Bulletin USAMV-CN, 64/2007 (1-2).

SHIGETA METHOD APPLICATION OF BLOOD TYPING IN AN CANINE POPULATION FROM TRANSYLVANIA AND EXTRAPOLATE OF THESE DATA IN THE DEA SYSTEM

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Key words: dog breeds, blood types, antigenic systems, transfusion compatibility.

Abstract: The adoption of a new canine blood type antigenic classification system and the verification of transfusion compatibility was the principal research objective of the physiology department of FMV, Cluj-Napoca, in collaboration with the Japanese Company "SHIGETA Animal Pharmaceutical Inc." For this purpose a heterogeneous population of dogs (n=129) from Transylvania central area, composed of 30 breeds were tested. The preliminary obtained data reveal the B antigen domination in the antigenic structure of the tested canine population. In the frequency evolution of the blood phenotype the dominant weight returned to the 1.1B (45,73%), 1(-)B (24,80%) and 1.2B (22,48%) blood types, while the association between the A and B antigens had a very low frequency, given by the 1.1AB (6,20%) and 1.2AB (0,77%) blood types. The correlation between the blood type frequency and the breed revealed the domination of the B antigen: 1(-)B (50%) and 1.1B (41,67%) in German Sheppard breed, respectively 1(-)B (46,15%) and 1.2B (41,67 %) in English Bulldog breed. Other breeds investigated had also the dominant blood type 1.1B with the following distributions: 100% for the Asian Sheppard, 77,78% for Rottweiller, 70% for Romanian Sheppard and 43% for half-breed dogs, A very heterogeneous sample of animals (n=37) represented "other breed" category, in which 1.2B and 1.1B blood types equally represented (32,43%) were also dominant, as well, the 1.2AB (2,70%) blood type has been signed. It was appreciated that the German Sheppard breed can be considered an important source of potential donors because of its high number of individuals 1(-)B positive (50%), blood type associated with the highest level of compatibility, based also on the large number of dogs DEA 1 negative found in its composition.

INTRODUCTION

The majority of animal species have a multiple number of antigenic systems that considerably increases the identification rhythm of new antigenic types and also subtypes, which enhances the difficulty of blood typing (Wagner et all.; 2002). The complexity and diversity of blood type systems in animals had determined the adoption of new principles that made possible the approach of antigenic erythrocyte configuration in some animal species (Wardrop et all., 2005). In this matter is sufficient to remind the new antigenic classification system of canine blood typing, adopted by the SHIGETA Company from Japan, which can also be a future alternative of the DEA system, which is extended in Europe and America (Ognean et all., 2006). The argumentation of this new knowledge accumulation in the blood typing field, justifies a fundamental principle update.

MATERIALS AND METHOD

In the first stage of the widely spread research plan were tested heterogeneous population of dogs (n=129) from Transylvania central area, composed of 30 breeds, with domination of the German Sheppard breed (n=24) and Romanian Sheppard (n=20). The animals tested were chose from our University Emergency Hospital clients and from 4 private

veterinary cabinets from Cluj-Napoca respective from the effective of a German Sheppard farm.

The biologic material used for blood type tests was exclusively represented by fresh whole blood, treated with an anticoagulant similar to heparin, blood typing were done with SHIGETA blood typing materials. Every blood sample was tested by tube agglutination method towards the 4 monoclonal antibodies produced by the SHIGETA Company. At the same time the first 20 blood samples were also tested by the slide agglutination method, following the fast test technique used in human blood and Rh typing (Ognean et all. 2006).

The individual data were extrapolate in the DEA system and processed for grouping the investigated dogs into categories with common characteristics, finally the data interpretation will primary refer to transfusion compatibility and therapy risks of blood products in canines.

RESULTS AND DISCUSSIONS

Analysis of the resulted data from the tested canine population ensemble, reveled a certain grade of homogeneity in the evolution of the blood type erythrocyte antigenic profile. From the 9 blood types, which compose the SHIGETA system, only 5 were identified, indicating the clear domination of B antigen in the antigenic structure of the signaled blood types: 1.1B; 1.2B; 1(-) B; 1.2AB; 1(-)AB.

The individual and average processed data reveled a high frequency of the 1.1B, (45,73%) blood type followed by the 1(-)B, (24,40%) blood type and 1.2B, (22,48%) blood type. The blood types that include both of the antigens, A and B were less represented; two of which were signaled with a low frequency: 1(-)AB (6,20%) and 1.2AB (0,77%). Notice that no blood types that have the A antigen, without the B antigen, were signed till now.

Depending on the breed, the distribution of blood types, reveal some particularities, in spite of the low number of animals tested (table 1).

