MICROSATELLITE DIVERSITY OF CROSSBRED HORSES RAISED IN BOSNIA AND HERZEGOVINA

Dunja Rukavina[1*], Danica Hasanbašić[1], Belma Kalamujić Stroil[2], Naris Pojskić[2]

[1]*University of Sarajevo, Veterinary Faculty, Sarajevo, Bosnia and Herzegovina*
[2]*University of Sarajevo, Institute for Genetic Engineering and Biotechnology (INGEB), Sarajevo, B&H*

***Correspondence**
E-mail: dunja.rukavina@vfs.unsa.ba

Received
February, 2017
Accepted
May, 2017
Published
June, 2017

Copyright: ©2017 Genetics & Applications, The Official Publication of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo

Research article

Abstract

The focus of this study was microsatellite diversity of crossbred horses raised in Bosnia and Herzegovina. Genomic DNA was extracted from blood samples of 20 individuals (KBA group – 7 individuals, crosses between Bosnian and Herzegovinian mountain horse and Arabian horse; KBR group – 9 individuals, crosses between Bosnian and Herzegovinian mountain and Belgian horses, crosses between Bosnian and Herzegovinian mountain horses and Holstein, crosses between Bosnian and Herzegovinian mountain and Lipizzaner horses and KBN group – 4 individuals, crosses between Bosnian and Herzegovinian mountain horse with an unknown origin of the other parent). The samples were profiled using 17 microsatellite markers. This method consisted of multiplex PCR procedure and generated reasonable amplification across all the loci. All samples were genotyped successfully. Considering all the observed parameters, VHL20 locus showed the highest microsatellite diversity. Locus HMS7 was the least variable in KBR group, while HMS1 locus was the least diverse in KBN group. The highest microsatellite diversity in KBA group was found at AHT5 locus while HTG6 locus was the least diverse. Obtained results suggest that the investigated populations of crossbred horses from Bosnia and Herzegovina are not affected by substantial loss of genetic diversity, as indicated by the presence of reasonably high level of genetic variation. An increase in the inbreeding coefficient and sufficient heterogenity in KBN group indicate occurrence of consanguineous mating. The present research contributes to the knowledge of current status of genetic structure of the investigated crossbred horses.

Key words: *crossbred horse, genetic diversity, molecular markers*

Introduction

Microsatellites (Short Tandem Repeats - STR) are a class of genetic markers, currently the most commonly used for diversity studies in livestock (Fornal et al., 2013; Semik and Zabek, 2013). Due to their high level of polymorphism, dispersion throughout eucariotic nuclear genome and Mendelian co-dominant inheritance, microsatellites are relatively easy to score and are considered the markers of choice in equine parentage testing and individual identification (Zabek and Fornal, 2009; Moshkelani et al., 2011). Microsatellites are

regions of repeated 2 to 7 nucleotide long units that occur primarily in non-coding regions of DNA (Moshkelani et al., 2011). Microsatellites have been employed to construct linkage maps, to examine population genetic structure and genetic variation, to explore molecular evolution, in studies of gene flow, in resolution of forensic cases and as parentage testing markers (Tozaki et al., 2003; Moshkelani et al., 2011). Microsatellites loci constitute an informative source concerning population history, structure and genetic diversity and microsatellite polymorphism still plays an important role in the assessment of genetic diversity of livestock (Semik and Zabek, 2013).

In this paper we report the results of the first analysis of microsatellite diversity of crossbred horses from Bosnia and Herzegovina (B&H) using 17 microsatellite markers currently recommended by International Society for Animal Genetics (ISAG) (ISAG, 2014). Number of different alleles, observed and expected heterozygosity, polymorphic information contents, inbreeding coefficient and deviation from Hardy-Weinberg equilibrium were estimated.

