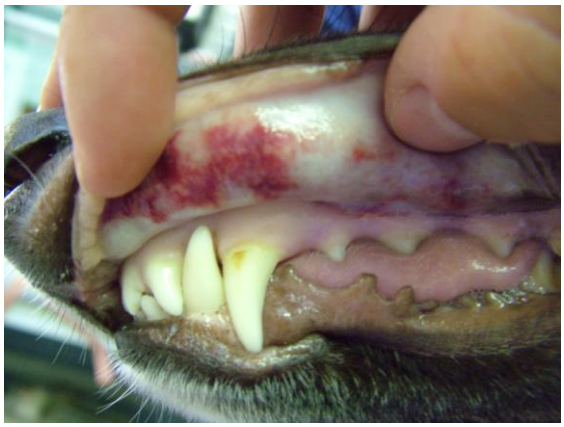
cpdsolutions

transferring knowledge through excellence in training

Anaemia and Bleeding Disorders Mini Series

Session 3: Approach to and Treatment of the Bleeding Patient

Simon Tappin MA VetMB CertSAM DipECVIM-CA MRCVS
European and RCVS Recognised Specialist in Veterinary Internal Medicine



Session 3: Approach and treatment of the bleeding patient

- Review coagulation in cats and dogs, including recent advances in understanding
- Assessment of laboratory and patient side tests of coagulation
- Primary coagulation disorders – thrombocytopenia, thrombocytopathia and von Willibrand's disease
- Secondary coagulation disorders – inherited diseases such as haemophilia A and B, and Hageman factor deficiency, and acquired disorders such as rodenticide toxicity
- Understanding disseminated intravascular coagulation and treatment options

Disorders of haemostasis are theoretically divided into three different phases. Whilst this helps with understanding of the mechanisms, it is important to appreciate that in life, all 3 events markedly overlap.

- Primary haemostasis – vasoconstriction and formation of platelet plug
- Secondary haemostasis – clotting cascade results in formation of fibrin
- Tertiary haemostasis – fibrinolysis

Primary Haemostasis

When a blood vessel is damaged, the initial response is vasoconstriction (mediated by thromboxane A_2, serotonin and adrenaline produced by platelets and the endothelium) followed by formation of a platelet plug. This requires functioning blood vessels, adequate numbers of functioning platelets and platelet adhesion factors (including Von Willebrand's Factor). Platelets adhere to sub-endothelial collagen via an interaction with von Willebrand's factor (vWF) and the platelet glycoprotein receptor GP1b. Binding of this receptor activates the platelet changing its shape to develop numerous pseudopods which project in numerous directions. Activation allows aggregation of platelets into a plug through the vWF binding to GP11a/IIIb receptors. Activation also exposes platelet factor 3 which provides a scaffolding onto which fibrin can then be deposited following secondary haemostasis.

Clinical presentation

Animals with disorders of haemostasis will vary in their presentation depending on the severity of the disease and any inciting trauma to blood vessels. In general, animals with defects in primary haemostasis present with petechial haemorrhages and bleeding from mucosal surfaces (epistaxis, gastrointestinal bleeding, haematuria). Mucous membranes (oral, ocular, rectal and the prepuce and vulva) should be carefully checked; clipping fur sometimes reveals an area of haemorrhage.

Differential diagnoses for animals with primary haemostatic disorders include

- Disorders of blood vessels e.g. vasculitis
- Reduced numbers of platelets (in general, spontaneous bleeding occurs if platelet count $<30x10^9$/l; bleeding following surgery etc occurs if platelet count $<50x10^9$/l)
  - Inadequate production (bone marrow disease)
  - Destruction of platelets (immune-mediated thrombocytopenia – primary or secondary)
  - Consumption of platelets e.g. DIC, vasculitis, haemorrhage, sepsis, haemangiosarcoma, lymphoma, FeLV, FIV, FIP, heartworm
- Abnormal platelet function
  - Inherited disorders e.g. von Willebrand's disease, canine cyclic haematopoiesis (grey collies), Chediak-Higashi syndrome (cats)
  - Acquired disorders e.g. DIC, hepatic disease, pancreatitis, Ehrlichia canis, lymphoma, multiple myeloma

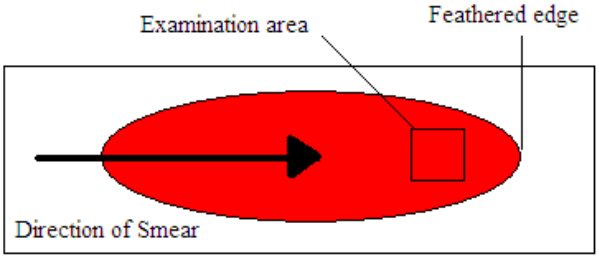
Tests for primary haemostasis

- Platelet count
  - A properly collected and mixed EDTA blood sample without clots is essential – check the underside of the lid. Machine counts should ALWAYS be checked on a smear – slight clumping, small red cells, and large platelets (e.g. CKCS) will all adversely (and unpredictably) affect the platelet count
  - Estimating numbers from a smear – check the feathered edge and body of the smear for clumps. If present, estimated counts cannot be performed. Examine the red cell monolayer on x1000 (100x oil immersion, 10x eyepiece) and count the number of platelets in each of ten fields; average this number.
  - Average platelet count (per x1000field) x 15 = platelet numbers x 10^9/l. This is an approximate guide with a margin around +/- 20%
  - Spontaneous bleeding not usually apparent unless platelet count <50x10^9/l and often lower

Manual Evaluation of Platelet Counts

Manual evaluation of a blood smear is essential for the accurate conformation of platelet numbers. Platelets clump easily and as a consequence may not be counted correctly during automated counting. Similarly machines that count platelets by size alone may be confused by the presence of small red cells (counting them as platelets) or macrothrombocytes (counting them as red blood cells). If clumping is a consistent problem then citrate anticoagulant, rather than EDTA can be used. Some breed variation occurs with greyhounds having normal platelet counts in a range lower than other breeds and Cavalier King Charles Spaniels normally have circulating macrothrombocytes.

