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Anaemia and Bleeding Disorders Mini Series

Session 1: Approaching the Anaemic Patient

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Session 1: Approaching the anaemic patient

- Review red cell physiology and the approach to patients with anaemia
- Assessment of laboratory findings; understanding red cell parameter, cytological morphology and classify causes of anaemia
- Understanding how, when and where to take a bone marrow biopsy
- Introduction to transfusion medicine

Red cell production and life cycle

Red cells are produced in the bone marrow of the long bones. However in times of increased demand for production the liver and spleen can also contribute (so called extra medullary haematopoiesis – EMH). Once produced red cells have a finite life span (110 days in the dog and 70 days in the cat) before being removed from the circulation by the spleen and their components recycled. Animals become anaemic if red cells are not produced in adequate numbers (bone marrow disease) or if excessive red cells are lost from the circulation either through haemorrhage or destruction.

What are the clinical signs of anaemia?

Result from reduced oxygen carrying capacity

- Weakness
- Lethargy
- Exercise intolerance
- Pale mucus membranes – lips/gums, conjunctivae, hairless skin
- Systolic heart murmur / haemic murmur (due to low blood viscosity and increased flow velocity with increased turbulence)
- Tachycardia
- Bounding femoral pulse
- Tachypnea
- Look for signs of external blood loss
    - Epistaxis
    - Melaena / Haematemesis
    - Wound
    - Ulcerated tumour
    - Haematuria

Useful Historical findings

- Breed sex and age of animal?
    - Young male Golden Retrievers sex linked inherited haemophilia
    - Immune-mediated haemolytic anaemia ↑ in females & Cockers
- Rate of onset?
    - Sudden onset suggests haemorrhage or haemolysis
    - Marked clinical signs with relatively mild anaemia
    - Insidious onset suggests primary bone marrow disorder or anaemia of chronic disease
    - Relatively mild clinical signs with severe anaemia due to physiological compensation
- Trauma? A possible cause of blood loss
- Exposure to toxins e.g. warfarin / zinc
- Exposure to drugs
    - Misalliance oestrogen injection -> bone marrow damage
    - Potentiated sulphonamides trigger IMHA

Taking the sample and making the smear

Samples should be taken from the jugular vein were possible. This avoids the slower sampling rate from peripheral veins, increasing the likelihood of platelet clumping and the risk of red cell damage from excessive suction. EDTA is the anticoagulant of choice enabling a

complete blood count and smear analysis. EDTA tubes should be filled quickly after sampling and filled to the line to avoid possible sampling abnormalities such as initial red cell shrinkage (which can be as much as 5%) followed by red cell swelling over time (for example if the sample is posted). A smear should always be made as soon as possible after sampling which will allow assessment of red cell shape, size and morphology. Before analysis the sample should be checked for the presence of a clot, which will significantly reduce the red, white and platelet cell counts.

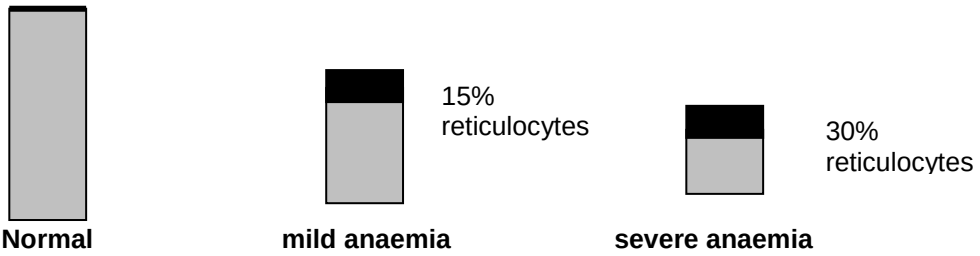
Diagnostic approach: is the anaemia Regenerative or Non-Regenerative?

Anaemia caused by the loss of destruction of red cells is regenerative as the bone marrow tries hard to replace the cells lost. This response takes 3 days to start and at least 7 days get to its maximal effect and is seen by an increase in polychromatic cells (reticulocytes within the circulation. This increases the MCV and decreases MCHC leading to a macrocytic, hypochromic anaemia. If this response is not seen and there are no polychromatic cells in the circulation, then the anaemia is non regenerative and the anaemia is normocytic and normochromic.

Counting reticulocytes gives a good indication of the bone marrows erythropoietic activity. In general the higher the reticulocyte count the more active the bone marrow response will be. As a percentage of circulating red cells the reticulocyte response is characterised as:

- mild - 2 - 4%
- moderate 5 - 20%
- marked > 20%

BUT since the reticulocyte percentage is a ratio of reticulocytes to mature cells it can be misleading for example:



Here both the mild and severe anaemia have same absolute number of reticulocytes circulating, but the reticulocyte percentage is higher for the severe anaemia, giving the false impression that the severe anaemia is more regenerative than the mild anaemia.

It is therefore preferable to use the absolute reticulocyte count, which corrects for variation in red cell number i.e. the degree of anaemia.

Absolute retic count (x 10^9 /l) = observed % reticulocytes x RBC count (x 10^{12} /l) x 10

If the regenerative response is appropriate (i.e. if the marrow is functioning normally) a severe anaemia should result in a marked reticulocytosis.

