Inflammatory bowel disease (IBD) in horses. A retrospective study identifying the diagnostic value of different approaches.

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Abstract

Reasons for performing study

Diagnosing IBD in horses is a challenge and requires a multimodal approach, since no conclusive diagnostic test is available. Prelevation of duodenal biopsies has become quite popular, however little is known about their diagnostic value.

Objectives

The objective of this study was to provide an overview of patient data in a group of 78 IBD suspected horses admitted to 4 large equine referral clinics, subjected to an oral glucose absorption test (OGAT) and to set out the enteral biopsy results against other diagnostic modalities.

Study design and methods

In this retrospective multi-centre study 78 IBD suspected horses were included. Case history, clinical findings, diagnostic test results including OGAT and enteral biopsies, therapy and outcome were mapped out for all horses. Clinical findings, diagnostic results and outcome were compared by means of Chi-Square analyses. P-values smaller than 0,05 were considered significant.

Results

80% of horses were admitted in the age range between 5-15 years. 'Lethargy', 'diarrhea', 'recurrent colic' and 'weight loss despite good appetite' were recorded in respectively 21,8%; 14,1%; 28,2% and 78,2% of cases. None of these symptoms showed a significant association with OGAT test results, low blood total protein or biopsy based diagnosis of IBD. The majority of IBD suspected horses had an abnormal OGAT (70,5%) There was no association between an aberrant duodenal or rectal biopsy result and a disturbed OGAT test. There was no association association between OGAT test results, or enteral biopsy test results on one hand

and blood TP, gastric ulcer disease or an aberrant endoscopic duodenal aspect. There was no association between any treatment and positive outcome.

Conclusions

Histopathological results of enteral biopsies, either duodenal or rectal should be interpreted with caution in cases suspected of IBD since they show no association with OGAT results, currently viewed as golden standard.

Introduction

Chronic inflammatory enteropathy in adult horses, often referred to as equine chronic inflammatory bowel disease (IBD), is a real challenge in horses both with respect to diagnosis and estimation of prognosis as to design an adequate management and treatment plan. Depending on the main type of inflammatory cells involved, several different types of IBD are histopathologically distinguished such as granulomatous enteritis (GE), multisystemic eosinophilic epitheliotropic disease (MEED), lymphocytic-plasmacytic enterocolitis (LPE), diffuse eosinophilic enterocolitis (DEE) and proliferative enteritis (PE), each of which can affect either small and/or large intestine [Kalck, Schumacher, Mair 2006]. Most often horses are presented for problems such as recurrent colic, weight loss often despite good appetite, poor performance, lethargy and sometimes even edema [Kalck, Schumacher 2000, Kemper, Mair 2006]. It has been described that equine IBD patients often show hypoproteinemia and hypoalbuminaemia and that on one hand the majority of horses is euthanized or dies within short time, but on the other hand it is possible to treat these patients successfully [Schumacher 2000]. However, no study has been focussing on the diagnostic work-up of IBD suspected patients and all available studies involve a maximum of 10-20 IBD patients [Kalck,

Schumacher 2000, Kemper, Merrit 1976, Scott 1999, Olofssen 2015, , Schumacher 1990, Southwood 2000, Kaikkonen,].

Due to the lack of a real diagnostic test, diagnosis of IBD often entails a multimodal approach and presumptive diagnosis is often based upon the combination of results of several different tests. Retrieving a complete medical case history is an important step, often followed by clinical examination including rectal exploration. Besides this, routine bloodwork, faecal parasitic egg count, transabdominal ultrasound, gastroduodenoscopy, abdominocentesis and a D-glucose or D-xylose absorption test are often performed. This multimodal patient approach ultimately leads to the presumptive diagnosis of IBD. Confirmation of the diagnosis can only be provided by full thickness biopsy of intestines performed during exploratory laparotomy or laparoscopy. However, at the moment most clinicians don't prefer this approach because performing this invasive procedure in a chronically ill patient doesn't seem to be a first choice option in the face of the presence of many other diagnostic modalities. However, very little is known about the real diagnostic value of these less invasive diagnostic approaches

The latest few years also duodenal biopsies are harvested during gastroscopy in the work-up of IBD suspected horses. They are thought to provide an additional "window" on the gastrointestinal tract besides rectal biopsies, of which the biopsy procedure has already been described in the 80's [Kalck, Kemper, Mair 2006, Divers, Trachsel, Kaikkonen, Lindbergh, Oloffsen 2015, Brown 1985]. Duodenal and rectal biopsies seem a very elegant diagnostic aid. They are technically quite easy to harvest and unharmful [Davis]. Duodenal biopsy collection requires some experience with gastroscope steering, but once passed the pyloric entrance, biopsy collection is quite easy using a long biopsy pinch inserted through the biopsy canal [Divers]. Due to the low threshold of both biopsy techniques, they are currently routinely performed during the work-up of IBD suspected patients in many clinics, however, little is known about how reliable they actually are as a diagnostic aid. Are they representative

