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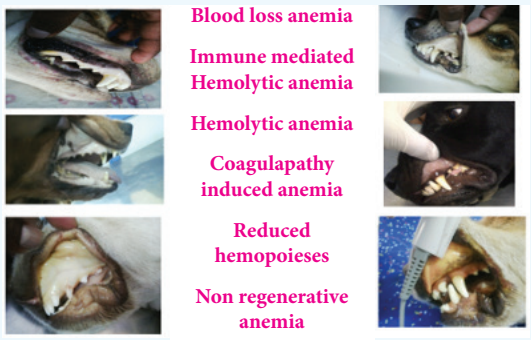
SAFE WHOLE BLOOD TRANSFUSION IN DOGS

Blood transfusion is an emergency procedure done in anemic dogs to improve the recipient red blood cells and the oxygenation capacity.

Common indications for whole blood transfusion

- Anemia due to hook worms.
- Anemia due to severe acariasis.
- Anemia due to Vector Borne diseases- Ehrlichiosis and Babesiosis.
- Blood loss in profuse epistaxis.
- Immune mediated haemolytic anemias.
- Disseminated Intravascular Coagulopathy(DIC).
- Bleeding disorders.
- Surgical blood loss.
- Anemia due to trauma.
- Anemia in kidney and liver diseases.

However, the safe use of blood component therapy requires knowledge of dog blood groups, and antibody prevalence, and knowledge of the means to minimize the risk of adverse reactions by including use of proper donors and screening assays.



Dog blood groups

Blood groups are identified based on the surface antigens of the erythrocytes and there are more than eight blood groups in dogs identified till date including DEA 1.1, 1.2, 3,4 5,7 and 8. Amongst these blood groups, the identification of the highly immunogenic Dog Erythrocyte Antigen (DEA) 1.1 in dogs has gained importance and momentum in the world. These groups are capable of causing acute hemolytic, potentially life-threatening transfusion reactions during subsequent unmatched transfusions. Generally, a first transfusion may often not cause any acute hemolytic reactions because dogs lack allo-antibodies. However, a repeated transfusion will cause reactions subsequent to antigen sensitization. It is recommended that a first

transfusion can be carried our at primary clinics and veterinary dispensaries and that the subsequent repeated transfusions can be carried out at referral institutions with blood bank and transfusion facilities.

CRITERIA FOR AN IDEAL DONOR DOG

To be a donor, the dog needs to be fit and healthy

- Between one and 8 years old
- Weigh more than 25kg
- Have a good temperament
- Have PCV < 30% and Platelet < 2,00,000/µl
- Vaccinated
- Not on any medication
- Negative for infectious / haemoprotozoan diseases
- Negative for ecto and endo parasites.

COMPATABILITY

BLOOD CROSS MATCHING

Major crossmatching = Recipient's Plasma + Donor's RBC

Minor crossmatching = Recipient's RBC + Donor's Plasma

Recipient's control = Recipient's Plasma + Recipient's RBC

Crossmatching is a simple technique that can be performed with standard laboratory equipment. A major, minor, and autocontrol crossmatch should be performed although the minor crossmatch is rarely used in dogs. The major crossmatch should always be compatible at room temperature and at 37^0C.

Procedure

1. Centrifuge EDTA or citrated DONOR blood at the LOWEST centrifugal rate possible on your centrifuge for about 10 minutes.
2. Remove 0.2 mLs of packed and place in 4.8 mLs of normal (0.9%) saline. Mix. (There are now 5.0 mLs in the tube; this essentially replaces washing step.)
3. Place 0.1 mL of this mixture into three smalltest tubes.
4. Place 0.1 mL of PATIENT serum or plasma into each of the three tubes described above. Each tube will now have 0.1 mL of the donor red cell-saline mixture and 0.1 mL of patient serum or plasma, a total of 0.2 mLs each.
5. Incubate-for 15 minutes-one tube at 37^0C, one at room temperature (25^0C), and one at refrigerator temperature (4^0C).
6. Centrifuge briskly for one minute.
7. Examine the supernatant for any hemolysis. Any hemolysis indicates crossmatch incompatibility.
8. Examine the cell button. Flick or swish the test tube. The fluid in the tube should redden as red cells disperse. If the button is agglutinated or micro agglutinated (examine a drop under low microscopic power), this indicates crossmatch incompatibility.