Degree of regeneration	Canine reticulocytes x 10^9/l	Feline aggregate reticulocytes x 10^9/l
None	60 (1%)	<15 (0.5%)
Slight	150 (4%)	50 (2%)
Moderate	300 (10%)	100 (4%)
Marked	>500 (>25%)	>200 (>5%)

Feline reticulocytes
Cats have two forms of reticulocytes which are the aggregate and punctate reticulocytes. Aggregate reticulocytes are immature and look like canine reticulocytes. They circulate for short periods and then mature into punctate reticulocytes. These circulate for up to 4 weeks. Normal feline blood will have low levels of aggregate reticulocytes but up to 10% punctate reticulocytes. Following blood loss the aggregate count will increase and peak around 4-7 days. Thus the aggregate count (rather than the punctate count) should be used to assess bone marrow response.

Red cell indices

Mean cell volume (MCV) indicates the average size of the red cells.
Normal size = normocytic, Enlarged = macrocytic, Small (flat) = microcytic

- Increased MCV is due to:
    - Regenerative anaemia. Increased numbers of reticulocytes / immature red cells are present in the circulation. Since these cells are larger than mature red cells the MCV is elevated.
    - MCV often increases in aged samples due to red cell swelling
    - FeLV may cause a non-regenerative anaemia in which enlarged red cells are formed
- Decreased MCV is due to:
    - Iron deficiency as rate of haemoglobin synthesis is reduced
    - Delayed nuclear degeneration
    - Congenital portosystemic shunts
    - Acquired shunts in severe liver disease

MCV may be directly measured by an automated cell counter or may be calculated from the PCV and RBC

$$\text{MCV (fl)} = \frac{\text{PCV (\%)} \times 10}{\text{Total RBC } (\times 10^{12}/\text{l})}$$

MCH and MCHC
Haemoglobin concentrations are calculated from the PCV, RBC & Hb.

$$\text{MCHC (g/dl)} = \frac{\text{Haemoglobin (g/dl)} \times 100}{\text{PCV (\%)}}$$

$$\text{MCH (pg)} = \frac{\text{Haemoglobin (g/dl)} \times 10}{\text{Total RBC } (\times 10^{12}/\text{l})}$$

Mean cell haemoglobin (MCH) indicates the weight of haemoglobin per average red cell. It does no take account of cell size. This parameter is not as useful as MCHC and so is not usually considered when evaluating haemograms.

Mean cell haemoglobin concentration (MCHC) indicates the average concentration of haemoglobin per red cell. Normal MCHC defines the RBCs as normochromic, decreased MCHC is synonymous with hypochromasia.

Decreased MCHC occurs in:

- Regenerative anaemia - larger, immature red cells / reticulocytes contain relatively less haemoglobin per cell than mature cells.
- Iron deficiency anaemia

Elevated MCHC

- Most commonly an artefact due to haemolysis.

The MCV and MCHC can be used to classify anaemia:

Morphological Classification	Possible Aetiology
Macrocytic, hypochromic	Haemorrhage or haemolysis
Normocytic, normochromic	Non-regenerative anaemia or following acute blood loss before erythroid regeneration occurs
Microcytic, hypochromic	Iron deficiency anaemia due to chronic external blood loss
Macrocytic, normochromic	FeLV, myeloproliferative disease

It needs to be noted that the MCV and MCHC are only altered large numbers of cells are altered in size or Hb concentration and that they are not sensitive indicators of a regenerative response. Looking for polychromasia +/- anisocytosis is invaluable in detecting small changes.

Red cell morphology changes

- **Macrocytes**
  These are larger than normal red cells (about the size of a neutrophil) that are often immature red cells (as a result they are usually polychromatic and may be hypochromic). They can be found due to cell swelling if tubes are under filled and in association with FeLV infection. Occasionally they can be seen as a breed related finding in miniature Poodles.

- **Microcytes**
  These are smaller than normal red cells and may contain normal or reduced amounts of haemoglobin. They can be a normal finding in some breeds including the Shar Pei, Chow Chow, Sheba Inu and Akita; often this is associated with an increased potassium content compare to other breeds. Microcytes are often seen associated with abnormal iron metabolism seen in liver disease (for example associated with portosystemic shunts) and iron deficiency secondary to blood loss.

- **Echinocytes**
  Echinocytes or Burr cells vary form irregularly shaped cells (type 1) to regularly spaced blunt (type 2) and sharp (type 3) projections. The classified can help determine there cause with a drying artefact being the most common cause for types 1 and 2. Other causes include lymphoma, doxorubicin administration, glomerulonephritis, chronic renal disease and snake envenomation (type 3).

- **Spherocytes**
  Spherocytes occur in immune mediated disease and are caused by a phagocytic cell taking a proportion of the cell membrane, leaving the same amount of content as a result the cell loses its normal biconcave disc shape and become round, appearing dark and small under the microscope. Whilst some spherocytes can be normal larger number indicate the presence of IMHA.

- **Codocytes**
  Codocytes or target cells, have an area of central density surrounded by a clearer zone and peripheral density. They are caused by an increase in cholesterol to phospholipid ratio. They are commonly seen as part of a regenerative response and

after steroid therapy. They can also be seen in dogs with liver disease, iron deficiency anaemia and after splenectomy.

