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Anaemia and Bleeding Disorders Mini Series

Session 2: The Causes of Anaemia and How to Treat Them Successfully

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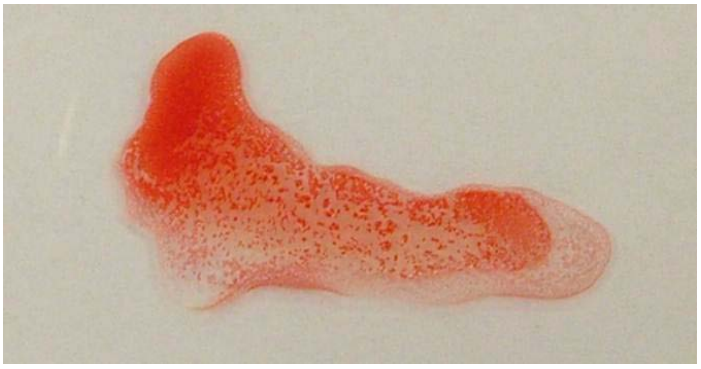

Anaemia and Bleeding Disorders

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Session 2: The causes of Anaemia and how to treat them successfully

- Review causes of regenerative anaemia; focusing on blood loss anaemia, haemolysis and immune mediated red cell destruction.
- Discussion of treatment options for immune mediated haemolytic anaemia (IMHA) including which immunosuppressive therapies should be considered.
- Review causes non regenerative anaemia, including chronic renal failure and bone marrow disease.
- Understanding how and when to use erythropoietin

Causes of Regenerative anaemia

Anaemia due to haemorrhage

Acute haemorrhage

- Trauma/surgery
- Bleeding gastrointestinal ulcers or tumours
- Internal haemorrhage - rupture of a vascular tumour e.g. splenic HSA
- Coagulopathy e.g. warfarin toxicity

Immediately following acute haemorrhage the red cell parameters including PCV are near normal because both red cells and plasma are lost in proportion. Compensatory mechanisms such as splenic contraction may further offset any fall in PCV. PCV falls only when blood volume is replaced by interstitial fluid and so does not indicate the full magnitude of blood loss for at least 24 hours after the onset of haemorrhage. When haemorrhage is Internal, there is an initial fall in plasma proteins, however these then rapidly increase due to resorption.

The response to acute blood loss is often accompanied by a neutrophilia and left shift; especially following haemorrhage into a body cavity. The presence of immature red cells and granulocytic precursors in the circulation is known as a leucoerythroblastic response. Mild thrombocytopenia may follow acute haemorrhage, reflecting increased consumption; this is followed by a rebound thrombocytosis often with large “shift” platelets or macrothrombocytes.

Following external blood loss the PCV rises quite rapidly and is usually low-normal within two weeks of a single haemorrhagic episode. PCV rises more quickly following intra-cavitatory haemorrhage (assuming the haemorrhage has stopped), since some of the red cells may re-enter the circulation via the lymphatics.

Iron deficiency anaemia – Chronic Haemorrhage

Chronic external blood loss will lead to iron deficiency anaemia. This is usually regenerative in the first instance but regeneration reduces over time and morphological changes occur. Due to iron deficiency there is reduced haemoglobin synthesis and as a result nuclear degeneration is delayed, this leads to the cell undergoing a further cell division and the presence of a macrocytic, hypochromic anaemia. Persistent thrombocytosis is often a feature of chronic haemorrhage and may be very marked i.e. $>1000x10^9$/l. It can be difficult to distinguish iron deficiency anaemia from anaemia of chronic disease, endocrine and inflammatory disease; in these cases evaluation of iron stores is helpful.

Serum iron is low in iron deficiency but also low in acute inflammation, chronic disease, hypoproteinaemia, portosystemic shunts and hypothyroidism. Thus other tests have been developed to evaluate iron stores. Most iron is found in red cells in haemoglobin. Non-haeme iron is stored primarily as haemosiderin and ferritin in cells of the mononuclear phagocytic system in various tissues including liver, spleen and marrow. Haemosiderin is a large protein composed of ferritin aggregates which is readily identifiable on Prussian blue (Pearls)-stained bone marrow aspirates as blue clumps and on routine Romanowsky stains as blue-black clumps (less obvious than on Pearls stain). Bone marrow iron is absent in iron deficiency anaemia. This is not useful in cats as normal cat marrow may have no stainable iron. Most ferritin is found within cells but a small amount is present in the serum and serum ferritin concentrations correlate with total body iron stores. Ferritin is an acute phase reactant and so serum levels increase in acute inflammation.

Transferrin is the protein that transports iron in the plasma from GI absorption or from red cell breakdown and delivers it ready for haemoglobin synthesis. The TIBC is an indirect measurement of serum transferrin and is determined by saturating an aliquot of serum with iron, removing the unbound iron and processing the sample for routine iron determination. The higher the circulating transferrin level the larger the amount of iron which binds, hence a higher TIBC; conversely the lower the transferrin concentration the lower the TIBC. The percentage iron saturation of transferrin is calculated:

$$\textbf{Percentage iron saturation of transferrin} = \frac{\textbf{Serum Iron}}{\textbf{TIBC}}$$

The normal percentage saturation is approximately 33%, meaning around one third of potential iron sites are filled. Less than 15% saturation is consistent with iron deficiency.

