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INVESTIGATION OF ERYTHROCYTE ANTIGENIC PROFILE AND BLOOD COMPATIBILITY BY BLOOD TYPING IN DOGS

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ABSTRACT. Blood typing of 139 dogs from north-west of Romania in SHIGETA antigenic system, as a basis for determining the frequency, distribution by race and also blood compatibility; respectively of the potential donors by extrapolating the data in DEA antigenic system. The high intensity reaction of the tube agglutination with monoclonal antibodies, showed a good detection of erythrocyte antigens, being the predominant groups with antigen B non-associated with antigen A: 1.1B (45.3%), 1(-)B (23, 7%) and 1.2B (20.8%). With a lower frequency were reported groups with the association of the 2 antigens: 1.1AB (8.6%), 1.2AB (0.7%) and 1-1A (0.7%). In most breeds predominated the group 1.1B associated in equal proportion with group 1.2B in category ''other races''. In German Shepherd (52%) and English Bulldog (46%) breeds the group 1 (-) B was the most common. The majority German Shepherd dogs were 1 (-) B positive and DEA 1 negative so they represented the major source of potential donors.

Keywords: dog, blood typing, compatibility, potential donors

INTRODUCTION

The blood group antigens are glycoproteins on erythrocytes membrane, synthesized during the fetal erythropoiesis, in relation to which there is natural antibodies (IgM). The formation of antibody anti-erythrocyte antigens can be the consequences of therapy with blood products. Post-transfusion antibodies are usually IgG, which can penetrates trough the placental barrier, causing the haemolytic disease of the foetus (Judd et al., 1997). The blood type encountered in most species of animals are more complex than the OAB antigen system known in humans, explaining the growth of considerable concern in the blood groups of non-human typing that is less easy (Stormont et al., 1982).

In this respect important results achieve the Japanese company SIGHETA, which has developed a new system of antigens classification and typing of canine blood groups, as an alternative to the DEA, expanded in Europe and America (Ognean et al., 2006). Concurrently were made important progress in testing blood compatibility, which facilitated the imposition of hemotansfusion procedure as an intensive care to pets and also diversifying the sources of blood products: blood banks, extern donors, blood substitute (Fischer et al., 2004; Hale, 1995; Hohenhaus, 2004; Lanevsschi et al., 2001).

MATERIALS AND METHODS

Testing blood groups in a heterogeneous population of dogs in the north-west of Romania has been the basis for evaluation of blood compatibility and for placing the hemotransfusion procedure as intensive care. Testing of blood groups and setting of the hemotransfusional compatibility were conducted in the Physiology laboratory of FMV Cluj-Napoca and the data were the basis for initiating blood transfusion treatments in the emergency hospital of our faculty and in a few veterinary offices in the central area of Transylvania.

Animals tested. During the period June 2005 - November 2006 were tested 139 dogs, coming from the hospital customers of FMV Cluj-Napoca, from some private Private Veterinary Practice and from a Romanian Shepherd Dog kennel. By summing tested sample dogs resulted a heterogeneous canine population, with majority German Shepherd (n = 25) and Romanian Shepherd (n = 20), but also contained many dogs (n = 37) of uncommon breeds (Boxer, Basset, Cocker Spaniel, Husky, Tossa prisoners, Chow-Chow, etc.), and a considerable number of crossbreeds (n = 18) (table 2).

Materials used for typing blood groups. The tests were conducted on heparinized blood with SHIGETA kits containing: a package with 5 micro tubes for monoclonal antibodies and control negative test, a set of 4 bottles with monoclonal antibodies, a vial of PBS (Phosphoric buffered Saline) for washing red cells. Other necessary materials included: special type of centrifuges and usual consumables (syringes with 18 G needles, protective gloves, microscope slides, alcohol, cotton wool or gauze).

Method of testing blood groups. It was used the tube agglutination method (SHIGETA variant), which consists of preparing a suspension of red cells in PBS and treating them with monoclonal antibodies (*Ognean* et al., 2008). The first pattern of samples (n = 25) was also tested using rapid method of agglutination on the slide with the 4 types of monoclonal antibodies, used in testing blood group and the Rh in humans (*Ognean* et al., 2006).

