UDC 575.
https://doi.org/10.2298/GENSR1902619R
Original scientific paper

USEFULNESS OF THE 17-PLEX STR KIT FOR BOSNIAN MOUNTAIN HORSE GENOTYPING

Dunja RUKAVINA[1*], Amir ZAHIROVIĆ[2], Ćazim CRNKIĆ[3], Mirela MAČKIĆ-ĐUROVIĆ[4], Adaleta DURMIĆ-PAŠIĆ[5], Belma KALAMUJIĆ STROIL[5], Naris POJSKIĆ[5]

[1*]University of Sarajevo-Veterinary Faculty, Department of Biology, Sarajevo, B&H
[2]University of Sarajevo-Veterinary Faculty, Department of Internal Diseases, Sarajevo, B&H
[3]University of Sarajevo-Veterinary Faculty, Department of Animal Nutrition, Sarajevo, B&H
[4]University of Sarajevo-Faculty of Medicine, Center for Genetic, Sarajevo, B&H
[5]University of Sarajevo-Institute for Genetic Engineering and Biotechnology, Sarajevo, B&H

Rukavina D., A. Zahirović, Ć. Crnkić, M. Mačkić-Đurović, A. Durmić-Pašić, B. Kalamujić Stroil, N. Pojskić (2019): *Usefulness of the 17-plex STR kit for Bosnian mountain horse genotyping.*- Genetika, Vol 51, No.2, 619-627.

In the present study modern technology of DNA extraction and automatic genotyping was applied in Bosnian and Herzegovinian autochthonous horse breed by using 17-Plex horse genotyping kit. The study was aimed at investigating usefulness of the 17-plex STR Kit for Bosnian mountain horse genotyping and establishing highly useful microsatellite markers system for genetic diversity studies in Bosnian mountain horse breed. Genomic DNA was extracted from whole blood collected from 22 unrelated Bosnian mountain horse specimens. A total of 95 alleles were detected. Average number of detected alleles per locus was 5.588, varying from 3 (HTG7) to 10 (ASB17). Average effective number of alleles was 3.603, fluctuating from 1.789 (HMS7) to 5.728 (HMS2). The observed heterozygosity ranged from 0.136 (HMS3) to 0.909 (ASB2) with a mean of 0.631. The results indicate that the studied population originates from the appropriate number of

Corresponding author: Dunja Rukavina, Biologist, Ph.D., Department of Biology, University of Sarajevo-Veterinary Faculty, Zmaja od Bosne 90, 71 000 Sarajevo, B&H, phone: + 38733 729-100, fax: + 38733 617-850, e-mail: dunja.rukavina@vfs.unsa.ba

parent generations. The mean expected heterozygosity was 0.690, varying from 0.441 (HMS7) to 0.853 (ASB17) indicating high genetic variability within Bosnian mountain horse population. The PIC values ranged from 0.409 (HMS7) to 0.837 (ASB17) with a mean of 0.643, suggesting that 94.12% markers were quite informative in terms of their suitability for genetic diversity studies .The most polymorphic locus was HMS2 and the least polymorphic locus was HMS7. The inbreeding coefficient ranged from -0.030 (HMS7) to 0.807 (HMS3) with a mean of 0.077. Inbreeding coefficient values indicated no shortage of heterozygotes in Bosnian mountain horses. Deviation from *Hardy-Weinberg* equilibrium ($p<0,05$) was found in three loci (HTG10, HMS3 and ASB17). The applied set of 17 microsatellite markers proved to be sufficiently specific for use in genotyping of Bosnian mountain horse. Considering the values of H_O, H_E and PIC over 0.6, five microsatellite markers system (HTG4, AHT4, AHT5, ASB2, HMS2) is considered to be highly useful for genetic diversity studies in Bosnian mountain horse breed.