Materials and methods

The study was performed on 20 blood samples of horse crossbreeds (KBA group – 7 individuals, crosses between Bosnian and Herzegovinian mountain horse and Arabian horse; KBR group – 9 individuals, crosses between Bosnian and Herzegovinian mountain and Belgian horses, crosses between Bosnian and Herzegovinian mountain horses and Holstein, crosses between Bosnian and Herzegovinian mountain and Lipizzaner horses and KBN group – 4 individuals, crosses between Bosnian and Herzegovinian mountain horse with an unknown origin of the other parent). All horses were raised in Bosnia and Herzegovina (Rukavina et al., 2015b). Blood samples were collected from *v. jugularis* using sterile venipuncture needles and EDTA vacuum containers. Genomic DNA was isolated using salting-out method that was originally developed for the isolation of DNA from human blood (Miller et al., 1988). Necessary modifications to the protocol were made in order to accommodate for different properties of horse blood as well as our laboratory conditions (3ml of blood; 10ml of Lysis buffer; 4ml of PBS; 4ml of Kern-lysis buffer; 150µl of 20% SDS; 100µl of protease and 0,5ml 6M NaCl). In total, 20 animals were genotyped for 17 microsatellite loci. This method consists of multiplex PCR procedure and shows satisfactory amplification of all analyzed fragments. Fragment separation and allele sizing were performed using ABI Prism 310 Genetic Analyzer. All the genotypes were successfully generated. Sizing of the amplified fragments was performed using GeneMapper ID v3.2 software. Number of different alleles (AN), polymorphic information content (PIC) (Botstein et al., 1980), observed heterozygosity (Ho), expected heterozygosity (HE) (Nei, 1987), inbreeding coefficient (F) (Weir, 1996) and deviation from *Hardy-Weinberg* equilibrium (HWE) (Guo and Thompson, 1992) was calculated using POWERMARKER 3.25 (Liu and Muse, 2005).

Results and discussion

The research described in this paper was the first analysis of microsatellite diversity of crossbred horses raised in B&H. All the equine microsatellite markers, reported in the study, were amplified successfully. Results for number of alleles (AN), observed heterozygosity (HO), expected heterozygosity (HE), polymorphic information content (PIC), inbreeding coefficient (F) and deviation from Hardy-Weinberg equilibrium (HWE) are given in Tables 1, 2 and 3. In KBR group the mean

Table 1. Number of alleles (AN), expected heterozygosity (HE), observed heterozygosity (HO), polymorphic information content (PIC), inbreeding coefficient (F) and deviation from Hardy-Weinberg equilibrium (HWE) at 17 microsatellite loci in KBR group

Marker	A_N	H_E	H_O	PIC	F	HWE
VHL20	7,0000	0,8333	1,0000	0,8119	-0,1111	1,0000
HTG4	5,0000	0,7222	0,6667	0,6800	0,1667	0,3010
AHT4	6,0000	0,7778	0,6667	0,7456	0,2308	0,4260
HMS7	3,0000	0,4861	0,3333	0,4235	0,3939	0,5050
HTG6	4,0000	0,5139	0,6667	0,4760	-0,2121	1,0000
AHT5	7,0000	0,7639	1,0000	0,7393	-0,2245	0,4350
HMS6	4,0000	0,5833	0,6667	0,5295	-0,0526	0,2290
ASB23	5,0000	0,7222	0,6667	0,6800	0,1667	0,3010
ASB2	4,0000	0,6528	0,6667	0,5994	0,0698	0,1430
HTG10	4,0000	0,7083	0,8333	0,6589	-0,0870	0,4800
HTG7	5,0000	0,7222	0,8333	0,6800	-0,0638	0,8100
HMS3	6,0000	0,7500	0,6667	0,7193	0,2000	0,2040
HMS2	5,0000	0,7361	0,8333	0,6920	-0,0417	0,7760
ASB17	7,0000	0,7639	0,8333	0,7393	0,0000	0,8910
LEX3	5,0000	0,7222	0,3333	0,6800	0,6000	0,0180
HMS1	3,0000	0,4861	0,6667	0,4235	-0,2903	1,0000
CA425	4,0000	0,6944	0,8333	0,6391	-0,1111	1,0000
Mean	4,9412	0,6846	0,7157	0,6422	0,0458	

Table 2. Number of alleles (AN), expected heterozygosity (HE), observed heterozygosity (HO), polymorphic information content (PIC), inbreeding coefficient (F) and deviation from Hardy-Weinberg equilibrium (HWE) at 17 microsatellite loci in KBA group