Evaluation of a blood smear is achievable with a practice microscope and in house stains. A blood smear should be prepared as soon after sampling as possible to reduce the possibility of the platelets clumping and stained once dry. This should be evaluated firstly under low power to evaluate the smear quality and overall pattern, then under oil emersion (x100) to evaluate the platelet numbers specifically. Platelets should be counted in the area just behind the feathered edge, where the red cells are just touching one another.



A manual platelet count can be evaluated as each platelet counted manually per high power field is equivalent to roughly 15x10^9/l platelets. Several areas should be examined and the count averaged. The edges of the smear should be examined for platelet clumps and the blood tube also examined for potential clots. If platelet clumps are present an accurate count can not be established but an overall impression of adequate platelet numbers may be achieved. Macrothrombocytes may be present and these indicate regenerative thrombopoiesis. These platelet precursors are also functional, with primary coagulation being more proportional to the volume of platelets rather than the actual number of platelets present.

- Platelet function
  - Many laboratory tests for platelet function are not readily available. Platelet function tests assessing aggregation (PFA-100) need to be performed on fresh blood immediately, so at present are more of a research tool than applicable to clinical cases. Flow cytometry assessing the expression of GPIIb/IIIa which is produced when platelets become activated is also useful as a research tool.
  - Buccal mucosal bleeding time
    - Should not be performed in patients with a severe thrombocytopenia
    - The upper lip is tied upwards reasonably tightly, as to slightly impair venous return. Shallow cuts in the lip are made with a standard spring-loaded device (e.g. simplate or the devices used to take heal blood samples from babies) and timing started. Excess blood is blotted with paper towel or filter paper from the edge of the cut. It is important not to touch the incision or to disrupt the clot.
    - Normal range is 1½ to 4½ minutes in dogs and 1 to 2½ minutes in cats. Slightly longer (but <5 minutes) in sedated or anaesthetised animals.
    - Tests the formation of the fragile platelet plug, but is independent of fibrin formation.
    - Will detect dogs with severe vWD, but may miss those that are slightly affected
  - Von Willebrand's factor testing
    - Available at the Animal Health Trust
    - The individual is tested for the amount of vWF by ELISA or immunoelectrophoresis and compared to normal pooled plasma. The value is expressed as a percentage.
      - <50% animal has von Willebrand's disease
      - >70% is normal
      - 50-70% is quivocal and the animal may be a carrier
    - Some breed specific genetic tests available for point mutations for example Scottish terriers, Manchester terriers, Kooikers. Only work for these breeds, and do not predict severity of condition
  - Nail clip bleeding time
    - Painful with unreliable results, and tests all parts of clotting system – not recommended
  - Clot retraction
    - Many non-platelet factors affect this test making it almost impossible to interpret – not recommended. Performed by putting blood in a glass tube and allowing it to clot, then waiting an hour to see if clot has retracted in size.

Disorders of Primary Haemostasis

Thrombocytopathia

Problems with platelet function are rare but occur when circulating platelets cant' become activated in the presence of normal platelet numbers and vWF levels. These patients will show signs of primary coagulation diseases as a result and have prolonged BMBT results. Acquired thrombocytopathia occurs with drug administration (the classic example of this is with aspirin) or in association with other disease such as liver failure, chronic renal disease or neoplasia.

Several inherited thrombocytopathias are reported, the best described is Ganzmann's thrombasthenia caused by an absence of GP IIb/IIIa. This leads to decreased platelet aggregation and clot retraction and is described in Otterhounds and Great Pyrenees. Signal transduction problems are reported in Basset Hounds, Spitz and Lanseers secondary to abnormal adhesion. Storage pool abnormalities are reported in Grey collies with cyclical neutropenia due to defective ADP and 5HT release.

Von Willebrands Disease (vWD)
VWD is caused by a deficiency of, or abnormality in, von Willebrand factor (vWf), and has been reported in many breeds of dogs in particular (in the UK) Dobermanns, Irish Wolfhounds, and German Shepherd Dogs. vWf is essential for effective primary haemostasis, where if acts as an adhesion molecule between platelets and the subendothelial matrix. In addition, it forms a complex with FVIII, protecting FVIII from degradation and transporting it to sites of vessel injury. Severe deficiency of vWf results in signs associated with impaired primary haemostasis. Drugs that impair platelet function or underlying disease such as hypothyroidism can worsen signs associated with vWD. Buccal mucosal bleeding test (BMBT) is a useful screening test for vWD but is not specific or particularly sensitive. The diagnosis should be confirmed by genetic testing or measurement of vWF antigen.

Treatment is palliative, with the aim of minimising haemorrhage.