Keywords: Bosnian mountain horse, genetic diversity, microsatellite markers, polymorphism, 17-Plex horse genotyping kit

INTRODUCTION

Bosnian mountain horse (Bosnian and Herzegovinian mountain horse, Bosnian pony), the only autochthonous horse breed in Bosnia and Herzegovina is an internationally recognized ancient breed that belongs to "warm-blooded" horses (DEKIC *et al*., 2014). Due to their size and type they belong to the largest groups of ponies. Their height at the withers ranges from 135 to 145 cm, and it achieves weight of 250-350 kg. They have a short muscular neck, long sloping shoulders, straight back, sloping quarters and wide and deep chest (DEKIC *et al*., 2014). The breed has been created long time ago by crossing the tarpan (*Equus caballus gmelini*) and the Asian wild horse (*Equus caballus prsewalskii*). Further infusions of oriental stock have probably been introduced into the breed by the Turks during the Ottoman Empire (ŽIGA and TELALBAŠIĆ, 2009). Bosnian mountain horse has been prized in its range for many centuries and it has been selectively bred since 1900s. Bosnian mountain horse is frequently used for light farm work, light draft, pack and riding and is very surefooted on the terrain unsuitable for motor vehicles (DEKIC *et al*., 2014).

During the war period in B&H (1992-1995) the total number of farm animals, including horses, has been reduced by more than half. The available statistical data indicate that the number of Bosnian mountain horses remains in decline ever since (STATE VETERINARY ADMINISTRATION of BOSNIA and HERZEGOVINA, 2003). According to horse breeding experts less than 140 Bosnian mountain horses were detected in whole B&H during 2017 (personal communication: enver_ziga@yahoo.co.uk.).

Microsatellites (short tandem repeat - STR) are a class of genetic markers, currently the most commonly used for diversity studies in livestock (FORNAL *et al*., 2013). The designation and the number of microsatellites that should be used in genotyping is yet a matter of discussion and depend on the characteristics of each locus and the variability of the breed under study (MOSHKELANI *et al*., 2011).

Data regarding genetic polymorphism in Bosnian mountain horse breed are generally limited for now. Understanding genetic diversity of this autochthonous Bosnian and

Herzegovinian horse breed is important for their conservation, characterization, registration, as well as, parentage controls.

The aims of the present study were to investigate usefulness of 17-plex STR Kit for Bosnian mountain horse genotyping and to establish highly useful microsatellite markers system for genetic diversity studies in Bosnian mountain horse breed.

MATERIALS AND METHODS

The study was performed on 22 blood samples of unrelated Bosnian mountain horses. Blood samples were collected from *v. jugularis* using sterile venipuncture needles and EDTA vacuum containers. Genomic DNA was isolated according to the modified protocol (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) for isolation of DNA from human blood by salting-out method (MILLER *et al*., 1988). The concentration of isolated DNA was determined by spectrophotometry, using UV mini-1240 (*Shimadzu*) spectrophotometer. Improved StockMarks® Equine Genotyping Kit (*Applied Biosystems*), designed for simultaneous amplification of 17 horse microsatellite markers, was used for the analysis of nuclear DNA polymorphism. PCR was performed according to the manufacturer's protocol. PCR products were analyzed on an ABI Prism^{TM} 310 Genetic Analyzer. Sizing of the amplified fragments was performed using GeneMapper ID v3.2 software.

Data analysis

Allele size range, allele frequencies, number of different alleles (A_N), polymorphic information content (PIC) (BOTSTEIN *et al*., 1980), observed heterozygosity (H_o), expected heterozygosity (H_E) (NEI, 1987), inbreeding coefficient (F) (WEIR, 1996), major allele frequency and deviation from *Hardy-Weinberg* equilibrium (HWE) (GUO and THOMPSON, 1992) were calculated using POWERMARKER 3.25 (LIU and MUSE, 2005). Effective number of alleles (A_E) was estimated as $1/\Sigma pi^2$, where p is allele frequency at given locus. Simple ratio between the effective and the detected number of alleles (A_E/A_N) was calculated as suggested by POJSKIĆ and KALAMUJIĆ (2015). Ratio indicates possible disproportion between the effective number of alleles and the number detected by direct counting. Ratio and its P values were calculated using Alleles Ratio, a Microsoft Excel workbook template (POJSKIĆ, 2015). A Z-score of $P<0.01$ was considered statistically significant.