- **Acanthocytes**
  These are irregularly speculated red cells with variably distributed and sized surface projections. They are also caused by cholesterol / phospholipid abnormalities and can be seen in regeneration as well as secondary to diseases which cause disruption to vascular beds, for example haemangiosarcoma, DIC and vasculitis.

- **Schistocytes**
  Schistocytes are red cell fragments caused by mechanical trauma. As such they are seen secondary to DIC, splenic neoplasia, secondary to thrombosis, valvular heart disease and myelofibrosis.

- **Eccentrocytes**
  These occur when oxidative damage leads to the 2 cellualr surfaces sticking together and fusing, with the fused membranes appearing as a semicircle in the cell membrane. They are a sensitive marker of oxidative damage in dogs (e.g. onion toxicity) in cats Heinz bodies are more commonly seen as the result of red cell oxidative damage then eccentrocytes.

Red Cell Inclusions

- **Infectious inclusions**
  Cellular inclusions occur in a range of parasites such as heamoplasma, Babesia and distemper – we will cover these in more detail in session 2.

- **Heinz bodies**
  Heinz bodies occur secondary to oxidative damage (e.g. secondary to paracetamol toxicity or severe inflammation for example pancreatitis) and are seen in cats more commonly than eccentrocytes. They are usually seen as protrusions from or accumulations just under the membrane.

- **Howell-Jolly Bodies**
  These are nuclear remnants and are circular inclusions seen in the centre of the cells. They are usually seen as part of the regenerative response but can also be seen in splenic or bone marrow disease.

- **Basophilic stippling**
  This is usually seen as part of the regenerative response but can also occur secondary to lead toxicity.

<u>Bone Marrow Biopsies</u>

Once the technique is mastered, collecting bone marrow biopsies is a relatively simple procedure and can give lots of very useful information about patients.

Indications for bone marrow sampling include:

- Non regenerative or inappropriately regenerative anaemia
- Pancytopenia or investigation of reduced production in more that than once cell line e.g. leucopenia, thrombocytopenia or anaemia. (thrombocytopenia is not a contraindication for sample collection)
- Suspected neoplastic diseases involving the bone marrow e.g. Lymphoma, leukeamia, primary thrombocytosis
- Evaluation of lytic bone lesions
- Staging of neoplasia e.g. lymphoma, mast cell tumours
- Investigation of unexplained hypercalcemia, hyperglobulinaemia or pyrexia of unknown origin
- Definitive evaluation of iron stores in the dog

Several different sites can be used including the proximal femur and the ilial wing, however the proximal humerus is preferred by most authors as it allows rapid and easy collection of both aspriates (used for cytology, giving information about cellular detail and structure) and core samples (used for histopathology, giving information about cellularity and architecture). If a 'dry' tap is obtained, a sample can be obtained from another site. Samples are usually collected with little lasting discomfort, however collecting the sample is uncomfortable. As a result most clinicians prefer to anaesthetise patients for this procedure.

Equipment Needed for Bone Marrow Biopsy

- Jamshidi bone marrow needle. These are available from a number of suppliers' e.g. Veterinary instrumentation and the size should be appropriate to the patient and the site being aspirated.

Animal size	Suggested Jamshidi Needle
Cats / Dogs <5kg	16 – 18 gauge
Dogs 5-15kg	15 – 16 gauge
Dogs 15-30kg	13 – 15 gauge
Dogs 30-50kg	11 – 13 gauge
Dogs >50kg	8 gauge

- 20ml syringe
- Local anaesthetic e.g. lidocaine
- No. 11 Scalpel blade
- Sterile anticoagulant. CPDA collected from a blood transfusion bag is best, however EDTA prepared form a CBC tube is adequate. EDTA is prepared by filling a CBC EDTA tube with the appropriate amount of sterile saline.
- Microscope slides (have between 15 & 20 microscope slides laid out prior to the procedure where possible on a 45° slope)
- EDTA tube for excess aspirated sample. This should be saved to make extra slides as needed or for performing further tests such as PCR for tick-borne disease or FIV
- Sterile gloves
- Sterile drape
- Assistant for positioning limb