- Infusion therapy
    - Cryoprecipitate (containing high concentrations of vWf and FVIII) is the ideal blood product for treatment of vWD, but is not widely available.
    - Fresh frozen plasma (6-10ml/kg) is the best alternative to cryoprecipitate
    - Fresh whole blood should be avoided as routine treatment for dogs with vWD, to prevent sensitisation to donor antigens in dogs that may well need further treatment with blood products in the future. However, typed and cross-matched fresh whole blood (13-22ml/kg) in an emergency for patients with severe anaemia.
- Desmopressin
    - Desmopressin is a synthetic analogue of arginine vasopressin (antidiuretic hormone; ADH). It causes release of vWf from endothelial cells, and will only be useful in dogs with Type 1 vWD i.e. those with endothelial stores, such as Dobermanns. It can be given pre-surgically to reduce the risk of bleeding, and can also be given to donor dogs before obtaining blood for transfusion purposes. It is administered at a dose of 1ug/kg by subcutaneous injection, 30 minutes prior to surgery, and its effects may last up to 4 hours, although response to treatment is unreliable.

Immune-mediated thrombocytopenia (IMT)
Primary IMT is characterised by severe thrombocytopenia (confirmed on a blood smear) in the absence of other potential causes of low platelet counts. Most other causes of thrombocytopenia (DIC, neoplasia, SLE, infections, splenomegaly, haemorrhage, haemolytic uraemic syndrome, breed-related thrombocytopaenia) cause mild-moderate rather than severe thrombocytopenia. IMT secondary to underlying diseases or drug treatment can result in severe thrombocytopenia. Treatment relies on a combination of immunosuppression and supportive care.

- Supportive care
    - Cage rest. Minimise trauma. Avoid S/C or I/M injections. Take any blood samples from cephalic or saphenous veins so that pressure can be applied effectively if haemorrhage occurs.
- Transfusion therapy
    - Blood transfusions are generally administered to dogs with IMT only in the event of life-threatening haemorrhage. For example if there is marked gastrointestinal or intracranial haemorrhage. In the UK at present platelet rich plasma is not available for veterinary use, however in US both freeze dried and DSMO preserved platelets have been used with some success. Whole fresh blood is the only readily available and effective way of transfusing platelets however is very inefficient. 10ml/kg of whole fresh blood which would be expected to raise the PCV by 5% will roughly increase the circulating platelet count by $10x10^9$/l. Although a small rise this may be enough to halt or reduce critical bleeding.

- Immunosuppression
    - Glucocorticoids inhibit macrophage destruction of antibody-sensitised platelets. They may also stimulate platelet production in some patients with IMT. It can take up to 7 days for platelet count to increase
        - Prednisolone 1mg/kg orally BID or dexamethasone 0.5mg/kg SID
        - This dose should ideally be continued until platelet count is within the normal range at which point it is gradually reduced by 25% every 4 weeks until it can be withdrawn after 6 months.
    - Additional/alternative immunosuppressants can be considered in patients in which glucocorticoids alone are ineffective or in which the side-effects are intolerable. There is little published evidence regarding efficacy of these other treatments.
        - Azathioprine 2mg/kg SID, tapering to EOD once a clinical response is obtained, then weaning off. Monitor haematology as can cause myelosuppression. Has been associated with pancreatitis.
        - Cyclophosphamide 200mg/m^2 weekly until a response is achieved. Monitor haematology as can cause myelosuppression.
        - Cyclosporine 5mg/kg SID or BID – trough levels can be measured to help guide therapy.
        - Splenectomy is performed in people if there is no response to glucocorticoids. However any potential advantages of splenectomy must be balanced against the risks of surgery in a thrombocytopenic patient.
        - Danazol is a synthetic androgen of which there are limited reports of use in ITP.
        - Human intravenous immunoglobulin (IVIG) is often used as emergency therapy in humans with IMT, and its use has been reported in dogs. It is administered as an infusion over 6-12 hours at a dose of 0.5-1.5g/kg.

    - Vincristine
        - Vincristine (0.5mg/m^2 or 0.02mg/kg I/V once) administered to dogs with IMT results in an increase in platelet numbers within a few days. One study by Rozanski and colleagues in 2004) demonstrated more rapid increases in platelet count and reduced hospitalisation times in patients given vincristine in addition to prednisolone compared to prednisolone alone. The mechanism of action is thought to be reduced phagocytosis of platelets by impairment of macrophage microtubule assembly, as well as stimulation of thrombopoiesis. There is controversy regarding whether or not the effects of vincristine on platelet microtubule function are clinically significant.
- Prognosis
    - Published studies suggest that over 70% of dogs with primary IMT will have a platelet count of >100x10^9/l following initial treatment, approximately 30% of dogs will die or be euthanased due to disease, and approximately 40% of dogs will suffer a relapse. The prognosis may be worse for dogs with both IMHA and ITP [Evans syndrome].

Management of Immune Mediated Thrombocytopenia

1. **Prevent further bleeding.** No jugular sampling – samples are taken form the distal limbs so bleeding can be controlled with pressure bandages as needed. Control local bleeding – pressure / surgery / packs / phenylephrine

2. Place large bore i/v catheter. Collect min database from catheter if possible.

3. Volume resuscitate, as appropriate. Consider whole blood to increase platelet numbers. 10ml/kg whole blood will raise the platelet count by approximately $10x10^9$/l. Transfuse as needed to replace blood loss (packed red cells/Oxyglobin).