RESULTS

All the loci reported in the study were amplified successfully. In the set of 17 microsatellite markers, 95 alleles were identified. Allele frequencies of microsatellite markers are shown in Table 1. PCR product size range varied from 79 – 95 bp at HTG6 locus to 233 – 251 bp at ASB2 locus.

Number of detected alleles (A_N), effective number of alleles (A_E), A_E/A_N ratio, P value for A_E/A_N, observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC), inbreeding coefficient (F), deviation from Hardy-Weinberg equilibrium (HWE) and major allele frequency (MAF) are given in Table 2. No statistical difference between effective and detected number of alleles were observed.

Table 1. Allele frequencies of microsatellite markers in Bosnian mountain horse

Locus	No. of alleles	Alele size (frequency)
VHL20	4	92bp (0.114), 94bp (0.409), 96bp (0.250), 98bp (0.227)
HTG4	5	126bp (0,045), 128bp (0.250), 130bp (0.318), 134bp (0.318), 136bp (0.068)
AHT4	6	144bp (0.250), 146bp (0.318), 148bp (0.023), 150bp (0.273), 158bp (0.068), 160bp (0.068)
HMS7	5	171bp (0.045), 173bp (0.159), 175bp (0.727), 179bp (0.045), 181bp (0.023)
HTG6	4	79bp (0.114), 85bp (0.364), 93bp (0.023), 95bp (0.500)
AHT5	6	130bp (0.295), 132bp (0.023), 134bp (0.091), 136bp (0.136), 138bp (0.023), 140bp (0.432)
HMS6	6	156bp (0.023), 158bp (0.045), 160bp (0.045), 162bp (0.159), 166bp (0.318), 168bp (0.409)
ASB23	5	186bp (0.045), 188bp (0.136), 190bp (0.591), 192bp (0.045), 204bp (0.182)
ASB2	8	233bp (0.227), 235bp (0.091), 237bp (0.318), 241bp (0.136), 243bp (0.114), 247bp (0.045), 249bp (0.023), 251bp (0.045)
HTG10	8	89bp (0.250), 91bp (0.050), 93bp (0.025), 95bp (0.100), 97bp (0.325), 99bp (0.150), 103bp (0.025), 105bp (0.075)
HTG7	3	118bp (0.364), 124bp (0.159), 126bp (0.477)
HMS3	6	148bp (0.068), 156bp (0.091), 158bp (0.045), 160bp (0.159), 162bp (0.159), 164bp (0.477)
HMS2	7	216bp (0.182), 218bp (0.045), 220bp (0.227), 222bp (0.205), 224bp (0.182), 226bp (0.091), 232bp (0.068)
ASB17	10	96bp (0.045), 98bp (0.045), 100bp (0.045), 104bp (0.023), 106bp (0.068), 108bp (0.136), 110bp (0.227), 112bp (0.136), 118bp (0.205), 120bp (0.068)
LEX3	4	139bp (0.341), 153bp (0.227), 155bp (0.386), 159bp (0.045)
HMS1	4	176bp (0.136), 178bp (0.568), 180bp (0.023), 182bp (0.273)
CA425	4	234bp (0.045), 236bp (0.273), 238bp (0.182), 240bp (0.500)

Table 2. Number of detected alleles (A_N), effective number of alleles (A_E), A_E/A_N ratio, P value for A_E/A_N, observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC), inbreeding coefficient (F), deviation from Hardy-Weinberg equilibrium (HWE) and major allele frequency (MAF) for 17 microsatellite markers in the sample of Bosnian mountain horse.

