INVESTIGATING WEIGHT LOSS IN THE ABSENCE OF APPARENT CAUSE

- ***First check adequate nutrition and ingestion of ration***
- ***Protein losing enteropathies are most common (with or without diarrhoea)***
- ***Important to rule out parasites and NSAID toxicity***
- ***Other main causes include hepatopathy, chronic inflammatory/neoplastic disease, PPID and renal disease and diabetes mellitus***

Assuming the horse is eating an adequate ration, the following are important conditions to consider:

Differential diagnoses for weight loss in the absence of apparent cause

Abnormal nutrient handling - digestion/metabolism/losses:
• Protein losing enteropathy
  ○ Parasitism
  ○ NSAID toxicity
  ○ Inflammatory bowel disease (IBD)
  ○ Neoplasia (lymphoma)
  ○ *Lawsonia intracellularis* infection
  ○ *Sand enteropathy*
• Hepatic disease
• PPID
• Chronic renal failure
• Diabetes mellitus
• Renal tubular acidosis

Increased demand for nutrients: • Increased physical activity, pregnancy, lactation • Neoplasia • Infection/Sepsis • Chronic pain
Neuromuscular wasting disorders • Equine motor neuron disease • Immune-mediated myositis • Polysaccharide storage myopathy

The following tests often provide a useful initial insight into differential diagnosis and should all be considered as a first clinicopathological step in all unexplained "examination negative" weight loss cases.

1. HAEMATOLOGY

Anaemia is commonly seen in weight loss cases. If it is marked or chronic a bone marrow aspirate and biopsy should be considered to investigate the type and therefore potential cause of the anaemia (see section on bone marrow). Mild non-regenerative anaemia is a very common, non-specific finding in chronic weight loss cases and is often unhelpful in the determining the diagnosis. A regenerative anaemia is more helpful diagnostically and is suggestive of a chronic source of blood loss or immune-mediated haemolysis (see separate section on Anaemia).

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Neutrophilia can be a feature of septic and non-septic conditions such as infectious diseases (viral, bacterial or parasitic), IBD, neoplasia, immune-mediated diseases and PPID. A band neutrophilia (left shift) is more suggestive of septic than non-septic conditions.

Neutropaenia is common in acute sepsis (especially when loss into effusions occurs – e.g. peritonitis) but uncommon in chronic inflammatory diseases.

Eosinophilia is a general indicator of inflammation in addition to being an indicator of allergic or infiltrative eosinophilic diseases. Parasitism and multisystemic eosinophilic epitheliotropic disease (MEED) are potential causes of weight loss which may be associated with peripheral eosinophilia.

2. INITIAL SCREENING BIOCHEMISTRY

- Serum Proteins (albumin and globulin)
- Serum amyloid A, fibrinogen
- AST, GGT, AP, GLDH, CK, bilirubin, creatinine, urea

Serum Proteins have a circadian rhythm and may vary by as much as 10-15g/L over the day (high evening, low noon) associated with hydration status.

Low serum albumin is the first thing to check in weight loss cases given how common protein losing enteropathy is. Marked hypoalbuminaemia (<20g/L) strongly indicates protein losing enteropathy although occasionally marked protein losses associated with renal failure will be seen. Mild to moderate hypoalbuminaemia (20-29g/L) may result from hepatopathy, malnutrition, chronic blood loss, chronic inflammation or protein-losing nephropathy.

Increased globulin concentrations is common in weight loss cases and may indicate a general state of chronic inflammation associated with parasitism, infections, neoplasia or immune mediated disease. Remember that *hepatic insufficiency* commonly causes high globulin concentration so always check liver enzymes. High, normal or low globulins may occur in protein losing enteropathy cases.

Acute Phase Proteins *comprise s*erum amyloid A (SAA), and to a lesser extent fibrinogen. They are sensitive indicators of inflammation or infection. Highest levels tend to suggest bacterial infectious processes with milder increases associated with viral disease and non-septic tissue inflammation (e.g. neoplasia).