4. **Give steroids** to reduce platelet destruction. Dexamethasone i/v 0.5mg/kg SID then switched to prednisolone (1mg/kg/BID p/o) as appropriate. Steroids are normally tapered once platelet numbers have returned to normal, and reduced over approximately 4-6 months (25% reduction each month, moving to EOD treatment at about ½ way through treatment – second line immunosuppressive drugs may help reduce the steroid dose more quickly).

5. **Consider antibiotics** if tick borne disease (e.g. Anaplasma) is possible. Submit blood for PCR or cover with doxycycline (10mg/kg/SID p/o).

6. **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.

7. **Consider vincristine** (0.5mg/m^2 or 0.02mg/kg) through a cleanly placed i/v catheter. Vincristine leads to the shattering of megakaryocytes and increased platelet numbers. It is not known if these platelets are functional but postulated that vincristine may then accumulate in macrophages, inhibiting their action. A recent paper reported that vincristine at admission reduced hospitalisation stays of ITP patients by 24 hours. **CARE** as the patient should now be considered cytotoxic.

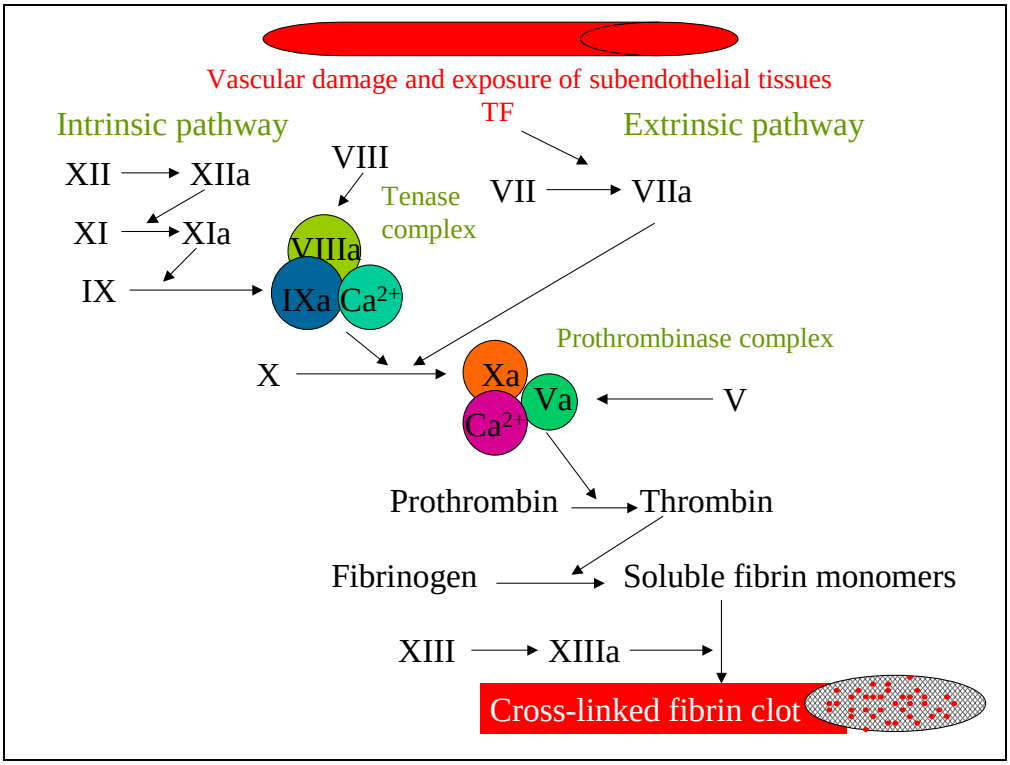
8. **Consider Gastro-protection.** Reduces blood loss as a result of ITP, also reduces risk of steroid associated GI haemorrhage. Sucralfate (0.5-1g/QID p/o) and cimetidine (5-10mg/kg/TID SLOW i/v) or Ranitidine (2mg/kg/BID SLOW i/v).

9. **Consider an i/v human IgG infusion** (0,5mg/kg over 4 hours). Human IgG is expensive but will act quickly to reduce platelet 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.

10. Consider splenectomy for chronic ongoing cases.

Secondary Haemostasis

Secondary haemostasis results in the formation of fibrin. Tissue factor initiates the clotting cascade on the extrinsic side, which is then amplified in the intrinsic pathway. Platelet factor 3 (PF3) is required to anchor and activate the process. Thrombin generation activates platelets and provides the major feedback loop for further activation of the clotting cascade (see diagram below):



Clinical presentation

Clinical signs in animals with defects of secondary haemostasis are often more dramatic than the primary defects. Haemorrhages into body cavities (pleural space, peritoneal space, lungs, joints) are often seen, as well as larger ecchymotic haemorrhages in subcutaneous tissues. Re-bleeding after an initial platelet plug has failed to stabilise may occur.