Marker	A_N	H_E	H_O	PIC	F	HWE
VHL20	6,0000	0,7551	0,8571	0,7186	-0,0588	0,7000
HTG4	6,0000	0,7653	1,0000	0,7308	-0,2353	1,0000
AHT4	5,0000	0,6735	0,8571	0,6319	-0,2000	0,5980
HMS7	4,0000	0,6633	0,7143	0,6003	0,0000	1,0000
HTG6	3,0000	0,4388	0,5714	0,3862	-0,2308	1,0000
AHT5	7,0000	0,8469	1,0000	0,8277	-0,1053	0,1720
HMS6	5,0000	0,7653	0,8571	0,7299	-0,0435	0,6170
ASB23	6,0000	0,6939	0,2857	0,6636	0,6364	0,0020
ASB2	7,0000	0,7959	0,7143	0,7719	0,1781	0,4420
HTG10	5,0000	0,7500	0,8333	0,7078	-0,0204	0,7110
HTG7	5,0000	0,7347	0,7143	0,6894	0,1045	0,1770
HMS3	6,0000	0,8163	0,8571	0,7898	0,0270	0,3980
HMS2	5,0000	0,7143	1,0000	0,6657	-0,3333	0,7890
ASB17	6,0000	0,7755	0,5714	0,7444	0,3333	0,2800
LEX3	7,0000	0,7959	0,4286	0,7681	0,5200	0,0060
HMS1	5,0000	0,7245	0,2857	0,6853	0,6522	0,0230
CA425	6,0000	0,6939	0,7143	0,6636	0,0476	0,2820
Mean	5,5294	0,7296	0,7213	0,6926	0,0892	

Table 3. Number of alleles (AN), expected heterozygosity (HE), observed heterozygosity (HO), polymorphic information content (PIC), inbreeding coefficient (F) and deviation from Hardy-Weinberg equilibrium (HWE) at 17 microsatellite loci in KBN group

Marker	A_N	H_E	H_O	PIC	F	HWE
VHL20	8,0000	0,8265	0,8571	0,8058	0,0400	0,8150
HTG4	4,0000	0,6429	0,5714	0,5849	0,1864	0,6240
AHT4	6,0000	0,7347	0,8571	0,7006	-0,0909	0,8260
HMS7	5,0000	0,7041	0,4286	0,6574	0,4545	0,0120
HTG6	5,0000	0,6224	0,5714	0,5874	0,1579	0,2930
AHT5	7,0000	0,8265	1,0000	0,8033	-0,1351	0,8150
HMS6	7,0000	0,8163	0,7143	0,7923	0,2000	0,0240
ASB23	7,0000	0,8469	0,4286	0,8277	0,5500	0,0060
ASB2	8,0000	0,8367	1,0000	0,8165	-0,1200	0,7510
HTG10	7,0000	0,7653	0,4286	0,7333	0,5000	0,0050
HTG7	5,0000	0,7755	0,5714	0,7397	0,3333	0,0310
HMS3	6,0000	0,7755	0,5714	0,7444	0,3333	0,0660
HMS2	5,0000	0,7245	0,5714	0,6853	0,2836	0,1980
ASB17	7,0000	0,8163	0,7143	0,7923	0,2000	0,0960
LEX3	6,0000	0,7755	0,4286	0,7444	0,5068	0,0060
HMS1	4,0000	0,6429	0,4286	0,5849	0,4000	0,1290
CA425	5,0000	0,5510	0,7143	0,5207	-0,2245	1,0000
Mean	6,0000	0,7461	0,6387	0,7130	0,2185	

number of alleles was 4.9412 and varied from 3 (HMS7, HMS1) to 7 (VHL20, AHT5, ASB17). The observed heterozygosity ranged from 0.3333 (HMS7) to 1.000 (VHL20, AHT5) with mean of 0.7157, while the expected heterozygosity ranged from 0.4861 (HMS7, HMS1) to 0.8333 (VHL20) with mean of 0.6846 (Table 1). The mean number of alleles in KBA group was 5.5294, varied from 3 (HTG6) to 7 (AHT5, ASB2, LEX3). The observed heterozygosity ranged from 0.2857 (ASB23, HMS1) to 1.000 (HTG4, AHT5, HMS2) with mean of 0.7213, while the expected heterozygosity ranged from 0.4388 (HTG6) to 0.8469 (AHT5) with mean of 0.7296 (Table 2). In KBN group the mean number of alleles was 6 and varied from 4 (HTG4, HMS1) to 8 (VHL20, ASB2). The observed heterozygosity ranged from 0.4516 (HTG6) to 0.8548 (ASB2) with mean of 0.6387 while the expected heterozygosity ranged from 0.4286 (HMS7, ASB23, HTG10, LEX3, HMS1) to 0.8469 (ASB23) with mean of 0.7461 (Table 3).

