

## 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: <ul style="list-style-type: none"> <li>• Protein losing enteropathy               <ul style="list-style-type: none"> <li>○ Parasitism</li> <li>○ NSAID toxicity</li> <li>○ Inflammatory bowel disease (IBD)</li> <li>○ Neoplasia (lymphoma)</li> <li>○ <i>Lawsonia intracellularis</i> infection</li> <li>○ Sand enteropathy</li> </ul> </li> <li>• Hepatic disease</li> <li>• PPID</li> <li>• Chronic renal failure</li> <li>• Diabetes mellitus</li> <li>• Renal tubular acidosis</li> </ul>
Increased	demand for nutrients: <ul style="list-style-type: none"> <li>• Increased physical activity, pregnancy, lactation</li> <li>• Neoplasia</li> <li>• Infection/Sepsis</li> <li>• Chronic pain</li> </ul>
Neuromuscular	wasting disorders <ul style="list-style-type: none"> <li>• Equine motor neuron disease</li> <li>• Immune-mediated myositis</li> <li>• Polysaccharide storage myopathy</li> </ul>



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.

**Neutropenia** 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 serum 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).

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**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  $\mu\text{mol}$ ) 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 > 250 $\mu\text{mol/L}$  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 LIVER DISEASE

### 1. SERUM ENZYMES

Where cases of liver disease are diagnosed it is worth considering screening liver enzymes in herd mates to establish if any others are suffering from subclinical liver disease. In establishing likely causes i.e. toxins in a common feed source, it can also be helpful to test horses that are kept on the same property but subject to different feeding and management.

#### ***Alkaline Phosphatase (AP)***

Increased serum AP concentration has the strongest association with failure to survive liver disease of any enzymes although increased serum AP concentration is neither consistently increased in liver disease nor liver specific. In addition to hepatobiliary sources, serum AP is known to be derived from bone, intestine, inflammatory cells and placenta and these possible sources should be considered in interpretation of increased serum AP concentrations.

#### ***Gamma-Glutamyl Transferase (GGT)***

Although mild to moderate increases in serum GGT (e.g. up to 100 iu/L) are of limited diagnostic or prognostic value, it is nevertheless very unusual to find significant hepatopathy in horses in the absence of increased serum GGT. Additionally, marked increases in serum GGT concentration (e.g. >400 iu/L) are associated with a poor prognosis. Modest increases in serum concentration of GGT should be interpreted with great caution as examination of liver biopsy specimens in such cases often fails to reveal significant underlying liver disease. The pancreas, or even kidneys, could potentially be the source of increased serum GGT in the absence of hepatopathy although renally-derived GGT is widely accepted to appear in urine and not serum. Increases in serum concentrations of liver-derived GGT may result from insults too minor to result in detectable histopathology. For example, horses with intestinal disease and particularly right dorsal displacement of the large colon are often reported with increased GGT perhaps as a result of direct pressure applied to the liver by the distended and heavy colon. Furthermore, cases of liver disease are not infrequently seen where increasing concentrations of serum GGT may be noted despite clinical evidence of improvement of the hepatopathy, perhaps as a consequence of reparative processes or biliary hyperplasia leading to increased serum GGT.

#### ***Aspartate Aminotransferase (AST)***

AST is derived from widespread tissue sources and has low specificity for liver disease although in the majority of liver disease cases it will be increased. Muscle is the main alternative source (same for LDH).



## INVESTIGATING LIVER DISEASE

### ***Glutamate Dehydrogenase (GLDH)***

Although serum GLDH is generally increased in liver disease, it only has moderate specificity probably due to fairly mild and innocuous hepatic insults resulting in increased serum GLDH concentrations. Its relatively short serum half-life might suggest an association between GLDH levels and the currently active degree of hepatic insult. The prognostic usefulness of GLDH is debatable and very high values may be encountered in horses that recover uneventfully.

