BLOOD TRANSFUSION IN DOGS

Köpeklerde Kan Transferi

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Özet: Veteriner hekimlikteki uzmanlaşmaya paralel olarak kan ve kan ürünlerine olan talep arttıkça bu alandaki bilgi ihtiyacı da artmaktadır. Bu derlemenin amacı köpeklerde kan gurupları, kan ürünlerinin elde edilmesi, saklanması, uygulanması ve karşı reaksiyonlarla ilgili bilgi vermektir. Köpeklerde tanımlanan 13 kan gurubundan antijenik yönden önemli olanlar DEA (Köpek Alyuvar Antijeni)1.1 ve DEA 1.2 pozitif olanlardır. Köpeklerde doğal olarak antijen meydana gelmediği için ilk transfüzyonda karşı reaksiyon riski bulunmamakta, tekrarlayan transfüzyonlar için ise çapraz karşılaştırma ve kan tiplemesi yapılarak risk önlenebilmektedir. Teknolojik gelişmeler kan ürünlerinin elde edilmesi ve saklanmasında önemli avantajlar sağlamıştır. Böylece hekim, hastanın ihtiyacına göre tedavi stratejisini oluşturmakta ürünleri daha yararlı ve ekonomik kullanabilmektedir.

Anahtar kelimeler: Transfüzyon, kan ürünleri, köpek, karşı reaksiyonlar, kan grupları

Abstratct: Parallel to increasing expertise in the field of Veterinary Medicine the demand for blood and blood components, thus the knowledge in this area has increased. The purpose of this review is to congregate information about blood groups, blood components and their preparation, storage, application and adverse reactions in dogs. Among the described 13 blood groups have been described in dogs, the ones having antigenic importance are DEA (Dog Erytrocyte Antigen) 1.1 and DEA 1.2 positive.

Dogs do not produce natural antibodies thus eliminating adverse effects risks during the first transfusion, but for pursuing transfusions the risk could be eliminated by blood typing and crossmatching. Technological improvements have provided important advantages for preparation of blood components and their storage. Thus, the veterinarian could choose a treatment strategy much more beneficially and economically according to the patient's needs.

Key words: Transfusion, blood products, dog, adverse reactions, blood groups

Introduction

Transfusion therapy has taken an increasingly important role in veterinary critical care and emergency medicine. The increasing usage of blood components is related to the technological improvements, which have facilitated their separation, storage and application (1,2,3,4,5). Benefits of the component usage could be listed as the followings: effective usage of the blood, prevention of transfusion reactions and limiting the transfusion time (5).

There have been important improvements in dog blood typing methods (6,7,8,9). On contrary of human beings and cats, the lack of natural antibodies in dogs provides important advantages for blood transfusion (9,10). Veterinary blood transfusion, especially to dogs is consciously and widely applied in advanced countries (4,10,11,12). As the interest to Transfusion Medicine has augmented, commercial blood banks (e.g. Animal Blood Bank, Dixon, California; Eastern Veterinary Blood Bank, Annapolis, Maryland) have been founded, and donor programs have been developed, thus enabling blood components to be ready for usage (2,6,13). It is a necessity to accommodate the developments in this field for contemporary Veterinary Medicine. In our country, blood transfusion is being performed following crossmatching, but since the usage of blood components is not improved and knowledge is limited, still notable problems occur during applications.

The purpose of this review is to report blood transfusions in dogs, their blood types, preparation, storage and application of blood components, reactions that might occur during and after transfusion, as well as the available medical treatments.

Blood Groups in Dogs and their Importance in Transfusion

Blood typing is made by identification of glicoproteins or glicolipids antigenic structures located on the surface of erythrocyte cell membrane (10,14). Dogs have over 13 identified blood groups, with 8 of them identified on international standards (6,15). An increase on blood group systems and factors could be expected as a consequence of future studies in this field. Although the naming of groups has undergone several changes by time, it is now referred to as DEA (Dog Erythrocyte Antigen) System (Table 1) (5). The International Society of Animal Genetics (ISAG) is responsible for group naming and for the standardization of the reagents for the identification of the blood groups.

Except DEA 1 blood group, dog erythrocytes could be either positive or negative. For example, a dog could be DEA 5 positive or negative. On the other hand, DEA 1 is separated into 3 subgroups, DEA 1.1 (A1), DEA 1.2 (A2) and DEA 1.3 (A3).