Collecting a Diagnostic Bone Marrow Sample

1. A blood sample should be collected to allow interpretation of bone marrow on the day the sample is collected. This allows interpretation of the bone marrow in light of the peripheral haematological status.
2. The easiest site to collect bone marrow is from the proximal humerus. This requires the patient to be anaesthetised for collection of the sample (both for positioning, but also due to the fact that collection of the marrow sample is uncomfortable.
3. To use this site the animal needs to be placed in lateral recumbency. This should be right lateral recumbency for a right handed operator and left lateral recumbency for a left handed operator.
4. The area over the shoulder joint is clipped and surgically prepared
5. An assistant positions the leg so that both the shoulder and elbow joints are flexed so that the humerus lies roughly parallel to the thorax. If possible the elbow should be rotated inwards which will bring the most prominent part of the shoulder joint upper most. The assistant will need to apply counter pressure to your needle placement, thus they will need to be strong!
6. The needle will be inserted into the most prominent palpable part of the humeral head which is the top of the humeral head. Local anaesthetic e.g. 2% lidocaine is instilled (0.25ml in cats and up to 1-2ml in dogs) in the area all the way down to the periosteum of the humerus.
7. A small stab insiscion id made over the site with the No. 11 blade
8. The needle then needs to be coagulated with the CPDA or EDTA. Bone marrow clots very quickly, thus an anticoagulant is essential. Even then once collected the smears should be made as quickly as possible. The stylet is withdrawn from the needle and with the 20ml syringe anticoagulant is flushed through the needle and emptied, thus the needle is coated with anticoagulant. The stylet is replaced and locked in place.
9. The Jamshidi needle is held in the palm of the hand using a pistol grip, with the heel of the needle in the palm of the hand and the needle being supported by the index finger.
10. The needle is inserted, with the stylet in place, into the greater tubercle of the humerus. The needle is inserted parallel to the humerus, aiming for the point of the elbow. A firm rocking motion should help advance the needle, however firm pressure is needed to advance the needle. Similarly firm counter pressure needs to be applied by your assistance to stop the animal moving off the table.
11. Once the medullary cavity is reached reduced resistance is felt. Once in the medullary cavity the needle should feel well seated, as if the animal could be lifted off the table by raising the needle.
12. Once in position the stylet is removed and the 20ml syringe attached. The syringe is then firmly aspirated and bone marrow (thick bloody material) should appear in the hub of the needle. Once present further aspiration should not be attempted as it will result in haemodilution.
13. Smears are prepared by running the bone marrow down the slides at a 45° angle. This allows excessive blood to run off and spicules of bone marrow should remain. These are then smeared as for FNA's or blood smears. Good smears once prepared should have fatty deposits and small spicles of bone marrow.
14. A core biopsy sample can then be obtained by advancing the needle a further 2-3cm down the humeral shaft without the stylet in place. Once advanced the needle is moved sideways quickly in different directions and withdrawn quickly. The core is then collected be inserting the blunt probe from the tip to the handle of the needle.
15. Impression smears can be made by rolling the core down a glass slide, before placing it in formalin for histopathological analysis.
16. A sterile dressing is placed and opioid analgesia given for 4-6 hours post procedure.

Transfusion medicine in Practice

Blood transfusion is most commonly performed in veterinary medicine to provide additional red blood cells to anaemic patients. It is also performed to correct coagulopathies such as those that occur secondary to anticoagulant rodenticide intoxication. Very occasionally they are performed if a patient is severely thrombocytopenic and they have life-threatening bleeding. Many transfusions performed in practice rely on blood donors that are located near to the practice, and these donors are brought into the practice so that blood can be taken. This blood is then immediately given to the patient. Increasingly, banked blood is used; this has the benefit of being split into components, so that only red blood cells or plasma are given. In addition, this allows the blood products to be stored so that a donor does not have to be located in an emergency. These notes will focus on blood donation and the administration of blood.

Blood Donation

Selection of the feline donor

The ideal cat donor should be larger than 4.5kg to ensure sufficient blood volume can be taken, and of course preferably be a friendly and good-natured one! Donors should be screened for feline viruses (FeLV, FIV and FIP) prior to giving blood and should ideally be indoor cats to avoid the risk of contracting these and other diseases. Cats with *Mycoplasma (Haemobartonella) felis* infection should not be used nor should any cat with obvious current disease (e.g. renal, hepatic, abscesses etc.). Where possible the donor should also be fully vaccinated and regularly wormed. The PCV of the donor should be established prior to donation to ensure it is within normal bounds. Cats will frequently require sedation to donate blood.

Ideally, the cat should be older than 18 months but less than 8 years of age. Pregnant queens should be avoided due to the risks imposed on the mother and unborn foetuses. However, previously pregnant but currently non-pregnant queens can be used as donors. Cats should not donate blood more than once every 3 months.

Blood groups Cats

Cats have different blood types and it is important that cross matching (finding the correct donor blood to go with the correct recipient) is performed. Blood groups are determined by the presence of specific antigens on the surface of the erythrocytes. These can trigger an immune-system response in the recipient which will result in haemolysis of the donated erythrocytes.

Cats are type A (most in the UK), type B, or very occasionally AB. There is **no** universal **donor** in cats as cats all have naturally occurring antibodies (unlike dogs and humans). Type B cats have high anti-A titres (95% have antibodies) so these should not be used as donors for either group A or group AB recipients otherwise a major transfusion reaction will occur. There is also a rare *Mik* antigen that cannot be screened for commercially. As such, all cats should not only be typed but also (ideally) be cross matched prior to transfusion if clinical circumstances commit.

In general:

1) Type AB recipient should receive type AB blood preferably although they can receive type A blood as such donors have a low anti-B titre (whereas type B donors have a very high anti-A titre)
2) Type A cats should receive type A blood
3) Type B cats should receive type B blood

The half-life of transfusion erythrocytes in the correctly cross matched recipient is 30-38 days. If incorrectly matched this can be much shorter (matter of hours in the case of A erythrocytes transfused into a B type cat or 1-2 days in the case of B erythrocytes transfused into an A type cat).

Breed	% Type A	% Type B
DSH (UK)	97.1	2.9
Abyssinian	84	16
Birman	82	18
Cornish Rex	67	33
Persian	86	14
Siamese	100	0
Somali	82	18
Sphinx	83	17
Turkish van	50	50

Procedure for blood typing cats

There is a simple rapid card-agglutination test (Rapid Vet-H Feline®, DMS Laboratories) available. Blood typing of both donor and recipient is necessary.

