CLINICOPATHOLOGIC ASSESSMENT OF WEIGHT LOSS CASES

– PART 1: INITIAL ‘SCREENING’ TESTS

In this chapter, initial ‘screening’ laboratory tests are discussed which might be first employed following a fruitless historical and clinical examination of the weight loss case. A further chapter follows that discusses further diagnostic tests that may be indicated after consideration and interpretation of the initial screening test results. Although clinical pathology ideally makes use of a very few selected tests, in weight loss cases with no clinical leads on which to base narrow and specific test selection one may have to cast a speculative net a little wider than is perhaps ideal. In these circumstances one must always be aware that some results will be outside the normal range even in completely normal horses and marginally abnormal results (especially if not supported by other clinical or clinicopathological data) should be repeated with possible confounding variables in mind.

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

a. Anaemia

Commonly seen in weight loss cases - if marked or chronic this should be considered in conjunction with a bone marrow aspirate to differentiate regenerative and non-regenerative types. Mild non-regenerative anaemia is a very common, non-specific finding in chronic weight loss cases and is not often very helpful in the differential diagnosis. This mild anaemia may result from chronic inflammatory conditions such as abscessation or inflammatory bowel disease (IBD), parasitism, chronic renal failure or many forms of neoplasia (especially lymphoma). A regenerative anaemia is more helpful diagnostically and is suggestive of a chronic source of blood loss (eg. parasitism, gastric scc, adenocarcinoma) or immune mediated haemolysis (eg. secondary to lymphoma).

b. Neutrophilia

Can be a feature of septic and non-septic conditions such as infectious challenge (viral, bacterial or parasitic), IBD, neoplasia, autoimmune diseases and Cushing’s disease. A band neutrophilia (left shift) is more suggestive of septic than non-septic conditions.

c. Neutropaenia

Common in acute sepsis (especially when loss into effusions occurs – eg. peritonitis) but uncommon in chronic bacterial diseases which have more relevance to this discussion.

d. Eosinophilia

The eosinophil is a general inflammatory cell but peripheral eosinophilia may suggest allergic or infiltrative eosinophilic diseases.
2. SERUM BIOCHEMISTRY

a. Blood proteins

Total serum protein has a circadian rhythm and may vary by as much as 10-15 g/L over the day (high evening, low noon) usually associated with hydration status and intestinal secretory activity.

i. Albumin

Albumin is one of the first parameters to check in weight loss cases. Marked hypoalbuminaemia (eg. <20g/L and as low as 6 g/L) strongly indicates protein losing enteropathy of various types (including IBD, lymphoma and PBZ-toxicity) although some wasting enteropathy cases can retain normal plasma albumin. Mild hypoalbuminaemia (eg. 20-25g/L) may result from hepatopathy, malnutrition, chronic blood loss (eg. gastric squamous cell carcinoma) and chronic inflammation (eg. abscesses or effusions) and very rarely protein-losing nephropathies may be seen.

ii. Globulins

Not as helpful as albumin but increased levels are common in weight loss cases. Hyperglobulinaemia generally indicates hepatopathy, parasitism or chronic inflammation although other causes are possible such as neoplasia. High, normal or low globulins may occur in protein losing enteropathy cases.

iii. Acute phase proteins (Fibrinogen and Serum amyloid A)

Sensitive indicators of inflammation/sepsis. Highest levels tend to suggest bacterial infectious processes with milder increases associated with viral disease and non-septic tissue inflammation (eg. neoplasia).

b. Serum enzymes and metabolites

i. AST (aspartate aminotransferase)

Can arise from many tissue sources but elevated plasma levels are usually of hepatic and/or muscular origin (check with CPK, GGT, and GDH). Long t½, slow to clear and can remain elevated for 1-2 weeks after resolution of the inciting cause.

ii. γGT (gamma glutamyltransferase)

A very sensitive indicator of hepatopathy but increased levels are sometimes misleading and not associated with severe liver 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. GGT may remain elevated for a long time after hepatic insult is resolving (?)due to biliary hyperplasia).

iii. AP (alkaline phosphatase)