## **2. MARKERS OF HEPATIC FUNCTION**

Serum concentrations of several biochemical substances have been reported to reflect the capability of the liver to perform its normal functions. These are primarily endogenous and exogenous substances that should either be eliminated or synthesised by the liver. They include various *amino acids, ammonia (NH<sub>3</sub>), bile acids, bilirubin (total (Tbil) and direct (dBil)), fibrinogen, globulins, glucose and urea.*

### ***Serum globulins***

Hyperglobulinaemia is a common finding in association with hepatic insufficiency, probably resulting from systemic immunostimulation by intestinal-derived antigenic material following loss of the protective barrier of K pffer cells in the liver. When increased serum globulins are found in liver disease cases, this is a strong indication that there has been a considerable liver insult and the magnitude of the increase in serum globulin concentration has prognostic relevance. Serum globulin concentrations greater than 45 g/L are concerning and values as high as 60-70 g/L are occasionally seen and warrant a guarded prognosis.

### ***Serum bile acids***

The main limitation of the usefulness of serum bile acid estimation is that liver disease must be quite severe before increased concentrations are detected and most liver disease cases will be found to have normal serum bile acid concentration at the time of initial presentation. A normal bile acid should not be taken as very reassuring as it only indicates that the liver is coping *currently*. Arguably this is the best time to explore the disease further as specific targeted treatment is likely to be more successful than when dealing with a failing liver. Anorexia and inappetance can increase serum bile acids as high as 20-30 mmol/L in the absence of liver disease. Hepatopathy cases with serum bile acid concentrations greater than 20 mmol/L are less likely to survive than those with lower values and chronic cases with bile acid concentrations above 100 mmol/L are almost invariably fatal. Occasional acute hepatopathy cases are seen with far higher bile acid values but this seems less prognostically helpful in acute cases.

### ***Serum albumin***

Although albumin is synthesised by the liver, it has a long serum half-life hence marked hypoalbuminaemia is uncommon in equine liver disease. Serum albumin concentrations below 20 g/L are very rarely encountered even in severe hepatopathies.

### ***Bilirubin***

Failure of the liver to take up, conjugate and excrete bilirubin may lead to increased serum concentrations of unconjugated and/or conjugated bilirubin. Anorexia and haemolysis are additional causes of unconjugated hyperbilirubinaemia. Horses may have serum unconjugated bilirubin concentrations greater than 200 mmol/L due to anorexia alone although more typically values of less than 150 mmol/L are expected.

## INVESTIGATING LIVER DISEASE

The magnitude of increased unconjugated bilirubin concentrations associated with acute haemolytic disease is very variable but can be greater than 500 $\mu$ mol/L. The majority of equine liver disease cases have normal or only moderate increases in serum bilirubin concentration (typically 50-150 $\mu$ mol/L) and the unconjugated fraction usually greatly exceeds the conjugated fraction. Cases of liver disease in the horse in which serum conjugated bilirubin represents greater than 25% of total bilirubin are likely to have obstruction of the biliary tract.

### *Urea and creatinine*

Low serum urea concentrations have been recognised previously in association with liver failure and have been suggested to indicate reduced hepatic synthesis of urea from ammonia. Although the majority of equine hepatopathy cases have normal serum urea concentrations, decreased serum urea is associated with more severe hepatopathies and has prognostic relevance. Creatinine is also sometimes low in hepatic disease cases for unknown reasons but perhaps due to washout associated with polydipsia.

### *Blood clotting times*

Hepatic insufficiency is associated with a decrease in the synthesis and function of the majority of procoagulant, anticoagulant and fibrinolytic proteins in addition to reduced platelet numbers and function. Despite the complexities of effects on individual proteins, the net effect of hepatic failure on haemostasis is invariably impairment of coagulation as determined by prolonged APTT and PT, although clinical evidence of coagulopathy is less commonly seen than is clinicopathological evidence of coagulopathy in horses with hepatic insufficiency. The incidence of bleeding disorders associated with performing a liver biopsy is very low and it is not considered necessary to check clotting times prior to performing the procedure.