Our data of microsatellite diversity are consistent with the data from previous studies. Indicators of microsatellite diversity reported in the literature for other horse breeds mostly ranged from 3.3 to 10.7 for number of alleles, from 0.45 to 0.78 for HO and from 0.47 to 0.82 for HE (Canon et al., 2000; Aberle et al., 2004; Galov et al., 2005; Solis et al., 2005; Plante et al., 2007; Di Stasio et al., 2008; Giacomoni et al., 2008; Shasavarani and Rahimi-Mianji, 2010). Based on all the observed parameters, in KBR and KBN groups VHL20 locus showed the highest microsatellite diversity. Locus HMS7 was the least diverse in KBR group, while HMS1 locus was the least diverse in KBN group. In KBA group the highest microsatellite diversity showed AHT5 locus and HTG6 locus was the least diverse. For the observed

heterozygosity, in KBR group values for VHL20 and AHT5 loci reached the maximum level (i.e. Ho = 1), in KBA group values for HTG4, AHT5 and HMS2 loci reached the maximum level and in KBN group values for AHT5 and ASB2 loci reached the maximum level. This result indicates that the studied populations originated from the appropriate number of parent generations. The average HE in all investigated groups indicated the existence of high genetic variability in populations of crossbred horses in B&H.

The greatest differences between HO and HE in our study were observed for LEX3 (KBR group) and ASB23 (KBA and KBN groups) loci. The same loci showed the highest inbreeding coefficient, the highest deviation from HWE and substantial heterozygote deficit. According to Galov et al. (2013) highly significant deviation from HWE combined with substantial heterozygote deficit is likely to indicate locus-specific genotyping problem due to null alleles. The largest disproportion between observed and expected heterozygosity was found in KBN group. According to Berber et al. (2014) larger disproportion between observed and expected heterozygosity could be an indicator of within-population inbreeding or conversely population subdivision reduction.

In population genetic analysis, genetic markers with PIC values higher than 0.5 are normally considered to be informative (Shasavarani and Rahimi-Mianji, 2010). The PIC values, detected in our study, suggested that most of the markers were quite informative (PIC > 0.5) in terms of their suitability for genetic diversity studies. An increased inbreeding coefficient was detected in KBN group (0.2185). High level of inbreeding coefficient in KBN group could be due to small sample size. Inbreeding coefficient values for KBR and KBA groups (0.0458, 0.0892, respectively) indicate no shortage of heterozygotes. Deviation from HWE in KBR group was found in one locus, in KBA group in three loci. In KBN group deviation from HWE was found in six loci. Possible causes for disequilibrium in KBN group were small population size and inbreeding. Overall observed heterozygosity within KBR group is higher then expected, indicating quite large number of heterozygotes probably due to expressed different genetic variants from parental breeds. This is not a case in KBA and KBN groups.

The numbers of detected alleles are within the range for Arabian horse (Rukavina et al., 2015a) and thoroughbred horse populations from Bosnia and Herzegovina (Rukavina et al., 2016). On the other hand, heterozygosity levels are higher in these three types of crossbreeds then in “pure” breeds observed in abovementioned studies.

Conclusions

The results of the present study suggest that the investigated populations of crossbred horses raised in B&H are not affected by major loss of genetic diversity. The applied set of 17 microsatellite markers proved to be specific enough for use in study of genetic structure of crossbred horses. An increase in inbreeding coefficient and sufficient heterogenity between animals in KBN group indicate occurrence of consanguineous mating. The present research contributes to the knowledge of population structure and current status of genetic structure of the investigated populations. Also, the results offer basic information that may be helpful to horse breeders in designing and managing future breeding strategies.

References

Aberle KS, Hamann H, Drogemuller C, Distl O (2004) Genetic diversity in German draught horse breeds compared with a group of primitive, riding

and wild horses by means of microsatellite DNA markers. Anim Genet 35:270-277.

Berber N, Gaouar S, Leroy G, Kididi S, Tabet Aouel N, Saidi Mehtar N (2014) Molecular characterization and differentiation of five horse breeds raised in Algeria using polymorphic microsatellite markers. J Anim Breed Genet 131:387-394.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314-331.

