INVESTIGATING ANAEMIA

Investigation of anaemia generally starts with determining whether anaemia is the result of haemorrhage, haemolysis or failure of erythropoiesis in bone marrow.

CLINICAL SIGNS

- **Behaviour** - Depression/lethargy/poor performance – depending on severity and acuity
- **Mucus membrane colour** - Peracute anaemias from haemorrhage or haemolysis usually show pallor. Pallor might also occur with chronic non-regenerative anaemias although this is variable. Subacute to chronic haemolytic anaemias are more likely to show jaundice.
- **Pyrexia** - Common in cases of immune mediated (e.g. idiopathic IMHA, neonatal isoerythrolysis, paraneoplastic) or infectious causes (piroplasmosis, EIA).
- **Tachycardia/tachypnoea** – as a compensatory response to poor oxygen carrying capacity
- **Pigmenturia** - Discoloured urine may be a result of intravascular haemolysis and clearance of free haemoglobin (haemoglobinuria), blood loss into urine (e.g. renal haemorrhage) or bilirubin excretion as urobilinogen.

BLOOD SMEARS

Erythrocyte size and morphology and haemoglobin concentration may give an indication of the cause of anaemia. Erythrocyte size also increases with age. If reference ranges for young adult animals are used, foals may appear to have a mild microcytosis whilst geriatrics may have a mild macrocytosis.

- **Normocytic anaemia** may be a feature of chronic disease.
- **Microcytic anaemia** may accompany iron deficiency or chronic disease
- **Macrocytic anaemia** typically accompanies haemolytic causes of anaemia or haemorrhage and is an indicator of regeneration. It is an inconsistent indicator of regeneration and, as reticulocytes are not released into the peripheral circulation in horses, assessment of bone marrow is the only reliable means of classifying anaemia sub-types.

Blood smears should also be examined for:

- Schistocytes - haemolysis, DIC, neoplasia
- Heinz bodies or eccentrocytes - oxidant toxicity
- Howell Jolly bodies – regeneration
- Parasitaemia – Babesia caballi, Theileria equi
- Haemosiderophages - immune-mediated haemolysis
- Spherocytes - immune-mediated haemolysis

SERUM BIOCHEMISTRY

- **Total serum proteins.** Blood loss may be associated with pan-hypoproteinaemia (i.e. total protein < 50 g/L). Immune mediated haemolysis is often associated with increases in globulins. Chronic inflammation, which is a frequent cause of non-regenerative anaemia, may be associated with mild hypoalbuminaemia and hyperglobulinaemia.
- **Acute phase proteins** Plasma fibrinogen and serum amyloid A (SAA) may be increased as a result of: inflammatory/neoplastic haemorrhagic lesions, immune-mediated haemolysis, infectious disease (e.g. piroplasmosis); inflammatory/neoplastic lesions causing bone marrow suppression.
- **Bilirubin** Haemolysis is typically associated with high total and indirect (unconjugated) bilirubin and normal direct (conjugated) bilirubin. Values are generally proportionate to the acuity of haemolysis and may be > 200 mmol/L in some cases. However, haemolysis is not the only cause of increased bilirubin. Anorexia in horses is associated with mild to moderate increases in total and indirect bilirubin (and normal direct bilirubin) that may occasionally be as high as 100 mmol/L. Hepatic insufficiency is a further cause of increased total and indirect bilirubin as well as increased direct bilirubin. If direct bilirubin accounts for > 25% of total bilirubin then hepatobiliary disease is likely.
Within 24 hours of haemorrhage there may be little change in haematological and serum biochemical parameters as all blood constituents are lost in equal proportions and the spleen provides a reserve supply of cells. By 24 hours, a decrease in PCV and RBC count may be observed and there is usually an accompanying panhypoproteinaemia. A neutrophilia can be seen in some horses within hours of haemorrhage. In horses with acute or sub-acute haemorrhage clinical signs may develop when PCV drops to 15-20%. In horses with more chronic haemorrhage clinical signs may not be seen until PCV drops to 12% or less.

Iron deficiency anaemia may occur with chronic haemorrhage over weeks to months (though it is very rare and far less common than iron excess as a result of over-zealous supplementation). Reduction in iron stores results in reduced erythrocyte production in bone marrow which may be evident as hypochromic microcytic anaemia on peripheral haematology. Measurement of low serum iron concentration and high total iron binding capacity may also provide evidence of iron deficiency. Iron binding capacity will be decreased if low iron levels are the result of chronic inflammation.