AST arises from many tissue sources but elevated plasma levels are usually of hepatic and/or muscular origin (cross-check with CK, GGT, and GLDH). It has a long half-life and can remain elevated for 1-2 weeks after resolution of the inciting cause.

GGT is the most sensitive liver enzyme and it is unusual to have significant liver disease in the absence of increased GGT. However, increased GGT concentrations are sometimes misleading and associated with mild liver disease or even non-hepatic disease (e.g. gastrointestinal disease). The pancreas contains high concentrations of GGT but pancreatic disease is rare in horses. Damaged renal tubules may also release GGT but this appears in urine rather than blood. Anecdotally, enteropathies and colics may often have raised GGT in the absence of liver disease – perhaps due to the close anatomic and vascular association between the gut and the liver. Colon displacements, particularly right dorsal displacements frequently have high circulating GGT concentrations. GGT may remain elevated for a long time after hepatic insult is resolving (possibly due to biliary hyperplasia).

INVESTIGATING WEIGHT LOSS IN THE ABSENCE OF APPARENT CAUSE

AP arises from many sources but high levels in adult horses are usually from hepatopathy or enteropathy. AP is also released from monocytes and may non-specifically reflect inflammation. Young, growing horses normally have high levels derived from bone sources. The placenta may also be a source in pregnant mares

GLDH generally indicates hepatic insult although very high levels are sometimes seen following relatively minor hepatic disease. It is very sensitive and primary intestinal insults sometimes cause increases in GLDH also (possibly from increased endotoxin reaching the liver?)

Bilirubin is usually increased in hepatic failure and also in other conditions such as haemolysis and anorexia. Very high levels (>300 µmol) often indicate biliary obstruction or haemolysis whereas increases of lesser magnitude could indicate hepatocellular disease (30-150) or anorexia (typically up to 100-150). In these equivocal cases direct bilirubin is more useful for indicating hepatopathy. Direct bilirubin should be no more than 10-20% (usually <5%) of total bilirubin, however if hepatobiliary disease and cholestasis are present then direct bilirubin may account for a greater percentage of total bilirubin. Occasionally horses are encountered with mysterious persistently high bilirubin levels and these may well have genetic bilirubin processing problems.

CK is useful to check for the site of tissue damage if AST is increased (i.e. determining muscle vs liver). Modest increases in CK are also seen in cases of polysaccharide storage myopathy or equine motor neuron disease, the latter may present as little more than weight loss. Muscle catabolism itself can also lead to mild increases in CK (e.g. 500-800 iu/L) as can increased recumbency or IM injections.

Creatinine and urea will be increased If renal disease is severe enough to be causing weight loss. Creatinine is the preferred marker for renal failure and will typically be > 250µmol/L in such cases. Mild increases in creatinine and urea may be due to dehydration (worth checking urine specific gravity). Urea may be low in hepatic failure.

3. FAECAL ANALYSIS

Parasite eggs/Larvae are often hard to find even in weight loss cases caused by parasitism as owners will invariably have dewormed a thin horse. Larval cyathostominosis is a common cause of acute (and sometimes chronic) weight loss usually but not necessarily always with diarrhoea. Overreliance on fenbendazole could lead to a significant parasite problem in horses which are reportedly 'well wormed' as it rarely has a great effect on adult or larval cyathostomes even when repeated for 5 days.

Sand ingestion may cause chronic weight loss from an abrasive enteropathy due to voluntary or involuntary sand consumption. Sand can easily be detected by sedimentation in a suspended faecal sample, but the quantity that is regarded as normal in a horse grazing sandy pasture is debatable. Colonic sand accumulation may be identified and quantified radiographically.