Canon J, Checa ML, Vega-Pla JL, Vallejo M, Dunner S (2000) The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. Anim Genet 31:39-48.

Di Stasio L, Perrotta G, Blasi M, Lisa C (2008) Genetic characterization of the Bardigiano horse using microsatellite markers. Ital J Anim Sci 7:243-250.

Fornal A, Radko A, Piestrzynska-Kajtoch A (2013) Genetic polymorphism of Hucul horse population based on 17 microsatellite loci. Acta Biochim Pol 60:761-765.

Galov A, Byrne K, Đuras-Gomerčić M, Gomerčić T, Nushol Z, Vincek D, Kocijan I, Tadić Z, Benković V, Bašić I, Funk SM (2005) Effectivenes of nine polymorphic microsatellite markers in parentage testing in Posavina, Croatian Coldblood and Lipizzaner horse breed in Croatia. Livest Prod Sci 93:277-282.

Galov A, Byrne K, Gomerčić T, Duras M, Arbanasić H, Sindičić M, Mihelić D, Kovačić A, Funk SM (2013) Genetic structure and admixture between the Posavina and Croatian Coldblood in contrast to Lipizzan horse from Croatia. Czech J Anim Sci 58:71-78.

Giacomoni EH, Fernandez-Stolz GP, Freitas TRO (2008) Genetic diversity in the Pantaneiro horse breed assessed using microsatellite DNA markers. Genet Mol Res 7:261-270.

Guo SW, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

ISAG 2014 Workshop Report. (http://www.isag.us/committees.asp), Accessed: February 17, 2016.

Liu K, Muse S (2005) Powermarker: Integrated analysis enviroment for genetic marker data. Bioinformatics 21:2128-2129.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Moshkelani S, Rabiee S, Javaheri-Koupaei M (2011) DNA fingerprinting of Iranian Arab horse using fourteen microsatellites marker. Res J Biol Sci 6:402-405.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Plante Y, Vega-Pla JL, Lucas Z, Colling D, deMarch B, Buchanan F (2007) Genetic diversity in a feral horse population from Sable Island, Canada. J. Hered 98:594-602.

Rukavina D, Hasanbašić D, Durmić-Pašić A, Kalamujić B, Zahirović A, Ramić J, Pojskić N (2015a) Genetic diversity of Arabian horse from stud “Borike” (Bosnia and Herzegovina) using microsatellite markers. Journal of veterinary sciences 1:21-25.

Rukavina D, Hasanbašić D, Pojskić N, Ramić J, Zahirović A, Ajanović A, Beganović K, Durmić-Pašić A (2015b) Analysis of genetic diversity among certain horse breeds from Bosnia and Herzegovina. Veterinaria 64:25-29.

Rukavina D, Hasanbašić D, Ramić J, Zahirović A, Ajanović A, Beganović K, Durmić-Pašić A, Kalamujić B, Pojskić N (2016) Genetic diversity of Thoroughbred horse population from Bosnia and Herzegovina based on 17 microsatellite markers. JJVR 64:215-220.

Semik E, Zabek T (2013) Application of 7STR markers for parentage testing and genetic distance study of Equidae. Ann Anim Sci 13:229-239.

Shasavarani H, Rahimi-Mianji G (2010) Analysis of genetic diversity and estimation of inbreeding coefficient within Caspian horse population using microsatellite markers. Afr J Biotechnol 9:293-299.

Solis A, Jugo BM, Meriaux JC, Iriondo M, Mazon LI, Aguirre AI, Vicario A, Estomba A (2005) Genetic diversity within and among four South European native horse breeds based on microsatellite DNA analysis: Implications for conservation. J Heredity 96:670-678.

Tozaki T, Takezaki N, Hasegawa T, Ishida N, Kurosawa M, Tomita M, Saitou N, Mukoyama H (2003) Microsatellite variation in Japanese and Asian horses and their phylogenetic relationships using a European horse outgroup. J Heredity 94:374-380.

Weir BS (1996) Genetic data analysis II, Sunderland, MA: Sinauer Associates, Inc.

Zabek T, Fornal A (2009) Evaluation of the 17-plex STR Kit for parentage testing of Polish coldblood and hucul horses. Ann Anim Sci 9:363-372.