IJAS Iranian Journal of Applied Animal Science

Microsatillate based Parentage Verification in Crossbred Sheep Herds

Research Article

K. Kasraei[1], S.A. Rafat[1*], A. Javanmard[1], J. Shoja[1], M. Aabdelsayed[2] and M. Khansefid[3]

[1] Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2] Department of Animal Bioscience, Faculty of Veterinary Science, University of Sydney, Sydney, Australia
[3] Department of Environment and Primary Industries, AgriBio, Ring Road, Bundoora, VIC, 3083, Australia

Received on: 1 Jan 2017
Revised on: 17 Apr 2017
Accepted on: 30 Apr 2017
Online Published on: Dec 2017

*Correspondence E-mail: rafata@tabrizu.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Parentage testing is an important tool in farm animals for genetically determining the accuracy of pedigree information. The objective of the current study was to implication of multiplexing 14 microsatellite markers for routine parentage testings. The twenty-four lambs were crossbred of Ghazel × Baluchi, Ghazel × Baluchi × Merinos, and Baluchi × Moghani × Merinos breeds. The genomic DNA was extracted from the whole blood samples and genotyped using fragment analysis method. The highest and the reproducible multiplex group appeared by grouping ILSTS0049, MCM512, BMC1009, BM148 and CSSM032 loci in 5-plex reaction. The markers were first evaluated based on the number of alleles, allelic frequency, polymorphism information content (PIC), expected heterozygosity (H_E), observed heterozygosity (H_O) and the individual exclusion probability using popgene and cervus software. The average heterozygosity, polymorphism index content (PIC) and number of alleles per loci were 0.60, 0.58 and 4.93, respectively. The total exclusion probability of 14 microsatellite loci was 0.9999 in the population by compatibility according to the Mendelian fashion. The pedigree was considered incorrect in one out of all the evaluated progeny, as the genotype of that progeny did not match to any of its parents. The results of our study suggest the multiplex microsatellite panel a fast, robust, reliable, and economically efficient tool to verify the parentage and hence it can be used in the routine parentage testing in sheep.

KEY WORDS microsatellite, parentage control, pedigree errors, sheep.

INTRODUCTION

Pedigree validation is an essential step in breeding programs in most livestock breeds. The pedigree errors have a large impact on the efficiency of a selection response of the breeding program (Parlato and Van Vleck, 2012) and raise the question of trusting in the issued pedigree certificates (Leroy *et al.* 2011). Although pedigree information has an important role in the breeding programs, its recording is not an easy task, especially in breeds which are raised under an extensive production system. There are many factors contributing to the difficulty of recording of pedigree information, notably costs involved in mating system control and registration of offspring at birth. Therefore, cost-effective parentage control systems that can be implemented under common production conditions are very important for both conservation and improvement programs in livestock (Leroy *et al.* 2011). Pedigree errors in animal breeding could happen due to several factors. Errors are commonly observed in extensive breeding systems where natural mating of multiple sires is practiced and recording systems are poorly established. Parentage misidentification may also occur in a controlled system due to human errors in mothering up, recording and in the artificial insemination process,

and also due to errors made by the animal itself for example animals jumping fences (Souza *et al.* 2012). DNA testing to identify the correct parentage is the best solution to overcome the effects of pedigree error on genetic evaluation (Parlato and Van Vleck, 2012; Souza *et al.* 2012). Microsatellites are extensively used in livestock parentage control because of their many advantages, including high variability due to high mutation rates, simple assays, high distribution in genome coverage and co-dominance, rapid detection (Goldstein *et al.* 1995). Mutations typically result in length variations, with the number of repeat units either increased or decreased in this sequence (Goldstein *et al.* 1995). Usha *et al.* (1994) and Heyen *et al.* (1997) suggested using at least five microsatellite markers in the same analysis to achieve a 0.99 probability of exclusion of an incorrect sire.

The validity of the pedigree records is a major concern in many countries such as Iran, where DNA testing is not routinely done to ascertain the parentage of the animals before registration. In many countries and especially in Iran, a rural enterprise, the males and females graze together and the pedigree of the offspring can be largely unknown. The lack of information from relatives in many Iranian sheep populations has hindered the development of optimum breeding strategies. To design an efficient improvement program and genetic evaluation system for Iranian sheep breed, as well as a crossbreeding program, accurate estimates of the population genetic parameters is pre-required and ideally all pedigrees and relationships should be correctly recorded. Otherwise, it can produce biased evaluations when pedigrees contain errors and procedures utilize wrong information from the relatives (Weller *et al.* 2004; Van Eenennaam *et al.* 2007). The most common mating approach in extensive sheep production systems is the use of multiple sires. The combination of these males makes the paternal origin of the new born individual as a challenging question. On the maternal side there might be a natural cross fostering. The challenged task included pedigree verification and parental identification using molecular marker information. Applying capillary electrophoresis since it has advantages of high separation efficiency, short analysis time, low sample and solvent consumption, low cost of the running and lower effect of matrices comparing with the other separation techniques (Stewart *et al.* 2011; Wang *et al.* 2009). The aim of the work could be addressed to the parentage control in the crossbred sheep herd, in order to discuss the problems of applicability of this method for parentage verification.

