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Practical and Safe Whole Blood Transfusion in Dogs and Cats

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Introduction

Blood transfusion is defined as the intravenous transfusion of blood or its components that have become more common in veterinary medicine and are considered as an important aspect of lifesaving and advanced treatment for critically ill patients. Historically, blood transfusion relied only on the transfusion of whole blood. However, after the advancements in component therapy of blood, such as pRBC's, frozen plasma and platelet rich plasma, have given clinicians the ability to employ the components according to the patient's needs.

The successful transfusion of blood from one dog to another was first reported in 1665 by Richard Lower [1]. With the advancement in techniques and equipment development after 1950, blood transfusion became more popular in veterinary medicine [2]. The 20th century was known for the discovery of anticoagulants and preservatives for blood products, the description of human blood groups, and the development of compatibility assays [3]. Donor Day is celebrated worldwide every year on 14th June by the World Health Organization (WHO) [4].

Transfusion therapy has increasingly become more practicable in small animal treatment [5,6]. Better access to blood products through on-site donors, the purchase of blood bank prod-

ucts, external donor programmes, or the availability of blood component substitutes and infectious pathogen screening have increased the safety of transfusion therapy for canine patients. The safe use of whole blood or blood component therapy, on the other hand, necessitates knowledge of blood groups and antibody prevalence, as well as the methods for reducing the risk of adverse reactions, such as the use of appropriate donors and screening assays that aid in the detection of serological incompatibility. Although blood transfusions may be life-saving, they are not a definitive treatment for disease but play a critical role in patients with acute blood loss, improving oxygenation capacity and the patients' ability to overcome the underlying diseases.

The hematological parameters such as hemoglobin, hematocrit, and total erythrocyte count should be evaluated concurrently with physical examination findings to determine whether the patient requires a transfusion or not [7]. The type of anemia, blood group, blood parameters, animal size, and blood products to be transfused all influence blood transfusion. A transfusion specialist should be well-versed in the transfusion method, as well as its complications and emergency procedures.

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This chapter reviews the practical aspects of whole blood transfusion and acts as a basic guide to blood grouping, typing in dogs and cats, as well as the standard technique for cross-matching before transfusion, optimal donor criteria, blood collection methods, storage, transfusion procedures, and adverse reactions and their care.

Indications

Blood transfusions are indicated for the management of anemia, coagulopathy, and, rarely, for other conditions such as thrombocytopenia, thrombopathia, and hypoproteinemia. 1) Anemia of various diseases, like due to infection or acute/chronic hemorrhage. 2) Bleeding disorders such as thrombocytopenia or coagulopathies. 3) Poisons like warfarin. 4) Hypoproteinemia is usually of parasitic or infectious origin. 5) Burns. 6) Parasitism, toxicosis, or immune-mediated anemia [8]. Transfusion is indicated in case of canine patients, when blood constituents like Packed Cell Volume (PCV) is 15% or less and haemoglobin is 5 gm/dL of blood or less [9]. Clinical signs of anemia include tachycardia, tachypnea, lethargy, weakness, respiratory distress, pale or discolored mucous membranes, and prolonged capillary refill time. Transfusion of blood and blood products were viewed as a life-saving measure in more than 80% of critically ill canine patients [7]. In cases of anemia resulting from hemorrhage, replacement of blood volume is of primary importance. The clinical signs of anaemic patients are pale mucus membranes, exercise intolerance, weak pulse, tachypnea, tachycardia, hypotension and these signs of anemia are associated to decreased oxygen delivery, because Hb plays an important role in delivering oxygen to cells [6].

Blood groups

The presence of glycolipids and glycoproteins on the surfaces of red blood cells allows classification into blood groups. These antigens can elicit an immunological reaction in a non-compatible recipient by causing the formation of circulating anti-erythrocyte antibodies. A hemolytic transfusion reaction can take the form of an early intravascular crisis triggered by IgM or high-titre IgG, or a delayed extravascular haemolytic event triggered by IgG binding to RBCs. During transfusion therapy, antigens combined with platelets, leukocytes, and plasma proteins may also cause immune-mediated reactions in the host animal.