   To complete the minor cross match, use patient red cells and donor serum or plasma.

   To complete the auto control use patient red cells and patient plasma**.** In dogs, the minor cross match is useful for a patient receiving multiple plasma product transfusions.

Blood crossmatch determines incompatibilities between donor blood and recipient blood. Crossmatch is performed in addition to blood typing, and is recommended prior to any transfusion.

Minor crossmatch examines donor plasma or serum for the possible presence of antibodies to recipient red blood cells. Though unlikely to cause a significant transfusion reaction as donor plasma is diluted substantially in the transfused recipient, this crossmatch could be important when transfusing small patients

Major Crossmatch examines recipient plasma or serum for the possible presence of antibodies to donor red blood cell antigens. These antibodies, if present, can cause a major, life-threatening transfusion reaction in an incompatible recipient.

BLOOD COLLECTION PROCEDURE

- Place the donor dog on the blood donation table and prepare the site around the jugular vein aseptically. A local anesthetic cream can be applied.
- Let the dog be settled down on the table and secure it properly on a lateral recumbancy. Mild sedation or tranquilizers can be used if necessary.
- It is preferred to put on a pair of protective gloves. A gentle digital pressure is applied on the thoracic inlet at the neck region till the jugular vein is palpated and visualized.
- Make sure that the blood collection bag is placed on a digital weighing machine or on a blood collection monitor and the donor tubing is clamped by a hemostat or clamps provided along with the blood collection bag, prior to expose the 16 gauze needle in the air. This is to prevent the entrance of air into the blood collection bag the moment the needle cap is twisted and opened.
- Remove the needle cap and perform the phlebotomy. Remove the clamp or the hemostat the moment a flash of blood flow is seen in the tubing. The needle should be repositioned if no blood flow is seen, but should never be withdrawn out. In case if withdrawal

of the needle is required, the donor tube should clamped back again.

- The blood bag should be positioned lower than the table to allow the blood flow by gravitation, and periodically tilted to make sure the uniform mixing of blood and the anticoagulant. Each standard bag contains 350ml of Whole Blood in 43 ml of CPDA anticoagulant solution.
- Once the blood bag is full, the donor tubing is clamped and the needle is withdrawn gently from the vein.
- Pressure is immediately applied thereafter over the venipuncture site for around 6 minutes to prevent hematoma formation.
- The donor tubing is refilled with the anticoagulated blood and clamped at the distal end. If there is no availability of tube sealer, a tight knot can be applied.
- The tube lining should be sealed/clamped at every 10 cm including the blood bag ID number, for future subsequent cross matchings.
- The blood bag is the labeled for the date of collection, product type, donor PCV (or Hb) and blood group, donor identifications, date of expiration and name of the phlebotomist.

CHOICES FOR DONOR SEDATION

Butorphanol 0.1-.02 ± Diazepam @ 0.5mg/kg IV (same syringe).

Acepromazine @ 0.04 mg/kg IM.

Diazepam @ 0.2mg/kg + Ketamine @ 5mg/kg (or 1:1 in ml) IV.

WHOLE BLOOD ADMINISTRATION

Always administer using a blood giving set or suitable inline blood filter. Carefully and gently prewarm Whole Blood to room temperature before administration. Gently resuspend Whole Blood by inversion prior to spiking bag.

When the transfusion is complete, flush the infusion site with 0.9% saline before initiating other infusions or drugs.

Saline (0.9%) is the most compatible fluid with RBC products; hypotonic solutions cause hemolysis, and calcium-containing solutions (eg, lactated Ringer's) can overcome the anticoagulant properties of citrate and lead to clot formation.

Thereafter, if fresh whole blood was used, the RBCs should have a normal lifespan (approximately 110 days in dogs). The lifespan of packed RBCs depends on the age of the unit: the longer the unit has been stored, the shorter the lifespan.

Attach the blood transfusion administration set (A) to the blood unit, and prime it with blood (B) to eliminate all air. Then connect it to the patient's catheter (C). Both IV and intraosseous (IO) catheters can be used. Smaller catheter sizes do not cause more hemolysis than larger ones but are the main factor in limiting the infusion rate.

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.