Cross matching cats

It is important to cross-match especially if there has been more than one transfusion for any individual recipient; as they can go on to develop new antibodies. In addition, with the recently identified Mik antigen, it further adds to the case that all cats should be cross-matched.

In cats the rapid slide test is generally used as a means of cross-matching blood. This is performed as follows:

1) A blood sample is taken from both donor and recipient
2) 2ml of blood from each cat is placed into a plain tube and allowed to clot and 1ml from each is placed in a potassium EDTA tube
3) All tubes (both potassium EDTA and plain) are spun down to separate the cells from serum/plasma
4) Prepare four clean glass slides and label them:
    a. Donor control
    b. Major cross match
    c. Minor cross match
    d. Recipient control
5) To the donor control slide add one drop of spun down potassium EDTA donor erythrocytes to two drops of donor serum or plasma.
6) To the major cross match slide add one drop of spun down potassium EDTA donor erythrocytes to two drops of recipient serum or plasma.
7) To the minor cross match slide add one drop of spun down potassium EDTA recipient erythrocytes to two drops of donor serum or plasma.
8) To the recipient control slide add one drop of spun down potassium EDTA recipient erythrocytes to two drops of recipient serum or plasma.
9) Rock the slides back and forth and examine with the naked eye within 2 minutes of mixing to observe for macroscopic agglutination reactions and examine under the microscope within 5 minutes for microscopic agglutination. It is important to differentiate between agglutination (clumps of haphazardly arranged erythrocytes) from rouleaux formation (stacks/columns of erythrocytes one on top of another) as the latter is not indicative of a cross-reaction.

Results of the above slide test in cats can be interpreted thus:

1) If you have mixed type A serum with type B or AB erythrocytes then this results in a weak naked eye (macroscopic) agglutination or no agglutination at all
2) If you have mixed type B serum with type A or AB erythrocytes then there is usually a strong macroscopic agglutination reaction.
3) If you have mixed type AB serum with type A or type B erythrocytes then there is no reaction.
4) Therefore a positive major cross-match (strong agglutination) suggests type B recipient and therefore type A or AB as the donor. To differentiate between the type A and type AB donor-look at the minor cross-match slide which will be little or no agglutination in the case of the type A donor and no agglutination with the type AB donor.

5) A positive minor cross-match slide test (strong agglutination) suggests type A or AB as the recipient and type B as the donor. To differentiate between the two, look at the major cross-match test where the type A recipient will be negative or weakly positive and the type AB recipient will be negative.
6) If the major and minor cross matches are both negative then the donor and recipient are likely to be the same blood type and therefore the donor is the most appropriate one to use. Occasionally negative results may still have a weak anti-B or anti-A titre and therefore mild haemolysis may still occur sometime after the transfusion.
7) If the donor or recipient controls show agglutination then it is usually due to operator error.

Blood typing and cross matching dogs

Selection of donor

As with cats, the larger the dog, and the more placid it is, the easier and more suitable a donor it will make. The dog should be health screened to ensure it is free of disease, should generally be more than 25-30kg in weight and between 18 months and 8 years of age. Ideally the donor should be fully vaccinated and regularly wormed. The PCV should be established prior to donation to ensure it is within normal limits. Donor animals should not give blood more than once every 3 months. Dogs should not need to be sedated for donation.

Pregnant bitches should not be used owing to the risk to dam and foetuses. In addition, bitches that have had previous pregnancies should not be used because of the development of antibodies in the dam e.g. if a DEA 1.1 negative bitch (see below) is bred to a DEA 1.1 positive dog, she may develop DEA1.1 antibodies and so increase the risk of any blood used from this bitch causing a minor transfusion reaction.

Donors should not have received previous blood transfusions and should be screened (although in the UK they are likely to be clear) of conditions such as *Brucella canis* and *Dirofilariaimmitis* (heartworm).

Blood groups dogs

In dogs there are 6 different DEA (dog erythrocyte antigen) blood groups (1.1, 1.2, 3, 4, 5 and 7) and over 20 subtypes. However most are not of significance. The important blood group is DEA 1.1. Breeds tend to follow trends; therefore Greyhounds are often used as blood donors as they are generally negative.

In dogs, unlike cats, there are no naturally occurring antibodies to the different blood groups. Therefore, it is rare for a reaction to occur the first time a patient is transfused. But, reactions can still occur - they just do so later on as the recipient makes antibodies. This commonly occurs when say a DEA1.1 negative dog receives blood from a DEA1.1 positive one - the antibodies in the recipient (DEA1.1 negative) take time to form and so a reaction may be seen 1-2 weeks after the transfusion. Thereafter, once these antibodies have formed, the recipient will then react quickly to any further transfusion of DEA1.1 positive blood, and the reaction will be seen within hours.

