjacent to the margo plicatus the stratum corneum contains pockets of neutrophils and has surface bacterial colonisation. There is an intense lymphocytic infiltrate within the lamina propria at the glandular margin. Throughout the section the epithelial layer is thickened and the elongated rete pegs showed spongiosis of the stratum basale and stratum spinosum.

SITE 3- slide 02/384-1

Normal

SITE 4 – slide 02/385-1

Fundic gland region. Normal

SITE 4a – slide 02/384-2

Fundic gland region - normal

SITE 5 – slide 02/386-1

Pyloric gland region. Normal

SITE 6 – slide 02/387-1

Proximal duodenum. There is a mild lymphoplasmacytic infiltrate within the lamina propria of the villi and two small lymphoid follicles within the superficial

Brunners glands.

CASE NUMBER 143791

SITE 1 – Slide 01/4262-1

The entire section shows a diffusely thinned *stratum corneum* overlying a thinned epithelium with prominent vacuolated basal epithelial cells. The *stratum corneum* is nucleated and caps shallow rete pegs with prominent lamina papillae. There is severe hydropic degeneration of many of the basal epithelial cells which are almost necrotic in appearance. The underlying lamina propria contains prominent congested capillaries and scattered lymphocytes and neutrophils. There is one other area of erosion showing ghost basal epithelial cells over an oedematous lamina propria with a mild infiltration of lymphocytes and bacterial adherence to the lamina propria

SITE 2 – slide 01/4263-1

Stratified squamous epithelium. There is almost complete loss of epithelium along the section with shallow rete peg remnants displaying basal cell hyperplasia. The lamina propria has an increase in scattered lymphocytes and capillary budding with vessel congestion. There is adherence of bacteria to the necrotic epithelial surface.

SITE 2a – slide 01/4269-1

This section shows complete ulceration of the stratified squamous

epithleium, leaving a lamina propria with congested capillaries and a scattered mixed inflammatory infiltrate.

SITE 2b – slide 01/4270-1

Stratified squamous epithelium. The section shows a thinned intact length of epithelium, with marked basal cell hydropic degeneration, superficial vessel congestion and a mild infiltrate of neutrophils and mononuclear cells within the lamina propria and exocytosing into the epithelium.

SITE 3 – slide 01/4264-1

Fundic gland region. There is some loss of epithelial surface (erosion) with a variable layer of necrotic tissue in its place and bacterial adherence. Focal areas of lymphocytic infiltrate and vessel congestion are found in the lamina propria between the gastric pits and there is one lymphoid follicle at the level of the gastric glands. The submucosal layer is diffusely oedematous with marked capillary proliferation.

SITE 4 – slide 01/4265-1

Fundic gland region . There is a mild mononuclear cell infiltrate within the lamina propria.

SITE 5 – slide 01/4266-1

Pyloric gland region. Artefactual damage prevents examination of the epithelial

surface. However there is a mild neutrophilic and mononuclear cell infiltrate present within the lamina propria at all levels.

SITE 6 – slide 01/4267-1

Duodenum. The section is autolytic.

143818

SITE 1 – slide 01/4387-1

The section shows regular parakeratosis of the stratum corneum measuring 11 cells thick on average with uniform shallow rete pegs.In places the basal epithelial cells appear vacuolated (hydropic change) .

SITE 2 – slide 01/4388

The section shows irregular parakeratotic hyperkeratosis of the stratum corneum and elongate lamina papillae extending towards it. Some of the basal cells show hydropic change and frequent mitoses are scattered through them.

SITE 3 – slide 01/4389-1

Fundic gland region shows a mild infiltration of eosinophils scattered through the lamina propria and lamina muscularis.

SITE 4 – slide 01/4390-1

Fundic gland region. Normal.

SITE 5 – slide 01/4391-1

Pyloric gland region. Towards the duodenal junction there is marked glandular hyperplasia with an intense lymphoplasmacytic infiltrate located in the lamina propria at the level of the gland isthmus. Below this there is a chronic inflammatory infiltrate and scarring of the lamina muscularis with oedema of the submucosa. The epithelium remains intact throughout.

