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Canine Blood Groups: a review

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Abstract. This literature review aims to discuss the major blood types of dogs and the particularities of each system, in order to help clinicians to carry out correct blood transfusion procedures and to ensure good survival chances to the patients. DEA groups (*Dog Erithrocyte Antigens)* 1.1, 1.2, 1.3, 3, 4, 5, 6, 7, 8, Dal and Shigeta will be reviewed, with their respective prevalences and its immunogenic characteristics. It is concluded that some canine erythrocyte antigens have higher antigenicity and may cause a major hemolytic reaction, reducing the therapeutic action of blood transfusion or its components in dogs.
Keywords: Immunhematology, canine erythrocyte antigen, transfusion medicine.

Context and Analysis

The discovery of the human blood group known as ABO system occurred in 1900 by an Austrian doctor named Karl Landsteiner. Driven by this finding, Dungern and Hirszfeld began investigations on the blood systems of domestic animals, obtaining recognition of the blood groups in 1910, through isoagglutination tests. These advances encouraged studies in veterinary medicine, which began to be clinically relevant in the 1990s (Castellanos et al., 2004).

Currently, blood transfusions are increasingly common in veterinary medicine and are often used in emergency and surgical procedures. It is not uncommon to have patients requiring several transfusions throughout their lives. The clinical consequences of blood types other than DEA 1.1 are controversial and determination of these blood groups is limited to specialized laboratories (Hohenhaus, 2004). In these cases, it is necessary to know about blood types and to use specific tests to avoid transfusion reactions. Fortunately, several commercial kits for typing DEA 1.1 antigen have been developed (Tocci, 2010).

Blood groups refer to species-specific antigenic components present on the surface of erythrocytes. Most antigens are composed of carbohydrates associated with lipids or proteins inserted into the erythrocyte membrane, which are called glycolipids or glycoproteins. These antigens can also be found on platelets, body fluids such as sweat and saliva, leukocytes and tissues (Brown, 2006; Allen et al., 2007; Rozanski and Rondeau, 2011).

Studies related to the characterization of the in vivo and in vitro behavior of these antigens allowed the recognition of eight different phenotypes expressed on the erythrocyte membrane (SWISHER and YOUNG, 1961; HALE, 1995). This expression is genetically controlled by a single gene locus and there is no evidence of the occurrence of gene interactions. A dog may express more than one antigen on its red blood cells simultaneously, with the exception of DEA 1.1, DEA 1.2 and DEA 1.3. The simplest genetic organization of a blood group antigen would be one in which there is only one controlling gene, so that the presence of the gene in the locus results in its expression. The absence of the gene means the absence of the blood group antigen on the erythrocyte. This is the case of the majority of the canine blood groups recognized to date. Complex groups are those that have more than one allelic expression of the gene within the locus, which are called blood group systems. Polymorphism, in this case, means variation of allele expression (Suzuki, 1975; Bull, 1992; Symons e Bell, 1992; Andrews, 2000).

Initially, scientists gave the antigens letters designation from A to G, according to the order in which they were discovered. The International Forum on Canine Immunogenetics in 1972 designated a new nomenclature being that "canine erythrocyte antigen (CEA)" followed by the number of blood group antigen indication. The same forum,

two years later (1974) redefined the nomenclature for "dog erythrocyte antigen (DEA)" followed by a number for part of the locus, followed by the dot, followed by a number for each allele recognized in the locus. For example, DEA 1.1 (Vriesendorp, 1976).

Currently, more than 13 blood groups have already been described and have remained with the nomenclature used in the United States, the DEA system, which includes DEA 1.1, 1.2, 1.3, 3, 4, 5, 6, 7 and 8 (Hale, 1995; Slater, 2003; BROOKS, 2006; Ford & Mazzaferro, 2007; Gonzalez & Silva, 2008). Blais (2010) studied a new group of antigens, based on the specific development of antibodies of the IgG class in Dalmatians previously sensitized by blood transfusions, called Dal group.

Regardless of the nomenclature, the importance of blood groups in dogs is based on three main factors, such as the incidence of the antigen, the incidence of natural antibodies and the effect of the antigen-antibody reaction in vivo (Hale, 1995).

Not all antigens are considered to be potentially important with regard to their ability to trigger a transfusion reaction, but among them, the DEA 1.1 is the most antigenic and, thus, it becomes the major responsible for triggering an acute hemolytic transfusion reaction in dogs (Jain, 1986; Rozanski & Rondeau, 2011).

Although studies have shown that there are no natural antibodies against DEA 1.1, and there is no risk of transfusion reaction in the first transfusion between a DEA 1.1 negative dog and a DEA 1.1 positive one, this is the most antigenic and most important blood type for blood transfusions (Giger et al., 1995). Once sensitized in prior transfusions, patients may develop severe hemolytic reactions following a subsequent incompatible transfusion. DEA 1.1 negative females may also develop anti-DEA 1.1 antibodies during pregnancy, if puppies are positive for DEA 1.1, what may occur in up to 25% of cases (Hale, 1995; Corato et al., 1997).