Dosage

Whole Blood is intended for intravenous use only. The amount of blood to be administered depends upon the specific blood type, desired effect and patient's response. Most of the dog patients require 10-22 ml/kg of whole blood. However a suggested formula for this purpose has been mentioned as follows:

$$V = 85\ (dogs) \times Body\ wt \times \frac{Desired\ PCV - Actual\ PCV}{Donor's\ PCV}$$

For packed RBC and FFP, the average volume required is usually 6-12 ml/kg. As a general rule 2 ml/kg of Whole Blood will raise the PCV by 1 percentage point or the haemoglobin level by 0.3 g/dl. A separate dose calculation form is included with this data sheet for individual patient dosage.

Rate of administration: The calculated dose should be given over 4 hours to normovolaemic cases. Hypovolaemic patients may tolerate transfusion over 2 hours. Acutely hypovolaemic patients may tolerate a rate of 4-6 ml/minute. Whole Blood should be used with extreme caution in patients with cardiac disease and other patients at risk for circulatory embarrassment. Ideally these patients should receive PRBC. Bags should not be at room temperature for more than 4 hours**.** If the transfusion cannot be completed in this time frame, the Whole Blood should be divided and the unused portion stored in the refrigerator until required, once opened any remaining product should be discarded after 24 hrs.

Precautions: Inspect the bag before use. If significant haemolysis, colour change compared to the tubing segments, cloudiness or presence of flocculent material is noted, then the bag should not be used.

Warning: Care should be taken to avoid volume overload.

Handling Precautions: In case of accidental contact with skin, wash affected areas thoroughly with soap and water. Unused product and empty pack should be discarded and disposed of as clinical waste.

When the transfusion is complete, flush the infusion site with 0.9% saline before initiating other infusions or drugs. Saline (0.9%) is the most compatible fluid with RBC products; hypotonic solutions cause hemolysis, and calcium-containing solutions (eg, lactated Ringer's) can overcome the anticoagulant properties of citrate and lead to clot formation

Check packed cell volume (PCV) and total solids 1 to 6 hours after transfusion. If there is no ongoing loss or hemolysis, 70% of the transfused RBCs are expected to remain in circulation. Thereafter, if fresh whole blood was used, the RBCs should have a normal lifespan (approximately 110 days in dogs).

The lifespan of packed RBCs depends on the age of the unit: the longer the unit has been stored, the shorter the lifespan.

MONITORING TRANSFUSION

The infusion should be commenced slowly and the patient monitored closely (see adverse reactions) every 15-20 min during the transfusion as well as pre and post transfusion. It is recommended that patients be monitored using the standard transfusion record form.

Parameters:

- Attitude
- Pulse rate and quality
- Rectal temperature
- Respiration rate and pattern
- Colour of the mucous membrane and urine

Check packed cell volume (PCV) and total solids 1 to 6 hours after transfusion. If there is no ongoing loss or hemolysis, 70% of the transfused RBCs are expected to remain in circulation. Using a Transfusion Monitoring Chart, carefully monitor physiologic parameters and adverse reactions, including fever, hypotension, urticaria, pruritus, pigmenturia, vomiting, and shivering. Record baseline vital signs before starting the transfusion, then q15min for the first 45 minutes and q30min until the end of the transfusion.

Adverse Reactions to Whole Blood:

Anaphylaxis can occur with blood transfusion. Careful monitoring, especially at the start and throughout the transfusion is essential. If tachycardia, increased temperature, hyperventilation, vomiting or trembling occurs, slow the transfusion or stop altogether. If signs abate within five minutes, then continue transfusing at a slower rate.

Consider the administration of dexamethasone (1 mg/kg IV) and chlorpheniramine (0.5 mg/kg IV, SC, IM depending on the preparation) If signs recur, stop the transfusion entirely. If severe or other signs occur such as pyrexia, cardiac arrhythmias, urticaria and collapse, stop the transfusion. If necessary, administer adrenalin (0.01 mg/kg IV), corticosteroids and intravenous saline. These emergency drugs should always be on hand.

DELAYED ADVERSE REACTIONS can include jaundice, haemoglobinuria, anuria, disseminated intravascular coagulation, acute renal failure and, in extreme cases, death. Delayed haemolytic reactions can occur days to weeks post transfusion.