Blood type percentage distribution breed dependent

Table 1

Breed	Subjects		blood type									
			1.2B		1.1B		1(-)B		1.1AB		1.2AB	
	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
German Sheppard	24	18,60	2	8.3	10	41.67	12	50	-	-	-	-
English Bulldog	13	10,07	5	38.46	2	15.38	6	46.15	-	-	-	-
Rottweiler	9	6,97	1	11.11	7	77.78	-	-	1	11.11	-	-
Romanian Sheppard	20	15,50	1	5.00	14	70.00	5	25.00	-	-	-	-
Asian Sheppard	6	4,65	-	-	6	100.0	-	-	-	-	-	-
Half-breeds	16	12,40	5	31.25	7	43.75	3	18.75	1	6.25	-	-
German Braque	4	3,10	3	75	1	25	-	-	-	-		
Other breeds (23)	37	28,68	12	32.43	12	32.43	6	16.21	6	16.21	1	2.70
GENERAL TOTAL	129	-	29	22.48	59	45.73	32	24.80	8	6.20	1	0.77

In the case of the German Sheppard breed the domination of the 1(-)B (50%) blood type was observed, followed by the 1.1B (41,67%) and 1.2B (8,3%). A similar situation was in the case of the English bulldog breed, which had also the dominant blood type 1(-)B (46,15%), followed by the 1.2B (41,67%) blood type and 1.1B (15,38%) blood type. Other breeds investigated had also the dominant blood type 1.1B with the following distributions: 100% for the Asian Sheppard (represented by one family), 77,78% for Rottweiller, 70% for Romanian

Sheppard and 43% for half-breed dogs (half of the cases from the Emergency Hospital). The situation was different for the German Braque breed which had the dominant blood type 1.2B representing 75%, while the 1.1B blood type had a weight only of 25%. A certain heterogeneity rank had the samples that represented the Rottweiller breed and the half-breed dogs at which the 1(-)AB blood type was signaled in a proportion of 11,11% respective 6,25%. "Other breed" category, was represented by a very heterogeneous population, that gathered 23 breeds (Boxer, Basset, Cocker Spaniel, Husky, Tosa Inn, Chow-chow etc.) and it was characterized by an even distribution of the blood types signed. In this canine population the 1.1B and 1.2B blood types had an equal representation (32,43%), and the 1(-)B and 1.1AB blood types proportion was also identical (16,21%) (Tab.1). Notice the presence of the 1.2AB blood type in only one Cocker Spaniel (2,70%).

Comparative tube and slide agglutination intensity reaction appreciation, revealed, that the samples that had the maximum agglutination intensity (++++) in tube had the same expression on slide. On the other hand, the reactions with a more weaker intensity (+++) on slide had an insufficient clarity, and those with weak intensity (++) and very weak intensity (+) could not be emphasized. This differences, between the agglutination reaction intensity obtained in tube and slide, can only confirm the research ascertain that methods based on antigen-antibodies reactions, are more sensible as the reaction is much more complex. This principle explains why a test simple as Crossmach (cross testing the donor and the recipient) can reveal plasma with high antibodies titer, and blood typing tests can reveal different antigenic types and subtypes. (Giger et all. 2005; Rejas et all. 1996; Harrell et all., 1995). After the shown data in the table 3, the scores obtained at the tube agglutination reaction intensity appreciation, were marked with +++ or ++++ at all the signed blood types, which indicates that the monoclonal antibodies used permit a very good detection of all the erythrocyte antigens from this antigenic system.

Slide agglutination tests offer, as it is known, good results in human Rh and blood typing, which shows that the 3 antigens that have to be detected (A, B and D) are highly immunogenic.

Using Crossmach as a primer test in checking canine transfusion compatibility it is recommended by the majority researchers in this field (Callan et all. 1996; Howard et all. 1992; Rejas et all. 1996; Waldrop et all. 2005). Between the diverse arguments brought in favor to the use of this test is the low cost and simplicity (Harrell et all. 1995; Rejas et all. 1996; Howard et all. 1992), the possibility of fast accomplishment in clinics (Howard et all. 1992), the after birth absents of alloantibody against blood type antigens in dogs and the presence of those after a previous blood therapy (Callan et all. 1995; Ejima et all. 1982; Wagner et all. 2002).

In spite of the fact that the values of the new Crossmach tests had increased significantly, the majority of researchers working in this field recommend blood typing to ensure transfusion compatibility in dogs, in order to prevent any transfusion reactions (Giger et all. 2005; Ognean et all., 2006).

In America and Europe it is well known the DEA (Dog Erythrocyte Antigen) system that includes 12 or more recently 13 blood types. In spite of the continuous evolution number of canine erythrocyte antigenic types and subtypes, typing sera are available only for 6 antigens (DEA 1.1, 1.2, 4, 5, and 7). Forward more, at clinic levels blood typing cards for DEA 1.1 blood type are frequently used (Ejima et all., 1982). There are already sufficient data to support the fact that the differential of dog in DEA 1.1 positive and negative is risky, suggesting to clinicians a better checking of transfusion compatibility in dogs. In this mater it is sufficient to remind only two high antigenic blood types (DEA 1.1, 1.2, and 7). To be

mentioned the particular antigenic structure of the DEA 1 system, which include two different genes (A1 and A2) determined by allelic genes.