Selected differential diagnoses for animals with secondary haemostatic disorders

- Inherited disorders
  - Factor VIII deficiency (Haemophilia A) in many pure and cross-bred dogs and various cat breeds. Sex-linked recessive
  - Factor IX deficiency (Haemophilia B) in severel dog breeds, British shorthair, Siamese and DSH-cross cats. Sex-linked recessive
  - Factor X deficiency. Reported in American cocker families and jack Russell terriers.
  - Factor XII (Hageman factor) deficiency in various cat breeds. Rarely clinically significant.
- Acquired disorders
  - Vitamin-K dependent coagulopathies
    - Coumarin-based rodenticide toxicity (antagonism of vitamin K)
    - Severe GI disease causing lack of absorption of vitamin K – EPI, severe intestinal inflammation, bile duct obstruction.
    - Hepatic disease – lack of activation of vitamin-K dependent factors, and lack of production of many clotting factors (as well as inhibitors of coagulation)
  - Disseminated intravascular coagulation

Tests for secondary haemostasis

"Patient-side" tests

- Whole blood clotting time
  - 2mls of whole blood are put in a glass (not plastic) tube and kept at 37°C. The time to beginning of clot formation is recorded.
  - Times of 3-13 minutes recorded in normal animals; platelet numbers may affect test. Best only used as a last resort, interpreted with platelet counts, and only grossly abnormal results heeded.
- Activated clotting time
  - Pre-prepared tubes with an aluminium salt which activates clotting are available (Actalyke ACT tubes, Helena laboratories). Whole blood (1-2mls) is added to the tube, kept at 37°C, and inverted at regular intervals until the first sign of a clot appears.
  - Up to 90 seconds reported in dogs.
  - The activating substance removes the impact of platelet numbers, making it more accurate than whole blood clotting time. It challenges the intrinsic pathway, but severe deficiencies are required to prolong the test (less than 10% of a factor remaining), so the APTT should be used in preference where possible.

Laboratory based tests

- Citrated plasma is required for APTT and PT testing. Good blood sampling technique is required as tissue damage and release of tissue factor will invalidate the sample. The sample needs to be spun within 20 minutes of collection, and separated from the cells. A correct anti-coagulant:blood ratio is extremely important, and check the tubes carefully for clots before submitting.
- Activated partial thromboplastin time (APTT)
  - Tests the intrinsic and common pathways. Prolonged if a factor reduced to <30% normal. Conditions that prolong it include coumarin poisoning, DIC, hepatic disease, haemophilias A or B, factor X deficiency, Hageman factor deficiency
  - Reference ranges vary widely and the test should be run against a pooled control sample for comparison.
- Prothrombin time (PT)
  - Tests extrinsic and common pathways. The short half-life of factor VII means that this is the first test to prolong in Vitamin-K dependent coagulopathies and is the test of choice for monitoring response to treatment with these conditions. Can also be prolonged in DIC and Factor X deficiency.
- Proteins involved in vitamin K antagonism (PIVKAs)
  - Misleading name, as interrogates activity of factors II, VII and X – any condition that reduced Vitamin K will result in prolongation, not just coumarin poisoning.
- Specific factor assays
  - Patient plasma is used to try to correct known deficiencies in test plasma. If the test comes back normal, the patient does not have that deficiency. A single factor deficiency of <30% of normal is likely to be significant.

The cell-based model of Coagulation

The classic model of haemostasis details specific and separate roles for platelets and the coagulation system when in reality there functions are more intertwined. A more complex and more physiologically accurate model described a cell based model for coagulation, enhancing the role of interaction of coagulation proteins and cell surfaces in particular the surface of the platelet. This then leads to three stages of initiation, amplification and propagation, ultimately leading to the formation of fibrin as per the classic common pathway.

Secondary Coagulation Disorders

Inherited coagulation defects

Many specific coagulation factor defects have been reported in veterinary medicine. Examples of breeds with reported inherited factor deficiencies

- Factor I – Bernese, St. Bernard, Borzoi, Lhasa Apso, Vizsla, Bichon Frise, Collie, DSH
- Factor II – Boxer, English Cocker Spaniel, Otter Hound
- Factor VII – Beagle, Boxer, Malamute, Alaskan Klee Kai, Bulldog, Miniature Schnauzer, Scottish Deerhound, DSH
- Factor VIII – German Shepherd, Golden Retrievers, DSH
- Factor X – American Cocker Spaniel, Jack Russell Terrier, DSH
- Factor XI – English Springer Spaniel, Kerry Blue Terrier, Great Pyrenees, DSH
- Factor XII (nonpathologic) – Miniature Poodle, Standard Poodle, German Shorthair Pointer, Shar Pei, DSH, DLH, Siamese, Himalayan Cats
- Vitamin K-dependent factor deficiency - Devon Rex cats