A dog's erythrocytes could be DEA 1.1 positive or negative and simultaneously DEA 1.1 negative blood cells could be DEA 1.2 positive or negative (9,10,15).

Groups bearing an importance for transfusions, and high antigenic characteristics in dogs are the groups that possess DEA 1.1 and DEA 1.2 positive factors. Transfusions of these groups might lead to the appearance of anti-DEA 1.1 and 1.2 antibodies (9,10,17,18). It has been stated that DEA 1.1 positive is much more antigenic and DEA 1.1 positive donors should only donate blood to recipients of the same blood group (5,10,17). DEA 1.1 negative blood can be transfused to patients with untyped and DEA 1.1 negative blood group (10). The lifespan of incompatible erythrocytes during an acute hemolytic reaction varies from minutes to hours and varies from 1 to 2 weeks in delayed reactions (17). DEA 1.1 and DEA 1.2 negative dogs are referred to as universal donors (3), such as Greyhound breed dogs (19). DEA 7 is reported as another probable factor that might cause transfusion reactions, but this cold agglutinin does not cause clinical impairment (3,6,10).

Preparations Prior to Transfusion

Contrary to humans and cats, dogs usually do not have clinically important naturally occurring alloantibodies (7,8,10,20). Although, DEA 3, 5 and 7 negative erythrocytes have naturally occurring antibodies in opposition to DEA 3, 5 and 7 positive erythrocytes, these blood types do not cause an important hemolytic reaction (6,7,10). The lack of naturally occurring antibodies in dogs provides some clinical advantages. Reactions do not occur if the dog is receiving blood transfusion for the first time (5), thus eliminating the need for a crossmatching (21). Yet, antibodies might be synthesized 4 to 14 days following the first transfusion (7,8,10,18).

Due to this reason, for repeating applications crossmatching ensures blood compatibility (7,20). However, in spite of crossmatching, there is still risk of adverse reactions due to the lack of evaluation of the WBCs and platelets by this test (19).

Crossmatching aims to demonstrate the existence of antibodies in the recipient against the antigens in the donor (5). This test will not determine blood groups but will divulge the serological compatibility between patient and donor (10,20). But blood typing specifies the blood group of the donor or the recipient (10). By using one of these two tests, one can prevent acute hemolytic reactions that might occur during or after transfusion, incompatible transfusions for following applications and neonatal isoerythrolysis, ensure optimum erythrocytes lifespan (10,14,22). Due to presence of unidentified antigen groups of red blood cells, crossmatching tests are recommended even if type-specific blood components are being used (5).

Crossmatching

The test consists of Major and Minor Crossmatch , which identifies the presence of antibodies, in the recipient serum or plasma against donor cells, and in the donor plasma or serum against recipient cells respectively (3,5,6,10,22).

Assay procedure:

- 2 ml blood collected in anticaogulated (EDTA) tubes is centrifuged for 1 minute at 3000 g; plasma is separated into tagged tubes.

- 2% of erythrocyte suspension is prepared (0.1 ml RBC+5 ml 0.9% saline solution) and allowed to interfere. The supernatant is separated following to centrifugation of the suspension for 1 minute. The same processes are repeated three times.

	Major	**Minor**	**Control**	
	1st Tube	**2nd Tube**	**3rd Tube**	**4th Tube**
Donor	2 drops RBCs	2 drops plasma	2 drops RBCs 2 drops plasma	-
Recipient	2 drops plasma	2 drops RBCs	-	2 drops RBCs 2 drops plasma

- All tubes well mixed, are then left for incubation in room temperature for 30 minutes. (For optimal results incubate at 4°C, room temperature and 37°C).

- Major, minor and control tubes are centrifuged for 1 minute at 3000g.

Microscopic agglutination, plasma color and the presence of hemolysis are evaluated. For suspicious conditions the test must be reevaluated for microscopic agglutination at a magnification of 40 times. The bloods are recognized to be compatible if there isn't any agglutination in all the tubes compared to the controls. But some antibodies coating the erythrocyte surface, especially IgG, do not cause agglutination. Indirect antiglobulin test (IAT, Indirect Coomb's test) might be necessary to identify these antibodies. The result of the major test must be compatible. The minor test bears a lesser importance since the transfused plasma is diluted in the recipient, but still its significance increases for plasma transfusions at great volumes (5,23).