Parameter	Chronic Haemorrhage	Anaemia of Chronic Disease
Serum iron	Low	Low
TIBC	Increased (Dogs no sig change)	Low/normal
% transferrin saturation	Low	Low/normal
Ferritin	Low	Normal/high

Anaemia Due to Red Cell Destruction

Immune Mediated Haemolytic Anaemia (IMHA)

Immune-mediated haemolytic anaemia (IMHA) is one of the most common causes of anaemia in general practice and is the most common immune-mediated disease. Causes are divided in to primary or idiopathic disease or secondary IMHA, which is triggered by an underlying disease process for example an infection or neoplasia. Primary disease is the most common form in and typically affects young-middle-aged dogs, with Cocker and Springer Spaniels, Poodles and Collies being over represented. It is important to realise it can affect animals of any age, breed or sex. Secondary IMHA does not respond well to treatment unless the primary cause is treated, thus it is important to be meticulous in ruling out underlying causes of disease before diagnosing primary IMHA. This is especially important now that endogenous babesiosis has been documented in the UK.

Red blood cell destruction results from the immune response marshalled by immunoglobulins and/or complement. The immune response in primary IMHA is directed against erythrocyte surface antigens and can result in either intra-vascular or extra-vascular haemolysis. Intra-vascular haemolysis occurs when there are high levels of antibody and/or complement fixation, and leads to acute, severe anaemia often with haemoglobinaemia and haemoglobinuria. Extra-vascular haemolysis occurs more commonly and happens when when antibody attachment and cell membrane damage lead to more rapid clearance of cells by tissue macrophages, primarily in the spleen and liver.

IMHA usually results in a regenerative anaemia. However, non-regenerative anaemia can occur for a variety of reasons. Early in the course of acute disease, there may not yet have been time for a regenerative response to occur (pre-regenerative response and can take up to 5-7 days). Additionally, in some cases antibodies are directed against red cell precursors within the bone marrow which results in non-regenerative immune mediated haemolytic anaemia (NRIMHA) or pure red cell aplasia (PRCA) (see later).

Clinical signs seen include lethargy, anorexia, pale mucous membranes, tachycardia, tachypnoea, icterus, pyrexia, systolic murmur, hepatomegaly, splenomegaly and mild lymphadenopathy. Additionally, it is important to realise that patients with IMHA are likely to be in a hypercoagulable state and that DIC and thromboembolic disease, especially pulmonary thromboembolism (PTE), are common.

There is no single test that allows absolute diagnosis of IMHA and generally diagnosed if there is regenerative anaemia in association with autoagglutination, spherocytosis, or a positive Coombs test. Full haematological analysis should be performed in cases with suspected IMHA. Classically the regenerative anaemia, is associated with reticulocytosis, spherocytosis, anisocytosis and polychromasia. Neutrophilia, which can be very marked, is often seen due to a leukoerythroblastic response. Thrombocytopenia may occur if there is concurrent immune-mediated platelet destruction (Evan's syndrome – IMHA & ITP) or if other causes of thrombocytopenia (e.g. DIC) are present. Biochemistry often shows elevated bilirubin, ALT and ALKp, which are most likely associated with hepatic hypoxia. Although severe haemolysis can overwhelm the liver's ability to metabolise bilirubin, in most cases it is more likely that hepatic hypoxia impairs the ability of the liver to cope with the increased demand.

A positive in-saline agglutination test suggests in-vivo agglutination. One drop of saline (2 in cats) is added to one drop of EDTA blood and the slide gently rotated back and forth for 2 minutes. The slide is observed for agglutination both grossly and microscopically. A positive Coombs test is seen in approximately 60-70% of cases of IMHA. Importantly, a negative Coombs test does not rule out IMHA. False negatives can occur if there is insufficient antibody or complement on RBCs, inappropriate antibody to antigen ratio, prolonged glucocorticoid treatment, or technical difficulties. False positives can occur with non-specific antibody coating RBCs, prior blood transfusions, or in vitro complement binding. Coombs test which give information regarding IgG, IgM and Complement titres at warm and cold temperature yield more information than tests which report only polyvalent antibody titres.

Treatment hinges around immunosuppression and supportive care.

- Blood transfusions may be required in severe cases. Ideally packed red blood cells should be administered but in general practice whole blood maybe more practical and accessible. Whether or not to transfuse is based on the clinical status of the patient (tachycardia, tachypnoea) and assessment of hypoxia. Oxyglobin (when available) may be considered if blood is not available.

- Glucocorticoids are the basis of immunosuppression in most cases. Prednisolone 2mg/kg PO SID (or divided daily dose) initially until normalisation of PCV, then this dose is gradually reduced by 25% every 3-4 weeks over a 4-6 month period. Every other day therapy is suggested as soon as possible. Dexamethasone 0.5mg/kg I/V SID can be administered to dogs where vomiting prevents oral therapy, or if prednisolone appears to be ineffective.

- Gastroprotectants (cimetidine, ranitidine or famotidine with or without sucralfate) are usually administered in an attempt to reduce the risk of gastrointestinal ulceration in patients which are hypoxic and being treated with corticosteroids.