Haemophilia

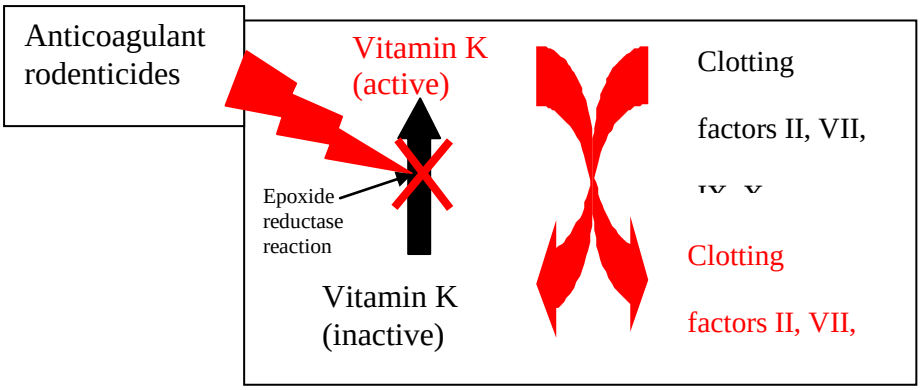
Haemophilia A (deficiency of Factor VIII) and haemophilia B (deficiency of Factor IX) are the most common inherited coagulation defects in dogs. (A is roughly 4 times more commonly reported than B. It is sex linked and usually seen in young males, who present with haematoma formation, lameness secondary to heamarthrosis or prolonged bleeding after the loss of deciduous teeth or minor surgery. Carrier females are often normal but produce roughly half the normal amount of factor. Bleeding can be a problem if there has been excessive blood loss for example after surgery or trauma. Common breeds for A include German shepherd dogs & Golden retrievers and for B German wire haired pointers, but a wide range of breeds for both reported. Diagnosis is usually made in the presence of clinical signs, an increased APTT & ACT, but normal PT and confirmed by factor analysis.Treatment is symptomatic and includes transfusion or FFP, cryoprecipitate or whole blood, however repeated transfusion will become more challenging over time. Human recombinant factors can be used and experimentally gene therapy has been used to correct the disorder.

Hagemann factor deficiency

Most common deficiency in cats. Dramatic increase in aPTT with a normal PT. Whilst factor XII is needed in vitro it is not required in vivo for coagulation to be activated thus it does not lead to bleeding disorders. It can be confirmed by measuring factor XII levels. It can also be associated with other factor deficiencies. As well as cats it has also been reported in dogs, including Standard Poodles and German Short Haired Pointers.

Rodenticide Toxicity

Activated Vitamin K is required for the activation of clotting factors II, VII, IX and X. Vitamin K is converted to its active form via the epoxide reductase reaction, which is inhibited by anticoagulant rodenticides.



First generation rodenticides (warfarin, dicoumarin, diphacinone, chlorphacinone) are generally less potent than second generation drugs. Repeated exposure or a massive single dose are usually required for clinical signs to become apparent, and bleeding may not occur until 4-5 days post ingestion. Half-life varies so that treatment may be required for between a week (warfarin) to a month (diphacinone). Second generation drugs are now more common, including brodifacoum, bromadiolone and difenacoum, and are highly potent. A single dose can be enough to cause bleeding within 24 hours, and secondary poisoning from ingestion of rodents is possible. Treatment may be required for up to a month.

Deficiency of active Vitamin K leads to bleeding due to inadequate secondary haemostasis. Animals often present with bleeding into major body cavities eg haemoperitoneum, haemothorax, or haematoma formation. However animals with no clinical signs should still be treated. Factor VIIa has the shortest half-life of the Vitamin K-dependent factors (about 6 hours), which can cause the prothrombin time (PT) to increase before the activated partial thromboplastin time (APTT). However in most cases, PT, APTT and activated clotting time will all be prolonged. It is important to note that, in general, primary haemostasis (ie platelet function as assessed by buccal mucosal bleeding time) will be intact, although some patients may have a transient thrombocytopaenia of unknown aetiology. The non-activated forms of vitamin K-dependent clotting factors may build up in hepatocytes and spill over into the circulation. These are known as “PIVKAs” (proteins induced by vitamin K antagonism) and assays are available in some centres.

Treatment:

- Emesis/gastric lavage/adsorbents if <3h post ingestion
- Vitamin K_1 is the treatment of choice
    - Give s/c for 1-2 days then continue with oral medication
    - Initial dose of 5mg/kg s/c followed by 1.25mg/kg BID s/c or orally
    - Avoid I/V administration due to the risk of anaphylaxis; I/M administration may lead to haematoma formation.
    - Continue treatment for 7-28 days depending on toxin; check PT 2days after stopping treatment and continue for another 2 weeks if necessary.
    - Vitamin K_3 is available and, although cheaper, is not nearly as effective
- Cage rest and drainage of cavity bleeding if necessary
- Blood transfusions may be required as functional clotting factors may not be generated for 1-2 days even with Vitamin K treatment. The vitamin-K dependent clotting factors are relatively stable and either fresh or stored whole blood can be used. Plasma products (fresh, stored, frozen, or fresh frozen plasma) may be used if available in the patient which does not require additional red blood cells.

Tertiary Haemostasis

Fibrinolysis is initiated by the same processes that activated the clotting cascade, with plasmin formation in the area local to the clot. Plasmin cleaves fibrinogen and fibrin, leading to the formation of various measurable fibrin degradation products, and cleaves cross-linked fibrin into D-dimers. Abnormalities of tertiary haemostasis result in an increased tendency to form a clot, with the main clinical presentations being DIC and thrombosis.

Tertiary Coagulation Disorders

Post-Operative Bleeding in Greyhounds

Racing Greyhounds are reported to have much increased risk of postoperative bleeding compared with other breeds. Those dogs will typically have completely unremarkable results of presurgical haemostatic screening. Recent studies suggest that use of Epsilon Aminocaproic Acid (EACA) will significantly reduce prevalence of postoperative bleeding in Racing Greyhounds. Protocol described in those publications advises initial dose of 15-40mg/kg administered IV immediately after surgery (1ml diluted in 15mlpf 0.9%NaCl over 30 min), followed by oral doses of EACA (total dose of 500-1,000mg every 8 hours, for 5 days). It might worth considering use of EACA in Racing Greyhounds scheduled to undergo a surgical procedure (Marín et al., 2012).