for the inflammatory status of the 15-meters of small intestine further down the line (in case of the duodenal biopsies) and of the 6- to 8-meters long large colon (in case of the rectal biopsies) that lie ahead? Is this enteral biopsy tissue in general of sufficient quality to be assessed by a histopathologist? Is their histopathological evaluation correlated with results of other diagnostic tests routinely applied in the work-up of the IBD suspected patient? How important is their predictive value for long term prognosis? To obtain a better view on these issues, the following retrospective study was set up, as a joint venture between the Utrecht University Equine Clinic (The Netherlands), The Wolvega Equine Clinic (The Netherlands), The Morette Equine Clinic (Belgium) and The Bosdreef Equine Clinic (Belgium). The objective of this retrospective study was dual: (1) to provide an overview of patient data such as breed, gender, age, symptoms, dietary management, diagnostic test results, applied treatment protocol, applied dietary adaptations and long term outcome in a group of 78 horses and ponies suspected of having IBD and subjected to an oral glucose absorption test (OGAT) between 2009 and 2013, and (2) to find out whether there are associations between duodenal and rectal biopsy test results and other diagnostic test results such as OGAT, gastroduodenoscopy, blood work and long term outcome. To our knowledge, this is the first large scale study to evaluate the association between diagnostic tests routinely applied in the work-up of the IBD suspected patient, also including histopathological evaluations of enteral biopsies.

Material and methods

Study population characteristics

The study population encompassed 83 horses and ponies that were suspected of having IBD and that were admitted to one of the four participating referral centres and

subjected to an OGAT. Horses that were diagnosed with malignancy (n=5) were excluded from the study.

Case features and history were mapped out for 78 horses, such as age, breed, gender, time of admission, dietary management at admission (pasture turn out, hay, haylage, alfalfa, concentrate feeding (pelletized versus muesli, oats, maintenance versus high energy), protein or fat rich). Symptoms were categorized as 'lethargy/poor performance', 'diarrhea', 'recurrent colic' and 'weight loss' [Kalck, Schumacher 2000, Kemper, Mair 2006].

Routine diagnostic tests

Blood work included packed cell volume (PCV), leucocyte count, total protein (TP), albumin, gamma globulin and gamma glutamyl transferase (GGT). An overview of applied reference ranges per participating referral centre for the different laboratory analyses is provided in Table 1.

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Following findings were recorded for **rectal examination**: 'palpation of small intestinal loops with increased wall thickness', 'palpation of large intestinal segments with increased wall thickness', 'other abnormal findings', 'no abnormal findings'. In case **transabdominal ultrasound** was performed, following findings were categorized: 'visualisation of small intestinal loops with increased wall thickness', 'visualisation of large intestinal loops with increased wall thickness', 'visualization of liquid content in the large intestine', 'no abnormalities'. Following ultrasonographic reference ranges for intestinal wall thickness were applied: small intestinal wall thickness $\leq$ 3 mm, colonic wall thickness $\leq$ 4 mm [Kalck, Freeman 2001].

All horses were subjected to an **OGAT** using the following protocol: The horses were starved 18-24 hours before testing. A baseline venous sodium-fluoride blood sample was taken and subsequently, 1 gram glucose anhydrous (20% solution) per kg BW was given by nasogastric intubation dissolved in 1L luke warm water. Next, venous blood (sodium fluoride) was collected at the jugular vein, every 30 minutes for eight consecutive times. OGAT test results were deemed normal when peak glucose level reached 85% above baseline, approximately 90 and 120 minutes after tubing and returned back to baseline within 6 hours. OGAT test results were recorded as partial malabsorption when peak glucose levels only reached 15-85% above baseline and as total malabsorption when peak glucose levels remained below 15% above baseline [Mair 2006, Davis].

Gastroduodenoscopy was performed after finalization of OGAT and gastroduodenoscopic findings were categorised as either: 'signs of equine gastric ulcer syndrome (EGUS grade 1 or more)', 'aberrant aspect of entrance of the duodenum (hyperaemia (either patchy or diffuse)), oedematous, striations)' or 'no aberrant findings to be reported'.

Enteral biopsies

The study includes two types of enteral biopsies: 'duodenal biopsies' and 'rectal biopsies'.

Duodenal biopsies were harvested by means of a 2.8 mm biopsy forceps inserted through the biopsy canal of a gastroduodenoscope [Kalck, Divers]. Briefly, in the sedated horse, the gastroscope was guided through the pylorus into the first part of the duodenum, after which at least two biopsy samples were taken from the first part of the duodenum and subsequently immersed in 10% neutral buffered formalin, to be sent to the pathology lab (Department of pathobiology Utrecht University (Utrecht University equine clinic and

Wolvega Equine Clinic) and Medvet Antwerp (De Bosdreef and The Morette Equine clinic). Haematoxylin and eosin (H&E) histopathological staining was performed.