- Heparin is being used increasingly in patients with IMHA, as a large proportion of the mortalities with the disease are due to thromboembolism, particularly PTE. Unfractionated heparin at a dose of 100IU/kg SC QID can be administered but requires monitoring of PT and APTT, with an increased risk of bleeding. Many

clinicians now use low molecular weight heparin (LMWH) such as dalteparin at a dose of 150IU/kg S/C BID which does not require monitoring of clotting times and does not appear to increase the risk of bleeding. However there is still little published evidence as to efficacy. Different forms of LMWH may require different dosing schedules.

- Ultralow-dose aspirin – there is a suggestion in a retrospective study that very low doses of aspirin (0.5mg/kg PO SID) in combination with prednisolone/azathioprine/+/- mixed molecular weight heparin may offer a survival advantage

Additional immunosuppressive drugs which can be considered at the start of treatment or if corticosteroids are ineffective after 7-14 days include:

- Azathioprine 2mg/kg SID initially moving to EOD therapy once the PCV normalises. It can allow the prednisolone dose to be reduced more rapidly, which is particularly useful in dogs suffering side-effects of corticosteroids. Generally azathioprine is used at the 2mg/kg dose until the prednisolone is withdrawn and then the azathioprine is gradually tapered too. Possible side-effects include myelosuppression and pancreatitis, but most dogs tolerate it well. Tablets should not be split or crushed and gloves should be warn to administer the medication. A re-compounding pharmacy (such as Nova laboratories) can reformat tablet sizes as required.

- Cyclophosphamide studies suggested that cyclophosphamide with prednisolone had no advantage over prednisolone alone in dogs with acute IMHA and is generally not suggested in dogs with IMHA. Side effects include myelosuppression, gastrointestinal signs, and haemorrhagic cystitis.

- Cyclosporine 5-10mg/kg/day PO (divided dosing). Cyclosporine is not myelosuppressive, and has the advantage of a licensed preparation being available for dogs. However it is expensive, especially if appropriate therapeutic drug monitoring is performed. Gastrointestinal upset in the first few days of administration is possible. It can take 7-10 days to have its full effect and is generally used when other medications are ineffective.

- Leflunomide 4mg/kg PO SID has been reported in an abstract to be effective in treatment of immune-mediated and inflammatory disease in dogs when other treatments are ineffective.

- Mycophenolate mofetil (12-17mg/kg SID PO) is a lymphocyte-specific immunosuppressive drug which has been reported as treatment for IMHA in conjunction with prednisolone.

- Human Intravenous Immunoglobulin (IVIG) 0.5-1.5g/kg given over 6-12 hours as an infusion. It works by saturating the macrophage antibody receptors and has been demonstrated to improve short-term survival (which may be of use in patients with concurrent ITP), but with no increase in long-term survival. It is VERY expensive, with limited availability.

Mortality rates with IMHA vary between 20-80%, with most referral centres reporting approximately 20-30% mortality. This may not reflect the situation in general practice. Various studies have highlighted some prognostic indicators including:

- Pulmonary thromboembolism is a negative prognostic indicator
- Severity of anaemia and effect on prognosis is controversial
- Moderate to severe leukocytosis is a poor prognostic indicator
- Hyperbilirubinaemia is usually considered a poor prognostic indicator
- For each 1s increase in PT there is a 30% increased risk of dying
- The presence of autoagglutination has been reported as a negative prognostic sign
- Hypoalbuminaemia may worsen prognosis

Management of Immune Mediated Haemolytic Anaemia (IMHA)
1. Place large bore i/v catheter and collect for minimum database, full haematology, biochemistry, Coombs test and Babesia/rickettsial PCR as needed. Perform in saline agglutination test (mix 1 drop of saline and I drop of EDTA blood on a glass slide, mix and look for agglutination – check microscopically that it is agglutination & not rouleaux formation). Diagnosis made on the basis of 1 or more of, agglutination, positive Coombs & spherocytes. 2. Increase oxygen content of the blood. Supply supplemental oxygen. Consider transfusing whole blood, packed red cells or Oxyglobin to increase oxygen carrying capacity. The decision when to transfuse is made on an individual patient basis, based on HR, RR, demeanour, speed of PCV drop, etc. Volume resuscitate, as appropriate. Care not to mix calcium contain fluids (e.g. Hartmann's) with CPDA containing blood products. Continue IVFT to support renal function, due to haemoglobinuria and bilirubinuria. 3. Give steroids to reduce red cell destruction. Dexamethasone i/v 0.5mg/kg SID then switched to prednisolone (1mg/kg/BID p/o) as appropriate. Steroids are normally tapered once anaemia, spherocytosis and agglutination, reducing them gradually over approximately 4-6 months (25% reduction each month, moving to EOD treatment at about ½ way through treatment – 2nd line immunosuppressive drugs help reduce dose more quickly). 4. Consider antibiotics if tick borne disease (e.g. Babesia) or over sea travel is a possibility. Submit blood for PCR and cover with doxycycline pending results (10mg/kg/SID p/o). 5. Consider a second line immunosuppressive. Azathioprine (2mg/kg/SID moving to every other day treatment once after 10-14 days – cheap but care with handling and long term myelosuppression) and cyclosporine (5mg/kg/SID – possibly more potent compared to azathioprine but more expensive) are good choices, these will help reduce the steroid dose and hence side effects in the longer term. Both are reported to take between 7-10 days for full immunosuppressive action, thus there will be a delay in onset of their immunosuppressive action. 6. Consider Gastro-protection. Reduces risk of steroid associated GI haemorrhage, especially given GIT hypoxia related to anaemia. Sucralfate (0.5-1g/QID p/o) and cimetidine (5-10mg/kg/TID SLOW i/v) or ranitidine (2mg/kg/BID SLOW i/v). 7. Consider anticoagulant therapy. Dogs with IMHA die of thromboembolic disease, hence treat with very low dose aspirin (0.5mg/kg BID – use dispersible aspirin and dilute in 10ml of water) or low molecular weight heparin e.g. Fragmin (100IU/Kg BID) to reduce risks. 8. Consider an i/v human IgG infusion (0,5mg/kg over 4 hours). Human IgG is expensive but will act quickly to reduce red cell destruction. Transfusion reactions are possible; largely because of the small percentage of human albumin contained in the product (premedication with chlorphenamine [4-8mg i/m as a one off] is suggested). Polyclonal human antibodies block macrophage Fc receptors reducing platelet destruction. They also dilute out anti-platelet antibodies and have long term feedback reducing antibody production. 9. Look for underlying trigger e.g. secondary disease, zinc foreign bodies etc.

