Parentage Tests in Khuzestan Buffaloes Populations Using Three Microsatellite Markers

Homayoun Banani Rad[1*], Jamal Fayazi[1], Cyrus Amirinia[2], HedayatolahRoshanfekr[1], Hamidreza Seydabadi[2]

[1] Department of Animal Science, Ramin Agriculture and Natural Resources University, Khouzestan, Iran

[2] Department of Biotechnology, Animal Science Research Institute, Iran

ABSTRACT

Pedigree information and phenotypic records are the key features in animal genetics and breeding. Other wise best methods for genetic evaluation and estimation of parameters will be tending to no correct result s. This study was aimed to investigate and verify pedigree relations among 76 individuals who were genot yped for 3 fluorescent labeled microsatellite markers picked up from FAO-ISAG joint recommended list as one multiplex PCR set. 11 sires, 11offspring and 11 dams with 43 unknown samples were analyzed. Ba sed on exact allele sizes, 33 alleles were observed within the studied population. The mean number of all eles per locus was 11. Polymorphism information content (PIC) values ranged from 0.3257 to 0.8043 with the mean value of 0.6213. The most informative loci were :BM2113 (16alleles,PIC=0.7338), BM 1818(3alleles,PIC=0.3257).The indices of genetic variability within population and polymorphism of loc i including average heterozygosis, the number of observed and effective alleles, polymorphic information content (PIC) and so on showed that the studied khouzestan buffaloes population and used markers have still a reliable diversity and polymorphism level, respectively. Also, combined probability of exclusion (P E) values obtained per all loci in both parentage and identification analysis was 0.9381 and 0/4483thatindi cate the high efficiency of studied marker set for parentage and identification test in this population

Key words: Parentage testing, Identification, khouzestan buffaloes population, Microsatellite markers

INTRODUCTION

In the USA and Canada during the 1970s and in the early 1980s. Each year approximately 80 young bulls are entered into the progeny-testing programmer by the Iranian animal breeding center, of which 12–20 b ulls would be selected as proven sires (Dadpasand et al., 2008). The increase in genetic progress depends highly on accurate evaluation based on the entire and correct pedigree (Zhang et al., 2010). Inaccurate ped igree information is a common problem in the livestock industr, and paternity pedigree errors always have

a substantial negative impact on the national genetic evaluation and estimates of inbreeding (Kioset al., 2 011). Van Vleck (1970 a, b) demonstrated that incorrect identification of sires, in cattle data, could cause biased estimates of heritability, evaluations of sires and estimates of genetic progress due to selection. Sel ection decisions based on the best linear unbiased prediction (BLUP) are more accurate because BLUP ta kes account of all available relationships and pedigree information. However, when pedigrees contain err ors, estimation of heritability is mostly doubtful. The use of genomic selection (GS) also depends on the a ccuracy of the GS models to predict the breeding values (BV). Improvements in BLUP BV can be obtaine d simply by correcting errors in the pedigree or using more complex approaches, such as applying a realiz ed relationship matrix (RRM) in the BLUP prediction as an alternative to the relationship matrix (A) base d on expected values derived from the pedigree (Munoz et al., 2011). Due to the above facts, there is a ne ed for tools or indicators to check the correct paternity relationships. Nowadays DNA analysis and micros atellite markers have become a powerful tool for verifying the parentage and identification of individual a nimals (Rehout et al., 2006). Microsatellite markers, because of several advantages such as their high poly morphism content, widespread distribution in the genome and easily interpretable results are markers of c hoice (Baron et al., 2002). Regardless of the importance of microsatellite markers for parentage test, it als o may be prone to errors in every step of the genotyping process, from initial sampling to allele scoring an d data entry, which could affect parentage analysis (Bonin et al., 2004). Applying advanced methods such as capillary electrophoresis and their many advantages, including separation efficiency, short analysis tim e, low sample and solvent consumption, low cost of running and lower effect of matrices compared with t he other separation techniques could determine accurate allele size (Mitchelson and Cheng, 2001). The ai m of the present study was to validate pedigree relationships using a multiplex microsatellite marker assa y as a critical step for Holstein cattle genetic evaluation and also to investigate the efficiency of 12 ISAG/ FAO recommended microsatellite loci used for parentage tests of Iranian Holstein cattle.