Faecal Occult blood generally indicates colonic bleeding rather than gastric or small intestinal - bleeding e.g. colitis, NSAID toxicity, neoplasia or merely prior rectal examination. High numbers of leucocytes in stained smears may be significant. There is a hand-held test available for faecal occult blood that it is claimed can identify the presence of gastric and colonic ulcers, although its diagnostic value is debatable.

Faecal bacterial culture is rarely helpful in chronic weight loss cases.

Clostridial toxin immunoassay (*C.difficile* Tox A/B, *C.perfringens* betatoxin) is useful to look for toxins typically in colitis cases. However, In the absence of diarrhoea, faecal samples positive for clostridial toxins have been associated with necrotic intestinal lesions including neoplasia.

INVESTIGATING WEIGHT LOSS IN THE ABSENCE OF APPARENT CAUSE

Lawsonia intracellularis PCR is worth checking especially in post-weaning foals of 3 to 13 months of age, although the disease has been seen rarely in older horses. PCR can be used to identify *Lawsonia* DNA in faeces but might occasionally be found in the absence of disease. Excretion of *Lawsonia* may also be short-lived after initiation of antimicrobial therapy and PCR may not be reliable once treatment has commenced.

4. FURTHER SIMPLE BLOOD TESTS DEPENDING ON INITIAL FINDINGS

Bile acids are a useful test of hepatic function. After excretion in bile, BAs are reabsorbed into the circulation via the ileum and should then be removed from the portal circulation by the liver for recycling. High BAs are suggestive of liver failure but can sometimes be increased to around 20µmol/L with anorexia of gastrointestinal problems without liver disease. Normal levels are slightly higher in foals.

Glucose is often not run in equine samples meaning that Diabetes mellitus (defined as a persistent hyperglycaemia) is easily missed in horses. Weight loss and polydipsia/polyuria may be the only signs and the latter are easily missed especially in turned out horses. Most, but not all cases are a result of PPID (see later).

Plasma ACTH concentration is worth checking especially in older horses. PPID is an uncommon cause of marked weight loss in isolation but may be a contributing factor. (See later chapter on the diagnosis of PPID).

Vitamin E concentration is a useful check in possible equine motor neurone disease cases where vitamin E will be almost invariably found to be low.

Acid-base balance and electrolytes are worth checking in vague weight loss cases with no specific findings. Hypercalcaemia may arise in some cases of neoplasia or chronic renal failure. Hypophosphataemia may occur in the latter cases too. An increased serum chloride along with metabolic acidosis is suggestive of renal tubular acidosis which may present as weight loss and lethargy without marked azotaemia.

***Lawsonia intracellularis* serology** may be a useful indicator of possible disease but merely indicates exposure rather than active infection. Seroconversion can occur within days of clinical signs developing and may persist for up to 6 months.

Serum protein electrophoresis is an exceedingly overinterpreted test that rarely, if ever, provides reliable evidence of cause of disease. Its only established value is in the detection of monoclonal globulin spikes caused by plasma cell myelomas which are obviously very rare. Its use in other conditions such as infection, parasitism neoplasia, liver disease lacks any evidence basis in horses.

5. OTHER WORTHWHILE TESTS/TECHNIQUES

ORAL GLUCOSE ABSORPTION TEST (OGAT)

The OGAT is a valuable test for the detection of small intestinal malabsorption syndromes and is generally performed if a protein losing enteropathy is suspected. This test has no relevance to large intestinal disease (see chapter on Intestinal Disease).

PERITONEAL FLUID ANALYSIS

Peritoneal fluid analysis may be useful in the investigation of intra-abdominal disease, particularly septic peritonitis or neoplasia. See separate section on this.

INVESTIGATING WEIGHT LOSS IN THE ABSENCE OF APPARENT CAUSE

ULTRASONOGRAPHIC EXAMINATION

Ultrasonography may provide evidence of peritoneal effusion, intestinal thickening (small and/or large bowel), pathology of other abdominal organs or the presence of neoplasms or abscesses. Small intestinal wall thickening (normal measurement 2-3mm) would be typical of problems such as Lawsonia infection, IBD and lymphoma. Large intestinal thickening is more typical of parasitism.