The same process occurs in a DEA1.2 negative recipient receiving DEA1.2 positive blood – only all the reactions listed above tend to take slightly longer to appear and are generally less severe. There is also some cross reactivity between DEA 1.1 and 1.2. e.g. if a patient is DEA1.1 negative and is given a DEA1.1 positive transfusion, it will produce antibodies to the DEA1.1 erythrocytes of the donor. If that same patient is then transfused at a later date with DEA1.2 positive blood - there will often be a delayed haemolytic reaction to the donor's blood. Just to confuse things even further, evidence suggests that there is a DEA1.3 group which again provides cross-reactivity with DEA1.1 and 1.2 after initial transfusions. This is why any dog receiving more than one blood transfusion must be blood typed and cross-matched with the donor.

The universal donor dog is negative for DEA 1.1, 1.2, 2, 3, 5 and 7. The most important DEA's to be negative to are DEA 1.1 and 1.2, particularly 1.1. There is no universal recipient for dogs.

Procedure for blood typing dogs

Commercial tests are currently available for DEA1.1 determination (Rapid Vet-H® and Alvedia).

Cross matching dogs

Cross-matching is important particularly if the recipient has received blood greater than 4 days prior to a second transfusion or has had a previous reaction, has been pregnant, or indeed if the previous history of the patient is unknown. In any case, the slide test as for cats may be used. In addition the test tube test is also recommended (where time allows). This can be performed as follows:

1) Blood samples are collected as for the slide test
2) The spun down erythrocytes from both the donor and recipient should be made into a 4% solution by taking 0.2ml of spun down erythrocytes from the potassium EDTA sample and adding this to 4.8ml of isotonic saline - this helps to prevent rouleaux formation
3) Four tubes are labelled as for the four slides above
    a. Donor control
    b. Major cross match
    c. Minor cross match
    d. Recipient control
4) Into each tube, 2 drops of the 4% red cell solution and 2 drops of serum/plasma is added as before i.e.
    a. To the donor control tube add two drops of donor erythrocyte suspension to two drops of donor serum or plasma.
    b. To the major cross match tube add two drops of donor erythrocyte suspension to two drops of recipient serum or plasma.
    c. To the minor cross match tube add two drops of recipient erythrocyte suspension to two drops of donor serum or plasma.
    d. To the recipient control tube add two drops of recipient erythrocyte suspension to two drops of recipient serum or plasma.
5) The tubes are then lightly centrifuged at low speed for 20-30 seconds to encourage the red cells to accumulate.
6) The tubes are gently mixed and held up to the light to examine for macroscopic agglutination or haemolysis.
7) Then examine any apparent clots microscopically to determine if they are associated with non-specific rouleaux formation or true clots.
8) The degree of clotting/cross reaction can then be graded from
    a. No clots microscopically or macroscopically (negative)
    b. Microscopic aggregates (+w)
    c. Small macroscopic aggregates (+1)
    d. Medium macroscopic aggregates (+2)
    e. Several large macroscopic aggregates (+3)
    f. One large clot (+4)
9) Technically speaking the process should be repeated again after 37°C incubation at for 30 minutes if the results above are negative, as anti-DEA1.1 and antiDEA1.2 antibodies have a maximum reactivity at 37°C.

The results are interpreted thus:

1) A positive major cross-match suggests a sizeable antibody titre in the recipient to the donor erythrocytes. Therefore that donor should not be used for that recipient and usually indicates that there are anti-DEA1.1 antibodies present.
2) A positive minor cross-match suggests that there are antibodies in the donor against the recipient's erythrocytes. Strongest reactions occur in the presence of anti-DEA1.1 antibodies and will preclude the use of the donor's plasma. The donor's erythrocytes could be used providing they are washed in saline before administration, but this is rarely possible in practice.
3) A positive recipient control suggests that the recipient is auto-agglutinating, probably due to non-specific anti-erythrocyte antibodies rather than specific DEA ones.
4) A positive donor control usually suggests a handling error, or rarely that the donor has auto-immune mediated haemolytic anaemia.

Equipment required for blood collection
The following is an example of the equipment required for collection of the donor blood sample:

1) Collection bags/human collection packs (for dogs)
2) 60ml syringe (for cat blood collection)
3) Anticoagulants

60ml Syringe
This can be used for collecting blood in cats. They should be pre-filled with CPD from a human collection bag prior to use (see below).

Collection bags
Human collection bags are generally used for dogs (see below). These are generally supplied as a 450ml capacity bag containing 63ml of citrate phosphate dextrose (CPD) or citrate phosphate dextrose adenine$_1$ (CPDA$_1$) as anticoagulant. The bag is connected to a length of drip tubing which is connected to 16 gauge thin walled needle.
If the intention is to separate the blood into plasma and packed red cells, then so-called 'triple' or 'quad' bags are available.

Anticoagulants
The anti-coagulant used for storing blood for any length of time is citrate. The citrate used is citrate phosphate dextrose (CPD) and is supplied in human collection bags as 63 or 70ml CPD per bag. This is too much for taking samples from smaller animals such as cats as the 63ml CPD bag will collect 450ml donor blood and the 70ml collection bag will collect 500ml blood. Therefore, for cat collections, 7.5ml of CPD is withdrawn from the bag into a 60ml syringe that is then used for the blood collection. For collecting erythrocytes only (i.e. separating blood post collection) the anticoagulant used should be citrate phosphate dextrose adenine$_1$ (CPDA$_1$) (which can be used to collect whole blood as well). As with CPD it is supplied in 450ml bags with 63ml of anticoagulant.

