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The Author

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List of Abbreviations

Abbreviation	Elaboration
F	Female
Hg	Hemoglobin
M	Male
PCV	Packed cell volume
TEC	Total erythrocyte count
TLC	Total leucocyte count
SE	Standard error

Symbols

Symbols	**Stands for**
&	And
>	Greater than
<	Less than
˚C	Degree centigrade
%	Percentage

Abstract

Transfusion therapy is a major resource that can improve the patient's capability to overcome the disease. Clinically it is very important for all species especially in traumatic cases. The objective of this study was to evaluate the efficacy of blood transfusion in native dogs. The study was performed in Teaching Veterinary Hospital, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. The study was conducted on five donors and five recipient's street dogs. 250ml blood was collected from each donor's jugular vein and 100- 125ml blood was transfused in 5 recipient's dog through the cephalic vein at the 5-6ml/kg/hr transfusion rate. Whole procedure was performed in aseptic condition under sedation and anesthesia. All donors were normal after collection of blood (250ml). Blood parameters were slightly increased in recipient case no 3 after blood transfusion. The highest PCV (2.2%), Hg (0.44%) and neotrophil (1.1%) were recorded after 3 days of post transfusion. The maximum heart rate was 90.2 ±1.7/min recorded after 10 minutes of transfusion. The mean control value of rectal temperature was 100.88 ± 0.23/min^0 F and maximum value was 102± 0.31/min ^{0}F after 20 minutes of blood transfusion. This study suggests that blood transfusion can be performed in critical condition to our native dogs without any blood grouping at least during first time.

Key word: Blood transfusion, Transfusion reaction, Cross matching

Chapter: 1

Introduction

Blood transfusion is the process to replace the liquid or cells found in blood (*eg*. red blood cells, plasma or cells called platelets) (Squires, 2002). Blood transfusions have become an important tool in veterinary critical care and emergency medicine and have taken an increasingly important role in the life support of an animal (Weingart et al., 2008). Over the past decade, the use of blood product in treating critically ill dog has drastically increased. Blood transfusions are usually practiced in acute condition like acute hemolysis or hemorrhage and in the treatment of acute or chronic anemia. Animals with hemostatic disorders often require repeated transfusions of blood. Blood products can be produced only by a living animal and production is limited to the donor's physiologic capability to produce blood. Availability of blood for transfusion is further limited by the small number of commercial canine and feline blood banks (Chohan and Davidow, 2015). Strict aseptic technique must be used during the collection process to prevent contamination of blood and donors should be large, quite and healthy free from any blood borne disease. Donors should be between 1-6 years, weight around 50 pounds and vaccinated.

A transfusion reaction consists of the range of immunologic and metabolic changes that occur during or after administration of a blood product. Transfusion reactions in dogs are limited (Wardrop, 1997). The most common acute immunologic transfusion reactions in the dogs are fever, restlessness, salivation, incontinence, vomiting. Sometimes shock, acute death may occur. A strict transfusion policy, with the use of blood typing and cross matching procedures and careful monitoring, minimize the risk of adverse reaction and maximize the benefits of transfusion. Blood types are classified according to specific antigens on the surface of erythrocytes. Platelets, leukocytes and body tissues and fluids may also consists of erythrocyte antigens (Brown and Vap, 2006). In immunogenicity and clinical significance these antigens can differ. They can serve as markers of disease in some cases and taking part in recognition of self. The clinical significance of blood group antigens is generally noted in transfusion reactions and neonatal iso-erythrolysis (NI) in veterinary medicine. These antigens can characteristically trigger a reaction caused by circulating anti-erythrocyte antibodies in the opposite host or donor.

The dog erythrocyte antigen types or blood types are categorized by the DEA (Dog Erythrocyte Antigen) system. DEA 1.1, 1.2, and 1.3 are termed A system. There are also DEA 3, DEA 4, DEA 5, DEA 6, DEA 7 and DEA 8 (Seth et al., 2012). In people, a hematocrit below 12 percent increases the probability of multiple organ failure (Cotton et al., 2008). Human and veterinary patient's hemoglobin levels, rate of decline and the ability to compensate are also considered before the decision to transfuse is made. Transfusions do not cure disease, they stabilize the patient to buy the clinician time to diagnose and treat the underlying cause of the anemia (Kirby, 1995). Cross matching detects antibodies in the serum of one patient against the RBC antigen of another. It does not predict future compatibility or confirm blood type. Two cross matching procedures are used: a major and minor. The major cross match looks for all antibodies of recipient plasma against the donor RBCs, whereas the minor looks for all antibodies of the donor plasma against the recipient's RBCs. The minor cross match is of less concern when pRBCs are being used because most of the plasma and donor antibodies have been removed (Malone et al., 2006).

Transfusion reaction is another important key note for blood transfusion. Proper monitoring during transfusion pretreatments measurements are necessary. The indication of transfusion reactions can be immunologic or non immunologic. They can be immediate or delayed. After transfusion a decrease in hematocrit between 2 days and 2 weeks resulted in suspicion of delayed hemolysis, dogs show few common clinical signs like (vascular hemolytic as follows: fever, tachycardia or bradycardia, hypotension, dyspnea, cyanosis, excessive salivation, tearing, urination, defecation, vomiting, collapse, opisthotonos, cardiac arrest, hemoglobinemia, and hemoglobinuria. However, with the help of cross matching test of blood, blood transfusion can be performed in our native dog during emergency case.

Blood transfusion especially in dogs is very new and gradually demanding in Bangladesh .Every day a good number of dogs are registered in our Teaching Veterinary Hospital, Chittagong Veterinary and Animal Sciences University (CVASU) where blood transfusion has a great demand especially for the treatment of accidental emergency cases. A strict transfusion policy, with the use of blood typing and cross matching procedures, and careful monitoring, minimize the risk of an adverse reaction and maximize the benefits of transfusion. The main goal of this study was to clarify the effects of whole blood infusion in dogs in regard of pulse rate, respiratory rate, rectal temperature, blood pressure, platelet count and PCV; to check the outcome in native dog.

The objective of this study was to evaluate the problems and possibility of blood transfusion in Native dogs.

Chapter: 2

Review of Literature

2.1. Blood transfusion

Blood transfusion is a process of receiving blood or blood products into one's circulation intravenously. Transfusions were used for various medical conditions to replace lost components of the blood. Early transfusions was used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets (Ferraris et al., 2007).

2.2 Blood transfusion in Dog

Blood transfusions are acute, as in acute hemolysis or hemorrhage; transfusions are also appropriate in treatment of acute or chronic anemias. Animals with hemostatic disorders often require repeated transfusions of whole blood, red cells, plasma, or platelets. Whole blood frequently is not the ideal product to be administered. Animals that require coagulation factors benefit most from administration of fresh-frozen plasma or cryoprecipitate if the need is specifically for factor VIII, von Wille brand factor, or fibrinogen. Platelet-rich plasma or platelet concentrates may be of value in thrombocytopenia, although immune-mediated thrombocytopenia usually does not respond to administration of platelets because they are removed rapidly by the spleen. Domestic animals have blood volumes of 7%–9% of their body weight; cats have a slightly lower volume of ~6.5%. Collection, storage, and transfusion of blood must be done aseptically. The anticoagulant of choice is citrate phosphate dextrose adenine (CPDA-1). Commercial blood bags containing the appropriate amount of anticoagulant for a “unit” (500 mL) are available. Heparin should not be used as an anticoagulant, because it has a longer half-life in the recipient and causes platelet activation; also, heparinized blood cannot be stored (Bagnagatti De Giorgi, 2013).