Allele	A_N	A_E	A_E/A_N	P (A_E/A_N)	H_O	H_E	PIC	F	HWE	MAF
VHL20	4.000	3.396	0.849	0.419	0.545	0.706	0.653	0.227	0.134	0.409
HTG4	5.000	3.681	0.736	0.218	0.864	0.728	0.678	-0.186	0.402	0.318
AHT4	6.000	4.033	0.672	0.125	0.818	0.752	0.710	-0.088	0.226	0.318
HMS7	5.000	1.789	0.358	0.030	0.455	0.441	0.409	-0.030	0.349	0.727
HTG6	4.000	2.527	0.632	0.179	0.591	0.604	0.527	0.022	0.477	0.500
AHT5	6.000	3.315	0.553	0.063	0.727	0.698	0.650	-0.041	0.646	0.432
HMS6	6.000	3.349	0.558	0.065	0.591	0.701	0.651	0.158	0.606	0.410
ASB23	5.000	2.469	0.494	0.066	0.545	0.595	0.554	0.083	0.194	0.591
ASB2	8.000	5.068	0.634	0.058	0.909	0.803	0.777	-0.133	0.486	0.318
HTG10	8.000	4.762	0.595	0.044	0.550	0.790	0.761	0.304	0.016	0.325
HTG7	3.000	2.595	0.865	0.510	0.636	0.615	0.536	-0.035	0.627	0.477
HMS3	6.000	3.408	0.568	0.069	0.136	0.707	0.673	0.807	0.000	0.477
HMS2	7.000	5.728	0.818	0.236	0.864	0.825	0.801	-0.046	0.227	0.227
ASB17	10.000	6.817	0.682	0.052	0.591	0.853	0.837	0.308	0.002	0.227
LEX3	4.000	3.133	0.783	0.324	0.545	0.681	0.617	0.199	0.124	0.386
HMS1	4.000	2.402	0.6	0.157	0.682	0.584	0.520	-0.168	0.370	0.568
CA425	4.000	2.782	0.695	0.230	0.682	0.640	0.580	-0.065	0.879	0.500
Mean	5.588	3.603	0.645	-	0.631	0.690	0.643	0.077	-	0.424

DISCUSSION

In this study, a routine DNA profiling at 17 microsatellite markers was performed in a sample of Bosnian mountain horse breed. Previously, 17-Plex STR kit was, also, used in genetic diversity studies in Arabian (RUKAVINA *et al*., 2015) and Thoroughbred (RUKAVINA *et al*., 2016) horse breeds raised in B&H. All loci in the StockMarks® Kit are dinucleotide markers and allelic size range at individual loci varies between 74 and 268 bp (USER GUIDE, 2014). Our detected allelic sizes (spanning between 79 and 251 bp) in general concur with the described range (USER GUIDE, 2014). Occasionally, we observed alleles that falls outside (ASB2), or rises above (HMS1) of expected size range. For ASB17 locus, expected size range was out of described ranges (96bp–120bp vs. 104 bp–116 bp).

Average effective number of alleles per locus observed in our study was similar to GUPTA *et al*. (2005) research on Marwari horse (3.3), DI STASIO *et al*. (2008) research on Bardigiano horse (3.60) and JISKROVA *et al*. (2016) research on Akhal-Teke horses from Russia (3.434), Estonia (3.161) and Switzerland (3.041). Our results of the average effective number of alleles per locus were lower than those previously reported for Zanskari horses (4.95) (BEHL *et al*., 2006), Caspian horse population (5.86) (SHASAVARANI and RAHIMI-MIANJI, 2010) and Akhal-Teke horses from Czech Republic (4.116) (JISKROVA *et al*., 2016).

The most variable locus in the present study was ASB17 with 10 alleles. In Lithuanian (JURAS and COTHRAN, 2004), Hucul (FORNAL *et al*., 2013) and Tunisian horse breeds (JEMMALI *et al*., 2017) locus ASB17 was also the most variable. The lowest number of allelic variants (3) was detected at HTG7 locus. The same locus showed the lowest number of alleles in Lipizzan (CURIK *et al*., 2003), Lithuanian (JURAS and COTHRAN, 2004), Posavina, Croatian Coldblood and Lipizzaner (GALOV *et al*., 2005), Iranian Caspian (SEYEDABADI *et al*., 2006), Bardigiano (DI STASIO *et al*., 2008), Sanfratellano (ZUCCARO *et al*., 2008), Akhal-Teke horses from Russia and Switzerland (JISKROVA *et al*., 2016) and Turkmen horses (SEYEDABADI and SAVOR SOFLA, 2017).