STORAGE: Whole Blood should be stored at normal refrigerator temperature (1-6ºC). Blood bags should be stored in a vertical (upright) position with adequate space between units to allow breathing. Ideally the bags should hang to allow full air contact. The refrigerated blood bags should be mixed by gentle inversion several times each week during the storage period. Temperature stability is very important. Use of a maximum/minimum thermometer is recommended that is checked daily and temperatures recorded to ensure products are stored within appropriate temperature ranges. Whole Blood should not be stored in a refrigerator that is opened frequently. Whole Blood breathes and therefore should not be kept in a refrigerator used for the storage of food or other possible contaminants.

WARMING: Whole Blood removed from refrigeration will warm quite rapidly. Where active warming is required do not use a microwave oven .Immerse the bag in warm water at not more than 37^0C. A water bath such as a sink full of domestic warm water is ideal. The Whole Blood should be warmed inside the provided zip lock bag to avoid contaminating ports while in the water bath. Avoid hot water, as it will damage the proteins and haemolyse the red cells. The unit of Whole Blood should be brought slowly to not more than 37ºC temperature before use. Room temperature is preferable. Remember, blood transfusions are mostly emergency procedures and the etiology of the anemia needs to be diagnosed to treat the dog further.

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RESEARCH HIGHLIGHTS (2016-17)

Parasitological and molecular identification of blastocystis in food animals

- A study was undertaken to identify Blastocystis from food animals such as small ruminants, poultry and pigs slaughtered. In direct wet mount examination, small ruminants (14.02 per cent), poultry (29.41 per cent) and pigs (94.44 per cent) were found positive. Some positive samples were cultured in Jones' medium. PCR of 76 samples targeting the small subunit (SSU) ribosomal RNA (rRNA) gene of Blastocystis, revealed specific amplification in 57 samples. Phylogenetic analysis grouped all the mammalian isolates in a monophyletic clade with one of the small ruminant isolates clustered with the pig isolates. The chicken isolates showed greater diversity by being clustered in two diverse clades. The study shows a very high prevalence of Blastocystis among the food animals. Parasitological procedures and in vitro culture in Jones' medium can be useful diagnostic tools, while PCR can unequivocally diagnose the presence of the organism and aid in genotyping. The presence of pig and chicken strains which include subtypes that are commonly infective to humans, indicate the high risk of transmission of the agent to humans.

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Effect of early chick nutrition on the production performance of Japanese Quail

- Effect of early chick nutrition regimens viz., glucose with probiotic (T1), glucose with threonine (T2), high density diet (28 per cent CP and 3100 Kcal ME/kg) alone(T3), and high density diet along with glucose and probiotic (T4), and high density diet along with glucose and threonine (T5) in hatcher tray and subsequent three days in pen, on the production performance of Japanese quail was studied. After three days, all birds were fed with Japanese quail diet (BIS 2007) upto 16 weeks of age. High density diet in combination with glucose either with probiotic or threonine had shown superior body weight, better cumulative feed efficiency ($P < 0.01$) at 6th week of age and higher ($P < 0.01$) villi height and width, crypt length and depth in duodenum, jejunum and ileum at 4th day and 3rd week of age. Higher ($P< 0.01$) eviscerated, ready to cook and breast yields at 6th week of age and more ($P< 0.05$) number of goblet cells at both 4th day and 3rd week of age were noticed in high density diet along with glucose and probiotic fed groups. High density diet alone fed quails exhibited significantly higher body weight and breast yield at 6th week of age. The birds fed with glucose and probiotic had shown significantly ($P< 0.05$) higher hen housed and hen day egg production (%) from 11th to 16th week of age. Better economic returns were recorded in early chick nutrition groups.