MATERIALS AND METHODS

Animals and location of study

The twenty-four animals in the experiment were obtained from a Khalat Poshan research farm in Basmanj located approximately 10 km from Tabriz city in the North West of Iran. The pedigree information used in this study were collected from the Breeding Station of Tabriz University during 2012-2013 and the experimental pedigree included 4 rams, 24 cross-bred lambs (14 males, 14 females) from Ghazel × Baluchi, Ghazel × Baluchi × Merinos and Baluchi × Moghani × Merinos cross-bred lambs. The selection of the animals for our study was based on their physical condition, general health and age. The breeding design was three two-way crosses, with one ram to 7-8 ewes ratio. The breeds were mated equally and each mating type was carried out in triplicate and all rams and ewes were allocated to the mating groups randomly. The mating was natural and went for a period of 51 days (three estrous cycles).

Blood collection and DNA extraction

The twenty-four blood samples were collected from the animals in the experiment at the research station through the jugular vein in tubes containing 10 µL 0.5 *M* EDTA (pH=8). The tubes were kept on ice before being stored at -20 ˚C. The DNA was then extracted from the thawed whole blood samples using the commercially available QIAGEN® (USA) DNA extraction kit, according to the manufacturers' instruction. An OD_{260} reading value of one corresponded to 50 mg double-stranded DNA/mL and OD_{260} 1 indicated 33 mg/mL single stranded DNA. The ratio of readings taken at 260 nm and 280 nm (OD_{260}/OD_{280}) was used as an indicator of the purity of the nucleic acid.

Loci characteristics

The 14 microsatellite markers were selected from the panels recommended by FAO (2004), as well as markers used in previous parentage verification studies on sheep and goats. Ten markers were selected from the ISAG panels. Four alternative markers were selected based on their usage in other studies and performance in the sheep population. The parameters for inclusion in the current test panel included previously reported (ISAG/FAO) levels of polymorphism, heterozygosity, null allele frequencies and fragment sizes. The 14 markers were grouped into different genotyping sets according to range and fluorescent labeling for cost-efficient genotyping (Table 1).

PCR mixture and program

The PCR for initial screening of the DNA samples from the twenty-four lambs for the 14 microsatellite loci was carried out in 25 µL volumes, comprising of 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTP, 0.01 m*M* of each primer (NEB®, UK), 50 ng of genomic DNA, and 0.2 U Taq DNA polymerase (Promega®, USA).

Table 1 Characteristics of microsatellite loci used for the present study

Locus	Motif	Primer sequence	Allele size range (bp)
ILSTS004	$(CA)_{16}$	F:CTTAAAATCTGTCTTTCTTCC R:TAGTGTGTATTAGGTTTCTCC	100-120
CSSM004	$(GT)_{10}(TA)_5$	F:ATGCGTCCTAGAAACTTGAGATTG R:GAAATCATCTGGTCATTATCAGTG	196-220
BM1312	$(CA)_{13}$	F:CCATGTGCTGCAACTCTGAC R:GGAATGTTACTGAACCTCTCCG	105-119
BM148	$(CA)_{11}$	F:AGGCACAGTACCACCCCTC R:CTCAGCCTCAGCACCATG	150-270
CSSM032	$(CA)_{19}$	F:TTATTTTCAGTGTTTCTAGAAAAC R:TATAATATTGCTATCTGGAAATCC	97-270
MCM512	$(GT)_{24}$	F:CTGAAGTGAAGGAAAGGGGACAC R:GGAATTAGAATATCATTCCTTCATCGTG	150-200
OarFCB5	$(GT)_3A(TG)_6TA(TG)_4$	F:AAGTTAATTTTCTGGCTGGAAAACCC R:GACCTGACCCTTACTCTCTTCACTC	70-100
BMC1009	$(AC)_{15}$	F:ACCGGCTATTGTCCATCTTG R:GCACCAGCAGAGAGGACATT	200-210
RM029	$(CA)_{12}$	F:ATATGTCTCTGCATATCTGTTTAT R:CTAATCCCATAGTGAGCAGACC	100-150
OarFCB11	$(GT)_{28}$	F:GCAAGCAGGTTCTTTACCACTAGCACC R:GGCCTGAACTCACAAGTTGATATATCTATCAC	121-143
OarFCB20	$(GT)_{14}$	F:AAATGTGTTTAAGATTCCATACAGTG R:GGAAAACCCCCATATATACCTATAC	94-128
BM415	$(TG)_{18}$	F:CAAATTGACTTATCCTTGGCTG R:TGTAACATCTGGGCTGCATC	100-150
BM1314	$(CA)_{17}$	F:TTCCTCCTCTTCTCTCCAAAC R:ATCTCAAACGCCAGTGTGG	100-150
TEXAN006	$(TG)_{17}$	F: AGGCAGTTACCATGAACCTACC R: ATTCCTGGTGGGCTACAGTCTAC	157-175

Unlabeled primers were used for this purpose. The touch-down PCR cycle was performed as follows: an initial denaturation for 3 min at 94 ˚C, followed by 10 cycles of denaturation for 45 s at 94 ˚C, annealing at temperatures from 65 ˚C with 1 degree reduction per cycle to 55 ˚C, and extension at 72 ˚C for 45 s, followed by 25 cycles of denaturation for 45 s at 94 ˚C, annealing at temperatures 55 ˚C, and extension at 72 ˚C for 45 s, and the final extension at 72 ˚C for 10 min.

Electrophoresis

The amplified products were separated by electrophoresis in 4% metaphor gels at 65 V for 2 to 3 h, depending on the expected allele size.