The mean number of alleles published for different horse breeds or populations mostly ranged from 4.70 to 7.86 (JURAS and COTHRAN, 2004; GALOV *et al*., 2005; GUPTA *et al*., 2005; BEHL *et al*., 2006; DI STASIO *et al*., 2008; FELICETTI *et al*, 2010; FORNAL *et al*., 2013; KUSZA *et al*,, 2013; BERBER *et al*., 2014; JISKROVA *et al*., 2016 and SEYEDABADI and SAVAR SOFLA, 2017) and were consistent with the data established in our study. Higher mean numbers of alleles were reported in Sanfratellano horse (10.09) (ZUCCARO *et al*., 2008), Caspian horse population (8.69) (SHASAVARANI and RAHIMI-MIANJI, 2010), Brazilian horses (14.36) (SILVA *et al*., 2012), Mediterranean horses (10.6) (FORNAL *et al*., 2013), Pantaneiro horse (9.1) (KUSZA *et al*., 2013), Akhal-Teke horses from Czech Republic (8.769) (JISKROVA *et al*., 2016) and Tunisian horse breed (9.31) (JEMMALI *et al*., 2017). Lower mean number of alleles was reported in Iranian Caspian horse (3.86) (SEYEDABADI *et al*., 2006). The observed differences among breeds might depend on the sample size, the analyzed number of alleles, the breed itself, as well as, the population structure. The high level of A_E/A_N ratio, observed in this study, indicates that large portion of detected alleles actually have major participation in genetic diversity of Bosnian mountain horses.

The average levels of observed and expected heterozygosity reported in the literature for other horse breeds mostly ranged from 0.45 to 0.78 for H_O and from 0.47 to 0.875 for H_E (CANON *et al*., 2000; TOZAKI *et al*., 2003; JURAS and COTHRAN, 2004; GALOV *et al*., 2005; GUPTA *et al*., 2005; BEHL *et al*., 2006; DI STASIO *et al*., 2008; GIACOMONI *et al*., 2008; ZUCCARO *et al*., 2008; SHASAVARANI and RAHIMI-MIANJI, 2010; SILVA *et al*., 2012; FORNAL *et al*., 2013; BERBER *et*

al., 2014; JISKROVA *et al*., 2016; JEMMALI *et al*., 2017; SEYEDABADI and SAVAR SOFLA, 2017). The values of the observed and expected heterozygosity for Bosnian mountain horse are consistent with the data from previous studies. We noticed disproportion between observed and expected heterozygosity. This result could be an indicator of converse population subdivision reduction.

Comparing the values of average number of effective alleles, observed and expected heterozygosity in our sample of Bosnian mountain horse with the results previously reported for domestic horse breeds (JURAS *et al*., 2003) we can conclude that average effective number of alleles in Bosnian mountain horse (3.603 compared to 3.531) is similar to the results for domestic horse breeds, observed heterozygosity is below the mean for domestic horse breeds (0.631 compared to 0.697), with the expected heterozygosity higher than the mean for domestic horse breeds (0.690 compared to 0.674). The results obtained in the present study showed that the studied population originated from the appropriate number of parent generations and indicated the existence of high genetic variation within Bosnian mountain horses.

Genetic markers showing PIC values higher than 0.5 are normally considered informative in population genetic studies (SHASAVARANI and RAHIMI-MIANJI, 2010). The PIC values, observed in our work, suggest that 94,12% markers (16 microsatellite markers) are informative in terms of their suitability for genetic diversity studies.

The values of H_O, H_E and PIC show that the most polymorphic locus in our study is HMS2 with HMS7 being the least polymorphic. Also, assuming values of H_O, H_E and PIC over 0.6, we consider loci HTG4, AHT4, AHT5, ASB2 and HMS2 as the most polymorphic.

The overall inbreeding coefficient value at locus HMS3 was significantly higher than zero indicating a certain level of heterozygote deficiency. Inbreeding coefficient values for the rest of the loci with the mean of 0.077 indicate no shortage of heterozygotes and absence of inbreeding in Bosnian mountain horse. Statistically significant deviation from *Hardy-Weinberg* equilibrium detected for only three loci (HTG10, HMS3 and ASB17), supports the starting assumption that there is no inbreeding among the animals in the observed group.