SITE 6 – slide 01/4392-2

Proximal duodenum. There is an increase in the numbers of eosinophils both within the lamina propria of the villi and scattered throughout the Brunners glands and the lamina muscularis.

143819

SITE 1 – slide 01/4417-1

Stratified squamous epithelium. The section shows diffuse irregular orthokeratotic hyperkeratosis and acanthosis and marked rete pegging of the epithelium.There is some evidence of basal epitheluim hydropic change and oedema of the adjacent lamina propria.

SITE 2 – Slide 01/4418-1

The section shows parakeratotic hyperkeratosis and markedly elongated rete pegging, with the vascular lamina papillae almost reaching the *stratum corneum.* There are scant areas of basal cell hydropic change.

SITE 2a - slide 01/4426-1 This section shows a region of stratified squamous epithelium taken from adjacent to the margop plicatus. It shows a punctuate ulceration extending to the lamina propria and surrounding reactive epithelium. There is a focal mixed inflammatory infiltrate.

SITE 3 – slide 01/4419-1

Fundic gland region. Normal

SITE 4 – slide 01/4420-1

Fundic gland region. Normal

SITE 5 – slide 01/4421-1

Pyloric gland region. There is a mild focal mononuclear/eosinophilic infiltration within the lamina propria at the level of the gastric glands which intensifies within the thickened lamina muscularis and submucosa. The epithelial surface remains intact.

SITE 6 – slide 01/4422-1

Proximal duodenum. There is a mild lymphoplasmacytic infiltration within the

lamina propria of the villi and scattered eosinophils throughout the lamina propria of the glandular area.

CASE NUMBER 143863

SITE 1 – slide 01/4457-1

The section shows regular parakeratosis of the stratum corneum . The rete pegs are shallow and blunt and there is mild spongiosis of the basal epithelium with some prominent mitotic figures visible.

SITE 2 – slide 01/4459-1

The stratum corneum is parakeratotic , but this is even and regular throughout the section.

SITE 3 – slide 01/4460-1

Fundic gland region. There is a mild infiltrate of mononuclear cells and eosinophils.

SITE 4 – slide 01/4458-1

Fundic gland region. The section contains a mild mononuclear cell infiltrate, although artefact and autolysis makes it difficult to interpret.

SITE 5 – slide 01/4461-1

Pyloric gland area. There is a mild mononuclear cell and eosinophilic infiltrate at the level of the gland neck

SITE 5a – slide 01/4462-2

Pyloric gland region: There is a mild mononuclear infiltrate at the level of the gland isthmus within the lamina. Here there are areas of glandular dilation and increased tortuosity (dysplasia)..

SITE 6 – slide 01/4462-1

Proximal duodenum. There is a mild lymphoplasmacytic infiltration within the lamina propria of the villi.

CASE NUMBER 143989

SITE 1 – slide 02/682-1

There is focal irregular parakeratosis of the stratum corneum throughout the section and marked cytoplasmic vacuolation of basal epithelial cells.

SITE 2 – slide 02/683-1

Margo plicatus. There is one area of ulceration within the stratified squamous epithelium which is capped with eosinophillic debris and degenerate neutrophils. This infiltrate remains superficial only extending partially into the lamina propria. The immediate surrounding epithelium has remnant tags of epithelium

supporting a patchy keratin layer which is infiltrated with neutrophils. Here, some keratinocytes appear markedly swollen and contain amorphous eosinophyllic fluid. The underlying lamina propria has a mild lymphocytic infiltrate and appears thickened. There is glandular tissue which underuns the stratified squamous epithelium in the region of the margo plicatus. The stratum corneum appears regular in thickness away from these lesions

SITE 2a – slide 02/692-1

Section from the margo plicatus, showing extensive areas of erosion of the stratum corneum containing pockets of neutrophils and areas of massive dilation of the keratinocytes which appear to be filled with a pink proteinaceous exudate. The rete pegs are dendritic and the underlying lamina propria contains a marked infiltration of neutrophils, and a dense congested network of capillaries. At the margo plicatus there is an intense lymphocytic infiltrate in the lamina propria with scattered eosinophils. PAS. There is associated spongiosis of the epidermis.