MATERIAL AND METHODS

Animals
Overall, the pedigree of 76 animals was tested for both parentage control and identity. Regarding the pre-assumption of pedigree, 11 sires along with 54 offspring and 11 dams were selected for paternity allocatio ns.

DNA extraction
Blood samples in offspring and dams were collected from the jugular vein, supplemented with 0.5 M ED TA (pH=8) and transferred to the laboratory freezer (-20°C). Genomic DNA was extracted by modified sa lting out method (Miller et al., 1988) and purity of all extracted DNA was assessed by calculating the 260 /280ODratio determined with the Nano drop (ModelND1000).

Primer sets and method of genotyping
In the present study, 3 microsatellite loci from an ISAG/FAO joint recommended list of markers in bovin e genotyping were co-amplified using 3 primer pairs. The forward primer of each locus was end labeled with a fluorescent dye. Table 1 shows the loci characteristics and the primers used in amplifying each loc us.

Table 1: chromosome number, Allele size range and fluorescent-labeled dye

Locus	Chromosome	Allele size range	Dye
BM1818	23	248–276	Green
BM1824	1	170–218	Black
BM2113	2	116–146	Blue

bp: Base pair

The amplification of microsatellite sequences was performed by multiplex PCR reaction using commercially available bovine genotype panel 1.3 (FINNZYMES DIAGNOSTICS, F-904, FINLAND) in a 25 µl of the reaction volume containing 2 µl (50-100 ng) of genomic DNA and 18 µl of master mix according to manufacturer's instructions. Polymerase chain reaction was carried out in a Master Cycle gradient PCR system (Eppendorf) with the following PCR programmer: initial denaturation for 1 min at 98°C, followed by 30 cycles of denaturation at 98°C for 20 s, annealing temperature at 60°C for 75 s and extension at 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products were run on ABI PRISM Genetic Analyzer 3130 (Applied Bio systems Inc., Foster City, CA) capillary electrophoresis in the presence of GeneScan-500 LIZ internal size standard (Applied Bio systems Inc., Foster City, CA), which is designed for sizing DNA fragments in the 35–500 bp range and provides 16 single-stranded labeled fragments. During the process and due to normalization of data we also used bovine genomic DNA at the 0.5 mg/µl concentration as a control for verification of acceptable PCR and electrophoresis conditions. The raw data were collected using Data Collection software version 4.0, which was installed on the ABI system. Fragment analysis of PCR products was then performed by Gene Mapper software version 4.0 (Applied Bio systems Inc., Foster City, CA).

Statistical analysis
After the adjustment and normalization process using binning alleles in Excel, measures of genetic variation including observed and effective number of alleles and their frequencies, observed and expected heterozygosis, and Shannon index were calculated using Gene Alex 6.4 software. Polymorphism information content (PIC) and exclusion probability (EP) value of each locus were obtained by CERVUS 3.0 software (Kalinowski et al., 2007). Parentage and identity test according to most likely candidate parent was also done with CERVUS 3.0.

Diversity of microsatellites
According to calculated diversity indices, all microsatellite loci were polymorphic and a total of 34 alleles were identified in the present study. The number of alleles per locus ranged from 4 (BM1818) to 17 (BM2113) with the overall mean number of 11 alleles per locus. The results of the microsatellite marker potential, expressed by expected heterozygosis, and polymorphism information content (PIC) are shown in Table 2.