Transfusion therapy is indicated in patients with many different diseases and conditions, including anemia, hemorrhage, coagulopathy, and hypoproteinemia. Dogs rarely have clinically significant preformed antibodies, so only those that have received repeated transfusions are at risk. The most common hemolytic reaction in dogs that have received multiple transfusions is delayed hemolysis, seen clinically as shortened survival of transfused RBCs and a positive Coombs’ test (Barr and Bowman, 2011). There are many indications for transfusion therapy in veterinary practice, and with the increased availability of blood

products, it is becoming more widespread. Fractionating whole blood into its component products has made it easier to treat a broad range of conditions when donors are not available. Blood components accessible to veterinarians include fresh and stored whole blood, packed red blood cells (pRBCs), fresh-frozen plasma (FFP), cryoprecipitate, and platelet products. However, administering blood products is not a benign procedure. Red blood cells (RBCs) are very antigenic and can promote a significant immune response. Administering foreign proteins, leukocytes, or platelets may also stimulate the immune system. The appropriate blood product should be selected based on maximum benefit with minimum risk to the patient (Callan et al., 1995).

2.3. Selecting Blood Donors

Blood donors should be healthy adult dogs. Dogs should weigh more than 30 kg; have a packed cell volume (PCV) of 40% or more; be fully vaccinated; and be free of heartworm infection, brucellosis, and tick-borne diseases (e.g., *Ehrlichia canis, Babesia canis, Rickettsia rickettsi, Borrelia burgdorferi* infections). It is also recommended that canine donors test negative for dog erythrocyte antigen (DEA), (universal donors would also need to test negative for DEA 1.2 and 7). Purebred and crossbred Akitas have high levels of intracellular potassium in their RBCs and should not be used as blood donors (Harvey, 1997).

Cats should weigh more than 5 kg, be lean, and preferably be shorthaired. Feline donors should have a PCV greater than 35%; be fully vaccinated; and be free from FeLV, FIV, *Toxoplasma gondii,* and *Hemobartonella felis* (now called *Mycoplasma hemofelis*) infections.

2.4. Canine Blood Groups

Nine blood groups (i.e., DEA 1.1, 1.2, 1.3, 3, 4, 5, 6, 7, 8) have been identified in dogs. The most clinically significant are DEA 1.1 and 1.2. Spontaneously occurring antibodies (i.e., alloantibodies) to these antigens have not been identified; thus immunologic reactions are rare for the first transfusion administered to dogs. However, if a transfusion of blood positive for DEA 1.1 is administered to a recipient with blood negative for DEA 1.1, antibody induction will occur. If a second transfusion of blood positive for DEA 1.1 is administered, an immune-mediated transfusion reaction may destroy all transfused cells in less than 12 hours. Pregnancy can also induce all antibodies to DEA 1.1 in up to 25% of dogs. All antibodies against DEA 7 are present in up to 15% of dogs. These antibodies cause a delayed-type immune response against RBCs positive for DEA 7, resulting in hemolysis 1 to 3 days

after transfusion. Delayed-type hemolytic reactions may also occur if the donor animal has all antibodies to DEA 3 or 5; however, these occur rarely in the general population.

In addition to being evaluated for an underlying cause, patients with severe anemia often require red blood cell (RBC) transfusion. Packed RBCs and fresh whole blood are the two main blood products for the treatment of anemia in dogs and cats. The choice of blood component should be tailored to the patient's needs. Although both packed RBCs and fresh whole blood are hemoglobin sources for improving oxygen-carrying capacity, whole blood has the advantage of providing clotting factors and platelets to help with hemostasis when necessary. However, this advantage is provided at the cost of increased infusion volumes, which all patients may not tolerate. Plasma-containing products were initially used to improve oncotic pressure in patients with hypo-albuminemia. With the widespread availability of synthetic colloids and their superior ability to improve oncotic pressure, however, there are fewer indications today for whole blood transfusions.

2.5. Improvement of transfusion medicine in Dog

Transfusion medicine has improve day by day become more feasible in small animal practice, with best access to blood products through either on-site donors, the purchase of blood bank products, external donor programs, or the availability of blood component substitutes. However, the safe use of blood component therapy requires knowledge of blood groups and antibody prevalence, and knowledge of the means to minimize the risk of adverse reactions by including the use of proper donors and screening assays that facilitate detection of serological incompatibility (Lanevschi and Wardrop, 2001).

Red blood cell (RBC) transfusions have become an important tool in veterinary critical care and emergency medicine (Goodnough et al., 1999). A strict transfusion policy, with the use of blood typing and cross matching procedures, and careful monitoring, minimize the risk of an adverse reaction and maximize the benefits of transfusion. Fresh whole blood (WB) is composed of RBCs, white blood cells (WBCs), platelets, all the coagulation factors, albumin and immunoglobulins (Chiaramonte, 2004). After 6 hours of storing, platelets, factor V, VIII and vWF become depleted (Abrams-Ogg, 2000). WB is indicated when several blood components are required or when the patient has acutely lost more than 50% of its total blood volume, in order to increase oncotic pressure and reestablish tissue oxygenation (Lanevschi and Wardrop, 2001). There are some studies concerning the indications and efficacy of whole blood transfusion in dogs and cats (Godinho-Cunha et al., 2011), although very few succeeded in establishing a relation with the outcome. A recent survey clarified the effects of

blood collection in cats (Iazbik et al., 2007), but, so far, some of the effects of whole blood infusion in patients are still unknown. The main goal of this study was to clarify the effects of whole blood infusion in dogs and cats in regard of pulse rate, respiratory rate, rectal temperature, blood pressure, platelet count and PCV; and establish an association with the outcome.

2.6. Importance of Blood Transfusion in Dog

Transfusion therapy is a major resource that can improve the patient's capability to overcome the underlying disease. However, the effects of whole blood infusion, and how they affect the patient's outcome, are not yet clear. One of the studies, a protocol was developed in order to monitor a group of 15 animals (9 dogs, 6 cats) that received a total of 19 transfusions; 3 animals received more than one transfusion each. The most common indications for blood transfusion included acute blood loss (47%), coagulopathy (33%) and other anaemias (20%). The mean pre-transfusion packed cell volume (PCV) of animals with acute blood loss (18%) was higher than in the group of coagulopathy (15%) or other anaemias (15%). The survival rates at 6 days after transfusion were greater in the coagulopathy (80.0%) and other anaemias (66.7%) than in the group of acute blood loss (42.9%). After transfusion, pulse rate ($p < 0.01$) and platelet count ($p < 0.05$) decreased signifiantly, and there was a signifiant increase in body temperature of the animals that suffered from hypothermia before the transfusion ($p < 0.05$). Overall survival was predictable based upon post transfusion body temperature, observed PCV change, the difference between the obtained and the calculated PCV, and administered transfusion volume ($p < 0.05$) (Godinho-Cunha et al., 2011).

2.7. Blood group of Dog

Blood groups are named according to the species-specific antigens present on the surface of erythrocytes. Theses antigens play an important role in inducing immune-mediated reactions and can cause complications while transfusing blood from different blood groups. Antigens coupled with platelets, leukocytes and plasma proteins may also induce immune mediated reactions in host animals during transfusion therapies. Plasma also has some naturally occurring all antibodies that can act against other blood groups without any prior exposure to the erythrocyte antigens (Schmitz et al., 1998). Erythrocyte antigens can induce production of antibodies when animals get exposed via blood transfusion, transplacental exposure or in the case of neonatal iso-erythrolysis (NI), through colostrum. Blood groups in the common domestic and pet animal species are described here. From a clinician's point of view, these are the antigens to which the veterinary practitioner should be most familiar. However, many

other blood group factors and systems have been described and the lack of commercially available typing sera does not diminish the potential significance of these other systems in transfusion medicine.

