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EFFICIENCY OF BLOOD PROTEIN SYSTEMS AS GENETIC MARKERS FOR PARENTAGE VERIFICATION IN YUGOSLAV SHEPHERD DOG

DIMITRIJEVIĆ V, JOVANOVIĆ S, SAVIĆ MILA and TRAILOVIĆ RUŽICA

Faculty of Veterinary Medicine, Belgrade

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The study aimed to evaluate the utility of blood protein systems of the Yugoslav Shepherd dog as genetic markers for parentage verification in this breed. Polymorphism of hemoglobin (Hb), acid phosphatase (Acp), superoxide dismutase (Sod), albumin (Al), and transferrin (Tf) was analysed by vertical polyacrylamide gel electrophoresis in 60 blood samples. Out of the five tested markers, Hb was the only monomorphic and, thus, of no value for parentage analysis in the Yugoslav Shepherd dog. The Acp, Sod and Al loci exhibited a certain degree of polymorphism, but their efficiency as single markers for parentage control was relatively low. The paternity exclusion probabilities established were 12.7%, 15.5% and 16.6% for Acp, Sod and Al, respectively. Tf as a genetic marker displayed a significantly higher efficacy since it was able to reach the 49% power of exclusion for parentage verification. Joined parentage exclusion probability for a panel of four protein systems displaying polymorphism, Acp, Sod, Al and Tf, was 68.5%. In comparison with results of previous studies investigating blood protein systems as markers for canine parentage testing, the panel of markers tested in our study displayed high discriminatory power and provided a substantial probability of resolution in parentage verification for the Yugoslav Shepherd dog.

Key words: dog, protein polymorphism, parentage testing

INTRODUCTION

A number of studies on genetic characterization of canine species and breeds have investigated tools for verifying the parentage and identification of individual animals (Juneja *et al.,* 1987; Muller *et al.*, 1987, Ostrander and Kruglyak, 2000; Ichikawa *et al.*, 2001; DeNise *et al*., 2003). Development of powerful and efficient tools for breeders and breed registries to provide parentage verification is of utmost importance. Study of parentage in different animal populations started with the utilization first of chromosomal polymorphisms and later of blood protein polymorphism (Jones and Ardren, 2003). The advent of DNA analyses provided a new powerful tool for verifying the parentage. Within the past several years microsatellites have established as the genetic markers of choice for paternity

testing in dogs (Fredholm and Wintero, 1996; Ichikawa *et al.*, 2001; DeNise *et al*., 2003; Parker *et al.,* 2004). Microsatellites consist of short repeats of one to six nucleotides reiterated 10 to 50 times, are well dispersed in the canine genome and highly polymorphic (Jouquand *et al.,* 2000). However, tests for evaluation of microsatellite polymorphism are not easily feasible and are still too costly for the majority of underdeveloped and developing countries.

The parentage analyses of some local cattle (Jovanović, 1988), goat (Savić, 1992), and horse (Trailović *et al*., 1994) breeds have already been performed, but local dog breeds have not been analyzed so far. Our study presents the first parentage analysis in the Yugoslav Shepherd dog, which is considered as the oldest autochthonous dog breed on the Balkan peninsula (Dimitrijević, 1999). The breed was recognized by the F.C.I (Federation Cynologique Internationale) in 1939 under the name Illyrian Shepherd dog, but in 1957 the name was changed into the Yugoslav Shepherd dog - Šarplaninac, after the Šarplanina Mountain Range. Both Serbia and Macedonia are currently listed as countries of origin of this breed by the F.C.I.

The purpose of this study was to evaluate the utility of five blood protein systems of the Yugoslav Shepherd dog as genetic markers for parentage verification in this breed. Polymorphism of the following five blood proteins and enzymes was analyzed: hemoglobin (Hb), acid phosphatase (Acp), superoxide dismutase (Sod), albumin (Al), and transferrin (Tf).

MATERIAL AND METHODS

Study population

A total of 60 Yugoslav Shepherd dogs bred in the Center for Dog Breeding, Niš, were included in the survey. The study population included 29 males and 31 females, forming 16 complete and 22 incomplete families. All samples were obtained from animals over six months of age.

Blood samples collection

The blood samples (10 mL) were collected with EDTA by venipuncture of *v. cephalicae antebrachii*. Samples of previously separated plasma and hemolyzed erythrocytes were stored at -20^{o}C until electrophoretical investigation.

Analysis of protein polymorphism

As previously described (Dimitrijević *et al*., 2005), the Al and Tf types were investigated by vertical polyacrylamide gel electrophoresis (PAGE) in continuous tris-citrat buffer system, while vertical PAGE in discontinuous tris-citrat buffer system was used for detection of Hb, Acp, and Sod polymorphism. Specific stainings to detect Acp, Sod, Al and Tf were applied to gels, while detection of naturally stained Hb did not require staining (Dimitrijević *et al.*, 2005).