Canine blood groups

Canine blood groups are classified by the DEA system. The abbreviation DEA stands for "Dog Erythrocyte Antigen," which is followed by the numerical identification of the blood group containing polyclonal alloantibodies [10,11]. Over 13 canine blood groups have been described and eight DEA (Dog Erythrocyte Antigen) types have been recognized [12]. Different blood types in dogs have been documented over time, and the terminology for canine blood groups has evolved.

Three antigens (1, 2, and 3, or 1.1, 1.2, and 1.3) have been identified within the DEA 1 system, although new research has identified DEA 1 as the dominant autosomal model for identifying dogs as DEA 1-positive or negative. DEA 1.1 and 1.2 are the most important blood groups and are found in 60% of the population of canines [5]. Dogs have been shown to have blood antigens other than DEA. A gel agglutination assay was used to identify the Dal blood group in an anaemic dalmatian dog in 2007 [13]. Anti-Dal alloantibodies are a feature of the Dal blood group; Dal is a red cell antigen linked to the development of anti-Dal alloantibodies [14]. Another blood group, Kai, was

studied in South Korea via the use of monoclonal antibodies, anti-Kai 1, and anti-Kai [15]. The clinical roles of these blood groups (Dal and Kai) in transfusion treatments still remain to be determined. Therefore, it is essential to perform blood typing and cross matching prior to blood transfusion to determine the compatibility between the donor and the recipient, to minimize the frequency of reaction and their severity [16].

Feline blood groups

Cats have three main blood groups A, B and AB based on the antigen they possess. All type A and type B cats, like humans, have naturally occurring alloantibodies against the blood group antigen they don't have [17,18]. Hemolytic transfusion responses to mismatched or incompatible RBC transfusions, as well as neonatal isoerythrolysis, are caused by these alloantibodies. Feline type A is the most common type in the world, and its prevalence varies by breed and geographic area. The Devon Rex and British Shorthair cats, as well as non-purebred. Australian cats, have the highest prevalence of type B cats. Mik, a novel antigen, was recently described [19]. Anti-Mik is a naturally occurring alloantibody in Mik antigen negative cats that has recently been described. All donors and recipients should have their blood typed, and a crossmatch is advised in addition to blood typing [20].

Type A cats may have weak anti-B alloantibodies, resulting in reduced RBC survival if type B donor is used. Type B cats, on the other hand, have strong anti-bodies and can have fatal reaction from as little as 1 mL of type A blood transfused. Because of the significant anti-A antibodies present in B serum, type AB cats should receive AB or A blood products if they require a transfusion [21].

Blood typing

The principle of all veterinary blood typing methods is a visible hemagglutination reaction between patient RBC surface antigens and known reagent monoclonal or polyclonal antisera. The International Society of Animal Genetics is responsible for the standardization of blood typing reagents. Prior to cross-match and transfusion, blood group testing can be done in the clinic to screen possible cat and dog blood donors and to type the recipient for proper donor selection. Blood typing is significant to identify the presence of dog erythrocyte antigen which are responsible for initiating approximately 70 to 80% of immune-mediated transfusion reaction [22].

A card-based agglutination assay, an immune-chromatographic cartridge, and a gel column diffusion assay are all commercially available blood typing kits. Antisera are lyophilized and placed in reaction wells on blood typing cards. Positive and negative control wells are included on the dog cards, while an auto control well is included on the cat cards. The availability of blood typing reagents for extended blood typing is limited. The procedure is simple, and the results can be obtained in less than 2 minutes without the use of any additional equipment. The auto control well on the cat typing card, as well as separate cards for dogs, provides for auto-agglutination testing. Auto-agglutination resembles a positive reaction and may make correct typing impossible.

The immune chromatographic kits use a plastic cartridge device and take around 2 minutes to complete. Simple preparation of a cell suspension and manipulation of the device to appropriately position the response strip in the suspension are all that is required for testing. By using capillary action, erythro-

cytes can migrate up the membrane. The antibody impregnated in the strip traps erythrocytes that are positive for the antigen in question, resulting in a visible line. Erythrocytes that do not have the antigen do not create a line and pass through the antibody. The strip is also impregnated with control material, which must read positive in order for the test to be valid.