International workshops met in 1972 and 1974 to standardize canine blood groups as defined by iso-immune sera and to standardize canine blood group system nomenclature. The fist workshop designated the terminology canine erythrocyte antigen (CEA) followed by a number to indicate the blood group antigen. The second workshop adopted the designation dog erythrocyte antigen (DEA). The new terminology was adopted to avoid confusion with the carcinoembryonic antigen (CEA) system. The blood group system in dogs includes DEA 1.1, DEA 1.2, DEA 3, DEA 4, DEA 5 and DEA 7. The DEA nomenclature system of canine blood group is not accepted worldwide, although some authors use the newer genetic nomenclature system in reporting new blood group specificities. In dogs, naturally occurring alloantibodies are of lesser clinical significance whereas in cats it is very important clinically. DEA 1.1 and 1.2 are the most important blood groups and are found in 60% population of canines. These blood groups can elicit severe transfusion reactions in previously sensitized dogs. DEA 1.3 has been described in German shepherd dogs in Australia.10 DEA 4 blood group of dogs is found in high frequency that can cause hemolytic transfusion reactions in DEA 4-negative dogs previously sensitized by DEA 4-positive blood transfusions. The DEA 3, 5 and 7 blood groups can cause delayed transfusion reactions in dogs lacking these antigens but are previously sensitized to these antigens.

2.8. Protocols of Blood grouping

Three protocols of blood transfusion were evaluated in a canine model for (1) the strength and breadth of leukocytotoxin induction, (2) the induction of cell-mediated immunity against the blood donors, (3) haemagglutinin production, and (4) any effect on kidney graft survival. At the end of the transfusion schedule, each dog received a kidney graft and was given azathioprine and prednisolone postoperatively. All dogs were unrelated and blood donors were not used as kidney donors. All three transfusion protocols, comprising i.v. injections of blood twice weekly or every 2 weeks from one or three donors, induced unacceptably strong and broad leukocytotoxins (Fabre et al., 1978). All transplants performed across a positive cross match failed to function. However, where a negative cross match was available, the trend of results was that the transfused dogs had better graft survival than nontransfused animals similarly treated with azathioprine and prednisolone. Only one dog produced haemagglutinins. Several animals had positive cell-mediated immunity against the blood

donors, but the response was not strong and was frequently not sustained. The transfusion of incompatible blood into dog is accompanied with or immediately followed by chills, fever, nausea and vomiting, acute pains in the muscles, dyspnea and a feeling of constriction in the chest. Signs of hemolysis *in vivo* may occur within a few hours. These include hemoglobinemia, hemoglobinuria and jaundice. If a relatively small amount of blood is hemolyzed, hemoglobinuria and jaundice may not be evident. DeGOWIN has shown that only about 10 per cent of the hemoglobin that disappears from the blood stream of the dog appears in the urine. The patient may recover with nothing more serious than the loss of the transfused erythrocytes and consequent hemoglobinuria for several days. The urinary excretion is immediately diminished, or ceases entirely, and the products of nitrogen metabolism increase rapidly in the blood. Vomiting continues, and generalized edema sometimes appears (DeGOWIN et al., 1938). Dogs injected intravenously with dog erythrocytes containing one or more antigenic factors lacking in their own red cells developed iso-hemagglutinins and hemolysins exhibiting characteristics of immune antibodies. Transfusions of incompatible whole dog blood and plasma were carried out under controlled conditions. Pretransfusion observations were made and followed by closely spaced post-transfusion measurements of serologic and hematologic alterations. The rate of destruction of incompatible donated corpuscles was determined by tagging the cells with radioactive iron and also by employing the technique of differential agglutination of erythrocytes. It was thereby shown that all of the incompatible donated cells disappeared from the recipient's circulation within the first thirty to ninety minutes following transfusion. The probable mechanisms and relative importance of intra- and extravascular destruction of erythrocytes are briefly discussed. Destruction of recipient dogs' corpuscles by donated immune plasma was relatively slow, and spherocytosis and increased osmotic fragility of the recipients' cells were evident for periods as long as twenty days (Ness et al., 2001). These observations are compared with those made in human beings after transfusions of plasma and of blood from dangerous universal donors. The titer of complement in the sera of recipient dogs was sharply reduced for at least five hours after all transfusions of incompatible whole blood, but isoagglutinin titers were less regularly reduced after such transfusions. Other notations of interest included estimates of the concentrations of serum bilirubin, sodium and potassium, determinations of clotting time, prothrombin concentration, and observations on red cell morphology, intravascular erythrophagocytosis, and shifts in distribution of leukocytes and in the electrophoretic patterns of plasma.

2.8. Storage of Blood for Dog

The pH of stored blood progressively decreases with lactic and pyruvic acid accumulation, promoting (2,3-DPG) reduction by 54% within the first 24 hours of storage in canine RBC (Williams et al., 1991) to undetectable levels within 2 weeks of human pRBC storage (Raat and Ince, 2007). ATP prevents erythrocyte membrane loss by microvesiculation which leads to the extracellular accumulation of negatively-charged pro-inflammatory and pro-coagulant microparticles (MPs). Negativelycharged phospholipids, specifically phosphatidyl-serine, are transported from the outer to inner surfaces of RBC, by ATP-mediated active transport, thus minimising macrophage clearance following transfusion (Hess et al., 2006) With decreasing ATP concentration, RBC shape degenerates with irreversibly reduced deformability.

2.9. Justification of Dog blood transfusion

Transfusion medicine has gradually become more feasible in small animal practice, with improved access to blood and blood products through either on-site donors, the purchase of blood bank products, external donor programs, or the availability of blood component substitutes. However, the safe use of blood component therapy requires knowledge of blood groups and antibody prevalence, and need to learn about knowledge of the means to minimize the risk of adverse reactions by including the use of proper donors and screening assays that facilitate detection of serological incompatibility (Lanevschi and Wardrop, 2001). Transfusion medicine, an update on canine and feline blood groups and known blood incompatibilities, laboratory testing for blood type or compatibility, donor selection and blood collection, storage of blood components, blood component and blood substitute therapy, and adverse reactions in small animal transfusion medicine (Griot-Wenk and Giger, 1995). Day by day dog was affected different blood related problems like canine parvo viral infection, blood protozoa etc. This type of disease can cause anemia related problems are parturition, abortion, animal biting and others (Singh and Somvanshi, 2013). That time need to transfer blood for saving animal life.

Chapter: 3

Methods and Materials

3.1 Study area and study period

The study was conducted from January to December, 2016 at Chittagong Veterinary and Animal Sciences University, Bangladesh. The experiment was performed at Shahedul Alam Quadery Teaching Veterinary Hospital, CVASU.

3.2. Study design

3.2.1. Selection of Donor

Subjected sample size for blood collection was five healthy dogs. We select our native dogs for this study. To select the donor animal, we had to undergo some criteria like all donors were healthy young's that have never been transfused. The criteria adopted for the selection were: minimum body weight of 13 to 25 kg; age between 1 and 2 years (Table-1) In addition, donors must have undergone routine physical, hematological and clinical chemistry evaluations examinations. All donors were vaccinated and also tested of blood parasites and other infectious diseases. After collection of blood, the donor, were transfused with 0.9% sodium chloride solution and orally supplemented with iron.

