Canine and Feline Transfusion Medicine – Indications, blood groups, blood donor screening, blood collection and administration, components and transfusion reactions

Medicina de transfuzie de câine și de transplant de animale - Indicatii, grupuri de sânge, screening al donatorilor de sânge, colectarea și administrarea sângelui, componente și reacții de transfuzie.

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Introduction

Veterinary clinicians play a key role in providing safe and effective transfusion therapy. Blood typing is clinically important to ensure blood compatibility and therefore is recommended for any dog and cat in need of a transfusion or considered to become a blood donor. Moreover, previously transfused dogs also should be crossmatched. In contrast in cats, there are naturally occurring alloantibodies which could result in acute hemolytic transfusion reactions on a first transfusion and type A and AB kittens may experience neonatal isoerythrosis if born to a type B queen. Unless blood typing is performed regularly in practice, blood may be sent to a clinical pathology laboratory for typing. Different viewpoints exist regarding the extent and methods used for compatibility testing.

Canine Blood Types

Blood types are genetic markers on erythrocyte surfaces that are antigenic and species specific. A set of blood types of two or more alleles makes up a blood group system. Dogs have likely more than a dozen blood group systems mostly known as dog erythrocyte antigens (DEA). However, there is no DEA 2 blood group and some may be rather labeled high frequency or common red blood cell (RBC) antigens (e.g. DEA 4) and some have not yet received a DEA designation (e.g. Dal). Canine erythrocytes are either positive or negative for a blood type (e.g., DEA 4+ or DEA 4-), and these blood types are likely codominantly inherited. The DEA 1 system was thought to be an exception with DEA 1.1 (A1), DEA 1.2 (A2) and potentially DEA 1.3 (A3) being allelic. Thus, a dog could apparently be DEA 1.1+ or DEA 1.1- and DEA 1.1- dogs can be DEA 1.2+ or DEA 1.2-. However, these studies were based upon weak polyclonal antibodies (DEA 1.1 and 1.X) requiring Coombs' reagents. Recent studies with a monoclonal antibody showed that the DEA 1 blood group is a continuum from DEA 1- to weakly to strongly DEA 1+; hence DEA 1.2 typing is no longer offered. The degree of DEA 1 expression is constant and DEA 1+ appears to be dominantly inherited. A recent survey in North America indicates that most dogs are either DEA 1- or strongly DEA 1+ with fewer dogs being weakly to moderately DEA 1+. The biochemical structure of the DEA 1 remains still unknown, but a genome wide association study has identified a likely single locus.

Recent surveys revealed that the Dal- type is not restricted to Dalmatians but is also seen in Doberman Pinschers, Lhasa Apsos and Shih Tzus and thus typing for this blood type is becoming more important particularly for those requiring multiple transfusions. In a related study dogs from North America were screened for two new blood types, preliminarily called Kai 1 and Kai 2. Most dogs were Kai 1+ and only few dogs were Kai 2+ or Kai 1-/Kai2-. The clinical importance is yet to be determined albeit anecdotally dogs can develop anti-Kai 1 alloantibodies. The PennGen Laboratory currently offers Dal and Kai 1 and Kai 2 typing.

The clinically most important canine blood type is DEA 1, which elicits a strong alloantibody response after sensitization of a DEA 1- dog by a transfusion and thus can be responsible for a transfusion reaction in a DEA 1- dog previously transfused with DEA 1+ blood. It is currently unknown if DEA 1- dogs are equally sensitized by weakly to strongly DEA 1+ blood, or if weakly DEA 1+ dogs are sensitized by strongly DEA 1+ blood. Furthermore, transfusion reactions against other blood types or common antigens have rarely been observed and reported. They include reactions against the DEA 4, Dal, Kai 1 and other common RBC antigens; other clinically important blood types may be found in the future. No reagents currently are available against several antigens or are only available on a limited basis, and additional blood types continue to be recognized. Only limited surveys on the frequency of these blood types have been reported, which suggest possible geographic and breed-associated differences.

Strongly antigenic blood types are of great clinical importance because they can elicit a potent alloantibody response. These alloantibodies may be of the immunoglobulin G (IgG) or IgM class and may be hemagglutinins or hemolysins. Based upon experimental and clinical data, dogs can become sensitized after receiving a mismatched transfusion (i.e., a blood unit positive for one or more blood types not found on the recipient's RBCs). There are no clinically important, naturally occurring alloantibodies (also known as isoantibodies) present before sensitization of a dog with a transfusion. Sensitizing dogs in experimental studies in the 1950s led to the documentation of some transfusion reactions caused by blood group incompatibilities and to the characterization of new blood types.