The gel tube typing kits require a basic cell suspension, a 10-minute incubation period, and a 10-minute centrifugation in a centrifuge designed specifically for holding the gel tube cartridges. At or near the top of a gel column, the reaction is apparent as a compact to moderately scattered layer of agglutinated cells. At the bottom of the column, non-reacting cells accumulate. Both these tests are simple to interpret and understand [23].

For the dog DEA 1.1, typing is commercially available can be determined. The commercial methods include typing cards (DMS Laboratories, Flemington, NJ, USA), gel column (DiaMed, Switzerland), and immune-chromatographic cartridges (Alvedia, France) (Figure 1).

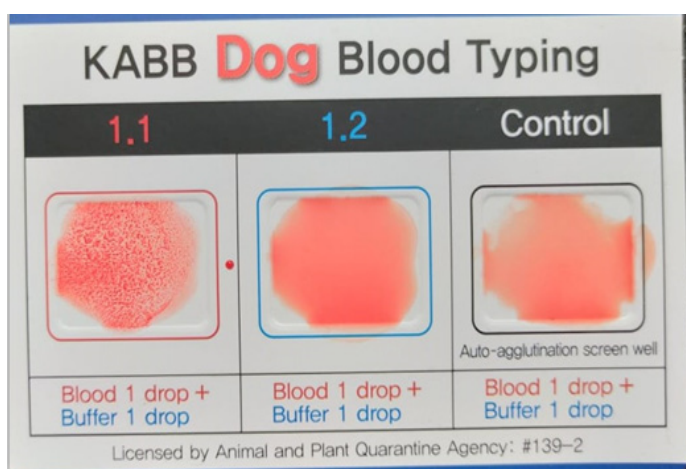


Figure 1: Dog blood typing (DEA1.1 positive).

	Major cross match	Minor cross match	Control Patient
Recipient	2 drops plasma	1 drop RBC solution	1 drop patient RBC solution + 2 drops patient plasma
Donor	1 drop RBC solution	2 drops plasma	1 drop donor RBC solution + 2 drops donor plasma

- Incubate tubes for 15 min at 37°C.
- Centrifuge tubes for 15 s.
- Reading results Note plasma color and record any hemolysis. Then gently resuspend the red cell button into the overlying plasma layer, noting the presence of agglutinating clumps. Next, place a drop of re suspended RBCs on a slide, apply a coverslip, and read at 10X and 40X. If the crossmatch is compatible, the RBCs should be individually distributed (Figure 2). Hemolysis (compared to control) or agglutination is seen with an incompatible cross-match.

8. Rouleaux, a physiological plasma-related phenomenon, may sometimes be observed. In order to distinguish this from agglutination, centrifuge the tubes again for 15 s, remove the serum, and add 2 drops of saline; then centrifuge the tubes once more and reexamine the RBC suspensions.

Cross matching

Cross-matching reveals the serological compatibility or incompatibility between donor and recipient. Blood typing tests reveal the blood group antigens on the red blood cell surface. However, cross-matching reveals the serological compatibility or incompatibility between donor and recipient. Cross matching, tests for the presence or absence of naturally and induced antibodies, does not replace blood typing.

Cross matching is similar to blood typing, except that specific antisera are not used, and consists of a major and minor part. The major and minor cross matching tests are performed for agglutination and/or hemolytic responses between donor and recipient. Animals with strong naturally occurring antibodies, such as cats, or those with induced antibodies, such as those from previous transfusions, should always have a substantial cross match performed. Even if the same donor blood is used for multiple transfusions over a period of days, the cross match should be performed. The major cross match test searches for alloantibodies in the recipient's plasma against donor cells, whereas the minor cross match test looks for alloantibodies in the donor's plasma against the recipient's RBC's.

The following is a simple major and minor cross match protocol.