The site of haemorrhage may be apparent if it is external or further examinations may be required:

- Rectal examination – possible masses associated with intraperitoneal haemorrhage. NB. Any faecal samples submitted for faecal occult blood (see later) should precede rectal examination.
- Gastroscopy – bleeding gastric ulcers. Not a common cause of anaemia but is possible – especially in foals/youngsters
- Endoscopy – for direct signs of URT or LRT bleeding and/or collection of tracheal wash/bronchoalveolar lavage to look for presence of haemosiderophages.
- Ultrasonography – haemothorax or haemoperitoneum may be evident as a cloudy, swirling effusion. Abnormal masses may also be evident.
- Abdominocentesis – for the presence of increased red cell content, phagocytosed red cells or haemosiderophages.
- Urinalysis – dipsticks will show positive blood if haemoglobinuria, haematuria or myoglobinuria is present. Centrifugation and examination of sediment should enable differentiation of haemoglobinuria from haematuria if the sample is fresh. Increased urobilinogen detected by standard urine dipsticks supports a diagnosis of haemolysis.
- Faecal Occult Blood testing is neither sensitive nor specific in horses. Experimental studies have suggested that several hundred millilitres of blood given intragastrically is required to produce a positive faecal occult blood test. Furthermore, false positive results are possible after routine rectal examination.

Although serial assessment of PCV may give an indication of recovery from haemorrhage, evaluation of bone marrow provides a more reliable assessment. Following haemorrhage, PCV is expected to increase at around 0.7% per day and regeneration should be complete within a month. However, other factors that affect PCV (hydration status, excitement etc) may confound assessment based on PCV alone.
**INVESTIGATING HAEMOLYSIS**

- Intravascular haemolysis is characterised by haemoglobinuria and during regeneration by an increased MCV.
- Extravascular haemolysis is considerably more common and the only indication that it is occurring may be progressive anaemia and the presence of spherocytes. Haemoglobinuria is not present.
- Immune mediated haemolytic anaemia (IMHA) is usually insidious in onset and clinical signs may include pyrexia, lethargy and weight loss. Haematological analysis may reveal reduced RBC count, increased spherocytes, macrocytosis, anisocytosis and biochemical analysis may reveal increased bilirubin concentration. Identification of antibodies on erythrocytes with a Coombs test and demonstration of a bone marrow response provides further evidence of IMHA.
- The Direct Coombs test is used to examine for antibody (IgG and/or IgM) or complement attached to erythrocyte membranes and may be positive in cases of immune mediated anaemia. False negative results are very common.

<table>
<thead>
<tr>
<th>Testing for Autoagglutination</th>
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<tr>
<td>Autoagglutination and increased fragility of erythrocytes in saline may also provide evidence of IMHA and is worthwhile given the limitations of the Coombs test. The test is performed warm and cold. A drop of whole EDTA blood is added to a drop of normal saline on a slide and gently rocked and observed for grossly visible agglutination.</td>
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</table>

- Primary autoimmune disease is very rare and if IMHA is identified then investigations should be performed into potential underlying causes. Many infectious diseases, drugs and neoplastic diseases have the potential to trigger IMHA.
- Oxidative injury is a rare cause of haemolytic anaemia but has been reported as a result of genetic defects or ingestion of Maple leaves and onions. On a blood smear, Heinz bodies (round, blue-black granules of precipitated haemoglobin at the edge of the cell) may be seen and erythrocytes may have increased fragility when mixed with saline.
- Equine infectious anaemia (NOTIFIABLE!) should also be considered as a potential cause of haemolytic anaemia in imported horses or horses which have received imported blood products. Clinical signs may be vague and can include recurrent pyrexia, weight loss and oedema in addition to anaemia. Thrombocytopenia is the most profound haematological abnormality. The diagnosis may be confirmed by the traditional Coggins test, by ELISA or by PCR.
- Piroplasmosis is another potential cause of haemolytic anaemia in horses imported from warmer climates (Mediterranean) and is caused by infection with Babesia caballi or Theileria equi and is a real threat to UK horses (see later Chapter on Piroplasmosis).
NONREGENERATIVE ANAEMIA

- Peripheral blood examination in horses rarely offers any specific evidence as to whether anaemia is regenerative or non-regenerative and thus whether there is a bone marrow disorder. Bone marrow sampling is a relatively straightforward procedure in horses and aids significantly in the investigation of anaemia (as well as cases showing other persistent abnormalities of leucocytes or platelets).