Table 1**- Criteria of donor animal for assessment of the animal**

Case no	Body Wt (Kg)	Age	Breed	Sex	Vaccination	Pcv %
1	17.10	1 year	ND	F	Vaccinated	40
2	23.80	2 year	ND	M	Vaccinated	40
3	21.9	1.5 Year	ND	M	Vaccinated	41
4	20. 7	1 year	ND	M	Vaccinated	40
5	21	1.5 year	ND	F	Vaccinated	39

PCV= Packed cell volume

3.2.2. Selection of Recipients

Five recipients were selected for blood transfusion. The recipients were selected on the basis of hypovolemic shock after external or internal hemorrhage, parvovirosis with clinically hemorrhagic gastro-enteritis, babesiosis, acute renal failure, chronic renal failure, coagulopathy. Most of them were between 10 months to 1 year old and weight between 12 to 21 kg. (Table 2). The recipients were undergone also routine physical (Fig-1), hematological and clinical chemistry evaluation examination.

Table 2**- Criteria of donor animal for assessment of the animal**

Case no	Body weight(kg)	Age	Breed	Sex	Problem
1	15	1 year	ND	F	Hemorrhage
2	15.7	1 year	ND	F	Anemia
3	13	10 Year	ND	M	Anemia
4	13.6	10 year	ND	M	Blood protozoa
5	15	1.3 year	ND	F	Hemorrhage

F-Female, M-Male

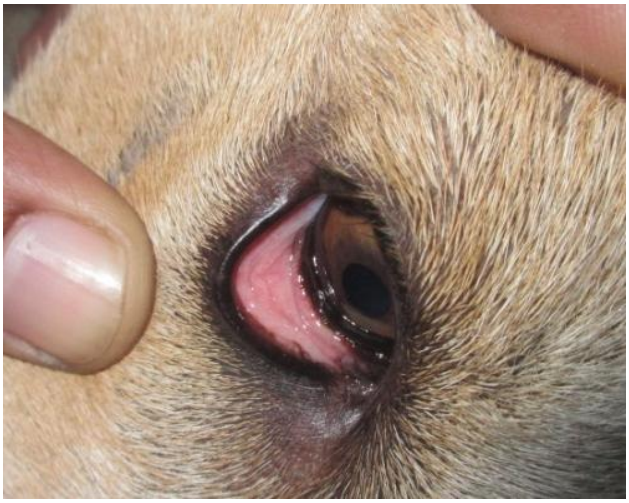

Fig 1**:** Picture of pale mucous membrane of recipient dog, where pale mucous membrane is the indicator of animal suffers in anemia.

3.2.3. Pre transfusion examination of blood

Blood samples were collected from both donor and recipient in the morning before transfusion and were undergone different hematological examinations.

For hematological examination, 4ml of blood were collected from both donor and recipient. Then 2 ml of blood were transferred into a vial without EDTA for collection serum and another 2ml into with anticoagulant for the following hematological study.

3.3. Hematological study

3.3.1 Haematocrit or Packed Cell Volume (PCV)

The haematocrit tube was filled up with well-mixed blood by special loading pipette. Then the tube was centrifuged at 3000rpm for half an hour and reading was taken. The PCV was determined as per method described by Coffin (1955).

3.3.2 Hemoglobin (Hb) concentration

The Hb estimation was performed by the acid Hematin method with the Shali's Hemometer. The result was read as per method described by Coffin (1955).

3.3.3 Total Erythrocytes Count (TEC)

Thomas red blood cell pipette was used and pipette was filled up to 0.5 marks with blood and diluting fluid (Hayem's solution). The dilution of the contents was 1:200. The counting chamber was filled with the contents and was placed under microscope. Counting of cells and calculation of TEC was performed as per method indicated by Coffin (1955).

3.3.4 Differential Leucocytes Count (DLC)

A thin blood film was made by spreading a blood drop evenly on clean grease free slide and modification of Romanowsky's stain namely Leishman's stain was used. For Giemsa's staining the air dried blood smears were prefixed with acetone free methanol for five minutes (Conn and Darrow, 1960).

3.3.5 Preparation of serum sample

After 30-60 minutes at room temperature the coagulated blood which was kept in vacutainer where no anticoagulant was centrifuged (Capp Rondo, CR-68X, Denmark) for 20 minutes at 3000rpm. Obtained serum samples were shifted to the eppendorf tube by using micropipette and given unique identification no. The obtained serum samples were stored in -20^{o}C until analysis for biochemical test.

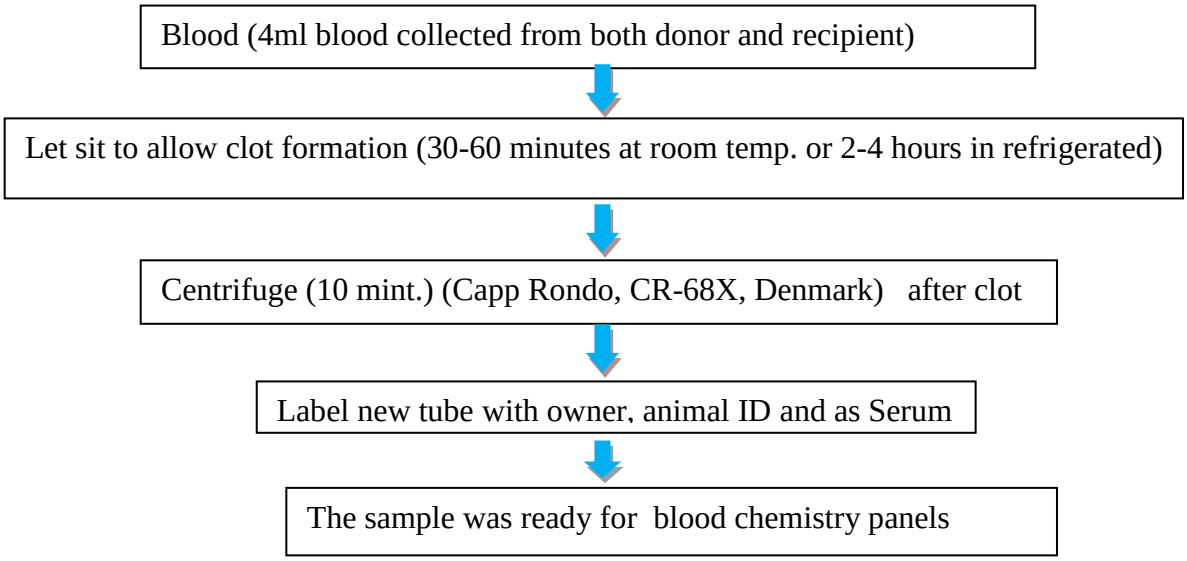

Fig 2: Preparation of serum for cross matching test whether sample collected from both donor and recipient animal after centrifuge

3.3.6 Cross matching procedure

A blood cross match (BCM) was performed to detect serological incompatibility between donor and recipient prior to a blood transfusion. A control BCM (recipient plasma + recipient cells) always performed because some recipients may have immune medicated hemolysis and/or agglutination that will interfere with results.

2ml blood sample was collected from both donor and recipient. After collection the blood was centrifuged at 3000 rpm for 5 min. to separate plasma from red blood cells (RBCs). The plasma was collected from each sample with a clean pipette and transfer to clean, labeled glass or plastic tubes. RBC was three times with normal saline solution; resuspend to make 3-5% RBC suspension (1 drop RBC: 20 drop saline). After those 3 tubes were labeled with Major, Minor, and Recipient control for donor. For crosshatching procedure 2 parts of plasma and 1 part of RBC suspension was mixed as follows:

Major BCM: recipient plasma + donor cells

Minor BCM: donor plasma + recipient cells

Recipient control: recipient plasma + recipient cells

Samples were mixed and incubate for 15 min. at room temperature. When macroscopic agglutination is not observed, a small amount was transferred onto a glass slide and examine for microscopic agglutination.

