

Case Report

Diagnosis of inflammatory bowel disease in a Hackney pony by gastroduodenal endoscopy and biopsy and successful treatment with corticosteroids

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Introduction

Chronic inflammatory bowel disease (CIBD) is a common cause of chronic weight loss and hypoproteinaemia in adult horses (Kemper *et al.* 2000; Shumacher *et al.* 2000). Eosinophils, plasma cells, lymphocytes, basophils and/or macrophages are found in abnormally large numbers in mucosal and submucosal layers (Roberts 1983). Diagnosis of CIBD has been based upon clinical signs, results of oral glucose and/or xylose absorption test, and small intestinal biopsy (Kemper *et al.* 2000; Shumacher *et al.* 2000). Previously, biopsy of the small intestine to diagnose CIBD had been performed via exploratory laparotomy (Kemper *et al.* 2000). There is only a single report of successful medical treatment of CIBD in the horse (Duryea *et al.* 1997).

This case presentation reports on the use of gastroduodenoscopy-assisted biopsy of the small intestine in a Hackney pony with CIBD and the successful medical treatment of the pony.

Case history and clinical findings

An 11-year-old Hackney pony was referred because of inappetence and weight loss of several weeks duration. The pony had a good appetite for grain but not for hay. He had been dewormed regularly (4–6 times/year) and most recently 2 weeks prior to admission. Faecal flotation was negative for parasites. All 12 of the other ponies on the farm were reported by the owner and referring veterinarian to be in excellent body condition. On clinical examination, temperature, pulse and respiratory rates were normal (37.4°C, 48 beats/min and 20 breaths/min, respectively). The pony appeared in poor body condition (body score 2.5/9), weighed 250 kg and had nonpainful, pitting oedema under the mandible and ventral abdomen. Ultrasound examinations of

the thorax and abdomen were normal except for thickening of the small intestinal wall (**Fig 1**). The only abnormal laboratory findings on complete blood count (CBC) and serum chemistry were mild neutrophilia (8.3×10^3 cells/ μ l), low plasma protein (36 g/l with 17 g/l albumin), hypocalcaemia (2.6 mmol/l), hypomagnesaemia (0.42 mmol/l), and low iron-binding capacity (TTBC = 34.0 μ mol/l). The plasma oncotic pressure was very low at 10.4 mmHg (normal 18–22 mmHg). Renal function tests were normal (BUN 5.6 μ mol/l, creatinine 140.4 μ mol/l) as was the urinalysis. Based upon the clinical signs, laboratory findings and ultrasound findings, a tentative diagnosis of infiltrative bowel disease was made.

The pony was held off feed overnight for a xylose absorption test and endoscopy of the stomach and duodenum. The xylose absorption test was performed by standard protocol (Brown 1992) and results indicated

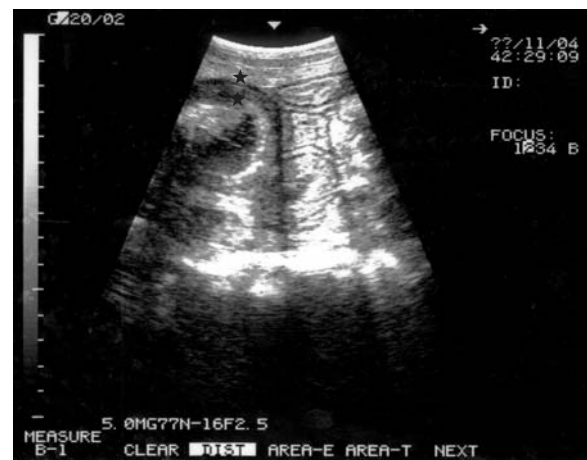


Fig 1: Ultrasonogram (5.0 MHz sector scanner) of the ventral abdomen of the 11-year-old Hackney pony. The jejunum is markedly thickened (0.5–0.8 cm, between *) in all segments visualised. Real time intestinal motility appeared to be normal and differences seen in luminal diameter are related to phase of contraction at the time of the image capture.

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malabsorption (**Fig 2**). Immediately following the completion of the xylose absorption test, the pony was lightly sedated with 75 mg of xylazine, and a 2.2 m endoscope (Olympus) was passed into the stomach and duodenum and 3 biopsies taken from different areas of the duodenum (**Fig 3**) and submitted for histopathology. Microscopic examination revealed a moderately severe mononuclear cell infiltrate of the duodenal mucosa. The large mononuclear cells were initially thought to be histiocytes, but after immunostaining were determined to be T-lymphocytes (**Fig 4**).