A potentially very useful enzyme. AP arises from many sources but high levels in adult horses are usually from hepatopathy and enteropathy cases. Might also non-specifically reflect inflammation. The majority of significant hepatopathies show raised levels as do many enteropathies. NB. young, growing horses have normally high levels derived from bone sources. The “intestinal isoform” may have dubious reliability.

iv. Creatinine and urea

Likely to be raised if renal disease is severe enough to be causing weight loss. Urea not uncommonly up to 9 or 10 in normal horses and both can be raised out of normal range by wasting, dehydration (check urine SG) or high protein diets. Urea may be low in hepatic failure. Urea is much higher in morning than evening (eg as much as 2 vs 9 mmol/L).
v. Glucose

A simple screening test for Cushing’s disease – persistent fasted hyperglycaemia is highly suggestive but not that common (NB. $\alpha_2$ sedatives or hard feed increase glucose). Usually normal in enteropathies and hepatopathies.

3. Faecal Analysis

a. Parasite eggs/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 cyathostominosis is a common cause of acute (and sometimes chronic) weight loss usually but not necessarily with diarrhoea. Overreliance on fenbendazole could lead to a significant parasite problem in horses which are reportedly ‘well wormed’.

b. Sand

Chronic weight loss may result 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. Sand accumulation can be identified radiologically.

c. Occult blood

In general, positive faecal occult blood indicates colonic bleeding rather than gastric / small intestinal – eg. colitis, NSAID toxicity, neoplasia or just prior rectal examination. Also high numbers of leucocytes in stained smears may be significant.

There has been a recent development in the analysis of faeces for the presence of occult blood for the investigation of various gastrointestinal diseases (“Succeed”). It is claimed that the test can help in the differentiation of gastric or colonic lesions where a positive result for faecal haemoglobin only indicates a gastric lesion (since albumin is digested in the small intestine) and a colonic lesion will give a positive result for albumin and haemoglobin. Further work is currently underway to assess the accuracy of this test further.

d. Culture

Rarely very helpful in chronic weight loss cases.

e. Clostridial toxin immunoassay (C.difficile Tox A/B, C.perfringens enterotoxin)

In the absence of diarrhoea, faecal samples positive for clostridial toxins have been associated with necrotic intestinal lesions such as neoplasia.
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– PART 2: FURTHER DIAGNOSTIC TESTS

Further tests may be required depending on the results of the initial screening tests (see part 1). These include:

1. Further simple blood tests

Bile acids
Test of liver function with prognostic relevance. After excretion in bile, BAs are reabsorbed into the circulation in the ileum and should then be removed from the portal circulation by the liver for recycling. The test is very specific for liver failure but can sometimes be up to 10 or 20 \( \mu \text{mol/L} \) from anorexia without liver disease (also often high in foals). Liver disease values can be up to 100-150 \( \mu \text{mol/L} \) exceptionally.

Bilirubin
Total bilirubin is usually increased in hepatic failure and also in other conditions such as haemolysis and anorexia. Very high levels (eg >300 \( \mu \text{mol/L} \)) often indicate biliary obstruction or haemolysis whereas increased but lower levels 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 as direct - should be no more than 10-20% total (usually <5%) – normal in anorexia, may be high in hepatopathy.

Creatine Kinase (CK)
Used to check for tissue source if AST or LDH are increased (ie. muscle vs liver). Modest increases in CK are also seen in cases of polysaccharide storage myopathies or motor neurone disease which may present as little more than weight loss. Muscle catabolism itself can also lead to mild increases in CK (eg 400-800 iu/L) as can increased recumbency or im injections.

Glutamate dehydrogenase (GLDH)
GLDH is liver specific (hepatocellular) with a short half-life (12 hours) and therefore persistently increased levels suggest ongoing hepatic insult. It is a bit oversensitive as primary intestinal insults sometimes cause increases in GLDH (possibly from increased endotoxin reaching the liver?) and even dramatically increased concentrations do not have prognostic relevance in cases of liver disease.

Serum protein electrophoresis
An exceedingly overinterpreted test that very rarely provides reliable evidence of cause of disease. Its only true usefulness is in the detection of monoclonal globulin spikes caused by plasma cell myelomas! In all other conditions such as infection, parasitism neoplasia, hepatopathy etc... results have a very poor sensitivity and specificity. There is certainly no point in doing this test if total serum globulins are within the normal range and very little point even if globulins are mildly to moderately increased.