Intrinsic Haemolytic Anaemia

Inherent metabolic defects (most frequently red cell enzyme deficiencies) have been reported in certain breeds. These defects lead to membrane damage and therefore accelerated extravascular haemolysis and consequential anaemia. The University of Pennsylvania (http://research.vet.upenn.edu/penngen) offers PCR analysis for both PK and PFK deficiency.

Red cell pyruvate kinase deficiency (PK) occurs most commonly in Basenjis but is also reported in West Highland white terrier, Cairn terrier, Miniature poodle, Abyssinian, Somali and DSH cats. This PK deficiency leads to a mild to moderate regenerative anaemia, which can progress to myelofibrosis. Due to persistent haemolysis bilirubin choleoliths have been reported in Somali cats.

Red cell phosphofructokinase deficiency (PFK) occurs in English Springer spaniels and Cocker spaniels. It leads to mild to moderate anaemia, as the red cells show a marked osmotic and alkaline fragility. As a result there is an increased rate of haemolysis, which is often associated with exercise or excitement.

Causes of Canine and Feline Haemolysis	
Mechanical damage	Haemangiosarcoma Vasculitis Disseminated Intravascular Coagulation Heart Valve Disease
Toxins	Onion Garlic Acetaminophen (Paracetamol) Zinc Naphthalene
Immune meditated destruction	Primary Immune Mediated Haemolysis Secondary Immune Mediated Haemolysis (e.g. Drugs, Neoplasia, Infection) Neonatal Isoerythrolysis Transfusion Reactions
Inherited disease	Osmotic Fragility Syndrome Phosphofructokinase Deficiency Pyruvate Kinase Deficiency
Infectious disease	Mycoplasma Babesia FIV / FeLV Leptospirosis
Miscellaneous	Hypophosphataemia Haemolytic Uraemic Syndrome

Feline Infectious Anaemia

Feline infectious anemia is caused by intracellular bacterial parasites. Three forms are recognised M. haemofelis (MHf), candidatus mycoplasma heamominutum (cMh) and candidatus mycoplasma turicensis (cMt). There pathogenicity varies, with MHf being able to cause severe illness, with both cMh and cMt having minimal pathogenicity unless other diseases (e.g. FIV, FeLV or renal disease) are present. There mode of transmission is unknown, however fleas and fighting are strongly suspected. The parasite attaches to red cell membrane which causes distortion and sequestration in the spleen. If the membrane damage is severe then there is intravascular haemolysis. Bystander antibody production directed against the red cell membrane can lead to immune-mediated haemolysis. The parasites first appear in the blood 7-8 days after infection and are followed by random cycles of parasitism. These are cleared from the blood quickly, which leads to rapid fluctuation in both numbers of parasites and PCV. Once animals recover they often become chronic carriers, even if treated with antibiotics.

Diagnosis can be made on the basis of finding the small coccoid organisms on the edges of red cells, however this is difficult and it can be hard to differentiate them from stain deposits and Heinz bodies; this is especially difficult when low levels of parasites are present. A specific PCR is available to at the University of Bristol which can detect very low levels of parasites and (http://www.langfordvets.co.uk/diagnostic_laboratories.htm) differentiate between the species present.

Treatment with doxycycline (10mg/kg PO SID) for a minimum of 28 days is usually recommended. Care should be taken to always follow doxycycline with food or water, to prevent delayed oesophageal transit and stricture formation. Enrofloxacin (Baytril 10 mg/kg PO SID) has some efficacy but does not always clear the infection. Immunosuppressive therapy may be indicated if immune-mediated destruction of altered red cells is a playing a large part in the anaemia. Prednisolone is usually recommended for this use, commencing at 2 mg/kg SID and tapering gradually over 2-3 weeks once a response is seen. Blood transfusions may be required in severe anaemia

Neonatal isoerythrolysis
Cats have 3 blood groups A, B and AB (see notes on transfusion medicine below for more detail). All type B cats have high titres of naturally occurring anti-A isoantibodies without prior exposure. Neonatal isoerthrolysis occurs if a type A tom mated with type B queen and has type A kittens. These will be born healthy, but when they feed from the type B queen they will receive anti- A antibodies in colostrum leading to haemolysis. This can be very severe and lead to severe anaemia and death. A subacute form with mild anaemia is also seen and these kittens, may recover. In-house blood typing kits (Rapid vet-H Lab Pack) used prior to mating help pre-empt the problem. If not done use on neonates to prevent problem; if B queen and A kittens, remove queen and feed milk replacer for first 48 hours of life, after which intestinal absorption of antibodies ceases.