- In normal horses 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.

- Failure of bone marrow may be caused by myelophthitic disease (destruction of normal bone marrow structure) or aplastic disease (functional failure of stem cells). Myelophthisis is most commonly caused by neoplastic disease (especially lymphoma) but may also be caused by proliferation of fibrous tissue within the bone marrow. Aplastic anaemia has been reported secondary to bacterial or viral infection, chronic renal or hepatic disease, neoplasia, irradiation, drug therapy or autoimmune disease.

- Whilst it is helpful to identify aplastic anaemia it is frequently impossible to determine the primary cause of the aplasia although a presumptive diagnosis may be made from the horse’s history.

- Aplasia associated with drug administration may be temporary or permanent. Phenylbutazone and chloramphenicol have been reported as causes of aplasia.

<table>
<thead>
<tr>
<th>Performing a Bone Marrow Biopsy</th>
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<tbody>
<tr>
<td>* Collected from the sternum on the midline at the level of the points of the elbows in a horse standing square.</td>
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<tr>
<td>* 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.</td>
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<tr>
<td>* A brief and gentle vacuum is then applied to the syringe in an attempt to obtain a bead of bone marrow in the hub.</td>
</tr>
<tr>
<td>* Air-dried smears are then prepared and submitted for evaluation.</td>
</tr>
<tr>
<td>* If a free flowing sample is obtained this is likely to be heavily blood contaminated and unsuitable (by contrast to collection for stem-cell harvesting when a free flowing sample is desirable) and if this is the case then a site slightly caudal or cranial to the original site is chosen and the procedure repeated. If no sample is obtained despite several attempts then the needle may be sitting in an intersternebral space rather than in a sternebra itself and the needle should be positioned a few centimetres in front or behind the previous site.</td>
</tr>
<tr>
<td>* Ultrasound can be utilised to determine the position of the sternebrae.</td>
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<tr>
<td>* Bone marrow biopsy is performed at the same site with an 8g Jamshidi needle which collects a small core of biopsy for a better evaluation of cell numbers within bone marrow. Biopsy provides considerably more information than aspirate alone.</td>
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# Differential Diagnoses of Anaemia in Horses

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<th>Hameorrhage</th>
<th>Haemolysis</th>
<th>Bone Marrow Suppression</th>
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<td>Guttural pouch mycosis</td>
<td><strong>Infectious</strong></td>
<td>Iron deficiency</td>
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<td>Ethmoid haematoma</td>
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<td>Chronic sepsis</td>
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<tr>
<td>EIPH</td>
<td>Equine Infectious Anaemia</td>
<td>Chronic hepatic disease</td>
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<tr>
<td>Neoplasia</td>
<td>IMHA</td>
<td>Chronic renal disease</td>
</tr>
<tr>
<td>Abscessation</td>
<td>Autoimmune</td>
<td>Neoplasia</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>C. perfringens infection</td>
<td>Myeloid neoplasia</td>
</tr>
<tr>
<td>Haemothorax</td>
<td>Streptococcal Infection</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Viral infection</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>Neoplasia (especially lymphoma)</td>
<td>Phenylbutazone toxicosis</td>
</tr>
<tr>
<td>Cystitis/Urolithiasis</td>
<td>Penicillin administration</td>
<td>Chloramphenicol toxicosis</td>
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<tr>
<td>Splenic rupture</td>
<td>Neonatal Isoerythrolysis</td>
<td>Radiotherapy</td>
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<tr>
<td>Mesenteric vessel rupture</td>
<td><strong>Oxidative Injury</strong></td>
<td>Erythropoietin administration</td>
</tr>
<tr>
<td>Verminous arteritis (S. vulgaris)</td>
<td>Phenothiazine administration</td>
<td>Idiopathic pancytopenia</td>
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<tr>
<td>Uterine artery rupture</td>
<td>Onion or Maple toxicosis</td>
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<td>Gastric/colonic ulceration</td>
<td>Familial methaemoglobinuria</td>
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<td>NDAID toxicoses</td>
<td><strong>Toxicity</strong></td>
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<tr>
<td>Granulomatous disease</td>
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<td>External parasites</td>
<td>Clostridial toxins</td>
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<td></td>
<td>Oak</td>
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<td>Burns</td>
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INVESTIGATING BLEEDING DISORDERS

Clinical signs of coagulopathies may include petechial or ecchymotic haemorrhages, unexplained persistent haemorrhage or multiple haematomas. Terminology usually describes problems with clotting factors as clotting disorders and problems with platelets as bleeding disorders.