Rectal biopsies were harvested by means of an Olympus Japan, K47062, 4 mm snare as follows: Horses were restrained in stocks, in most cases without sedation. After removal of faeces a lubricated biopsy catheter was introduced into the rectum with the hand guarding the catheter tip and 2 to 4 pieces of rectal mucosal tissue were taken at 10 h. Tissues were carefully removed with a small gauche needle and immersed in 10% neutral buffered formalin, processed by standard methods for paraffin-embedding, sectioned at 4.0 μm, and stained with H&E. Rarely discomfort was shown during the procedure and no complications were encountered in all 45 cases [Kemper, Kaikkonen, Lindbergh, Gibson, Rickketts].

The histopathological quality of the biopsies was labelled as either 'useful diagnostic material', 'less than ideal' meaning: sample size too small, solely mucosa sampled and/or pinch artefacts, and 'insufficient to establish a clear diagnosis'. The enteral layers (depth) that could be evaluated histologically were categorized as either 'only mucosa or mostly mucosa', 'mucosa and submucosa' or 'obscure'.

The enteral biopsy results were categorized by an equine veterinarian as either "aberrant" or "normal". In case of an aberrant biopsy result, one of the following IBD types was recorded: 'granulomatous enteritis', 'eosinophilic enteritis, 'lymphocytic-plasmacytic enteritis' or 'mixed enteritis' in case of a duodenal biopsy and 'lymphocytic proctitis', 'eosinophilic proctitis' and 'neutrophilic proctitis' in case of a rectal biopsy.

Applied medical therapies and dietary adaptations

Medical therapy implied after diagnostic work-up was categorized as either 'corticosteroids' (prednisolone 1 mg/kg BW po q24h), dexamethasone (0,06 mg/kg BW po q24h) or 'omeprazole' (2-4 mg/kg BW po q24h). It was also recorded whether and which

dietary adaptations were applied after diagnostic work-up ('laxative', 'increased fibre content in the diet', 'low carbohydrate diet', 'high fat diet', 'high protein diet', 'increased pasture turn-out', 'against bloating', 'supplementation with probiotics').

Long term follow-up

For horses that returned to the clinic more than once throughout the five years the study encompassed, the following categories were created: 'control visit', 'visit due to recurrent problems', 'colic surgery' or 'euthanasia'.

Long-term follow-up was performed by means of a telephone questionnaire executed at least one year after establishment of diagnosis. Horse owners were asked for input concerning: mean features of dietary management since diagnosis (pasture turn out, amount of hay/haylage, type of concentrate feed provided); applied medical therapy (type, therapy loyalty, necessity for repeated therapy); amount of weekly exercise performed with the horse (how many hours per day/per week, performance before and after treatment). Outcome was categorized as either 'performing to expectation', 'no amelioration despite therapy' 'euthanasia or natural death due to recurrent GI related problems', 'euthanasia or natural death due to other reasons'.

Statistical Analyses

Data were analysed by means of Chi-Square analyses. P-values smaller than 0,05 were considered significant. Since only three horses showed total malabsorption, it was decided to merge this group with the partial malabsorption group throughout all statistical analyses.

Results

Study population characteristics

The study population encompassed warmblood horses (n=60), standardbreds (n=4), Friesian horses (n=8) and ponies (n=6). Geldings (47.4%) were most commonly represented followed by mares (35.9%) and stallions (16.7%). There was no gender predisposition (p=0.549), nor seasonality (p=0.364) in the pattern of admittance. Figure 1 gives an overview of admission pattern of cases throughout the year with the average admission age per month. The average age at admission was 8.95 years (range 1-25 years, SD 4.895).

Of 47 horses, the dietary management prior to referral was recorded: hay (68.1%), haylage (19.1%), combination of hay and haylage (6.4%), pellets (74.5%), muesli (31.9%), oats (4.3%) and alfalfa (4.3%). The majority of horses had access to pasture turn-out (66.7%). A combination of pellets and muesli were fed in 10.6% of cases. The majority of horses had pellets in their diet (74.5%). Horses receiving concentrate feed in our study population were on average younger (6.66 years (SD 5.507) than horses receiving no concentrate feed in their diet (15 years (SD 9.539)). There was no positive predictive value found for pellets in the diet and either a disturbed OGAT (p=0.174), aberrant gastroscopic findings (p=0.690) or a disturbed duodenal (p=0.893) or rectal biopsy (p=0.42)

Clinical symptoms in horses suspected of IBD

'Weight loss', 'recurrent colic', 'lethargy' and 'diarrhea' were recorded in respectively 78.2%, 28.2%, 21.8% and 14.1% of cases. 'Weight loss' was recorded in 61 horses, the majority of which showed 'weight loss despite good appetite' (41 horses, 67.2%). There was no significant association between either of these symptoms and OGAT test results (weight loss p=0.249, recurrent colic p=0.334, lethargy p=0.518 and diarrhea p=0.611).