Babesiosis
Babesiosis is a tick borne parasitic disease which leads to infection of erythrocytes and can result in severe and life threatening anaemia in dogs. It is particularly prevalent in France due to *Babesia canis*, with increasing incidence in the south (particularly south of the Loire valley), however tick vectors are widespread and the disease in endemic in most of Southern Europe. In early 2016, several cases of *Babesia canis* infection in dogs from the Essex area were reported in both the media and veterinary press that had not travelled outside the United Kingdom. These cases, combined with a fatal case of *Babesia vogeli* infection in an untraveled dog from Kent and unpublished reports of *Babesia* in untraveled dogs identified by the Acarus Laboratory in Bristol, suggest that canine babesiosis is becoming established within specific areas of the United Kingdom.

Babesia canis (previously called *B canis canis*) is transmitted by *Dermacentor reticulates* (the ornate cow or marsh tick) and to a lesser degree by *Rhipicephalus sanguineus* (the Brown dog tick). *Dermacentor reticulatus* has historically been found in the southern parts of Europe, with 45-70% of French practices reporting confirmed infection each year. Several studies have also documented pockets of *Dermacentor reticulatus* within the United Kingdom, mainly in west Wales, parts of Essex and coastal areas of both North and South Devon, although until recently they were not thought to harbour *Babesia* species. The geographic incidence of *Babesia canis* infection, largely mirrors the distribution of its tick vector, with outbreaks of babesiosis seen in polish sled dogs and the recent cases in the UK and Norway reflecting a northerly increase in its geographic range.

Babesiosis is transmitted as a result of high numbers of *Babesia* sporozoites being passed in the host circulation within tick saliva during feeding. This happens relatively late after attachment with the tick needing to be attached for 48-72 hours for transmission to occur. These infectious sporozoites infect erythrocytes, allowing differentiation in to merozoites allowing reproduction and production of more infectious merozoites.. These either infect further erythrocytes or the life cycle is completed by infecting ticks during feeding. After feeding the organisms appear in the tick gut about 10 hours later and differentiate into gametes, which penetrate the tick gut and undergo sexual reproduction, fusing to form zygotes. Zygotes then penetrate the tick salivary gland allowing infection of host on feeding and the ovary transforming to infectious merozoites within eggs produced by the tick. As female ticks can produce many eggs over her life time (for example a fully fed *Rhipicephalus sanguineus* can lay over 5000 eggs in her life time), one infected tick may quickly become and infected population.

Babesia infection results in an array of clinical signs which vary between the infecting strain and species, however host factors such as age, and the immunogenic response of generated again the parasite and/or its tick vector play a role. Most signs result from anaemia or the systemic inflammatory response this generates, leading to tissue dysfunction, resulting in eventual multiple organ failure.

Antigens from the parasite become incorporated into the erythrocyte surface and soluble antigens can also adhere to platelets and erythrocytes which are not infected with parasites, host antibodies focus on these antigens, leading to the removal of infected erythrocytes by the splenic mononuclear-phagocytic system. In addition antibodies again normal erythrocyte

and platelet targets can also be produced leading to the immune mediated haemolytic anaemia and/or thrombocytopenia, which doesn't correlate to the number of parasites present.

Presenting clinical complaints are very variable and may be compounded by concurrent disease also transmitted by the tick vector, for example *Ehrlichia canis*. Acute illness, with pyrexia, pallor and splenomegaly are frequently noted with *Babesia canis* infection, with progression of signs to collapse in severe cases. Haemoglobinuria and bilirubinuria can also be reported secondary to excessive red cell breakdown. The systemic inflammatory response caused can leads organ dysfunction and a more severe syndrome often referred to as 'complicated babesiosis', which includes acute kidney injury, hepatic dysfunction, acute lung injury, cerebral dysfunction and coagulation defects secondary to disseminated intravascular coagulation and immune mediated thrombocytopenia; these signs are also frequently associated with *Babesia rossi* infection.

Diagnosis of canine *Babesia* is most convincingly made by demonstrating the presence of organisms within infected erythrocytes, with *Babesia canis* usually forming pairs of pyriform organisms. The level of parasitism is often low, especially in chronic cases, which can make detection difficult. Collecting blood from peripheral capillary beds with lower flow rates (e.g. the ear tip or nail bed) can yield a higher number of infected cells. Yield can also be improved by examining cells from spun microhaematocrit tubes, taken from just below the buffy coat, as the parasite is present in higher numbers in immature red cells. PCR on EDTA anticoagulated blood is the most sensitive and specific way of diagnosing infection, and also allows determination of the species present. Serology is possible and can support exposure; however PCR and direct identification of the parasite are preferred.