Table 2: Informativeness of analyzed markers in the khouzestan buffaloes

Locus	NA	Effective Allele	Ho	He	PIC
BM1818	4	1.527	0.85	0.345	0.3393
BM1824	13	5.74	0.982	0.827	0.8043
BM2113	17	4.356	0.986	0.77	0.7338

Multiplex PCR

In the present study ,angle multiplex PCR using primer pairs of 3 microsatellite markers first evaluated for each individual locus by the sharpness of band and easy optimization and then the PCR products of the loci were mixed and based on fragment size they were grouped for multiplex system. Using multiplex PCR allows the target sequence to be amplified simultaneously by using several pairs of primers in the reaction which substantially saved time and cost in this study to prevent overlapping among loci with the same color labeling, a suitable distance should be considered between loci, which was done for 3 microsatellites in the present study. A comparison between the allelic size ranges of the studied loci and ISAG allelic size range showed the following differences: markers were in a different and slightly lower range than ISAG reported range. Table 3 shows this comparison between allele size rang in study with I SAG.

Table 3: comparison between allele sizes rang in study with ISAG

locus	ISAG	Buffalo (present Study)
BM1818	248–276	256-266
BM1824	170–218	171-212
BM2113	116–146	118-145

Fragment analysis

Fluorescently labeled PCR fragments were detected in Genetic Analyzer 3130 and then analyzed in Gene mapper software. This software uses the size standard (Genes can 500 LIZ) to create a standard curve for each lane and then determines the length of each dye-labeled fragment by comparing it with the standard curve for that specific lane. An example of individual genotyping in loci labeled with NED and VIC are shown in Figure 1. Each single peak in the Figure shows a homozygote genotype and double peaks indicate the heterozygote genotype of the sample.

RESULTS AND DISCUSSION

Threat incidence of genotype at Buffalo

Position BM2113 has the most account and Genotypes were determined at 89% of Buffalo. The lowest ac

count is for Position BM1818 that Genotypes were determined at 25% of Buffalo. The table 4 shows the percent determination of genotyping of 3 markers used.

Table 4: Threat incidence of genotype at Buffalo

locus	Buffalo	Percentage%
BM1818	20	25
BM1824	27	36
BM2113	68	89

Place BM1818

This place Buffalo Genotypes were determined in 20 genotypes of the two individual heterozygous and 3 homozygous genotype for these loci showed. Number of alleles, effective number of alleles at these loci 1 7 and 527/1 respectively. The allele size range was 256 to 266 bp,. The most frequent allele frequency bet ween 0/789 respectively. Null allele (s) for this position +0 /5421 was calculated. Like Carolina and colle agues (2009) analyzed 10 microsatellite markers with the highest number of alleles and allele status equal to 11 Brdbaka and colleagues (1999) reported in their study of 10 alleles for this position. Hashemi 1390 number of alleles at these loci, 6 alleles effective 95/2 reported the results are inconsistent.

Place BM1824
Genotypes were determined at 27 Place, 19 Buffalo genotype heterozygous and 8 homozygous genotype s for these loci showed. Number of alleles Views At this location 13 and effective number of alleles 5.74 were estimate. The size of alleles was In the range of 171 to 212.The most frequent allele wasBetween0/2 778.Null allele frequency for this location +0 / 0705 was calculated .Hashemi for This location, 8 alleles o bserved in 1390.Frequency of The most frequent allele wasBetween0.2554.The size of alleles was in the r ange of 178 to 214 and average of frequent Null allele for Null location calculated. Ozkan et al, 8 alleles r eported for these loci in the range of 192-178 in 2009. Pvtnvva et al (2011) alleles ranged 125-139 reporte d for this   location. Zhang et al, (2010) six alleles reported for this indicator in Iran Holstein cows and the research was reported in the allele ranges between 202-176.Astyvanvykand colleagues (2010), allele rang e reported for this position 190-176. These differences could be due to difference in the strains studied.