GASTROSCOPIC EXAMINATION

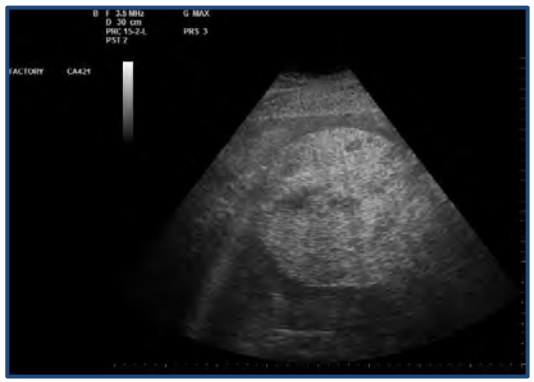
Gastroscopy may provide evidence of equine gastric ulcers or (rarely) gastric neoplasia. It also provides a means of examining and biopsying the duodenum. Gastric ulcers are an unlikely primary cause of marked weight loss.

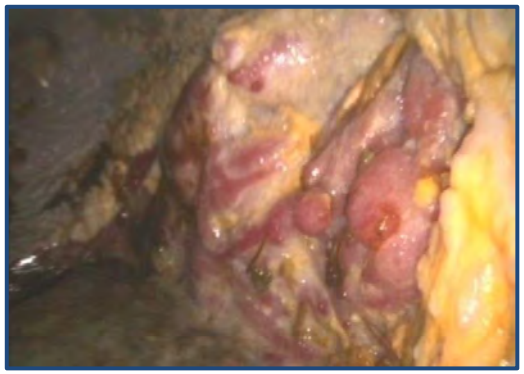
URINALYSIS

Urinalysis is worthwhile in cases of polydipsia/polyuria, or in horses with an abnormal pattern of urination (dysuria/stranguria/pollakiuria), unexplained hypoproteinaemia or cases with significant increases in serum urea and/or creatinine (see separate section on urinalysis).

TISSUE BIOPSIES

Rectal or intestinal biopsies are generally required to provide a definitive diagnosis if disease has been localised to the intestinal tract (see intestinal biopsy section).





INVESTIGATING INTESTINAL DISEASE

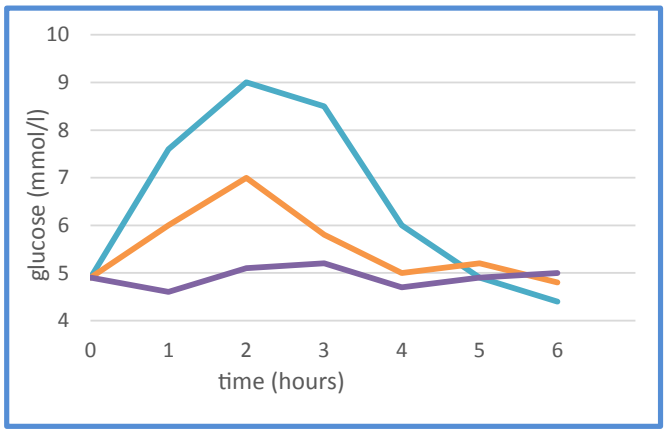
Clinicopathological examinations are an important adjunct to a thorough clinical examination, including diagnostic imagining, for the investigation of intestinal disease. Important examinations include:

- Rectal examination which may allow palpation of neoplasms, abscesses or thickened intestine.
- Ultrasonographic examination may provide evidence of peritoneal effusion, intestinal thickening, diffuse pathology of parenchymatous organs or the presence of neoplasms or abscesses.
- Gastroscopic examination may provide evidence of equine gastric ulcer syndrome or (rarely) gastric neoplasia and provides a means of examining and biopsying the duodenum.