CONCLUSION

The applied set of 17 microsatellite markers proved to be adequate for use in genotyping of Bosnian mountain horse. Almost all the microsatellite markers were sufficiently polymorphic and suitable for genetic diversity studies in this horse breed. Only the locus HMS7 is not sufficiently informative. Also, five microsatellite markers system (HTG4, AHT4, AHT5, ASB2, HMS2) is found to be highly useful for genetic diversity studies in Bosnian mountain horse breed and we can recommended this system for the service laboratories.

Received, June 06th, 2018
Accepted May 18th, 2019

REFERENCES

BEHL, R., J., BEHL, N., GUPTA, S.C., GUPTA, S.P.S., AHLAWAT, M., RAGNEKAR, Z., AHMED (2006): Genetic characterization of Zanskari breed of horse. J. Genet., *85*(3): 199-203.

BERBER, N., S., GAOUAR, G., LEROY, S., KIDIDI, N., TABET AOUEL, N., SAIDI MEHTAR (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., R.L., WHITE, M., SKOLNICK, R.W., DAVIS (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., *32*: 314-331.

CANON, J., M.L., CHECA, J.L., VEGA-PLA, M., VALLEJO, S., DUNNER (2000): The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. Anim. Genet., *31*: 39-48.

CURIK, I., P., ZECHNER, J., SOLKNER, R., ACHMANN, I., BODO, P., DOVC, T., KAVAR, E., MARTI, G., BREM (2003): Inbreeding, Microsatellite Heterozygosity, and Morphological Traits in Lipizzan Horses. J. Heredity, *94*(2): 125-132.

DEKIC, R., A., IVANC, D., CETKOVIC, Z., DOLICANIN, S., OBRADOVIC (2014): Hematology of Bosnian pony. Bulgarian J. Agricult. Sci., *20*(5): 1237-1244.

DI STASIO, L., G., PERROTTA, M., BLASI, C., LISA (2008): Genetic characterization of the Bardigiano horse using microsatellite markers. Ital. J. Anim. Sci., *7*: 243-250.

FELICETTI, M., M.S., LOPES, A., VERINI-SUPPLIZI, A., DA CAMARA MACHADO, M., SILVESTRELLI, D., MENDONCA, O., DISTL (2010): Genetic diversity in the Maremmano horse and its relationship with other European horse breeds. Anim. Genet., *41*(2): 53-55.

FORNAL, A., A., RADKO, A., PIESTRZYNSKA-KAJTOCH (2013): Genetic polymorphism of Hucul horse population based on 17 microsatellite loci. Acta Biochim. Polon., *60*(4): 761-765.

GALOV, A., K., BYRNE, M., ĐURAS-GOMERČIĆ, T., GOMERČIĆ, Z., NUSHOL, D., VINCEK, I., KOCIJAN, Z., TADIĆ, V., BENKOVIĆ, I., BAŠIĆ, S.M., FUNK (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.

GIACOMONI, E.H., G.P., FERNANDEZ-STOLZ, T.R.O., FREITAS (2008): Genetic diversity in the Pantaneiro horse breed assessed using microsatellite DNA markers. Genet.Mol. Res., *7*(1): 261-270.

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

GUPTA, A.K., M., CHAUHAN, S.N., TANDON, S.N., SONIA (2005): Genetic diversity and bottleneck studies in the Marwari horse breed. J. Genet., *84*(3): 295-301.

JEMMALI, B., M.M., HADDAD, N., BARHOUMI, S., TOUNSI, F., LASFER, A., TRABELSI, B.B., AOUN, I., GRITLI, S., EZZAR, A.B., YOUNES, M.H., EZZAOUIA, B., REKIK, H.O., AHMED (2017): Genetic diversity in Tunisian horse breeds. Arch. Anim. Breed., *60*: 153- 160.

JISKROVA, I., I., VRTKOVA, M., PRAUSOVA (2016): Genetic diversity of populations of Akhal-Teke horses from the Czech Republic, Russia, Estonia and Switzerland. Acta Univ. Agric. Silvic. Mendelianae Brunn., *64*(2): 461-466.