General haematology results will typically reveal a normocytic normochromic anaemia which becomes regenerative a few days after infection. Autoagglutination is seen in around 20% of cases with 85% reported to have positive Coombs test results. Thrombocytopenia is usually not particularly severe and overt signs of bleeding are uncommon. Concurrent infection with *Ehrlichia canis* may also worsen the risk of bleeding due to platelet dysfunction. A leucocytosis with neutrophilia and left shift is usually present, although a moderate neutropenia has been reported in *Babesia canis* cases. Biochemistry results are not specific and include elevated bilirubin, globulins and liver enzymes, with hypoglycaemia, azotaemia and marked acid-base imbalances associated with in *Babesia rossi* infection.

Treatment for babesiosis is based on providing symptomatic supportive care and parasite clearance. Supportive care usually consists of cautious intravenous fluid therapy with balanced crystalloid solutions to correct fluid balance, support renal function and improve acid-base imbalances. Symptomatic treatment of specific clinical signs such as vomiting, may require specific antiemetic therapy. In cases of severe anaemia blood transfusions can be required. The decision to perform a transfusion is made on an individual patient basis, with it being strongly considered in any patient with a haematocrit less than 0.15l/l or showing clinical signs associate with poor oxygen carriage and delivery (tachyponea, tachycardia, systemic weakness, etc). Steroid use is controversial and usually avoided unless signs of severe agglutination or immune mediate thrombocytopenia are present and in these circumstances therapy can often be tapered rapidly over 10-14 days. The monocyte-macrophage system is important in removing the *Babesia* parasites and usually does so quickly after definitive treatments have been given; inhibiting its action through immunosuppression can result in a more severe parasitaemia post administration.

In general, imidocarb dipropionate is suggested as the most effective drug for clearance of *Babesia canis*. Imidocarb is used off label, under the cascade, as there is no licensed product for the treatment of canine babesiosis available in the UK, but it is readily available in injectable format as a treatment for *Babesia divergens* which causes redwater fever in cattle. If imidocarb is not immediately available high dose clindamycin is suggested until definitive treatment can be given (25 mg/kg q12h po). Various dose regimes have been reported but two treatments (5mg/kg) given intramuscularly 14 days apart is most commonly recommended. In general improvement is normally seen within 24 to 72 hours of treatment, but some dogs can respond more slowly taking up to 7 days to respond.

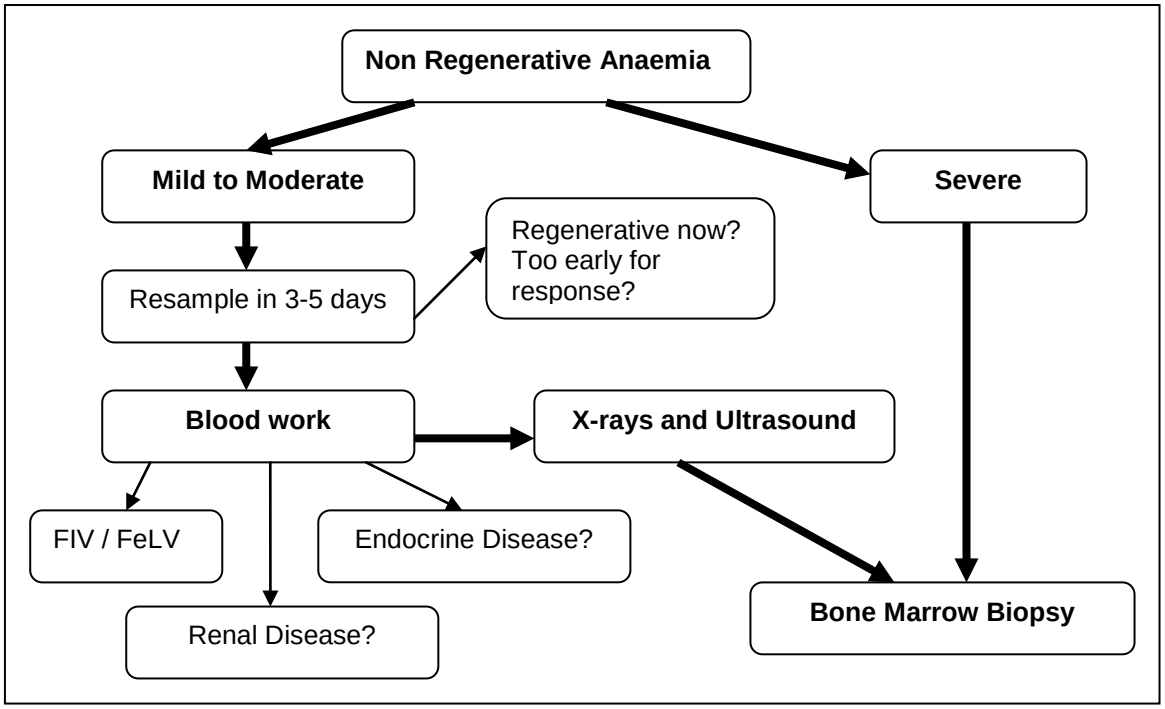
Imidocarb is an aromatic diamidine derivative and interferes with parasite DNA metabolism and aerobic glycolysis. It also has anticholinesterase activity which leads to commonly seen side effects relating to stimulation of muscarinic cholinergic receptors. These include excessive salivation and or lacrimation, nausea and vomiting, tachycardia, diarrhoea and unsettled or agitated behaviour. Premedication with atropine (0.02-0.04mg/kg i/m) is suggested to prevent these signs occurring; an alternative strategy is to monitor closely and administer atropine (0.02mg/kg) intravenously should side effects be noted. Pain at the injection site is also commonly reported. Transient increases in liver enzymes will be seen post administration. Acute hepatic and renal tubular necrosis are rarely reported with high doses (>10mg/kg). It is suggested that dose reductions should be made in animals with hepatic or renal insufficiency.