ORAL GLUCOSE ABSORPTION TEST

The OGAT is a valuable test for the detection of small intestinal malabsorptive syndromes. This test has no relevance to large intestinal disease such as parasitism. **It is important to not confuse the sampling protocols for the OGAT and the oral glucose test for assessment of insulin resistance (see endocrine chapter).**

Performing an OGAT
• 12 hour fast prior to testing (allow water)
• Take 'baseline' oxalate-fluoride blood sample
• Give 1g/kg glucose as warm 20% solution by stomach tube. Take oxalate-fluoride bloods hourly for 5-6 hours or until there is a return to baseline • Oxalate-Fluoride blood can be taken once at 2 hours for a shortened version of the test that is still quite accurate.
• Analyse samples for glucose and calculate percentage increases above baseline



Interpretation

'Normal' response (blue) is an approximate doubling of baseline blood glucose at 2 hours post-dosing. However, severely hypoalbuminaemic (<15 g/L) cases may have depressed peaks in the absence of small intestinal pathology possibly as a result of bowel oedema. Normal horses peak between 90-150 minutes and the peak may only be 60-70% above baseline.

A **'partial malabsorption'** (15-65% rise, orange)) is often significant and merits a retest at a later date.

A **'total malabsorption'** (purple) is regarded as a no greater than 15% rise in blood glucose at 2 hours post-dosing. This is almost always a highly significant finding leading to a poor prognosis although occasional cases of total malabsorption have been known to improve.

Following a OGAT it is recommended to undertake further diagnostics which can include a duodenal biopsy and ultrasonography of the abdomen.

INVESTIGATING INTESTINAL DISEASE

PERITONEAL FLUID ANALYSIS

Performing an abdominocentesis
• **Insert a 2 inch, 19 gauge needle through the lowest point of the linea alba or just to the right of midline. (Some prefer to use a blunt-ended teat cannula and a small stab incision to perform this procedure).** • **Prior ultrasound examination maximises the chances of successful collection.**
Common reasons for failure to collect a peritoneal tap include: • **Dehydration (whereupon successful taps are usually obtained following rehydration and fluid therapy)** • **Splenic tap (ultrasonography will identify the position of the spleen and allow repositioning of the peritoneal tap site)** • **Deep retroperitoneal fat layers (which can be up to 10 cm thick and occasionally require a spinal needle to obtain a tap and this can again be predicted on the basis of abdominal ultrasonography).** • **Enterocentesis can occur and ultrasonography should be used to assess a better location.**

Interpretation

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Normal **peritoneal fluid has a total nucleated cell count of <2 x 10^9/L (with approximately two-thirds of the cells being PMNs) and a total protein concentration of <20g/L. Peritoneal fluid glucose concentration is normally slightly greater than blood glucose in the range of 4-7mmol/L.**

With **septic** peritonitis cell counts are generally >50 x10^9/L and total protein concentration >50g/L. Glucose is metabolised by bacteria and concentration decreases to <2mmol/L.

Borderline total nucleated cell counts around 5-10 x 10^9/L represent **a modified transudate** and are more difficult to interpret but infer intra-abdominal disease. Intra-abdominal neoplasia in horses is rarely identified by a peritoneal tap and exfoliated neoplastic cells. However, mild to moderate increases in total nucleated cell counts and protein concentrations are often found though they may be confused with low-grade septic peritonitis or equine grass sickness. Horses with inflammatory bowel disease and other causes of hypoalbuminaemia with peritoneal effusions may have dilute peritoneal fluid with low cell counts and protein concentrations.

INTESTINAL BIOPSY

The site for biopsy (rectal vs. duodenal) will depend on the clinical presentation and whether disease of large or small intestine is suspected to be predominant.

