Blood Donation and Blood Transfusions in Dogs and Cats
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Blood-banks
Historically blood was collected as needed (i.e. it was “stored in the donors”) and transfused. Blood-banking developed as a means of providing immediate access to whole blood and blood components, which is especially useful in emergencies. Blood may be purchased from a commercial blood bank and/or collected in-house. If a clinic collects and processes its own blood, this gives the technicians a great opportunity to develop technical skills and further contribute to patient care. With a client-based blood donor program there is also the potential to attract new clients.

Blood donor selection
Donors may be clinic or client-owned. OVC uses a client-owned canine donor program but clinic-owned feline donors, as the latter are typically anesthetized for donation. However, some OVC feline donors are fostered, especially type B cats, as they are bled less frequently. Screening and prevention of infectious diseases varies geographically (see below). At OVC donors are excluded if they are fed a raw food diet, have surgical implants, or have advanced periodontal disease as these increase the risk for bacteremia. Donors must be up-to-date on vaccination. (Vaccination is typically not performed 10-14 days before collection because of unknown effects on blood during storage and potential effects on platelet number and function. This is not important if the blood is to be immediately transfused for anemia.) Donors have a routine history taken to identify any health risks, physical examination, and PCV/TP/BUN stick prior to each donation, and annual CBC, serum biochemistry profile, urinalysis, fecal floatation and potentially re-screening for infectious diseases. In addition to free health screening, potential client-owned donor incentives include free vaccination and a bag of treats or dog food at each donation. Potential sponsorship from a company may be explored for the latter.

Dogs
The general criteria for OVC canine blood donors are: healthy and free of parasites and infectious diseases (see below); good temperament; 1-7 years of age; minimum 25kg body weight, although >28kg preferred; easily accessible jugular veins; pre-donation PCV >40%. Greyhounds are ideal donors for these criteria, and also because of their long necks higher RBC counts as most transfusions are given to treat anemia. However, they have lower platelet counts than other breeds; this makes them less suitable donors for platelet products. Also, the lifespan of their RBC is shorter than in other breeds, but it is not known if this affects post-transfusion RBC lifespan in the recipient. Acquisition to blood donor programs provides a home for retired track dogs. Some breeds are not ideal donors for various reasons: short necks and long hair (Samoyed, Chow Chow); thick skin (Shar Pei); small RBCs (low MCV) and higher RBC potassium content (Akita, Shiba Inu).

Canine donors at OVC receive routine regular heartworm screening and prophylaxis. We do not have a high prevalence of tick-borne diseases (although tick exposure is increasing), but canine donors are screened for Ehrlichia canis, Anaplasma phagocytophilium, Anaplasma platys (recent addition), Borrelia burgdorferi (Lyme disease), Mycoplasma haemocanis, and Mycoplasma haematoparvum (previously Haemobartonella). Dogs imported from endemic areas, or with known risk for specific diseases, will be tested for Babesia canis and Babesia gibsonii, rocky mounted spotted fever (Rickettsia rickettsii), and Leishmania. Retired track Greyhounds are considered at increased risk for B. canis and RMSF, American pit and Staffordshire bull terriers at risk for B. gibsoni, and foxhounds and Neapolitan Mastiffs at risk for Leishmania. Breeding dogs are tested for Brucella canis.

Splenectomy was once recommended for clinic-owned donors to reveal Mycoplasma and Babesia, but this is no longer practiced and not applicable to client-owned donors. The costs of infectious disease screening are a significant contributor to the costs of a blood bank, and risk-benefit must be carefully assessed.

Donors should not have been transfused because of the risk for developing antibodies that may cause incompatibility reactions. In the past bitches that had whelped were not used because of the risk of the dam being sensitized by fetal red cells. However, the placenta of the dog should not allow this, and a recent study found no evidence of pregnancy-induced anti-RBC antibodies. We have used many donors at OVC that have whelped without any apparent incompatibility problems. We prefer to avoid the use of intact females because it is logistically difficult to work around heat cycle ± pregnancies.

Hypothyroid dogs receiving appropriate replacement therapy are accepted as donors at OVC. Donors are excluded while receiving and for 2 weeks after receiving most other medications including antibiotics or NSAIDS; an exclusion period for 6 weeks after treatment is used for corticosteroids. An exclusion period of 2 days after treatment is used for most intermittent heartworm and ectoparasite treatments. Most of these exclusion are based on caution rather than evidence.

