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TRANSFUSION MEDICINE

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Since the first blood transfusion was carried out on a dog at Oxford University in 1666, the science of transfusion medicine has advanced a great deal. To perform transfusion medicine successfully, there must be an understanding not only of when and how to administer whole blood and its products but also of safe ways of obtaining and potentially storing these blood products. It is vital that blood used for transfusion is collected, processed, stored and used in a way that minimises the chance of any harm occurring to either the recipient or donor.

To allow the use of human blood collection systems that facilitate sterile collection and separation of blood components, canine donors should weigh more than 25 kg. This allows collection of a full 450ml unit. They should be adult animals but not old – the maximum age for a donor is variable and dependent on the breed, however as most donors are large breed dogs, we commonly use 8 years as our upper age limit. All dogs should be fully vaccinated and receive regular veterinary preventative health care. A full physical examination should be performed by a veterinary surgeon prior to each donation and should be unremarkable. Donors should be screened for infectious diseases such as Dirofilariasis, Babesiosis, Leishmaniasis, Ehrlichiosis, and Rocky Mountain Spotted Fever. The dog should have a good temperament which will allow a full unit to be collected. Dogs such as greyhounds with long thin necks and easily visible jugulars make excellent donors. Dogs with blood type DEA 1.1 negative are the most versatile donors however as long as recipients are typed, dogs that are DEA 1.1 positive dogs can be used too.

Generally speaking, feline blood is collected into a syringe and a full unit is about 60ml. Feline donors should thus be fairly large cats (5-7kg). Although brachycephalic breeds may be used, it is often harder to access their jugular veins reliably. Invariably cats require sedation for donation and thus the donor must be healthy and similarly to dogs should be fully vaccinated and receive regular veterinary preventative health care. There are several feline diseases that may be transmitted to the recipient via transfusion. The most important of these are FeLV, FIV and *Mycoplasma haemofelis* (Haemobartonellosis). Ideally donor cats should test negative for these diseases and then should be kept in an environment where subsequent infection is unlikely (i.e. an indoor environment with exposure only to other negative cats).

With both species, the jugular vein should be clipped and the area prepared aseptically. With canine blood collection, the dog can be restrained either sitting or lying in lateral recumbency and blood is collected either by gravity or suction. Suction is much quicker taking around 5 minutes to collect a unit; with gravity flow a full unit commonly takes 15-20 minutes to collect. Cats generally need sedation – various protocols can be used and should be chosen to suit the cat and the practice. Blood is collected into a 60 ml syringe (which already contains anticoagulant) by gentle suction with rocking of the syringe during collection. Cats are usually placed on intravenous fluids for a period of several hours following donation as they recover from sedation.

Most commercial blood bags used for canine donations contain CPDA 1 (citrate phosphate dextrose adenine) as their anticoagulant. This allows storage of whole blood for 28 days. When adding anticoagulant to a syringe for feline blood collection, it is recommended that either CPDA 1 or ACD (acid citrate dextrose) is used at a ratio of 1ml anticoagulant to 7ml of blood. If these are unavailable, 3.8% citrate can be used at a ratio of 1ml to 9ml of blood but the blood must be transfused immediately. Heparin is not recommended.

An animal's blood type is a genetically determined characteristic that does not change during their lifetime. Blood types simply represent proteins or glycoproteins present on the surface of the red cell. Similar to any antigenic stimulus, it is thus possible for animals to have or develop antibodies to blood types that are different to their own. Interaction between these antibodies and the red cells form the basis of immunological transfusion reactions. Dogs have at least 12 blood groups the most important of which are DEA (dog erythrocyte antigen) 1.1 and 1.2. Many of these blood groups can only be evaluated on a research basis, however luckily, dogs do not have preformed antibodies to other blood types therefore they are highly unlikely to have a transfusion reaction on a first transfusion. A transfusion from a dog of a different blood type will however sensitise the recipient's immune system to that blood type meaning there is a risk with subsequent transfusions. Crossmatching should thus be carried out for all second and subsequent transfusions in dogs even if the transfusions occur many years later. However, as DEA 1.1 appears to be the most important blood type in clinical transfusion reactions, it is best practice, whenever possible, to type all donors and recipients for this prior to their first transfusion. DEA 1.1 negative blood can be given to either DEA 1.1 negative or positive recipients, however DEA 1.1 positive blood should only be given to DEA 1.1 positive recipients wherever possible. This reduces the risk of future transfusion reactions. This has led some authors to consider DEA 1.1 negative dogs to be considered as "universal donors" – this is a slight misnomer as it does not appreciate the fact that there are many canine blood groups that we are not regularly assessing.