The allele sizes were estimated by comparison to a 25 bp ladder of molecular weight markers. This step was carried out to confirm the success of the PCR amplification, estimate the amplicon sizes, and identify the heterozygous loci for each ram. The microsatellite loci that did not show a satisfactory pattern of amplification or which were uninformative, were excluded from the analyses. The remaining 14 microsatellite loci were used for the subsequent analysis.

Multiplex PCR and capillary electrophoresis

Multiplex PCR allows more than one target sequence site or loci to be amplified simultaneously by using more than one pair of primers in the reaction. This has the potential to save time and reduce the lab works. In the present study, PCR was performed for each locus individually, the PCR products of the loci for each designed multiplex system were mixed, and the fragment sizes analyzed as a multiplex system. The fragment analysis or genotyping of the PCR amplicon were done using the genetic analysis system (CEQ, Beckman Coulter). The labeled PCR products can be deteriorated quickly on exposure to light, and so fragment analysis was performed on the same day as PCR. The PCR products, however, may be stored at 4 ˚C up to a week by wrapping the PCR tubes/plates with aluminum foil. The PCR products (0.4-1 µL of each loci) were transferred to the CEQ sample plate with a 20 µL multi-channel pipette. The size Standard (0.5 µL) and the sample loading solution (SLS) buffer were mixed together and 10 µL was transferred to each of the eight tubes of the eppendorf PCR strip, and subsequently aliquot to the wells in the sample plate. For identification of the fragment sizes, the CEQ DNA size Standard Kit-400 was used.

The DNA marker fragments were also labeled with well RED fluorescent dye and were from 60 to 100 bp in 10 bp increments and from 100 to 420 bp in 20 bp increments. The 14 microsatellite loci were grouped into PCR multiplex systems (3- to 5-plex systems) based on the above criteria.

Statistical analysis

The estimation of genotype and allele frequencies was performed using POP GENE software (Yeh *et al.* 1999). Allelic frequencies and number of alleles per locus were estimated by direct counting from observed genotypes. Heterozygosities, polymorphic information contents and exclusion probabilities were computed using the CERVUS (Ver. 2.0) software (Marshall *et al.* 1998). The theoretical powers for identity and parentage exclusion of these markers were calculated based on allelic frequency data. The combined powers of parentage exclusion (PE) were calculated in different scenarios; considering the situations where both parents are genotyped but only one parent is evaluated for exclusion (PE_1) or both parents are evaluated for exclusion (PE_2) and when only parent is genotyped and evaluated for exclusion (PE_3). The parentage analysis was conducted applying the levels of confidence of 95% and 80%. The simulation was run to estimate the resolving power of a series of loci given their allele frequencies, and to estimate critical values of the log-likelihood statistics logarithm of the odds (LOD) or Delta, so that the confidence of parentage assignments made using the parentage analysis module could be evaluated statistically. In the simulation analysis, we considered: 1000 offspring, 100 mothers and 10 fathers, 95% of sampled typed, 92% of loci typed, and 1% of genotyping error. The simulation was performed for either paternal or maternal side, and for both parents with known sexes, each time for two levels of confidence.

The assumption was that one of the parents was known. At first, the dam was assigned as known, then in a second run the sire was assigned as known. The wrongly recorded dams and sires in the pedigree file were put to missing. Parentage analysis was carried out in three steps: first assuming known dams and unknown sires; second, assuming known sires and unknown dams; and thirdly assuming that both of the parents were unknown. During this analysis to test each offspring, parentage was either assigned to the most-likely candidate parent (or parent pair) with a pre-determined level of confidence or was left unassigned.

RESULTS AND DISCUSSION

Genotypes were scored from all individuals based on the co-dominant nature of microsatellite loci (Figure 1).

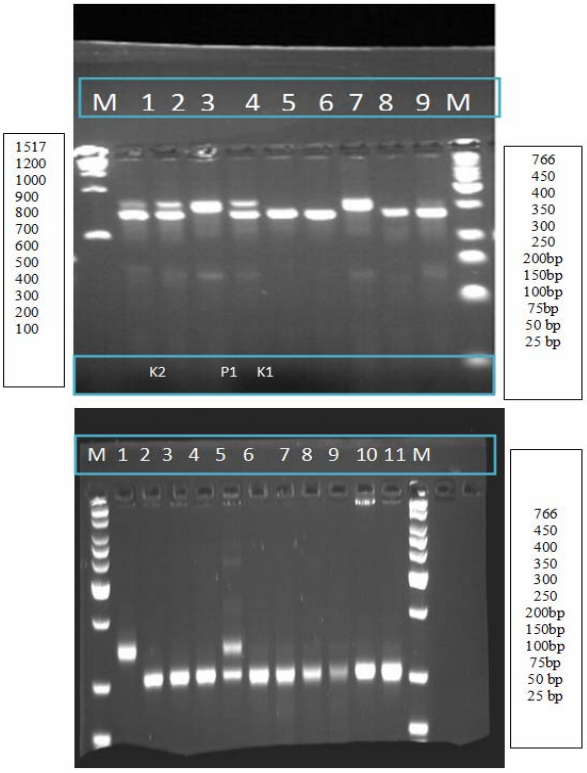

Figure 1 Genotype pattern and range of allele size of ILSTS0049 locus (100-120 bp) in upper gel and OarFCB5 (70-100 bp) in lower gel
P1 candidate's father for k1 and k2 carry similar allele size

Observed allele sizes at each locus were within the reported ranges of earlier studies (Table 2). Highest and the reproducible multiplex group was included as follows: ILSTS0049 (100-120 bp), MCM512 (150-180), BMC1009 (200-210 bp), BM148 (250-270 bp) and CSSM032 (270-290 bp) loci (Figure 2).