Doberman pinschers and other breeds at risk are tested for vWF deficiency. Deficient dogs with no bleeding are not excluded as donors (RBCs and albumin are not affected) but blood products should be identified as having low vWF levels.
Cats
OVC feline donors are friendly cats, up to 8 years of age, with a lean body weight >4.5 kg, and a pre-donation PCV >35%. Most are males because of size. The thick skin of mature toms makes jugular venipuncture difficult, but this resolves within 4-6 weeks of neutering. In-clinic and fostered cats are kept strictly indoors to minimize exposure to infectious diseases via fighting and vectors. Infectious disease screening includes FeLV/FIV, Mycoplasma haemofelis, and Mycoplasma haeminominatum, Bartonella henselae and B. clarridgeiae and other diseases based on exposure risk. OVC feline donors are not routinely tested for Toxoplasma gondii or FIP. Previous pregnancy is not an exclusion.

Blood compatibility testing (immunohematology)
Blood groups (blood types) refer to inherited antigens of RBC membranes that may cause an immune reaction in another animal. If we did not give blood transfusions we would not know about them. There are 3 components to testing if a donor and recipient are compatible: blood typing, antibody screening, and crossmatching. All are based on immunologic reactions, i.e. antigen and antibody interactions, which result in agglutination (clumping together of RBCs) or hemolysis. Blood-typing tests for antigens on RBCs by using antibodies. Antibody screening and cross-matching test for antibodies in serum by using RBC antigens. Antibody screening is a sub-type of crossmatching used extensively in humans, and crossmatching is no longer routinely performed.

Dogs
Most blood groups are known as DEA (dog erythrocyte antigen). The most clinically important is DEA 1.1. If a DEA 1.1 negative (−ve) dog receives a 1.1 positive (+ve) transfusion it will become sensitized (form antibodies to it) within 4-7 days and will have an acute hemolytic reaction after the next 1.1 +ve transfusion. Therefore 1.1 –ve dogs should be transfused with 1.1−ve blood and 1.1 +ve dogs may receive 1.1 +ve or –ve blood. Other blood groups that can be tested for include DEA 1.2, DEA 3, DEA 4, DEA 5, DEA 7, and Dal. Many other blood groups exist (estimated 13 DEAs) but cannot be tested for by blood-typing. Dogs negative for the other DEA blood groups will also become sensitized if transfused with positive blood. Reactions to DEA 1.2, 3, 5 and 7 are usually mild and will result in a delayed transfusion reaction – the transfusion does not “last” as long as expected. Reaction to DEA 4 can be acute, but is rare since 98% of dogs are positive. Dal is newly discovered and we do not know if it corresponds to a DEA. Reactions after sensitization to Dal can be acute or mild. There are no naturally-occurring antibodies, i.e. there is no risk of an RBC incompatibility reaction in a dog that has not been previously transfused. The older term “A –ve” refers to DEA 1.1/1.2 –ve because antisera used to exist to type for this.

Options for blood-typing dogs are: 1) Submission to Animal Blood Resources International (ABRI), Stockbridge, MI and Dixon, CA; serum which contains specific antibodies to the various DEA is mixed with RBCs being tested; testing is available for all DEA above. 2) Typing card, Rapid Vet H Canine DEA 1.1, DMS Laboratories Inc., Flemington, NJ. There have been some reports suggesting a 10% false positive result. If true, the card is best used for selecting 1.1 –ve donors because if used to select recipients some 1.1 –ve dogs may become sensitized. 3) Cartridge kits, Quick test for DEA 1.1, Alvedia, Lyon, France. 4) A previous microtube gel column agglutination test, ID-Gel test Canine DEA 1.1, DiaMed-Vet, Switzerland, was excellent but is no longer available. Dal antigen typing is not commercially available. Current minimum standard-of-care is donor typing for DEA 1.1.