### *Glucose*

Despite the central gluconeogenic role of the liver, plasma glucose typically remains within normal limits in adult horses with hepatopathy. Hypoglycaemia is more common in foals with hepatopathy.

### *Ammonia*

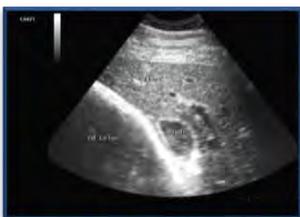
Although plasma ammonia concentration is increased in nearly all cases of hepatic encephalopathy, the concentration does not necessarily correlate with severity of the disease. This apparent paradox may be explained by increased permeability of the blood brain barrier to ammonia in cases of hepatic encephalopathy. Ammonia has to be assayed within minutes and is therefore not offered via our referral laboratory unless you can arrange immediate delivery of a chilled sample to us.

## **3. LIVER BIOPSY**

There are three fundamental aims of the investigation of cases of suspected liver disease which liver biopsy remains the best technique by which to answer.

- To confirm the definite presence of liver disease
- To determine the type of liver disease (and therefore select specific therapy).
- To determine prognosis.

## INVESTIGATING LIVER DISEASE



### Performing a liver biopsy

- The subject is sedated
- Biopsy site and depth are chosen based on ultrasound and site is prepared for a sterile procedure
- 5-10 mL local anaesthetic is infiltrated subcutaneously and through the intercostal muscles to the parietal peritoneum using a 21 gauge 1½" needle
- A small stab incision is made through the skin using a no.15 (or 11) scalpel blade
- A 14 gauge 16 to 20 cm biopsy needle is advanced perpendicularly to the skin to the predetermined depth and the biopsy is collected (angling cranially creates a larger target but makes automatic needles harder to fire)
- A total of 2-3 cm of biopsy specimen is required. The procedure is repeated if a suitable biopsy specimen is not obtained (2 or 3 attempts sometimes required)
- Biopsy specimens are placed in 10% neutral buffered formalin for histopathologic examination and/or plain sterile containers for bacteriologic culture
- Samples from at least 2 separate sites are preferable
- Topical antiseptic spray is applied to the skin incision
- A single dose of 2 mg/kg phenylbutazone IV is administered

### Interpretation

A prognostic biopsy scoring system has been developed at Liphook based on scoring 5 histopathologic features (Table 1). This results in a prognostic biopsy score between 0 (best prognosis) and 14 (worst prognosis). As a general rule horses with biopsy scores of 0-2 will survive, horses with scores above 8 generally die and those in between merit aggressive treatment.

	Absent	Mild	Moderate	Severe
Fibrosis	0	0	2	4
Irreversible cytopathology	0	1	2	2
Inflammatory infiltrate	0	0	1	2
Haemosiderin accumulation	0	0	0	2
Biliary hyperplasia	0	0	2	4

Table 1. Prognostic biopsy scoring system. Each individual parameter is scored and the total calculated.

#### FURTHER READING:

Durham, A.E. *et al.* (2003) An evaluation of diagnostic data in comparison to the results of liver biopsies in mature horses. *Equine Veterinary Journal* 35, 554-559.

Durham, A.E. *et al.* (2003) Development and application of a scoring system for prognostic evaluation of equine liver biopsies. *Equine Veterinary Journal* 35, 534-540.

Durham, A.E. *et al.* (2003) Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. *Equine Veterinary Journal* 35, 542-547.

## INVESTIGATING INTESTINAL DISEASE

Clinicopathological examinations are an important adjunct to a thorough clinical examination, including diagnostic imaging, 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.