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Effect of dietary supplementation of Vitamin E and selenium on semen characteristics and reproductive performance of male breeder turkey

- A study was conducted using 24 male and 48 female Belts-ville small white cross variety of turkeys to find the effect of dietary supplementation of Vit-E and selenium on semen characteristics and reproductive performance following AI. Twenty eight turkey toms were selected at 32 weeks of age and initially screened for seminal parameters. Twenty four superior toms were randomized into four treatment groups viz.,TI-control (basal male breeder turkey diet - 2,700 k.cal. ME /kg and 16 % CP), T2 , T3 and T4 groups were fed with basal diet supplemented with 300 IU Vit-E/kg diet, 0.3 mg of inorganic selenium/kg diet and 150 IU Vit-E and 0.15mg selenium /kg diet, respectively. Hens were fed with standard female turkey breeder diet (2,900 k.cal. ME /kg and 16 % CP). Pooled semen samples were collected from each treatment group twice a week and analyzed for seminal parameters. There were no significant differences in semen volume, hydrogen-ion concentration and spermatozoa motility (%). Dietary addition of either Vit-E or combination of Vit-E and selenium significantly ($P\leq0.01$) improved the spermatozoa livability (%), spermatozoa concentration, spermatozoa activity and HOST reactive sperm percentage, significantly ($P\leq0.01$) reduced the per cent spermatozoa mid-piece abnormality. Selenium supplemented group shown a highly significant ($P\leq0.01$) improvement in the per cent spermatozoa livability, reduction in the per cent spermatozoa head, per cent spermatozoa tail abnormality. All the anti-oxidant supplemented groups significantly ($P\leq0.01$) reduced the total spermatozoa abnormality. Dietary

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supplementation of anti-oxidants did not show significant (P>0.05) effect on seminal plasma activity of ACP and GPT while combination of Vit-E and selenium had shown a highly significant (P≤0.01) increase and a significant reduction (P≤0.01) in the seminal plasma activity of ALP and GOT respectively.

- A dose 0.2 ml of BPSE diluted (1:4) pooled semen was artificially inseminated into the hens of respective groups. All the three anti-oxidant supplemented groups had shown significant (P≤0.01) improvement in the total fertility and total hatchability percentage. Fertile hatchability (%) and late embryonic mortality (%) did not differ (P>0.05) among treatments. The toms supplemented with combination of Vit-E and selenium had significantly (P≤0.01) better fertility, total hatchability (%) and reduced early embryonic mortality.
- Highly significant (P≤0.01) positive correlation existed between the seminal plasma GOT level, spermatozoa abnormality and the Methylene blue reduction time and negative correlation with spermatozoa motility, spermatozoa concentration and HOST reactive sperm percentage. Seminal plasma ALP level had highly significant (P≤0.01) positive correlation (r=0.470) with spermatozoa concentration and negative correlation (r=-0.279) with MBRT while seminal plasma GPT level had significant (P≤0.05) positive correlation with spermatozoa abnormality (r=0.206). Fertility of the turkey eggs had highly significant (P≤0.01) positive correlation with spermatozoa livability (r=0.675), HOST reactive sperms (r=0.437), seminal plasma ACP (r=0.508) and ALP level (r=0.424) while seminal plasma level of GPT had highly significant (P≤0.01) negative correlation (-0.098) with fertility.

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Enhancing fertility response in postpartum anestrous cows with estradiol and insulin

- Negative energy balance reduces insulin and insulin like growth factor-I (IGF-I) and delays onset of estrous cycle in cows. Healthy pluriparous crossbred cows in four groups (Each 6 animals) were used in this study. Group I cows were treated with GnRH (day 0 - 10 µg) + PGF2α (day 7 - 500 µg) + GnRH (day 9 - 10 µg) and Timed Artificial Insemination (TAI) on day 9 and 10 respectively. In group II, the second dose of GnRH (10 µg) was replaced with 1mg of estradiol benzoate 24 hours after PGF2α (day 8) in the protocol of group-I cows. In group III – along with treatment protocol of Group I insulin - 0.2 I.U/ kg body weight subcutaneously was administered on day 0, 1 and 2 of the experiment. In group IV - Group II treatment was followed and insulin - 0.2 I.U/ kg body weight subcutaneously was given on day 0, 1 and 2 of the experiment. Cows in group IV followed by cows in group II had significantly (P<0.01) high scoring in behavioural, intensity of estrus and ovulatory response (83.33 and 66.66 per cent). The cows from group IV had significantly higher (83.33 per cent) conception rate and the conception rate in all other groups (group I, II and III) were 50.00 per cent. In conclusion, it was found that the protocols followed in this experiment have improved the ovulation rate and conception rate to a considerable level in postpartum anestrus cows. Hence, heatsynch with supplementation of insulin can be used to improve conception rate in postpartum anestrus cows under field condition.

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