- Centrifuge (1000 x g or 3400 rpm) donor blood from an EDTA vial (purple top) or citrated vial (blue top).
- Washing RBCs: Re suspend 0.25 mL of RBCs in 2 to 4 mL of saline; centrifuge for 1 min, remove supernatant, and repeat the procedure twice; remove supernatant.
- Resuspend 0.1 to 0.2 mL of RBCs in approximately 4.8 mL of saline in order to obtain a 2% to 4% RBC solution.
- In 3 tubes identified as "Major," "Minor," and "Control," add the following:

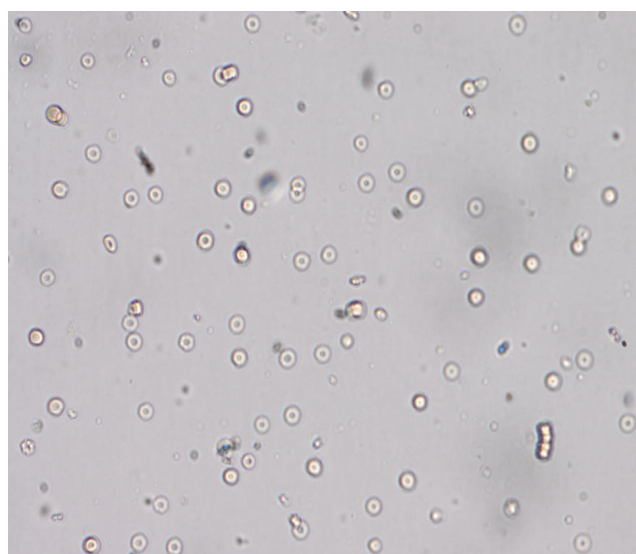


Figure 2: Compatible cross match.

Canine donor selection

Donors should be typed and screened for general health and for endemic infectious diseases. The donor selected for blood collection should be an adult (2-8 years), healthy (neutered male or spayed female are preferred), weighing more than 30 kg, with a PCV 40% for dogs and or more, fully vaccinated and free of various diseases such as heartworm infection, tick-borne diseases (*Ehrlichia canis*, *Babesia*, *Borrelia burgdorferi*, *Rickettsia rickettsii*), brucellosis [24]. Ideally, donor should have taunt neck skin that permits easy access to the jugular vein. Moreover dogs that have thick skin (Rottweilers) or skin fold on the neck (Basset hound, Mastiffs) are less preferred than long-necked dogs (Greyhounds). The ideal donor should have a good temperament, fit in condition, no previous history of transfusion or pregnancy. Regular blood donors should be sufficiently tractable to allow donation with either manual restraint or light sedation, especially if they are client-owned. Blood can be collected from an uncooperative dog using sedation or general anesthesia, but regular blood donors should be sufficiently tractable to allow donation with either manual restraint or light sedation. The common used sedatives for canine transfusion are shown in Box 1.

Bacteremia-prone dogs should not be utilized as donors. Wounds, abscesses, surgical implants, widespread skin lesions, advanced periodontal disease, and diarrhea are examples of these. Blood should also not be collected from dogs whose collection sites have been affected by pyoderma. Dogs with immune-mediated illnesses, cancer, organ failure, and other systemic conditions should not donate blood because of the possibility of undesirable stress on the donor and detrimental consequences on blood quality. Heartworm prophylaxis is required for donor dogs residing in heartworm-endemic areas, although it is debatable whether these treatments should be administered right before the donation. Flea treatments may be given to donor dogs on a regular basis, although this is not recommended right before donation. Donors should not be on any drug therapy because of the potential for negative effects on the recipient and blood quality.

The donors who donate on a regular basis should receive a well-balanced, high-performance diet that may be supplemented twice weekly with oral ferrous sulfate if the donor is bled every 4 to 6 weeks.

Feline donor selection

A blood donor must be large (>4 kg) and non-obese, have a calm disposition, and be between the ages of 1 and 8 years (preferably 1 to 5 years). Healthy (indoor, fully vaccinated cats are preferred) and clinically sound. In practice, this necessitates a number of (initial and ongoing) assessments: Thorough clinical examinations and a thorough donor history should be obtained; haematology (complete blood count) and biochemistry (urea, creatinine, total protein/albumin/globulin, ALT, ALP, blood glucose, Na, K, Cl) should be confirmed to be normal. The donor cat should be checked for blood borne infectious illnesses (FeLV-, FIV-, and Haemoplasma-negative important; Bartonella screening).