3.4. Anticoagulant

Citrate-phosphate-dextrose adenine (CPDA-1) was used for storage of blood. The single blood bag contains 35 ml CPDA-1 per 250 ml blood bag. CPDA-1 was considered better anticoagulant, stored for approximately 35 days

3.5. Blood collection bag

CPDA 1 whole blood bag (TERUFLEX , Terumo corporation, 250 ml , contain 35 ml CPDA-1) with 16 were used for collection of blood from donor. The blood holding capacity of the bag was 250 ml. The bag was clean, sterile and was kept in dry until used.

3.6. Technique of blood collection

3.6.1. Acquire a Blood Donor

First step was to determine a dog to use as the blood donor Then a complete blood count (CBC) was performed to make sure the dog donating is healthy enough to donate either 250 ml of blood. Initial temperature, pulse, respiration rate (TPR) was taken. After collection of blood, IV infusion set was placed in cephalic vein of the patient to given fluids for mantainence body's normal fluid volume and nutritional supplementation.

3.7. Prepare Donor

Shaved hair on left or right side of neck to prepare for obtaining blood from jugular vein. After shaving, alcohol was used to sterilize skin for blood collection.

3.8. Collection of blood

The blood collection bag was put in position that easily blood drawn into the bag. Needle was inserted into donor dog's jugular vein as until blood begins to flow into blood collection bag. Under handling, the needle was in the jugular vein after blood flow has been established. The blood bag has to hold lower than dog and gently agited blood collection

3.9. Complete Collection

After complete blood collection the needle was removed very carefully and holds off the entry point of needle into the vein for few minutes to clot the blood. The line was closed by tying simple knot on the line close to needle for preventing blood fall down from the tube. And needle was removed by cutting the remaining part.

3.10. Transfusion technique

The cephalic vein was preferred to transfuse the blood. After proper cleaning and shaving the area, the infusion set was fixed with vet wrap. Then the blood bag was hanged above the patient level for proper drawn of blood.

The transfusion rate was fixed by the formula-

Volume (mL)=Recipient weight(Kg)×

88(dog) × (Recipient desired PCV– Current PCV) / PCV of donor blood

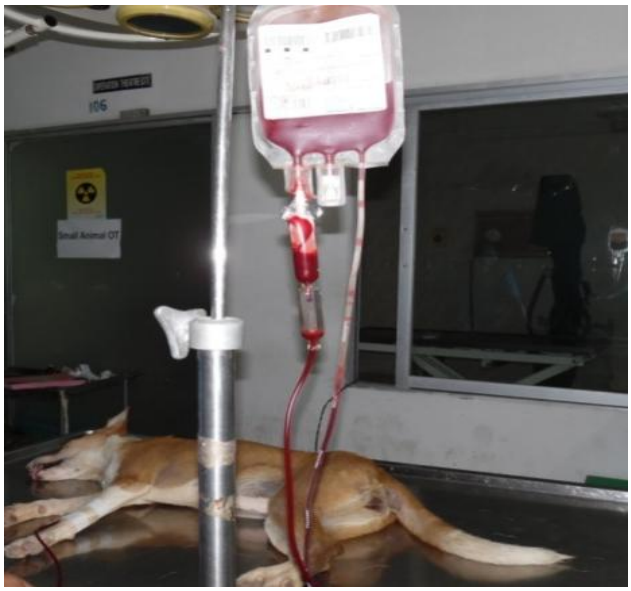

Fig 3- Picture of blood transfusion. Where CPDA-1 whole blood bag (250 ml bag, contain 35 ml CPDA-1) was choose to transfusion of blood. The transfusion rate was 5-6 ml/kg/hour. During the transfusion process temperature, heart rate, respiration rate were monitoring for evaluation of transfusion reaction of recipient.

3.11. Monitoring the patient

Temperature, pulse rate and respiration were monitored for every 5, 15 and 30 minutes. Facial swelling, vomition and increased respiration rate were also noticed during monitoring.

3.12. Post transfusion assessment

Assessment of recipient was performed until few hours of blood transfusion for monitoring blood transfusion reaction. The process was performed by matching of baseline values of attitude, rectal temperature, pulse rate and quality, respiratory rate and character, mucous membrane color, capillary refill time, hematocrit before and after transfusion. The majority of these parameters were checked for every 30 minutes throughout the transfusion and evaluated routinely post-transfusion to ensure the desired effect has been achieved.

3.13. Statistical evaluation

The data were put into the MICROSOFT EXCEL 2007 and ANOVAs test and student T test were used for analysis.

Chapter: 4

Results

4.1 Effect of blood transfusion on the basis of haemato-biochemical parameters

4.1.1 Packed cell volume

The mean packed cell volume was increased by 2 % by 1 hour after transfusion. The highest increased (2.2%) packed cell volume was recorded at 3 days after post transfusion (Table 3). The changes however were statistically significant ($p<0.05$).

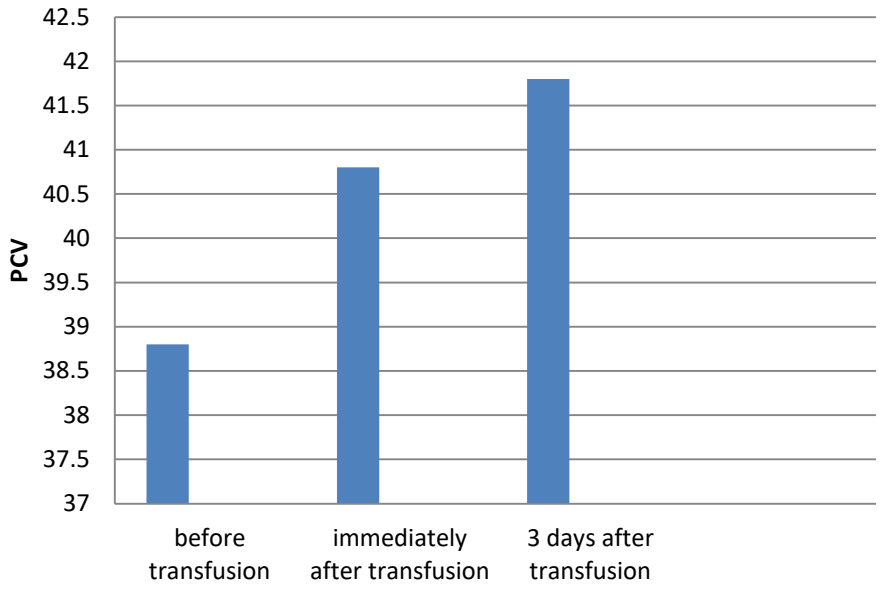

Fig 4- Percentages changes of various PCV in recipient dog before, immediate and after blood transfusion. Values represent the mean value of PCV before, immediate and after blood transfusion. Here the changes are statistically significant ($p<0.05$)

4.1.2 Hemoglobin

The mean hemoglobin was increased by 0.84% 1 hour after transfusion. The highest increased (0.44%) hemoglobin value was recorded at day 3 post transfusion (Table 3). The value was not significant after statistical analysis ($p> 0.05$).

4.1.3 Total erythrocyte count (TEC)

The mean total erythrocyte count was increased by 0.15 % by 1 hour after transfusion. The highest increased (0.54%) of total erythrocyte count was recorded at 3 days after post transfusion. The changes however were not statistically significant ($p > 0.05$).

4.1.4 Total leukocyte count (TLC)

The mean of total leukocyte count was increased by 0.61 % by 1 hour after transfusion. The lowest point (0.58 %) of packed cell volume was recorded at 3 days after post transfusion. The change after one hour of blood transfusion was statistically significant ($p<05$).