JURAS, R., E.G., COTHRAN, R., KLIMAS (2003): Genetic Analysis of three Lithuanian native horse breeds. Acta Agricult. Scandinavica, Sect. A, Animal Sci., *53*: 180-185.

JURAS, R., E.G., COTHRAN (2004): Microsatellites in Lithuanian native horse breeds: usefulness for parentage testing. Biologija, *4*: 6-9.

KUSZA, S., K., PRISKIN, A., IVANKOVIĆ, B., JEDRZEJEWSKA, T., PODGORSKI, A., JAVOR, S., MIHOK (2013): Genetic characterization and population bottleneck in the Hucul horse based on microsatellite and mitochondrial data. Biol. J.Linnean Society, *109*: 54-65.

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

MILLER, S.A., D.D., DYKES, H.F., POLESKY (1988): A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., *16*: 1215.

MOSHKELANI, S.,S., RABIEE, M., JAVAHERI-KOUPAEI (2011): DNA fingerprinting of Iranian Arab horse using fourteen microsatellites marker. Res. J. Biol. Sci., *6*(8): 402-405.

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

POJSKIĆ, N., B., KALAMUJIĆ (2015): Simulations based on molecular-genetic data in detection of expansion Salmo trutta allochtonous population in the Neretva River's tributaries. In: Proceedings of the 27th International Congress

for Conservation Biology and 4th European Congress for Conservation Biology. Visconti P, Game E, Mathevet R, Wilkerson M, editors. Montpellier, 2-6 August,. p.539-540.

POJSKIĆ, N. (2015): Alleles ratio: Excel program for analysis of relation between detected and effective of alleles. Sarajevo: Institute for Genetic Engineering and Biotechnology, University of Sarajevo. 2015. Available from: http://www.ingeb.unsa.ba/lbb/alleles-ratio.html

RUKAVINA, D., D., HASANBAŠIĆ, A., DURMIĆ-PAŠIĆ, B., KALAMUJIĆ, A., ZAHIROVIĆ, J., RAMIĆ, N., POJSKIĆ (2015): Genetic Diversity of Arabian Horse from Stud "Borike" (Bosnia and Herzegovina) Using Microsatellite Markers. J. Vet. Sci., *1*: 21-25.

RUKAVINA, D., D., HASANBAŠIĆ, J., RAMIĆ, A., ZAHIROVIĆ, A., AJANOVIĆ, K., BEGANOVIĆ, A., DURMIĆ-PAŠIĆ, B., KALAMUJIĆ, N., POJSKIĆ (2016): Genetic diversity of Thoroughbred horse population from Bosnia and Herzegovina based on 17 microsatellite markers. Jpn. J. Vet. Res., *64*: 215-220.

SEYEDABADI, H., C., AMIRINIA, M.H., BANABAZI, H., EMRANI (2006): Parentage verification of Iranian Caspian horse using microsatellites markers. Iranian. J. Biotechnol., *4*(4): 260-264.

SEYEDABADI, H.R., S., SAVAR SOFLA (2017): Microsatellite Analysis for Parentage Verification and Genetic Characterization of the Turkmen Horse Population. Kafkas Univ. Vet. Fak. Derg., *23*(3): 467-471.

SHASAVARANI, H., G., RAHIMI-MIANJI (2010): Analysis of genetic diversity and estimation of inbreeding coefficient within Caspian horse population using microsatellite markers. Afr. J. Biotechnol., *9*(3): 293-299.

SILVA, A.C.M., S.R., PAIVA, M.S.M., ALBOQUERQUE, A.A., EGITO, S.A., SANTOS, F.C., LIMA, S.T., CASTRO, A.S., MARIANTE, P.S., CORREA, C.M., MCMANUS (2012): Genetic variability in local Brazilian horse lines using microsatellite markers. Genet. Mol. Res., *11*(2): 881-890.

TOZAKI, T., N., TAKEZAKI, T., HASEGAWA, N., ISHIDA, M., KUROSAWA, M., TOMITA, N., SAITOU, H., MUKOYAMA (2003): Microsatellite variation in Japanese and Asian horses and their phylogenetic relationships using a European horse outgroup. J. Heredity, *94*(5): 374-380.