Acid-base balance and electrolytes
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 only as weight loss and lethargy and are often not very azotaemic.
2. Dynamic blood tests

**Oral glucose tolerance test** (see separate chapter on OGTT on p32 or call LEH for one to be faxed)

**Testing for Cushing’s disease** (see separate chapter on p1)

Cushing’s disease alone is unlikely to cause profound weight loss. Most Cushing’s disease cases will be diagnosed clinically rather than clinicopathologically as there is no test that has absolute accuracy. There are numerous options but the overnight dexamethasone suppression test and resting ACTH are perhaps the most reliable.

3. Peritoneal fluid

**Technique**

Peritoneal fluid can be easily collected in most horses using a 2 inch, 19 gauge needle through the lowest point of the linea alba or possibly just to the right of midline. Some prefer to use a blunt-ended teat canula and a small stab incision to perform this procedure. 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) and 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). This author routinely employs ultrasound examination prior to peritoneal tap to maximise the chances of a successful procedure.

**Interpretation**

Peritoneal fluid analysis from normal horses generally results in a total nucleated cell count of between 1-2 x 10^9/L with approximately two-thirds of the cells being PMNs. The total protein concentration of the peritoneal fluid is usually less than 12 g/L. Peritoneal fluid glucose concentration is normally slightly greater than blood glucose usually in the range of 4-7 mmol/L.

Septic peritonitis, a fairly common problem in equine practice, is easily diagnosed by peritoneal fluid analysis and usually shows cell counts greater than 50 x 10^9/L, total protein concentration greater than 50 g/L and a very low glucose concentration (less than 2 mmol/L). Borderline total nucleated cell counts around 5-10 x 10^9/L represent a modified transudate and are more difficult to interpret but clearly do infer intra-abdominal disease. Intra-abdominal neoplasia in horses is rarely specifically identifiable by a peritoneal tap and exfoliated neoplastic cells. However, mild to moderate increases in total nucleated cell counts and protein concentrations are often found but this is easily confused with low-grade septic peritonitis. A similar pattern is often the case in equine grass sickness. Relevantly, but fairly non-specifically, it is common to find horses with inflammatory bowel disease and other causes of hypoalbuminaemia that produce profuse quantities of peritoneal fluid that runs freely following the peritoneal tap procedure and tends to be quite dilute in terms of low cell counts and protein (a subclinical ascites).

4. Urinalysis

Worth investigating in cases of polydipsia/polyuria, dysuria/stranguria/pollakiuria, unexplained hypoproteinaemia or significant increases in serum urea and/or creatinine.

**Specific Gravity**

Simple but very useful test. Can be anywhere from 1.005 to 1.060 in normal horses but if SG >1.020 then the kidneys must have concentrating ability and chronic renal failure is therefore not present. SG 1.008-1.015 could be normal but is consistent with chronic renal failure. Very dilute urine (SG < 1.006) usually indicates psychogenic polydipsia but or rarely *diabetes insipidus*. 

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Protein
Normally negative (or possibly a trace). May find significant protein loss in urine in rare cases of chronic renal failure or plasma cell myelomas or in cases of urinary tract haemorrhage, infarction or inflammation.

γGT
Released from the proximal renal tubules into urine in higher quantities with tubular damage – very sensitive (almost too sensitive) and may be raised by subclinical renal insult. Normally similar to plasma range ie. up to 30-50 iu/L and unlikely to be significant unless >100 iu/L. Should be interpreted in light of urine SG or creatinine – ie. slight elevation in concentrated urine not significant (GGT: creatinine ratio usually used to correct for urine concentration and is usually <3 iu GGT/ mmol creatinine – eg. normal urine may have 20 iu/L GGT and 10,000 µmol/L creatinine creating a ratio of 2 iu/mmol).

Cells
Increased numbers of blood cells and/or casts is associated with urinary tract infection/infarction /inflammation/neoplasia (NB dipsticks don’t differentiate between blood, haemoglobin or myoglobin).