Before each donation, the donor's Packed Cell Volume (PCV) should be assessed and donors with approximately 35% are considered as ideal donors. Before each donation, donor's blood pressure should be normal (120-180 mmHg). Low blood pressure, which can be aggravated by anaesthesia and blood donation, can be associated with occult heart disease and other disorders. To rule out occult heart disease, all cats should have an echocardiogram conducted prior to donation.

Feline blood donors should have a pleasant temperament for simple handling and confinement, but it is more vital that they do not get stressed or sad. The more pleasurable the experience, the more relaxed and cooperative feline donors will be throughout future donation visits. Feline pheromone diffusers or sprays, the use of the donors' own blankets, and a peaceful, relaxing setting can improve the quality of the visit while also promoting a relaxed donation process for the donors.

Sedation of feline blood donors is now widely acknowledged as a prerequisite for blood collection, implying that temperament is solely examined in order to safely provide sedation.

Feline blood donor health assessments to be performed prior to each blood collection.

Table 1: Sedation protocol for donor sedation.

Protocol for donor sedation
1. Butorphanol 0.1-.02 ± Diazepam @ 0.5mg/kg IV
2. Acepromazine @ 0.04 mg/kg IM
3. Diazepam @ 0.2mg/kg + Ketamine @ 5mg/kg (or 1:1 in ml) IV

Table 2: Adapted from manual of veterinary transfusion medicine and blood banking [26].

Assessment	Action
Cardiac auscultation	Rate and rhythm: Compare to previous recordings Presence or absence of a heart murmur or gallop(Barfield and Adamantos 2011)
Body condition	Suitable body condition score Suitable weight: Compare with previous measurements(Helm and Knottenbelt 2010)
Dermatologic	No obvious wounds or injuries Pyoderma should delay a donation due to potential bacterial contamination of collected blood Evidence of ectoparasites should also delay donation accordingly(Wardrop <i>et al.</i> 2005)
Abdominal palpation	Evidence of abnormality or discomfort Bladder size: If large/full ensure no evidence of urinary tract obstruction
Vital signs	Measurements within normal limits Compare with previous recordings
Respiratory system	Rate and effort Abnormalities during lung field auscultation
Lymph nodes	Ensure lymph node sizes are within normal limits
Eyes	No discharge or abnormalities
Mobility	Ambulatory without lameness
Temperament	Ensure there is no evidence of distress or pain
Oral cavity	Mucous membrane color Capillary refill time Presence of dental or periodontal disease

Anticoagulants

The two anticoagulants are used for administration as well as storage: Citrate-Phosphate-Dextrose-Adenine (CPDA-1) and Acid-Citrate-Dextrose (ACD). Citrate is present in the solutions used to collect blood. It acts as an anticoagulant by binding Ca. These preservatives help RBCs maintain their viability during storage by supporting their glycolytic energy metabolism (adenosinetriphosphate synthesis). Because it keeps larger amounts of 2, 3-disphosphoglycerate (2,3-DPG) and Adenosine Triphosphate (ATP) in collected blood, CPDA-1 is a better anticoagulant. Blood can be kept in the CPDA-1 system for about 35 days. Blood can be stored for 21 days in acid-citrate-dextrose (ACD). For every 7mL of blood, 1mL of anticoagulant (CPDA-1/ACD) can be used. Heparin is not routinely preferred for blood transfusion due to the fact that it potentiates the activity of anti-thrombin, resulting in the inactivation of thrombin, although 5 U per mL of blood is adequate. The shelf life of CPDA is 28 days. However, the addition of SAGM (saline adenine glucose mannitol) increases the shelf life to 42 days.

Blood collection

Blood collection is done over a 5-10 minute period. Blood is drawn aseptically from the jugular vein by gravity and a bag is usually placed on the blood collection monitor. These commercial blood bags represent a closed collection system in which the blood does not come into contact with the environment at any time during collection or separation into blood components, thus minimizing the risk of bacterial contamination and allowing storage of the blood products (Figure 3). Dogs can donate every 3 weeks as long as they receive good nutrition. Client owned dogs usually donate blood every 2-3 months. The maximal blood volume to be donated is 20 ml blood/kg or one regular blood bag unit of 450 ± 45 ml per ≥ 25 kg dog. Fifteen to 20% of estimated blood volume can be safely donated.