About 40-60% of dogs are 1.1 +ve. Breeds that are usually +ve include Rottweilers, Golden Retrievers, Bernese Mountain Dogs, German Short-Haired Pointers, and chocolate Labrador Retrievers. Breeds that are usually 1.1 −ve include Boxers, Irish Wolfhounds, Greyhounds, G. Shepherd Dogs, Dobermans, Pit bulls, Flat-Coated Retrievers, and Huskies. Dal −ve dogs identified so far include Dalmatians (hence the name), Dobermans, Shi Tzus, and mixed-breeds.

Because not all blood groups are known or can be typed for, a major cross-match should be performed before a dog receives another transfusion after 1 week because it may have been sensitized. A major cross-match is performed by mixing together donor RBCs and recipient serum, and watching for agglutination or hemolysis which indicates the recipient has antibodies in its serum against the donor RBCs. An EDTA blood sample is used (same as CBC); the sample should be <24hours as older RBCs are more fragile and may give false positive reactions. A complete major cross-match is usually performed in an external lab as follows (the procedure will vary a bit from lab to lab):

1. Place 0.5 – 1 mL EDTA blood in a small plastic tube (e.g. 12 x 75mm) and wash the donor RBCs three times. Washing involves adding saline to the blood, mixing, centrifuging, and then pouring off the saline.
2. Add 0.5 – 1 mL saline to the washed RBCs to make a donor RBC suspension. The purpose of washing the RBCs is to remove proteins in the donor blood which contribute to rouleaux and hide the RBC antigens. The purpose of making a saline suspension is to dilute the proteins in the recipient serum which may cause rouleaux. Rouleaux is the enemy of the cross-match, because it may be easily confused with agglutination.
3. Add two drops of donor RBC suspension and 2 drops recipient serum to a small tube, and mix by flicking the bottom of the tube. Centrifuge at low speed for 15-30 sec, just enough to bring the RBCs together but not to pack tightly.
4. Gently shake the tube (“wiggle and tip”) to resuspend the RBCs and hold up to light to grade the reaction as: neg (no clumping); trace (tiny or microscopic clumping, verify microscopically); 1+ (small clumps, turbid reddish background; 2+ (medium clumps, clear background); 3+ (several large clumps); 4+ (one solid clump of RBCs, clear background).

5. If no clumping is seen, repeat steps 2-4, but incubate the tube for 30-60 min before centrifugation. Ideally incubation should be at 37.0 - 38.5 ºC (water bath) and room temperature, and optionally at 4 ºC.

6. If no clumping is seen, add Coomb’s reagent and steps 2-4 with incubation for 30 min. This is known as an indirect antiglobulin test. Coomb’s reagent contains antibodies against canine antibodies. If the recipient serum has a low level of antibodies against RBCs, the donor RBCs may not stick together. By adding antibodies against the recipient’s antibodies, it makes it more likely for the donor RBCs to stick together. Several donors are usually being crossmatched to a recipient at the same time, and it is very important to keep all tubes well-labelled, to avoid contamination between tubes, and to run controls (donor RBC + serum, recipient RBC + serum). If the lab reports a donor as incompatible, ask if incompatibility was after immediate mixing, after incubation, or after adding Coomb’s reagent. The sooner the incompatibility shows up, and the stronger the reaction, the more likely there is to be an acute hemolytic reaction. A donor with an immediate 4+ reaction will likely cause an acute hemolytic reaction (this cross-match incompatibility has been seen with DEA 1.1 and Dal), while a donor with a trace reaction after Coomb’s reagent will likely result in only mild delayed hemolysis, with a spectrum in between.

Most clinics will not keep Coomb’s reagent and will only do steps 1-5. In an emergency situation there is no time to do a complete crossmatch with incubation, and if no incompatibility is seen after steps 1-4 the transfusion may be given if it is deemed life-saving. An even quicker alternative is to mix omit washing the RBCs and to directly mix a drop of RBC suspension and a drop of recipient serum on a microscope slide, place on a cover slip, and observe the wet mount under low-power for agglutination. It is not reliable to look at the slide with the naked eye – rouleaux may form quickly which cannot be distinguished from agglutination. Any donor that agglutinates on a slide should not be used under any circumstance. Dogs with chronic anemia requiring multiple transfusions should have a complete major cross-match (including Coomb’s) prior to each transfusion. The value of crossmatching when treating IMHA is controversial, and incompatibilities detected may not be present when IMHA has resolved. Crossmatching in IMHA has been performed frequently at OVC with success in an effort to identify the most compatible donor.