SITE 3 – slide 02/685-1

Cardiac gland region. There is a mild infiltration of lymphocytes scattered throughout the section.

SITE 4 – slide 02/684-1

Fundic gland region. Normal

SITE 5 – slide 02/686-1

Pyloric gland region. The section shows diffuse gland dysplasia (hypertrophy, dilation), glandular hyperplasia and a mild superficial mononuclear cell infiltrate. The lamina muscularis appears thickened and is infiltrated with mononuclear cells and extends up between the glands.

SITE 5a – slide 02/688-2

The section shows massive glandular hyperplasia and marked areas of gland dysplasia. There is a diffuse moderate mononuclear cell infiltrate extending into the thickened lamina muscularis. There are punctuate shallow erosions showing neutrophilic infiltration, fibrin exudation and haemorrhage.

SITE 5b – slide 02/689 –2

The section shows erosion to half the height of the glands and superficial glandular necrosis, haemorrhage, fibrin leakage capped with eosinphilic debris and degenerate neutrophils. The glands appear hypertrophic and distorted in places (dysplasia). A large glandular dilatation is present within the lamina muscularis

SITE 6 – slide 02/687-1

Proximal duodenum. Normal except for goblet cell hyperplasia.

CASE NUMBER 144056

SITE 1 – slide 02/99-1

The section shows areas of diffuse erosion.The stratum corneum is mostly anucleate and dark pink in colour (necrotic) and is separated from the underlying stratum transitionale by an infiltrate of clusters of neutrophils. These also invade the stratum spinosum and basale and are present at the apex of adjacent lamina papillae. There is marked spongiosis and some hydropic change in the basal epithelium. Where the epithelium is thinnest the underlying lamina propria is oedematous and diffusely infiltrated with lymphocytes and neutrophils. In areas where there are no neutrophils, there is oedema of the stratum spinosum

SITE 2 – slide 02/100-1

The section shows areas of normal epithelium interspersed with ulcers and erosions. The ulcers are full thickness with pink, necrotic remnants of epithelium capping a lamina propria filled with neutrophils. Beneath this in the lamina propria is a mucus secreting gland lined with plump cuboidal cells with a foamy cytoplasm. There are several of these structures present throughout the section ,. Some are associated with erosions and underunning of the *stratum corneum* with neutrophils. There are areas of elongated rete pegs and hydropic change of some epithelial basal cells. The lamina propria has focal increases of neutrophils and a generalised increase in lymphocytes.

SITE 3 – slide 02/102-1

This is a section from the cardiac area showing a diffuse mild lymphocytic infiltrate in the lamina propria which is mainly concentrated about the gastric glands and infiltrating the surface epithelium in some places which

appears congested. There is no evidence of erosion or ulceration..

SITE 4 - slide 02/101-1

Section from the fundic area shows a mild mononuclear cell infiltrate.

SITE 5 – slide 02/103-1

The section shows scarring of the lamina muscularis and a lymphocytic infiltrate which extends into the lamina propria between the gastric pits and glands. There is patchy gland hyperplasia and dilation (dysplasia).

SITE 5a – slide 02/105-1

There is a concise area where an erosion of necrotic debris and neutrophils cap eroded glandular tissue. The lamina propria is congested and infiltrated with neutrophils and lymphocytes.!

SITE 6 – slide 02/103-1

Duodenum normal

CASE NUMBER 144095

SITE 1 – slide 02/227-1

There is diffuse regular parakeratosis of the stratum corneum with some nuclear loss towards the surface where there is patchy attachment of bacteria. The

stratum spinosum appears pale and oedematous focally.

SITE 2 – slide 02/228-1

There is parakeratotic hyperkeratosis with loss of nuclear detail towards the epithelial surface and bacterial adherence. The stratum spinosum appears pale and oedematous in patches.