USER GUIDE (2014): StockMarks® Kits for Horses, Cattle, and Dogs. Equine, Bovine, and Canine Genotyping Kits, Applied Biosystems. Publication Number 4346135, Revision F, Chapter 3, (2014): 21-22.

WEIR, B.S. (1996): Genetic data analysis II, Sunderland, MA: Sinauer Associates, Inc.

ZUCCARO, A., S., BORDONARO, A., CRISCIONE, A.M., GUASTELLA, G., PERROTTA, M., BLASI, G., D'URSO, D., MARLETTA (2008): Genetic diversity and admixture analysis of Sanfratellano and three Italian horse breeds assessed by microsatellite markers. Animal, *2*(7): 991-998.

ŽIGA, E., R., TELALBAŠIĆ (2009): The Bosnian Mountain horse – a monograph. IP Šahimpašić, Sarajevo,. p. 350.

STATE VETERINARY ADMINISTRATION OF BOSNIA AND HERZEGOVINA (2003): Animal genetic resources in Bosnia and Herzegovina. Sarajevo, 2003. ftp://ftp.fao.org. Accessed: February 14, 2016.

KORISNOST 17-PLEX STR KITA ZA GENOTIPIZACIJU BOSANSKOG BRDSKOG KONJA

Dunja RUKAVINA[1], Amir ZAHIROVIĆ[2], Ćazim CRNKIĆ[3], Mirela MAČKIĆ-ĐUROVIĆ[4], Adaleta DURMIĆ-PAŠIĆ[5], Belma KALAMUJIĆ STROIL[5], Naris POJSKIĆ[5]

Univerzitet u Sarajevu-Veterinarski fakultet, Katedra za biologiju, Bosna i Hercegovina,
Univerzitet u Sarajevu-Veterinarski fakultet, Klinika za interne bolesti, Bosna i Hercegovina
Univerzitet u Sarajevu-Veterinarski fakultet, Katedra za hranu i ishranu životinja
Univerzitet u Sarajevu-Medicinski fakultet, Centar za genetiku, Bosna i Hercegovina
Univerzitet u Sarajevu-Institut za genetičko inženjerstvo i biotehnologiju, Bosna i Hercegovina

Izvod

U radu je primenjena savremena tehnologija ekstrakcije DNK i automatska genotipizacija u bosanskohercegovačkoj autohtonoj pasmini konja koristeći 17-Plex STR kit. Cilj rada je bio istražiti korisnost 17-Plex STR kita za genotipizaciju Bosanskog brdskog konja i ustanoviti najkorisniji sistem mikrosatelitnih markera za studije genetičke raznolikosti na ovoj pasmini. Genomska DNK je ekstrahovana iz pune krvi sakupljene od 22 Bosanska brdska konja koji nisu bili u srodstvu. Ukupno je detektovano 95 alela. Prosečan broj alela po lokusu je iznosio 5.588, a prosečan broj efektivnih alela po lokusu je bio 3.603. Rezultati srednje vrednosti uočene heterozigotnosi (0.631) su pokazali da je istraživana populacija potekla od odgovarajućeg broja roditeljskih generacija. Srednja vrednost očekivane heterozigotnosti (0.690) je ukazala na postojanje visoke genetičke varijabilnosti unutar Bosanskih brdskih konja. Vrednosti testa o sadržaju polimorfizma su pokazale da je 94.12% markera informativno i podesno za proveru genetičke raznolikosti. Najveću polimorfnost je pokazao lokus HMS2, dok je za lokus HMS7 ustanovljena najmanja polimorfnost. Primenjeni set od 17 mikrosatelitnih markera se pokazao koristan za upotrebu u genotipizaciji Bosanskog brdskog konja. Kada se uzimu u obzir vrednosti standardnih parametara heterogenosti preko 0.6 (uočena i očekivana heterozigotnost, kao i test o sadržaju polimorfizma), pet mikrosatelitnih markera (HTG4, AHT4, AHT5, ASB2, HMS2) se pokazalo kao najkorisniji za studije genetičke raznolikosti na pasmini Bosanskog brdskog konja.

Primljeno 01.VI.2018.
Odobreno 18. V. 2019.