Rectal biopsy

This is undoubtedly the easiest and most accessible part of the gastrointestinal tract but is only likely to be pathologically affected in cases showing signs of distal intestinal tract disease (i.e. diarrhoea). In the absence of diarrhoea it is questionable whether this test is justified. It is easy and relatively safe to perform with light sedation and stocks. Although pathologic change is fairly commonly found in rectal biopsies taken from cases of chronic weight loss and diarrhoea, the changes are often highly non-specific and sometimes quite misleading. Overall there is a fairly poor correlation between rectal biopsy findings and intestinal pathology confirmed later at postmortem. Nevertheless, it is a simple and straightforward test to use and is certainly justifiable in many cases.

INVESTIGATING INTESTINAL DISEASE

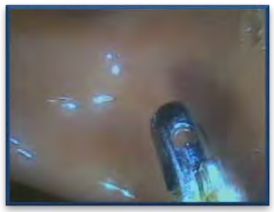
Performing a rectal biopsy
Biopsy forceps are the best and most appropriate tool with which to collect the rectal biopsy. The site to choose is in the dorsal midline. Some prefer to take a true rectal biopsy with the hand inside the rectum no more than "wrist deep", others prefer to take a colonic biopsy by performing the biopsy at full arm's length. Whilst the latter technique may give results that are more representative of the large intestine, any inadvertent penetration through the rectum is likely to result in intraperitoneal rather than retroperitoneal infection. The biopsies should be placed in 10% formalin for histopathologic analysis and/or cultured for enteropathogenic bacteria such as Salmonellae.

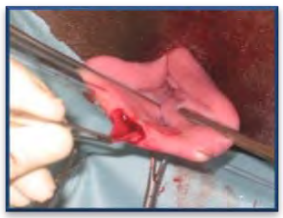
Small bowel biopsies

There are three methods for taking small intestinal biopsies:

- Duodenal pinch biopsies can be taken via **duodenoscopy** by passing a gastroscope through the stomach and pylorus. The resultant samples are superficial mucosal biopsies that are often damaged by crush artefact in the collection procedure. However, they are relatively simple to take and can sometimes be diagnostic in suspected small intestinal disease especially if grossly abnormal mucosa is identified endoscopically.
- **Laparoscopic** full thickness biopsies may be collected from the small intestine in the standing sedated horse.
- Full thickness biopsies from all levels of the intestinal tract may be collected via laparotomy under general anaesthetic. Exploratory **laporotomy** also enables thorough examination of the intestinal tract and whilst it is the most invasive technique in most cases it offers the best chance of achieving a definitive diagnosis.

Duodenoscopic biopsy





Biopsy under laparoscopic guidance

DIAGNOSIS OF PARASITISM FROM BLOOD SAMPLES??

Nematode infections in the adult horse were once typified by intra-luminal adult worms and intra-arterial larval migration associated with *Strongylus vulgaris*. These were often associated with an eosinophilia detectable in blood samples in response to intra-arterial larvae and also, in some instances, a detectable increase in γ-globulins (especially IgG(T)). *S. vulgaris* has declined and cyathostomins now account for virtually all nematode eggs detected in equine faecal samples in this country. Cyathostomin infection results in encystment of larvae locally in the caecal and colonic wall but is not associated with larval parasitic migration outside the intestinal tract. An eosinophilia is not associated with cyathostomin infections and a raised ß1-globulin fraction is a very occasional and non-specific finding.

Several research studies have failed to confirm any clinically useful relationship between serum protein electrophoresis and parasitism in horses. Normal concentrations of IgG(T) and ß1-globulins are usually found in parasitised adult horses and ponies although changes may be more likely in young horses. In an investigation of horses with chronic diarrhoea, less than half of horses with parasitic diarrhoea had raised ß1-globulins and this finding was also common in horses with non-parasitic disease. 'Cyathostominosis', the acute diarrhoea and weight loss syndrome associated with en masse larval emergence, is consistently associated with a neutrophilia, hypoalbuminaemia and hyperfibrinogenaemia (all non-specific findings). Blood samples taken from parasitised horses show no consistent abnormalities in haematology or protein analyses therefore and are only one piece of the diagnostic jigsaw.