Blood Typing

Dogs can be classified as DEA 1.1 positive or negative with the practical test kits developed recently. The principal of these commercial kits (RapidVet-H Canine 1.1, DMS Laboratories Inc. Flemington, USA) is based on an agglutination reaction, which occurs in 2 minutes between DEA 1.1 positive erythrocytes and DEA 1.1 specific murine monoclonal antibodies (5,9). Even though polyclonal antisera have been developed for some other blood types, the procedure demands expertise and experience. *Anticoagulants and Additives*

Commercial bags containing several different solutions are being used in order to extend the storage period of blood and blood components. Commonly, acid citrate dextrose (ACD), citrate phosphate dextrose (CPD, CP2D) and citrate phosphate dextrose-adenine (CPDA-1) maintain to keep erythrocytes viable for 3 to 5 weeks (19,26). Commercial nutrient solutions such as Adsol (Fenwal Division of Baxter Healthcare Corp., Deerfield, Ilinois) and Nutricel (Cutter Biological, Division of Miles Lab. Emeryville, California) could be added to erythrocytes separated from plasma in order to maintain energy metabolism and viability. One unit of blood for dogs (450 ml) could be collected in bags containing CPDA-1. Heparin has no properties as an anticoagulant and therefore is not suitable for use in stored blood (24).

Donor Selection

The donor must be between the ages of 1 to 8 years old, more then 25 kg body weight (24), possess a good temperament, have at least a 40% pack cell volume (14), a hemoglobin concentration of 13,5g/dl, an adequate level of von Willebrand Factor (vWF) and should be DEA 1.1, 1.2 and 7 negative (5,19). Female donors must be nulliparous and spayed (22). Donors must be examined on a regularly basis; the inspection should include a regular vaccination program, hemogram, clinical chemistry profile and must be screened for infectious diseases (Erlichia canis, Brucella canis, Babesia canis, Dirofilaria immitis) every year, as well as routine heartworm prophylaxis (5,19,22,25). There must be sufficient vitamins, minerals and proteins in their diet (24).

Blood Collection

In case of necessity 10 to 15 minutes prior to collection, Butorphanol (0.1 mg/kg BW, IV) may be applied to the donor (27). The collection area must be prepared according to surgical rules, and blood could be collected by way of a catheter into commercial plastic bags.

Blood and anticoagulant must be sufficiently mixed during collection and the system must be closed against contaminations (5,10,24). Hospitalized dogs could donate 15-25ml/kg of blood every 3 to 4 weeks. Nine ml/kg of blood could safely be collected from a dog every 2 to 3 months without any fluid therapy. If the dog receives fluid therapy, the collected amount could be increased (5,24).

Blood Components

Whole blood is usually applied for situations requiring more then just one-blood component, such as acute blood loss in operations or trauma, and anemias caused by thrombosytopenia and coagulopathies (5,20). Whole blood must not be used for patients with chronic anemia, due to the increase in blood volume by time whole blood transfusion might result with an overload (1,3,5,24).Component treatment limits the use of whole blood and increases the use of component reserves advantageously (1,5,12,13,28). Component treatment possesses a big part of the transfusion practice in humans (6). Whole blood could be transfused in order to increase oxygen capacity and plasma volume, to supplement coagulation proteins, non-functional or deficient trombocytes, proteins, leukocytes, or for a combination of all (3,22,28).

A specific component treatment also prevents non-immunologically and immunologically mediated reactions, such as overload and erythrocyte antigens incompability, respectively, thus preventing time loss (1,5,12).

Fresh whole blood, (FWB); this product is collected into anticaogulated bags just before the transfusion. Due to the loss of functional coagulation factors in the course of time the product must be applied as soon as possible (1,20).

Stored whole blood, (SWB); stored at 1-6°C within 6 hours of collection. This product has non-functional trombocytes and leukocytes, and possesses low levels of labil coagulation factors (5,29,30).

Packed red blood cells (pRBCs); subsequent to the collection into bags containing anticoagulant and additives, and to the cold centrifugation (5000 g, 4°C for 5 minutes) or sedimentation processes, erythrocytes and plasma are separated with the help of an extractor. To prevent contamination and adverse reactions caused by leukocytes, filters can be used to obtain erythrocyte suspensions with diminished leukocytes. In addition, frozen and washed erythrocyte suspensions are also in use (3,20,31).