Species		Distribution	Tick Vector	Clinical Findings
Babesia canis	**Large Forms**	Southern & Central Europe	*Rhipicephalus sanguineus* *Dermacentor reticulatus*	Moderate clinical disease Haemolytic anaemia Thrombocytopenia Fever
Babesia rossi		South Africa	*Heamaphysalis eliptica*	Very virulent haemolytic anaemia Immune mediated disease
Babesia vogeli		Africa Asia Australia Central America North America South America Northern & Central Europe	*Rhipicephalus sanguineus*	In affected animals fever, anaemia and thrombocytopenia Often unapparent clinical disease. Young animals may have more severe signs
Babesia gibsoni	**Small Forms**	USA Asia North & East Africa Australia Likely extending through southern Europe	*Rhipicephalus sanguineus* *Heamaphysalis bispinosa*	Haemolytic anaemia Thrombocytopenia Chronic subclinical infection leading to weight loss and progressive debilitation
Babesia Annea (Theileria annae)		North west Spain	*Probably Ixodes hexagonus*	Severe haemolytic anaemia A proportion develop renal failure

Common Babesia species, their vectors and distributions.

Non - Regenerative Anaemia

Non regenerative anaemia is usually insidious in onset and so animal is well adapted to anaemic state, thus animals may be severely anaemic with surprisingly mild clinical signs. Non regenerative anaemia is either caused by primary bone marrow disease or suppression of bone function by systemic disease (e.g. anaemia of chronic disease or endocrinopathies). Bone marrow disease is usually accompanied by other cytopenias. The anaemia will appear normocytic, normochromic, with an inappropriate reticulocyte count.



Aplastic anaemia

Is a basket term for the appearance of severely damaged marrow, where all cell lines are reduced. It is characterized by a pancytopenia i.e. anaemia, thrombocytopenia and neutropenia, with the anaemia often presenting last due to the longer life span of red cells (dogs 11days, cats 70 days). Sometimes the damage is reversible, if not the prognosis is poor

Causes of Aplastic Anaemia
Drugs – many agents reported including: • Oestrogen • TPMS • Phenylbutazone • Chemotherapy agents • Griseofulvin, Immune mediated disease Infection – parvo virus, ehrlichia Idiopathic

Pure Red Cell Aplasia

Pure red cell aplasia (PRCA) is caused by immune mediated destruction of the red cell precursors within the bone marrow. PRCA affects mainly the very early red cell precursors leaving the marrow with sparse erythroid activity. Myeloid precursors and thrombocyte production are unaffected. Immunosuppression is effective in approximately 50% of cases; however it may take the bone marrow a long time to recover if the very early precursors are destroyed. Supportive treatment with blood transfusions will be needed through this period.

Iron deficiency anaemia
Iron deficiency anaemia is relatively uncommon in small animal patients, and is characterised by microcytic, hypochromic anaemia with an absence of haemosiderin (iron stores) in the bone marrow. Causes of iron deficiency include blood loss and rapid erythropoiesis (e.g. during therapy with erythropoietin). The normal total daily iron loss from the body is about 1-2mg, which is easily replaced from dietary sources. Blood contains about 0.5mg of iron/ml, so chronic blood loss eventually leads to iron deficiency.

If iron deficiency anaemia is identified, and there is no history of repeated blood sampling or erythropoietin therapy, then investigations should be focussed on determining a source of haemorrhage. Skin should be examined for external parasites, and a faecal sample should be submitted for endoparasites such as hookworm. Testing for faecal occult blood should be performed only after feeding a meat-free diet for 3 days to prevent false-positive results. Further investigations for GI blood loss include abdominal ultrasound, radiographic barium studies, and endoscopic investigations, depending on the case and available facilities. Further confirmation of a patient's iron status might include serum iron concentration, total iron binding capacity, transferrin saturation, serum ferritin, and bone marrow smears, as described above.

Long-term treatment of iron deficiency requires treatment of the underlying disease. In addition, iron can be supplemented orally at a dose of 2-10mg/kg per day. Ferrous sulphate is the treatment of choice, and absorption is maximal when administered on an empty stomach. Side-effects of oral iron administration include vomiting, diarrhoea, abdominal pain and constipation. Injectable iron dextran (25mg/kg IM weekly as required) can be administered to patients with malabsorption or when side-effects of oral treatment are unacceptable. Note that I/M injection can be painful. The PCV should start to increase within a week of starting iron supplementation, and should reach normal levels by about 4 weeks. It is recommended that supplementation is continued for 4-9 months after the PCV has returned to normal, in order to effectively replenish body stores.