Immune-mediated hemolytic transfusion reactions involving type 1.1 are considered to be of major clinical importance, as they can cause removal of incompatible red blood cells within 12 hours, inducing hemoglobinuria and hyperbilirubinemia (Giger et al., 1995). However, the reactions that involve negative DEA 1.2 dogs that received successive blood transfusions with DEA 1.1/1.2 blood type are also considered hemolytic, but can take up to 24 hours and usually cause extra vascular hemolysis (Hale, 1995).

Initially, it was believed that DEA 1.2 and 1.3 were blood types; however, these are now known to be variants of DEA 1.1. The first indicative arose when it was found that anti-DEA 1.2 antibodies could not be produced using DEA 1.2 cells, but rather DEA 1.1 cells. With the emergence of the DEA 1.1 kits, the results indicated that only the DEA 1.1 was actually found. When the same samples were evaluated in laboratories it was observed that the degree of agglutination of DEA 1.2 was different from that presented by DEA 1.1 ("Animal Blood Resources International", 2012).

The DEA 1 system is composed of three factors (1.1, 1.2, 1.3) and four phenotypes (1.1.,1.2, 1.3 and negative). The researchs suggest an autosomal dominant inheritance pattern, with the dominance order DEA 1.1, DEA 1.2, DEA 1.3 to DEA 1 negative. Each individual exhibits only one of the phenotypes for the DEA 1 group. As a characteristic, the isoimune antiserum produced against one of the antigens may exhibit degrees of cross-reactivity with the other antigens of that group (Wardrop, 2000). The anti-DEA 1.X antiserum (anti-DEA 1.1, 1.2, 1.3) is produced by immunizing a negative DEA 1 dog with erythrocytes from another positive DEA 1.1 individual, thus being a mixture of anti-DEA 1.1, 1.2 and 1.3. The anti-DEA 1.1 antiserum is produced by immunizing a positive DEA 1.2 dog with erythrocytes from another DEA 1.1 positive animal. Therefore, it only reacts with red cells DEA 1.1. Attempts to produce specific anti-serum anti-DEA 1.2 and anti-DEA 1.3 were not successful (Callan et al., 1995).

Andrews et al. (1993) reported that the erythrocyte antigen DEA 1.1 resides in two membrane proteins with molecular weight of 50 and 200 KDa, respectively. The same authors developed a cardboard agglutination test using anti-DEA 1.1 IgM monoclonal antibodies. Experiments using polyacrylamide gel electrophoresis and anti-DEA 3 monoclonal antibody identified five bands with molecular weights of 34, 53, 59, 64 and 71 KDa (Hara et al., 1991). A study on the biochemical characterization of DEA 1.2, DEA 4 and DEA 7 antigens described molecular weights of 85, 32 to 40 and 53 to 66 KDa for these antigens, respectively (Corato et al., 1997).

The DEA 3 group has been poorly considered because of its low incidence in the US canine population (6%). However, Greyhound dogs had a prevalence of 23%. Naturally occurring antibodies are found in 20% of DEA 3 negative dogs, and studies indicate that they may elicit late transfusion reactions characterized by sequestration and destruction of red blood cells in the spleen within 72 hours. However, repeated exposure to DEA 3 cells results in antisera that are strongly reactive with all three cell types of the DEA 1 group (Weiss, 2011). Thus, DEA 3 positive dogs should not be used as donors of blood, except for DEA 3 positive dogs (Hale, 1995). It is not currently possible to classify this blood antigen since no animal serum is available ("Animal Blood Resources International", 2014).

The DEA 4 group is highly prevalent in the canine population, reaching rates of 100%. Dogs that are negative for all other groups and are only positive for DEA 4 are considered "universal donors", since the natural anti-DEA 4 antibodies do not occur and, in addition, the sensitized DEA 4 negative dogs show antibodies with a variation of time between 4 and 40 days and this sensitization does not show loss or intra- or extra-vascular

hemolysis after being transfused with DEA 4 positive blood. Thus, dogs that only present positive reactions to the DEA 4 group are the best blood donors (Hale, 1995).

The DEA 5 group is of low prevalence and the natural antibodies are found in about 10% of the 5 negative AED dogs. This antibody is capable of causing a late transfusion reaction, similar to that triggered by the anti-DEA 3 antibody. Consequently, 5 DEA positive dogs are not good donors (Hale, 1995).

The DEA 7 system is composed of two factors and three phenotypes. It is believed that DEA 7 is not an integral membrane antigen, but produced by lymphocytes, secreted in plasma and adsorbed on the surface of the red blood cells (Bull, 1992).