The pony was discharged the following day and, after biopsy results were known, a tapering treatment regimen of dexamethasone administered i.m. was commenced (20 mg daily for 1 week, 10 mg/day for the second week, 5 mg/day for the third week, 2 mg/day for the fourth week, and 2 mg every other day for an additional 4 weeks). The pony's appetite returned to normal during treatment and the pony was reported by the referring veterinarian and owner to have regained much of the lost weight. Two years after treatment, the pony is still normal. One year after the hospital admission, the serum albumin was 30 g/l.

In order to increase our confidence of the diagnosis of lymphocytic IBD in the horse in this report, full thickness

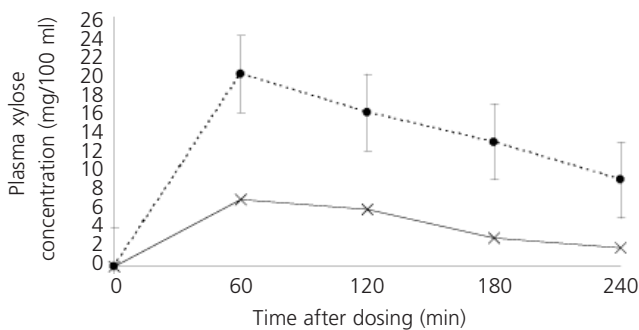


Fig 2: Xylose absorption test (0.5 g/kg bwt per os) of the affected pony. • = Range for normal horses (Brown 1992), x = Patient.

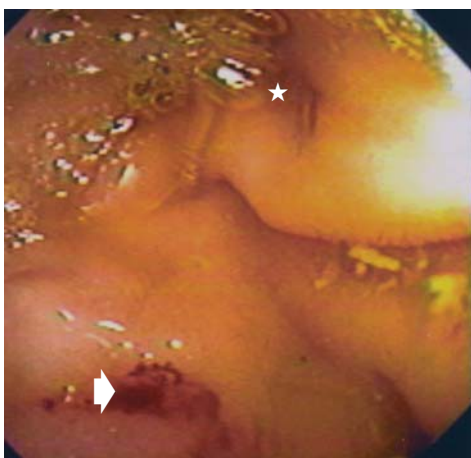


Fig 3: Duodenum and biopsy site with bleeding (arrow head) photographed immediately after first of 3 biopsies. The major duodenal papillae and secretions (*) are shown dorsal to the biopsy site.

duodenal biopsies were harvested at necropsy from 3 warmblooded horses that were subjected to euthanasia for reasons unrelated to gastrointestinal disease. A 5 cm length of duodenum midway between the pylorus and the hepatopancreatic ampulla was harvested from each horse within 4 h of death and fixed by immersion in 10% neutral buffered formalin for 24 h. For each section, 10 villus-crypt units selected at random were examined at 400x. Only those crypt-villus units where the architecture was intact from the tip of the villus to the base of the crypt were evaluated. The number of cells that were stained with each antibody was counted and the results of all 3 horses were averaged. Solitary lymphoid nodules were rare but when encountered were excluded from the counts. Similar to Packer *et al.* (2005) CD79a+ cells excluded plasma cells, which showed cytoplasmic rather than cell surface labelling. Monoclonal antibody BLA.36 labels both equine B-cells and a mononuclear cell population with extensive cell processes, compatible with dendritic cells (personal observations). Marker Mac387 labels neutrophils and a subset of equine macrophages (personal observations). The results are summarised in **Table 1**.

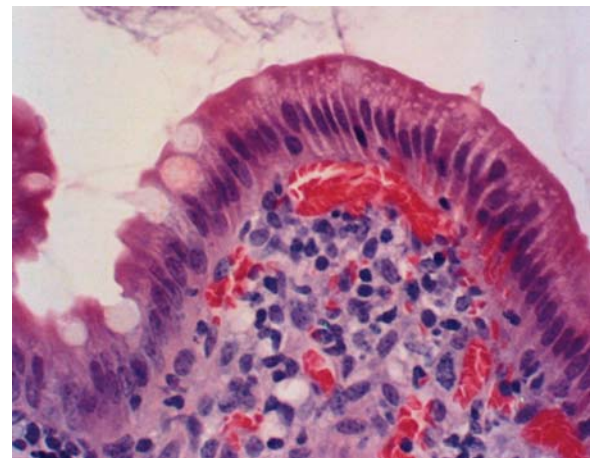


Fig 4a: Haematoxylin-eosin stain (60x) of duodenal biopsy demonstrating villus atrophy and mononuclear cell infiltration of the lamina propria.