Cats have only two major blood groups (A and B). Importantly, cats do have preformed antibodies to the other blood type and thus MUST be blood typed prior to the first transfusion. Otherwise there is a significant risk of a serious transfusion reaction even with the first transfusion. The relative frequencies of the two blood groups vary dependent on geographical region and

breed. For example Siamese and other Oriental breeds are almost invariably type A whereas British Shorthairs may be up to 50% type B. It is often quoted that only 5-10% of Domestic Shorthairs are type B, however this is variable and at our hospital, it seems that closer to one third of the DSH patients we see are type B. Type AB has been rarely reported. Blood typing can be carried out using commercially available typing cards or performed by external laboratories.

A crossmatch is a laboratory test that determines serologic compatibility between a donor and recipient at that time point. Essentially, blood from both donor and recipient is centrifuged and the cells and plasma separated with the red cells subsequently being washed. A major crossmatch is then performed by adding 1 drop of the donor's cells to 2 drops of the recipient's plasma and a minor cross match by adding 1 drop of the recipient's cells to 2 drops of the donor's plasma. The samples are then incubated for 15 minutes and observed for any evidence of agglutination.

Traditionally transfusions have been given as fresh whole blood. However the development of component therapy allows tailoring of transfusion therapy to the patient's needs, allows a single unit to help more than one patient and storage of blood products. Component therapy involves spitting a unit of whole blood into its parts, most commonly packed red cells and plasma. A human multi-bag blood collection system is used to allow sterile separation of products and in human medicine one unit may be split into 20 or more components. Many human bags are now being supplied with a leucocyte reduction filter built into the line that the blood must pass through with the aim of reducing human transfusion reactions. The effect of these filters on canine blood is as yet unknown. Blood centrifuges are refrigerated and have 500ml buckets that hold a full unit. To maximise preservation of plasma proteins (especially the labile clotting factors), blood should be spun down and the plasma frozen within 6 hours of collection. Generally, whole blood is centrifuged at 5000 g for 5 minutes and then separated into packed red cells and plasma.

Fresh whole blood can be stored for up to 28 days at 1-6°C with an appropriate anticoagulant (e.g. CPDA-1). It should be noted however that many of the important plasma proteins will degrade fairly rapidly and be gone within 12-24 hours. Packed red cells commonly have a PCV of around 80%. They should be stored in a fridge at 1-6°C and can be stored for 21 days. Some blood bags contain an additional preservative (e.g. Nutricel/Adsol) that extends the red cell's shelf life to 35-37 days. Ideally the fridge used should be one that is not opened frequently – if this must be the case then the cells should be stored in a small cooler inside the fridge. The cells should be gently mixed twice weekly.

Fresh frozen plasma represents plasma that has been separated and frozen within 6 hours of collection. It contains all the plasma proteins including all clotting proteins. It should be stored at -18°C which is colder than most household freezers, however at this temperature the activity of all the plasma proteins is maintained for up to one year. In an ordinary household freezer, it is thought that the activity of the plasma proteins may be maintained for 2-3 months. Frozen plasma represents plasma that has either been frozen more than 6 hrs after collection, has been thawed and refrozen or has been frozen for more than 1 year. It has lost the activity of many of the important plasma proteins including the labile clotting factors (Factors V and VIII, vWF). It may still be useful however as it contains albumin (and thus may be used as a colloidal fluid) and importantly it still contains the vitamin K dependent clotting factors.