Statistical analyses

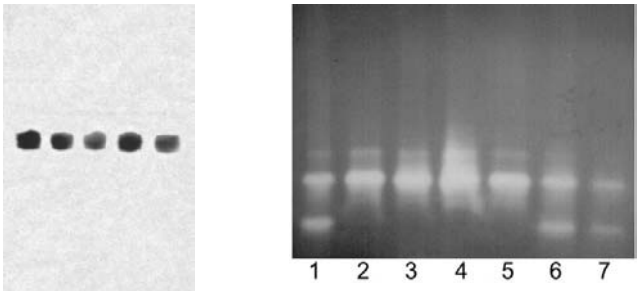
Number of alleles and gene frequencies were established by direct counting from the phenotypes. Paternity exclusion probability was determined for each

individual protein system as well as for the whole panel of protein markers tested according to methods by Jamieson and Wiener, respectively (Avers, 1984; Jones and Ardren, 2003).

RESULTS AND DISCUSSION

All five blood protein systems evaluated as markers for parentage testing in the Yugoslav Shepherd dog are considered as closed codominant systems, with each phenotype defining a unique genotype. Four of these five protein systems (Al, Tf, Acp and Sod) have been recognized as reliable markers for parentage testing in dogs (Dostal and Stratil, 1994) and, thus, recommended for parentage studies. The electrophoretical analyses was aimed to explore the five selected markers on their information content and to determine their power to exclude non-parents within the Yugoslav Shepherd dog breed. The representative monomorphic (Hb) and polymorphic (Sod) electrophoresograms obtained by the analyses are shown in Figure 1. The established gene frequencies and parentage exclusion probabilities obtained by analysis of the five blood protein systems in the Yugoslav Shepherd dog are summarized in Table 1.

Evaluation of Hb polymorphism in the blood of Yugoslav Shepherd dog revealed only one allelic product, namely phenotype B (Figure 1a), which presents phenotypic expression of HbBB homozygous type. Out of the five tested markers, Hb was the only monomorphic and, thus, of no value for parentage analysis in the Yugoslav Shepherd dog. This finding is consistent with results of previous studies, since Hb locus showed only limited polymorphism or no polymorphism at all in European dog breeds tested (Braend, 1987; Muller *et al.*, 1987). Two distinct phenotypes, FS and SS, of erythrocyte Acp were found in the Yugoslav Shepherd dog, with Acp^S allelic gene displaying a significantly higher frequency than Acp^F (Table 1). Results of previous studies investigating Acp polymorphism in dogs are somewhat conflicting, since they have been ranging from substantial polymorphism (Braend and Austad, 1973) to complete homogeneity (Scherer and Kluge, 1993). Nevertheless, the usefulness of this protein as single marker for parentage analysis in the Yugoslav Shepherd dog is



a b

Figure 1. Representative results of electrophoretical analyses of blood protein systems of the Yugoslav Shepherd dog: 1a – hemoglobin (phenotype B in all lanes); 1b – superoxide dismutase (phenotype AB in lanes 1, 6 and 7; phenotype A in lanes 2 - 5)

doubtful due to clear predomination of Acp^S allelic gene and, thus, low information content as shown in Table 1. As far as Sod polymorphism in the Yugoslav shepherd dog is concerned, homozygous SodAA and heterozygous SodAB phenotypes (Figure 1b) controlled by allelic genes Sod^A and Sod^B were found. Predomination of Sod^A allele was evident within the study population (Table 1), although Sod^B frequency established in the Yugoslav Shepherd dog was higher than those of the allelic gene found in other dog breeds tested (Muller *et al*., 1987; Scherer and Kluge, 1993). Still, this protein system also showed low information content as a single marker for parentage analysis (Table 1). It was able to reach a 15.5% power of exclusion for parentage verification. Three Al phenotypes, namely F, FS and S, were revealed in the Yugoslav Shepherd dog. This system has been recognized as reliable polymorphic marker in dogs (Christensen *et al.*, 1985; Scherer and Kluge, 1993), and showed moderate to high degree of polymorphism in the Yugoslav Shepherd dog. However, it displayed relatively low separate paternity exclusion probability of 16.6% (Table 1). This was most probably due to a significantly higher frequency of Al^S within the tested population.