Routine diagnostic test results

Rectal palpation was performed in 72 (92.3%) horses and in a minority of cases (22 horses (30.6%)) thick-walled small intestinal loops were identified. Of 19 of these cases (86.4%) a duodenal biopsy was available and showed an aberrant histopathological evaluation in 9 cases (47.37%). Interestingly, thick-walled small intestinal loops were identified during rectal palpation in less than half (36.5%) of the cases with an aberrant OGAT result. No association was found between identifying thick walled small intestinal loops during rectal palpation and either a disturbed OGAT result (P=0.094) or an aberrant duodenal biopsy result (p=0,491).

Thick walled large intestinal loops were identified in only 9.7% of cases during rectal palpation. Of 5 of these cases (71.4%) a rectal biopsy was available and showed an aberrant histopathological evaluation in all cases.

Faeces were tested for sand accumulation in 44 cases and parasitic egg count was performed in 62 cases. In 4 horses (9.1%) an abnormal accumulation of sand in the faeces was reported and 10 horses (16.1%) had a positive parasitic egg count for strongylus type eggs.

Transabdominal ultrasound was performed in 57 horses and revealed thick walled small intestinal loops in 16 cases and thick walled large intestinal segments in 9 cases. In 72.2% (n=39) of cases with an aberrant OGAT result transabdominal ultrasound was performed and 33.3% (n=13) revealed presence of thick-walled small intestinal loops. There was a significant association between finding thickened small intestinal loops on rectal palpation and visualizing them during abdominal ultrasound (81.8%; p <0.001). No association was found between visualization of thick walled small intestinal loops during

transabdominal ultrasound and either a disturbed OGAT (p=0.193), a low blood TP (p=0.798) or an aberrant duodenal biopsy (p=0.294).

Blood work was available for 69 cases. Blood work included packed cell volume (PCV) and leucocyte count (n=67), total protein (TP) (n=69), albumin (n=69), gamma globulin (n=66) and gamma glutamyl transferase (GGT) (n=61). An overview of applied reference ranges for the different labs is provided in Table 1 together with an overview of the percentage of the study population out of reference range for the different parameters.

The majority of IBD suspected horses (70.5%, n=55 horses) had an abnormal **OGAT** result (Table 2) and in 33 of these horses (60%) an aberrant duodenal (n=15) or rectal biopsy (n=21), was identified. No positive predictive value could be identified for a disturbed OGAT on one hand and on the other hand presence of thick-walled small intestinal loops on either rectal palpation or transabdominal ultrasound (p=0.550 and p=0.697), a low blood total protein (p=0.601), aberrant duodenal (p=0.478) or rectal biopsies (p=0.522) and negative outcome (p=0.937). There was no significant gender predisposition for a disturbed OGAT (p=0.549).

Gastroduodenoscopy together with OGAT was performed in 60 IBD suspected horses. In 5 horses (8.3%) an aberrant aspect of the duodenum was found, and in two of these cases also signs of EGUS were identified. Only a minority of horses with an abnormal OGAT result (n=42) showed abnormalities during gastroduodenoscopy (47.6%, n=20). Likewise, nearly half (41.4%, n=24) of the horses showing an aberrant enteral biopsy showed abnormalities during gastroduodenoscopy. There was no significant association between finding abnormalities (EGUS or abnormal aspect of the entrance of the duodenum) during

gastroscopy and a disturbed OGAT result (p=0.296). Interestingly, all horses with an aberrant aspect of the duodenum (n=5) also had an abnormal OGAT result and 2 of these cases showed an aberrant enteral biopsy.

There was no significant association between finding abnormalities (EGUS or abnormal aspect of the entrance of the duodenum) during gastroduodenoscopy and either a disturbed OGAT test result (p=0.296) or an aberrant enteral biopsy test result (either duodenum or rectum) (p=0.446).

Enteral biopsies

Rectal biopsies were evaluated as moderate quality diagnostic material in 93.3% of cases (Table 3). Duodenal biopsies were labelled as moderate quality in only 59.5% of cases (Table 3). Noteworthy, the quality assessment of the duodenal biopsies significantly differed between clinics: biopsies taken at the Utrecht University Equine Clinic or Wolvega Equine Clinic were deemed useful quality in 50% of cases, whereas 76.9% of duodenal biopsies taken at The Morette Equine Clinic or The Bosdreef Equine Clinic were labelled as of useful quality. The majority (86.5%) of the duodenal biopsies consisted of only mucosa. Rectal biopsies contained more often deeper enteral layers: 64.4% consisted of only mucosa or mostly mucosa (superficial), 31.1% consisted of both mucosa and submucosa and 4.5% were labelled as obscure.