Platelet rich plasma is prepared from fresh whole blood by centrifugation at a lower speed to maximise platelet recovery. It must be constantly agitated and used within 8-12 hours. It should be remembered that even fresh whole blood only contains a very small number of functional platelets. Blood from approximately 6 donor dogs is required for sufficient platelet recovery to help severely thrombocytopaenic dogs – this limits the use of platelet rich plasma in veterinary medicine.

Cryoprecipitate is not commonly prepared but may be useful in the management of patients with von Willebrand's disease as it is a concentrated source of von Willebrand factor as well as fibrinogen, factor VIII and fibronectin. It is produced by slow-thawing fresh frozen plasma at 4°C, followed by centrifugation at a similarly cool temperature. The proteins mentioned above precipitate at this temperature and are maintained in a very small amount of remaining plasma (approximately 1/10 of the starting volume of FFP). Cryoprecipitate is stable for 1 year from the date of collection of the whole blood for transfusion purposes (not the date of preparation of the product) if maintained at or below -18°C. Cryo-poor plasma/cryosupernatant represents the plasma remaining following preparation of cryoprecipitate as above. It is a source of all coagulation and plasma proteins, except for factor VIII, fibrinogen, von Willebrand factor and fibronectin. and is stable for 5 years if stored at or below -18°C.

All blood products should be warmed gently to room temperature prior to administration. Products containing red cells should be given through a filter (270µm) to facilitate removal of any small clots and other debris that may be present. These filters may be present within the lines of blood giving sets or attached separately if using a syringe for the transfusion (common with cats). Peristaltic type infusion pumps may be used safely however some forms of fluid pump may damage the red cells - if in doubt the manufacturer should be contacted to check the pump's compatibility with blood products. With stable patients, an initial infusion rate of 0.25-0.5 ml/kg/hr should be used for the first 15-30 minutes. During this time the patient should be monitored for any evidence of a transfusion reaction. As long as no problems are identified the rest of the unit should be delivered over 4-6 hours dependent on the animal's intravascular volume status. In an emergency (e.g. severe acute haemorrhage), red cells can be given

as fast as necessary. As the PCV of packed red blood cells is so high, they must be resuspended in a non calcium containing crystalloid (ideally 0.9% NaCl) before administration to reduce sludging. Resuspension of packed cells to which plasma extenders such as Adsol has been added is not required.

Plasma products must be thawed slowly. A standard intravenous infusion set may be used for administration. The rate of administration is dependent on the reason for transfusion but commonly 4-6ml/kg/hr is used. The total dose of plasma necessary will depend on the reason for its administration however in most patients a daily dose of 20ml/kg is recommended – this should represent sufficient plasma proteins to have a beneficial effect for the patient. Cross matching is not required prior to plasma administration.

Anaemia is one of the most common reasons for transfusion in veterinary medicine. Patients with anaemia resulting from many causes including haemorrhage, intravascular haemolysis and bone marrow disease will benefit from additional red cells. In patients with a normal intravascular volume (commonly those with intravascular haemolysis, and non regenerative anaemias), packed red cells are the best choice, whereas in patients with blood loss (especially those that are also coagulopathic) fresh whole blood is optimal. It is not possible to give a precise PCV where transfusion becomes necessary. Factors to take into consideration other than simply the PCV include the rapidity of onset of the anaemia, the clinical signs of anaemia that the patient is displaying and whether the red cell loss is ongoing. Generally patients with rapid onset anaemia require transfusion at a higher PCV than patients with chronic anaemia. Any patient with a PCV of less than 20% should be considered a potential candidate.