The most extensive polymorphism was established within the Tf locus. Seven Tf phenotypes controlled by five allelic genes, Tf^A, Tf^B, Tf^C, Tf^D, and Tf^E, were revealed. It is noteworthy that the Yugoslav Shepherd dog is the only dog breed which displayed all five possible Tf loci (Braend and Andersen, 1987; Tanabe, 1990) and, thus, exhibited the maximal polymorphism of this genetic marker. Therefore, the effectiveness of this marker in parentage exclusion was expected to be high. The Tf system alone was able to reach a 49% power of exclusion for parentage verification for the Yugoslav Shepherd dog (Table 1). Similarly high discriminatory power of Tf system for parentage control has already been shown in other canine breeds (Arnold and Bouv, 1985; Juneja *et al*., 1987).

Table 1. Gene frequencies and parentage exclusion probabilities obtained by analysis of five blood protein systems in the Yugoslav Shepherd dog

Locus	Allele	Frequency	Exclusion probability	Joined probability
Hb	Hb^B	1.000	0	
Acp	Acp^F Acp^S	0.183 0.817	0.1273	
Sod	Sod^A Sod^B	0.742 0.258	0.1548	
Al	Al^F Al^S	0.300 0.700	0.1659	
Tf	Tf^A Tf^B Tf^C Tf^D Tf^E	0.133 0.350 0.059 0.408 0.050	0.4882	
Acp Sod Al Tf				0.6851

(Hb - hemoglobin; Acp - acid phosphatase; Sod - superoxide dismutase; Al - albumin; Tf - transferrin)

Joined parentage exclusion probability for the panel of four protein systems displaying polymorphism, Acp, Sod, Al and Tf, was 68.5%. As far as previous studies investigating protein systems as genetic markers for parentage verification in canine breeds are concerned, the battery test including four protein systems was able to reach only 42% and 52% power of exclusion for the

Dachshund and German Shepherd dog, respectively (Reetz, 1985). Muller *et al*. (1987) established joined parentage exclusion probability of 67% for five protein systems in Austrian canine breeds. Only the panel of seven protein systems was able to reach the 75% to 85% power of exclusion in different dog breeds (Juneja *et al*., 1987). Therefore, it is apparent that the panel of markers tested in our study provided a high probability of resolution in parentage testing for the Yugoslav Shepherd dog. It was shown that 68.5% of wrong parentage cases can be solved by only four tested systems which clearly indicates that these genetic markers can be applied for parentage verification in the Yugoslav Shepherd dog.

Address for correspondence:
Vladimir Dimitrijević
Department of Animal Breeding and Genetics
Faculty of Veterinary Medicine
University of Belgrade
Bulevar oslobođenja 18
11000 Belgrade, Serbia
E-mail: vanja@vet.bg.ac.yu

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EFIKASNOST PROTEINA KAO GENSKIH MARKERA ZA KONTROLU RODITELJSTVA KOD JUGOSLOVENSKOG OVČARSKOG PSA

DIMITRIJEVIĆ V, JOVANOVIĆ S, SAVIĆ MILA i TRAILOVIĆ RUŽICA

SADRŽAJ

Cilj ove studije je bilo ispitivanje efikasnosti proteinskih sistema krvi jugoslovenskog ovčarskog psa kao genskih markera za proveru roditeljstva kod ove rase pasa. Polimorfizam hemoglobina (Hb), kisele fosfataze (Acp), superoksid dismutaze (Sod), albumina (Al) i transferina (Tf) analiziran je metodom vertikalne elektroforeze na poliakrilamidnom gelu u 60 uzoraka krvi. Od pet ispitivanih markera, Hb je bio jedini monomorfan tako da primena ovog sistema kao markera za kontrolu roditeljstva kod jugoslovenskog ovčarskog psa nije moguća. Acp, Sod i Al lokusi su pokazali izvestan stepen polimorfizma, ali je njihova efikasnost kao pojedinačnih markera za kontrolu roditeljstva bila relativno niska. Verovatnoća isključenja pogrešnog roditeljstva za Acp je iznosila 12.7%, za Sod 15.5%, a za Al 16.6%. Tf je kao genski marker pokazao značajnu višu efikasnost, jer je verovatnoća isključenja pogrešnog roditeljstva primenom samo ovog markera iznosila 49%. Četiri proteinska sistema u kojima je ustanovljen polimorfizam, Acp, Sod, Al i Tf, su kao panel markera dostigli verovatnoću isključenja pogrešnog roditeljstva od 68.5%. U poređenju sa rezultatima prethodnih studija koje su ispitivale proteine krvi kao markere za kontrolu roditeljstva kod pasa, panel markera testiran u našoj studiji pokazao je veliku diskriminatornu moć i omogućio pouzdanu proveru roditeljstva kod rase jugoslovenski ovčarski pas.