Platelet rich plasma (PRP) / Platelet concentrate (PC); are the products obtained with successive centrifugation processes (1000 g at 20°-24°C for 4 minutes) (37). Although platelet viability is only about 1 to 3 days, if prepared with classical methods, advanced techniques increases their lifespan (36).

Fresh plasma (FP); plasma is extracted from red blood cells within 6 hours and used within 24 hours of collection (4,6,12).

Fresh frozen plasma (FFP); plasma is extracted from red blood cells within 6 hours and frozen at -20°C. This product should be relabeled as frozen plasma in 1 year (31,33).

Cryoprecipitate (CP); fresh frozen plasma is slowly thawed at 1-6°C then centrifuged at 5000 g for 5 minutes, the remaining liquid plasma is extracted (6,29,30).

Cryofree plasma (CFP); is the plasma product left over after cryoprecipitate preparation. There are no coagulation factors in this product (23).

Adverse Reactions

Transfusion of blood and blood components carries several risks, which can be classified as immune or non-immune, as acute or delayed, or hemolytic or non-hemolytic (Table 3).

Other immune and non-immune reactions such as disease transmissions, hemostatic abnormalities (e.g. dilution of coagulation factors, thrombocytopenia), neonatal erythrolysis, hypothermia, immune suppression, and improper preparation and application of blood components causing hemolysis, must also be taken in consideration (5,14,19,28,29,40). Storage, monitoring the patient during transfusion, applying the correct doses, using filters, crossmatching before transfusion and if possible transfusing blood of a donor with a previously identified blood type, and a delicate preparation will prevent the complications stated above and allow intervention if necessary (19).

Conclusion

The importance of blood transfusion and the request for blood and blood components has a parallel increase with pet stockbreeding in our country. Technological developments have provided an appropriate and economical usage of blood and blood products, also provided the possibility to determine a transfusion strategy to the veterinarian according to the patient. The adverse reactions are minimized by filtration and irradiation, by blood typing and crossmatching of the products, thus implying for expertise in the field of transfusion. Currently, researches in this field still continue in the world, and studies tend to provide synthetic products (e.g. colloid solutions, erythropoietin, hemoglobin solutions) to replace the naturals.

In conclusion, we believe it is an urgent necessity to establishment blood banks and donor programs in our country, and hope that this review will provide acceleration to the studies to be conducted in the field of transfusion.

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Table 1: Blood Groups in Dogs

Old nomenclature	Canine blood type	Natural antibody	Significance
A1	DEA 1.1	No	Acute hemolytic reaction
A2	DEA 1.2	No	Acute hemolytic reaction
B	DEA 3	Yes	Delayed hemolysis
C	DEA 4	No	None
D	DEA 5	Yes	Delayed hemolysis
F	DEA 6	No	Unknown
Tr	DEA 7	Yes	Delayed hemolysis
He	DEA 8	No	Unknown

Based on References: 7,8,9,15,16

Table 2: Blood components and administration.