A minor cross-match refers to mixing donor serum with patient RBC. Minor incompatibilities are minor because donor antibodies are so diluted in the recipient that they are usually unimportant. Minor crossmatching should be considered and should be obtained if a dog has a history of unexplained transfusion reactions.

Cats

There are 3 main blood types: A, B, and AB (rare). Unlike dogs, cats have naturally-occurring antibodies. Anti-A antibodies of type B cats are typically very strong, such that a type B cat transfused with type A or type AB blood will have an acute and often fatal transfusion reaction, which can occur after transfusion of as little as 0.5 mL of blood. Anti-B antibodies of type A cats are weak, and will cause delayed hemolysis. A new antigen “Mik” (named after the cat’s name, Mike) was recently reported. Breeds with reported type B prevalence include British shorthair (40%), Devon Rex (41%), Cornish Rex (34%), Birman (16%), Abyssinian (14%), Persian (14%), Himalayan 7%, Norwegian Forest Cat (7%), Main Coon (2%), domestic shorthair/longhair (1-5%), Siamese (0%), and Tonkinese (0%).

Cats may be blood-typed using a the Rapid Vet H Feline typing card (DMS Laboratories Inc) or the Quick Test cartridge kits (Alvedia). If a typing card or cartridge is not available, then steps 1-4 or a rapid slide crossmatch may be performed. A type B recipient will rapidly agglutinate type A donor cells, however, a type B donor – type A recipient incompatibility is not detectable. If the blood-type of the donor is known, the crossmatch is an antibody screen for antibodies against that blood-type. This is also referred to as back-typing.

The value of routine crossmatching beyond blood-typing in cats is not known, but should be considered if a cat is receiving repetitive transfusions, and should be performed if a transfusion reaction has occurred.

Blood donation

Dogs

Commercial human 450 mL collection bags are used, which contain an anticoagulant-preserve solution (APS), and a sterile collection line with a 16-gauge thin-walled needle, attached to the bag. This is a “closed system”. The bags are available as single to pentad packs, which indicate whether zero to four satellite bags, respectively, are attached via tubing to the collection bag. These satellite bags, some of which may contain red blood cell nutrient additive solutions (AS), are used for the separation of components. The various APS in the bags are composed of citrate-citric acid and dextrose ± phosphate or adenine, and the various AS are composed of saline, adenine and dextrose ± mannitol ± citrate-citric acid. Blood collection packs are also available with leukocyte filters. Leukocyte reduction reduces the risk for febrile transfusion reactions and immunization.
Blood is collected by jugular venipuncture with the donor in lateral or sternal recumbency, or sitting, on a table or in some cases on the floor. Most donors habituate and do not need sedation; mild sedation may be given with butorphanol 0.4 mg/kg IM/IV. The venipuncture site is clipped and surgically prepared. Further palpation of the jugular vein must be done above or below the prepared site to avoid contamination.

Blood may be collected by gravity and donor blood pressure, in which case the bag should be continuously gently rocked by an assistant during collection; blood collection typically takes 5 – 15 min. Light suction may also be used, in which case the procedure typically takes 3 – 10 min. For suction collection, the collection bag is hung from a hook inside a clear cylinder with a flat lid such that the blood is forced to pass through the APS. The collection line with attached needle is brought through a notch between the cylinder and lid, and a tube clamp is place on the line near the needle. A vacuum source is connected via tubing to an inlet in the chamber. The cylinder is placed on a scale, tared to zero, and the suction adjusted to ≤ 5-7 inches Hg. With one hand, the phlebotomist puts gentle pressure on the jugular vein below the prepared skin site, and the needle held in the other hand is inserted through the prepared site into the donor's jugular vein in either direction. When the tubing clamp is removed, blood flows into the collection line to the bag as the scale measures the grams of blood collected. When the desired amount has been obtained, pressure is released from the jugular below the venipuncture site, the line is clamped, and the needle is removed from the vein as pressure is applied over the site.