The average heterozygosities, polymorphism index content (PIC) and number of alleles per loci were 0.60, 0.58 and 4.93, respectively (Table 2). The total exclusion probability of 14 microsatellite loci was 0.9999 in the population by compatibility according to the Mendelian inheritance (Figure 3). The exclusion probabilities, as shown in Table 3, are values based on the allele frequencies of each of the markers alone, and can thus be computed in any family structure.

The alleles of the offspring were found to be compatible with those of the parents in all cases. The pedigree was considered incorrect in 1 out of all the evaluated progeny, as the genotype of that progeny did not match to any of its parents (Table 3).

Accurate pedigree information is required for a successful breeding program and improvement of productivity in the animal industry. Misidentification of parentage can lead to breeding inaccuracy, causing great financial losses in herd management and this is of more interest in beef industries (Cervini *et al.* 2006).

Table 2 Molecular diversity descriptive statistics of loci used in the present study

Marker	N_a	N	Ne	MFA (bp)	MFA	H_e	H_o	PIC	SI
ILSTS004	7	32	3.293	116	0.421	0.707	0.969	0.647	1.427
CSSM004	4	32	3.969	200	0.281	0.760	0.844	0.701	1.382
BM1312	3	32	2.018	104	0.641	0.512	0.281	0.433	0.841
BM148	3	32	1.857	101	0.656	0.469	0.469	0.368	0.707
CSSM032	4	32	2.379	217	0.531	0.589	0.719	0.499	0.991
MCM512	7	32	3.779	82	0.406	0.747	0.906	0.698	1.549
OarFCB5	5	30	3.100	112	0.467	0.692	0.833	0.632	1.304
BMC1009	7	31	3.219	288	0.468	0.701	0.742	0.647	1.440
RM029	4	30	2.748	95	0.533	0.647	0.733	0.587	1.181
OarFCB11	3	30	2.052	78	0.533	0.521	0.500	0.397	0.763
OarfCB20	3	29	2.154	130	0.552	0.545	0.586	0.437	0.848
BM415	6	31	3.308	115	0.484	0.709	0.935	0.663	1.449
BM1314	7	30	4.054	115	0.417	0.766	0.967	0.724	1.639
TEXAN006	6	30	4.018	153	0.333	0.764	0.733	0.709	1.508
Mean	4.93	30.93	2.996	-	-	0.652	0.601	0.582	0.688

Na: number of alleles; N: overall number of genotype; Ne: effective number of allele; MFA: minor frequent allele; He: expected heterozygosity; Ho: observed heterozygosity; PIC: polymorphism information content and SI: shanon index.