Place BM2113
Genotypes were determined by the position of the 68 buffalo That 63 individuals heterozygous genotype and 5 individuals homozygous genotype Showed to this position .At this position , Number of Views allel es 16 and effective number of alleles 4.356 Were estimated. allele sizes were in the range of 118 to 145. Hashemihave estimated the number of alleles observed 9 and effective allele number 4.58 at this location .The size of alleles have reported in the range of 113 to 137.In a study was by Zhang et al (2010), 8 alleles was observed for this position .Ozkan et al (2009) reported 12 alleles and allele size range of the 123-149 for this position. Also Pvtnvva and colleagues (2011) reported 7 alleles in the range of 139-125 for this p osition. AmiriNia reported six alleles for this position in 1390 and allele range reported between 150-126

.Astyvanyk and colleagues (2010) reported allele range of 122-142 for this position. Zhang (2010) reported 122-137allele range for this position. The results of many researchers are close to results of this study in this position.

Allelic average of positions

Checking allelic frequency of 18 positions of review genotype was determined in 76 samples in this research, showed 241 allele in this population. Between microsatellite status of surveyed, the position BM2113 with 17alleles, largest number of alleles and the position BM1818 with 4 alleles, the lowest number of alleles had assigned to own .Allelic average for every position was estimated In this population. Allelic spectrum was observed between 2-22. Zhang has reported this spectrum for 13 marker 6-10In 1390. PositionSPS115 with10allelehas the most alleles .The same position has 12 alleles in this research. Astyvanyk and colleagues (2010) did genealogy test with11 marker at cow and reported domain6-14that the most alleles is for positions TGLA53. Zhang et al (2010) reported domain 6-16 by using 17 markers that the most alleles for position TGLA122 and the same position was observed 18 alleles.

The number of effective alleles

The number of effective alleles showed a wide range, so that the minimum effective number of alleles that are relevant to the position BM1818 and the most effective alleles of the BM1824 place 5/740 indicated.

Allele range

Compared to the range of allele status with allege fragment range listed by the International Association of Animal Genetics, There is very little difference in the way that some standing range with a small band-band range reported by the International Society of Animal Genetics showed. The main reason for this difference could be related to the small population and the low number of samples. Other researchers have confirmed that this is due to the small size of the population has an impact on allele size range (Hashemi, 1390, Amin Afshar, 1387).

Shannon index

Level of genetic diversity can be determined by Shannon's information index. Since the heterozygosis for each   number of Alleles is the final level to 1, heterozygosis values Is not as a measure of variation within a population for accurate Micro satellite Markers and differences of Views Does not provide true information at this level.

Wright index

The values obtained is for Wright Index at the table 5 that Negative values shows Increase the proportion of heterozygous than homozygous. Negative values of Wright index shows decrease of inbreeding in the population and increase of heterozygous of views is seconder for this reason.

Table 5: Values obtained for Wright Index

Position	Wright Index
BM1818	-1.464
BM1824	-0.213
BM2113	-0.308

Hardy - Weinberg equilibrium

In this study, 3 position of the microsatellite examined by Chi-square test and CERVUS software also Gene Alex the table 6 shows the results.

Table 6: Test for Hardy - Weinberg equilibrium CERVUS software

Position	Chi-square	Degrees of freedom	p-value	Degree of Significant
BM1818	2.6063	1	0.1064	NS
BM1824	9.153	3	0.0273	NS
BM2113	56.2212	6	<0.00000001	***

Chi-square values listed at the table 6 has been reported by Yates correction1 and degree of Significant by correction Bnfrvny and Only Position BM2113 showed Significant deviation from the equilibrium.

Table 7: Test for Hardy - Weinberg equilibrium Gene Alex software.

Position	Degrees of freedom	Chi-square	Probability	Degree
BM1818	6	43.828	0.000	***
BM1824	78	134.077	0.000	***
BM2113	120	537.789	0.000	***

As at table 7 can be viewed using this software did not none of the position in balance. The reason of this is that the software reports degree of significant without correction Bnfrvny. Power to detect each of the 3 positions microsatellite of review in this study that as normative for show the ability of a marker issued at exception and identify the correct parent at genealogy test and was estimated by CERVUS software.