Calculation of blood volume required
The total volume of blood required for the recipient depends of course on the size of the recipient and on its current packed cell volume (PCV) in relation to the donor's PCV.Generally as a rough rule of thumb 2ml of transfused whole blood/kg of the recipient's weight raises the recipient's PCV by 1% and 1ml/kg of packed red blood cells will raise the PCV by 1%. This rule is fairly accurate (as supported by a recent paper!) however a more specificformula can also be used if needed:

$$Volume\ of\ Donor\ Blood = \frac{80\ (dog) * BW\ (kg) * desired\ change\ in\ PCV}{PCV\ of\ donor}$$

Where BW is the body weight of the recipient and for cats the factor in the equation above is 60.

Collection techniques for blood (cats)
In cats, sedation isfrequently required as the site of venepuncture is the jugular vein and most cats will not sit still for long enough to allow collection. Recommended sedation combinations include standard combinations such as ketamine and midazolam/diazepam. Alternatively full anaesthesia with propofol induction and isoflurane/sevoflurane maintenance can be used. The alpha 2 agonists should be avoided.The cat is maintained in lateral recumbancy and the neck over the jugular vein is clipped and surgically prepared. A 19 gauge winged (butterfly) catheter attached to a 60ml syringe is inserted aseptically into the cats jugular. The blood is collected into a 60ml syringe containing anticoagulant (either citrate phosphate dextrose adenine or acid citrate dextrose used at ratio 1ml to 7ml blood. Approximately 11-13mL/kg of blood is collected from the donor.

Pressure should be applied to the jugular vein after removal of the required volume of blood to prevent haematoma formation. This may take up to 5 minutes. Cats should be offered food and kept quietly cage rested for the first 3-4 hours and then kept indoors for the next 24-48 hours. Intravenous fluids will not usually be necessary following transfusion.

Collection techniques for blood (dogs)
Most dogs will not require sedation or anaesthesia, but instead can be restrained preferably in lateral recumbency. If sedation is needed, then you should reconsider your choice of donor. The jugular vein is the only vein that should be used for blood collection. The site is clipped and surgically prepared. The needle on the human collecting bag is used and placed, as for the cat into the jugular. The needle should be well sited into the vein to ensure it does not become dislodged during the collection process. The maximum amounts which can be collected from a dog safely are around 16-18m/kg. In reality it is more usual to collect one unit (450ml) from the donor. It is useful to weigh the blood bag before collection as a full unit of 405ml (the minimum amount which should be withdrawn to mix with 63ml of anticoagulant) up to 495ml blood (maximum amount of blood which should be withdrawn to mix with 63ml of anticoagulant) weighs from 429-525g respectively. This allows monitoring of the volume of blood extracted. The time to collect a unit of blood is usually around half an hour.If the needle becomes dislodged or a haematoma forms then it is best to re-site the venepuncture location. The donor should have manual digital pressure applied at the site of venepuncture for at least 5 minutes after removal of the needle to prevent haematoma formation. Dogs should be kept quiet for the following evening and night and only very lightly exercised for the following 72 hours. Dogs will not usually require intravenous fluids following donation.

Blood and blood product storage and the use of blood banks.
Blood banks are now present in UK with changes in the interpretation of the existing legislation, which now allows the collection and storage of blood and blood products. This allows storage and therefore increased availability of blood products. In addition, it allows the clinician to tailor the therapy according to the needs of the patient. For example, anaemic patients may require red blood cells but not the plasma, and similarly, coagulopathic patients will require plasma (coagulation factors) and not the red blood cells.

Administering the Transfusion
If the blood has been directly removed from the donor or only been refrigerated before administration to the recipient then it does not need to be warmed, as it will come to room temperature quite quickly. If preferred though it can be warmed in water ensuring that the water is no hotter than 37°C. Blood and plasma products should NEVER be warmed in the microwave as this will damage the contents.

Whole blood should be gently inverted to mix its components prior to administration. For platelets to be functional, the fresh whole blood should be kept at room temperature and used within 4 hours of collection.

Any vein may be used to administer the transfusion. If venous access is not possible then blood may be administered intraosseously following placement of an intraosseous needle. Specific administration sets are used for blood which contain a microfilter to remove microclots. The rate of administration is 1 ml/kg/hour for the first 30 minutes to allowing the recipient to be checked for any signs of a transfusion reaction, and the remainder within the next 3.5 hours (transfusion complete within 4 hours). Faster rates of administration may be used if the patient is decompensating rapidly, although it is then not possible to monitor for adverse reactions.The rate of blood administration is usually controlled purely via drip rate. Alternatively for smaller patients, a syringe driver can be used (e.g. cats and neonates).

Transfusion monitoring and possible problems

<u>Post transfusion monitoring</u>
The patient should be closely monitored during the initial slow phase of blood transfusion for signs of an acute reaction. However, delayed reactions can also occur. Therefore it is important to keep the patient under observation for the following clinical signs which may indicate a possible transfusion reaction:

1) Fever (most common in veterinary medicine)
    a. Rule out contamination of blood and haemolysis
    b. Treatment is supportive and fever is usually self-limiting (anti-histamines will not improve this)
    c. Stop/slow down the transfusion

2) Vomiting (second most common)
    a. Slow down the transfusion
3) Urticaria
    a. Acute hypersensitivities are 3rd most common
    b. Treat with anti-histamine and steroids only if necessary

In addition, the patient should be monitored for the following:
1) Tachycardia (although cats will occasionally show bradycardia)
2) Dyspnoea
3) Weak pulses
4) Pale mucous membranes
5) Weakness, lethargy, decreased mentation
6) Muscle tremors
7) Evidence of haemolysis e.g. haemoglobinuria and haemoglobinaemia.