Recording and processing of data. Based on individual data has been set the frequency of blood groups in the investigated population and their distribution on race. The data were extrapolated in the DEA system, comparing in this way the impact of the

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two antigenic systems in the analysis of the opportunities and the risks of a transfusion therapy in dogs.

Setting the blood transfusion compatibility. Using SHIGETA scale allows direct reading of the compatibility level based on the recipient and the donor blood group (table 1): maximum compatibility, when the two partners have the same blood group (O); compatibility between partners with different blood groups, with at least 2 common antigens (▲); compatibility accepted only in emergencies cases, without exclusion of the risks of possible transfusion side effects (+). According to this charts for the recipients with 1 (-) A and 1 (-) B groups it is possible only ideal level of compatibility, while the other 7 groups, there may be two or more alternatives.

Table 1

Levels of transfusional compatibility in dogs, based on blood groups from SHIGETA antigenic system

Recipient Group	Donor Group: 1-1A	1-1B	1-1AB	1-2A	1-2B	1-2AB	1(-)A	1(-)B	1(-)AB
1-1A	O	—	—	—	—	—	▲	—	—
1-1B	—	O	—	—	—	—	—	▲	—
1-1AB	☼	☼	O	—	—	—	☼	☼	▲
1-2A	—	—	—	O	—	—	▲	—	—
1-2B	—	—	—	—	O	—	—	▲	—
1-2AB	—	—	—	☼	☼	O	☼	☼	▲
1(-)A	—	—	—	—	—	—	O	—	—
1(-)B	—	—	—	—	—	—	—	O	—
1(-)AB	—	—	—	—	—	—	☼	☼	O

O = Maximum level of compatibility; ▲ = Transfusion is possible;
☼ = Avoid transfusion, except emergencies; — Maximum level of incompatibility.

RESULTS AND DISSCUTIONS

Comparative assessment of the reaction intensity of agglutination in tube and on the slide, put in evidence that maximum agglutinate intensity (++++) in the tube were similar on the slide. Instead, agglutination on the slide were insufficiently clear in the samples with an average intensity of reaction (+++) and invisible for those with weak (++) and very weak (+) intensity in the tube. According to the average intensity of the agglutination reaction in tube (+++/++++), the use of monoclonal antibodies have allowed a good detection of the erythrocytes antigen types, components of blood groups in the SHIGETA system.

Red blood cell antigen profile of the investigated population has reached the maximum degree of diversity, as of 9 blood group of this antigenic system 3 have still remained unidentified. It also confirms the dominance of B antigen in the sample investigated, contained in 5 of the 6 groups reported: 1.1B, 1.2B; 1 (-) B; 1.2AB and 1.1AB (figure 1). Moreover, as the chart in figure 1 shows, antigenic configuration of this population is represented in the highest proportion (89.8%) of 3 groups, with B antigen unrelated with antigen A: 1.1B (45.32%), 1 (-) B (23.74%) and 1.2B (20.86%). This structure is also completed by 3 groups with antigen A, but with a very low representation: 1.1.AB (8.63%), 1.2.AB (0.71%) and 1-1A (0,71%).

The size and structure of the tested sample also allowed a delineation of the blood groups distribution in breeds commonly encountered in the central area of Transylvania (table 2). In this respect trends revealed the predominance of groups 1 (-) B and 1.1B to German Shepherd Dog breed (52 respectively 40%) and English Bulldog (46.15 respectively 41.67%). These groups were also often encountered in German Pointer breed, the major proportion returning to group 1.2B (75%), followed by group 1.1B (25%). In general the blood group 1.1B is predominant in most investigated races, with the following distribution: 100% in Asia Shepherd Dog (a family), 83.33% in Rotweiller, 70% in Romanian Shepherd Dog and 44.44% in half blood dogs.

Less homogeneous have been shown patterns of Rotweillers and half blood dogs, because they included in low proportions also groups with antigen A: 1.1AB (11.11% respectively 8.33%). Finally, the pattern other

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races, with a good representation, has the most heterogeneous antigenic configuration, including 5 of the 6 groups reported. Changes in the frequency of blood groups in this pattern followed the same general trend seen throughout the population. Thus, the major share (64.86%), distributed equally, reverted to groups 1.1B and 1.2B, followed by group 1 (-) B and 1.1.AB, with equal representation (16.21%) and group 1.2AB, seen only in one case, a Cocker Spaniel (2.70%).