SITE 3 – slide 02/229-1

There is a mild lymphocytic infiltrate within the superficial epithelium and at the level of the gastric pits within the lamina propria.

SITE 4 – slide 02/230-1

Fundic gland region. There is a mild lymphocytic infiltrate at the level of the gastric pits.

SITE 5 – slide 02/231-1

There is a mild infiltrate within the lamina propria of lymphocytes, eosinophils and the occasional neutrophil. In places the gastric glands and pits appear distorted with puckered lumen (dysplasia).The epithelium overlying these areas shows shallower branched glands, more similar to cardiac glands than pyloric.The lamina muscularis is thickened and contains scattered lymphocytes.

SITE 6 – slide 02/232-1

Duodenum. There is a scant lymphoplasmacytic infiltrate within the lamina propria with focal aggregates of lymphocytes.

CASE NUMBER 144241

SITE 1 – slide 02/1583-1

The sections shows diffuse regular parakeratosis of the stratum corneum with areas of bacterial adhesion to surface flaking keratin. Beneath this there is swelling and oedema of two to three layers of cells within the *stratum* transitionale.

SITE 2 – slide 02/1584-1

Much of the section shows orthokeratotic hyperkeratosis throughout, with scattered pockets of bacteria contained within the superficial keratin layers. Only in one area do the bacteria appear in conjunction with neutrophils so forming an intraepithelial microabscess. The lamina propria has a mild increase in mononuclear cell infiltration and some new capillary formation. The rete pegs appear lengthened.

SITE 2a – slide 02/1589-1

The section shows diffuse parakeratosis with some cytoplasmic vacuolation of keratinocytes, luminal flaking and bacterial adherence. The stratum spinosum appears thickened in places but there is no exaggerated rete pegging.

SITE 2b - slide 02/1589-2

The section of keratinised epithelium shows two areas of erosion. These appear as intracorneal pustules (degenerate neutrophils, eosinophilic debris), one extending half way and the other to the level of the stratum transitionale. Ventral to these there is marked oedema of the lamina propria at the margin of the stratum basale and a mild mononuclear cell infiltrate. The remaining section shows parakeratosis and patchy oedema of the stratum corneum which has clumps of bacteria adherent to it.

SITE 3 – slide 02/1586-2

Cardiac gland region. Normal.

SITE 4- slide 02/1586-1

Fundic gland region. Normal

SITE 5 – slide 02/1587-1

Pyloric gland region. The sections shows a mild increase in mononuclear cells and eosinophils superficially and within the lamina propria between the glands, some of which appear mildly dysplastic. Changes are associated with areas where the glands appear dysplastic and resemble shallower branching cardiac glands.

SITE 6 – slide 02/1588

Duodenum. The section shows a scant infiltrate of chronic inflammatory cells within the lamina propria, with scattered lymphoid follicles.

CASE NUMBER 144292

SITE 1 – slide 02/450-1

The stratum corneum shows areas of parakeratosis but it is generally anucleate (orthokeratotic) and regular in thickness. The section is otherwise normal.

SITE 2 – slide 02/451-1

The section shows diffuse parakeratosis, with some swelling and cytoplasmic pallor of keratinocytes within the stratum corneum towards the margo plicatus. There is a cluster of lymphocytes and a few eosinophils at the margo plicatus within the lamina propria, but otherwise the section is normal.

SITE 2a – slide 02/456-1

This is a section showing the region of the margo plicatus and surrounding epithelium. The stratum corneum appears nucleated with patchy oedema of keratinocytes towards the stratum transitionale . There is a shallow erosion towards the margo plicatus (3 cells thick) with adherence of bacteria to the surface keratin and a microabscess adjacent to this. Underlying this in the lamina propria is a cluster of lymphocytes, which have a mild infiltration throughout the rest of the section along with a mild infiltration of neutrophils.