Glucose
Glycosuria in some cases of Cushing’s disease, acute stress or α₂ sedation (renal threshold about 9-12 mmol/L in plasma).

5. Tissue biopsy
Liver biopsy (see separate chapter on p16)

Intestinal biopsy
Various parts of the intestine may be affected by disease processes and the site of choice may well depend on the clinical presentation e.g. cases associated with weight loss and diarrhoea would justify rectal biopsy whereas weight loss and hypoalbuminaemia without diarrhoea would not.

i) Rectal biopsy
This is undoubtedly the easiest and most accessible part of the gastrointestinal tract but clearly it 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 either in stocks or perhaps with light sedation. Biopsy forceps are the best and most appropriate tool with which to collect the rectal biopsy although anecdotally beer bottle caps have been used by some practitioners with success by pinching mucosa between the cap and the thumb. 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. The biopsies may be put into formal saline for histopathologic analysis and/or cultured for enteropathogenic bacteria such as *Salmonellae*. Interpretation of rectal biopsies is often difficult. Although pathologic change is fairly commonly found in rectal biopsies taken from cases of chronic weight loss and diarrhoea, the changes are unfortunately often highly non-specific and sometimes quite misleading. Generally speaking there is a fairly poor correlation between rectal biopsy findings and intestinal pathology confirmed later at post-mortem. Nevertheless it is a simple and straightforward test to use and is certainly justifiable in many cases.
ii) **Small Intestinal Biopsy**

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. These inevitably tend to be fairly superficial mucosal biopsies and can quite often be damaged by crush artefact in the collection procedure. However, they are relatively simple to take and can sometimes be diagnostically very helpful in suspected small intestinal disease especially if grossly abnormal mucosa is seen endoscopically. Full thickness forms of intestinal biopsies require surgery. They can be relatively easily collected **laparoscopically** in the standing sedated horse or alternatively during **laparotomy** under a general anaesthetic.

ii) **Colon Biopsy**

For full thickness colon biopsies laparotomy under general anaesthetic is undoubtedly the technique of choice.

**Bone marrow aspirate and biopsy**

Peripheral blood examination in horses may well establish a diagnosis of anaemia but rarely offers any specific evidence to the practitioner on the origin of that anaemia as regards regenerative/non-regenerative sub types. Mean corpuscular volume (MCV) is always worth looking at in anaemia cases as there is a tendency for higher MCVs to be associated with regenerative anaemias and lower MCVs with non-regenerative anaemias although this is a great generalisation. Bone marrow sampling is a relatively straightforward procedure in horses and adds significantly useful information to the investigation of cases of anaemia and also horses showing other persistent abnormalities of leucogram or platelets.

**Method**

The preferred site in adult horses is the midline sternum level with the points of the elbows in a horse standing square. Following sedation and sterile preparation a 4 inch, 18 gauge spinal needle can be slowly “drilled” into the sternum and then the stylet removed and a 10 ml syringe attached containing a bead of EDTA solution in the hub. Very short and gentle vacuum is then applied to the syringe in an attempt to obtain a bead of bone marrow in the hub. Air dried smears of this are then prepared and submitted for evaluation. If a free flowing sample is obtained this is almost invariably heavily blood contaminated and unsuitable and if this is the case then a site slightly caudal or cranial to the original site is chosen to repeat the procedure. Very occasionally no sample at all is obtained despite several attempts in which case, again, another site is chosen as the needle may well be sitting in an intersternbral space rather than in an a sternebra itself. Bone marrow biopsy is performed at the same site with a Jamshidi needle which collects a small core of biopsy for a better evaluation of cell numbers within bone marrow.

**Interpretation**

Normally there are similar numbers of cells from myeloid (WBC) and erythroid (RBC) series. The reference range of the ratio of myeloid to erythroid cells is typically 0.5 to 1.5. Higher numbers of erythroid series (low M:E ratio <0.5) infers a regenerative condition whereas lower erythroid series (high M:E ratio >1.5 ) infers non-regeneration. Occasionally other notable abnormalities are found in the bone marrow such as myelophthistic conditions, leukaemias, cell phagocytosis etc...