In 37 horses (47.4%) a duodenal biopsy was harvested. These duodenal biopsies showed in slightly more than half of the cases (56.8%: 21 out of 37 cases) histopathological features fitting with inflammation. In 45 horses (57.7%) a rectal biopsy was harvested. The majority of rectal biopsies (84.4%: 38 out of 45 rectal biopsies) showed abnormal histopathological features fitting with inflammation (Table 2). In 10 out of 78 horses (12.8%)

both duodenal and rectal biopsies were harvested. Interestingly, in 7 of these horses both types of enteral biopsies were aberrant and 3 of these horses (42.9%) had an abnormal OGAT result. In 29 out of 55 horses (52.7%) with an aberrant OGAT result, a duodenal biopsy was harvested and showed an aberrant result in slightly more than half of the cases (51.7%). A rectal biopsy was taken in 26 horses (47.3%) with an aberrant OGAT result and 84.6% showed an aberrant biopsy result. A schematic overview of abovementioned data can be found in figure 2.

Aberrant duodenal and rectal biopsy results were both significantly correlated to weight loss (duodenal biopsy p=0.029 and rectal biopsy p=0.035), but not to recurrent colic (duodenal p=0.639 and rectal biopsy p=0.239), lethargy (duodenal p=0.209 and rectal biopsy p=0.434) and diarrhea (p=0.776 and p=0.491)

There was no association between either duodenal biopsy or rectal biopsy test result on one hand and on the other hand, one of the following specific features of the blood work such as aberrant white blood cell count (duodenal p=0.247 and rectal biopsy p=0.677), PCV (rectal biopsy p=0.596), TP (duodenal p=0.306 and rectal biopsy p=0.386), albumin (duodenal p=0.436 and rectal biopsy p=0.498), gamma globulin (duodenal p=0.405 and rectal biopsy p=0.495) or GGT (duodenal p=0.906 and rectal biopsy p=0.102). Likewise, there was no association between either type of enteral biopsy and reported findings during rectal palpation (p=0.580).

Follow up

A dietary advice was provided in 62.8% (n=49) of the 78 IBD suspected cases. In most cases these horses had an aberrant OGAT result. In 15 horses a dietary advice was provided despite a normal OGAT test result. The different kinds of diets that were advised were either 'laxative' (n=8: 16.3%), 'high-protein' (n=18: 36.7%), high-fat' (n=31: 63.3%), 'high-fiber'

(n=40: 81.6%), 'against bloating' (n=9: 18.4%), 'pasture turn out' (n=15: 30.6%) and 'probiotics' (n=10: 20.4%). Dietary changes aiming for an increase in total fat content (63.3%) and fiber content (81,6%) were advised most often. There was no association between applying dietary changes and positive outcome (p=0.745).

Medical treatment was applied in 68 IBD suspected cases. Prednisolone, omeprazole and dexamethasone oral therapy were instituted in respectively 82.4%, 39.7% and 2.9% of these cases. An overview of the applied **treatments** is provided in Table 4. Only 10 out of 78 horses didn't receive any medication, interestingly 70% (n=7) of these cases had an aberrant OGAT result.

No association between one or either of the applied treatments and positive outcome could be found (prednisolone p=0.174, dexamethasone p=0.653 and omeprazole p=0.725)

Only a minority of cases was presented to the clinic sometime after diagnosis, either as control visit (n=17) or as a recheck because of recurrence of problems (n=9, of which 4 horses were euthanized shortly after admission). Combined with the telephone questionnaire long term information was provided for 39 horses. In total, 12 out of 39 (30,8%) horses died, 10 of which were actively euthanized (83.3%) because of poor prognosis, whereof 8 of these cases (80%) had a disturbed OGAT. 3 of the 10 euthanized horses had an aberrant duodenal biopsy and 3 had an aberrant rectal biopsy. 1 case had both an aberrant duodenal and rectal biopsy. None of the euthanized horses showed signs of malignancy on autopsy.

Discussion

The objective of the current retrospective study was dual. On one hand to provide an overview of population characteristics, diagnostic test results, applied treatment modalities

and outcome in a large group of IBD suspected horses presented to one of four large equine referral hospitals and on the other hand to look for possible associations between the applied diagnostic modalities, including enteral biopsy test results, since more and more clinicians tend to include them in their diagnostic work-up of the IBD suspected horse.

There are currently no epidemiological studies available reporting on an increased prevalence of IBD over the past 20 years, though it seems that the disease is encountered with increasing frequency. Inflammatory bowel disease (IBD) in horses is mainly classified into 6 subtypes based upon histopathological features, being lymphocytic-plasmacytic enterocolitis (LPE), granulomatous enteritis (GE), multisystemic eosinophilic epitheliotropic disease (MEED), diffuse eosinophilic enteritis (DEE), idiopathic focal eosinophilic enteritis (IFEE) and proliferative enteritis (PE) [Kalck, Schumacher, Mair 2006, Kaikkonen]. Depending on the type of IBD, either small intestine or large intestine or a combination of both can be attained.