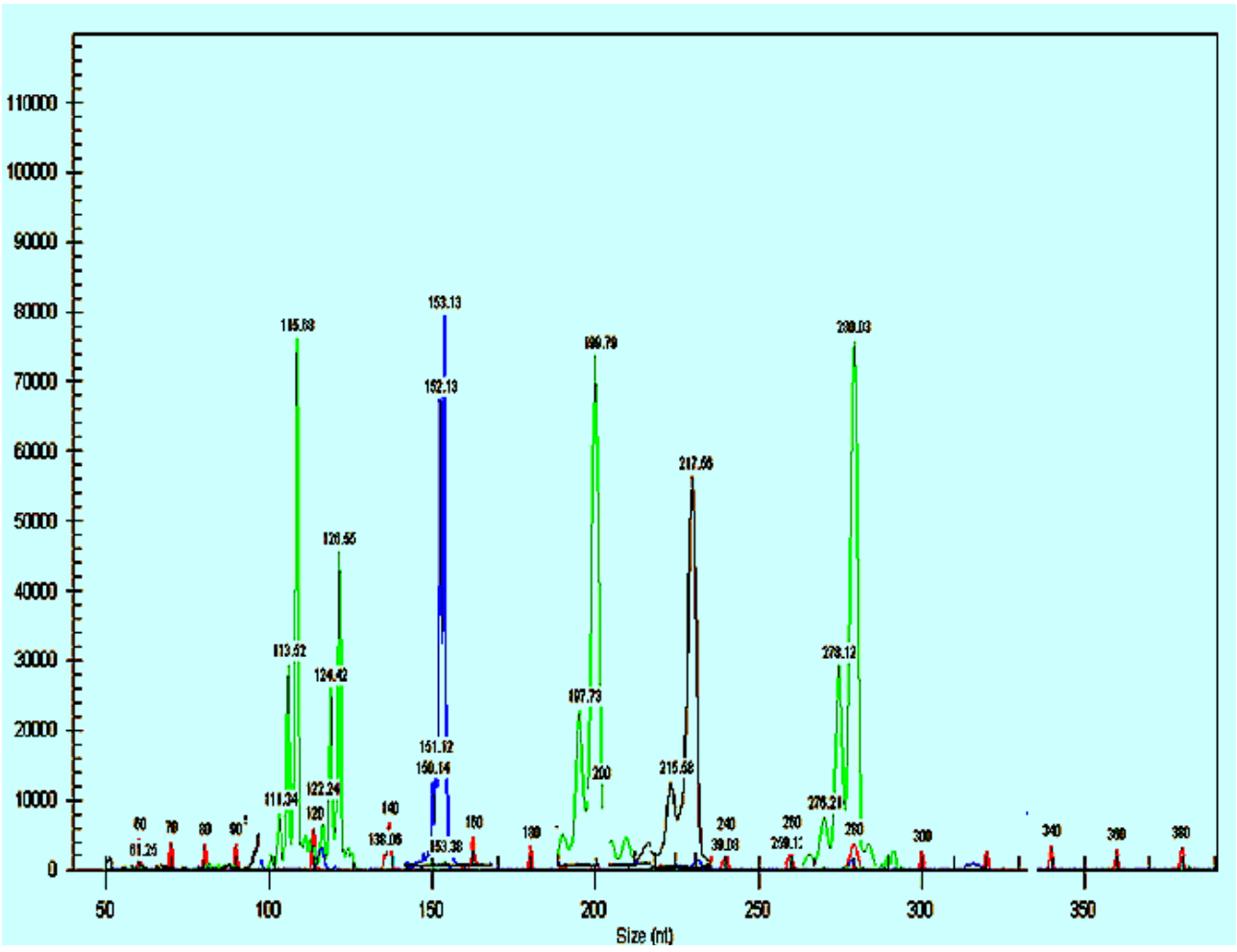

Figure 2 Capillary electrophoresis pattern of 5-plex microsatellite assay
(1): ILSTS0049 locus (100-120 bp); (2): MCM512(150-180); (3): BMC1009(200-210 bp); (4): BM148(250-270 bp) and (5): CSSM032(270-290 bp)

A small rate of percentage misidentification can excessively endangers genetic patterns estimation. The paternity misidentification rate of 11% would result in a decrease of 11-15% in the genetic trend for milk traits (Banos *et al.* 2001). Moreover, the pedigree errors may reflect on the structure of selection indexes (Přibyl *et al.* 2004). Inaccurate pedigree information is a common problem in livestock production and paternity pedigree errors have a substantial negative impact on the national genetic evaluation and estimates of inbreeding (Pollak, 2005; Dodds *et al.* 2007). The proportion of misidentified progeny varies between 2.9 and 23% of cattle worldwide (Weller *et al.* 2004; Van Eenennaam *et al.* 2007).

The parentage verification is crucial as accurate selection and genetic improvement rate has relied on valid pedigree information (Dodds *et al.* 2005) and it becomes more critical after widespread use of artificial insemination, embryo transfer and multiple sire breeding schemes in animal breeding.

The genetic relationships among individuals in the population can be determined based on either pedigree or marker information. In the pedigree approach, founder animals are assumed to be unrelated to each other. Also, there is a possible occurrence of recording errors in the pedigree file. Therefore, marker based inference to genetic relationships seems to be an alternative approach.

Since related individuals share more alleles than unrelated ones, relationships can be estimated from marker genotypes in every pair of individuals, including the founders. However, determination of the relationship which only based on the marker information is not an easy exercise (Weller *et al.* 2004; Van Eenennaam *et al.* 2007).

In a classical analysis of genetic relationship, one of the useful parameters is the power of exclusion (PE), the power of a genetic marker in excluding a non-related individual chosen by chance in a specific population, as an alleged father in a paternity investigation. The PE is a parameter to solve problems of some genetic markers in a population and is most commonly used as molecular markers in pedigree verification.

The paternity PE is the expected average probability that a polymorphic locus shows the exclusion of a male without kinship with the biological sire (Marshall *et al.* 1998). This index depends on the informative content of a locus, which depends on its number of alleles and its respective frequencies. Since the probabilities of exclusion of several loci, it is possible to calculate the combined PE (PEC), and the value of PEC is a function of the examined locus number, as well as of the informative content of each locus. The PE and CEP for each loci can be used in an analysis of genetic relationship. The genetic paternity testing can provide sire identity data for offspring when females have been exposed to multiple males. However, correct paternity assignment can be influenced by factors determined in the laboratory and by size and genetic composition of breeding groups.

When parents are assigned on the basis of LOD scores, the most likely candidate parent is the candidate parent with the highest LOD score. The likelihood ratio is the likelihood that the candidate parent is the true parent divided by the likelihood that the candidate parent is not the true parent. A negative LOD score shows that the candidate parent is less likely to be the true parent than an arbitrary randomly chosen individual. A LOD score of zero, indicates that the candidate parent is equally likely to be the true parent as an arbitrary randomly chosen individual. A positive LOD score means that the candidate parent is most likely to be the true parent than arbitrary randomly chosen individual (Marshall *et al.* 1998). Jakabova *et al.* (2002) have also revealed that at least, five microsatellites with the highest individual PE values that have a 97% total exclusion probability should be used to obtain a high degree of incorrect parentage exclusion. Usha *et al.* (1994) reported for cattle parentage control, a total PE of 0.88 for two microsatellite loci can be used. Marklund *et al.* (1994) analyzed eight microsatellite loci in paternity testing to reach at total exclusion probability of 0.96 to 0.99 in different sheep breeds. The pedigree errors were shown to occur in 1% of the cases tested in this study. This is out of the pedigree errors ranges recorded by Barnett *et al.* (1999) in sheep (8.7 to 15.5%) and by Visscher *et al.* (2002) in cattle (2 to 22%) using both the microsatellite markers. The 7 microsatellites evaluated in this study provide sufficient power (75.3%) to be useful in a parentage determination panel to confirm parentage of offspring which are going to be selected for future breeding animals. The possibility of multiple paternities from sheep may require a greater power of exclusion, which would be provided by using more loci. The 1% mismatch rate found among pedigree in this study was relatively low and comparable to pedigree errors reported for four sheep breeds in France (1 to 10%, Leroy *et al.* 2011) and for New Zealand sheep flocks (0.5 to 9.4%, Crawford and Buchanan, 1993).