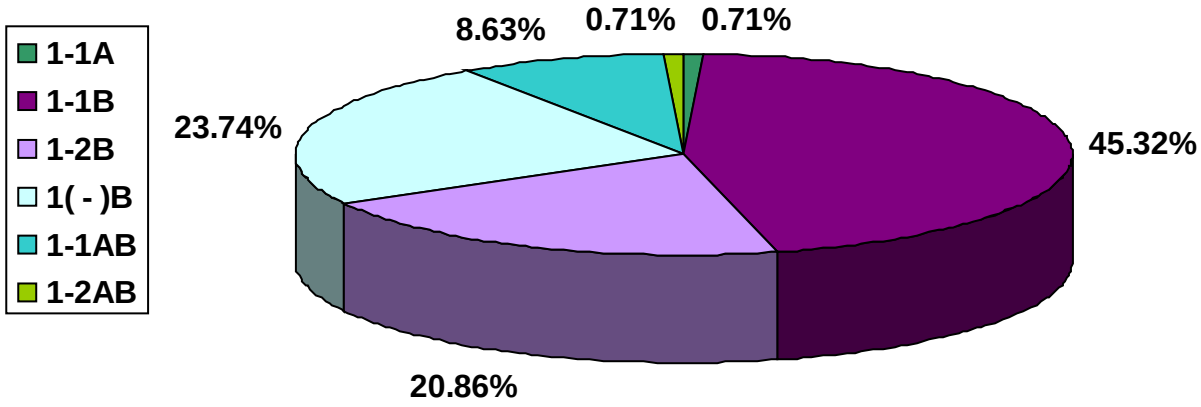

Fig. 1 Frequency of blood groups in the investigated population of dogs (n=139)

Table 2

In races distribution of the blood groups in a heterogeneous canine population from the central area of Transylvania

Breed	Subjects		Blood Group											
			1-1A		1-1B		1-2B		1(-)B		1-1AB		1-2AB	
	NR.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
German Shepherd	25	17.98	-	-	10	40.00	2	8.00	13	52	-	-	-	-
English Bulldog	13	9.35	-	-	2	15.38	5	38.46	6	46.15	-	-	-	-
Rotweiler	12	8.63	-	-	10	83.33	1	8.33	-	-	1	8.33	-	-
Romanian Shepherd	20	14.38	-	-	14	70.00	1	5.00	5	25.00	-	-	-	-
Asia Shepherd	6	4.31	-	-	6	100.0	-	-	-	-	-	-	-	
Half blood	18	12.94	-	-	8	44.44	5	27.77	3	16.66	2	11.11	-	-
German Pointer	4	2.87	-	-	1	25.00	3	75.00	-	-	-	-	-	-
Westie	4	2.87	1	33.33	-	-	-	-	-	-	3	75.00	-	-
Other breeds	37	26.61	-	-	12	32.43	12	32.43	6	16.21	6	16.21	1	2.70
TOTAL	**139**	**100**	**1**	**0.71**	**63**	**45.32**	**29**	**20.86**	**33**	**23.74**	**12**	**8.63**	**1**	**0.71**

According to statistical data, German Shepherd , Romanian Shepherd and half blood dogs have made up nearly half of the tested population, characterized by dominance of 1.1B (45.32%) and 1 (-) B (23.74%) groups. Extrapolation of the SHIGETA groups in the DEA system has allowed us a better assessment of the sources of potential donors. The group 1.1B corresponds to group DEA 1.1, DEA 4 and DEA 6, and the group 1 (-) B corresponds to DEA 4 and DEA 6. Through these correlations it has confirmed that German Shepherd Dogs, with 52% of tested dogs, 1 (-) B positive and DEA 1.1 negative, were the most important source of potential donors. A good potential about the purchase of compatible blood donors or compatible blood was also awarded at English Bulldog breeds with 46.15% of dogs 1 (-) B positive and

Romanian Shepherd Dog, with 25% of dogs 1 (-) B positive.