With all their abundance, data regarding the structure and antigen of the DEA system are still controversial and are reflected in the clinical results. As an alternative to this canine blood type and classification controversy, we assist to a preoccupation increase for the understanding and identification of antigenic systems proposed by Japanese investigators based on blood typing with monoclonal antibodies (SHIGETA Company).

In the data absences of the SHIGETA system of blood typing distribution on family and breed level, we will remained some observation regarding the DEA system phenotype incidence. This show the tendency of dogs from Labrador, Golden Retrievers and Rottweiller breeds for DEA 1.1 and 1.2 positive blood type. While dogs from Greyhound and German Sheppard breeds manifest the tendency for negative DEA1 blood type that makes them possible “ideal donors”. In the population ensemble tested by us the 1.1B (45,73%) blood type dominated, followed by the 1(-)B (24,80%) blood type, including 70,5% of the tested dogs. After it is showed in the presented statistics a group of 3 breeds had included almost half of the dogs examined, which coincide with their tendency of grouping the individuals in 1.1B or 1(-)B positive blood type.

This observation corresponds for the German Sheppard breed with 41,67% of the 1.1B positive individuals and 50% 1(-)B positive individuals, respective for the Romanian Sheppard breed with 70% of the individual 1.1B, unlike the English Bulldog breed case which presented less individuals 1.1B positive (15,30%), dominant over 1(-)B positive (46,13%) and 1.2B positive (38,46%).

In order to extrapolate our data in the DEA system, we resort to the correlation diagram of the two antigenic systems (SHIGETA web site). After consulting this, the 1.1B blood type may correspond to DEA 1.1, 4 and 6 blood types; and 1(-)B blood type with DEA 4 and 6 blood types. Under the reserve that the number of tested individuals that we have tested is still small, we can observe that in the tested population, the 1(-)B positive dogs (24,8%) are well represented which are at the same time DEA 1 negative, this supports much more their appreciation as “ideal donors”. The same consideration can be applied to the German Sheppard breed in which we had found the higher percent of dogs 1(-)B positive (50%), this can be associated also with researchers observation that had found a high weight of dogs DEA 1 negative, in other words potential donors, of this breed (Van der Merwe et all., 2002).

To ensure the safety of the blood therapy, in dogs, it is also necessary the knowledge of the antibodies prevalence and blood types, in order to minimize the risks. There is a large palette of mechanisms involved in producing of transfusion reactions which can be immune or non immune. (Harrell et all., 1995). Regardless of their nature, such reactions can be avoided if the transfusion compatibility is rigorous checked and if the blood therapy it is used rationally (Giger et all., 2005). Some researchers had observed the lack of blood typing sera, adequate donors and of commercial blood bank on the market of countries with tradition in dog blood transfusion therapy, finding also an improvement of this situation recently. Haword et all. (1992), brings new important observations after a market study made on 25 small animals cabinets which had done at least 6 transfusion in the last 12 months. According to this study the primer source of blood was represented, in equally proportion, by borrowed dogs and donor dogs maintained by the cabinets, and in one case dogs from the Veterinary Faculty nearby. It was also found out that only 8 cabinets were testing the donor for blood type and the testing of the recipient was not done at all. On the other hand it was found out that only 10 from the 25 cabinets practiced the Crossmach test, but only one practiced this test in every case.

CONCLUSIONS

The SHIGETA system of canine blood typing and classification allows differentiation based on clear criteria of 9 blood types, components of two antigenic systems, assuring at the same time a good difference of antigenic types and subtypes;

In the tested blood phenotype frequency evolution of the dogs (n=129), the major weight returned to the 1.1B (45,73%) blood type, followed by the 1(-)B (24,80%) blood type and 1.2B (22,48%) blood type;

The B antigen domination in the antigenic structure of the tested canine population and the association between the A and B antigens had a very low frequency, represented by the blood types 1.1AB (6,20%) and 1.2AB (0,77%);

The correlation between the blood type frequency and the breed revealed the domination of the same blood types in breeds such as German Shepard and English Bulldog: 1(-)B (50%) followed by 1.1B (41,67%) and 1.2B (8,3%) blood types; respectively 1(-)B (46,15%) followed by 1.2B (41,67 %) and 1.1B (15,38%) blood types;

The majority of breeds investigated had the dominant blood type 1.1B with the following distributions: 100% for the Asian Shepard, 77,78% for Rottweiller, 70% for Romanian Shepard and 43% for half-breed dogs;

In Rottweiller breed and in half-breed dogs was also signaled the 1(-)AB blood type in a proportion of 11,11% respective 6,25% and in “other breed” category (n=23), the 1.1B and 1.2B blood types had an equal representation (32,43%), the proportion of the 1(-)B and 1.1AB blood types was also identical (16,21%);

The German Shepard breed can be considerate an important source of potential donor because of its high number of individuals 1(-)B positive (50%), blood type associated with the highest level of compatibility, based also on the large number of dogs DEA 1 negative found in its composition.

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