Studies carried out on cattle (Geldermann *et al.* 1986; Banos *et al.* 2001; Baron *et al.* 2002; Visscher *et al.* 2002) have recorded higher mismatch rates (4-36%) based on microsatellite markers. These results indicated that more accurate ways of animal identification and parentage information are required. Several studies in cattle with simulation and real data showed that mismatch rate up to 5% have minimal effect on the estimation of genetic values, but the effect increased by increasing error rates (Van Vleck, 1970b).

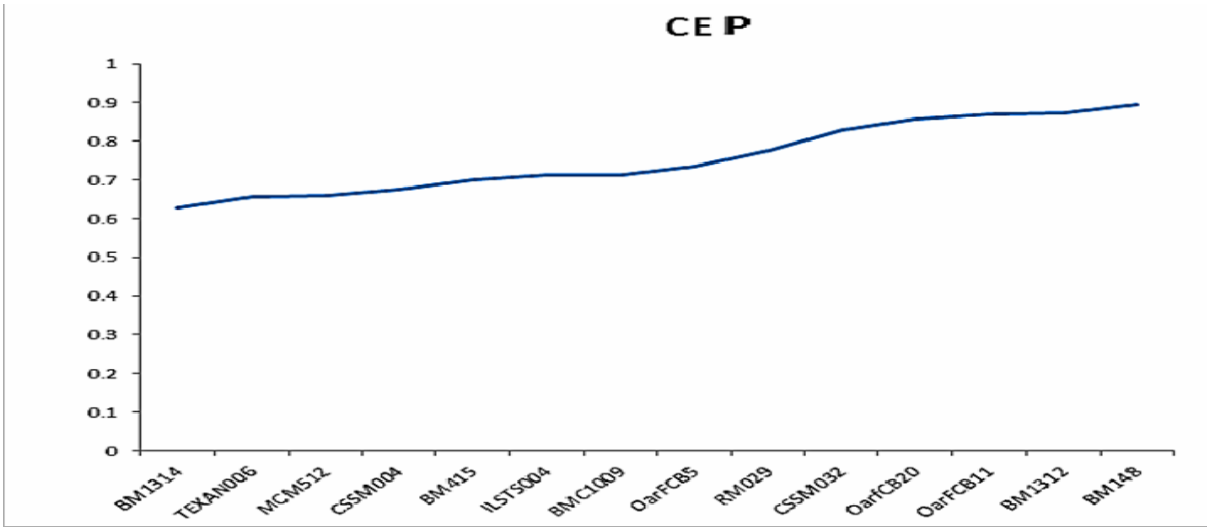

Figure 3 Combined probability of exclusion (CEP) in parentage testing with increasing number of microsatellite markers

Table 3 Combined powers of exclusion and probability of identity for a panel with different numbers of markers*

Locus	PE	PE_1	PE_2	PE_3
ILSTS004	0.356	0.141	0.713	0.542
CSSM004	0.325	0.111	0.674	0.498
BM1312	0.618	0.317	0.873	0.752
BM148	0.706	0.383	0.894	0.808
CSSM032	0.555	0.257	0.829	0.701
MCM512	0.291	0.108	0.661	0.483
OarFCB5	0.381	0.151	0.734	0.564
BMC1009	0.347	0.139	0.714	0.539
RM029	0.429	0.181	0.777	0.609
OarFCB11	0.687	0.353	0.869	0.792
OarfCB20	0.633	0.314	0.857	0.756
BM415	0.321	0.126	0.701	0.519
BM1314	0.249	0.090	0.629	0.446
TEXAN006	0.297	0.104	0.655	0.478
Cum. EP	0.99999	0.9999	0.9821	0.99932

* The combined powers of parentage exclusion (PE) were calculated, considering the situations where both parents are genotyped but only one parent is evaluated for exclusion (PE_1) or both parents are evaluated for exclusion (PE_2) and when only parent is genotyped and evaluated for exclusion (PE_3).

Table 4 A summary of some reported results for parentage control in different livestock species

Species	n	Country	SSR	Na	Electrophoresis	H_{obs}	H_{exp}	PIC	CEP (%)	Misidentification	Source
Sheep	32	Iran	14	3-7	Capillary electrophoresis	0.28-0.96	0.46-0.76	0.36-0.72	0.999	1%	Present study
Canine	9561	USA	17	2.9-8.4	ABI	0.57-0.64	-	0.53-0.61	99	-	DeNise *et al.* (2004)
Cattle	175	Kenya	11	7-13	ABIPRISM Genetic Analyzer	0.61-0.81	0.72	0.6901	0.999	0-5%	Kios *et al.* (2012)
Cattle	475	Portugal	10	8.20	ABI310	-	0.69 to 0.79 (0.73)	0.70	0.999	2%	Carolino *et al.* (2009)
Cattle	371	China	17	8.35	Capillary electrophoresis	0.68	0.711	0.667	0.999	3%	Zhang *et al.* (2010)
Sheep	50	Iran	7	2-7	Methaphor agaros	0.22-0.68	0.35-0.64	0.31-0.59	0.933	12%	Saberivand *et al.* (2011)
Buffalo	212	India	10	5-12	Automated DNA sequencer.	0.57-.82	0.64 to 0.89 (0.76)	0.60-0.80	0.993	19%	Jakhesara *et al.* (2012)
Goat	209	Poland	23	4-10	ABI3130 Genetic analyzer	0.37-0.79	0.37-0.77	0.36-0.76	0.955	17%	Siwek *et al.* (2010)
Sheep	83	Italy	24	2-30	ABI	0.48-0.83	0.50-0.93	0.736	0.999	20.3%	Rosa *et al.* (2013)

n: number of animals; Na: number of alleles; Ng: overall number of genotype; Ne: effective number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; PIC: polymorphism information content and CEP: combined probability of exclusion.