According to data obtained by us and by other researchers in the field, the agglutination reaction is expressed more relevant in the tube than on the slide explaining the difference between the simple test like Crossmatch (crossed), which detects only plasmas with increased titer of antibodies and the blood group tests, which are typing even types and subtypes of antigens (*Arndt* et al., 2004). On the slide are relevant only blood typing and Rh tests in humans, witch involve the detection of strong immunogenic antigens (A, B and D) (*Langston* et al., 1999; *Ognean* et al., 2008).

In clinical use the Crossmatch is often the first hemotransfusional compatibility test in dogs, the basic arguments being the low cost and the simplicity (*Howard* et al., 1992) along with the accessibility and the speed tests (*Chabanne* et al., 1994; *Howard* et al., 1992). Although Crossmatch's relevance has increased significantly lately, the blood group typing remains the test of certainty for confirmation blood compatibility in dogs (*Giger* et al., 2005; *Lanevsschi* et al., 2001).

At present, there were not made important progress in typing antigens in the DEA system and the antiserums diponiblility on the market has not increased. Moreover, of the 12, most recently 13, blood groups in DEA system, only 6 have a good spread of the identification kits on the market (1.1, 1.2, 3, 4, 5 and 7). According to market studies neither testing them is not sufficiently broad at the clinical level; practitioners commonly use monovalent DEA 1.1 cards.

Van Der Merwe (2002) reconfirms the high frequency (42-46%) and the clinical major importance of the DEA 1.1 antigen, involved in producing the reaction that may have fatal posttransfusional development.

The author also shows that, even if the antibodies to the DEA 1.1 do not occur naturally, they are rapidly synthesized after the first incompatible transfusion. Continuing studies in the Onderstepoort (Gauteng Province, South Africa) on donors (n = 90) and potential dog donors (n = 146), Van Der Merwe finds the dominance of DEA 1.1 group (47%), the 47% frequency in pure breed dogs and 48% in half breed dogs. In races distribution showed variations of the DEA 1.1 positive dogs under 20% in German Shepherd Dog and Boxer and over 70% to Rottweilers, Great Denmarks, Saint Bernards and Dalmatians.

However, we believe that a simple division of dogs in DEA 1.1 positive and negative is not sufficient to exclude any risk. Accordingly it requires a more complex verification of the blood compatibility, testing at least the three DEA groups with high antigenicity (1.1, 1.2, and 7) (*Daniels et* al., 2002).

Abundance of data about the structure and antigenicity of DEA system includes enough controversy, which led to the growth of research in the field of canine red cells antigenic systems. Thus, Marie-Claude Blais and col. (2007) suspected the existence of a new type of red cell common antigen based on the reporting, at 40 days posttransfusional, of specific aloantibodies to a Dalmatian dog; in witch 55 crossmatch tests were incompatible. At this patient, DEA 1.1, 3, 4 and 5 positive, and DEA 7 negative, were identified alloantibodies IgG type. In the absence of whelp it has appealed to testing a lot of Dalmatian adult dogs (n = 25), of which 4 unrelated to each other had compatible crossmatch, suggesting the involvement of one type of red cell antigens. The authors believe that the Dalmatians who not possess this new antigen (called by them "Dal'') are predisposed to acute or delayed posttransfusional reaction.

With convincing arguments and more important, however, should be the new classification system and typing of canine blood groups, developed by Japanese company SIGHETA. A careful analysis of our data on the racial distribution of blood groups Sigheta, must be preceded by a few references to the frequency of DEA fenogroups. Thus, it is noted the trend of Labrador, Golden Retrievers and Rottweilers breeds to group DEA 1.1 or DEA 1.2 positive individuals. While Greyhounds and German Shepherd Dog shows tend to be negative for DEA 1 groups, also gives them quality of very good donors. Moreover, some of the researchers in the field consider German Shepherd dogs as a source of "ideal donors" (*Ognean* et al., 2006; *Van Der Merwe* et al., 2002). According to our data, in the investigated population are predominantly groups 1.1 B (45.73%) and 1 (-) B (24.80%), together representing 70.5% of the sample tested. Consulting correlation diagram established between the two antigen systems (site SIGHETA), to group 1.1.B is corresponding DEA (1.1, 4 and 6) groups and to group 1 (-) B is corresponding the two DEA groups: 4 and 6. Although the sample tested is still limited, dogs 1 (-) B positive are well represented and also considered by us available donors and with a high compatibility potential, as they are also DEA 1 negative.