Since there is no single definitive diagnostic test, diagnosis requires a multimodal approach, during which special attention is paid to certain typical features such as recurrent colic, weight loss despite good appetite, low blood total protein content, low to normal PCV, identification of thick walled intestinal segments during rectal palpation and/or transabdominal ultrasound and a disturbed OGAT. At the same time, presence of certain pathologies that are part of the differential diagnosis, such as malignancies, right dorsal colitis and hepatic and renal disease need to be excluded.

Especially OGAT test results are deemed to be very useful for diagnosing IBD. The specificity and sensitivity of the oral glucose absorption test are 90-100% and 40-45% respectively [Mair 1991,Murphy]. In the current study only IBD suspected horses that underwent an OGAT were included. Still it is important to realize that it has been shown before that a normal OGAT does not mean the horse is not suffering from IBD or another infiltrative enteral disease. [Davis]. We are well aware of the fact that the xylose absorption

test would have been a better and more precise method [Mair 1991, Batt], however in none of the participating referral centres it is applied.

Recurrent colic and weight loss despite good appetite are two notorious signs reported in horses suffering from IBD [Kalck, Schumacher, Kemper, Mair 2006]. In our study, the majority of horses showed weight loss, and in most cases this was manifested despite presence of good appetite. This is in accordance with previous studies. [Kalck, Kaikkonen 2014, Metcalfe] No association between either of the recorded symptoms and any applied diagnostic test could be found in the current study. However, aberrant duodenal and rectal biopsy results were both significantly correlated to weight loss. No other symptom showed a significant association. This is a quite interesting finding, which should encourage research into further fine tuning histopathological interpretation of enteral biopsies.

In comparison with earlier reports hypoproteinemia and hypoalbuminemia were not highly prevalent in our study population and overall prognosis was reasonably well: 30,8% of cases being euthanized or diseased after 1 year. Kaikkonen et al [Kaikkonen 2014] used hypoproteinemia and hypoalbuminemia as essential inclusion criteria, thereby possibly excluding early, or milder IBD cases. Likewise, Kemper et al [Kemper 2000] looked retrospectively at horses that exclusively showed lymfoplasmacytic enterocolitis on autopsy, so focussing on the worst cases of this subtype of IBD. Increased GGT and leucocyte count were quite often encountered in our study population (respectively 23% and 23.9%), though no association with either of the other diagnostic modalities, nor outcome could be demonstrated.

In the current retrospective study, no gender predisposition could be identified. In human medicine ulcerative colitis, a subtype of human IBD, is diagnosed with a higher incidence in females when compared to males. [Subasinghe 2011, Betteridge 2013].

The average age at which horses were presented to the clinic was 8.95 years (range 1-25 years, SD 4.895). So horses were predominantly admitted during their active sportive life, and apparently predominantly consuming a diet containing concentrate feed. Indeed, horses receiving concentrate feed in their diet were on average younger than horses receiving no concentrate feed in their diet. Although this was only a trend and no significant difference in age between both groups could be shown, this could be an interesting point of attention for future large scale epidemiological studies. In humans, IBD is caused by a combination of genetic predisposition and presence of certain antigenic stimuli. There are ample studies demonstrating that certain dietary habits are more often encountered in human IBD patients [Kostic, Schaubeck].

Interestingly our study shows that there is a significant association between finding thickened small intestinal loops on rectal palpation and visualizing them during abdominal ultrasound and that abdominal ultrasound is a more sensitive technique when compared to rectal palpation to identify these intestinal loops, which was to be expected. However, and this is quite important to remark, finding thick walled small intestinal loops has no positive predictive value for a low blood TP content, a disturbed OGAT, nor for aberrant enteral biopsies. Another interesting finding is the fact that there is no significant association between visualizing abnormalities during gastroscopy and an abnormal OGAT result. Even more important to mark is the lack of association between histopathological evaluation of duodenal biopsies and visual endoscopic appreciation of the duodenal entrance. Since it is most probable that a clinician is predominantly triggered to perform a duodenal biopsy upon visualizing an aberrant aspect of the duodenum. The fact that there was no association

between either duodenal biopsy or rectal biopsy test result and any specific features of the blood work, reported findings during rectal palpation or gastroscopy and OGAT results needs some critical thoughts. First of all the quality of duodenal biopsies is often disappointing, as has been demonstrated in the current retrospective study. Most probably this has its effect on the value of its histopathological interpretation. On top of that there is the lack of a standardized protocol to interpret these biopsies both for pathologists and veterinary clinicians that need to link the pathology reports to their patient. The difference in quality assessment of duodenal biopsies between the participating referral centers supports this lack of a standardized protocol.