Studies on the occurrence of natural antibodies against this antigen suggest that the anti-DEA 7 antibody has a prevalence of 20-50% in negative DEA 7 dogs. However, this naturally occurring antibody produces low hemolytic reactions in a dog transfused with DEA7 red blood cells that show sequestration and red cell loss within 72 hours. Gibson (2007) reports that there are risk factors associated with transfusion reactions in dogs even after the first transfusion, as with anti-DEA 3 and anti-DEA 5. Due to the above, DEA 7 positive dogs are not recommended as donors.

The DEA 6 and DEA 8 groups, previously described and recognized at the Second International Meeting on Canine Immunogenetics, have not been studied due to the lack of antisera for these antigens (Vriesendorp et al., 1976).

The DAL blood group was identified after studies with a Dalmata breed dog that had previously been sensitized by means of an isoagglutination test with 56 dogs of different races and all presented agglutination. Blood typing results indicated the presence of an antibody against a red cell antigen that was different from any DEA. Considering that, following transfusion sensitization, the development of anti-Dal antibodies can result in ineffective transfusions and acute hemolytic and life threatening transfusion reactions, if Dal-positive blood products are repeatedly transfused to a Dal-negative dog. Thus, performing the cross-reaction test of Dalmatian dogs previously transfused with other breed dogs is critical to selecting compatible and viable blood for transfusion (Blais, 2007). Natural antibodies were not found in negative dogs (Kesller et al., 2010).

The SHIGUETA system (SGT) consists of two systems and nine subgroups. These systems are easily differentiated between type A and B and blood subtype 1-1A, 1-1B, 1- 1AB, 1-2A, 1-2B, 1-2AB, 1 (-) A, 1 (-) B and 1(-). This system is independent of the DEA system and was adopted in Japan (Ognean et al., 2006). To date, only the SGT A system has correlated with the DEA system, which is the SGT A group that is equivalent to the DEA 3.

Recently two monoclonal antibodies were produced by mouse hybridoma techniques and examined by ELISA isotyping, immunoblotting and affinity chromatography. They were named Kai 1 and Kai 2, respectively. It was demonstrated that either Kai 1 and Kai 2 can be expressed but not both in an individual dog and there were no naturally occurring anti-Kai 1 or Kai 2 alloantibodies. In addition, Kai 1 and/or Kai 2 dogs developed Kai 1 and Kai 2 alloantibodies, respectively, when transfused with mismatched blood (Lee et al., 2017).

In spite of other canine blood systems reported in literature recently, canine blood antigens were internationally recognized as the DEA system, and none of the other systems have been standardized.

The prevalence of canine blood groups has been studied by researchers in many countries, in both pure breed and crossbred dogs, once transfusion medicine has become very usual in the practice.

Table 1 presents the prevalence of canine erythrocyte antigens described by different authors in the literature.

Table 1. Prevalence of internationally recognized canine erythrocyte antigens in dogs from different countries, according to several authors.

Authors	Number of dogs	Dog Erythrocyte Antigen DEA (%)					
		1.1	1.2	3	4	5	7
SWISHER & YOUNG (1961)	332	40	20	6	98	22	45
SUZUKI et al. (1975)	217	36	51	10	nd	nd	nd
VRIESENDORP (1976)	31	37	4	5	56	8	31
EJIMA et al. (1986)	545	44	22	24	nd	nd	nd
GIGER et al. (1995)	224	33	7	nd	97	nd	8
NOVAIS et al. (1996)	150	51	40	nd	nd	nd	nd
VAN DER MERWE (2002)	233	47	nd	nd	nd	nd	nd
NOVAIS et al. (2003)	200	60	38	10	96	10	9
GIGER et al. (2005)	23	39	17	13	91	22	nd
SOUZA (2005)	300	53	nd	nd	nd	nd	39
VILAR (2006)	72	69	31	nd	nd	nd	nd
GRACNER et al. (2007)	30	66	nd	nd	nd	nd	nd
ARIKAN et al. (2009)	198	61	nd	23	100	55	71
FERREIRA et al. (2011)	274	57	nd	nd	nd	nd	nd
ESTEVES (2011)	100	61	22	7	100	9	16
SOUZA et al. (2014)	300	53	nd	nd	nd	nd	39

nd = not described

Final Considerations

It was possible to conclude that for procedures involving blood and its components it is necessary to perform the blood typing and compatibility test, aiming at the health of the animal and the viability of the transfused cells.

Canine erythrocyte antigens are present in various tissues and further studies on their immunogenicity are necessary for a better understanding of existing reactions linked to procedures in which there is antigen-antibody interaction.

In dogs, the absence of natural antibodies decreases the chance of a clinically relevant reaction in a first transfusion. However, after sensitization, the patient may be at risk of hemolysis, hemagglutination or early removal of transfused red blood cells.

To date, there is no consensus on a universal donor for dogs. Indeed, blood donors should have the serological status for DEA 1.1 determined and the compatibility test between donor and recipient must be performed.

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