SITE 3 – slide 02/452-1

Fundic gland region. Normal

SITE 4 – slide 02/453-1

Fundic gland region.This shows a layer of mucous containing sloughed epithelial cells which is laminated and closely adhered to the surface epithelium. This is a normal finding.

SITE 5 – slide 02/454-1

The surface epithelium appears intact with no areas of acute inflammation. The lamina propria is thickened with fibrous tissue and there is a mild lymphocytic infiltrate which forms intraglandular follicles in two places. The lamina muscularis appears thickened and has a mild lymphocytic infiltrate. There is a focal area of glandular hyperplasia.

SITE 6 – slide 02/455-1

Duodenum with a mild lymphoplasmacytic lamina proprial infiltrate.

CASE NUMBER 144439

SITE 1 – slide 02/639-1

There is diffuse irregular hyperkeratotic parakeratosis of the stratum corneum with adherence of bacteria to many of the surface keratinocytes. There are

areas of cytoplasmic vacuolation (hydropic change) within the basal epithelial cells and acanthosis and acanthosis and rete peg formation.

SITE 2 – slide 02/645-1

Section shows a fungi present within the stratum corneum and acanthosis.. Due to artefact, it is impossible to determine the state of the epithelium at the margo plicatus. Within the lamina propria at this point there are two large areas of glandular tissue surrounded by a mononuclear cellular infiltrate. The rest of the section shows diffuse parakeratosis but is otherwise normal.

SITE 3 – slide 02/640-1

Cardiac/fundic transitional gland region. Artefact prevents visualisation of much of the epithelial surface otherwise normal

SITE 4 – slide 02/641-1

Fundic gland region. Normal although there is some post mortem change.

SITE 5 – slide 02/642-1

There is a mild lymphocytic infiltrate within the lamina propria at the level of the gastric glands.

SITE 5a – slide 02/644-1 – Pyloric gland region. There is some sloughing of surface epithelial cells with tip necrosis, fibrin leakage haemorrhage and neutrophils scattered throughout the surface debris. There is also

thickening of the lamina propria and a lymphocytic infiltrate in this area.

SITE 5b – slide 02/644-2

This section shows a thickened lamina muscularis containing a patchy lymphocytic infiltrate. This extends into the lamina propria between which some of the glands appear tortuous and dysplastic. Towards the epithelial surface there are a few crypt abscesses capped by masses of neutrophils, blood and eosinophyllic debris. There is a focal area of gland hyperplasia.

SITE 6 – slide 02/643-1

Duodenum. Normal

CASE NUMBER 144468

SITE 1 – slide 02/1184-1

The section shows diffuse regular parakeratosis of the stratum corneum. Some of the basal epithelial cells appear vacuolated with oedema of adjacent cells in the lamina propria. There is a mild lymphocytic infiltrate in the lamina propria.

SITE 2 – slide 02/1185-1

The *stratum corneum* is parakeratotic and orthokeratotic and there is cytoplasmic pallor and swelling of the cells in the stratum transitionale. As the rete pegs elongate towards the margo plicatus, so the lymphocytic infiltrate intensifies

within the lamina propria.There is also a thickening of the lamina muscularis at this point.

SITE 3 – slide 02/1186-1

Fundic gland region. Part of the epithelium is missing due to artefact. The rest of the section appears normal.

SITE 4 – slide 02/1187-1

Fundic gland region. Normal.

SITE 5 – slide 02/1186-2

Pyloric gland region. Normal.

SITE 6 – slide 02/1189-1

Duodenum. Lymphocytic infiltrate within the lamina propria.

SITE 6a – slide 02/1189-2

Duodenum – there is a mononuclear infiltrate within the lamina propria

This stomach was suspended with helicobacter.

CASE NUMBER 144476

SITE 1 – slide 02/863-1

The section shows irregular hyperkeratotic parakeratosis of the stratum corneum which measures 15 cells deep. Adjacent to this, cells in the stratum transitional are swollen with cytoplasmic pallor. The rete pegs are short and blunt and the lamina propria appears normal.