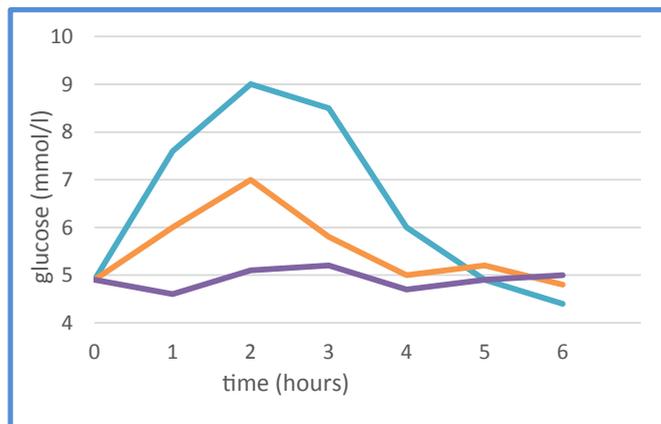


### ORAL GLUCOSE ABSORPTION TEST

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

Normal peritoneal fluid has a total nucleated cell count of  $<2 \times 10^9/L$  (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  $>50 \times 10^9/L$  and total protein concentration  $>50g/L$ . Glucose is metabolised by bacteria and concentration decreases to  $<2mmol/L$ .

Borderline total nucleated cell counts around  $5-10 \times 10^9/L$  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 **laparotomy** 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  $\gamma$ -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  $\beta$ 1-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  $\beta$ 1-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  $\beta$ 1-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.



## DIARRHOEA IN HORSES AND FOALS

Although diarrhoea is common in equines of all ages the percentage of horses with diarrhoea in which an ante mortem diagnosis is made has been variably reported to be as low as 10 to 20%. In many cases the cause may be undeterminable as the inciting factors may no longer be present by the time the horse is examined or may be related to dietary or other factors. However, infectious agents and/or their associated toxins are of major pathogenic importance in equine diarrhoeas and there are several test procedures now available to improve our diagnostic rate and help select more targeted therapy and prevention strategies.

### **NEMATODE EGGS AND LARVAE**

An adult parasite burden is greatly overestimated as a cause of weight loss (especially as owners will invariably have dewormed a thin horse!) but cyathostomiasis is a common cause of acute (and sometimes chronic) weight loss and in many cases, diarrhoea. Over-reliance on fenbendazole could lead to a significant parasite problem in horses which are reportedly 'well wormed' therefore the de-worming protocol should be critically appraised.

### **CLOSTRIDIAL TOXINS**

Testing is available for toxins of *Clostridium difficile* (toxins A&B) and also *Clostridium perfringens* enterotoxin and beta toxin. These toxins are readily detectable in faecal samples using enzyme immunoassay tests and positive results infer either Clostridial enterocolitis or areas of severely compromised or necrotic bowel with secondary Clostridial infection (e.g. neoplasia). Clostridia are a common cause of post-antimicrobial diarrhoeas but are often also seen in the absence of prior antimicrobial treatment.

### **SALMONELLA**

Salmonella can be a primary cause of diarrhea in horses although is not common in the UK. Intermittent shedding of Salmonellae may lead to false negative results and repeat samples are always advisable. Confirmation of Salmonella can be made by either PCR or culture and to ensure a horse is negative the convention is to collect 5 samples from different defaecations over a period of up to 5 days. A positive result in an otherwise healthy horse can be due to shedding of the bacteria and does not always confirm its involvement in the diarrhea.

### **BACTERIAL CULTURE**

Anaerobic culture for *Clostridia* is often uninformative as *Clostridium difficile* is very difficult to culture *in vitro*. *Clostridium perfringens* is a normal gut constituent of horses and therefore identification of this bacterium does not imply aetiologic significance hence the value of ELISA tests for toxins. Similarly, many other bacteria such as *E. coli*, *Bacteroides* and *Enterococcus* are also of highly equivocal relevance when cultured. By contrast, aerobic culture of *Aeromonas*, *Campylobacter* and *Salmonella* spp. are probably relevant to diarrhoea. The gastrointestinal tract is normally very heavily populated by an extensive and diverse bacterial population creating significant difficulty and confusion when faeces are subject to culture or PCR in suspected bacterial enteritis cases. Even unequivocal enteropathogens such as *Salmonella* spp. may sometimes be shed secondary to other primary disease processes such as cyathostomiasis.