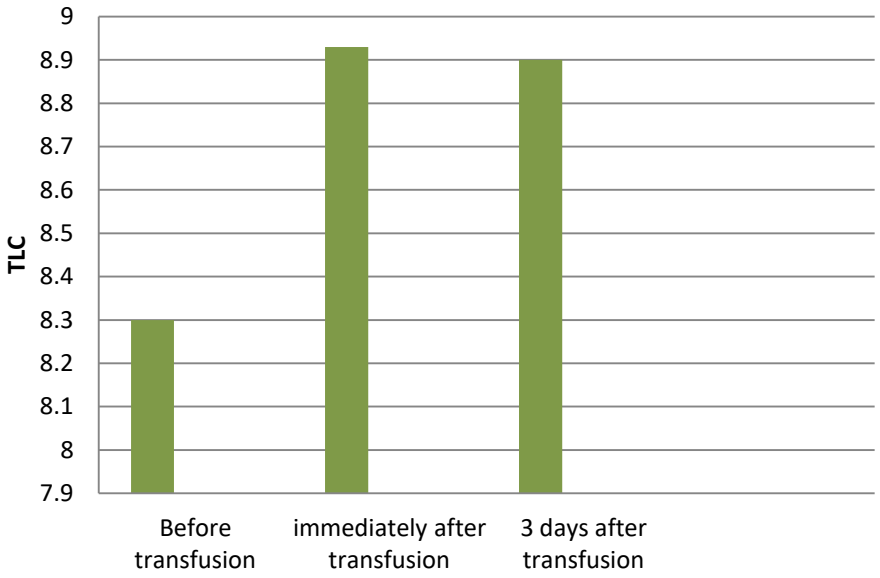

Fig 5- Percentages changes of TLC in recipient dog before, immediate and after blood transfusion. Values represent the mean value of TLC before, immediate and after blood transfusion. Here the changes are statistically significant ($p<0.05$)

4.1.5 Lymphocyte

The mean of lymphocyte was increased by 5 % by 1 hour after transfusion. The lowest point (0.54%) of lymphocyte was recorded at 3 days after post transfusion (Table 3). The change after one hour of blood transfusion were statistically significant ($p <0.05$).

4.1.6 Neutrophil

The mean control value of neutrophil was decreased by 3 % by 1 hour after transfusion. But the highest increased (1.1 %) of neutrophil was recorded at 3 days after post transfusion (Table 3). The changes however were not statistically significant ($p >0.05$).

4.1.7 Eosinophil

The mean value of Eosinophil was decreased by 0.4 % by 1 hour after transfusion. The lowest decreased (0.54%) of Eosinopil was recorded at 3 days after post transfusion (Table 3). The mean value was higher before blood transfusion. The changes however were not statistically significant ($p > 0.05$).

4.1.8 Monocyte

The mean of total erythrocyte count was increased by 0.2 % by 1 hour after transfusion. The lowest decreased (0.54%) of Monocyte was recorded at 3 days after post transfusion (Table 3). The changes however were not statistically significant ($p > 0.05$).

4.1.9 Basophil

The mean control value of Basophil was increased by 0.4% by 1 hour after transfusion. The lowest decreased (0.2%) of Total basophil was recorded at 3 days after post transfusion (Table 3). The changes however were not statistically significant ($p > 0.05$).

Table 3**- Effect of blood transfusion on various Hematobiochemical parameters in recipient dog**

Parameters	Pre-transfusion values (control) (mean±SE)	After transfusion (mean±SE)	P value	3 day after transfusion (mean±SE)	P value
Packed cell volume%	38.8± 1.01	40.8 ± 1.24	0.003	41± 0.00	0.09
Hemohlobin gm%	8.78± 0.69	9.62 ± .097	0.21	9.2 ± 0.2	0.60
TEC Million /cumm	4.76±0.35	4.91 ±0.25	0.68	5± 0.00	0.54
TLC thousan/cumm	8.32 ±1.07	8.93 ±0.97	0.006	8.9± 0.9	0.14
Lymphocyte	48.6 ± 3.5	53.6 ± 2.6	0.03	52 ±2	0.38
Neutrophil%	45.4 ±4.98	42.2 ± 4.4	0.58	46.6 ±1.12	0.83
Eosinophil%	9.6 ± 2.9	9.2 ± 2.1	0.80	6.8 ± 1.6	0.14
Monocyte%	4.2 ± 0.73	4.4 ± 0.50	0.62	3± 0.00	0.17
Basophil%	0.6 ± 0.24	1 ± 0.31	0.18	0.8 ±0 .2	0.37

S E= Standard error

Significant variation (p <0.05)

4.2 Effect of blood transfusion on clinical parameters in recipient

4.2.1 Heart rate and Respiration rate

The mean control value of heart rate and respiration rate were 84.2 ±1.8 and 19.2 ±0 .2 / min respectively. This values increased to 89 ±1.9 (SE) and 20.2 ±0 .37 / min respectively 5 minutes after transfusion. The maximum heart rate was 90.2 ± 1.7 min was recorded after 10 minutes of transfusion (Table 7). The increased heart rate gradually returned to control level in 3 days after post transfusion. The increased in heart rate was significant (p< 0.05). And increased respiration rate also significant (p<0.05).

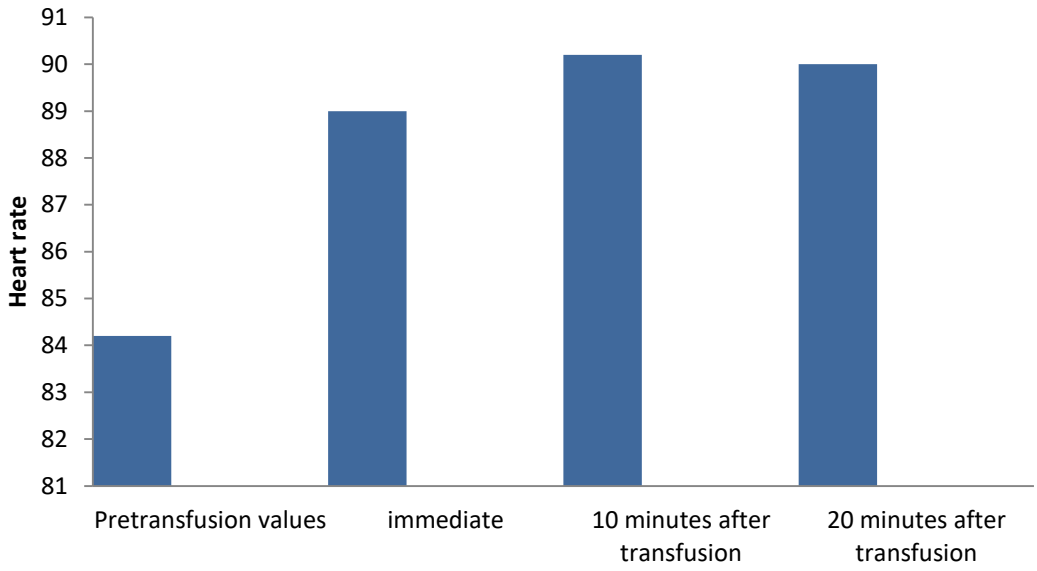

Fig 6- Changes of Heart rate in recipient dog before, immediate and after blood transfusion. Values represent the mean value of heart rate before, immediate and after blood transfusion. Here the changes are statistically significant ($p<0.05$)