SITE 2 – slide 02/864-1

The stratum corneum shows hyperkeratotic parakeratosis and orthokeratotis. It is absent towards the the margo plicatus (erosion) where the rete pegs are elongate and the lamina papillae have increased vascularity. Beneath this are two areas of glandular tissue within the lamina propria. They are surrounded by increased amounts of fibrous tissue and a mild lymphocytic infiltrate. There is a lymphoid follicle within the glandular epithelium at the margo plicatus and a mild lymphocytic infiltrate along the length of the lamina propria.

SITE 2a – slide 02/869-1

The section shows a sparsely nucleated stratum corneum with fine rete pegging and a vascular lamina propria. At the margo plicatus nests of glandular tissue are present in a body of squamous tissue. There is a mild lymphoplasmacytic infiltrate in the lamina propria.

SITE 3 – slide 02/865-1

Fundic gland region. There is some sloughing of surface epithelial cells with capillary congestion. There are dilated lymphatics within the submucosa, otherwise the appearance is normal.

SITE 4 – slide 02/866-1

There is congestion of many of the surface capillaries throughout the section. Otherwise the glandular area appears normal.

SITE 5 – slide 02/867-1

Pyloric gland region. There is loss of surface epithelium due to artefact. Where present the surface epithelial cells appear proliferative and swollen and PAS stain shows increased mucus production throughout the section. In intact areas there is superficial vessel congestion and scattered neutrophils and mononuclear cells. The lamina muscularis appears thickened and shows infiltrating glandular structures and a generalised mild lymphocytic infiltrate. Here, a single lymphoid aggregation is centred around a foreign body. There is dilation of lymphatics throughout the gastric gland area.

SITE 6 – slide 02/868-1

Duodenum with a mild lymphoplasmacytic infiltrate.

CASE NUMBER 145037

Site 1 – slide 02/1446-1

The section shows two areas of abrupt erosion, bordered by a full thickness epithelium with a parakeratotic stratum corneum. These areas show mild, blunt rete pegging with the lamina papillae extending into the stratum spinosum. There

is marked clear vacuolar change within the cytoplasm of several of the basal epithelial cells and there is diffuse hydropic change within the adjacent cells of the lamina propria beneath. The rest of the lamina propria appears mildly oedematous with dilated lymphatics and a scattered lymphocytic infiltrate. The erosions consist of the tips of the rete pegs (stratum basale) capped by a discontinuous single layer of keratinocytes. Scant numbers of neutrophils and lymphocytes infiltrate the area and are accompanied by proliferating capillaries. The basal epithelial cells are plump but are not affected by such severe vacuolar change in this region. There is one focus of mucus producing (PAS positive) acini in the lamina propria directly underlying the edge of one erosion.

SITE 2 – slide 02/1447-1

The section shows two areas of erosion bordered by a parakeratotic stratum corneum and elongate rete pegs with vacuolated basal epithelial cells and oedema of the adjacent lamina propria. The areas of erosion consist of a single layer of keratinocytes over rete peg remnants (basal epithelium) with a high mitotic rate, infiltrated with numerous capillaries but few inflammatory cells. There is a moderate lymphocytic infiltrate within the lamina propria and mild capillary proliferation throughout the section.

SITE 3 – slide 02/1448-1

Fundic gland region. There is gland hyperplasia and slight dilation of the gastric glands with several apoptotic cells present at the isthmus of the glands. There is oedema of the submucosa and a mild lymphocytic infiltration.

SITE 4 – slide 02/1449-1

Fundic gland region. Normal

SITE 5 – slide 02/1450-1

Pyloric gland region. There is a diffuse mononuclear cell infiltrate from the level of the gastric pits to the epithelial surface within the lamina propria and associated gland atrophy. The lamina muscularis is thickened with increased amounts of fibrous tissue and has scattered foci of lymphocytic infiltrate which extend down into the submucosa and muscle layers. There is cutting artefact along the length of the section making accurate interpretation difficult.