Estimated blood volume (litres) = $0.08-0.09 \times$ Body weight (kg)

Blood collection procedure

- The donor is positioned in lateral recumbency on the blood donation table and the venipuncture site (jugular vein) is clipped and prepared with surgical scrub to minimize the chances of bacterial contamination. A local anesthetic cream can be applied.
- The donor is allowed to settle down and secured properly on a lateral recumbency.
- The jugular vein is punctured with a needle provided with the bag.
- The progress of blood collection is monitored via the blood collection monitor.
- Once the desired amount is collected, the blood line is re-clamped close to the needle and the needle is removed from the donor.
- Pressure is applied digitally or with a pressure wrap on the venipuncture site until haemostasis is achieved.
- The collected bag is labelled with the date of collection, product type, donor PCV (or Hb) and blood group, donor identification and date of expiry.

- The donors are monitored further for any signs of hypotension or hypovolemia, such as weakness, pale mucous membranes, prolonged capillary refill time, weak pulses, or altered mentation etc.

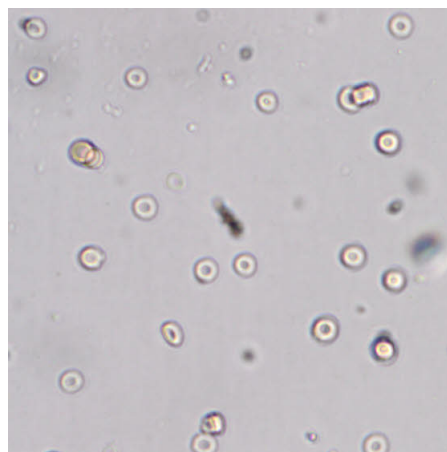


Figure 3: Collection of blood from a donor dog.

Dosage and volume of blood administered

The actual requirement of blood is calculated by using either of the following formula (Slatter, 2003):

1) Blood required to raise PCV by 1% is 2.2 ml when assumed anticoagulated donor PCV 40% for dog and 30% for cats.

2) Amount of donor whole blood = Body wt. (kg) \times Recipient volume (dog 90 ml or cat 66 ml) \times (Desired PCV–PCV of recipient) PCV of Donor As a general rule, 2ml/kg of whole blood will raise the PCV by 1 percentage point or Hb level by 0.3g/dL

Example of estimation of volume

A 25 kg dog with a PCV=10% desired PCV =20%. Donor blood PCV 50% as per formula

$$[25 \times 90] \times \frac{[20\% - 10\%]}{50\%} = 2250 \times \frac{10\%}{50\%} = 450 \text{ mL.}$$

Blood administration to recipient

Blood should be transfused, preferably using a commercial blood infusion set that has an in-line microfilter (Figure 4). A long (85 cm) blood infusion set with a dripping chamber and a short infusion set for small dogs to connect with syringes are available. Microfilters with 170 μ m pores are commonly used to remove clots and larger red cell and platelet aggregates. Finer filters with 40 μ m pores will remove most platelets and micro-aggregates and clog after 100 ml.

Sufficient gravity flow is required for easy administration. The preferred route of transfusion is slow intravenous, but sometimes intra intramedullary (or intraosseous) infusion at the trochanteric fossa (or other sites) may be used when no venous access can be obtained, while intraperitoneal administration is not recommended because absorption time is delayed and RBCs get damaged in the peritoneal cavity. The initial infusion rate should be approximately 0.25 ml/kg for the first 30 minutes, after which the rate can be increased if no reactions are seen. The entire volume should be administered within 4 hours to prevent functional loss or bacterial growth.

Blood is transfused using a commercially available blood infusion set with an in-line microfilter. A long (85 cm) blood infusion set with a dripping chamber is preferred. Clots and bigger red cell and platelet aggregates are routinely removed using

micro filters with 170 μm pores. Most platelets and microaggregates are removed using finer filters with 40 μm pores. Concurrent administration of fluids other than normal saline should be avoided to prevent erythrocyte lysis or coagulation. Thus, during the transfusion, fluids containing calcium or glucose, as well as those that are hypotonic or hypertonic, should not be administered through the same intravenous line. Food should also not be provided during a transfusion.