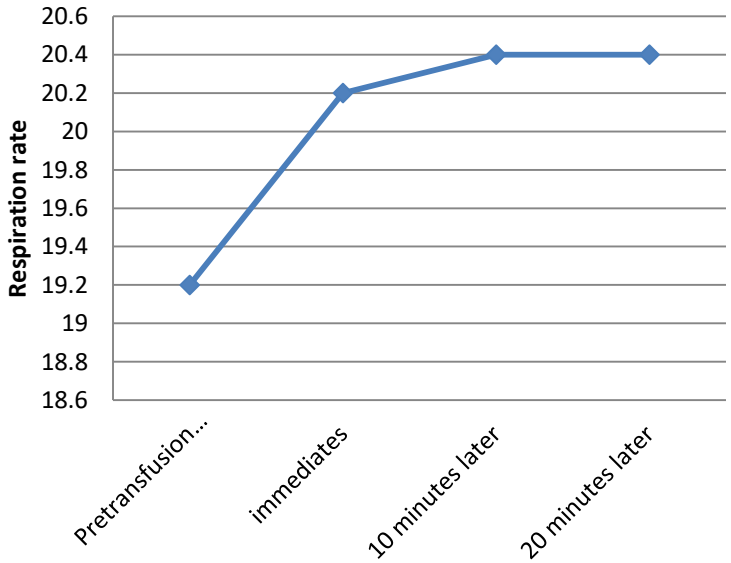

Fig 7- Changes of Respiration rate in recipient dog before, immediate and after blood transfusion. Values represent the mean value of respirationt rate before, immediate and after blood transfusion. Here the changes are significant ($p<0.05$)

4.2.2 Temperature

The mean control value of rectal temperature was 100.88 ± 0.23 (SE) ^{0} F maximum value was 102± 0.31(SE) ^{0}F after 20 minutes of blood transfusion (Table 7). The increase temperaturrate was significant (P<0.05).

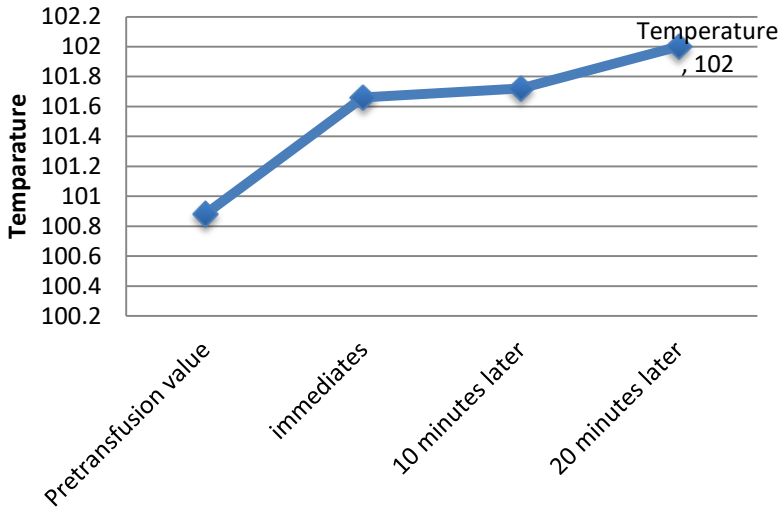

Fig 8- Changes of Temperature in recipient dog before, immediate and after blood transfusion. Values represent the mean value of heart rate before, immediate and after blood transfusion. Here the changes are significant (p<0.05)

4.3 Clinical evaluation after blood transfusion

The clinical intra-transfusion status of the patients was monitored during therapy for early detection of the possible transfusion reactions. Monitoring parameters included measurements of temperature, heart rate, pulse strength and synchronicity, mucous membranes and respiratory rate, 3 times in the first 30 minutes and each half hour to the end of the procedure. The patients were carefully observed if vomiting, tachycardia, dyspnoea, diarrhea, salivation, angioedema, urticaria, facial oedema or hemoglobinuria had developed during transfusion. Fever, restlessness and salivation were found in case no 3, after 30 minutes later of blood transfusion. But it decreased and come in normal level after 3 days after transfusion. (Table 4)

Table 4**: Effects of blood transfusion monitoring during blood transfusion**

Case No.	Before transfusion	10 minutes later	30 minutes later	60 minutes later	3 days later
Case no 1	No reaction sign	No reaction sign	No reaction sign	No reaction sign	No reaction sign
Case no 2	No reaction sign	No reaction sign	No reaction sign	No reaction sign	No reaction sign
Case no 3	No reaction sign	No reaction sign	Fever, Restlessness, salivation	No reaction sign	No reaction sign
Case no 4	No reaction sign	No reaction sign	No reaction sign	No reaction sign	No reaction sign
Case no 5	No reaction sign	No reaction sign	No reaction sign	No reaction sign	No reaction sign

Chapter: 5

Discussions

Blood transfusion was practiced after proper blood grouping and cross matching the donor's group with the recipient's to prevent the transfusion reactions. In my study the mean control value of packed cell volume was increased by 2 % by 1 hour after transfusion and gradually its increase the value according to the time. The highest increased (0.44%) of hemoglobin value was recorded at 3 days after post transfusion on statistical value. The mean control value of Total Leukocyte Count was increased by 0.61 % by 1 hour after transfusion. So, immediately after transfusion, the value of TLC increases but after few days its back into the normal value. The mean control value of Lymphocyte was increased by 5 % by 1 hour after transfusion. It's also back into the normal value after 3 days of transfusion. Temperature, heart rate and respiration rate increased within 1 hour after transfusion but it's returned into the normal value 3 days after transfusion.

The mean control value of packed cell volume was increased immediately after transfusion. The highest increased (2.2%) packed cell volume was recorded at 3 days after transfusion. Transfer of whole blood to the anemic patient, body gets immediate oxygen after transfusion. After that the oxygen initiates to produce more RBC production and increase the packed cell volume in the body. Other studies showed that a rise in PCV was documented after 13 transfusions, a decline was seen after five, and in one case there was no change (Luis f et al, 2010) which is very similar to my study result. But decreases in PCV are not rare and have been described before (Weingart *et al* .2004). Causes for this phenomenon include hemodilution by crystalloid fluids and persistence of the primary cause (blood loss or hemolysis) (Rozanski and Laforcade 2004). Patients with more efficient transfusions achieved higher PCV and had better oxygen-carrying capacity to overcome the underlying disease (Luis F et al, 2010). When these patients receive a RBC transfusion, blood volume and oxygen-carrying capacity are reestablished ((Jutkowitz et al., 2002). There was a significant PCV increase after transfusion, platelet count decreased significantly. This phenomenon is consistent with the findings of Jutkowitz et al. (2002). This phenomenon also satisfies the study.

The Mean value of hemoglobin was increased immediately after transfusion and after 3 day of transfusion the value was maximum. After transfusion of whole blood recipients get direct oxygen from the whole blood and increase the hemoglobin level in the body. Hemoglobin-based oxygen carriers have been under investigation as a type of blood substitute, principally to augment oxygen delivery to tissues (Moore,2003). The oxygen-carrying effects of hemoglobin last up to 3 days in circulation. However, in the absence of hemolytic processes, transfused RBCs should remain in circulation more than 28 days (Driessen et al, 2003). Hemoglobin and hematocrit values rapidly equilibrate after transfusion in normovolemic patients who are recovering from an acute bleeding episode (J. Ignasi Eligalde Md et al, 2003). This phenomenon also satisfies the study.