This finding is particularly relevant for the German Shepherd Dog breed, in which we found a high percentage of dogs 1 (-) B positive (50%), and other researchers have found significant proportion of dogs DEA 1 negative. Thus, *Giger* et al. (2005) compares several methods of typing blood groups, testing them on healthy dogs (n = 23). The results of this study show that CARD method allows rapid identification of DEA 1.1 group, but in the case of DEA 1.2 positive dogs the reaction results are very weak. On the other hand, GEL method is fast and faithful to identify the DEA 1.1 group, and MSU test (Michigan State University) requires Coombs reagent to identify DEA 1.2 and 1.1 groups. According to data presented by these authors, 9 samples have strong agglutinate with DEA 1.1 reagent in CARD, GEL and MSU tests, and 4 other gave weak agglutination in CARD test and have it be the DEA 1.2 positive at MSU test. They also agglutinate all samples with reagent B antigens with tube technical and 21

have strong reacted positive to the DEA 4 reagent to MSU test.

The positive response were also recorded in the case of 20 samples to the reagent E at TUBE test and to DEA 3 reagent at MSU test, respectively in the case of 3 samples with the reagent A at tube test. Strong agglutination reaction also gave the DEA 5 reagent in 5 samples at MSU test.

To check the compatibility of the hemotherapy in dogs it could resort at certainty test, represented by typing blood groups and / or tests versatile value expressed, as Crossmatch. The existence of such differences it can also correlate with various immune or non-immune mechanisms involved in the production of immediate or delayed posttransfusional reactions (*Giger* et al., 2005; Judd et al., 1997; *Merlez* et al., 2003).

Callan et al. (1996) reconfirms in their study the possibility for alloantibodies production to the red cell common antigens in dogs with more than one hemotransfusion, even if present compatible crossmatch with several donors. It also confirms that the most reliable sources of compatible blood are represented by young dogs at first transfusion.

According to these authors, alloantibodies to the red cells antigens are very commonly seen in canine population (92 - 99%), usually being formed after incompatible transfusions in DEA 1.1 negative dogs. Their presence correlates with the production of hemolytic reaction, avoided only by correct checking of the transfusion compatibility and rational use of hemotherapy.

According to the observations in practice there are many areas, even in developed countries, which face problems in providing kits for typing, compatible donor or blood products (*Hohenhaus* et al., 2004; *Vânătu* et al., 2007).

CONCLUSIONS

Agglutination of maximum intensity (++++) have expressed very clearly in the tube and on the slide, while those with average intensity (+++) were not always sufficiently clear on the slide and those with weak (++) and very weak (+) intensity were not visible on the slide.

In the investigated canine population regarding erythrocyte antigenic configuration, antigen B predominated in the in the frequently encountered groups: 1.1B (45.32%), 1 (-) B (23.74%) and 1.2B (20.86%), antigen A is missing.

The frequency of blood groups with antigen A associated or not with antigen B were very low: 1.1AB (8.63%), 1.2AB (0.71%) and 1.1A (0.71%).There were no reported group without antigen B and those with associated antigens A and B have very low frequencies and were represented by groups.

The frequency of blood type is related to race and revealed a trend of dominance of the group 1(-) B in German Shepherd Dog (52%) and English Bulldog (46.15%) and the group 1.1B to Asia Shepherd (100%), Rotweiller (83.33%), Romanian Shepherd (70%) and half blood dogs (44.44%).

In the heterogeneous category "other races" (n = 37), the frequency of blood type was characterized by domination of groups 1.2B and 1.1B, represented in equal proportion (32.43%), followed by groups 1 (-) B and 1.1AB , also found in equal proportion (16.21%).

German Shepherd Dogs, Romanian Shepherd Dogs and half blood dogs predominated in tested population, being assessed as sources of potency donors, often in groups engaged 1.1B (45.32%) and 1 (-) B (23.74%).

Extrapolating data into the DEA system, 1 (-) B positive dogs have been also shown DEA 1.1 negative and their increased frequency in German Shepherd breed (52%) reconfirmed the known tendency of this breed to group "ideal donors" of compatible blood.

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