Component	Content	Storage	Preparation	Dose	Rate	Indication	Comment,Caution
Fresh whole blood (FWB)	RBCs; plasma proteins, clotting factors, WBCs, Platelets	<12 hours CPDA1	Use immediately after collection.	20ml/kg will PCV of 10%	3-4 ml/kg/h	Anemia (blood loss, hemolysis, nonregenarative), coagulopathy, trombocytopenia, no stored blood available, hemangiosarcoma	Restores blood volume and oxygen-carrying capacity
Stored whole blood (SWB)	RBCs, plasma proteins some clotting factors, platelets (if less than 72 hours)	37 days (ADSOL), 4°C 21 days (CPDA1), 4°C	Allow to come to room temperature	20ml/kg will PCV of 10%	3-4 ml/kg/h	Anemia with hypoproteinemia, hypovolemic shock, unaviability of equipment to prepare components	Restores blood volume and oxygen-carrying capacity
Packed red blood cells (PRBCs)	RBCs	37 days (ADSOL), 4°C 20 days (CPDA1), 4°C	Allow to come to room temperature	10ml/kg will PCV of 10%	4-6 ml/min	Anemia	Oxygen-carrying capacity (less than whole blood)
Platelet–rich plasma (PRP)	Platelets, few RBCs and WBCs, plasma	1-3 days (CPDA1), 22°C 2 hours, 4°C	Use immediately after collection and preparation	1 unit/10kg	2 ml/min	Trombocytopenia (bleeding in patients with decrease in platelet number or function)	Do not refrigerate (check platelet count 1 hour prior and following transfusion)
Fresh plasma (FP)	Platelets, clotting factors, plasma proteins, electrolytes	24 hours room temperature	Use within 24 hours of collection	6-10 ml/ kg	4-6 ml/min	Trombocytopenia, hypovolemia hypoproteinemia, coagulopathy	For patients with chronic hypoproteinemia
Fresh frozen plasma (FFP)	Plasma proteins, clotting factors, complement	1 year -30°C 3 months -18°C	Thaw in 37°C water bath	10 ml/kg 2-3 times for 3-5 days	4-6 ml/min infuse within 3-6 hours of thawing	Coagulation and liver disorders, DIC, cumarin toxicity, prior to surgery patients with coagulopathy, pancreatitis	Should be frozen within 6 hours after collection. Treatment or prevention of secondary edema
Frozen plasma (FrP)	Plasma, albumin, stable coagulation factors	5 years at -20°C or below	Thaw in 37°C water bath	6-8 ml/kg	2 to 3 times per day	Stable coagulation factor deficiencies, acute hypoproteinemia (parvoviral enteritis)	Administer as soon as thawed, do not use as volume expanders
Cryoprecipitate (CP)	Factor Vlll, vWF, fibrinogen, fibronectin	1 year -30°C 3 months -18°C	Thaw in 37°C water bath	1 unit/10kg (25-50 ml)	Repeat until bleeding is controlled	Coagulopathy (congenital or acquired), prior to surgery in patient with coagulopathy	Administer as soon as thawed (as many units can be administered)
Cryofree Plasma (CFP)	Lacks of coagulation factors	5 years, -30°C	Thaw in 37°C water bath mix 1:1 in 0,09% NaCl	10 ml/kg	Repeat until bleeding is controlled	Hypoproteinemia, coagulopathy, with loss of factor ll, Vll, IX, X, Hypofibrinogenemia, F IX eficiency	Administer as soon as thawed

(Based on references: 3,4,5,12,19,20,23,24,29,30,31,32,33,34,35,36 DIC: Disseminated intravascular coagulation WBC: White blood cell PCV: Packed cell volume

Table 3: Adverse reactions

	Reaction	Cause	Clinical Signs	Treatment
Immune reactions	Acute Hemolytic Reaction	Blood type incompatibility. Antibody secretions in the host serum against donor (occurs within 2-4 hours PT)	Fever, dyspnea, tachycardia, weakness, tremors, salivation, nausea, vomiting, coollaps,, hemoglobinemia, hemoglobinuria	Stop transfusion, supportive therapy for hypotension and shock (IV fluids, corticosteroids)
	Delayed Hemolytic Reaction	Blood type incompatibility in the first transfusion. Occurs within 2-21 days after 2nd transfusion	Decreased PCV, hyperbilirubinemia, fever, anorexia, icterus	Specific treatment is generally not required. Coombs' test (becomes positive)
	Febrile Reaction	A multitude of factors-infectious	Fever	Antipyretics, antibiotics
	Anaphylactic Reaction	Foreign proteins	Pruritis, facial edema, wheals, urticaria, hypotensive shock, bronchoconspasm, cardiopulmonary arrest	Stop transfusion, administration of antihistamines and corticosteroids
Non-immune reactions	Septicemia	Overheating blood products causes protein denaturation and increases bacterial growth	Pyrexia, chills, nausea, vomiting, diarrhea, shock	Antipyretics, antibiotics
	Air embolism	Air infused with components	Dyspnea, coughing	Place patient on the left side
	Citrate Toxicity	Disproportionated citrate-blood volume ratio, massively transfused patients	Tremors, cardiac arhythmias, cardiac output	Slow transfusion, administer calcium gluconate
	Circulatory Overload	Massively whole blood transfusion to normovolemic patient or to those with liver, cardiac dysfunction	Coughing, dyspnea, cyanosis, tachycardia , vomiting	Slow transfusion, supportive therapy, diuretics

Based on references: 3,5,10,13,17,18,19,22,30,38,39,40 PCV: Packed Cell Volume PT: Post Transfusion