Disseminated Intravascular Coagulation (DIC)

DIC is a complicating feature of many diseases. It involves activation of both coagulation and fibrinolytic pathways, with tendencies towards both thrombosis and haemorrhage depending on the interplay between the processes in the individual patient. Thrombosis can lead to multiple organ failure. It can be acute or chronic in nature. Diagnosis requires identification of at least three of the following: thrombocytopaenia, prolonged PT and APTT, reduced antithrombin concentrations, and increased FDPs or d-dimers.

Aggressive, prompt treatment is required for successful management of DIC.

- Diagnose and treat underlying condition - This is the single most important aspect of treatment!
- Improve blood flow in microcirculation -Fluid therapy as indicated to maintain blood volume. Supportive treatment for cardiac and renal function as required. Treat metabolic acidosis/hypoxia when appropriate.
- Treat coagulopathy -There is much controversy over treatment with fresh frozen plasma (FFP) and, in particular heparin. From a clinical point of view, it is easier to divide patients into those which are actively bleeding, and those which are at high risk of thrombosis. This judgement may be difficult to make in many patients.
    - Bleeding patients: FFP is probably the treatment of choice for all cases of acute DIC, in particular those with active haemorrhage, in order to replenish clotting factors. The recommended dose rate is 2-3 doses daily of 10-15ml/kg, which is now possible (although expensive!) with recent developments in blood-banking in the UK. It is debatable whether even this level of supplementation results in administration of clinically useful amounts of individual clotting factors.
    - Clotting patients: There are no stage III trials of the use of heparin in DIC in human or animal patients, and over the last few years it has been a field of increasing controversy. The current thoughts are that heparin should certainly be used in patients at high risk of thromboembolism in the absence of significant inflammatory disease. However, heparin should be avoided in patients with active bleeding or with significant inflammatory disease, as heparin has been shown to significantly impair the anti-inflammatory activity of antithrombin, which may outweigh any benefits of heparin administration.
    - Unfractionated heparin has traditionally been used to treat DIC. Dosages of 200-250IU/kg S/C QID have been recommended by some authors, but will lead to an increase in PT/APTT and an increased bleeding tendency. Other authors recommend a more conservative dose of 75-100IU/kg S/C QID which doesn't usually prolong the coagulation times, but we have no evidence whether or not this actually has a clinically significant effect.

Disorder	Platelets	BMBT	PT	aPTT	ACT	Fibrinogen	D-dimer
Thrombocytopenia	↓	↑	N	N	N	N	N
Thrombocytopathia	N	↑	N	N	N	N	N
vWD	N	↑	N	N/↑	N/↑	N	N
Haemophilias	N	N	N	↑	↑	N	N
Rodenticide toxicity	N/↓	N/↑	↑↑	↑	↑	N/↓	N/↑
Hepatic failure	N/↓	N/↑	N/↑	↑	↑	N/↓	N
DIC	↓	↑	↑	↑	↑	N/↓	↑

Angiostrongylosis

The cause of bleeding in patients with Angiostrongylosis is not well understood and likely has many factors. Patients commonly have mild increases in prothrombin and activated partial thromboplastin times, with slight reductions in platelet numbers; although bleeding can be seen in patients with no documented changes in coagulation parameters. These clinical findings together with experimental work, suggests *Angiostrongylus vasorum* triggers a form of compensated state of disseminated intravascular coagulation (DIC).
Immune mediate thrombocytopenia, reduced levels of factors V and VIII and acquired von Willebrand factor deficiency have also been reported. Where bleeding is clinically significant, whole blood or fresh frozen plasma may be required. In dogs where DIC has been documented transfusions products and heparin may also be considered.

Angiostrongylus vasorum was first documented in France in 1853; as a result is often referred to as French heart worm as the adults live in the pulmonary arteries and the right side of the heart. Infection leads to varied clinical signs, with respiratory, coagulopathic and neurological signs most commonly reported. *Angiostrongylus vasorum* has been increasingly diagnosed in the United Kingdom over the last two decades, with cases reported within the classic geographic hotspots of the south west and south east England, and the south of Wales, as well as in lower numbers through most geographic areas of the UK. Several online maps are regularly updated, providing local information as to the risk of infection for example www.lungworm.co.uk or www.angiodetect.co.uk.

Angiostrongylus vasorum is a metastrongylid nematode that primarily infects canidea, especially domestic dogs and foxes, although infection has been reported in wolves, coyote, otters, ferrets amongst others. Dogs become infected by L3 larvae after eating infected intermediate (molluscs such as slugs and snails) or paratenic hosts (e.g. frogs). Over 25 different species of snails and slugs have been identified as intermediate hosts for *Angiostrongylus vasorum* and these vary considerably depending on geographic location. It has also reported that the L3 larvae can leave the host and be ingested directly from the environment, however the epidemiological significance of this is currently unclear. Once ingested, L3 larvae migrate through the intestinal wall to the mesenteric lymph nodes where they develop to the L4, then L5 larvae. From here, they migrate via the lymphatic and venous systems to mature in the pulmonary vasculature. The pre-patent period is variable with reports ranging from 28 to 108 days. Ova released from the adult worms are washed into the pulmonary capillaries, where they develop into L1 larvae and migrate into the alveoli. These are coughed up and swallowed and are passed in the faeces, infection molluscs by direct contact. Dogs with untreated asymptomatic infection are thought to excrete larvae for prolonged periods after infection; this has been documented experimentally with untreated dogs excreting L1 larvae for nearly 2 years post infection.