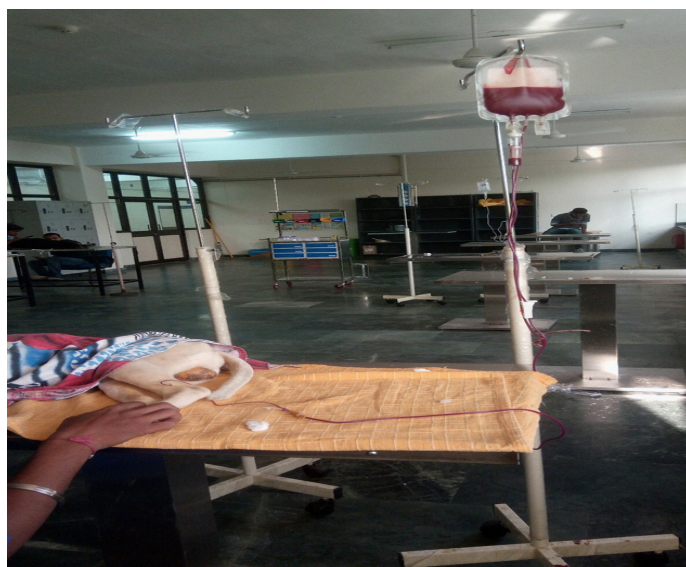


Figure 4: Administration of blood.

Monitoring transfusion

The rate of transfusion is dependent on the recipient's cardiovascular status, hydration status, degree of anaemia, comorbidities, and general condition. Even with blood-typed or crossmatched transfusions, the initial rate should be slow, to observe for any transfusion reactions. The transfusion should be given more slowly (i.e., 4 ml/kg/hr) in animals with cardiac illness, and close monitoring is essential. To avoid functional loss or bacterial growth, a single bag of blood should be transfused within 4 hours. The amount of blood components required is determined by the type of deficit and the animal's size. The patient is monitored closely every 15-20 min during the transfusion as well as pre and post transfusion. The transfusion should be monitored using the standard transfusion record form. The parameters like attitude, pulse rate and quality, rectal temperature, respiration rate, and pattern and colour of the mucous membrane, and urine. The packed cell volume (PCV) and total solids 1 to 6 hours should be monitored after transfusion. All the parameters should be noted on the transfusion monitoring chart. All the physiological parameters or any kind of adverse reactions like monitoring physiologic parameters and adverse fever, hypotension, urticaria, pruritus, pigmenturia, vomiting, and shivering must be recorded. All the base line values should be carefully entered before starting the transfusion, then q15min for the first 45 minutes and q30min until the end of the transfusion.

Adverse reactions to the transfusion

Adverse reactions usually occur during or shortly after the transfusion and can be caused by any component of the infused blood. Most transfusion reactions can be prevented by carefully selecting only healthy donors, utilising suitable collection, storage, and administration protocols, completing blood type and cross matching, and only providing the blood components that

are required. Fever is the most common symptom of a transfusion reaction, followed by vomiting and hemolysis; any reaction should result in the transfusion being stopped immediately.

Transfusion reactions have adverse effects and are classified as immunologic or non-immunologic and acute or delayed. Most reactions are acute and some can be delayed reactions, such as delayed immune-mediated hemolysis, anuria, neonatal iso erythrolysis, post-transfusion purpura and transmission of an infectious disease to the patient. Delayed hemolytic reactions can occur days to weeks post transfusion. Acute non-immunologic reactions can include potential hemolysis due to blood product storage issues, bacterial contamination volume overload and citrate toxicity [27]. Delayed hemolytic transfusion reactions, immunogenic or non-immunogenic reactions, hypothermia, citrate toxicity and heart failure are delayed reactions and can be seen later.

Treatment of a suspected transfusion reaction initially involves stopping the transfusion, at least temporarily. Diphenhydramine, glucocorticoids, epinephrine, and isotonic crystalloid fluid administration are most commonly used to treat these reactions.

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