The mean control value of TLC was increased immediately after transfusion and the value was increase until 3 days of transfusion. After transfusion, clinically silent inflammatory response to blood transfusion might be present; because many transfusion recipients have ongoing inflammation associated with the underlying illness. It is also likely that any possible negative impact of transfusion on the status of a critically ill dog would be underestimated or attributed to the primary disease process. The fatality rates of dog undergoing transfusion has been reported to range from 39 to 53%, with most deaths attributed to the underlying disease process (Garratty *et al*, 2002)

The mean control value of heart rate was 84.2 ±1.8 / min respectively. These values increased to 89 ±1.9 / min respectively 5 minutes after transfusion. The maximum heart rate was 90.2 ±1.7 /min was recorded after 10 minutes of transfusion. The increased heart rate gradually returned to control level in 3 days after post transfusion. Patients with acute anemia's have a compromised oxygen-carrying capacity, presenting different states of hypoxia or anoxia (Carson and Hébert 2009). The body tries to increase oxygen delivery to vital organs by activating the sympathetic and adrenergic nervous systems, which increases heart rate and cardiac output (Carson and Hebert, 2009). When these patients receive a RBC transfusion, blood volume and oxygen-carrying capacity are reestablished that supports my result. Pulse rate decreased significantly after transfusion. Patients with acute anaemias have a compromised oxygen-carrying capacity, presenting different states of hypoxia or anoxia (Carson and Hébert, 2009). That supports my result.

The mean control value of rectal temperature was 100.88 ^{0} F maximum value was 102± 0.31 ^{0}F after 20 minutes of blood transfusion. The body tries to increase oxygen delivery to vital organs by activating the sympathetic and adrenergic nervous systems, which increases heart rate and cardiac output and temperature (Carson and Hébert 2009). After transfusion, the mean rectal temperature of these animals was considered normal (37.5 ± 1.2ºC). This could be a consequence of successful transfusion therapy and correction of hypovolemia. Moreover, before transfusion, the mean rectal temperature in the 9 survivors (37.4 ± 1.4) was similar to the 6 non-survivors (37.0 ± 1.9) (Luis, 2011).

The clinical intra-transfusion status of the patients was monitored during therapy for early detection of the possible transfusion reactions. Monitoring parameters included measurements of temperature, heart rate, pulse strength and synchronicity, mucous membranes and respiratory rate, 3 times in the first 30 minutes and each half hour to the end of the procedure. In my studies case no 3 showed fever, restlessness, and salivation. Transfusion reactions include vascular overload and fever. Vascular overload may result in clinical signs of dyspnea, cough, and vomiting. Nonimmunologic fever results from break in aseptic technique during collection, administration, or storage of blood (Willer et al, 1985). Nonimmunologically mediated transfusion reactions include vascular overload and fever. Vascular overload may result in clinical signs of dyspnea, cough, and vomiting (Killingsworth CR et al, 1984). This phenomenon absolutely supports the study result. Nonimmunologic fever results from break in aseptic technique during collection, administration, or storage of blood. Blood transfusions can produce various adverse reactions. The most effective means of decreasing the incidence of transfusion reactions is to avoid transfusion therapy unless it is absolutely indicated (Turnwald et al, 1985). It is important to carefully monitor the patient during transfusion therapy and follow the guidelines for proper administration of blood components.

This study is limited by the small number of animal's involved and simultaneous analysis of dogs, which made difficult to determine the transfusion effects in each species. The small and heterogeneous studied population may lead to inevitable miscalculations, and further studies should be undertaken to reinforce our conclusions. Blood grouping is another barrier of this study. We done the study without blood grouping. However, post transfusion body temperature, observed PCV change, the difference between the obtained and the calculated

PCV, and transfusion volume determined overall survival and may function as prognostic factors of transfusion outcome.

Chapter: 6

Conclusion and Recommendation

Transfusion aims to replace the missing component of blood and, in the case of anaemia, haemorrhage, haemolysis or ineffective erythropoiesis, to increase oxygen carrying capacity. In my study, I'm trying to checking any difficulties from blood transfusion in critical and emergency cases in our native dog. So I transfuse whole blood to the anemic patient and evaluate them by their hemobiochemical reports. The result under the physical evaluation and blood tests were really satisfactory.

For further extension of this work, its need to increase the studied population and the homogenous population group. This study suggests that the blood transfusion can be performed in critical condition to our native dogs without any blood grouping at least during first transfusion.

Chapter: 7

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Chapter 8

Appendix

Table 5- Percentages changes of various Hematobiochemical parameters in recipient dog after blood transfusion

Parameter	Donor					Recipient									
	Case 1	Case 2	Case 3	Case 4	Case 5	Case1		Case2		Case3		Case4		Case4	
						BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
Packed cell volume%	40	40	41	40	39	41	43	39	41	35	36	39	42	40	42
Hemoglobin gm %	11.2	12.2	11.5	12	11.9	11.2	13.2	8.2	7.3	7	9.2	9.2	9.4	8.3	9
TEC Million/cumm	5.4	4.67	5.3	5.2	5.3	5.11	5.36	4.57	4.37	3.49	4.49	5.2	5.6	4.7	4.9
TLC thousand/cumm	8.6	9.3	9	9.1	8.9	9.8	10.15	5.5	6.55	10.1	10.6	10.3	10.6	5.9	6.6
Lymphocyte%	63	64	62	60	62	36	47	57	60	47	49	50	53	53	59
Neutrophil%	25	54	30	34	32	63	44	35	25	37	50	48	47	44	45
Eosinophil%	8	3	6	5	4	8	4	5	8	21	17	6	8	8	9
Monocyte%	3	2	3	3	3	3	4	3	3	7	6	4	4	4	5
Basophil%	1	0	1	1	1	0	0	1	2	1	1	0	1	1	1

Table 6: Physiological parameter of donor and recipient before and after transfusion

1	Temperature F	101	102	102	102	102	101	101
	Heart rate beat/minutes	84	96	90	89	89	89	85
	Respiration , beat/minutes	19	20	20	20	20	20	19
2	Temperature F	101.4	101.6	101.6	103	103	103	101.4
	Heart rate Beat/minutes	78	85	85	85	82	82	78
	Respiration rate/minutes	19	20	20	20	20	19	19
3	Temperature F	101	102	102	102	102	101	100
	Heart rate Beat/minutes	86	87	89	89	86	86	87
	Respiration rate/minute	20	21	21	21	21	20	19
4	Temperature F	101	102	102	102	102	101	100
	Heart rate Beat/minute	84	87	96	96	93	93	85
	Respiration rate Rate/ minute	19	21	21	21	21	21	18
5	Temperature F	100	100.7	101	101	101	101	100
	Heart rate Beat/minute	89	90	91	91	90	90	88
	Respiration rate Rate/minute	19	19	20	20	20	20	18

Table 7-Effets of blood transfusion on various clinical parameters in recipient dog

Parameters	Pre-transfusion values (control) (mean±SE)	Immediate (mean±SE)	10 minuts (mean±SE)	20 minutes (mean±SE)	30 minutes (mean±SE)	60 minutes (mean±SE)	3 day (mean±SE)
Respiration rate	19.2 ± .2	20.2 ± .37*	20.4 ± .24*	20.4 ± .24*	20.4 ± .24*	20 ± .31	18.6 ± .24
Temperature	100.88 ± .23	101.66±.25*	101.72 ± .19*	102± .31*	101.8± .37*	101.4 ±.4	100.48 ±.30
Heart rate	84.2 ±1.8	89 ±1.9	90.2 ± 1.7*	90 ±1.78*	88.8 ±2.3	88 ± 1.87	86.2 ±2.43

SE= Standard error

Significant variation (p <0.05)

Brief Biography

Ireen Sultana passed Secondary School Certificate (SSC) examination from Bangladesh Bank Colony High School in 2006 and then Higher Secondary Certificate (HSC) examination from Govt. City College in 2008. She obtained her Doctors of Veterinary Medicine (DVM) Degree in 2015 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a Candidate for the degree of MS in Surgery under the Department of Medicine and surgery, Faculty of Veterinary Medicine, CVASU. She has immense interest to practice in small animal medicine.