In patients showing signs of moderate/severe hypovolaemia secondary to haemorrhage, the PCV may initially be normal, however whole blood may be the optimal fluid for intravascular volume replacement. Volume expansion with crystalloids or colloids will lead to a marked fall in PCV that can be lessened or avoided if whole blood is available as the initial resuscitation fluid. Whole blood however is rarely available in this scenario and fluid therapy should NEVER be withheld from a hypovolaemic patient due to concerns about the haematocrit decreasing. Severe hypovolaemia is much more likely to lead to death than the anaemia that volume replacement with an asanguinous fluid will create.

Coagulopathy is another common reason for transfusion. It is necessary to have some understanding of the mechanism of the coagulopathy to determine the optimal transfusion product. Fresh whole blood and fresh frozen plasma contain all the clotting factors and thus are suitable for most coagulopathies – fresh whole blood is preferred if the patient is anaemic from concurrent haemorrhage whereas FFP is preferred if the patient is not anaemic. The use of FFP allows delivery of large amounts of clotting factors with less risk of volume overloading the animal with red cells that it does not need. For rodenticide toxicity, frozen plasma is also adequate as the vitamin K dependent factors are maintained. In patients bleeding from von Willebrand's disease, large volumes of vWF may be needed and cryoprecipitate is the most appropriate product to allow delivery of large amounts of vWF in a small volume of fluid. It should be remembered that in patients with haemorrhage from severe thrombocytopaenia, transfusions are principally used to support the red cell count as the number of functional platelets delivered even in fresh whole blood is very low.

Fresh frozen plasma may be useful in treating patients with sepsis or systemic inflammatory response syndrome (e.g. severe pancreatitis). The plasma may contain a number of beneficial substances including the clotting factors, antithrombotic proteins (e.g. antithrombin III), inhibitors of circulating proteases (e.g. α2-macro-globulin) and albumin.

Transfusion reactions can be split into immunologically mediated and non-immunologically mediated and acute and delayed reactions. Acute immunologically mediated reactions represent severe and often fatal reactions caused by preformed antibodies that the patient has against the donor cells. The most common scenario where this occurs in veterinary medicine is when a type B cat is transfused with type A blood. Acute reactions can be detected by carefully monitoring the patient's temperature, respiratory and heart rate during the early part of the transfusion. Patients undergoing an acute transfusion reaction will commonly become febrile, tachypnoeic and tachycardic. They may vomit and ultimately they will progress into DIC, multi organ failure and death. Occasionally milder acute transfusion reactions are suspected (e.g., urticaria). These may result from recipient antibodies against donor plasma proteins or white cells. If a transfusion reaction is suspected, the transfusion should be stopped and the patient treated with intravenous fluids and possibly antihistamines or corticosteroids. Acute reactions can generally be avoided by using typed and cross matched blood. Although the vast majority of acute immunologic transfusion reactions occur secondary to administration of products containing red cells, occasionally patients receiving plasma show signs consistent with an acute reaction e.g. facial oedema. This presumably occurs secondary to an immunological response to foreign plasma proteins and is generally mild.

This may occur even when compatible cross matched blood is given to a patient but where over the subsequent few days, the recipient develops antibodies to the transfused cells. This results in early destruction of the transfusion and may manifest as haemoglobinuria. The reaction is rarely as serious as an acute reaction. Another concern is immunosuppression; there is good evidence from human medicine that transfusions are immunosuppressive. The clinical significance of this phenomenon in veterinary medicine is unclear.

There are various nonimmunologic complications that can occur acutely following transfusion. Hypocalcaemia may occur resulting from the citrate used in the anticoagulant. Embolism is a risk from clots in the transfusion product. Circulatory overload may occur with rapid administration of fluid in a euvolaemic patient. Others include bacterial infection from contaminated blood and haemolysis from physical or thermal damage to the red cells. Delayed nonimmunologic transfusion reaction largely relates to infections (often viral) that are transmitted with the transfusion. In the UK, it is much more likely to occur with feline transfusions. Possible transmissible infections include FIV, FeLV and *Mycoplasma haemofelis.*