Clinically the most antigenic blood type in dogs is the DEA 1. Transfusion of DEA 1+ RBCs to a DEA 1- dog invariably elicits a strong alloantibody response. Following a first transfusion, anti-DEA 1 antibodies develop after more than 4 days and may cause a delayed transfusion reaction (rarely clinically documented). However, a previously sensitized DEA 1- dog can develop an acute hemolytic reaction after a second transfusion of DEA 1+ blood. Transfusion reactions also may occur after a sensitized dog receives blood that is mismatched for a RBC antigen other than DEA 1 (e.g. DEA 4 and Dal). However, in most cases the incompatible blood type has not been determined. Because administration of a small (<1 ml) amount of incompatible blood can result in life-threatening reactions, the practice of giving small "test volumes" of donor blood to assess blood-type compatibilities is unacceptable. In contrast, pregnancy does not cause sensitization in dogs, because of a complete placenta, and does not induce alloantibody production; thus dogs with prior pregnancies can be used safely as blood donors.

Canine Blood-Typing Procedures

Because of the strong antigenicity of DEA 1, typing of donors for DEA 1 is recommended. Whenever possible, the recipient also should be typed to allow the use of DEA 1+ blood for DEA 1+ recipients. Canine blood typing tests are generally based on serologic identification by agglutination reactions but chromatographic strip methods are also offered. Originally serum from sensitized dogs has been used for typing, but such polyvalent alloantibodies vary from batch to batch, may require Coombs' reagent to enhance agglutination, and may not be always available and are therefore not optimal. Two monoclonal antibodies against DEA 1 have been developed. The gel column technology, widely used in human blood banking, was found to be an excellent standardized laboratory method (DiaMed), but is unfortunately no longer commercially available. A blood typing card has been available with modifications since the mid-1990s as a simple in-practice kit to classify dogs as DEA 1- or DEA 1+ (degree of reaction can vary). a standardized simple immunochromatographic technique became available in the mid-2000s from Alvedia. Another cartridge with a similar strip technique was introduced by DMS/AgroLabo, but has not been evaluated. Moreover, a third cartridge method in which blood flows through the cartridge is also available (DMS/Abaxis) but seems to produce inconsistent results.

Polyclonal reagents against other DEA types are currently only available on a limited bases for DEA 3, 4 and 7 from Animal Blood Resource International (prior Michigan state University and Midwest Blood Services). And only limited anti-Dal reagents from sensitized dogs are currently available in a couple of laboratories like Montreal University and PennGen, monoclonal anti-Kai 1 and anti-Kai 2 alloantibodies have been developed in South Korea. DEA 1 typed and matched patients in need of a transfusion may be typed for DEA 4, Dal and Kai 1/2, which may then permit the localization of a type-matched donor dog.

Caution should be exercised whenever the patient's blood is autoagglutinating or has a low hematocrit (<10%). If autoagglutination is not too severe, it does not appear to affect the Alvedia strip technique because only free RBCs are moving up the strip. Clinicians and technicians should check for autoagglutination of blood with buffer/saline on a slide or the card. Autoagglutinating blood may be first washed three times with ample physiological saline to overcome the apparent autoagglutination similar to what is done for the Coombs' and crossmatch testing. However, if autoagglutination after three washes persists at more than 1+, it is considered to reflect true autoagglutination, which may preclude typing (as well as Coombs' testing and crossmatching), because it always looks like DEA 1+ blood. In such circumstances, DEA 1- blood should be used, until the patient does not agglutinate anymore and can be retyped. DEA 1+ blood from severely anemic animals may not agglutinate when exposed to the anti-DEA 1 or other reagents because of a prozone effect. In these cases, some of the patient's plasma may be discarded before applying a drop of blood onto the card. Finally, recently transfused dogs may display a mixed field reaction, with only the transfused or recipient cells agglutinating if they were DEA 1 mismatched.

Canine Blood Crossmatching Test

Whereas blood typing tests reveal the blood group antigens on the red blood cell surface, blood crossmatching tests assess the serologic compatibility or incompatibility between donor and recipient. Thus the crossmatch test checks for the presence or absence of naturally occurring and induced alloantibodies in serum (or plasma) without determining the blood type and thus does not replace blood typing. These antibodies may be hemagglutinins and/or hemolysins and can be directed against known blood groups or other RBC surface antigens. Many laboratories commonly use a standardized tube crossmatching procedure, but the interpretation of the agglutination reaction is highly variable. The crossmatching test requires some technical expertise, may be accomplished through a veterinary laboratory along with blood typing, and is done with washed EDTA-anticoagulated blood from recipient and potential donor(s). The DiaMed gel column technique and more recently the in-clinic DMS gel tube assay have been evaluated and were found to be simple, sensitive, and standardized methods to crossmatch dogs and cats. In addition, Alvedia introduced a simple strip crossmatch test with a Coombs' phase.