Parentage and identity tests

In parentage testing, the usefulness of any co-dominant marker is defined as the probability of it making e xclusion and called exclusion probability (EP). The EP values of this population were calculated in CER VUS 3.0. For each offspring, CERVUS calculates the likelihood of parentage of every candidate parent or parent pair.

Table 8: Parentage and identity tests

Locus	first parent	Second parent	Parent pair	identity	sib identity
BM1818	0.9381	0.8099	0.6743	0.4483	0.6896
BM1824	0.5066	0.3364	0.1556	0.0509	0.3505
BM2113	0.6124	0.4336	0.2404	0.0861	0.3893
PE	71	99.89	99.98	99.99	0.99.90

REFERENCES

Baron E.E., Martinez M.L., Ver neque R.S., CoutinhoL.L. (2002). Parentage testing and effect of misidentification on the estimation of breeding value in Gir cattle. Genet. Mol. Biol., 25 (4): 389–394.

Bonin A., Belle main E., B r o n k e n -Eideen P., Pompanon F., BrochmannC., Ta berle t P. (2004). How to track and assess genotyping errors in population genetics studies. Mol. Ecol., 13 (11): 3261–3273.

Carolin o I., Sousa C.O., Ferreira S., Carolin o N., Silva F.S., Gam a L.T. (2009). Implementation of a parentage control system in Portuguese beef cattle with a panel of microsatellite markers. Genet. Mol. Biol., 32 (2): 306–311.

Dadpasand M., Miraei - Ashtiani S.R., Moradi Shahrebabak M., VaezTorshizi R. (2008). Impact of conformation traits on functional longevity of Holstein cattle of Iran assessed bya Weibull proportional hazards model. Livest Sci., 118: (3) 204–211.

Kalinowski S.T., Taper M.L., MarshalT.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol., 16 (5): 1099–1106.

Kios D., Marle - Koster E.V., VisserC. (2011). Application of DNA markers in parentage verification of Boran cattle in Kenya. Tropical Animal Health and Production. Trop. Anim. Health Pro., 44 (3): 471–476.

Miller S.A., DykesD.D.,Polesky H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res., 16 (3), p. 1215.

Mitchels on K.R., Cheng J. (2001). Capillary Electrophoresis of Nucleic Acids (Volume I: Introduction to the Capillary Electrophoresis of Nucleic Acids). Humana Press, Totowa, New Jersey, 484 pp.

MunozP., Resende M., Peter G., HuberD., Kirst M., Quesada T. (2011). Effect of BLUP prediction on genomic selection: practical considerations to achieve greater accuracy in genomic selection. Tree Biotechnology Conference: From Genomes to Integration and Delivery, Brazil, 49.

Ozakan E., Soysa l M.I., Ozder M., Koban E., Sahin O., Togan I. (2009). Evaluation of parentage testing in the Turkish Holstein population based on 12 microsatellite loci. Livest Sci., 124 (1): 101–106.

Putnova L., VrtkovaI., Srubarova P., StehlikL. (2011). Utilization of a 17 microsatellites set for bovine traceability in Czech cattle populations. IJAS., 1 (1): 33–38

Rehout V., Hradecka E., Citek J. (2006). Evaluation of parentage testing in the Czech population of Holstein cattle. Czech. J. Anim. Sci., 51 (12): 503–509.

Van VleckL.D., (1970 b). Misidentification and sire evaluation. J. Dairy Sci., 53 (12): 1697–1702.

VanVleck L.D. (1970 a). Misidentification in estimating the paternal sib correlation. J. Dairy Sci., 53 (10): 1469–1474

Zhang Y., Wang Y., SunD., YuY., ZhangY. (2010). Validation of 17 microsatellite markers for parentage verification and identity test in Chinese Holstein cattle. Asian Austral. J. Anim., 23 (4):425–429.