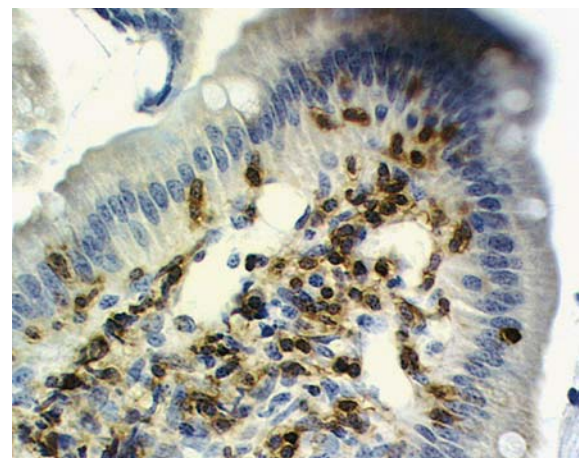


Fig 4b: Immunostaining of the biopsy revealed the mononuclear cells to be mostly T-cells (CD3+).

Discussion

Chronic inflammatory bowel disease in horses has been reported due to lymphocytic, lymphocytic/plasmacytic, eosinophilic, granulomatous or neoplastic infiltration of the bowel or as part of a multisystemic eosinophilic epitheliotropic disease. The distinguishing features of each have been reviewed recently (Shumacher *et al.* 2000). Knowledge of the cell type in horses with CIBD may permit specific treatments and provide a more accurate prognosis. Clinical signs, laboratory findings, and ultrasound exam do not usually distinguish between the different disorders. Rectal biopsy is helpful in the diagnostic work-up in less than 50% of the cases since the disease is often limited to the small intestine (Lindberg *et al.* 1996; Schumacher *et al.* 2000). If diarrhoea is a clinical sign, the chance of the rectal biopsy being informative may be increased, since this would suggest the colon is also involved. Performing a laparotomy to obtain the intestinal biopsy can be expensive, may occasionally result in peritonitis or incision infection (possibly associated with the catabolic condition of the patient), and may delay introduction of immunosuppressive therapy until the incisional sites are healed. Performing a laparotomy-assisted intestinal biopsy does permit manual assessment of the most abnormal bowel prior to biopsy, sampling of any and/or multiple areas of the bowel, and gathering of complete thickness and larger pieces of bowel. Small intestinal biopsy via endoscopy has been used for several years in both man and small animals to diagnose inflammatory bowel disease (Alcantara *et al.* 1993; Zoran 2001). The procedure is recognised as relatively safe and one recommendation in dogs and cats is to obtain multiple pieces of the small intestine in order to provide diagnostic specimens (Williard *et al.* 2001). In this Hackney pony, 3 samples were obtained and all appeared suitable for diagnosis. Two samples may be adequate for the horse since larger pieces can be gathered than in small animals. Observation of bleeding at the biopsy site is one indication that an acceptable sample may have been obtained.

Biopsy of the small intestine via gastroduodenoscopy offers a relatively noninvasive method of determining cell type in horses with inflammatory bowel disease. In adult horses, a

3 m endoscope may be required in order to obtain a duodenal biopsy. The method for performing the gastroduodenoscopy and collecting mucosal biopsy specimens in normal mature horses has been described (Brown *et al.* 1985). A brief summary follows. After the scope enters the empty stomach, the stomach is mildly dilated with air, the endoscope is moved around and down the greater curvature until the scope is retroflexed on itself, pointing upwards, and the cardia is visualised (with the scope entering). The scope is then rotated and advanced slightly to the right to visualise the pyloric canal and pyloric sphincter. The scope is then gently manipulated into this region and advanced with the aid of peristaltic activity. The biopsy probe can be used to grasp the pyloric sphincter, if necessary, to facilitate movement of the scope towards the duodenal opening. The duodenal mucosa can be recognised by its pale pink colour, and occasionally by the presence of villi and clear yellow, bile-stained fluid in the lumen. The duodenal diverticula can be seen which normally has an intermittent discharge of bile. The biopsy(ies) are collected with a standard biopsy instrument that passes through the biopsy channel of the scope. The duodenal sample is placed in formalin or other fixative. As in this case, the biopsy can be performed following a 2–6 h carbohydrate (glucose or xylose) absorption test which will help confirm malabsorption. Withholding feed for 12 h is required for both the gastroduodenoscopy and absorption test.

Focal small intestinal infiltrative diseases, especially focal eosinophilic strictures, have been reported in the horse and would likely not be detected by duodenal biopsy, but the focal eosinophilic disorders generally cause acute or chronic colic and are not accompanied by hypoproteinaemia and weight loss, which are characteristic of more diffuse chronic inflammatory bowel disease. Lymphocytic-plasmacytic enteritis usually has diffuse small intestine involvement (Kemper *et al.* 2000) such that abnormalities would be expected on the duodenal biopsy specimen.