Causes of Bleeding Disorders:

Coagulopathies (all rare!)

- **Hepatic disease.** Although the liver produces a large number of factors important in coagulation bleeding disorders are uncommon as a consequence of hepatic disease,
- **Vitamin K deficiency** may occur as a result of coumerol toxicity
- **Factor VIII deficiency (classic haemophilia)** has been identified in Thoroughbreds, Standardbreds, Quarterhorses and Arabs
- **deficiency of Von Willebrand’s factor** has been identified in Thoroughbreds and a Quarter horse
- **Prekallikrein deficiency** has been identified in Belgian horses, American Miniature horses and a Quarter horse
- **Glanzmann’s thrombasthenia** is a rare congenital disorder of platelets

Vasculitis

- **Infectious, immune-mediated, toxic and neoplastic diseases** may all lead to vasculitis and result in coagulopathy (relatively common)
- **Equine Infectious Anaemia (NOTIFIABLE!)**
- **Equine Viral Arteritis (NOTIFIABLE!)**
- **Anaplasmosis**
- **Piroplasmosis**

Thrombocytopaenia

- **Hereditary defects (rare)**
- **Bone marrow disease (see anaemia)**
- **DIC**
- **Excessive haemorrhage**
- **Thrombosis**
- **EIA**
- **Anaplasmosis**
- **Immune-mediated (primary or secondary to infection/neoplasia/drugs)**
- **Vitamin K deficiency (rare)**
Diagnostic Tests

The diagnostic tests that are available in practice are crude and comprise prothrombin time (PT) activated partial thromboplastin time (aPTT) and bleeding time. Further functional tests are used as research tools but are not available commercially. All tests should be compared contemporaneously with a control horse as reference ranges are unreliable due to the number of external factors that may affect the test. Values greater than 20% above the control are considered abnormal. Horses rarely develop bleeding diathesis and coagulopathies are more likely to manifest as excessive clotting and thrombus formation than excessive and uncontrolled haemorrhage.

- **PT** - (extrinsic pathway) is usually around 10-12 secs and depends on factors I, II, V, VII, and X and will be affected by coumarin-type anticoagulants, vitamin K deficiency, liver damage and general consumptive coagulopathies (DIC). Factor VII has the shortest half-life of all clotting factors so generalised clotting disorders may be detected by prolonged PT prior to prolonged aPTT.

- **aPTT** - (intrinsic and common pathways) is usually around 30-45 secs and depends on factors I, II, V, VII, IX, X, XI, & XII and will be affected by heparin treatment, general consumptive coagulopathies (DIC), von Willebrand disease, haemophilia and probably severe hepatic failure.

- **Bleeding time** – is a crude test of platelet function whereby a small stab incision is made in the skin and blood removed every 30 secs with tissue (without touching the skin). Bleeding should cease within 5 minutes in normal horses. Bleeding time is affected by several processes including thrombocytopaenia, thrombasthenia, DIC, vasculitis, aspirin, liver and kidney failure.

- **Platelet count** – is usually > 100 x 10^9/L but is very susceptible to artefactually low measured values. If thrombocytopaenia is identified in EDTA samples then measurement should be repeated on a citrate sample as the likelihood of artefactual platelet clumping is reduced when citrate is used as an anticoagulant. Bleeding disorders are generally associated with platelet counts < 20 x 10^9/L. Primary differentials for thrombocytopaenia include immune mediated disease, DIC and liver failure.

- **D-Dimer** is a product of fibrin breakdown and is increased in horses with colic, laminitis, jugular thrombosis and other inflammatory disorders. Increased concentrations may therefore give an indication of coagulopathy. D-dimer concentrations in peritoneal fluid may also be measured as an indicator of intra-peritoneal fibrinolytic activity in horses with intestinal disease. In plasma, concentrations above 1000 ng/ml are considered to indicate a coagulopathy and in colic cases a concentration > 4000 ng/ml is associated with reduced likelihood of survival.