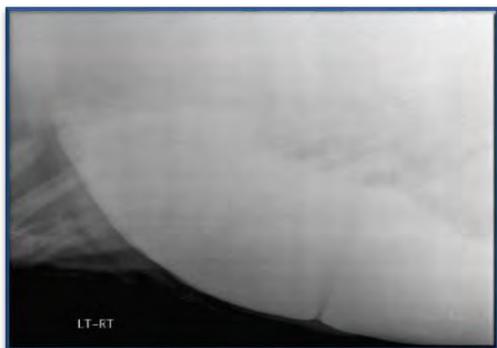
## DIARRHOEA IN HORSES AND FOALS

### **SAND**

The significance of sand in faecal samples is difficult to determine. Sand enteropathy is commonly suspected in certain parts of the country as a cause of weight loss syndromes, diarrhoea and colic. However, the presence of fairly large quantities of sand may be normal in some horses on certain pastures and the finding of sand in faecal samples only really indicates that the horse is ingesting large quantities of sand and doesn't always necessarily imply aetiologic significance. To further diagnose sand as a cause of colic radiography of the abdomen should be performed.

### **FAECAL OCCULT BLOOD**

Faecal occult blood tests can be performed in horses to investigate distal intestinal bleeding; however blood loss in the proximal intestinal tract such as stomach and duodenum is highly unlikely to be detected in faecal samples due to degradation in the colon. Claims that horse-side test kits for faecal blood and protein can differentiate gastric and colonic bleeding or even detect bleeding from any gastrointestinal source have little evidence-base and our experiences of using such kits have been very disappointing. There seems to be little, if any, relationship between test kit results and gastroscopy findings when diagnosing gastric ulcers. If a rectal examination has been performed then the test should not be run afterwards as it will always be positive.



*Sand impaction in colon*



## TAKING CONTROL OF DEWORMING – TARGETED PARASITE CONTROL

It is concerning that most equine parasite control programmes are run without consultation with a veterinary surgeon under whose care the horses are placed. Cyathostomin resistance to fenbendazole is now widespread, resistance to pyrantel is recognised and preliminary warning signs of resistance to ivermectin (and therefore moxidectin) are also now present.

Many factors contribute to anthelmintic resistance but the prime factor is overuse of anthelmintics. **Targeted dosing** involves checking the horse's faecal worm egg counts (FWECs) and only those that need deworming are dewormed leading to the preservation of refugia. **Refugia** are the parasites that remain both in the horse and in the environment which are sensitive to anthelmintics upon which a selection pressure is not placed. The fundamental aim therefore is not to eliminate parasites but to control egg output. The threshold that decides the level at which to treat is debatable but around 150-250 eggs per gram (epg) is reasonable – preventing high levels of egg-shedding whilst maintaining the refugia population. When checked prior to deworming, the vast majority of adult horses fall below this threshold and do not need treating. Recent studies indicate that over 80% of FWEC results will be below this cut-off and only 1 in 5 horses are likely to require treatment.

The targeted dosing programme can be customised to particular circumstances. On small premises with only a few (e.g. 2-6) mature horses in a static population then FWECs performed initially every 3 months and perhaps eventually every 4-6 months can be used to decide whether or not deworming is needed. On larger yards FWECs initially every 2-3 months, followed by 3-4 month intervals when a 'track record' is established, might be more appropriate.

There are 3 main limitations to targeted dosing. Firstly, tapeworms are not reliably detected by FWECs and therefore a routine annual (or possibly biannual) cestocide (double dose pyrantel or praziquantel) should be factored into the programme. Secondly, the increased susceptibility of young horses to parasitism (especially cyathostomin encystment) means that a routine interval-type programme may be more suited to these horses especially if their previous deworming history is questionable. Thirdly, *Oxyuris equi* infection may develop in the absence of regular worming. However, with anthelmintic resistance becoming more common in *Oxyuris equi* avoiding blanket use of anthelmintics that may potentiate resistance is likely to be beneficial in the long-term.