In this context, Sherman *et al.* (2004) and Van Eenennaam *et al.* (2007) recommended a non-exclusion probability value of 0.001 as an acceptable level for correctly identifying the real sire. In our present study the pedigree error rate observed was 4.9%, which is expected to have minimal effect on the genetic evaluation of the population. The microsatellite based parentage tests for relationship verification or assignment in case of unrecorded mating or multiple sires have been developed for many species, including dogs (DeNise *et al.* 2004), cats (Lipinski *et al.* 1999), horses (Tozaki, 2001a), cattle (Van Eenennaam *et al.* 2007), goats and sheep (Glowatzki-Mullis *et al.* 1995). Aiming to reach the minimum recommended combined non-exclusion probability of 0.001 suggested as an acceptable level for correctly identifying the real sire (Sherman *et al.* 2004; Van Eenennaam *et al.* 2007).

Many factors related to animal species, breed and geographic area could explain the differences between our present results and those reported in the literature. Likewise, it is supposed that genetic tools used such as electrophoresis methods, DNA markers and even the difference in candidate SSR locus as well as human error of technical staff and genoytyping errors should be taken into consideration in the analysis (Table 4).

CONCLUSION

These results show that the developed microsatellite marker panel has sufficient power for either paternity exclusion or assignment and for individual identification in crossbred lambs. The microsatellites multiplex ILSTS0049, MCM512, BMC1009, BM148 and CSSM032 microsatellites showed the highest and reproducible results. Therefore, the results of this study can be used in the parentage testing in practice to provide parentage verification and is expected to contribute to the quality control in the breeding system.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the university of Tabriz academic staff and Khalat Poshan research farm, Basmanj staff for collaboration during blood sampling and providing the pedigree information. This work was funded by Tabriz University Research Grant.

REFERENCES

Banos G., Wiggans G.R. and Powell R.L. (2001). Impact of paternity errors in cow identification on genetic evaluations and international comparisons. *J. Dairy Sci*. **84,** 2523-2529.

Barnett N., Purvis I., Van Hest B. and Franklin I. (1999). The accuracy of current dam pedigree recording strategies employed by stud Merino breeders. *Proc. Adv. Anim. Breed. Gen*. **13,** 373-376.

Baron E.E., Martinez M.L., Ver neque R.S. and 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.

Carolino I., Sousa C.O., Ferreira S., Carolino N., Silva F.S. and Gama 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.

Cervini M., Henrique-Silva F., Mortari N. and Matheucci E.J. (2006). Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). *Genet. Mol. Biol*. **29,** 486-490.

Crawford A.M. and Buchanan F. (1993). Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. *Anim. Genet*. **24,** 145.

DeNise S.E., Johnston J., Halverson K., Edwards J., Marshall K., Rosenfeld D., McKenna S. and Sharp T. (2004). Power of exclusion for parentage verification and probability of match for identity in American kennel club breeds using 17 canine microsatellite markers. *Anim. Genet*. **35,** 14-17.

Dodds K.G., Tate M.L. and Sise J.A. (2007). Genetic evaluation using parentage information from genetic markers. *J. Anim. Sci*. **83,** 2271-2279.

Dodds K.G., Tate M.L., McEwan J.C. and Crawford A.M. (2005). Exclusion probabilities for pedigree testing farm animals. *Theor. Appl. Genet*. **92,** 966-975.

FAO. (2004). Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended microsatellite markers initiative for domestic animal diversity. FAO, Rome, Italy.

Geldermann H., Pieper U. and Weber W.E. (1986). Effect of misidentification on the estimation of breeding value and heretability in cattle. *J. Anim. Sci*. **63,** 1759-1768.

Glowatzki-Mullis M.L., Gaillard C., Wigger G. and Fries R. (1995). Microsatellite-based parentage control in cattle. *Anim. Genet*. **26,** 7-12.

Goldstein D.B., Linares A.R., Cavallil-Sforza L.L. and Feldman M.V. (1995). Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc. Natl. Acad. Sci. USA*. **92,** 11549-11552.

Heyen D.W., Beever J.E., Da Y., Evert R.E., Green C., Bates S.R.E., Ziegle J.S. and Lewin H.A. (1997). Exclusion probabilities of 22 bovine microsatellite markers in fluorescent multiplexes for semi-automated parentage testing. *Anim. Genet*. **28,** 21-27.

Jakabova D., Trandzik J. and Chrastina J. (2002). Effectiveness of six highly polymorphic microsatellite markers in resolving paternity cases in Thoroughbred horses in Slovakia. *Czech J. Anim. Sci*. **47,** 497-501.