Apparently, quality of rectal biopsies is greater than that of duodenal biopsies, which was to be expected since there is much more freedom of manipulation when harvesting a rectal biopsy, whereas clinicians need to depend upon the biopsy pinch to harvest a duodenal biopsy. Probably further optimizing the form of the beak of the biopsy pinch can help in reducing traumatization of the biopsy tissue. To further tackle this problem, clinicians should be encouraged to always take multiple biopsies per horse. For human patients for example, a minimal amount of biopsies per patient, specific location and a minimum need of amount of villi present in one sample (i.e. 3-4 villi with correct orientation) are standardized [Walker 2011]. A system of standardization has been proposed but it has not been universally used yet and much progress was booked since then on the histological interpretation of endoscopically taken biopsies in humans, cats and dogs [Lindbergh]. There is need for a systematic study of both duodenal and rectal biopsy findings in horses, until then the histological diagnosis of IBD in horses suffers from inter-observer variability, especially concerning severity of cellular infiltration which is a subjective assessment until now. Packer et al started with collecting data of jejunal lamina propria immune cell populations by studying full thickness jejunal biopsies taken post mortem from adult horses and provided in that way an essential

view on the normal equine jejunal plasma cell, T cell, B cell, eosinophil, neutrophil and macrophage populations [Packer 2005]. Tossens aimed to determine the diagnostic value of duodenal and rectal biopsies by comparing control horses and horses admitted for recurrent colic, weight loss or diarrhea. In that study also a post-mortem was available for 15 horses. However, again, no straight forward conclusions could be drawn [Tossens 2012 ECEIM]. Another important finding is the fact that if a rectal biopsy is taken, there is an important chance that it will be aberrant (82,2% of IBD suspected cases), in comparison to 56.8% of duodenal biopsies. However, in our study population no significant association with other routine diagnostic tests that are classically deemed as an important support for the diagnosis of IBD, such as a disturbed OGAT, signs of recurrent colic and weight loss, low blood total protein could be shown for these enteral biopsies. Rectal mucosal biopsies seems a valuable technique as histopathological examination may reveal cellular infiltration, reflecting changes at more proximal, inaccessible, locations. However, again, evaluation is not standardized and it cannot be assumed that the inflammation, if noticed, is consistent throughout the intestinal tract. Therefore, although rectal mucosal biopsies can be useful in a proportion of cases, a lack of standardisation in histopathological interpretation limits their usefulness [Kaikkonen, Lindbergh, Ricketts]

Up until now, most equine IBD studies used post mortem diagnosis of full thickness biopsies to start from [Kemper, Mair 1991]. And an important criticism on the current study could be that these were not available in most cases. However, our goal was to sketch the clinical situation with which clinicians are confronted, together with the plethora of results of diagnostic tests based upon which decisions are made. We are well aware of the fact that full thickness biopsies taken during elective laparotomy or laparoscopy probably would have provided better quality diagnostic material. However, still, also with respect to those procedures there is a lack of standardization. As we all know, IBD can manifest itself at

certain specific locations alongside the GI tract. No studies are available to support the fact that visible or tactile differences are present at the level of attained intestinal loops to guide performance of full thickness biopsy at specific locations.

There are several shortcomings in this study, such as its retrospective character, the heterogeneous group (many different types of IBD), and the absence of a control group, 4 different referral centers. Nevertheless we do believe that this study holds enough power to encourage clinicians to be careful with the interpretation of enteral biopsy results. It is important to view enteral biopsy results in the face of the results of all other applied diagnostic tests during the work up of an IBD suspected horse. Therefore, in case of a clearly aberrant histopathological biopsy assessment, one should be very careful with associating this with a presumptive negative prognosis.

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Tables and Figures

	Utrecht University	*De Morette / De Bosdreef Equine Clinic*	*Wolvega Equine Clinic*	*% of study population out of reference*
Total Protein	52-79 g/L	52-67 g/L	62-94 g/L	11.6% ↓
Albumin	26-37 g/L	25-39 g/L	23-35 g/L	8.7% ↓
Gammaglobulin	6-19 g/L	7,9-17,9 g/L	4,6 -13,4 g/L	4.5% ↑
Gamma-GT	< 20 U/L	< 22 U/L	16-56 U/L	23.0% ↑
PCV	0,32-0,42 L/L	0,30-0,46 L/L	0,32-0,52 L/L	17.9% ↓
Leucocytes	7,0-10,0 10^9/L	5,0-10,0 10^9/L	5,5-12,5 10^9/L	23.9% ↑

Table 1: Overview of reference ranges of the different laboratory analyses and % of the study population being out of range for each of the performed analyses, taking into account the applied reference range for each participating referral centre.

	OGAT >85% (normal)		OGAT <85 % (abnormal)	
	Duodenum	Rectum	Duodenum	Rectum
Normal	1	4	4	1
Granulomatous enteritis	0	0	4	0
Eosinophilic enteritis	1	9	2	13
Lymphocytic plasmacytic enteritis or proctitis for rectal	5	15	11	21
Neutrophilic inflammation	0	1	0	2

Table 2: Histological findings of the duodenum and rectum of 72 IBD suspected horses,

together with their respective OGAT results

	Duodenal biopsies	**Rectal biopsies**
Good quality diagnostic material	0 % (n=0)	0% (n=0)
Moderate quality	56.8% (n=21)	93.3% (n=42)
Poor quality	18.9% (n=7)	0% (n=0)
Insufficient quality to assess	24,3% (n=9)	6,7 % (n=3)
Total number of biopsies	100% (n=37)	100% (n=45)

Table 3: An overview of the quality assessment of the duodenal and rectal biopsies.