During blood collection the dog should be monitored for mucous membrane color, pulse rate and strength, respiratory rate and donor attitude. Any indication of donor distress is cause for stopping the collection. Once the needle is removed from the donor, the blood in the tubing is stripped into the bag using a tube stripper/sealer, the bag is gently rocked to ensuring adequate mixing, and the tube is sealed with aluminum clips or a thermal sealer. Alternatively, the bag is manually compressed to refill the tubing which is sealed at several points at the "X" marks to provide small samples for quality and compatibility testing. A full unit contains 450 mL of blood, but 10% variation (405-495 mL) is acceptable. Blood volume is estimated by weight. The specific gravity of whole blood at 37 °C is 1.053, so an ideal unit weighs 474 g and the acceptable range is 426-521 g. The minimum acceptable underdraw for blood to be used to prepared pRBCs is 300 mL (316g), in which case the plasma is discarded as it contains excessive anticoagulant.

After the donation moderate pressure is placed over the venipuncture site for 2-5 minutes. A neck bandage is optional but recommended for greyhounds, and for dogs that are going home shortly after the donation who may pull on the leash. The leash should be looped around 1 leg of the side where the venipuncture was performed, so as not to pull tightly on the venipuncture site. The dog is observed for 15-30 minutes for signs of weakness, pale mucous membranes, weak pulses and other signs of hypotension. We have only had 2 cases of hypotension after collection, in which case we replaced the blood loss volume by three times with crystalloids (e.g. 1500mL) given at a rate of 90ml/kg/hr. Other potential adverse effects are bleeding/bruising from the venipuncture site, skin irritation from the clippers and scrub, and sometimes hotspots (especially Golden Retrievers). The dog can have a small snack (e.g. cookies) after the collection. We do not have owners fast their dogs prior to collection. Even though lipemia may increase rouleaux formation making crossmatching more difficult, we find the dogs are better behaved for collection when they have eaten breakfast. Activity should be restricted to leash walks only for about 24 hours post-donation.

**Cats**

Closed systems designed for feline blood donation are not commercially available. Open systems involve collecting blood by jugular venipuncture using a 19-ga butterfly needle attached to a syringe or collecting bag. The systems are open because APS must be transferred (using aseptic technique) using a syringe and needle from a multi-use container to the collecting syringe or bag. The APS used most often is CPDA-1 at a ratio of 1 mL: 7 mL blood (e.g. 7.5 mL CPDA-1 + 52.5 mL collected blood for a final volume of 60 mL). Heparin at a ratio of 5-12.5 U:mL blood (300-750 U for a 60-mL donation) may be used if the blood is to be promptly transfused. Open systems include: 1) Collection into 35 mL or 60 mL syringes, and then careful injection (to avoid hemolysis) into a transfer pack, either 75 mL (Pedi-Pak Pediatric 75 mL Transfer Pack, Genesis BPS, Hackensack, NJ), or 150 mL (Fenwal Inc, Deerfield, IL); 2) Commercial 100 mL bags and collection tubing with attached butterfly needle for gravity collection; or attached 3-way stopcock, 60-mL syringe and butterfly needle for syringe collection and subsequent transfer to bag (ABRI, Dixon, CA); 3) Collection into 35 mL or 60 mL syringes, and then careful injection (to avoid hemolysis) into a transfer pack, either 75 mL (Pedi-Pak Pediatric 75 mL Transfer Pack, Genesis BPS, Hackensack, NJ), or 150 mL (Fenwal Inc, Deerfield, IL).

Cats are fasted and sedation/anesthesia is given using ketamine ± midazolam (IM or IV at standard doses) or sevoflurane by facemask. Pre-medications may be given with butorphanol (0.4 mg/kg IM) ± low-dose acepromazine (0.05 mg/kg IM). Oxygen is delivered via endotracheal tube or face mask. The cat is positioned in lateral or sternal recumbency, and the jugular venipuncture site is prepared as for the dog. If a syringe or vacuum chamber is used, only gentle suction (≤ 3-in Hg) is applied to avoid collapsing the vein or causing hemolysis. Blood collection is typically completed within 3-5 minutes. Careful monitoring of the cat is essential because hypotension may be encountered despite all precautions, and oscillometric monitoring should be considered. Some routinely give donors intravenous and/or subcutaneous saline at a total dose of 2 to 3 times the volume of blood drawn beginning prior to and/or during and/or immediately after donation to avoid poor recovery. Others only treat the donors if hypotension or signs of hypotension occur. Pressure is placed on the venipuncture site for 2 min after donation; a neck bandage is not usually applied.
Blood separation and storage