SITE 5a – slide 02/1452-1

The section shows a moderate superficial mononuclear cell infiltrate. It is also visible in the lamina muscularis which appears thickened in places and contains nests of glandular material.

SITE 6 – slide 02/1451-1

Duodenum. There is a mild lymphoplasmacytic infiltrate within the lamina propria of the villi. A marked fasciitis involving the deep fat layers takes the form of a chronic inflammatory cell infiltrate surrounding patches of fat necrosis.

CASE NUMBER 145216

SITE 1 – slide 02/1551-1

The stratum corneum displays diffuse parakeratosis measuring 9 cells in depth throughout the section. The stratified squamous epithelium shows mainly mild, blunt rete pegging, but in one area they become elongated and irregular in shape. This is associated with a perivascular lymphocytic infiltrate in the underlying lamina propria.

SITE 2 – slide 02/1552-1

Where present, the *stratum corneum* forms an anucleate amorphous pink layer above an epithelium with distinct rete pegging and lamina papillae extending to over half its width. Here, the lamina propria has a scant lymphocytic infiltrate. Towards the margo plicatus where the keratin layer is lost, the lamina papillae appear wider and almost reach the epithelial surface. In these areas the *stratum basale* appears proliferative and thickened. The lamina propria contains increased numbers of lymphocytes which appear primarily concentrated within the lamina papillae The lymphocytic infiltrate is at its greatest at the margo plicatus where aggregations of lymphocytes extend the entire width of the lamina propria.

SITE 3 – slide 02/1553-1

Fundic gland region. Autolytic but normal.

SITE 4 – slide 02/1554-1

Fundic gland region. Normal.

Site 4a – slide 02/1557-1

There is lymphatic dilation and oedema of the lamina propria underlying the gastric pits.

SITE 5 – slide 02/1555-1

Pyloric gland region. There is an increase in the number of lymphocytes within the lamina propria. Lymphocytes are also found in the lamina muscularis .PM change prohibits accurate assessment of this slide.

SITE 6 – slide 02/1552-1

The section shows pyloric gland region not duodenum.

CASE NUMBER 145235

SITE 1 – slide 02/1560-1

The stratum corneum shows diffuse irregular parakeratosis and focal hyperkeratosis with pyknosis and flattening of these keratinocyte nuclei. .It averages 16 cells deep. Scattered areas of surface keratin with a basket weave appearance are accompanied by adherent bacterial colonies. There is mild, blunt, rete pegging of the squamous epithelium throughout the section, with scattered mitotic figures present in the stratum basale. The underlying lamina propria

appears mildly oedematous with dilation of lymphatic vessels.

SITE 2 – slide 02/1561-1

The stratum corneum displays diffuse parakeratosis which increases in thickness and irregularity towards the margo plicatus (20 cells deep). Patches of flaking keratin are covered in clusters of bacteria. The rete pegs are elongated with scattered mitotic figures in the stratum basale, and the interdigitating lamina papillae extend further towards the stratum corneum. This pattern becomes more exaggerated towards the margo plicatus and is interrupted by two foci of glandular tissue. Towards the base, the gastric pits are mostly lined with plump mucous producing cells, but in areas adjacent to surrounding squamous epithelium, the cells appear flattened and darker staining, resembling stratified squamous epithelium or mucous neck cells. These glandular areas are supported by a fibrovascular connective tissue framework with a chronic inflammatory cell infiltrate. The underlying lamina propria also appears scarred with a lymphoplasmacytic infiltrate.

SITE 3 – slide 02/1562-1

Fundic gland region. Normal.

SITE 4 – slide 02/1563-1

Fundic gland region. Normal.

SITE 5 – slide 02/1564-1

Pyloric gland region. The section shows patches of mild lymphocytic infiltrate within the lamina propria from the level of the gastric glands to the gastric pits. There is no evidence of erosion, ulceration, or intraepithelial inflammatory cells.

SITE 6 – slide 02/1565-1

Duodenum. There is a mild lymphoplasmacytic infiltrate within the lamina propria of the villi. There are two lymphoid follicles visible in the lamina propria.

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