Although horses with inflammatory bowel disease have generally been given a poor prognosis for recovery (Schumacher *et al.* 2000), this case and at least one other (Duryea *et al.* 1997), provide evidence that some cases of inflammatory bowel disease patients may undergo complete

TABLE 1: Distribution of leucocyte subsets in the duodenum of 3 horses

Leucocyte marker	Number of lymphocytes (per villous-crypt unit)		
	Villous <i>lamina propria</i> (mean \pm 1 s.d.)	Intraepithelial lymphocytes* (mean \pm 1 s.d.)	Intercryptal <i>lamina propria</i> (mean \pm 1 s.d.)
Plasma cells	3 (\pm 2.8)	0	17 (\pm 0.7)
CD3	37 (\pm 10.0)	20 (\pm 2.4)	34 (\pm 10.0)
CD79a	5 (\pm 4.2)	0	19 (\pm 10.3)
BLA.36	28 (\pm 2.7)	0	28 (\pm 4.8)
Mac 387	11 (\pm 7.6)	0	14 (\pm 6.2)

* Greater than 95% of all IELs expressed CD3.

TABLE 2: Distribution of leucocyte markers in pony with lymphocytic inflammatory bowel disease (IBD)

Leucocyte marker	Number of lymphocytes (per villous-crypt unit)		
	Villous <i>lamina propria</i> (mean/10 villi)	Intraepithelial lymphocytes* (mean/10 villi)	Intercryptal <i>lamina propria</i> (mean/10 intercryptal areas)
Plasma cells	0.2	0	2
CD3	50	9	47
CD79a	0	0	0
BLA.36	8	0	17
Mac 387	0.2	0	1.5

* Greater than 95% of all IELs expressed CD3.

recovery following corticosteroid treatment. A previous report suggested administering dexamethasone 0.1 mg/kg bwt i.m. every 96 h for 4 weeks, followed by a gradually declining dosage for an additional 10–12 weeks (Duryea 1997).

The diagnosis of idiopathic inflammatory bowel disease (IBD) is based largely on the degree of inflammation present and the predominate type of infiltrating leucocyte. The inflammatory infiltrate can be difficult to assess because the numbers of different classes of leucocytes present in normal intestine varies between species, individual animals and level of intestine. Subjective assessments of the severity of the inflammatory infiltrate lead to large interobserver variation because knowledge of the size of the normal immune cell populations is often lacking. Immunostaining can be most helpful in determining the specific cell types and the percentage of each cell type in the infiltrate. In this case the more routine haematoxylin and eosin stain suggested the large mononuclear cells were histiocytes but immunostaining revealed their true identity to be T lymphocytes. T lymphocytes were reported to be the most common inflammatory cell type observed in the jejunum of 14 adult horses without signs of gastrointestinal disease (Packer *et al.* 2005). There are no previous reports of cell types in the normal equine duodenum.

Although the number of control animal duodenum studied is small, all had similar trends. T cells were uniformly distributed in approximately equal numbers between the *lamina propria* of the villi and intercryptal areas. In contrast, B cells were preferentially localised to the intercryptal areas but at about equal numbers with T cells. Almost all of the B cells in the villous *lamina propria* were located at the base of the villi where they blended imperceptibly with those in the intercryptal areas. Neutrophils and eosinophils, based on examination of H&E stained sections were rare. Macrophages, as determined by Mac 387 staining were equally distributed between the villous and intercryptal *lamina propria*.

In contrast, the results obtained from the horse in this study differed in a number of ways (**Table 2**). The total number of T cells in the villi were increased. This was especially striking since the villi in this pony were only one-third to one-half the length compared to the normal controls (villous atrophy). Additionally, the number of other leucocytes was reduced. Infiltration of large numbers of T cells is suggestive of alimentary T cell lymphoma. However, the infiltrating lymphocytes respected normal anatomic boundaries and the response of the horse to anti-inflammatory therapy supports the conclusion that the infiltrating T cells were reactive rather than neoplastic.

The cause of lymphocytic enterocolitis is unknown, but a normal or abnormal immune response to bacterial, viral, parasitic or dietary antigens is proposed (Schumacher *et al.* 2000). T cells appear to play an important role in IBD of human patients with Th1 cells predominant in Crohn's disease and Th2 cells predominating in ulcerative colitis (Weigmann and Neurath 2002). Without specific cytokine staining, we were unable to determine which immune

response may have been occurring in this pony. Although changes in the parasite control programme or intentional dietary changes did not occur in this pony, they would be reasonable recommendations for inflammatory bowel disease in the horse; especially young horses in hopes of alleviating the immune response. Previous attempts at treating lymphocytic-plasmacytic enterocolitis in horses with steroids have been unsuccessful (Kemper *et al.* 2000), but in 2 of 4 horses, oral prednisone was used (information was not provided on the other cases). Prednisone has recently been shown to be poorly absorbed and/or metabolised in most horses (Jackson *et al.* 2000). Inflammation of the bowel wall would further decrease absorption. Corticosteroids should be administered parenterally in horses with inflammatory bowel disease.

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