Separation of whole blood into components requires a centrifuge that is not available to most clinics – techniques are given in the references. Most blood banking in clinics is for in-house collected or purchased whole blood or purchased packed red cells (pRBCs) and plasma. Units of WB and pRBCs should be stored in a refrigerator. Warming alarms, a regularly-checked thermometer and HemoTemp II blood bag thermometer labels (Biosynergy, Inc, Elk Village, IL) are means of identifying warming. Red cell viability and function decrease during storage. Shelf life is defined as the number of days after collection at which 75% RBC viability is maintained. Shelf-life for canine and feline whole blood collected into CPD is 4 weeks, and 5 weeks for CPDA1. Shelf-life for canine and feline pRBCs in CPDA1 is 3 weeks and in AS (Adsol, Nutricel, Optisol) is 5 weeks. Plasma products should be stored at -18 ºC or lower in a cardboard plasma box which protects the bag from breakage. Fresh-frozen plasma (FFP) is plasma frozen within 8 hours of collection, and has a shelf-life of 1 year if frozen at -18 C. Expired FFP may be relabelled as frozen plasma and stored for an additional 4 years. The main value of FFP is for vWf deficiency. Expired FFP is used for treatment of hypoproteinemia and vitamin-K antagonist poisoning. Other products available from blood banks (some produced by hemapheresis) include cryoprecipitate, platelet-rich plasma, and platelet concentrate.

Transfusion

Blood products are usually given IV but may also be given IO. Visually inspect the product for discoloration, presence of clots or hemolysis, cracked bags, evidence of warming and discard if noted. Refrigerated WB or pRBC do not need to be warmed unless they are being given to pediatric or patients at risk of hypothermia, or when large volumes are planned (to avoid arrhythmia). Excessive warming may decrease RBC viability and increase bacterial growth. To warm blood, it can sit at room temperature for 30-60 min prior to transfusion, or the IV line can be passed through a bowl of warm water or sandwiched between oat bags warmed to 37C. Stored whole blood should be mixed well by gentle inversion. If pRBC’s were not stored in AD, add 100mL saline to canine, and 20-30 mL to feline, RBCs and gently mix. Frozen plasma is thawed with intermittent gentle agitation in a 37 ºC warm water bath preferably in the bag in which it was stored. All products should be transfused using a blood filter set, although in an emergency situation you can give fresh unfiltered blood as long as there are no visible clots and the collection was smooth and rapid. Transfusion can be given by gravity and using an infusion pump. Pediatric filter sets are available to infuse smaller volumes in a syringe, which can be given by intermittent injection of with a syringe pump. Transfusion rate depends on the cardiovascular status and the emergent nature of the transfusion. In general start at 0.25-0.1 ml/kg/hr for the first 15 min to observe for an immediate transfusion reaction, and then increase the rate (maximum 10 mL/kg/hr), ideally to finish transfusion within 4 hours, but at OVC we will transfuse over a longer period, but strict attention has been given to prevent bacterial contamination during the set up. Alternatively some blood products may be divided into subunits in transfer packs and then refrigerated until needed. Patients should not receive food or medication during a transfusion and the only fluid that can pass through the same catheter as the blood is 0.9% saline. Attitude, TPR and blood pressure are initially monitored q5min for the 1st 15 min, and then q15min. Any change in these parameters, vomiting, or agitation should be taken as a sign of a transfusion reaction, the transfusion stopped, and the clinician contacted. If a transfusion reaction occurs, the blood bad should be saved for compatibility and quality testing. Patient PCV/TP and plasma colour (to assess hemolysis) may also be assessed prior to and 15 min after the start of the transfusion as well as 12 and 24 hours after the transfusion. Urinalysis is also helpful to detect hemolysis. It is helpful to design a transfusion monitoring sheet with time points and monitoring parameters noted. A transfusion log book should be kept with patient’s name, donor’s name, date of transfusion and product given, as well as any reactions. This should also be noted in the patient’s medical record.

References