The major crossmatch tests search for alloantibodies in the recipient's plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor's plasma against the recipient's RBCs. Generally tube segments from collection bags are used for this purpose in dogs. The presence of autoagglutination or severe hemolysis may preclude the crossmatch testing. A major crossmatch incompatibility is of greatest importance, because it predicts that the transfused donor cells will be attacked by the patient's plasma, thereby causing a potentially life-threatening acute hemolytic transfusion reaction. Because fatal reactions may occur with less than 1 ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate; this has been shown in experimental studies to potentially result in fatal reactions. A minor crossmatch incompatibility should not occur in dogs if canine donors have not been transfused previously and is of lesser concern because donor's plasma volume is small, particularly with packed red cell products, and is diluted markedly in the patient. Do not use previously used dogs as donors.

The initial blood crossmatch between two dogs that have never before received a

transfusion should be compatible, because dogs do not have naturally occurring alloantibodies. Therefore, a crossmatch may be omitted before the first transfusion in clinical practice for dogs. Because the crossmatch does not determine the blood type of the patient and donor, a compatible crossmatch does not prevent sensitization of the patient against donor cells within 1 to 2 weeks. Thus, previously transfused dogs should always be crossmatched, even when receiving again blood from the same donor. The time span between the initial transfusion and incompatibility reactions may be as short as 4 days and the induced alloantibody can last for many months to years (i.e., years after the last transfusion alloantibodies may be present). Again, a blood donor never should have received a blood transfusion to avoid sensitization. The practice of transfusing patients with the least compatible unit does not have any scientific basis. Nevertheless, some minor agglutination results in crossmatching a patient may be unrelated to alloantibodies and unspecific (e.g., patient's RBC damage by uremia and other illnesses, donor cells after extended storage of unit in the refrigerator). Of course, any patient with true/persistent autoagglutination may not be matched to any donor.

Although transfusion of blood and its components is usually a safe and temporarily effective form of therapy, there is always a risk for potential hazards. Adverse reactions usually occur during or shortly after the transfusion and can be due to any component of whole blood. Most transfusion reactions can be avoided by carefully selecting only healthy donors; using appropriate collection, storage, and administration techniques; performing blood typing and crossmatching; and administering only the needed blood components.

Transfusion Reactions In Dogs

While transfusion of blood and its components is usually a safe and temporarily effective form of therapy, there is always a risk for potential hazards. Adverse reactions usually occur during or shortly after the transfusion and can be due to any component of whole blood. Most transfusion reactions can be avoided by carefully selecting only healthy donors, using appropriate collection, storage, and administration techniques, performing blood typing and crossmatching, and administering only needed blood components. The most common clinical sign of transfusion reaction is fever, followed by vomiting and hemolysis. Hemolytic transfusion reactions can be fatal and are, therefore, most important, while fever and vomiting are usually self-limiting. Adverse effects of transfusions can be divided into non-immunologic (pyrogen-mediated fever, transmission of infectious agents, vomiting, mechanical hemolysis, congestive heart failure, hypothermia, citrate toxicity, pulmonary complications) and immunologic reactions (acute and delayed hemolytic transfusion reactions, urticaria to anaphylaxis, acute respiratory distress, graft versus host disease). Note that some clinical signs may be caused by both mechanisms. Despite the variety of blood types and the limited degree of compatibility testing in clinical practice, transfusion reactions are rarely reported.

Feline Blood Typing

The major feline blood group system is known as the feline AB blood group system and contains 3 alleles: type A, type B, and the extremely rare type AB (fairly common in Ragdolls). Type A is dominant over B. Thus, cats with type A blood have the genotype a/a or a/b, and only homozygous b/b cats express the type B antigen on their erythrocytes. In the extremely rare AB cat, a third allele (C) recessive to the a allele and/or codominant to b allele leads to the expression of both A and B substances. Noteworthy, AB cats are not produced by mating of a type A to a type B cat unless the A cat carries the rare AB allele. Cats with type AB blood have been seen in many breeds and domestic shorthair cats but particularly in Ragdolls.

Most domestic shorthair cats have type A blood, but the proportion of type B cats can be substantial in certain geographical areas. The frequency of A and B blood types varies greatly between different breeds, but likely not much geographically in purebred cats. Kitten losses due to A-B incompatibility and changes in breeding practices influence the frequency

of A and B in various breeds. Most blood donors have type A blood, but some places also keep cats with the rare type B and type AB as donors. All blood donors must be typed. Naturally-occurring alloantibodies have been well documented in type A and type B cats and absolutely require that blood typing be performed prior to both blood transfusion and breeding to assure appropriate blood compatibility.