The distribution of *Angiostrongylus vasorum* is patchy but fairly widespread through most of Western Europe, with spasmodic reports in the United States of America and Canada. In the United Kingdom there are geographical differences in case numbers with historically well-defined areas of infection in Cornwall and south Wales. Within these areas the parasite was common in foxes and frequently caused disease in dogs. The parasite has since spread to the south east of England and gradually spread northwards with cases more recently being reported in northern England and in Scotland.

A recent retrospective questionnaire based study of United Kingdom practices documented that a third of practices were aware of Angiostrongylus infections locally and just under 21% had seen at least one clinical case in the previous year. This study also confirmed the geographic hot spots of the south Wales and south-east England, with practices in these areas around 5 times more likely to have seen a clinical case of Angiostrongylus infection compared to the rest of the United Kingdom. Within the cluster in southern England younger dogs, Cocker, Springer and Cavalier King Charles Spaniels, Jack Russell and Staffordshire Bull Terriers were found to be at higher risk of infection within a referral population of cases.

Causes of the change to the reported distribution of *Angiostrongylus vasorum* are unclear but are thought to be due to multiple factors including changes to environmental conditions which have changed the distribution, or increased the abundance, of the mollusc hosts, and better awareness of clinical signs associated with the presence of the parasite. Furthermore it has recently been suggested that the increasing prevalence of Angiostrongylus infection in dogs mirrors increased prevalence in the fox population, with the fox acting as a reservoir for canine infection. Movement of animals may also have important epidemiological implications with cases being reported in the United States of America in a greyhound imported from Ireland and in Australia in a Cocker Spaniel imported from Surrey in England.

Definitive diagnosis is made on confirming the presence of the parasite or an immunological response to it. Traditionally this has been done using the modified Baermann floatation; however this is a time consuming test and of low sensitivity as the parasite is intermittently shed. Pooled faecal samples over a 3 day period or repeated sampling increases accuracy. A direct faecal smear is simple and quick to perform, with sensitivity of 54-61% depending on the experience of the assessor. Analysis of tracheobronchial secretions or tracheal wash samples can also confirm the presence of infection. PCR tests have been developed, however their sensitivity is disappointing. Recently an in-house ELISA test (Idexx Angio Detect) has been developed to document an antigen produced by adult *Angiostrongylus vasorum* using blood samples. Although this test appears to give good results (sensitivity (85 - 96%) and specificity (94 - 100%) compared to Baermann flotation) compared a diagnosis of Angiostrongylosis should be made in conjunction with clinical signs and supportive evidence such as imaging findings.

Vascular changes are rarely documented on radiographs and a mixed pattern is more common. A patchy alveolar pattern in the peripheral lung fields is commonly reported. Haematology and serum biochemistry findings are variable although eosinophilia is reported in between a quarter to a half of cases and hyperglobulinaemia in three-quarters. Hypercalcaemia has been reported in some cases; the exact mechanism of this elevation is unclear but is thought to be secondary to granuloma formation.

Treatment for *Angiostrongylus vasorum* infection has two focuses killing the parasites and supportive therapy. In the United Kingdom both imidacloprid/moxidection (Advocate®) and mibemycin/praziquantel (Milbemax®) are licensed to treat Angiostrongylosis; Advocate® as a monthly spot-on and Milbemax® tablets orally once a week for four weeks (Note: the datasheet suggests single agent therapy with mibemycin for the last three treatments which is not currently available in the UK). Imidacloprid/moxidectin has been shown to be effective against both immature and mature Angiostrongylus stages with an efficacy of 85% in a blinded randomised controlled field study. Milbemycin has also been used successfully to treat field infections. Although not a licensed product for treating *Angiostrongylus vasorum*, fenbendazole has traditionally been used as the drug of choice with doses of 25-50mg/kg SID suggested for 3-21 days, with similar efficacy to imidacloprid/moxidectin reported.

Supportive care depends on the clinical signs and presentation of individual patients. Cardiorespiratory signs are most commonly seen with coughing occurring due to the physical presence of the parasite. Non-steroidal anti-inflammatory drugs may be help limit the associated inflammatory response. Occasionally patients will develop worsening of their respiratory signs after adulticides are administered especially if worms are killed rapidly; these signs may need to be treated with steroids. Antimicrobial therapy is not usually needed unless there is secondary bacterial infection. Severe pulmonary hypertension is occasionally seen

and leads to signs of right sided heart failure (cor pulmonale). Sildenafil is occasionally indicated, however signs of pulmonary hypertension usually resolve quickly with treatment.

Studies have shown imidacloprid/moxidectin used in the pre-patent period clears infection with L4 and L5 (immature adult) worms. Advocate® carries a license in the United Kingdom for four weekly administrations for the prevention of Angiostrongylosis. Milbemycin can reduce parasite numbers if used in the prepatent period. Milbemax® is also licensed for preventative use with four weekly administration reducing the immature adults (L5) and adult parasite burden. The clinical relevance of the difference between the two products is unclear and requires further research. In an endemic area regular treatment with an effective drug is a sensible strategy to reduce the risks of developing disease. Other measures such as removing toys from the garden overnight, limiting access to areas were slugs and snails may be present and the regular removal of faecal matter will further reduce the risks of exposure.