Jakhesara S.J., Rank D.N., Kansara J.D., Parikh R.C., Patel V.M., Vataliya P.H. and Solanki J.V. (2012). Parentage verification in field progeny testing program of Mehsana buffalo. *J. Anim. Sci. Dec*. **90(13),** 4723-4728.

Kios D., Estè van Marle K. and Carina V. (2012). Application of DNA markers in parentage verification of Boran cattle in Kenya. *Trop. Anim. Health Prod*. **44,** 471-476.

Leroy G., Danchin-Burge C., Palhiere I., Baumung R. and Fritz S. (2011). An ABC estimate of pedigree error rates: application in dog, sheep and cattle breeds. *Anim. Genet*. **43,** 309-314.

Lipinski M.J., Amigues Y., Blasi M., Broad T.E., Cherbonnel C., Cho G.J., Corley S., Daftari P., Delattre Luikart G., Biju-Duval M.P., Ertugrul Y., Zagdsuren C., Maudet C. and Taberlet P. (1999). Power of 22 microsatellite markers in fluorescent multi- plexes for parentage testing in goats (*Capra hircus*). *Anim. Genet*. **30,** 431-438.

Marklund S., Ellegren H., Eriksson S., Sandberg K. and Andersson L. (1994). Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. *Anim. Genet*. **25,** 19-23.

Marshall T.C., Slate J., Kruuk L.E.B. and Pemberton J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol*. **7,** 639-655.

Parlato E. and Van Vleck L.D. (2012). Effect of parentage misidentification on estimates of genetic parameters for milk yield in the Mediterranean Italian buffalo population. *J. Dairy Sci*. **95,** 4059-4064.

Pollak E.J. (2005). Application and impact of new genetic technologies on beef cattle breeding: a "real world" perspective. *Australian J. Exp. Agric*. **45,** 739-748.

Přibyl J., Šafus P., Štípková M., Stádník L. and Čermák V. (2004). Selection index for bulls of Holstein cattle in the Czech Republic. *Czech J. Anim. Sci*. **49,** 244-256.

Rosa A.J.M., Sardinae M.T., Mastrangelo S., Tolone M. and Portolano B. (2013). Parentage verification of Valle del Belice dairy sheep using multiplex microsatellite panel. *Small Rumin. Res*. **113(1),** 62-65.

Saberivand A., Javanmard A. and Safdari M. (2011). Parentage verification and identity test of Ghezel sheep using microsatillate markers. *African J. Biotechnol*. **10(31),** 5815-5819.

Sherman G.B., Kachman S.D., Hungerford L.L., Rupp G.P., Fox C.P., Brown B., Feuz M. and Holm T.R. (2004). Impact of candidate sire number and sire relatedness on DNA polymorphism-based measures of exclusion probability and probability of unambiguous parentage. *Anim. Genet*. **35,** 220-226.

Siwek M. and Knol E.F. (2010). Parental reconstruction in rural goat population with microsatellite markers. *Italian J. Anim. Sci*. **9(3),** 500-512.

Souza C.A., Paiva S.R., McManus C.M., Azevedo H.C. and Mariante A.S. (2012). Genetic diversity and assessment of 23 microsatellite markers for parentage testing of Santa Inês hair sheep in Brazil. *Genet. Mol. Res*. **11(2),** 1217-1229.

Stewart S.D., Wickramasinghe A., Dorrance E. and Robertsonc A.E. (2011). Comparison of three microsatellite analysis methods for detecting genetic diversity in *Phytophthora sojae* (Stramenopila:Oomycete). *Biotechnol. Lett*. **33(11),** 2217-2223.

Tozaki T. (2001a). Characterization of equine microsatellites and repetitive elements, and validation of paternity testing for Thoroughbreds. Ph D. Thesis. Showa Univ., Japan.

Usha A.P., Simpson S.P. and Williams J.L. (1994). Evaluation of microsatellite markers for parentage verification. Pp. 41 in Proc. 24th Conf. Int. Soc. Anim. Genet. Prague, Czech.

Van Eenennaam A.L., Weaber R.L., Drake D.J., Penedo M.C.T., Quaas R.L., Garrick D.J. and Pollak E.J. (2007). DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. *J. Anim. Sci*. **85,** 3159-3169.

Van Vleck L.D. (1970b). Misidentification and sire evaluation. *J. Dairy Sci*. **53,** 1697-1702.

Visscher P.M., Woolliams J.A., Smith D. and Williams J.L. (2002). Estimation of pedigree errors in the UK dairy population using microsatellite markers and the impact on selection. *J. Dairy Sci*. **85,** 2368-2375.

Wang X., Rinehart T.A., Wadl P.A., Spiers J.M., Hadziabdic D., Windham M.T. and Trigiano R.N. (2009). A new electrophoresis technique to separate microsatellite allele. *African J. Biotechnol*. **8(11),** 200-208.

Weller J.I., Feldmesser E., Golik M., Tager-Cohen I., Domochovsky R., Alus O., Ezra E. and Ron M. (2004). Factors affecting incorrect paternity assignment in the Israeli Holstein population. *J. Dairy Sci*. **87,** 2627-2640.

Yeh F.C., Boyle T. and Yang R.C. (1999). POPGENE Ver. 3.31. Microsoft Window-based freeware for population genetic analysis. University of Alberta, Edmonton, Canada.

Zhang Y.W., Sun D., Yu Y. and Zhang Y. (2010). Validation of 17 microsatellite markers for parentage verification and identity test in Chinese Holstein cattle. *Asian-Australasian J. Anim. Sci*. **23(4),** 425-429.