Cats have naturally-occurring alloantibodies. All type B cats have very strong naturally-occurring anti-A alloantibodies, which can be detected by hemolysis and hemagglutination assays. Kittens receive alloantibodies through the colostrum from type B queens and all type B cats develop high alloantibody titers (>1:32) after a few weeks of age. These alloantibodies are strong hemolysins and hemagglutinins, and are of the IgM and, to a lesser extent, IgG classes. They are responsible for serious transfusion reactions and neonatal isoerythrolysis in type A or AB kittens born to type B queens. Type A cats have weak anti-B alloantibodies, and their alloantibody titer is usually very low (1:2), nevertheless they can also cause hemolytic transfusion reactions, but have not been associated with NI. Type AB cats have no alloantibodies. Furthermore additional blood group systems have been identified such as the common Mik red blood cell antigen in domestic shorthair cats and Mik- cats may also produce naturally occurring alloantibodies.

Blood typing relies on identification of surface antigens, leading to agglutination and hence can distinguish A, AB or B phenotypes. Several different reagents may be used but monoclonal antibodies against the type A and type B antigen are currently used in typing kits versus sera and lectins from the past. A genetic test has also been offered for identification of the b allele, but more recent research shows a more complex pattern and requires a panel of markers allowing precise identification of type A,B, and AB phenotypes in cats.

Noteworthy, there are no feline universal donor cats. All donors and patients need to be typed, even if it is “only” a domestic shorthair cat. Simple AB blood typing cards (DMS Laboratories, Flemington, NJ) and chromatographic strip cartridges (Alvedia DME, Lyon, France and recently DMS) are available for in practice use beside less well established cartridge methods.

Blood crossmatching tests: Blood incompatibilities have been recognized related to the AB blood group system, following blood transfusion and even on a first transfusion in cats through crossmatchin or as a result of observing acute hemolytic transfusion reactions. Standard laboratory tube and gel column crossmatching techniques, but also in-clinic gel tube (DMS and Alvedia) kits are now available. Screening feline blood donors and patients for the presence of naturally occurring (AB and Mik systems) or induced alloantibodies prove necessary in clinical practice. The presence of severe persistent autoagglutination or severe hemolysis may preclude the crossmatch testing.

Table. Examples of blood type A and B frequency in cats in certain countries and breeds*

	Percentage (%)			Percentage (%)	
DSH cats	**Type A**	**Type B**	**Purebred cats**	**Type A**	**Type B**
USA Northeast	99.7	0.3	Abyssinian	84	16
North Central	99.6	0.4	Am. shorthair	100	0
Southeast	98.5	1.5	Birman	82	18
Southwest	97.5	2.5	British shorthair	64	36
West Coast	95.3	4.7	Burmese	100	0
Argentina	97.0	3.0	Cornish rex	67	33
Australia	73.7	26.3	Devon rex	59	41
India (Bombay)	88.0	12.0	Exotic shorthair	73	27

Europe			Himalayan	94	76
Austria	97	3	Japanese Bobtail	84	16
England	97	3	Maine Coon	97	3
Finland	100	0	Norwegian Forest	93	7
France	85	14	Oriental shorthair	100	0
Germany	94	6	Persian	86	14
Greece	79	21	Scottish Fold	81	19
Italy	89	11	Siamese	100	0
Netherlands	96	4	Somali	82	18
Scotland	97	3	Sphinx	83	17
Switzerland	100	0	Tonkinese	100	0
Turkey	75	25	Turkish Angora/Van	50	50

*Ignoring the rare AB cats in many breeds with type B cats

The major crossmatch tests for alloantibodies in the recipient's plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor's plasma against the recipient's RBCs. Mixing a drop of donor/recipient blood with recipient/donor plasma will detect A-B incompatibilities, if typing is not available. However, proper techniques for crossmatching and experience are required to detect other less severe incompatibilities. A major crossmatch incompatibility is of greatest importance because it predicts that the transfused donor cells will be attacked by the patient's plasma, thereby causing a potentially life-threatening acute hemolytic transfusion reaction. As fatal reactions may occur with <1-2 ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate. This has been shown in experimental studies to result in fatal reactions. The major and minor crossmatch can show incompatibilities prior to any transfusion due to the presence of naturally occurring alloantibodies in cats, not only for the AB but also the Mik and possibly other blood group systems.

Previously transfused cats should always be crossmatched, even when receiving blood from the same donor. The time span between the initial transfusion and incompatibility reactions may be as short as 4 days and lasts for many years (i.e., years after the last transfusion alloantibodies may be present). Obviously, a blood donor should never have received a blood transfusion to avoid donor sensitization.

Xenotransfusion

Occasionally anemic cats are given canine blood because either no feline blood is available or the feline blood is incompatible (AB, Mik and other mismatch). In our recent study, we determined that canine blood is incompatible and very short-lived (<4 days) in cats. Therefore, we do not recommend such xenotransfusions (Euler et al 2016). Apparently, Oxyglobin, a highly purified bovine hemoglobin solution, should be again shortly available as it has been FDA approved and found to be extremely helpful when feline compatible blood is not available.

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