Possible problems

There are four main reactions that may be seen after a transfusion:
1) Acute immunological transfusion reaction
2) Delayed immunological transfusion reaction
3) Acute non-immunological transfusion reaction
4) Delayed non-immunological transfusion reaction

Acute immunological transfusion reaction

This is where the recipient reacts to the erythrocytes or plasma proteins administered. It can be manifested as an acute anaphylactic reaction or acute haemolytic crisis. There is often pyrexia and haemoglobinuria and haemoglobinaemia indicative of intravascular haemolysis. In dogs this is mediated via the immunoglobulin IgG. In cats it is usually more severe and is due to IgM with anaphylactic shock being the main clinical sign. This can include collapse, apnoea, hypotension and bradycardia. In milder cases in cats, tachycardia and polypnoea may be seen with cardiac arrhythmias.

Reactions to plasma proteins can also occur and may be seen as acute anaphylactic shock, with urticaria, angioedema, salivation, vomiting, diarrhoea and even bronchoconstriction (particularly in cats) similar to an allergic reaction. It is believed to be mediated by IgA antibodies. This may occur within minutes of starting the blood/plasma transfusion. Collapse and weak thready pulses with signs of hypotension are also seen. Such reactions are uncommon in dogs and cats but may be seen. There is usually no pyrexia in such cases and they do tend to respond to administration of antihistamines and corticosteroids whereas the acute reactions to miss-matched erythrocytes do not.

Delayed immunological transfusion reaction

As its name suggests this reaction occurs some days after the transfusion (anything up to 5 days after). At this point the PCV starts to fall dramatically (PCV levels should stabilise for around 3 weeks in dogs and 5 weeks in cats due to the normal half life of the transfused erythrocyte).

Acute non-immunological transfusion reaction

Acute circulatory overload from too rapid a rate of blood transfusion can occur leading to cardiac abnormalities and pulmonary oedema/pleural effusion. In addition an anaphylactoid type reaction (similar to an anaphylactic reaction), whereby non-IgE-mediated mast cell degranulation occurs triggered by plasma proteins which can lead to shock in worst case scenarios.

Hypothermia is another potential side-effect from rapid administration of cold blood products, particularly in smaller patients/neonates.Hypocalcaemic tetany may also occur due to chelation of blood calcium by the presence of large amounts of citrated blood products with muscle tremors, collapse, seizuring vomiting and arrhythmias. Ionised calcium levels will be lowered. This can be managed with calcium gluconate at 0.5-1.5ml/kg IV given slowly over 10 minutes.

Delayed non-immunological transfusion reaction
Sepsis is a potential problem in stored blood products. This may ensue over the first 24-36 hours post transfusion with signs similar to those seen with immunological reactions (e.g. vomiting, diarrhoea, tachycardia, pyrexia, and haemolysis). The bacteria or fungi responsible are usually those that can survive in chilled storage such as *Yersinia* spp. and *Pseudomonas* spp. in the case of whole blood products.

Use of Oxyglobin®
Oxyglobin® is a polymerised bovine haemoglobin solution. It is used where a blood transfusion is not possible, but the oxygen carrying capacity of the patient's blood is compromised. Its advantages are that it is easy to use, it has excellent penetrative powers through small or damaged capillary beds as the haemoglobin molecule is about 100 times smaller than the erythrocyte and it rarely causes any reactions after administration. Sadly it is not currently in production, which makes its availability very limited.

The disadvantages include interference with a number of biochemical tests and the development of haemoglobinaemia, haemoglobinuria and jaundice. It also does not last very long within the bloodstream with a half-life of 18-26 hours and a maximum duration of 82 hours whereas red cell transfusions last 4-6 weeks. Oxyglobin® has significant oncotic potential and will act as a plasma volume expander as well as causing some vasoconstriction, both of which can lead to an increase in blood pressure, which may be beneficial or detrimental depending on whether the patient is hypovolaemic or not. Oxyglobin® dose rate is 10-30mg/kg with a maximum rate of administration of 10ml/kg/hour in dogs.

Basic in house cross matching

- Collect 0.5-1ml blood into EDTA from both donor and recipient
- Centrifuge samples
- Separate plasma into new plain tubes and label
- Label 4 glass slides as below

Donor control Donor RBC + Donor plasma	Major X-match Donor RBC + Recipient plasma
Recipient control Recipient RBC + Recipient plasma	Minor X-match Recipient RBC + Donor plasma

- One drop of RBCs is mixed with 2 drops of plasma
- Mixed the 2 immediately
- Gentle rock slides back and forth
- Observe for macroscopic agglutination within 2 minutes
- Apply coverslip and observe under x40 lens for microscopic agglutination within 5 minutes
- Equivocal results – repeat with diluted RBCs
- 0.2ml packed RBCs with 4.8ml saline