Therapy	Number of horses
Only prednisolone (1 mg/kg BW)	36
Only dexamethasone (0.06 mg/kg BW)	0
Only omeprazole (2-4 mg/kg BW)	7
Prednisolone in combination with dexamethasone	1
Prednisolone in combination with omeprazole	19
Dexamethasone in combination with omeprazole	1
Other	4
Total	**68**

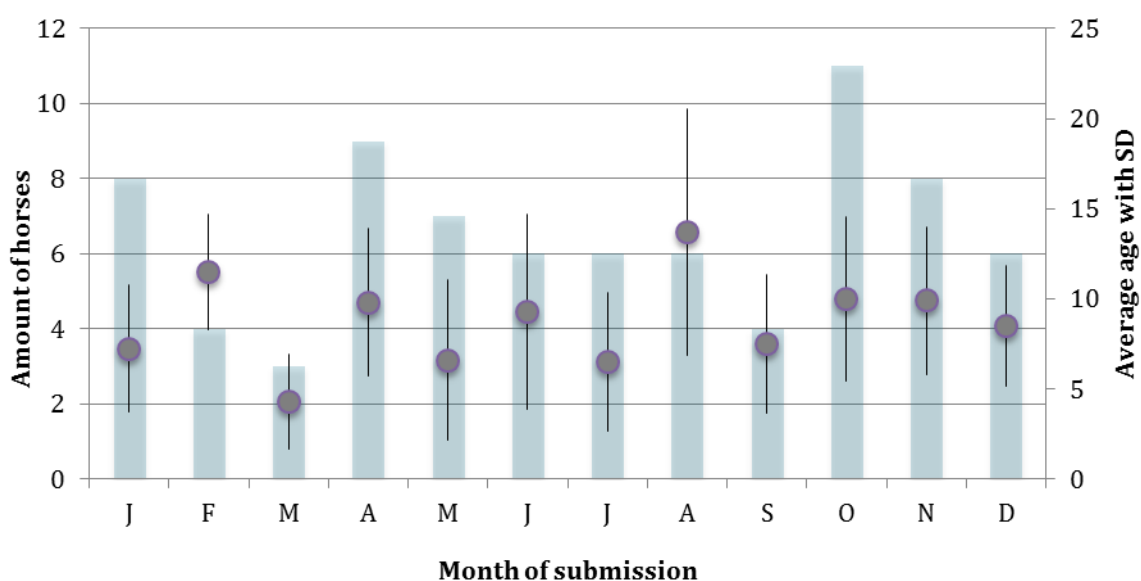*Table 4: An overview of the applied treatments.*

Figure 1: An overview of the admission pattern of cases throughout the year with the average age per month (grey dots)
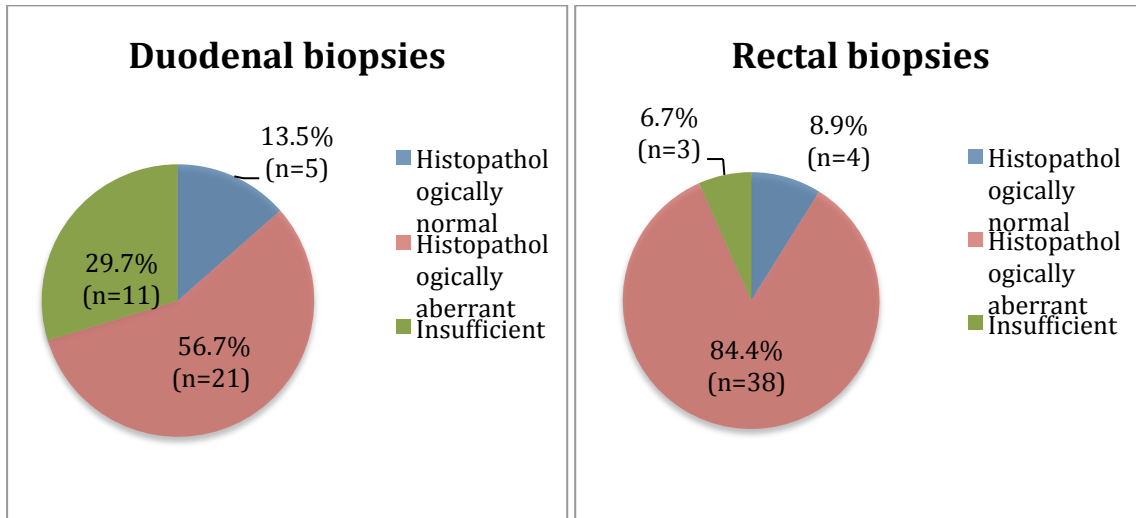
Figure 2: A schematic overview of the duodenal and rectal biopsies.