Renal Disease
Anaemia is a common complication of chronic renal disease in dogs and cats (IRIS stage III & IV) and may lead to severe anaemia. There are various mechanisms which can lead to development of anaemia in these patients:

- Gastrointestinal bleeding. Probably the most common cause of blood loss anaemia in uraemic patients. Gastric levels rise due to impaired renal excretion, and can lead to gastric ulceration. In addition, uraemia results in impairment of platelet function.
- Erythropoietin deficiency ability to produce erythropoietin is impaired in uraemic patients and there is a blunted response in the bone marrow.
- Hyperparathyroidism is common in uraemic dogs. Increased PTH can lead to myelofibrosis, and may also have a direct negative effect on erythropoiesis, as well as causing a reduction in red cell survival time.
- Iron deficiency anaemia. This may be due to decreased intake, decreased absorption, or increased loss.
- Increased osmotic fragility of RBCs. In man, increased osmotic fragility of RBCs is a feature of renal disease, but is less clear in cats and dogs

Clinical evaluation of anaemia in patients with renal disease should include a complete blood count (including a reticulocyte count) and investigation of any source of blood loss (hypoalbuminaemia may suggest haemorrhage, and a high BUN:creatinine ratio may suggest GI blood loss). Determination of PTH may be useful in some cases. Arterial blood pressure measurement is important in management of chronic renal failure, and especially if treatment with erythropoietin is to be considered, as pre-existing hypertension is a relative contra-indication to therapy.

Treatment of anaemia of chronic renal disease must be tailored to the individual patient, as it is often multifactorial in origin. The aims of treatment are to minimise ongoing blood loss and to maximise red blood cell production. Gastric ulceration is treated with a antacid such as cimetidine or ranitidine and sucralfate (which also acts to a small degree as a phosphate binder). Recombinant human erythropoietin (rHuEPO) can be used in dogs and cats. rHuEPO

is administered by subcutaneous injection 100IU/kg three times a week (Epogen; Roche); a slightly lower dose 50IU/kg is used in dogs with hypertension or milder anaemia. Once a normal PCV is reached the dose is reduced to 75IU/kg once a week. rHuEPO. Darbepoetin has also been used recently as it has a longer halflife (cats 6.25ug/cat or dogs 1.5IU/kg both once a week initially). Both therapies reliably increase the PCV, which results in improved well-being, appetite and activity. However it is expensive and antibodies develop against the EPO, making it refractory to treatment; at this stage blood transfusion is the only viable treatment. EPO is also very expensive.

Leukaemia

Leukaemia refers to neoplasia arising in the bone marrow and any of the cell lines may undergo neoplastic transformation, however this most commonly occurs with the lymphoid lines. Leukaemia leads to the clonal expansion of neoplastic cells, which results in the crowding of the rest of the bone marrow, competition for nutrients and the release of inhibitory factors. These inhibitory factors reduce haematopoiesis and lead to anaemia, thrombocytopenia, and neutropenia. Neoplastic cells are usually released into the circulation, often in very large numbers. For example, CBC from a dog with acute lymphoid leukaemia may reveal neutropenia, anaemia and thrombocytopenia + a marked leucocytosis due to large numbers of circulating lymphoblasts.

In acute leukaemia the marrow contains numerous blasts which do not mature and differentiate; this carries a very poor prognosis. In chronic leukaemia the marrow contains numerous well differentiated cells and their precursors, with less than 30% of the cells being blasts; these are often responsive to chemotherapy

Myelofibrosis

Myelofibrosis can occur as a primary condition but is also the end point of many disease processes and described ‘scarred’ bone marrow. The normal haemopoietic tissue is replaced with fibrous tissue, which results in reduced erythropoiesis and a marked non-regenerative anaemia but usually normal white blood cell and platelet numbers.

Anaemia of Chronic or Inflammatory Disease

Anaemia of chronic disease is usually mild to moderate in nature (Dog PCV 25 - 35%, cat 18 - 26%) and normocytic, normochromic. There may also be an inflammatory white cell picture. In inflammatory disease iron availability is reduced (although total body iron stores are normal) and inflammatory cytokines down regulate the bone marrows response to cytokines such as EPO.

Endocrine Disease

Both cortisol and thyroxine have stimulatory effects on erythropoiesis, and there reduction in hypothyroidism and hypoadrenocorticisim leads to mild anaemia. In hypothyroidism the anaemia may also inpart be due to a physiological adeaption to the reduced metabolic rate. This is due to decreased oxygen consumption in tissues and an increase in 2,3 DPG concentrations leading to more efficient oxygen delivery. Increased number of target cells (leptocytes) may be present as a result of increased cholesterol within the erythrocyte membrane.

Feline Leukaemia Virus

FeLV causes anaemia by several mechanisms:

- Macrocytic, non-regenerative anaemia is probably due to a direct myelodysplastic effect of the virus. Nuclear maturation is depressed so is out of step with haemoglobinisation of the cytoplasm. Haemoglobinisation is complete before nucleus is mature, thus nucleus is extruded before cell has undergone its usual number of divisions resulting in a macrocytic red cell.
- Aplastic anaemia or pure red cell aplasia
- Leukaemia typically causes an anaemia as well as leucopenia and thrombocytopenia.
- FeLV and FIV-related immunosuppression may lead to infectious / inflammatory disease which in turn may lead to anaemia of chronic disease.
- FeLV may also induce haemolytic anaemia by inducing the formation of antibodies directed against virus antigen on the red cell surface.